Phytochemistry of *Eucalyptus* spp. and Its Role in Insect-Host-Tree Selection

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STATMENT

This thesis contains no material which has been accepted for the award of any other higher degree or graduate diploma in any tertiary institution, and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Haifeng Li

ABSTRACT

Leaf oils and waxes of 29 native Tasmanian *Eucalyptus* species were studied from primarily a taxonomic aspect and their effects on insect-plant selection were evaluated. *E. nitens*, an important introduced pulpwood species that is not indigenous to Tasmania was also investigated in detail.

The chemical compositions of leaf oils and waxes of both juvenile and adult leaf samples of each species were examined by GC-MS. Results indicated that the distribution of both oil and wax components within Tasmanian eucalypts and their relative proportions between subgenera and species have taxonomic importance. A chemotaxonomic classification based on these data was constructed and this classification more functionally describes insect-host-tree relationships as opposed to the classification based on morphological differences.

The results of this study indicated that differences in leaf oils of eucalypt species are correlated to genetic divergence in the biosynthesis of terpenoid compounds and that biochemically related terpenoid compounds provide sensitive taxonomic characteristics for the identification of eucalypt species. A study, using material from species/provenance trials, indicated that seasonal variation in leaf oil chemicals was influenced by time, ontogenetic and physiological aging effects. The variation pattern of leaf oils between different species was found to be under strong genetic control and environmental factors tended to affect leaf oils quantitatively but not qualitatively.

For the first time the chemical composition of eucalypt leaf waxes of trees from a large number of species localities has been determined by the direct analysis of crude wax. In a representative number of species localities, wax morphology was examined by scanning electron microscopy (SEM). Two general wax categories, amorphous and structured were found, the latter including three types. The results indicated that wax structure was correlated with chemical composition and that the distribution and variation in both wax chemicals and wax types has relevance at all levels of the taxonomic hierarchy.

The chemical composition of leaf oils and waxes among most provenances and localities of *E. nitens* were examined. The differences in leaf oil characteristics of two forms of *E. nitens* has supported the separation of the two morphological forms into separate species, *E. nitens* and *E. denticulata*. Results indicated that the chemical

composition of leaf oils and waxes of *E. nitens* was similar to most *Symphyomyrtus* species but that oil yields from all *E. nitens* populations were significantly lower.

Commercially important eucalypt species are attacked by two paropsine defoliating beetles: *Chrysophtharta bimaculata* and *C. agricola* (Coleoptera: Chrysomelidae, Paropsini). The feeding and oviposition preferences of these beetles and the feeding response of their larvae to eucalypt leaves were examined in laboratory bioassay in which the effects of leaf oils and waxes were evaluated. The results indicated that leaf acceptance or rejection by *C. bimaculata* and *C. agricola* was correlated to differences in leaf oils and waxes which were chemotaxonomically correlated among Tasmanian eucalypt species. The toxic effects of 1,8-cineole and related chemicals on *C. bimaculata* beetles were confirmed by leaf extract and chemical bioassay. It was found that the leaves of *E. nitens* were successfully attacked by *C. bimaculata* through the lack of chemical defence, notably low levels of 1,8-cineole. The wax bloom on juvenile foliage acts also as a physical defence against adult *C. bimaculata* beetles. Results also indicated that leaf preference of *C. agricola* was related more to variation in the triterpenoid class of wax chemicals.

The inheritance of leaf oils and waxes in interspecific F1 hybrids of *Eucalyptus* was analysed. Results indicated that both oil and wax chemicals were strongly inherited in F1 hybrids and were valuable for identification of tree hybrids. Bioassay results indicated that feeding resistance was related to the inheritance of leaf oils in interspecific F1 hybrids. Hybrids between high and low cineole species had intermediate cineole levels and were therefore more resistant to *C. bimaculata* attack than the low cineole parent.

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SECTION 1

Chapter 1
Introduction

The genus *Eucalyptus,* which belongs to the family Myrtaceae, is one of the largest and most complex genera of woody plants in the world and is the dominant feature of the Australian vegetation except in drier desert areas and the closed rainforests. The genus contains 513 accepted species (Chippendale 1988) while many species contain either hybrids, clinal or other forms. Eucalypts are used mainly for pulpwood, poles, fuelwood, charcoal and sawn timber with minor forest products such as floral nectar for honey, bark for tannin, besides being extensively planted in certain areas for harvesting of their essential oil for pharmaceutical and industrial purposes.

Since the taxonomic history of eucalypts began in 1788 with Charles L' Heritier, it has been the subject of study by many botanists. Description of the large genus *Eucalyptus* has long been regarded as taxonomically difficult and, the delimiting of species within this genus is wrought with difficulties, many of which are inherent in the genus itself. The most recent treatment of *Eucalyptus* and angophoras in the 'Flora of Australia' series (Chippendale 1988) bring together all currently accepted species and presents a formal classification at the series level. However, in the past 20 years taxonomic research into the genus has been concerned mainly with the structure of the genus and inter-specific relationships. 'A classification of the *Eucalyptus*' by Pryor and Johnson (1971) recognised seven subgenera within the genus *Eucalyptus*. Moreover, some consideration has been given to re-organising *Eucalyptus* into eight genera, by raising the subgeneric groups to generic level (Johnson and Briggs 1983), although, a detailed revision along these lines awaits publication, and there may be resistance, for morphological or practical reasons, to its widespread acceptance (McAlpine 1986). Whereas such studies for eucalypt taxonomy are based on the premise that species, as currently accepted, are valid biological taxonomic groups. Many species of eucalypts are poorly defined and knowledge of their biology is limited. This is apparent when one considers that over 1200 names have been applied to species (including binomials applied to hybrids) of *Eucalyptus* (Chippendale 1976).

Many of the taxonomic problems of *Eucalyptus,* particularly at the species level, can be attributed to the large amount of genetic variability maintained in most eucalypt populations, and the ability of species to perform reproductive manoeuvres of

considerable complexity. The patterns of variation in many cases have been defined to mainly comprise hybridisation between species with systematic (i.e. taxonomic) affinities and the clinal variation that can occur within individual species and between closely related species. However, there is a limitation in the range of available characteristics and a lack of knowledge regarding their variability under natural conditions. Where variation has been described, it represents only a small proportion of the total genus.

Eucalyptus trees are widely distributed throughout Tasmania. Members of the genus occupy virtually all habitats from the sub-alpine environments on very poor soils found in south-west Tasmania with an annual rainfall in excess of 2,500 mm, through to the tall wet sclerophyll forests where tree heights may exceed 100m, and to the dry sclerophyll woodlands with an annual rainfall of less than 500mm per annum (Davidson *et al.* 1981).

There are twenty nine species of eucalypts native to Tasmania, of which seventeen are considered to be endemic. According to the classification of Pryor and Johnson (1971), the genus *Eucalyptus* is divided into eight subgenera, only two of which have been recorded to occur in Tasmania. They are the subgenera *Monocalyptus* and *Symphyomyrtus*. The *Monocalyptus* species in Tasmania all fall into the two series of the *Obliquae* (commonly known as the ashes) and the *Piperitae* (commonly known as the peppermints). The *Symphyomyrtus* species also come from only two series of the subgenus, the *Ovatae* and the *Viminales,* and are commonly known as the gums. The 29 Tasmanian eucalypt species comprise only five per cent of the number of *Eucalyptus* species occurring in Australia (Duncan 1989). However, variation in some morphological characteristics of eucalypts is possibly more pronounced in Tasmania than elsewhere. While most of the ashes exhibit less pronounced clinal variation and less intergradation between species allowing individual species to be readily identified in almost all situations, extreme variation occurs in the peppermint and gum groups in which many clinal intergradations and hybridisations between species have been found. These variations in morphological characteristics do not completely match with those of species described or illustrated in texts (e.g. Chippendale 1988). Difficulty is often experienced, therefore, in positively identifying trees in the peppermint and gum groups.

Eucalypts support many insect species, and many of these depend completely on one or more subgenera for their survival. In Tasmania, eucalypt species from both subgenera *Monocalyptus* and *Symphyomyrtus* are attacked by species of leaf feeding paropsine beetles of the family Chrysomelidae. *Chrysopharta bimaculata,* the major pest of eucalypt forestry in the state, is associated predominantly with the 'ash' group and some species of the 'peppermint' group of *Monocalyptus*. The *Symphyomyrtus* species are commonly defoliated by another major pest, *C. agricola,* among others. However, *E. nitens* (of the Subgenus *Symphyomyrtus,* series *Viminales*), which is not indigenous to Tasmania, is

extensively attacked by both paropsine species. *C. agricola* preferentially attacks the juvenile foliage and *C. bimaculata* the adult foliage. Although food preferences of *C. bimaculata* and *C. agricola* have been documented, the factors influencing host plant selection by them have not been examined. *C. bimaculata* is reported to favour eucalypts of the ash group but in recent times extensive defoliation of *E. nitens* of the 'gum' group has raised a question as to the general host plant specificity of *C. bimaculata*. Furthermore, the utilisation of *E. nitens* as a host by *C. bimaculata* has resulted in a reassessment of its economic impact. Because *E. nitens* is a potentially important introduced pulpwood species in the forest industry of the state and is the subject of intensive tissue culturing and breeding (crossing with *E. globulus* etc), an appreciation of the factors involved in host selection has relevance.

This thesis describes a study of the essential oils and leaf surface waxes of eucalypts and their influence on the host plant relationships of *C. bimaculata* and *C. agricola*. This study includes two major parts:

(1) A chemotaxonomic study of *Eucalyptus* based on analysis of leaf oils and surface waxes.

a. The first step of this study was to investigate the genetic differentiation and the seasonal, developmental and environmental variation in leaf essential oils of several important eucalypt species in Tasmania. This provided a basic profile of sources of variation in oil and wax characteristics in eucalypts and information which was useful for further analysis of chemotaxonomy in Tasmanian eucalypts and the study of host plant - insect relationships.

b. A chemotaxonomic study of all eucalypt species native to Tasmania based on essential oil and leaf waxes. Populations encompassing the major localities and important varietal forms of each eucalypt species in Tasmania were sampled. The variation patterns of their characteristics were elucidated by the application of numerical pattern analysis techniques. Variation patterns provide information which can be useful for taxonomic adjustments to *Eucalyptus* while the chemotaxonomic structure of Tasmanian eucalypts were obtained. Gene flow both within and between taxa can be indicated by gradations in characteristics. A biochemical relationship of terpenes in eucalypt essential oils was proposed and this proposal can reveal variation in oil character states which indicate taxonomic differentiation involving divergence in the biosynthesis of terpenes. This approach can be useful to demonstrate the genetic differentiation of essential oils among taxa and attributable to biogenetic divergence in terpene biosynthesis. The relationships that may occur between taxa in the wax chemistry, morphology and their visual glaucousness of their leaves were also evaluated.

c. Examination of leaf oils and waxes among an extensive range of mainland provenances of *E. nitens*. Samples from *E. nitens* provenance trials in Tasmania were used. The oil and wax characteristics of the *E. nitens* were compared with Tasmanian eucalypts.

d. Investigation into the heritability of oil and wax traits in hybrids. Samples from natural stands and hybrid trials were used. Oil and wax characteristics in eucalypts appear to be strongly inherited. The heritability of oil and wax characteristics provide information that is useful for the identification of hybrids. Moreover, the possibility is discussed that substantial increases in total oil yield and yield of individual compounds could be realised by establishing plantations using genetically improved trees.

(2) Study of the chemical ecology of eucalypt oils and leaf waxes involved in host-plant selection by insects (beetles).

Based on a comparison of chemical differentiation between eucalypt species, further research was undertaken to investigate the possible role of these chemicals in host-plant selection by *C. bimaculata* and *C. agricola* beetles. In this study, a twofold approach was adopted: (i) the effects of essential oils and waxes in various *Eucalyptus* species from different subgenera related to host-plant relationships was investigated by laboratory bioassay, while (ii) in several series of feeding tests the effects on beetles of the major components of different essential oils were evaluated by use of pure terpene compounds.

In general, the aims of this thesis are further the to understanding of the distribution of essential oils and leaf waxes in Tasmanian eucalypts and their effects on insect-plant selection and, furthermore, to provide information for chemotaxonomic study of *Eucalyptus* and to guide selection in future breeding program towards species, provenances or trees less preferred by major pests.

The body of the thesis is divided into twelve chapters:

Chapter 1 is the introduction.

Chapter 2 reviews the literature relevant to the thesis. The Tasmanian eucalypts and their major pests are described. The philosophies underlying chemotaxonomy based on essential oils and leaf waxes and the principles of insect-plant selection are discussed.

Chapter 3 describes the sources of variation in oil characteristics that may occur in *Eucalyptus*. The oil characteristics of two provenances of each of four commercially important eucalypt species, *E. regnans, E. delegatensis, E. globulus* and *E. nitens,* growing in provenance trials were examined.

Chapter 4 describes the variation in leaf oils within and between Tasmanian eucalypt species and the taxonomic significance of the patterns observed.

Chapter 5 describes the variation in leaf wax chemistry and morphology and their taxonomic significance.

Chapter 6 is a general discussion of leaf oils and waxes. A chemotaxonomic structure based on both oil and wax data was constructed. The relationships between leaf chemicals and current taxonomy of *Eucalyptus* were discussed.

Chapter 7 evaluates the feeding and oviposition preference of *C. bimaculata* and *C. agricola* adult beetles to *Eucalyptus* leaves in laboratory bioassay in which both chemical and physical effects of leaf oils and waxes were evaluated.

Chapter 8 evaluates the feeding response of *C. bimaculata* and *C. agricola* larvae to *Eucalyptus* leaves in laboratory bioassay where effects of leaf oils and waxes were evaluated.

Chapter 9 describes bioassays of *C. bimaculata* and *C. agricola* larvae in which the effects of the principal components of leaf oils were evaluated.

Chapter 10 describes the inheritance of feeding resistance in interspecific F1 hybrids of *Eucalyptus*.

Chapter 11 describes the variation in oil and wax characteristics among a wide range of provenances of *E. nitens*.

Chapter 12 is a general discussion and conclusion.

Chapter 2
Literature Review

2.1. Introduction to Tasmanian *Eucalyptus* spp.

2.1.1. Taxonomic affinity

The genus *Eucalyptus* belongs in the family Myrtaceae and the infra-familial classification is:

family: Myrtaceae
subfamily: Leptospermoidene
tribe: Leptospereae
subtribe: Eucalyptinae
genus: *Eucalyptus*

Eucalyptus is mainly endemic to Australia, but several species occur in parts of Indonesia, New Guinea, and the Philippines. According to Chippendale (1988), there are approximately 550 species and many varieties of *Eucalyptus* recognised but the relationship and status of many of these taxa are still undergoing change.

Species within the genus *Eucalyptus* were grouped into seven subgenera by Pryor and Johnson (1971) and then into eight by Johnson (1972, 1975), and some consideration has been given to re-organising the eucalypts into eight genera, by raising the subgeneric groups to generic level (Johnson 1975; Johnson and Briggs 1983). However, McAlpine (1986) considered that a detailed revision along these lines awaits publication, and there may be resistance, for morphological or practical reason, to its widespread acceptance. In the most recent treatment of eucalypts in the 'Flora of Australia' series, Chippendale (1988) maintains the status quo, and *Eucalyptus* remains the genus status. He brought together all currently accepted species and presented a formal classification at the series level where eucalypt species in Australia are divided into 92 series although the relationships of these series were not defined.

In contrast with Chippendale's (1988) classification, 'A classification of the *Eucalyptus*' by Pryor and Johnson (1971), was concerned with intra-generic relationships with species in the genus grouped at the levels of subgenus, section, series, subseries, superspecies, species and subspecies (incl. cline-form). However, their classification is

considered informal as they did not presented an account of characters upon which they based their classification.

In this thesis, the informal classification of Pryor and Johnson (1971) was used in conjunction with the classification of Chippendale (1988) which does not include grouping above the series level. The scientific names and authorities follow Chippendale (1988).

Eucalyptus species from two of the seven subgenera described by Pryor and Johnson (1971) occur in Tasmania. They are the two largest subgenera, *Monocalyptus* and *Symphyomyrtus* (Table 2.1). These two subgenera differ in many respects such as reproduction, physiology, morphology and their response to environmental stress (Florence 1981, 1982; Noble 1989). The Tasmanian *Monocalyptus* species all fall into the two series, the *Obliquae* (commonly known as the ashes) and the *Piperitae* (commonly known as the peppermints) (Table 2.1). The *Symphyomyrtus* species also come from only two series of the subgenus, the *Ovatae* and the *Viminales,* and are collectively known as the 'gums'. Within *Symphyomyrtus*, species were divided into five subgroups by Jackson (1965). All four species from the series *Ovatae* corresponded to the 'black gum' subgroup and species of the series *Viminales* were divided into four subgroups: 'white gum', 'yellow gum', 'blue gum' and 'alpine white gum'.

In all, twenty nine species of eucalypts have been recognised to naturally occur in Tasmania (Chippendale 1988), 12 from the subgenus *Monocalyptus* and 17 from *Symphyomyrtus*. Sixteen of these species are endemic to Tasmania (Duncan 1989, Fig. 2.1). All five 'ash' species are not endemic, although the Tasmanian form of *E. delegatensis* is recognised as being sufficiently distinct from its mainland counterpart to be designated as *E. delegatensis* var. *tasmaniensis* (Boland 1985). All seven *Piperitae* and ten of the seventeen *Symphyomyrtus* species are endemic (Fig. 2.1).

In Chippendale's (1988) classification, all seven Tasmanian peppermints from Pryor and Johnson's series *Piperitae* were maintained in a series, named as *Radiatae* and the black gums, from the series *Ovatae* were classified into the series *Foveolatae*. The status of the series *Viminales* was also maintained, however, the yellow gums, which were classified as subspecies by Pryor and Johnson (1971) were returned to species level. The other two subspecies of *E. gunnii*, ssp. *gunnii* and ssp. *archeri*, recognised by Pryor and Johnson were also returned to the species level (*E. gunnii* and *E. archeri* respectively). The five 'ash' species, which were included in Pryor and Johnson's series *Obliquae*, were classified into three different series by Chippendale (Table 2.1).

Table 2.1. The classification of Tasmanian *Eucalyptus* by Pryor and Johnson (1971) and Chippendale (1988), the species groups of Jackson (1965) and the glaucousness phenotype proposed by Barber (1955).

Pryor and Johnson's classification			Chippendale's classification		Jackson's	Barber's
Subseries	Superspecies	Species	Species	series	group	types
Subgenus *Monocalyptus*						
Series *Obliquae*						
Obliquinae						
		obliqua	*obliqua*	*Eucalyptus*	Ash	Green
Delegatensinae						
		delegatensis ssp. *tasmaniensis*	*delegatensis*	*Eucalyptus*	Ash	Clinal
Regnantinae						
	Regnans	*regnans*	*regnans*	*Regnantes*	Ash	Green
Considenianinae						
	Consideniana	*sieberi*	*sieberi*	*Psathyroxyla*	Ash	Clinal
Pauciflorinae						
	Pauciflora	*pauciflora* Seiner ex Sperengel	*pauciflora*	*Eucalyptus*	Ash	Clinal
Series *Piperitae*						
Amygdalininae						
	Risdonii	*risdonii*	*risdonii*	*Radiatae*	Peppermint	Glaucous
		tenuiramis	*tenuiramis*	*Radiatae*	Peppermint	Glaucous
	Amygdalina	*pulchella*	*pulchella*	*Radiatae*	Peppermint	Green
		amygdalina	*amygdalina*	*Radiatae*	Peppermint	Clinal
		nitida	*nitida*	*Radiatae*	Peppermint	Clinal
		radiata ssp. *robertsonii*	*radiata*	*Radiatae*	Peppermint	Clinal
		coccifera	*coccifera*	*Radiatae*	Peppermint	Clinal
Subgenus *Symphyomyrtus*						
Series *Ovatae*						
Ovatinae						
	Ovata	*ovata*	*ovata*	*Foveolatae*	Black gum	Green
		brookeriana	*brookeriana*	*Foveolate*	Black gum	Green
		barberi	*barberi*	*Foveolatae*	Black gum	Green
	Aggregata	*rodwayi*	*rodwayi*	*Foveolatae*	Black gum	Green
Series *Viminales*						
Globulinae						
	Globulus	*globulus* ssp. *globulus*	*globulus*	*Viminales*	Blue gum	Glaucous
Vernicosinae						
		vernicosa				
		ssp. *vernicosa*	*vernicosa*	*Viminales*	Yellow gum	Green
		ssp. *subcrenulata*	*subcrenulata*	*Viminales*	Yellow gum	Green
		ssp. *johnstonii*	*johnstonii*	*Viminales*	Yellow gum	Green
Viminalinae						
		viminalis ssp. viminalis	*viminalis*	*Viminales*	White gum	Green
Cordatinae						
	Rubida	*dalrympleana*	*dalrympleana*	*Viminales*	White gum	Subglaucous
		ssp. *dalrympleana*				
		rubida	*rubida*	*Viminales*	White gum	Glaucous
	Gunnii	*gunnii*				
		ssp. gunnii	*gunnii*	*Viminales*	Alpine white	Gl/Subgl*
		ssp. archeri	*archeri*	*Viminales*	Alpine white	Subgl/Green
		morrisbyi	*morrisbyi*	*Viminales*	Alpine white	Glaucous
		urnigera	*urnigera*	*Viminales*	Alpine white	Glaucous
		perriniana	*perriniana*	*Viminales*	Alpine white	Glaucous
		cordata	*cordata*	*Viminales*	Blue gum	Glaucous

* Gl = glaucous, Subgl = subglaucous

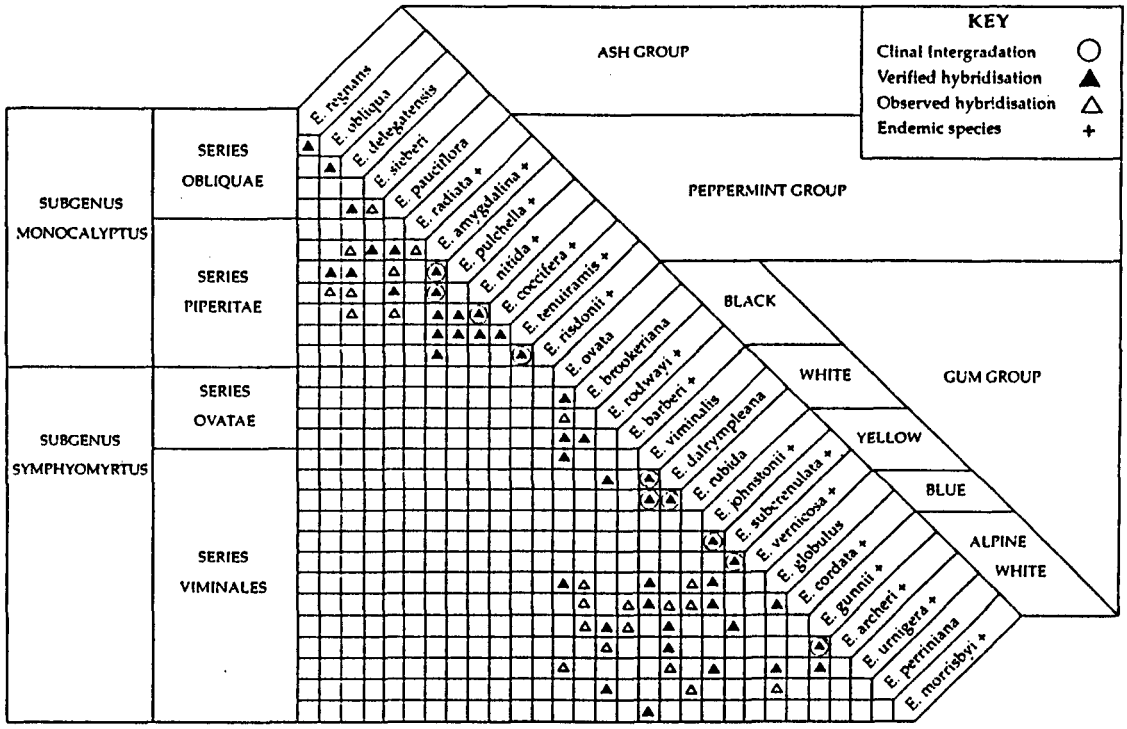


Figure 2.1. Species of *Eucalyptus* native to Tasmania, showing those species forming clines and hybrids.

*For each species, follow the horizontal and vertical axes. For example, *Eucalyptus barberi* is known to hybridise with *E. ovata* and *E. brookeriana* (horizontal axis) and possibly hybridises with *E. gunnii* and *E. cordata* (vertical axis). (from Duncan 1989).

A series of studies aimed at phylogenetic reconstruction and biogeography analysis of the informal subgenus *Monocalyptus* of Pryor and Johnson (1971) have been undertaken by Ladiges and co-workers (Ladiges and Humphries 1983, 1986; Ladiges *et al.* 1983, 1984,1987, 1989). They suggest that the subgenus *Monocalyptus* is a monophyletic group, and that the subgenera *Idiogenes* and *Gaubaea* are its sister taxa (Ladiges and Humphries 1983). They also suggest that the Western Australian species of *Monocalyptus* are a paraphyletic group and have revised the informal classification of Pryor and Johnson (1971) recognising three sections, five subsections, five infrasections, five superseries, five series and two subseries (Ladiges *et al.* 1987). Their cladistic analysis suggested that the 'ash' group of *Monocalyptus* are not monophyletic and that they probably consist of two groups, 'green ashes' and 'blue ashes' (Ladiges *et al.* 1987). The major differentiating characters upon which they based their classification are that the 'green ashes' have prominent oil glands and brown seeds in contrast to the 'blue ashes' which have dull, blue-green seedling and juvenile leaves and black seeds. The similarity in leaf arrangement was interpreted as a convergent feature of the 'ashes'.

Ladiges *et al.* (1989) classified the green ashes as a superseries *Eucalyptus* (Table 2.2). The major differences between the series *Obliquae* of Pryor and Johnson (1971) and their superseries *Eucalyptus* is that the latter consists of only 'green ashes' but the former includes both blue and green ash species. In the superseries *Eucalyptus, E. regnans* and *E. obliqua* were classified into the series *Regnaninae* and series *Eucalyptus* respectively, with *E. regnans* and *E. fastigata* as sister species. In Chippendale's classification (1988), the five Tasmanian ashes were classified into three series: the green ash *E. regnans* in series 30, *Regnantes,* the green ash *E. obliqua* and the blue ashes *E. delegatensis* and *E. pauciflora* in series 32, *Eucalyptus*, and the blue ash *E. sieberi* in series 33, *Psathyroxyla* (Table 2.1). However, Ladiges *et al.* (1989) pointed out that the three series of Chippendale (series 30, 32 and 34), which includes the green ashes, are not arranged in a phyletic sequence and the classification, if it is intended to reflect phylogenetic relationships, is misleading because the green and blue ashes are mixed up.

Table 2.2. Informal classification of the green ashes by Ladiges *et al.* (1989)

1 Superseries *Eucalyptus*	
1.1 series *Regnaninae*	
	E. regnans
	E. fastigata
1.2 series *Eucalyptus*	
	E. obliqua
1.3 series *Strictinae*	
1.3.1 subseries *Dendromorphitae*	
	E. dendromorpha
1.3.2 subseries *Strictitae*	
	E. triflora
	E. strica
	spp. *strica*
	spp. *obtusiflora*
	E. burgessiana
1.3.3 subseries *Approximanitae*	
	E. kybeanensis
	E. paliflormis
	E. approximans
	spp. *approximans*
	spp. *codonocarpa*
	E. rupicola
	E. apiculata

Ladiges and Humphries (1983) also considered the series *Piperitae* of Pryor and Johnson (1971) to be heterogeneous and follow Brooker (1977) in transferring subseries *Piperitinae* and *Haemastominae* to *Obliquae*. They recognise the peppermints, subseries *Amygdalininae*, as a series, *Amygdalinae* whose sister taxon is the series *Pilulares*, and monophyletic groups within it as subseries and superspecies (Table 2.3). In their revised classification of the *Amygdalininae* of Pryor and Johnson (1971), the peppermints were classified into four subseries. A notable change of species relationships for the

Tasmanian peppermints in their revised classification is that the superspecies *Amygdalina* of Pryor and Johnson (1971), which linked *E. amygdalina, E. nitida* and *E. radiata,* is not a natural group. *E. nitida, E. coccifera, E. risdonii* and *E. tenuiramis* were grouped into a subseries *Cocciferinae* while *E. pulchella, E. amygdalina* and *E. radiata* were classified into subseries *Pulchellinae, Amygdalininae* and *Radiatinae* respectively.

Table 2.3. Revised classification of the *Amygdalininae* of Pryor and Johnson (1971) by Ladiges *et al.* (1983).

Subgenus *Monocalyptus*	
Series *Amygdalinae*	
Subseries *Pulchellinae*	
	pulchella
Subseries *Amygdalininae*	
	amygdalina
Subseries *Cocciferinae*	
	nitida
	coccifera
Superspecies *Risdonii*	
	risdonii
	tenuiramis
Subseries *Radiatinae*	
Superspecies *Radiata*	
	radiata
	spp. *radiata*
	spp. *robertsonii*
	elata
Superspecies *Dives*	
	willisii
	dives

Ladiges *et al.* (1984) have investigated the relationships among members of series *Ovatae* of *Symphyomyrtus* (Pryor and Johnson 1971). Although the sister group to the *Ovatae* for polarisation of characters and establishment of monophyly were not identified readily, they suggested that this series is not monophyletic and needs taxonomic revision. Based on seedling characters, they found that the ovata subgroup (includes species *E. ovata, E. barberi and E. brookeriana*) identified is monophyletic in the subseries *Ovatinae* of Pryor and Johnson (1971), except that they included *E. aromaphloia* (classified by Pryor and Johnson in the subseries *Manniferinae*) and excluded *E. rodwayi* (classified by Pryor and Johnson in the subspecies *Aggregata* of the *Ovatinae*).

2.1.2. Distribution and ecology

The relationships between vegetation and Tasmanian's environment have been discussed and described at a general level and for specific vegetation types or areas (e.g. Gilbert 1959; Jackson 1968, 1973; Kirkpatrick 1977, 1981; Brown and Podger 1982; Ellis 1985). The natural distribution of Tasmanian eucalypts was provided by Jackson (1965),

Boland *et al.* (1984) and Kirkpatrick and Backhouse (1985, 1989). The distribution of major vegetation types in Tasmania was mapped by Kirkpatrick and Dickinson (1984) (Fig. 2.2) and the distribution of endemic eucalypt species is indicated on a 10 Km x 10 Km grid by Brown *et al.* (1983).

In spite of a temperate oceanic climate, the natural vegetation of Tasmania is varied due to the mixing of Australian and Southern Oceanic ('Antarctic') components, and a wide diversity of habitats. Jackson (1965) classified the natural vegetation that developed in Tasmania into three main ecological formations of austral-montane, temperate rain forest, and sclerophyll forest with the sclerophyll forest being completely dominated by eucalypt trees. However, the distribution of eucalypt species in the dominant sclerophyll forest in Tasmania is broadly determined by the climate with a steep rainfall gradient from west to east across the state producing a range of environments from wet to semi-arid, while Tasmanian's latitude (41-43^0 south) provides a cool temperate climate resulting in an extensive subalpine environment (Jackson 1965). Imposed upon the climatic influence in Tasmanian forest is the effect of fire. Jackson (1968) considered that fire is an important factor in maintaining eucalypt or mixed (eucalypts plus rainforest) forest in higher rainfall areas which would be expected to support, climatically, a temperate rainforest. For example, stands of *E. regnans* tend to be killed by intense wild fires resulting in extensive seedling regeneration. This does not, however, appear to be the case for other species which tend to be more fire resistant and vegetatively regenerate from either lignotubers or epicormic shoots following wildfire (e.g. *E. risdonii,* Potts 1986; *E. delegatensis*, Bowman and Kirkpatrick 1986) and seedling recruitment tends to be much more continuous, although still fire dependent. Davidson *et al.* (1981) suggested that climate and fire frequency, therefore, play a major role in determining the geographical region that the genus *Eucalyptus* can occupy.

According to Davidson *et al.* (1981), the eucalypt forests of Tasmania tend to be divided into three categories as determined by water availability (e.g. rainfall) and temperature gradients to give dry sclerophyll, wet sclerophyll and subalpine forests (Table 2.4 and Fig. 2.2). Species of the subgenus *Monocalyptus* series *Obliquae* (the ashes) tend to dominate wet sclerophyll forests and species of the subgenus *Monocalyptus* series *Piperitae* (the peppermints) tend to dominate dry sclerophyll forests and woodlands. The species from the subgenus *Symphyomyrtus* (the gums) tend to occur as subdominant or minor species in both forests while the subalpine forests may be dominated by species of either subgenus depending on the specific environments.

Fig. 2.2.

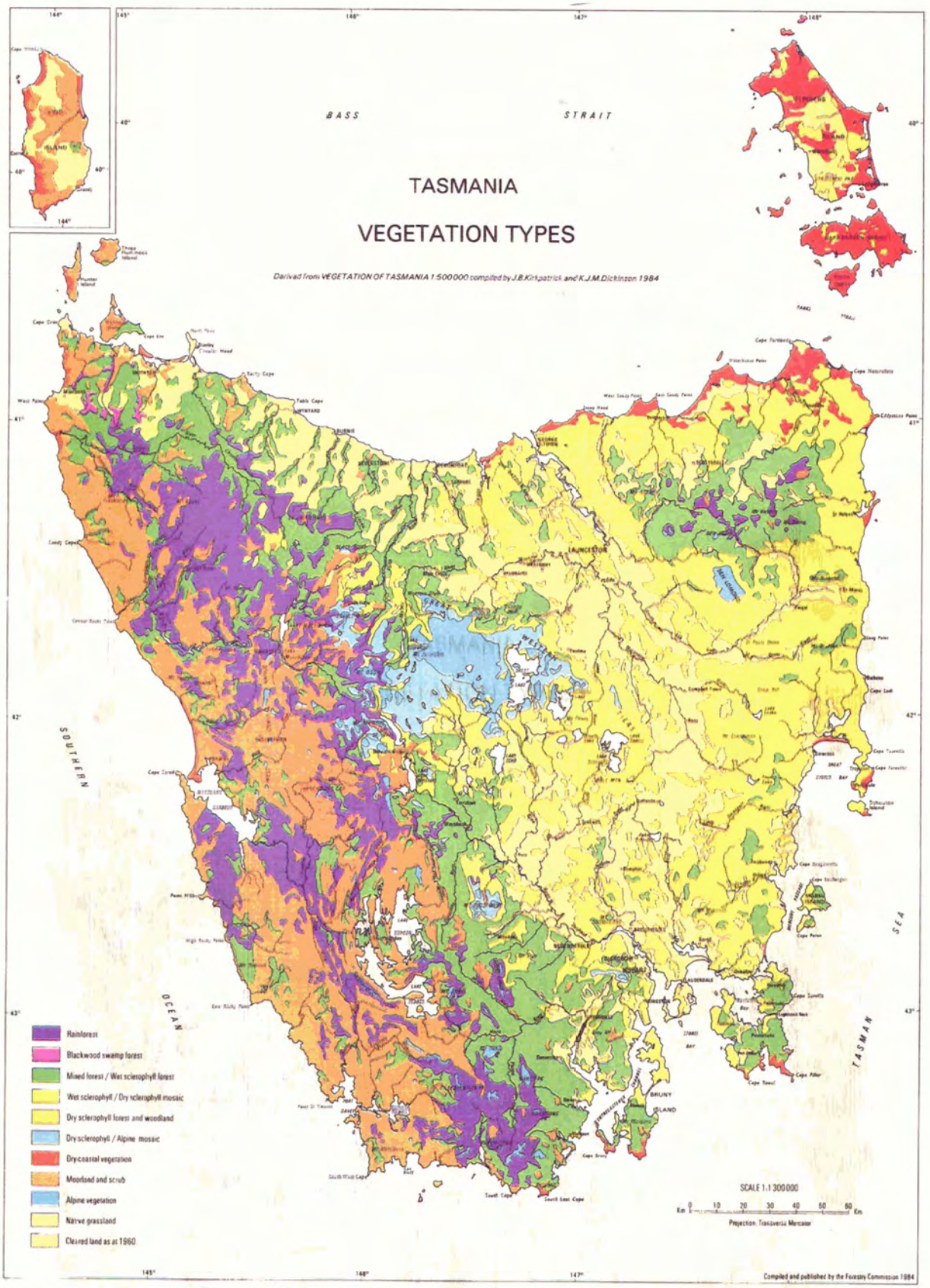

TASMANIA
VEGETATION TYPES
Derived from VEGETATION OF TASMANIA 1:500000 compiled by J.B.Kirkpatrick and K.J.M.Dickinson 1984
BASS
STRAIT
SOUTHERN
OCEAN
TASMAN
SEA
Rainforest
Blackwood swamp forest
Mixed forest / Wet sclerophyll forest
Wet sclerophyll / Dry sclerophyll mosaic
Dry sclerophyll forest and woodland
Dry sclerophyll / Alpine mosaic
Dry coastal vegetation
Moorland and scrub
Alpine vegetation
Native grassland
Cleared land as at 1960
SCALE 1:1 300 000
Km 0 10 20 30 40 50 60 Km
Projection: Transverse Mercator
Compiled and published by the Forestry Commission 1984

Table 2.4. The preferred habitat of each eucalypt species occurring in Tasmania. (following Davidson *et al.* 1981).

Dry Sclerophyll	Preferred Habitat
E. sieberi	dry infertile soils normally over granite in the north-east
E. amygdalina	widely distributed on dry sandy podsolic soils in the north and east
E. pulchella	dry soils developed on dolerite in the south-east
E. tenuiramis	dry soils developed mainly on mudstone and dolerite in the south-east
E. risdonii	restricted on very dry, skeletal soils developed on mudstone to the east of the River Derwent
E. radiata	very restricted distribution on Ordovician gravels in the Mersey and Forth River valleys
E. viminalis	widespread in coastal and riverine corridors in dry habitats, often subdominant
E. rubida	replaces *E. viminalis* on very dry, cold sites at intermediate altitude, frequently on mudstone
E. ovata	sites prone to occasional waterlogging at low to intermediate altitude
E. barberi	mallee scrub on dry, rocky dolerite hills in the Eastern Tiers
E. perriniana	restricted distribution on poorly drained, infertile shallow soils derived from Permian sandstone in frosty sites at intermediate altitude
E. morrisbyi	endangered species on poor coastal soils in the Clarence municipality
Wet Sclerophyll	
E. regnans	sites of low fire frequency, on moist, deep, well-drained soils to 700m
E. obliqua	widespread, replacing *E. regnans* on slightly drier sites with a higher fire frequency through to dry coastal areas
E. globulus	relatively moist coastal sites and in well-drained frost free valleys to 500m
E. cordata	local in the south-east, mainly on dolerite extending from dry coastal cliffs to poorly drained sub-alpine sites to 600m
E. dalrympleana	replaces *E. viminalis* in colder, wetter sites to 1050m
E. brookeriana	well drained rocky soils or alluvial deposits adjacent to streams
E. rodwayi	replaces *E. ovata* on poorly drained soils at intermediated altitudes
E. delegatensis	replaces *E. obliqua* and *E. regnans* on well-drained sites at higher altitudes about 600m
Subalpine	
E. coccifera	cold well-drained sites, forms treeline on most dolerite capped mountains in the centre and south-east
E. nitida	infertile skeletal soils or peaks in high rainfall areas of west and south-west, wide altitudinal range
E. pauciflora	well-drained sites in cold habitats in centre and east, wide altitudinal range
E. vernicosa	replaces *E. vernicosa* on less exposed sites and on waterlogged sites towards the south-east
E. johnstonii	replaces sub-alpine *E. subcrenulata* at lower altitudes towards the south-east
E. urnigera	cold sites on well-drained dolerite soil mainly in the south-east
E. gunnii	cold, waterlogged sites, generally on dolerite or basalt in central regions
E. archeri	upper woodlands, moderate exposure, forms treeline in the north-east

2.1.3. Genetic variation and clinal variation

It has been pointed out by Pryor and Johnson (1971) that a common characteristic of most eucalypts is the close adaptive response of the population to the environment where a large amount of genetic variability is often found within and between species. In cases where species are widely distributed, clinal variation commonly occurs, either continuously or more often in a stepped series (Pryor and Johnson 1971). Such clinal variation occurs when morphological and physiological differences within a species are correlated with particular changes in the physical environment (Pryor 1976) such as altitude (as a surrogate for temperature or rainfall), latitude (as a surrogate for temperature or day length) or environmental stress (such as moisture availability or frost susceptibility).

Numerous examples of clinal variation have been reported within and between the Tasmanian eucalypts and between disjunct populations and in cases appears to be maintained by selection despite considerable gene flow in some cases (e.g. Barber and Jackson 1957; Davidson *et al.* 1981). Within species, clinal patterns of variation have been reported both within continuous populations and between disjunct populations. For example, a continuous clinal variations in frequency of glaucous phenotypes have been reported along altitudinal gradients within *E. urnigera* (Barber and Jackson 1957; Thomas and Barber 1974a,b) and *E. gunnii* (Potts and Reid 1985a,b). Clines have also been reported in *E. regnans* (Ashton 1975, 1981), *E. delegatensis* (Boland and Dunn 1985), *E. globulus* (Kirkpatrick 1975) and between disjunct populations of *E. cordata* (Potts 1989) and *E. perriniana* (Wiltshire and Reid 1987).

In addition to the marked amount of differentiation which occurs within species, many of the 29 species have been reported to intergrade in various combinations (Figure 2.1). However, clinal variation between species tends to only involve species which are relatively closely related. For example, species within the series *Piperitae*, and the subgroup of the series *Viminales* tend to intergrade within each group but not between groups (Figure 2.1). In contrast, no interspecific clinal variations have been reported in the series *Obliqua* and the series *Ovatae*, although hybridisation does occur.

Clinal variation between species which vary in height and form or other morphological characteristics have been observed in geographic or environmental extremes. The Tasmanian yellow gums (*E. johnstonii, E. subcrenulata* and *E. vernicosa*) are continuously linked in a complex clinal array which is strongly correlated with exposure to the alpine environment and which may vary with geography, altitude (e.g. Mt Arrawsmith) and aspect (Jackson 1960; Potts and Jackson 1986). Altitudinal clines have also been reported between the two white gums, *E. viminalis* and *E. dalrympleana*

(Phillips and Reid 1980) and the alpine white gums, *E. gunnii* and *E. archeri* (Potts and Reid 1985a,b).

Within the Tasmanian *Piperitae*, a complex variation pattern occurs among species which are markedly different at the morphological level. The species intergrade in virtually all possible combinations, but also variation is common within species. Clinal variation occurs between *E. amygdalina* and *E. pulchella* (Davidson *et al.* 1981; Kirkpatrick and Potts 1987) and between *E. tenuiramis* and *E. risdonii* (Davidson *et al.* 1981; Wiltshire 1991, 1992). *E. amygdalina* also intergrades into *E. nitida* which in turn intergrades into *E. coccifera* (Davidson *et al.* 1981; Shaw *et al.* 1984).

Clinal variation between species in some cases may involve changes in the pattern of ontogenetic variation as in the case between *E. risdonii* and *E. tenuiramis* (Wiltshire *et al.* 1991, 1992). When differences in ontogenetic pattern were removed the clinal variation in morphological variation is also continuous and the morphological variation between *E. risdonii* and *E. tenuiramis* populations in the same geographical region is much smaller than that within *E. tenuiramis*. It has been suggested that *E. risdonii* may be the product of relatively recent changes in developmental timing (heterochrony) from *E. tenuiramis* (Wiltshire *et al.* 1992).

In contrast to the peppermints and some gum species in which extreme variation and intergradation has been reported, some species, such as the ash species, *E. regnans, E. obliqua* and *E. delegatensis* and the gum *E. globulus,* which occupy wet environments over an extensive geographic range, exhibit less pronounced clinal variation within species and less intergradation between species. Davidson *et al.* (1981) considered that the habitats where these species occur are buffered against environmental fluctuations, and therefore, selective pressures change less rapidly than that for the peppermints.

2.1.4. Hybridisation

In the genus *Eucalyptus*, hybridisation does not occur between the subgenera *Monocalyptus* and *Symphyomyrtus,* but hybridisation between species, even from different series within the same subgenus, may be extensive (Griffin *et al.* 1988). Hybridisation can be induced artificially in eucalypt breeding programs when barriers to natural hybridisation between species are circumvented.

The patterns of natural hybridisation between the Tasmanian eucalypt species have been reviewed by Duncan (1989, see Figure 2.1). He compared the frequency of hybrids amongst the Tasmanian species with the Australian wide results presented by Griffin *et al.* (1988). The Tasmanian figure ranged from 34 percent (using verified hybrids only) to 51 per cent (if reliably observed hybrids are also included) of potential hybrid

combinations among Tasmanian eucalypt species in the subgenus *Monocalyptus*. A similar figure was also recorded in *Symphyomyrtus*. In contrast, the figures of potential hybrid combinations within the genus *Eucalyptus* in Australia have been found to be only about 11 per cent in the subgenus *Monocalyptus* and six percent in *Symphyomyrtus* species. Duncan (1989) considered that these higher figures of potential hybrid combinations in Tasmania were not only due to the extent of the botanical survey and collection but the environmental heterogeneity that occurs over relatively short distances in Tasmania. The large environmental heterogeneity arising through the rugged topography results in species with substantially different ecological requirements often occurring close to each other.

It appears that the presence of hybrid individuals in Tasmanian eucalypts is common between species within series, such as the hybrid swarms of *E. amygdalina x E. risdonii* (Potts and Reid 1985c, 1988) in the series *Piperitae* and the hybrids between species within the series *Ovatae* or *Viminales* of gum (Duncan 1989; see Fig. 2.1).

Numerous interseries hybrids in Tasmanian eucalypts have been also reported, such as *E. delegatensis x E. coccifera* and *E. delegatensis x pulchella* (Davidson *et al.* 1987), *E. obliqua x E. pulchella* (Potts and Reid 1983) and *E. globulus x E. ovata* (McAulay 1938). These do not, however, tend to be as common as hybrids between species within series.

In addition to the natural hybrids, artificial hybrids have been produced amongst many of the Tasmanian *Symphyomyrtus* species, such as the interspecific hybrids of *E. globulus* (Potts and Savva 1988), *E. gunnii* (Potts *et al.* 1987) and *E. nitens* (Tibbits 1988, 1989). A major post mating barrier has been identified which is unilateral and associated with the flower size (Gore *et al.* 1990). Large flowered species are unable to act as female parents as pollen tubes of smaller flowered species cannot grow the full length of the style of the larger flowered species such as *E. globulus*.

The problems in identification of hybrids on morphological characteristics have been discussed by Duncan (1989). It is considered that most hybrids tend to be intermediate in several morphological characteristics between the two parents. In such cases, hybrids are relatively easy to identify, particularly when the putative parents are observed or known to occur in the general vicinity , e.g. *E. ovata x E. globulus* hybrids are intermediate in the shape and size of capsules, and in the shape of juvenile leaves between their parents (McAulay 1938). However, identification is relatively difficult when parents are morphologically similar, e.g. *E. viminalis* and *E. dalrympleana* and the clinal variation between the two species may also confuse the issue. Thus, it is difficult to tell whether

intergradation between species is a result of introgression (advanced generation hybridisation) or primary differentiation (Potts and Jackson 1986; Potts and Reid 1985b).

2.2. The *Eucalyptus*-defoliating paropsine beetles and host specificity

2.2.1. The major insect defoliators of Eucalyptus and their effects

The major defoliating insects in Australian eucalypt forests have been reviewed by several authors (Greaves 1961, 1966; Newman and Marks 1976; Carne and Taylor 1978; Ohmart 1990, Ohmart and Edwards 1991). These reviews have considered that three eruptive insect groups, which cause severe damage or tree death, are represented by three leaf feeding insect orders, notably Phasmatodea, Coleoptera, and Lepidoptera.

The phasmatids (Phasmatodea: Phasmatidae) have been reported mainly in south eastern Australia where the three species, *Didymuria violescens* (Keach), *Podocanthus wilkinsoni* (Macleay) and *Ctenomorphodes tessulata* (Greg) represent insects that cause serious defoliation to a range of eucalypt species, especially to ash species i.e. *E. regnans* and *E. delegatensis* (Hadlington and Hoschke 1959; Campbell 1960, 1961, 1964; Readshaw 1965; Mazanec 1966, 1967, 1968; Browne 1968; Readshaw and Mazanec 1969; Neumann *et al.* 1977).

The serious lepidopteran defoliators of eucalypts consist of the jarrah leaf miner, *Perthida glyphopa* (Lepidoptera: Incurvariidae) in jarrah forests (i.e. *E. marginata*) in Western Australia (Wallace 1970; Mazanec 1974, 1978, 1985, 1987; Casotti and Bradley 1991; Bennett *et al.* , 1992), the gum leaf skeletonizer, *Uraba lugens* (Walker) (Lepidoptera: Nolidae), on a wide range of eucalypt species in many parts of Australia (Brimblecombe 1962; Campbell 1962, Harris 1974; Morgan and Cobbinah 1977; Cobbinah 1985), and the autumn gum moth, *Mnesampela privata* (Guen.) (Lepidoptera: Geometridae), on Tasmania eucalypts (Anon 1977; Elliott and Bashford 1978, 1983; Elliott *et al.* 1980).

The paropsine leaf beetles (Coleoptera: Chrysomelidae) are serious pests of regeneration, plantations and mature forests and are regarded as an ecologically and economically important group of eucalypt herbivores. *Chrysophtharta bimaculata* (Olivier) is considered to be one of the most important forest insect pests in Tasmania (Carne 1966; Greaves 1966; Kile 1974; Carne *et al.* 1974; de Little and Madden 1976; de Little 1983; Elliott and de Little 1985). Many species of chrysomelid beetles have been also reported as defoliators of a range of eucalypt species (Ohmart *et al.* 1983a,b; Strauss and Morrow 1988) and *Paropsis atomaria* Olivier is a common species in south eastern Australia

which may cause occasional death of woodland eucalypts (Carne 1966; Tanton and Epila 1984; Larsson and Ohmart 1988; Edwards and Wanjura 1990). Christmas beetles (Coleoptera: Scarabaeidae) are also severe defoliators in eastern Australia (Carne 1957, 1981; Allsopp and Carne 1986; Carne *et al.* 1981)

Eucalypt defoliating insects in Tasmania have been reviewed by Elliott and de Little (1985). They concluded that leaf beetles (chrysomelids), gum leaf Skeletonizer Moth, Autumn Gum Moth, Termites, and the Fireblight Beetle, *Pyrgoides orphara,* and the *Chrysophtharta bimaculata* (Olivier), were major pests of Tasmanian eucalypt plantations and mature native forest. Severe defoliation of plantation and naturally regenerating eucalypts by *C. bimaculata* has caused reduced growth rates and poor form and is considered the major limiting factor to growth of fast-growing eucalypt species.

C. bimaculata severely defoliates regeneration and mature forest of *E. regnans, E. obliqua* and *E. delegatensis* (Greaves 1966; Kile 1974; de Little and Madden 1976; Elliott and de Little 1985) resulting in a noticeable effect on height growth. In areas with a high population of *C. bimaculata,* trees protected from defoliation by continual spraying with insecticide during a season were compared with unprotected trees (Kile 1974). The mean height increments of trees which were sprayed were approximately twice as great as those of unsprayed control trees. Moreover, paropsine defoliation may predispose eucalypts to attack by other organisms by contributing towards a condition of stress in the host trees. More recently, *C. bimaculata* has also been reported to heavily defoliate the potentially important pulp wood species, *E. nitens.*

2.2.2. Host specificity among insects which graze on *Eucalyptus*

The major tree genera *Acacia* and *Eucalyptus* in Australia, support very many species of insects, and many of these species depend completely on one or other plant genus for their survival. The species of phytophagous insects found on *Eucalyptus,* though, are almost entirely separate from those found on *Acacia.* Moreover, surveys on specialisation in the community of eucalypts and their herbivorous insects have shown that host specificity on eucalypts by grazing insect pests occurs at somewhat different levels of differentiation in which different insects exhibit different specificities to different species even in different populations of a species (Moore 1970; Carne 1965; Morrow 1977a,b).

Noble (1989) suggested that differences in herbivores and parasite damage to leaves is one of the major difference in ecological traits between *Monocalyptus* and *Symphyomyrtus.* He hypothesised that the *Monocalyptus* species carry a lower diversity of leaf herbivores and pathogens and suffer less leaf loss and damage by them than *Symphyomyrtus* species. However, comparison of the insects feeding on different

eucalypts reveals some host specificity of insects among the two major subgenera. Thus, some insect groups are specific to particular eucalypt subgenera (Noble 1989). For example, surveys of insect defoliation on two major subgenera *Monocalyptus* and *Symphyomyrtus* of eucalypts in south eastern Australia (Burdon and Chivers 1974) indicated that of seventeen species of insects, including both grazers and sap-suckers, four species showed a preference for *Symphyomyrtus,* and three species showed a preference for *Monocalyptus*. Morrow (1977b) showed that of insect population association on two *Monocalyptus* and one *Symphyomyrtus* species, 62 per cent of the collected insects (grazers and sap-suckers) attacked only one eucalypt species, 30 per cent attacked two species, and only 8 per cent fed on all three species. These results indicated that insect species varied in their host preference among *Eucalyptus* species.

Among the major stick insects which are associated with eucalypts in south eastern Australia, the polyphagous *Ctenomorphodies tessulatus* (Gray) feeds on a wide range of species among the different genera of *Eucalyptus* plus *Syncarpia, Acacia* and *Casuarina* species (Hadlington and Hoschke 1959), however, *Didymuria violescens* feeds on a range of both *Monocalyptus* and *Symphyomyrtus* species of the genus *Eucalyptus* but not on species of the other genera (Readshaw 1965). de Little (1979) found that among the leaf beetles of Chrysomelidae in Tasmania, *Chrysophtharta nobilitata, Paropsis aegrota* and *P. porosa* defoliated species over the full taxonomic range of eucalypts, however, *Chrysophtharta bimaculata, C. agricola* and *Paropsis charybdis* usually occurred only on eucalypts within a distinct sub-genera.

Some studies indicated that the host specificity could differ between species, between provenances within eucalypt species or between trees in some localities of a species. Strauss and Morrow (1988) reported that the distributions of *Chrysophtharta hectica* on the two host plant species, *E. stellulata* and *E. pauciflora* from the series *Obliquae*, were generally clumped which was partially due to their preference for *E. stellulata* over *E. pauciflora.* Steven (1973) assessed the performance of larvae of *Paropsis charybdis* in the laboratory on foliage from three localities of *E. obliqua* and also on foliage of some other species. Steven found that one source of *E. obliqua* foliage ranked second in palatability compared to the other species whereas the other two sources of *E. obliqua* were the least palatable of all foliage tested. Mazanec (1985) found that although the jarrah leafminer, *Perthida glyphopa,* occurred at chronically high levels on *E. marginata*, three different levels of tree susceptibility to *P. glyphopa* were identified. One type of tree was found to be totally resistant, another one resistant by preventing egg hatch or causing larvae to die but was still fed on by adult females, while the third was attacked and susceptible to *P. glyphopa.*

Studies into host specificity of insects on eucalypts have shown that host specificity could also differ between different growth stages of a defoliating insect species, hence, larval feeding preferences did not necessarily reflect adult feeding or oviposition preferences. For example, 150 tree species were acceptable for oviposition by the gum leaf skeletonizer, *Uraba lugens* female, but larvae could be successfully reared to the fourth instar on only seven species (Morgan and Cobbinah 1977). In contrast, the females of autumn gum moth, *Mnesampela privata* (Guen.), prefer to oviposit on the glaucous juvenile-form foliage of the blue gum group of eucalypts, but larvae can feed on a wide range of other eucalypts species (Elliott and Bashford 1978). In laboratory trials, larvae of leaf beetles of *Paropsis atomaria* performed best on *E. macrorhyncha* foliage which is a species that *P. atomaria* females do not oviposit on in the field (Carne 1966).

The host specificity of individual insect species may also differ with the different growth stages of a eucalypt trees. For example, *C. bimaculata* did not defoliate juvenile foliage of *E. nitens* but defoliated and oviposited on the non waxy adult-form foliage (de Little 1989).

2.2.3. Factors influencing host-plant selection in *Eucalyptus*

Noble (1989) reviewed the differences between the two largest subgenera, *Symphyomyrtus* and *Monocalyptus,* and suggested that these can be summarised as differences in leaf chemistry, in root morphology, chemistry and activity, and in early growth rates. Noble (1989) considered that variation in concentrations of secondary compounds may provide a basis for understanding the variation in herbivory levels.

Eucalyptus leaves usually have high levels of secondary compounds, including essential oils, phenolics, tannins and surface waxes which vary between species and subgenera (Hillis 1966a,b, 1967a,b,c,d; Pryor 1976, Hallam and Chambers 1970; Boland *et al.* 1991). Many of these secondary compounds occurring in eucalypt foliage have been demonstrated to significantly influence insect-plant relationships. However, their actual effects in insect-eucalypt interactions have not yet been demonstrated. Investigations into the effects of secondary compounds in eucalypt defoliators are few and show only a small effect on insect herbivory.

One of the most characteristic features of eucalypt leaves is the presence of oil glands; the secretions of which may comprise up to 5% of the fresh weight of leaves. Penfold and Willis (1961) suggested that oils may provide some protection from insect and fungus attack. However, there is little direct evidence to suggest that eucalypts are themselves protected by oils. Few studies have shown a relationship between foliar oil concentration and level of insect herbivory. Morrow and Fox (1980) examined the effect of leaf oils of

five eucalypt species which were growing in monospecific stands and found that the high oil yielding trees of *E. dives* and *E. viminalis* showed a significant negative correlation between oil content and insect damage, although the other three species had no effect on patterns of herbivory. Nevertheless, these authors have suggested that some insects overcome the oil defences of eucalypt leaves by toleration, passing it through the gut without change or by sequestering it in special body structures (Morrow *et al.* 1976; Morrow and Fox 1980), or by detoxifying it (Ohmart and Larsson 1989). It has been suggested that a threshold level of oil is needed to influence attack by adapted insect herbivores (Morrow and Fox 1980).

Some reports have claimed that tannin and phenolic compounds in eucalypt foliage also appear to have little influence on patterns and amounts of defoliation. Such an investigation by Fox and Macauley (1977) indicated that the variation in tannin and phenol contents of 11 eucalypt species did not appear to affect the growth of *Paropsis atomaria* and these compounds did not interfere with the efficiency of nitrogen utilisation of this insect species.

However, most publications appear to be concerned with the effects of N content of the leaves rather than that of secondary compounds or oils (Fox and Macauley 1977; Morrow and Fox 1980; Ohmart *et al.* 1985). Fox *et al.* (1977) investigated the insect response to variation in eucalypt leaf nitrogen and found that the nitrogen appears to affect the growth of eucalypt defoliators. She suggested that the growth and development of eucalypt defoliators depends upon obtaining sufficient nitrogen, which, however, is low in the foliage; therefore they must consume large amounts of foliage. Edwards and Wanjura (1990) demonstrated that *P. charybdis* is more efficient at converting nitrogen in *E. viminalis* foliage. Moreover, the nitrogen level of *E. blakelyi* foliage has been demonstrated to directly affect the growth of *Paropsis atomaria* larvae, the growth of which proceeds at an optimum rate regardless of the level of nitrogen ingested (Ohmart *et al.* 1985). Furthermore, variation in nitrogen level of foliage has been found to be an important factor which causes dieback in *E. blakelyi* (Landsberg 1990a,b,c). Thus, foliage of mature *E. blakelyi* trees suffering from dieback was more heavily grazed than that of neighbouring healthy trees, because the foliage of dieback trees was younger and higher in nitrogen content, even when corrected for leaf age with the healthy trees. However, these findings only indicated that insect growth and development may relate to the nitrogen percentage, but have not shown evidence of a systematic difference between the subgenera in leaf N contents and their relationship with host specify.

A striking feature of eucalypts is the different leaf types produced during the development of the tree. In many species, juvenile leaves differ markedly from adult leaves. This difference is marked in species of the blue-gum group, in which juvenile leaves are

opposite, sessile, ovate, and glaucous, whereas adult leaves are alternate, petiolate, lanceolate, and dark green. Tree height, distribution and growth habitats are features which differ in eucalypts. Studies of insect-plant interactions have indicated that several of these characteristic features may influence the interactions between the trees and their herbivorous insects (Ohmart and Edwards 1991).

Physical characters of eucalypt leaves have been reported to affect insect herbivory in some studies. The leaf waxes, particularly the glaucous bloom on the juvenile leaves of some blue gum species, have been suggested to confer resistance against *P. charybdis* adults due to their inability to grip on to wax on juvenile foliage of *E. globulus* and *E. nitens* (Edwards 1982). The glaucous wax surface of juvenile *E. globulus* foliage has been found to provide also a physical protection from *C. bimaculata* by preventing adult beetle attachment (Leon 1988). These findings have led to the speculation that *C. bimaculata* adults which defoliate adult but not juvenile foliage of *E. nitens* may be deterred by the physical effect of *E. nitens* juvenile foliage which is similar to the glaucous condition of *E. globulus* juvenile foliage.

The role of visual cues in host location of eucalypt defoliators has been investigated by Leon (1988) and Madden (unpublished data). Colour traps indicated a preference for yellow - light green, then yellow, then orange and lastly red. This has corresponded to field observation that *C. bimaculata* adults fed and oviposited preferentially on foliage of some "ash" species with a range of colours close to yellow and yellow-green (Madden, unpublished data). It has been suggested that the location of host plants by ovipositioning adults of *C. bimaculata* is aided by an attraction to 'orange' or anthocyanin-rich foliage (Leon 1988).

Entomologists have also paid attention to the tough and leathery nature of eucalypt leaves which may influence the population dynamics of specific eucalypt defoliators (e.g. Ohmart *et al.* 1987; Larsson and Ohmart 1988). Ohmart *et al.* (1987) suggested that toughness of foliage influenced the survival of *P. atomaria* larvae. *P. atomaria* fed preferentially on newly flushed eucalypt foliage which is high in nitrogen, however, as the foliage aged, leaves became low in nitrogen and very tough, and mortality probably resulted from the inability of first instar larvae to feed rather than by nitrogen starvation. Moreover, he proposed that nitrogen and foliage toughness influence the population performance of *P. atomaria*. Thus, during years of normal or enhanced growth of *E. blakelyi*, new foliage that is suitable for females to oviposit is plentiful and suitable for larval growth and development, favouring population growth. However, during years of drought, new foliage production would be minimal or absent, and old foliage which is tough and low in nitrogen would not be suitable for either adults or larvae, and therefore overall population performance would be reduced.

It has been found that population distribution of a insect species on eucalypt species was affected by a complex of factors. Strauss and Morrow (1988) found that the clumping of *Chrysophtharta hectica* within species *E. stellulata* and *E. pauciflora* was related to nutritional, spatial and biomass attributes of plants with plant height and foliage production the best predictors of beetle numbers.

2.2.4. The *Eucalyptus* -defoliating paropsine beetles

The *Eucalyptus*-defoliating paropsine beetles belong to a sub-tribe (Paropsina) within the tribe Chrysomelini of the sub-family Chrysomelinae which is one of 10 sub-families in the large cosmopolitan family Chrysomelidae (Briton 1970). Paropsine species feed mainly on foliage of *Eucalyptus* but also fed on foliage of related Myrtaceae genera : *Angophora, Tristania, Leptospermum* and *Melaleuca.* Some species also feed on the foliage of *Acacia* species (Cumpston 1939 in de Little 1979).

In a survey of eucalypt forest and woodlands through most of the island of Tasmania, de Little (1979) recorded that of the 36 paropsid species found to feed on foliage of eucalypts, 25 have been positively identified and classified as species belonging to five genera of the subfamily Chrysomelinae, *viz: Paropsis* Olivier, *Trachymela* Weise, *Chrysophtharta* Weise, *Paropsisterna* Motschulsky, and *Sterromela* Weise. He considered that the isolation of Tasmania from mainland Australia has led to the evolution of a distinct Tasmanian paropsine fauna, but that there are close affinities with the fauna of mainland, south-eastern Australia. For example, some endemic Tasmanian species have closely related species in the mainland south-east, e.g. *Paropsis incarnata* Erichson and *P. atomaria* Olivier.

The genus *Chrysophtharta* was established by Weise and *Chrysophtharta bimaculata* (Olivier) and *C. agricola* (Chapuis) were identified by de Little (1979) according to Blackburn's key and description (1899).

Adult *C. bimaculata* beetles are oval shaped approximately 9 x 7mm in size and are most easily recognised by the two distinct and invariable black marks on the pronotum. Live adults are variable in colour, changing from pale green to dark red-brown while hibernating. In their second season they change through a brilliant "brick" red to pale green with faint golden tessellation. The larvae of this species are highly gregarious and form large feeding colonies. *C. bimaculata* is extremely common in wet eucalypt forest and feeds preferentially on 'ash' species (genus *Monocalyptus*, series *Obliquae*).

In contrast, adult *C. agricola* beetles show more distinct differences in size between female and male with females approximately 9.6 x 7.5mm in size and males 8.8 x 7.1mm. It is the only Tasmanian paropsine species with a totally melanic form (de Little

1979). The *C. agricola* adult is easily distinguished from *C. bimaculata* by its body form which is much more convex in *C. agricola*. The colour of adult *C. agricola* are variable. In the spring season, after emergence from pupation adults are black with brilliant red prothoracic and elytral margins. After a short period of feeding, the red pigmentation is lost and the adults are entirely black. The larvae of *C. agricola* are strongly gregarious, feeding and resting in large groups. *C. agricola* is mostly encountered on 'gum' species (genus *Symphyomyrtus*, series *Ovatae* and *Viminales*).

C. bimaculata and *C. agricola* are closely related, both having adopted the "exposed" strategy having gregarious larvae, high fecundities, and closely synchronised phenologies (de Little 1979). However, they show strong host preferences in the field. Surveys of insect defoliation showed that *C. bimaculata* and *C. agricola* exhibited host specificity at any locality where there was a choice of potential host (Kile 1974; de Little and Madden 1976) with *C. bimaculata* preferring *Monocalyptus* and *C. agricola Symphyomyrtus* species.

The distributions of both *C. bimaculata* and *C. agricola* is not totally restricted to their preferred hosts. In a survey of the Tasmanian eucalypt defoliating paropsids, de Little (1979) encountered *C. bimaculata* and *C. agricola* on fifteen eucalypt species as listed in Table 2.5 and found that both insect species co-occurred on foliage of eucalypt species of both subgenera. However, in the non-preferred sub-genus, only adult *C. bimaculata* were found on *E. ovata* and *E. vernicosa* and adult *C. agricola* on *E. obliqua* and *E. delegatensis*. No larvae were found on the non-preferred subgenus.

In a comparison of the gross conversion ratio of larvae of *C. bimaculata* larvae fed on *E. delegatensis*, and *C. agricola* on *E. dalrympleana*, de Little (1979) suggested that *C. bimaculata* was a less efficient feeder than *C. agricola*. However, *C. bimaculata* developed significantly faster than *C. agricola* on *E. delegatensis*. Therefore, it was concluded that *C. bimaculata* was a more highly *"r"* selected species than *C. agricola*, because it had a selective advantage on *E.delegatensis*. In contrast, *C. agricola* has adapted to *E. dalrympleana* which was as suitable to it as *E. delegatensis*, but on which it did not face competition from a more highly *"r"* selected species.

de Little also suggested that the host preferences of these two insect species may be quite distinct between the species of the two different subgenera. However, their host specificities are still not clearly defined, as in some cases the findings of different workers conflict. Greaves (1966) reported that *E. regnans* and *E. viminalis* were the most favoured hosts for *C. bimaculata* in his study, *E. delegatensis* was also readily attacked, but *E. obliqua* and *E. simmonsii* (=*E. nitida* Pryor and Johnson 1971) were not attacked under natural conditions. However, *E. viminalis* was recognised to be an unsuitable host

for *C. bimaculata* in both field and laboratory, and *E. obliqua* a suitable host in de Little's study (1979). More recently, de Little (1989) observed heavy attack by *C. bimaculata* on the *Symphyomyrtus* species *E. nitens* which is commonly planted in Tasmania. Only the adult foliage was attacked, the glaucous juvenile foliage being avoided by *C. bimaculata* but attacked by *C. agricola.*

Table 2.5. The distributions of *C. bimaculata* and *C. agricola* in *Eucalyptus* spp. (de Little 1979).

	C. bimaculata		*C. agricola*	
	Adult	Larvae	Adult	Larvae
Monocalyptus species				
E. obliqua	+	+	+	
E. delegatensis	+	+	+	
E. regnans	+	+		
E. pauciflora	+	+		
E. amygdalina	+			
E. nitida	+	+		
E. coccifera	+			
Symphyomyrtus species				
E. ovata	+		+	
E. vernicosa	+			
E. nitens			+	+
E. globulus			+	+
E. viminalis			+	+
E. darympleana			+	+
E. rubida			+	
E. gunnii			+	+

Again, in a species trial where *E. globulus, E. nitens, E. delegatensis* and *E. regnans* were grown together, *C. bimaculata* preferred foliage of *E. delegatensis, E. regnans* and the adult foliage of *E. nitens* but not *E. globulus*. In contrast, *C. agricola* preferred foliage of *E. globulus* and *E. nitens* but not the 'ash' species *E. delegatensis* and *E. regnans* (Madden pers. comm.). Large numbers of adults, eggs and larvae of *C. bimaculata* occurred on the adult foliage of *E. nitens* as well as on the two 'ash' species. Once again, a large population of *C. bimaculata* was found to defoliate the *E. nitens* provenance trial in which trees had almost complete adult foliage. Similar findings have also been reported for other *E. nitens* plantations in Tasmania. These indicated that adult *E. nitens* was a suitable host for *C. bimaculata* as it supported larval growth, development and adult reproduction.

Southwood *et al.* (1974) suggested that the adventitious phytophagous insects which colonise transient habitats are selected for maximal food intake rather than their ability to utilise food efficiently. Thus highly *"r"* selected species are favoured in such situations where rapid population growth is at a premium rather than the conservation of food

resources and are more successful in establishing in new geographical regions. As a highly r-selected species, *C. bimaculata* has the potential to erupt in Tasmania in contrast to other paropsid defoliators.

The spatial dispersion of the host plant population and the species composition had been found to influence patterns of forest herbivory (Kareiva 1983; Stanton 1983; Schowalter *et al.* 1986 and reference therein). It has been suggested that eucalypt species that are the preferred hosts of only a few herbivores may do better if they occur in mono-specific stands (Morrow (1977a), and that *Monocalyptus* species tend to have a less diverse herbivore fauna. It is notable that *C. bimaculata* host trees are more likely to occur in mono-specific (Florence 1981; Kirkpatrick 1981) or mono-subgeneric stands (Austin *et al.* 1983).

In Tasmania, the wet sclerophyll forest, is dominated by subgenus *Monocalyptus* series *Obliquae* (the ashes). In those temperate habitats extensive continuous stands of ash species may occur (Davidson *et al.* 1981). Therefore, de Little and Madden (1976) speculated that *Monocalyptus* species are more susceptible to outbreaks of specialist herbivores and suffer high damage because they tend to occur in mono-specific stands. In addition, most species of Tasmanian eucalypts are resistant to intense fires, recovering from burning by sprouting epicormic buds and coppice shoots from lignotubers. However, the 'ash' species are killed by intense fires but due to their high seeding capacity, heavy seeding into ash-beds occurs after a hot burn. Since most of the undergrowth vegetation is killed by the fire, a dense, monospecific seedling stand results. de Little (1979) suggested that this ecological strategy of 'ash' species provided an ecologically unstable and ruderal habitat which favours the most highly *"r"*-selected pest *C. bimaculata*. Furthermore, 'ash' species of eucalypts have historically been the commercial timber species in Tasmania. A regeneration technique, which has been commonly employed, is the burning of logging slash followed by aerial seeding of the same species on to the ash-bed, to follow the natural ecological strategy of the 'ash'. Thus, they have provided a succession of large, monospecific regeneration stands highly attractive to *C. bimaculata*. Again, recent trends in large-scale planting of *E. nitens* has also attracted *C. bimaculata* to this new food resource.

In contrast, *C. agricola*, which is a lesser *"r"* species than *C. bimaculata*, preferred hosts of *Symphyomyrtus* species which are more fire-resistant and which recover from burning by sprouting epicormic buds and coppice shoots from lignotubers (de Little 1979). These species tend to occur in mixed stands with other fire-resistant *Monocalyptus* species and provide a more stable ecological strategy for *C. agricola*. Although *C. agricola* has been found to also favour *Symphyomyrtus* species in plantations, it does not appear to have the capacity to outbreak as does *C. bimaculata*. Moreover, *C. agricola* has never been

reported feeding on 'ash' plantation although it's larvae can feed on *E. delegatensis* in the laboratory (deLittle 1979) and adults were reported to attack natural stands of *E. delegatensis* (Greaves 1966).

Nevertheless, with the exception of *E. nitens, C. bimaculata* does not attack *E. globulus* and other *Symphyomyrtus* species which occur in many plantations in Tasmania, even where these plantations are established in the same way as 'ash' species plantations or where *E. globulus* is mixed with 'ash' in mixed trials. This indicates that the preference of *C. bimaculata* is specific rather than just the location of mono-specific stands or forest composition.

The differences in the ecological traits, and especially early growth rate is one of the most distinctive features between *Monocalyptus* and *Symphyomyrtus* species. *Monocalyptus* species have slower germination, resprouting and growth than *Symphyomyrtus* species but appear to catch up relatively quickly (Davidson and Reid 1980). Coley *et al.* (1985) suggested that slower growing species should have a greater investment in anti-herbivore defences than faster growing species. *Symphyomyrtus* species tend to grow slower after the initial establishment or re-establishment period, and to have higher levels of condensed tannins and phenolic compounds (Coley *et al.* 1985). However, Noble (1989) considered that the high production of secondary compounds has developed for reasons other than defense against herbivores or even as a response to the damage caused by herbivory because *Symphyomyrtus* species tend to suffer more damage from herbivores than *Monocalyptus*. But in contrast to Noble's conclusion, ash species from *Monocalyptus* species in Tasmania suffer more damage from pests than *Symphyomyrtus* species. Thus, *Symphyomyrtus* species must have a greater investment in defence against the major pest, *C. bimaculata.* While *Symphyomyrtus* species have tannins and phenolic compounds, the leaf oils have been found to differ markedly between the major pulp species of the *Symphyomyrtus* and the 'ash' species of *Monocalyptus* in Tasmania. Leaves of *E. nitens*, especially adult leaves, have very low oil contents compared to other *Symphyomyrtus* species (e.g. *E. globulus* and *E. viminalis*) (Li *et al.* 1990). *E. nitens* is one of the faster growing species among eucalypts. *E. nitens* not only has faster establishment and early growth, but also grows faster during the initial establishment than does *E. globulus* and other *Symphyomyrtus* species (Beadle and Turnbull 1986; Turnbull *et al.* 1988). If leaf oils of the *Symphyomyrtus* species play a role against *C. bimaculata*, then *E. nitens* lacks the defenses predicted by Coley *et al.* (1985).

The survey of the Tasmanian eucalypt defoliating paropsids by de Little (1979) showed that *C. bimaculata* adults occurred on juvenile leaves of the non-glaucous gum species, *E. ovata* and *E. vernicosa* but not on glaucous gum species. The glaucous juvenile leaves of

some gum species provide a physical protective barrier against attack by *C. bimaculata* (Leon 1988). This indicates that leaf waxes are also involved in host plant selection.

This review above indicates that both *C. bimaculata* and *C. agricola* appear to be strongly host specificity. However, since the highly "*r*"-selected species *C. bimaculata* has adapted to the *Symphyomyrtus* species *E. nitens,* their host selection can no longer be attributed to gross subgeneric differences.

It is known that secondary compounds play a mayor role in host selection of many other systems of insect herbivory. As suggested by de Little (1979), if the essential oils of *Eucalyptus* have a role in protecting them from defoliation by paropsids, some oil components in the foliage of eucalypt species (e. g. Penfold and Willis 1961) may also play a role in host tree selection. In the case of host specificities of *C. bimaculata* and *C. agricola* the investigation of leaf oils and waxes has relevance.

2.3. The principles of host-plant selection by insects

2.3.1. Factors involved in herbivory in forested ecosystems

Recent reviews of host-plant interactions on herbivorous insects are contained in several books, such as Ahmad (1983), Denno and McClure (1983), Crawley (1983), Hedin (1983) Barbosa and Schultz (1987) and Smith (1989). Reviews and discussion that focus on herbivory in forest systems have also been made by Schowalter *et al.* (1986) and Landsberg and Ohmart (1989).

These authors considered herbivory to be a function of size or biomass of herbivore populations. They reasoned that several majors factors influenced herbivory by affecting the natality, mortality and dispersal of such populations. These factors consist of abiotic factors, plant biochemistry, physical defenses, nutrients, predation and parasitism, and forest structure. The effects of these factors described by Schowalter *et al.* (1986) are re-summarized below.

a). Abiotic factors

Abiotic variables can influence herbivory directly through effects on insect feeding and survival and indirectly through effects on host susceptibility. The major controlling factors in forests are weather, soil conditions, and industrial pollution. Weather appears to be a dominant factor which influences insect activity, dispersal, defoliation, host choice and mortality.

b). Chemical defences

The chemical defenses are of two types: nitrogenous and nonnitrogenous. They considered the nitrogenous type to consist of non protein amino acids, cyanogenic metabolically glycosides, glucosinolates, alkaloids, and lectins which are relatively 'expensive' chemical defenses closely related to plant nutrition. Thus insect herbivory requires a potentially limiting nutrient for its construction.

Nonnitrogenous defenses include two major chemical compounds types: chemical products of the terpenoid pathway, tannins and related compounds of the phenolic pathway (see Harborne 1982). Terpenoid defences consist of mono-, di-, tri- and sesquiterpene derivatives, including their cardiac glycosides, saponins, and phytohormones, which are formed by the condensation of five-carbon isoprene units produced from mevalonic acid (see section: biosynthesis of terpenoids).

c). Physical defences

Physical or morphological defences often deter insect feeding and oviposition. They include characteristics of the leaf surface and margins, such as surface hairs or trichomes and epicuticular waxes, cutin, and suberin.

The leaf trichomes affect herbivores in several ways, such as to deter herbivore feeding, attachment or chewing on leaves, or eventually causing death of larvae. Plant leaves may be protected against insect herbivores by a layer of surface waxes over the epicuticle which functions as a physical or chemical defence. Leaf waxes, cutin and suberin act as physical defences making the surface slippery and camouflaging taste, while structural components such as calcium, cellulose, and lignins increase leaf toughness (Levin 1973). Leaf waxes containing allelochemicals may also affect insect feeding and oviposition (Thibout *et al.* 1982). Incorporation of silica and oxalate crystals lower palatability physically, whereas gums, resins, pectins, and milky sap are sticky and may also contain chemical defenses (McClure 1977).

d). Nutrients

Qualitative and quantitative levels of nutrients in food are important for phytophagous insects. The nutrients include nitrogen and inorganic irons. Thus, nitrogen levels of foliage have been found to be positively correlated with herbivory and nitrogen in foliage can limit insect populations. Some plants protect themselves from herbivores by producing less nutrious foliage instead of investing in feeding deterrents. Foliage levels of inorganic ion, such as K, and Ca, have also been found to correlate with herbivore biomass.

Schowalter *et al.* (1986) claimed that the effects of forest fertilisation on herbivory are still poorly understood because only limited studies have included the examination of plant allocation of nutrients. However, they referred to other workers suggestions that 'inducible responses of trees to herbivores is a by-product of mechanisms that rearrange the plant carbon/nutrient balance in response to nutrient stress' (Tuomi *et al.* 1983) and 'excess carbon that cannot be diverted to growth due to nutrient stress is diverted to the production of plant defenses' (Haukioja *et al.* 1985).

e). Predation and parasitism.

Forest herbivores are subject to a complex array of hymenopteran, dipteran, arachnid, and vertebrate predators and parasitoids. Meanwhile, predation and parasitism have a large effect on herbivore population size and therefore on the amount of herbivory in forested ecosystems. Pathogens may become epizootic in dense defoliator populations, resulting in population decline (Leonard 1981; Torgersen and Dahlsten 1978). Trees may 'buy' protection from herbivores by encouraging residence by predators (Tilman 1978; Price *et al.* 1980) and folivores engage in a number of predator-avoidance behaviours that effect herbivory in forested ecosystems (Holmes *et al.* 1979; Heinrich and Collins 1983).

f). Forest structure

Forest structure determines the spatial distribution of acceptable resources and influences the efficiency of resource discovery by dispersing herbivores. Aspects of forest structure also affect host condition, which may influence survival of feeding herbivores. On a set of host plants tree age, size, density, species composition, and vertical stratification, across stands and through time, could influence patterns of forest herbivory.

The effects of these factors described by Schowalter *et al.* (1986) and discussed by above other authors indicated that factors involved in regulating insects in forested ecosystems are very complex. Insect/plant interactions in forest system can be fully understood only when approached as a holistic phenomenon in community ecology. Competition and predation, evidently play a major role in the final realisation of an insect's feeding niche. However, there is little doubt that plant chemicals, are the important or key factor in the establishment of these associations, in particularly the host plant selection (Smith 1989).

2.3.2. Definition of plant chemicals involved in host-plant selection

An important definition of the host range of phytophagous insects was provided by Dethier (1954), who suggested that definitions of host range should be made on the basis of the chemical interaction between insect and plant. However, he regarded host range as

being determined by the attractant range of plant chemicals to the insect. This view was later criticised by several authors when more research was carried out on this field (Ishikawa *et al.* 1969).

Thorsteinson (1960) enlarged the view of Dethier and considered host range to be determined by all the varieties of stimuli offered by a plant and not just the chemical ones. He suggested that host range was determined by a 'signal pattern' which is capable of inducing feeding by an insect when close to the plant and that food-plant preference was subdivided on the basis of the distribution of chemical stimulators or inhibitors among plant taxa.

The separate stages in the behavioural pattern of an insect finding and recognising an acceptable host plant were modified by Beck (1965) and are, (i) directed movement towards a plant, (ii) cessation of movement on reaching the plant, (iii) the initiation of biting or piecing, and finally (iv) continued feeding. He classified stimuli into a sequence which may exist in either a positive or negative response. Thus, the positive stimuli are positive attractants, arrestants, incitants and stimulants. In contrast, the repellents, suppressants and deterrents are negative stimuli. Hsiao (1985) further described chemical stimuli affecting insect feeding behaviour and classified them on the basis of the behavioural patterns they elicit (Table 2.6).

Table 2.6. The chemical stimuli involved in insect feeding behaviour (after Hsiao 1985)

Behavioural effect	Positive Stimulus	Negative Stimulus
orientate insect to habitat or patch	attractant	repellent
elicit biting or probing	arrestant	locomotion stimulant
induce ingestion or engorgin	feeding stimulant phagostimulant	feeding deterrent
In addition:		
Feeding cofactors -	chemicals which do not elicit feeding responses by themselves but synergise with other feeding stimulants	
Toxicants/toxins -	chemicals producing acute neurotoxicity	
Metabolic inhibitors -	chemicals causing chronic adverse effects on growth and development	

Whittaker (1970) reviewed the biochemical ecological aspects of higher plants, and introduced the term *allelochemical* to replace *secondary plant compounds*. From an

ecological standpoint, an allelochemical was defined as a secondary substance produced by an organism that has the effect of modifying the growth, health, behaviour or population dynamics of other species, often having an inhibitory or regulatory effect (allelopathic substance) (Lincoln *et al.* 1982). An allelochemical, which is regarded as a non-nutritional chemical involved in host selection by insects, has been shown to affect insect feeding behaviour, oviposition, and survival in either a positive or negative manner. Thus, allelochemicals function to affect insects in a positive manner and can function as *kairomones*, when the chemical benefits the recipient insect and is represented by attractants, arrestants, and feeding or oviposition stimulants. Conversely, in a negative manner as allomones, when the chemical benefits the plants, and represented by plant-produced deterrents, repellents, or inhibitors of feeding and oviposition (Smith 1989). Allomones may also be produced by the insect themselves (Evans 1984).

2.3.3. Mechanisms of chemical defenses involved in insect host plant selection

The effects of chemical defences on herbivorous insects results in differential host plant selection through their effects on both insect sensory and metabolic systems (Visser 1983).

Visser (1983, 1986) suggested that visual and chemical stimuli are perceived simultaneously during the orientation of an insect to a potential host plant. He described that during long-range orientation, an insect may use vision for recognition of the shape of an object or utilise olfaction to perceive a plant attractant. Chemical defences may affect the responses elicited by insect herbivory. Odours emitted by plants that stimulate insect olfactory receptors involved may result in an insect moving toward or away from plants such as described by Visser (1983, 1986).

Andow suggested that plant volatile chemicals could influence host finding and thus affect insect populations (1986 and reference there in). Some workers found that insect response to host odours varies with concentration and is influenced by non-host vegetation near the host (Kareiva 1983; May and Ahmed 1983; Stanton 1983). Moreover, Morrow *et al.* (1989) postulated that non-host plant may affect host finding indirectly through decreasing host plant density and thus concentration of host volatiles or directly, by altering host quality through competitive interactions, making host less attractive or changing the way that host volatiles are perceived by insect.

Prokopy and Owens (1983) suggested that the final contact with the plant surface by many foliage feeding insects is due to a positive response to yellow or yellow-green

pigments in plant foliage where pigment compounds play an important role forming the colour characteristics of foliage.

The activity of chemical defences can affect an insect's taste where several different types of gustatory receptors detect both qualitative and quantitative differences in the chemical content of the plant tissues tested, as described by Hanson (1983). Therefore, chemical defences may act as deterrents or stimulants for insect feeding, resulting in feeding preferences, such as phenolic (Levin 1971) and tannic (Zucker 1983) compounds.

Chemicals involved in host plant selection may act through antibiosis, which is the negative effect of a plant on the biology of an insect herbivore attempting to use that plant as a host. The antibiotic effects of plants range from mild to lethal. Smith (1989) suggested that generally, lethal effects mainly affect young larvae and eggs while chronic affects can lead to mortality in older larvae, prepupae, pupae, and adults through failure to moult, pupate or eclose. Antibiosis may also result in a reduction of body size and weight, prolonged periods of development in the immature stages, and the reduction of fecundity of surviving adults. Beside the chemical defenses antibiosis may also be due to the lack of nutrients in plants.

2.3.4. The role of phytochemistry in host specificity

Many plant chemicals, nutrient and non nutrient, are widely recognised to elicit behavioural responses in insects. However, the nature of the role played by these chemicals in host selection is the subject of a long running debate amongst entomologists. The two major proposals that have been developed are the *token stimuli* and *dual discrimination theories*. These theories are reviewed by Smith (1989).

The *token stimuli theory* was first proposed by Fraenkel (1959) and further developed in a major review by Ehrlich and Raven (1964) in which a new theory of biochemical coevolution between animals and plants was proposed. Subsequent to the publication of Ehrlich and Raven's review, several important reviews which extensively explored the coevolution of plants with their insect herbivores were provided by Dethier (1972), Feeny (1975), Fraenkel (1969), Meeuse (1973) and Schoonhoven (1968, 1972). The symposium contribution of Visser and Minks (1982) are also relevant.

Harborne, a major contributor to this topic (1977, 1982, 1988), continued to review the proposals of Fraenkel (1959) and Ehrlich and Raven (1964) and extensively explored the coevolutionary aspects between insects and plant biochemicals. He considered the evolution of host plant selection within an overall evolutionary context between both insects and their potential host plants and, suggested that host plant specificity by insects is the result of the coevolution of insects and plants with plant secondary compounds

(terpenoids, alkaloids, flavonoids *et al.*) all probable major factors controlling relationships between insects and plants.

Essentially, the coevolutionary theory considers the development of insect specificity paralleling the coevolution of insects and plants. In the long course of evolution that has occurred in plants, plants have responded to insect herbivory attack by the use of chemicals as defensive agents. Insects may overcome these defences by behavioural and metabolic changes that have undergone adaptation to host plants which underscores the genetic plasticity of each of the participating organisms. Some of the species of insects which have overcome plant defence were able to utilise such chemicals as specific sensory cues in the identification of their host plants. Fraenkel (1959, 1969) suggested that phytophagous insects were essentially similar in nutritional requirements being adequately provided by most plants and, therefore, postulated that these nutritional substances could not be a factor in determining host plant specificity. Thus, '....host selection is guided by the presence or absence of secondary plant substances, and that qualitatively or quantitatively nutrients can play only a very minor role, if one at all, in this context' (Fraenkel 1969). He also considered that the frequent occurrence of monophagy or oligophagy among phytophagous insects was therefore the result of a long evolutionary chemical struggle between insects and their host plants. Thus, the insect segment of plant-insect coevolution is a shift from polyphagy (general feeding) to oligophagy (specialised feeding) or monophagy (highly specialised feeding on a single plant species).

There has been much debate in the past whether preferential feeding behaviour of insects is determined by nutritional requirements or solely by their response to hostile chemicals, since many studies have also demonstrated that feeding stimulants such as amino acids, carbohydrates, and vitamins could frequently be nutritionally important substances, (e.g. Beck 1965; House 1969, Hsiao 1969, 1985). Such information, although mostly based on laboratory experiments, provided evidence for the *dual discrimination theory,* which was proposed by Kennedy (1965). Kennedy's proposal views host selection as based on insect responses to both nutrient and non nutrient phytochemicals with host selection determined by a combination of both nutrients and secondary chemicals.

An important review on the effects of different proportions of nutrients on insects was provided by House (1969), who discussed the conversion of food material into insect performance as measured by growth, development, survival, reproduction and other vital functions but without concern for the stimuli to which an insect responds when locating food and recognising its suitability. He considered all insects to have quite similar qualitative nutritional requirements as it is likely that all plants contain the 25 or so nutrients required by insects. Therefore, he suggested that these qualitative factors for insect requirements play a less decisive role in the relations between insects and plants

and emphasised quantitative aspects as more important. Thus, whether the food plant is suitable for insect requirements 'depends on how much of each nutrient digested can provide for insect nutrition; and on how well the amounts and proportions of the nutrients made available fit the quantitative nutritional requirements, including especially proportional relationships, of insects.'

A important review of the chemosensory basis of host-plant selection was provided by Schoonhoven (1968) who considered that monophagy and oligophagy can be well based on a fairly subtle combination of a number of common plant components, combined with absence of several plant substances. He gave a more balanced view of the interaction of both nutritional and non nutritional chemicals in determining the host selection of a phytophagous insect. Thus, "when combining the present knowledge about the type of sensory information which the insect central nervous system receives from its gustatory receptors, it appears that sapid nutrients as well as secondary plant substances together characterise a particular plant for its commensal....In some insect-plant relations the accent may lay on the stimulating or deterrent effect of odd substances, in other cases the amounts or ratios of the nutritive constituents may form the decisive factors. Several volatile factors, acting as sign stimuli to release feeding behaviour or oviposition belong to the category of the secondary plant substances.'

At present, few insects have been studied in detail for the factors controlling all the various facets of host selection and ingestion to be known. The chemical basis of host selection of the chrysomelid *Leptinotarsa decemlineata* Say, Colorado potato beetle (CPB), an oligophagous insect naturally occurring on members of the plant family Solanaceae, is an example of the different chemical facets of host selection. The series of studies on feeding behaviour undertaken by Hsiao and Fraenkel (1968a,b,c,d) represent the major contributions to the chemical basis for host selection by this insect which is supported by many similar findings in other oligophagous insects.

First, the nutrient chemicals, including sucrose, several amino acids and phospholipids, have been recognised to stimulate feeding in CPB while several inorganic salts were regarded as feeding co-factors. However, the function of these stimulating chemicals has to be regarded in terms of their chemosensory influence on the insect rather than their nutritional value (Hsiao and Fraenkel 1968a). Hsiao and Fraenkel (1968a,b) also considered that these feeding stimulants and co-factors were widely distributed in the plant kingdom and therefore, could not be regarded as specific token stimulants for the potato beetle.

Second, Hsiao and Fraenkel (1968b) found that CPB larvae were able to discriminate between different species of solanaceous and non-solanaceous plants. Thus, the recognition of the host plant and initiation of feeding by this insect appeared to require a

botanically restricted chemical for the presence of active substances in various fractions of potato-leaf extract and an active flavonoid.

Further experiments showed that both the leaf powder of 9 solanaceous and 9 nonsolanaceous plant enhanced feeding and growth. Thus, as both solanaceous and nonsolanaceous were effective, it suggested that the active substance was generally distributed in plants. However, the active substance was not confined to a particular fraction of these powders when each of them were separated into different extract fractions and it was concluded that several kinds of plant substances were responsible for the increase of larval growth. Hsiao and Fraenkel (1968c) therefore suggested that a diverse group of plant chemicals can serve as feeding stimulants for potato beetle larvae which is in agreement with findings on the influence of nutrient chemicals (Hsiao and Fraenkel 1968a) and the occurrence of feeding stimulants in the host plant of this insect (Hsiao and Fraenkel 1968b).

Again, Hsiao and Fraenkel (1968c) found that substances that affected the degree of feeding inhibition varied with species with effects ranging from reduced weight gains by beetle larvae to complete suppression of feeding. These substances were confined to particular fractions, and in most cases the inhibitory substances occurred in water extracts of leaf powder, with few in other fractions. The results also showed that the presence of feeding deterrents was responsible for the resistance of solanaceous and nonsolanaceous plants. Since their results imply that a large number of chemically and botanically unrelated plant substances are responsible for the inhibition of feeding by the potato beetle, Hsiao and Fraenkel (1968c) concluded that:

"the high degree of sensitivity to deterrent chemicals stresses the important role of secondary plant substances in the resistance of plants to feeding by the beetle."

Moreover, the acceptability and suitability of foliage for CPB larvae were tested for leaves of 104 species by Hsiao and Fraenkel (1968d). They found that 36 were accepted to some degree, but only 15, which belonged to 5 unrelated families, supported growth of four-instar larvae to pupation. Four nonsolanaceous plants also supported growth from egg to adult, and 2 of them supported reproduction and continuous culturing. However, preference experiments indicated discrimination by larvae between solanaceous and nonsolanaceous plant. Thus, when potato leaves were present, the non-host plants (nonsolanaceous) were not selected. With non-hosts they found that larvae required a considerable period of adaptation before normal feeding proceeded but this was not observed with solanaceous plants. These results indicated that nonsolanaceous plants lack a chemical stimulus which is required by larvae to recognise the plant. From this evidence they suggested that:

"feeding and growth do not require the presence of host-specific substances"... however, "the host selection of the potato beetle is determined not only by the availability of adequate feeding stimuli and nutrients of the plant, but also by the presence of a host-specific substance which releases the initial feeding behaviour."

Furthermore, in the oviposition preference of the adults for potato and other plants where 11 solanaceous and 6 nonsolanaceous plant were tested, the potato plant was preferred. However, one species, *Solanum nigrum,* which was highly attractive for oviposition, was never grazed by adults and larvae. Hsiao and Fraenkel (1968d) suggested that host specific stimuli were required for oviposition and that selection of an oviposition site was the first and primary step in host selection by the CPB. They also showed evidence to suggest that oviposition stimuli (both positive and negative) were chemical and not physical in nature.

Hsiao's (1969) review of the available information from studies of CPB and the alfalfa weevil [*Hypera postica* (Gyll.)], concluded that host specificity in both species occurred and, that many different types of plant chemicals served as regulating factors for host selection. The main points of Hsiao's conclusion were:

"The host finding and oviposition of both species require the presence of host specific olfactory and gustatory stimulants and the absence of repellents and deterrents. Recognition of host and initiation of biting and feeding also require the presence of host specific sign stimulants....On the basis of available evidence, it appears that the resistance of botanically unrelated species to oligophagous insects is largely due to the presence of a diverse group of repellents and deterrents and the absence of attractants and feeding stimulants. Among the botanically related plant species or varieties, the presence of physiological inhibitors, and the deficiency and imbalance of nutrient factors would also be important determining factors."

Later, Visser and Nielsen (1977) found evidence to explain the host finding and oviposition preference of *L. decemlineata* adults, and demonstrated that unfed, newly emerged females and unfed post-diapause beetles were attracted by the volatiles of several solanaceous and nonsolanaceous species. They concluded that olfactory orientation would mainly lead adult beetles towards solanaceous species. Thus, secondary plant substances play a major role in the host selection and oviposition preference of *L. decemlineata* adults.

Moreover, Hsiao (1978) found evidence for four distinct, geographically isolated and host adapted populations of *L. decemlineata* in North America. He considered that this finding implied that the formation of host races or biotypes among oligophagous insects must be preceded initially by geographical isolation of the preferred and non-preferred

hosts, so as to allow sufficient time for the insect population to develop adaptation to the less preferred host.

Nevertheless, detailed study on one of the most typical examples of a monophagous insect, the silkworm *Bombyx mori* L., has demonstrated the inter-relationships of the various chemical factors that control the various facets of host selection and ingestion of larvae (Hamamura 1959, 1970).

Hamamura (1970) found that different chemicals were essentially controlling the complexity of the insect-plant interaction in which the volatile secondary plant substances may be involved in the initial phases of host location while nutrients act during later stages. Thus, the isoprenoids present as secondary plant compounds in mulberry leaves (e.g. citral, linaly acetate, linalool and terpinyl acetate *et al.*) were all found to be attractive to *Bombyx mori* larvae while foreign isoprenoids were unattractive. Factors initiating biting were also the secondary compounds ß-sitosterol, sioquercitin and morin. However, the next step in the feeding sequence, which was termed 'swallowing factors', was stimulated by a range of different chemicals, such as inositol, sucrose, silica and potassium phosphate. These substances when combined with several nutrients in an agar base, evoked feeding in *Bombyx* larvae. However, it was found that growth and development of larvae were poor unless twenty per cent of mulberry leaf powder was added to the agar diet. Thus, some secondary plant compounds, such as chlorogenic acid, linolenic acid and oleic acid were identified as growth promoting factors present in mulberry leaves. When additional nutrient chemicals, e.g. acetylcholine, gallic acid or protocatechuic acid, were added to the artificial diet without leaf powder growth and survival were improved, but the average weight of the cocoon produced was still below that of larvae fed a leaf-based diet.

Currently it is generally agreed that host selection is based on insect responses to both nutritional and non nutritional phytochemicals because the relative importance of nutrition versus secondary chemistry may vary from insect to insect (Harborne 1988).

Harborne (1988) stated that 'secondary chemistry is usually the controlling factor, since all plants are relatively similar in nutritional value'. He pointed out the two important facts in support of this view. Thus, 'Since the primary biochemical processes within the leaf are practically identical in all green plants, the relative amounts of sugar, lipid, polysaccharide, amino acid and protein are inevitably very similar. Again, physiological processes (e.g. senescence) are more likely to affect nutritional status in the plant than anything else' (Harborne 1988). In addition, little concrete evidence has been provided from insect feeding studies to suggest that certain plants are avoided because they are nutritionally inadequate (van Emden 1973).

From this review it can be seen that the host specify by insects is very complex. The diversity of insect/plant interactions related to plant chemicals cannot be explained by one universal model. However, both theories on this subject have reasoned that the plant chemicals are one of the most important factors controlling host specificity regardless of nutritional or non nutritional phytochemicals. It appears that volatile secondary plant compounds are more important in the initial phases of host selection, and that non-volatile phytochemicals (nutrients or non nutrients) operate principally in the later stages of the process. However, as complex relationships have not evolved in, nor operate in a void, it must be considered that influences affecting the life and development of both partners must operate within the framework of a dynamic equilibrium existing between insects and their potential host plants.

2.4. Introduction to chemotaxonomy

Plant taxonomy (botanical systematics) is the scientific study of (i) delimiting and naming any group of plants considered to represent a distinct unit (taxonomic unit or entity = taxon); (ii) arranging all recognised taxa in a logical classification (system); (iii) the relationships between taxa; (iv) the dynamics of evolution of characters and taxa and (v) the dynamics of the evolution of characters and taxa in the past and present. Chemotaxonomy (or chemosystematics) is the use of chemical characteristics for taxonomic assessment. Comprehensive reviews of this discipline are provided by Harborne (1968a,b), Smith (1976) and Harborne and Turner (1984).

The principles of chemosystematics have early beginnings. The early history is largely the history of the medicinal use of plants. Early herbalists began grouping together those with similar medical properties. By doing this they based their taxonomy upon the chemical attributes of plants. Toward the end of the 17th century the groupings began to resemble some of the modern plant taxa. The writings of 17th century taxonomists refer to the importance of chemical properties of plant groups (Smith, 1976). The relationship between chemistry and plant taxonomy has moved away from the pharmacological aspects toward providing a source of new characteristics which may improve classification and elucidate phylogenies.

Today, the most common feature to be used as a chemical characteristic has been biochemical including micro- and macromolecules and metabolic pathways (Harborne and Turner 1984; Hegnauer 1986). The investigation of macromolecular characters involves a broader spectrum of chemical characteristics, such as amino acid sequences of proteins, which are more closely related to the genetic make-up of plants than secondary substances

and provide valuable information about phylogeny and relationships at the higher taxonomic categorical level (families, orders, classes) (Boulter and Parthier, 1982). The investigation of micromolecular characters uses 'secondary plant products' or 'secondary metabolites' which also provide an insight into evolutionary processes and, in general, have been applied in the study of lower taxonomic categorical levels, such as species and genus (Turner 1969; Seigler 1981).

2.4.1. Secondary plant products as chemical characters for chemotaxonomy

Secondary plant products are micro-molecular organic compounds which are non-polymeric metabolic products. Included in this group are non-protein amino acids, alkaloids, flavonoids, betalains, glycosides and terpenoids, etc.. Secondary compounds can be defined as "compounds that have no recognised role in the maintenance of fundamental life processes in the organisms that synthesise them" (Conn 1981). This definition excludes the intermediates and end products of primary metabolic pathways and compounds such as the photosynthetic pigments of green plants and the oxygen-carrying pigments of mammalian blood (Bell 1981).

Many studies have indicated that secondary plant products, not less than the morphological features of plants, are subjected to the selection pressures of the environment (Harborne 1988 and references therein). Seigler (1981) suggested that, in plant evolution, all characters of a plant are related and self-consistent while the rate of change in the genetic material of a plant in large part depends on the function of products produced. Therefore, the evolution of morphological and chemical features of a plant must be interrelated but, significantly, the forces of natural selection do not have the same effect on each type of genetic expression. And therefore the evolution of chemical constituents may differ from morphological evolution through differences in selection so that the two processes are not parallel from a taxonomic standpoint (Turner 1970). Furthermore, for chemical constituents, the structure of any compound may be determined by different biosynthetic procedures under different selective forces. This suggests that not only the structure of a compound itself but the biochemical pathway from which it has arisen may be of taxonomic significance (Harborne and Turner 1984). Many chemotaxonomic studies have shown that secondary plant products, in conjunction with their metabolic pathways, have been valuable characters for resolving many problems of speciation and evolution (Harborne and Turner 1984 and references therein).

The rationale for the application of secondary plant products has been discussed by several authors (Hegnauer 1976, 1986; Seigler 1974, 1981; Harborne and Turner 1984). From these, several viewpoints should be noted in mind before the determination of

secondary compounds and their biosynthesis at what point has the sequence diverged and what subsequent changes have come to pass. First, as discussed by Hegnauer (1976), the biosynthesis and the accumulation of secondary products are distinct processes. These authors suggested that the concentration of secondary plant products in plants may be affected by natural selection operating on different processes such as the storage of products which one observes as the accumulation or disappearance of an altered product (e.g Seigler 1981). Second, one of the fundamental problems inherent in the taxonomic application of secondary plant products is the determination of the origin of homologues (Hegnauer, 1967). Analogues and homologues of secondary plant products should be recognised with enough biogenetical information or sufficiently plausible biogenetical hypotheses (Hegnauer 1986). Third, two taxa may synthesise or accumulate the same products by different pathways, therefore a relationship between these taxa is not necessarily indicated by the mere presence of a compounds (Seigler 1974, 1977, 1981). Fourth, even the structural characters of homologues is often not sufficient to indicate phylogenetic relationships due to metabolic convergence which is a very common feature and therefore should be examined carefully and compared with many types of characters (Hegnauer 1986). Finally, many secondary plant products may perform diverse ecological roles. This aspect should not be neglected because some of these compounds may be more ecologically important so occur in unrelated taxa but less deviate from essential metabolites.

2.4.2. Observable variation in secondary plant products and data collection for chemotaxonomic studies

A relationship between plant taxa is established by summarising the similarities among groups of plant populations and contrasting their differences. From this procedure, the plants can be considered to be either similar or closely related if they have many common characters, and only distantly so (or at higher categorical levels) if differences outweigh the similarities. In contrast to this, the important factors in evolution are change and the ability to maintain variability (Seigler 1981). Many studies have indicated that secondary plant products within the plant may vary in their distribution such as the amount and composition of classes of compounds which may be governed by age of either the plant or its parts, by the plant's locality and its habitat, etc.. Furthermore, chemical character data may also vary from experimental operations. The variability in chemical characters found among individuals in an given chemotaxonomic study, as summarised by Harborne and Turner (1984), may reflect several variables: (1) developmental differences among the organs or organisms being studied; (2) environmental conditions prevailing at the site or sites from which the samples are taken; (3) genetic differences among the

individuals and populations studied; and (4) inconsistency of sampling techniques and inconsistent techniques of extraction and analysis.

The amount and composition of phytoconstituents usually vary from different parts of an individual plant at various developmental stages or ages during a plant's growth and development which reflects the expression of enzymes. For example a given part of plant, such as leaves of some eucalypt species, may have different morphological types between juvenile and adult growth stages and essential oils may vary in these different leaves (e.g. Boland *et al.* 1982; Boland *et al.* 1990). The variability in the phytoconstituents of leaves is also commonly associated with leaf age and seasonal variation (Zavarin *et al.* 1971; Adams 1979). Harborne and Turner (1984) suggested that samples for chemotaxonomic work can only come out of studies in which multiple comparisons are made of chemical characters taken from comparable parts of plant at comparable stages of plant development.

Furthermore, the phytoconstituents may also vary between plants within a population associated with variation from genetic and environmental sources as do plant height, flowering time or size of vegetative features (Cranmer and Turner 1967; Nowacki 1963; Harborne and Turner 1984). Consistent sampling techniques and careful experimental design are needed to avoid this variability in a chemotaxonomic study. In addition, techniques for extraction and analysis should be carefully chosen. As has been pointed out by Harborne and Turner (1984), if experiments in chemotaxonomy had recognised these problems, most of the qualitative variability encountered would probably be due to genetic variation while quantitative variability would probably reflect environmental factors.

The principles and practices of describing the environmental and genetical variation of plants were developed using morphological characters. Therefore, some important aspects of morphological classification and basic definitions relating to chemotaxonomy are summarized here.

Variation in natural populations of plants can be partitioned into genotypic, environmental and environment x genotype components of variation. The genotypic component is that component of the phenotypic variation which is heritable and may arises through mutation and recombination of genes (Davis and Heywood 1963). Environmental variation arises through external factors such as spatial, temporal, physical and general ecological factors etc.. The environmental x genotype interaction arises through the differential expression of the genotype in different environments. Samples of natural populations confirm these sources of variation. However, environmental components are minimised when samples are taken from the same locality.

The way to partition the phenotypic variation into causal components is either through growing clones or families collected from field populations in a common environment to determine the magnitude of the genotype x environment interaction. The genetic material must be planted in trials established across a range of environments (e.g Potts 1985a,b). The genetic components of variation can be further partitioned into additive and non-additive components using various pedigreed mating designs (Lawrence 1984). From these, the proportion of the phenotypic variation which is due to additive genetic effects is termed the narrow-sense heritability whereas the proportion which is due to total genetic variation is termed the broad-sense heritability.

In nature, species populations affected by geographical, ecological, or reproductive barriers may exhibit clinal variation which tends to differentiate into a series of populations with gradually accruing differences or ecotypic variation which is stepwise variation associated with ecological differences (Davis and Heywood 1963). Where these differences among populations increase sufficiently and especially when reproductive barriers arise, these differentiating populations may be recognised as separate species (Seigler 1981). In contrast, where the genetic variation within a local population is discrete rather than continuous and individual variants are able to become new races by migration and selection, the genetic variation in the population is termed a polymorphism. Furthermore, species which comprise several races or subspecies, i.e. units with their own combination of characters and area and (or) habitats are called phlytypic (Hegnauer 1986).

Variation in plant chemicals may be inconsistent with morphological characters (Pryor and Bryant 1958), however, there is no reason to think that variation in chemical characters has not occurred and is not maintained in a similar manner. Furthermore, secondary compounds with specific structures and different biosynthetic pathways and steps may better identify genetic control (Seigler 1981). Many studies have indicated that variation in secondary compounds are also largely under the control of environmental and genetic factors. Occurrence of variation in secondary plant products bearing on these aspects, mainly with respect to flavonoids, terpenoids and alkaloids, have been discussed by Hegnauer (1976), Seigler (1981), Harborne and Turner (1984). The reader is referred to these comprehensive reviews.

2.5. Essential oils - a background

2.5.1. Definition of essential oils

Steam distillation of plant material produces an extract known as 'essential oil' or 'volatile oil' (Dell and McComb 1978). For the study of this thesis, the definition of essential oil is the biologically oriented definition of Hegnauer (1982):

"Essential oils are complex mixtures of odorous and steam-volatile compounds which are deposited by plants in the subcuticular space of glandular hairs, in cell organelles, in idioblasts, in excretory cavities and canals or exceptionally in heartwoods".

From a chemical and biogenetical standpoint, essential oils are heterogeneous chemicals commonly belonging to three different classes of compound: (1) mono-, sesqui- and di-terpenoids which are derived from mevalonic acid; (2) phenylpropanoids derived from cinnamic acid and (3) alkane derivatives (alkanes, alkenes, alkynes, alkanols, alkanals, alkanoic acids: mostly acetogenins). An essential oil may be formed by components from one, two or all three biogenetic families of compounds (Hegnauer 1982). It has to be noted that many publications dealing with chemotaxonomic studies based on the analysis of essential oils often use the term 'terpenoids' or 'terpenes', such as 'terpenoids are the main components of essential oil' (Dell and McComb 1978), in fact to include only mono-, sesqui- and di-terpenoids but no other class of terpenoid compounds.

2.5.2. Chemistry and biosynthesis of terpenoid compounds

Terpenoids are the main components of essential oils (Dell and McComb 1978). Terpenes, a generic term, can be recognised as compounds based on multiples of 5C isoprene units (C_5H_8). Terpene compounds possess 5, 10, 15 or 20 carbon atoms, to form hemi-, mono-, sesqui-, and di-terpenes respectively. The term 'terpenoid' covers a wide range of plant substances derived from isoprene units via common biosynthetic pathways. Terpenoids can show modified structures which result in deviations from exact multiples of the 5C structure (Dell and McComb 1978).

Monoterpenoids and sesquiterpenoids are compounds which contain a sequence of two or three isoprene units joined together in the structure indicating their origin from either head to tail or head to head units or derived from these initial condensations due to a range of secondary chemical transformations (Loomis and Croteau 1981). They are biosynthesized from mevalonic acid which is itself formed from photosynthates through the biologically active isoprene isopentyl pyrophosphate plus dimethylallyl pyrophosphate. The monoterpenes arise from a precursor, geranyl pyrophosphate, through the coupling of two isoprene units, while the head-to-tail coupling of three isoprene units provides

farnesyl pyrophosphate to serve as the progenitor of the sesquiterpenes (Dell and McComb 1978).

Since the 1960's separation and quantification of essential oils by Gas- Liquid-Chromatography (GLC) has been the major technique and the number and chemical typing of terpenes known to occur in plants has increased dramatically (Loomis and Croteau 1973). The structures and occurrence of terpenoids in plants have been reviewed by several authors (Devon and Scott 1972, Newman 1972, Nicholas 1973 and Robinson 1980; Erman 1985). The chemistry and biochemistry relating to terpenoids in this thesis, such as chemical names and genetic category of structure, are based on those proposed by Devon and Scott (1972).

Monoterpenoids may be classified simply into acyclic (open chain), monocyclic (one ring) and bicyclic (two ring) compounds, where the monoterpenes of each group may be further chemically subdivided into hydrocarbons and other functional groups such as alcohols, aldehydes, ketones, acid and esters (Harborne and Turner 1984). However, they can be structurally divided into four categories: acyclic, cyclopentanoid, cyclohexanoid, and irregular monoterpenes. The first three categories are constructed by the typical head-to-tail fusion of isoprene units and the last is atypical (Loomis and Croteau 1981). Furthermore, the monoterpenes are genetically classified into acyclic, menthane (monocyclic), pinane, bornane, carane, thujane and iridane classes according to their original genetic skeleton (Devon and Scott 1972; Charlwood and Banthorpe 1978).

Sesquiterpenoids are also mevalonate derived but their structures are based on three isoprene units so that they have C_{15} skeletons (Harborne and Turner 1984). They may be classified into four major groups; acyclic, monocyclic, bicyclic and tricyclic (Erman 1985). Because sesquiterpenes contain one more isoprene unit than monoterpenes, this allows much greater flexibility in the construction of sesquiterpenoid compounds. Almost 200 different carbon skeletons and several thousand individual sesquiterpenes have been identified. Most are formed by the typical head-to-tail fusion of isoprene units while the many atypical types can be regarded as rearrangement products of a more typical head-to-tail structures (Loomis and Croteau 1981). Details of these structures and genetic skeleton types of sesquiterpenoids, are provided by Devon and Scott (1972).

Plant monoterpenes and sesquiterpenes are usually synthesised and accumulated in complex secretory structures. These specialised structures appear only in particular organs at particular stages of plant development. Their formation is greatly influenced by environmental conditions. The biosynthesis of monoterpenes and sesquiterpenes may be restricted to certain times and places in the life of the plant as it is a regulated process. The biosynthesis of monoterpenes in plants has two stages: (i) the early stage results in the

biosynthesis of geranyl pyrophosphate (GPP) from acetyl coenzyme A (acetyl-CoA) and (ii) the later stage by the metabolism of monoterpenes from GPP. The early stage of the biosynthesis of sesquiterpenes is the biosynthetic process from acetyl coenzyme A to farnesyl pyrophosphate (FPP) and the later stage proceeds from FPP to essential sesquiterpene metabolism. Detailed information on the origin and biosynthesis of monoterpenes and sesquiterpenes are contained in Banthorpe *et al.* (1972); Roberts (1972); Banthorpe and Charlwood (1972, 1977); Rucker (1973); Cordell (1976); Charlwood and Banthorpe (1978); Anderson *et al.* (1978); Cane (1980); Loomis and Croteau (1981) and Dev *et al.* (1982) and more recent works in conjunction with early research in this field are discussed by Croteau (1987). The most recent review dealing with the regulation of monoterpene biosynthesis is that of Gershenson and Croteau (1990). Based on these reviews, aspects of the biosynthesis of monoterpenoids and sesquiterpenoids, which relate to this thesis, will be summarised and discussion in conjunction with the results of oil analysis in Chapter 4.

2.5.3. Function and ecological significances of leaf oils to insects.

The major functions of volatile terpenoids in plants are ecological. They are commonly ascribed to play an *allelopathic* or *allelochemic* role while recent evidence has demonstrated that they are either rapidly synthesised or catabolized and thus may play a dynamic role in metabolism. Studies in this field have been reviewed by Rice (1974), Hanover (1975), Harborne (1977, 1982, 1988), Deverall (1977), Swain (1977), Erman 1985, Croteau 1988). Recently, reviews dealing with the interactions between plant essential oils and terpenes have been provided by Saxena and Koul (1982) and Brattsten (1983). Based on these reviews in conjunction with other references, the major aspects of ecological functions of volatile terpenoids are summarized as follows.

The most common function that has been attributed to volatile terpenoids of plant is an allelopathic role in the plant-insect relationships. The function of volatile terpenoids has been suggested to make a major contribution to floral fragrances which serve as attractants to animal pollinators to plants by odour, such as the pheromone-flower scent interaction that attract bees to orchid flowers (Harborne 1988 and references therein). They may also serve as allelopathic agents in a defensive role against either predators and pathogens (Gershenson and Croteau 1990), or as non-specific toxicants produced in the defensive secretions of many insects (Harborne 1988). Terpenes have received the most attention from entomologists working on insect-tree relationships, and the specific composition of terpene vapours emitted by trees is particularly relevant to the problem of host selection by insects (Hanover 1975). Some fundamental roles of many volatile terpenoids to insect-plant relationships have been explored by many workers. They have shown that the many volatile terpenoids involved in insect-plant relationships may act as

attractants/repellents, feeding stimulants/deterrents, insecticides, ovicides, synergists, insect growth regulators and chemosterilants, etc. (Saxena and Koul 1982).

Communication between insects is known to be controlled by chemical signals, which take the form of volatile organic constituents released by one insect to affect another (Birch and Haynes 1982; Bell and Carde 1984). These substances are active in very small amounts and have been named as *pheromones* which can be defined as chemicals excreted by an organism that conveys information to another individual of the same species (Lincoln *et al.* 1982). In higher plants, volatile chemicals (mostly terpenoids) are involved in interactions between organisms, such as the detrimental allelopathic effects involving monoterpenoids and other compounds that occur between one higher plant and another (Harborne 1988).

Like pheromones, volatile terpenoids may act as (i) a sex attractant which elicits sexual displays by providing sex stimulant signals to male insects or attracting either sex (Vogel 1963; Bowers and Bodenstein 1971; Satoshi *et al.* 1975; Nishino and Tsuzuki (1975), and (ii) trail pheromones, such as feeding attractants, which are used by social insects to lay down an odour trail which other members of the species follow to guide them to a food source and back to the nest (Hamamura and Naito 1961; Rudinsky 1966; Gueldner *et al.* 1970; Payne 1970; Werner 1972; Birch *et al.* 1972; Sumimoto *et al.* 1974; Hedin *et al.* 1974; Reiter *et al.* 1977). Moreover, volatile terpenoids may also act as oviposition stimuli (e.g. Wiklund 1975). In contrast, some volatile terpenoids repel the insects and produce alarm and therefore act as alarm pheromones. For example, many volatile terpenoids have been reported to repel or mimic alerting or alarm pheromones of some insect species (Mayer 1952; Oksanen *et al.* 1970; Moore 1974; Hedin *et al.* 1974; Bordasch and Berryman 1977; Dabrowski and Rodriguez 1971; Hubbell *et al.* 1983; Saxena and Okech 1985).

Volatile terpenoids have been found to play an important role in the chemical defences of plants, thus they act not only as alarm pheromones as above but also deterrents which hinder the trophic relationships existing between insects and their host plants. For example, mixtures of mono- and diterpenes in pine resin, α– and β–pinene are probably major deterrents as they are known to be highly obnoxious to most arthropods (Harborne 1988). Many workers have reported that some volatile terpenoids inhibit feeding (e.g. Wada and Munakata 1967, 1971; Wada *et al.* 1968; Beck and Reese 1976, Rhoades and Cates 1976) and some monoterpenoids, such as *p*-cimol, terpinen-4-ol and α–terpineol, may exert an antifeedant effect by contact action (e.g. Gombos and Gasko 1977).

Furthermore, many instances have shown that volatile terpenoids may act as toxicants (insecticides) killing the insects after contacting the body or following ingestion.

Alternatively they may act as ovicides to kill the embryo in the egg stage of insects. Essential oils from many plant species have been reported to possess an insecticidal property against insect pests (e.g. Su *et al.* 1972; Saxena 1973; Ohigashi and Koshimizu 1976; Tipnis 1976; Oda *et al.* 1977 in Saxena and Koul 1982; Brattsten 1983). Pure compounds from essential oils, such as the monoterpenoids cineole, α–pinene, camphene, carvacrol, carvone, *d*- and *l*-limonene, citral, citonellol, geraniol, and β–phellandrene and the sesquiterpenoids farnesol and eugneol have been found to possess a lethal action against house flies with the mortality rate ranging from 0-37% for adults and 28-63% for larvae (Sharma 1974 referenced by Saxena and Koul 1982). More recently, Shaaya *et al.* (1991) assessed the fumigant activity of 28 essential oils extracted from various spices, against the adult beetles *Rhyzopertha dominica, Oryzaephilus surinamensis, Tribolium castaneum,* and *Sitophilus oryzae.* They found that three groups could be distinguished within the 28 essential oils, similarly, the major oil constituents were active against the four major stored-product insects. Thus, the compounds terpinen-4-ol, 1,8-cineole, and the extracted essential oils characterized by these components were most active against *R. dominica* while the compounds linalool, α–terpineol, and carvacrol and the essential oils predominated by these components were most active against *O. surinamensis.* Similarly, the compound 1,8-cineole and the essential oils of anise and peppermint were active against *T. castaneum.*

On the other hand, the toxicant effect of volatile terpenoids exhibited as ovicides to kill the insects at the egg stage consisted of preventing exchange of gases, hardening the ehorion thus preventing hatching, imbalance in water content, softening or dissolving of shell, coagulation of protoplasm or interference with enzymes and hormones (Saxena and Koul 1982 and references therein), Reports have shown that citrus oil is effective against *Callasobruchus chinensis* (Vogel 1963; Tipnis 1976). The effects of many identified monoterpenoids and sesquiterpenoids have also been recorded (Saxena and Sharma 1972).

2.5.4. Distribution and chemotaxonomy of the essential oils

The distribution and chemotaxonomy of natural plant products throughout the Plant Kingdom has been reviewed by Hegnauer (1962, 1963, 1964, 1966, 1969, 1973), Thorne (1976) and (Seigler 1981) and for angiospermous plants by Gibbs (1974) and Young and Seigler (1981). The distribution and chemotaxonomy of essential oils in plants has been discussed by Hegnauer (1982) and many aspects of chemotaxonomic studies using volatile terpenoids by Harborne and Turner (1984). For detailed information of distribution of essential oils, the reader is referred to these reviews. Important aspects relating to this study are briefly reviewed below based on these sources of information in conjunction with other published works.

The essential oils widely occur throughout the Plant Kingdom but the bulk of essential oil-bearing plants belongs to spermatophytes (seed plants). In Gymnosperms, essential oils are ubiquitous in *Coniferopsida* and *Taxopsidae* which possess schizogenous cavities and canals and, in *Cycadopsida,* essential oils appear to be restricted to *Ginkgo biloba* L. (lysigenous cavities). Chlamydospermutes seem to be devoid of essential oils. Within the angiosperms, which are classified into 62 orders according to Engler's Syllabus (1964), essential oils occur in 46 orders (Hegnauer 1982).

Essential oils have been extensively used as taxonomic markers in studies of gymnosperms due to their wide distribution in the leaves of conifers. Today, most available knowledge on chemotaxonomic studies using essential oils has been provided by these studies. In contrast, although many medicinally and industrially useful angiosperms are rich in essential oils much less work has been done on their chemotaxonomy. This may, partly, be due to the irregular distribution of essential oils in angiospermous families (Harborne and Turner 1984). For example, *Myrtiflorae* (=*myrtales*) contains 17 families but only the family *Myrtaceae* are essential oil-bearing plants throughout (Hegnauer 1982). For these reasons, the following review of chemotaxonomic study using essential oils is drawn from the information and ideas from studies in gymnosperms and, for angiosperms, is concentrated on the genus *Eucalyptus.*

2.5.5. Chemotaxonomic studies using essential oils in gymnosperms

Chemotaxonomic studies using essential oils in gymnosperms have focussed on conifers and many results have shown that terpenes from essential oils certainly meet the four basic requirements, as indicated by Harborne (1967), for chemotaxonomic study. First, many essential oils contain a large number of components some with over 100 individual terpene components and therefore possess both chemical complexity and structural variety. Second, in many plant species, the terpene composition remains unchanged during certain stages of plant ontogeny and this can be regarded as physiological stability. Third, the wide variety and distribution of volatile terpenoids found in many plant families such as discussed above. Finally, GC-MS apparatus is available for the identification of most volatile terpenoids while recent GLC apparatus provides high resolution for quantitative assessment so that the compound could be easily and quickly identified and quantified (von Rudloff 1975).

Moreover, recent biochemical research has provided greater understanding of the biogenetic and biosynthetic pathways of terpenoid synthesis and, especially monoterpenoids. Many studies of coniferous essential oils indicate that it is possible to arrive at biosynthetic relationships between various monoterpenes by observing their co-occurrence and in many cases, certain terpenes are closely linked in biogenetically related

groups which have agreed to the principals of current hypothesis and theories, e.g. Zavarin (1970), von Rudloff (1975), Rhoades *et al.* (1976), Lincoln and Langenheim (1976, 1981), Bailey *et al.* (1982), Zavarin and Snajber (1985). A chemotaxonomic study of volatile leaf oils of North American conifers by von Rudloff (1975) has provided an excellent over-view of the suprafamilial value of biochemical relationships of terpenes as systematic markers. Different biogenetically related terpenoid groups concurred with hypothetical pathways that were closely correlated to genetic variation of Conifers at the species and subspecies levels. These findings indicated that chemotaxonomic studies using essential oils could be based on biogenetic pathways when they are supported by sufficient biogenetic hypotheses and statistical correlations.

However, as a tool for chemotaxonomic study, essential oils have many inherent problems owing to their reactive nature toward developmental and environmental factors. As has been discussed by Harborne and Turner (1984), during the early developmental stages plants and their organs are likely to show considerable variation in terpenes especially. In the foliage of conifers, many studies have indicated that quantitative and qualitative variation in essential oils is affected by seasonal age and the most serious errors in chemotaxonomic studies are those relating to seasonal variation (von Rudloff 1968; Zavarin *et al.* 1971; Adams 1970a, 1972a,b).

A point of controversy is the actual cause of seasonal variation; whether it is genetic or environmental and whether losses and gains are due to catabolism, biosynthesis or interconversions.

Adams (1970a) found that the most volatile terpenoids in the leaves actually increased during the hottest months and the least volatile decreased and suggested that the volatile terpenoids were either being produced at a greater rate than they were being lost, or that non-volatiles were being produced at a lower rate than in winter. Some studies suggested that the variability of volatile terpenes during the flush period was due to the diluting effect of young leaves. However, over the same period of time, only minor changes in the older leaf material were observed (von Rudloff 1972a). Thus seasonal variation in volatile terpenoids are mainly due to changes in the young leaves during the growth flushes.

Adams (1970a) also found that variations in terpenes occur during the aging process of leaves and this was due to the physiological age of leaf material which is of utmost importance when studying the essential oil of any given tree. Oil components have been found to change rapidly in young actively growing leaves and is evident during growth flushes as it is then that a large amount of young material is present (Adams 1970a; von Rudloff 1972a; Zavarin *et al.* 1971).

In contrast, in the dormant period oil composition stabilises due to the small amount of young, actively growing material. There is little or no change in terpene composition in conifer leaves in Canada from the fall through the winter and this period can be regarded as one of physiological stability (von Rudloff 1975). Adams (1970a,b) found that the distillates of 'bulk' foliage may show measurable quantitative variation in the winter months which is less than that reported for the summer. Adams and Hagerman (1976), claimed that failure to separate bud material and developing needles from mature foliage may add considerable variability into samples. Furthermore Adams (1974) and Adams and Powell (1976) found that the winter foliage was least variable from month to month and from year to year, presumably because, during this period, the metabolic activity of plants is at a minimum.

Therefore, Harborne and Turner (1984) suggested that some standardisation involving season and age should be considered to minimise these variances, such as omitting those data which were detected to be associated with random changes through computation and omitting very young leaves from samples. Studies on some conifer species have shown that the quantitative composition of volatile terpenoids of leaves remain constant during later summer, fall and winter, such as those samples reviewed by Harborne and Turner (1984), e.g. von Rudloff (1962, 1966, 1967a,b, 1972a,b), Pauly and von Rudloff (1971), Juvonen (1966), and Zavarin *et al.* (1971).

The genetic make-up of the individual plant determines the types of components which can occur. Generally, the environment cannot alter this fact but it can cause variation in levels by triggering innate biochemical processes to varying extents. Environmental conditions such as temperature and photoperiod could cause changes in enzyme activity resulting in either decreased or increased synthesis of some components (Adams 1970a,b; Flake *et al.* 1973; von Rudloff 1975; Lincoln and Langenheim 1978). Although the potential yield of an individual tree is determined genetically, however, yield is more affected by environmental factors than oil composition (von Rudloff 1972a), for satisfactory rainfall and warm temperatures in the dormant and post-dormant periods were very important for the attainment of maximum yield of oil during the growth period.

In fact, the flush period varies annually in commencement, intensity and duration being dependent upon climatic conditions and rainfall. von Rudloff (1972a) found that while oil yield fluctuated seasonally it did not vary significantly over a five year period when samples were collected in the winter of each year. This showed that certain environmental conditions always affect oil yield in a similar manner.

Studies in gymnosperms had indicated that the variation of leaf oils were under strong genetic control and even though environmental factors were found to induce quantitative

variation in these terpenes almost all quantitative variation was under fairly strong genetic control (Mirov 1956; Hanover 1966, Squillace 1971; Rockwood 1973 and von Rudloff 1972a). The genetic control of oil compositional variation among *Satureja douglassi* had been demonstrated by growth of plant under controlled conditions and by hybridization (Lincoln and Langenheim 1976, 1981). Comer *et al.* (1982) compared volatile consitituent between juvenile foliage collected from 78 *Juniperus virginiana* and 28 *J. scopulorum* seedling sources growing in a "common garden" environment. They found that the species differences were maintained and the clinical patterns were indicated.

The genetic variation in essential oils of gymnosperms has been found to show interspecific and intraspecific variation and therefore these essential oils have been used to define species, detect hybridisation, confirm the presence of geographical races and to confirm generic and tribal limits.

Use of oil patterns for defining species has shown that species-specific patterns in leaf volatile are the rule rather than the exception, e.g. Bothriochloa (de Wet and Scott, 1965), Monarda (Scora 1967), Salvia (Emboden and Lewis (1967) and Single-Needle pines (Zavarin *et al.* 1990). Leaf oils could indicated interspecific taxa between species (Adams and Hogge 1983) or infraspecific taxa (Adams and Turner 1970; von Rudloff *et al.* 1988). Individual trees which were difficult to identify on the basis of morphology could be identified on the basis of their oil chemistry (Mirov *et al.* 1966a,b; Adams and Turner 1970). On the other hand, studies on essential oil of conifers have also demonstrated that variations in chemical characteristics may parallel morphological differentiation in some fir species (Zavarin *et al.* 1978) and *Pinus* species (Snajberk and Zavarin 1986).

Oil characteristics have been used to define some complex infra-species variation. The differentiation of oil chemistry between populations within a species may enable identification of populations which are apparently diverging from the "core" of species of junipers (Adams and Turner 1970). The variation in oil chemistry between populations of *Juniperus ashei* and *J. pinchotii* could be explained in relation to the pre-history of the area (Adams 1975a). The leaf oils were used to identify whether or not variation observed in *J. virginiana* was due to introgression of *J. ashei*. These work indicated that *J. virginiana* showed clinal variation and that there was no biochemical evidence to suggest that *J. ashei* was involved (Flake *et al.* 1973; Flake and Turner 1973).

The difference between species may be such that hybrids can be identified by an oil chemistry that is intermediate of the suspected parent species (Adams 1970b). Essential oils have been used to detect hybrids occurring in natural populations of conifers especially where morphological characters overlap and can not be used (Harborne and Turner 1984 and references therein). Terpenoid data has successfully demonstrated the

introgression of *Pinus monophylla* into *P. quadrifolia* by natural hybridisation (Snajberk *et al.* 1982) and *P. cembroides* into *P. discolor* (Bailey *et al.* 1982).

Some chemotaxonomic studies of gymnosperm essential oils dealing with geographic variation undertaken by von Rudloff (1972b) and Zavarin *et al.* (1975, 1982, 1990, 1991), have shown that essential oils did demonstrate geographic population groups and geographic relationships. Moreover, geographic patterns of terpenoid composition have been found to involve different biogenetic relationships of terpenoids (Lincoln and Langenheim 1976; Zavarin and Snajberk 1985).

The specific nature of gymnosperm essential oils has proved useful to determine gene flow within and between taxa and for the identification of hybrids. Clines within species have been detected, as have areas of divergence from species "cores" and introgression. In addition, analysis of leaf oils of North American conifers has provided good examples that demonstrate that essential oils of conifers are useful in determining differentiation of species and varieties, in population studies and, in favourable instances in the study of hybrids, intermediates and introgressives (von Rudloff 1975). von Rudloff (1975) has reviewed the chemotaxonomic studies of the essential oils of conifers from which a number of generalisations can been made.

2.5.6. Chemotaxonomic studies using essential oils in angiosperms, especially *Eucalyptus*

In contrast to gymnosperms, very few of the essential oils of angiosperms have been investigated chemotaxonomically and no genus has been investigated chemotaxonomically using essential oils to the extent of the conifers. Studies of eucalypt essential oils which have been made have been for commercial purposes rather than biological. With these limitations, information which relates to this thesis are reviewed below.

Studies on essential oils of Eucalyptus

Essential oils from the leaves of eucalypts with commercial value have attracted interest since the first settlement of Australia. Early studies into the identification of the composition of individual oils and their classification into groups according to their suitability for medicinal, industrial and perfumery purposes were commenced by a French chemist, Close, in 1870. In these initial studies, Close named the principal constituent of *E. globulus* oil as 'eucalyptol' (Read 1944) which was later isolated and correctly identified as 1,8-cineole by Jahns in 1884 (Boland 1991). A eucalypt oil industry was well established by 1900. A number of species were harvested in the southeastern states

of Australia and the country became the largest eucalypt oil supplier to the world market until about the end of World War II (Small 1977).

Baker and Smith (1920), two famous early researchers now regarded as the fathers of organic chemistry in Australia, investigated the chemical composition of the eucalypt oils of more than 300 known species growing in all parts of Australia, although they had difficulty in obtaining samples from more remote localities. Their results were published in 1902 and this was perhaps the first publication which dealt with the chemical characters of species of this large genus. From that time, they continued research and identified 40 different constituents from eucalypt oils. They had a profound and lasting impact on eucalypt oil chemistry and produced a revised edition of their 1902 edition in 1920 (Baker and Smith 1920; McKern 1968; Boland 1991).

Following Baker and Smith (1920), research into essential oils in Australia continually emphasised the commercial exploitation of essential oils for pharmaceuticals and industry and the main concern was to locate areas where the commercially viable chemical forms dominated (McKern 1968). It was considered up to the end of the 1940s that the essential oils from Western Australia had been largely overlooked and today a similar situation exists with eucalypt species growing naturally in the remoter tropical parts of Australia (Boland 1991).

Meanwhile, during this period, some chemical researchers had also made other studies relating to biology, such as work by Penfold and Morrison (Boland 1991). These workers investigated the variation of oil received from bush distilleries and made a significant finding. They found that although chemically distinct forms of a species existed, in many cases adjacent trees contained different chemical forms such that "several varieties or forms of *Eucalyptus dives* existed, distinguished only by chemical means" (Penfold and Morrison 1927). Their studies, mainly with peppermint eucalypt species, indicated a number of chemical forms, which they termed 'physiological varieties', existed in a range of distinct species which could be interpreted biologically as being chemical forms of the same species (Penfold and Morrison 1927, 1935). Furthermore, investigations of the seasonal variation of oil composition occurred during that time, e.g. Penfold and Morrison (1935) and Berry (1947). Beside that, the inheritance of oil characteristics was investigated by Pryor and Bryant (1958), although they have not been able to predict the chemistry of hybrids and very little correlation between morphological characters and terpenoid contents were found.

The early chemical investigations of eucalypt oils were limited by several factors, which have been summarised by Boland (1991), e.g. a notable lack of knowledge of terpene chemistry provided an obstacle to further investigations and often led to erroneous results

in the identification of oil constituents and also the lack of sufficiently accurate analytical techniques. Although Bellanto and Hidalgo (1971) published infrared spectra for the essential oils of a wide range of plants, infrared spectroscopy was not featured as a primary means of identification of essential oil composition and did not result in any major breakthroughs in chemotaxonomic studies. This deficiency was overcome by the development of GLC analysis, particularly when GC-MS was employed (Boland 1991).

After 1960, examination of eucalypt oils considered many aspects beside just commercial investigation. Biological studies of eucalypt oils involved both ecological and genetic studies and the heritability of specific oil traits. Investigations of the different aspects of these studies have been summarised in Boland *et al.* (1991) in which most available published and some unpublished references are included. In this book, many investigations relating to biology have been reviewed by Doran (1991) while the principal components of essential oils from all eucalypt species which have been examined have been summarised by Lassak *et al.* (1991). The composition of essential oils from leaves of 111 species, including some Tasmanian species provided by this studies of this study are included (Brophy *et al.* 1990). Although the main aim of the book, *Eucalyptus* leaf oils, was for commercial purposes a clear profile of the distribution of essential oils among eucalypt species is given. Based on this book, some aspects relating to this study are summarised, in conjunction with other studies, below.

Distribution of essential oils in Eucalyptus

The essential oils of the genus *Eucalyptus,* which is classified into seven subgenera according to Pryor and Johnson (1971), are restricted to six subgenera with the exception of the subgenus *Gaubaea.* In the subgenus *Corymbia,* the essential oils are characterized by the bicyclic monoterpenoids α– and β–pinene as principal components while the sole species, *E. citriodora,* is characterized by citronellal which distinguishes this species from nearly all other eucalypt species. Only the essential oils of four species from the subgenus of *Eudesmia* have been reported and they are characterized by high levels of either α– and β–pinene or *d*- borneol and 1,8-cineole. The subgenus *Idiogens* contained only one species but two forms of essential oils have been reported, one characterized by α– and β–pinene and the other by the phenolic compound, Tasmanone, as principal components. The subgenera *Monocalyptus* and *Symphyomyrtus* contain most species of eucalypts with 134 in *Monocalyptus* and 483 in *Symphyomyrtus* (Pryor and Johnson 1971)

Of the 134 species in the subgenus *Monocalyptus*, essential oils have been recorded for about half of them (Lassak *et al.* 1991). However, there was no regular distribution of chemical types of essential oils restricted to this subgenus or series within the subgenus. The essential oils found indicated a wide variation among species and also within species.

Within the series *Piluquae,* some species produced leaf oils characterized by α–pinene and/or 1,8-cineole as major components, some by the sesquiterpenoids α–, β– and γ–eudesmol and some by other compounds. In only two species, *E. aggregata* and *E. mckieana,* were the essential oils found to be characterized by high percentages (90% and 60%) of the phenylic ester β–phenylethyl phenylacetate as the principal component. Nevertheless, the essential oils of most species of the series *Obliquae* tended to be characterized by α– and β–phellandrene, piperitone, *cis*- and *trans*- piperitol and *p*-cymene and a few by either α–, β– and γ–eudesmol or 1,8-cineole as principal components. Moreover, the essential oils of series *Piperitae* showed mainly three major chemotypes, piperitone, α–phellandrene or 1,8-cineole predominant types. Notable variation in essential oils within species was found in this series where species occur in a wide range of habitats, thus *E. dives* has multiple chemotypes of essential oils which have been named by Lassak *et al.* (1991) as: (1) 'type' chemotypes [piperitone (53%), α–phellandrene (20%)]; (2) 'var. A' chemotype [piperitone (2-8%), α–phellandrene (60-80%)]; (3) 'var. B' chemotypes [α–phellandrene, 1,8-cineole (25-45%), piperitone (12-18%)]; and (4) 'var. C' chemotype [1,8-cineole (68-75%), terpineol, geraniol and citral]. In addition, five chemotypes of essential oils for this species were proposed by Johnstone (1984).

In the subgenus *Symphyomyrtus,* the largest subgenus of the genus *Eucalyptus* with over 480 species, essential oil compositions for over 170 species have been determined (Lassak *et al.* 1991). In contrast to the subgenus *Monocalyptus, Symphyomyrtus* shows less variability in its composition of essential oils. The essential oils of most species of *Symphyomyrtus* contain high amounts of 1,8-cineole plus α–pinene as principal components with few other prominent compounds. It is considered that the essential oils of *Symphyomyrtus* species are characterized by 1,8-cineole and related oil compounds.

Variation in essential oils of Eucalyptus

Like gymnosperms, sources of natural variation in essential oils of *Eucalyptus* may be attributed to three major factors, genetic, developmental and environmental factors.

(1) Genetic variation studies in essential oils of eucalypts and other angiosperms

As in conifers, the essential oils of *Eucalyptus* tend to show more variation at low taxonomic levels. Doran (1991) suggested that the leaf oils of *Eucalyptus* have assisted botanists in the interpretation of patterns of variation within and between species and in detecting hybrids. Thus, "qualitative differences are most common between species while variability within species, although by no means exclusively, is quantitative in nature" (Doran 1991).

Some early investigations into the essential oils of a number of eucalypts were concerned with the description of essential oil composition of specific samples and did not consider the variability of composition within species (e.g. Willis *et al.* 1963; Hellyer *et al.* 1964, Hellyer and McKern 1966; Lassak and Southwell 1969). More recently, the publication of the essential oils from a large numbers of eucalypt species based on GC-MS analysis has shown that the essential oils of many eucalypt species that are closely related taxonomically differ substantially in chemical composition (Brophy *et al.* 1991). Some studies of genetic variation of eucalypt essential oils were concerned not only with variability between species but also within species. For example, a study into variation and relationships between numbers of the *"ovata"* group of eucalypts, which considered both geographic morphological and chemical variation, has indicated that variation in essential oils is related to gene flow within and between species (Simmons 1974).

Within a given eucalypt species there may also exist a number of physiological forms which can be separated on the basis of their essential oil composition. This variation is most common in species which have a large geographic distribution. Local populations evolve particular adaptations to their specific environments and this results in apparent geographic variation which can lead to genetic divergence between populations through limited gene exchange (Johnstone, 1984). Even in species with no marked physiological forms there are often significant differences between individual trees grown at the same location. This indicates that biochemical processes resulting in oil formation are subject to separate genetic control.

Many chemical forms have been reported for eucalypt leaf oils and they are defined as 'plants in naturally occurring populations which cannot be separated on morphological evidence, but which are readily distinguished by marked differences in the chemical composition of their essential oils' (Penfold and Willis 1953, cited by Doran 1991) . These chemical forms did not appear to be correlated with seasonal, geographic, leaf age variation or hybridisation and appeared to be under strong genetic control. The chemical forms have now been simply referred to as biochemical variants, forms, chemoforms, chemovars or chemotypes, etc.. Generally, they could be well defined and readily distinguished from each other, such as the five chemical forms of *E. dives,* six of *E. radiata,* four of *E. citriodora* and *E. racemosa* (syn. *E. micrantha*), three in *E. elata* (syn *E. andreana*), and two in *E. piperita* (Doran 1991). Studies on variation in essential oils within a complete population of *E. radiata* indicated that chemically distinct forms of the species exist and that they are not correlated to any environmental parameters within a restricted locality (Bouchier 1976). However, study in *E. punctata* spp. *punctata* indicated that there was a wide variation in the leaf oil composition between individual trees and no grounds for the establishment of chemical varieties within this subspecies (Southwell 1974).

During crossing or hybridisation, the progeny will adopt characteristics of each parent and the way they interact will determine the final oil composition. Recent studies have indicated that oil characteristics in *Eucalyptus* appear to be strongly inherited, such as the high family heritabilities of 0.83 for 1,8-cineole yield in *E. kochii* (Barton *et al.* 1991) and individual tree heritabilities for 1,8-cineole yield of 0.5 in *E. camaldulensis* (Doran and Matheson in Doran 1991). Moreover, eucalypt essential oils have been reported to be also strongly inherited in hybrids in which the parents belong to different species and the oil characters have been used to detect hybrids (e.g. Simmons 1985). The strong heritability of oil traits in eucalypts suggests the possibility of increasing oil yield or yields of particular components by establishing plantations of genetically improved trees.

While the information of genetic studies in eucalypt essential oils is limited, studies in essential oils of other angiosperms, and particularly the family Labiatae, have provided many examples which indicate that essential oils are under strong genetic control. Some studies have involved the biosynthetic characteristics of essential oils, such as that for *Mentha* (Labiatae) (e.g. Burbott and Loomis 1969; Croteau *et al.* 1972) and reviewed by Hefendehl and Marry (1976). For example, studies of *Mentha* (Labiatae) have demonstrated the genetic control of oil inheritance through a series of controlled crosses in which a single incompletely dominant gene controls the production of menthol or menthofuran in *Mentha aquatica* (Murray and Hefendehl 1972), whereas another gene largely prevents either the conversion of limonene into isopiperitone or α–terpineol into terpinolene (Murray and Hefendehl 1973). Thus, Hefendehl and Marry (1976) have claimed that the biosynthesis of the compounds of *Mentha* essential oil must be genetically controlled and qualitative variation determined by controlling genes while environmental influences normally affect oil yield. Moreover, genetic studies into polyploidal strains and gene-inhibited strains derived from convergent backcrosses in *Mentha* have shown that the systematic creation of proper gene combinations could lead to the accumulation of large amounts of specifically selected components.

Studies in essential oils of other members of the family Labiatae have also shown similar results to that in *Mentha*. For example, a study of natural populations of *Thymus vulgaris* showed that different chemical forms of the species when established in progeny trials were stable and clearly genetically determined features of the plants (Granger and Passet 1973). Backcrosses in three taxa within the genus *Hedeoma* also led to possible genetic models of inheritance of monoterpenes (Irving and Adams 1973).

Chemical forms of essential oils have also been found in some other angiosperms, such as chemical forms found in *Bursera microphylla* (Burseraceae) which corresponded to morphological variation patterns (Mooney and Emboden 1968). A number of chemical forms were detected in *Chrysanthemum vulgare* (Compositae) and controlled crosses of

different forms suggested that every step of terpenoid biosynthesis may be under genetic control (Forsen and von Shants 1973). Furthermore, Hefendehl and Forces (1976, 1978) have found that a number of chemical forms of *Elyonurus viridulus* (Poaceae) could be attributed to divergences in the early stages of terpenoid biosynthesis.

(2) Developmental variation

In contrast to conifers, five distinct morphological types of leaves may occur during the life time of different eucalypts species: (1) cotyledons; (2) 'seedling' leaves; (3) 'juvenile' leaves; (4) 'intermediate' leaves; and (5) 'adult' leaves (Doran 1991). Perhaps, the most typically distinct morphological types of eucalypt leaves are between adult and juvenile leaves. Nevertheless, it should be noted that these leaf types recognised are the different developmental stages of tree but not the physiological leaf age commonly denoted by the terms 'young leaf', 'mature leaf' and 'aged leaf' as described by (Penfold and Willis 1961 in Doran 1991). Therefore, the variation in essential oils of eucalypt leaves attributed to developmental factors may reflect to both distinct morphological types (e.g. juvenile and adult) of leaves which related to the development of tree and physiological leaf age which related to the development of leaves within a developmental stage of tree.

Doran (1991) stated that few studies of variation of eucalypt essential oils have adequately define sampling controls such that the data can be on identity assumed to be free of the confounding influences of ontogeny and of physiological leaf age. Some studies suggested that the effects of ontogeny on eucalypt essential oils are mainly quantitative variation rather than qualitative (e.g. in *E. dives* Penfold and Willis 1961; in *E. delegatensis* Weston 1984; in *E. polybractia* Brooker *et al.* 1988), while one study showed marked qualitative differences between leaf types of *E. camphora* (Coorey *et al.* in Doran 1991). Studies on six eucalypt species in Tasmania comparing juvenile and adult leaves of the same physiological leaf age in samples of each species from the same locality have shown that there are limited qualitative differences in essential oils between leaf types while similar quantitative differences between leaf types were found in species within subgenera. Juvenile oil yields of two *Symphyomyrtus* species, *E. globulus* and *E. nitens,* were higher than adult leaves. In contrast, the oil yields of adult leaves of three *Monocalyptus* species, *E. obliqua, E. regnans* and *E. delegatensis,* were higher than juvenile leaves (Li *et al.* 1990). Studies on the variation in essential oils of eucalypt leaves between three age classes showed that contents of the compound cineole in leaf oils of *E. kochii* and citronellal in leaf oils of *E. citriodora* increased steadily in seedling trees and at 3 years had attained stable levels (Doran 1990 and references therein).

In contrast to leaf types, the variation in essential oils due to the physiological age of leaves is more complex. As with findings in conifers, studies into the variability of

eucalypt essential oils with respect to leaf age have indicated that oil yields between young and mature leaves are often marked but there is a lack of agreement in regard to the trends in this variation. Thus, the variability in the direction and extent of these differences occur not only between species but also between individuals of the one species in the same population (Doran 1990 and references therein). Some studies found that young, actively-growing leaves have a higher percentage oil yield than mature or aged leaves (Berry 1947) or older leaves (Berry 1947; Dzhnashiya and Fogel 1973).

Aged leaves have been found to generally yield less oil than recently matured leaves (Doran 1990 and reference therein). Furthermore, a study by Kapur *et al.* (1982) separated mature leaves of *E. citrodora* into three categories: mature young (mean age three months); mature adult (mean age six months) and mature aged (mean age nine months). The oil yield and citronellal content was found to be appreciably higher in mature young and mature adult leaves than in mature aged leaves. On the other hand, some studies have reported that there were no significant differences in the oil yield between different physiological ages, such as in *E. smithii* (Bryant 1950 in Doran 1990).

It can be summarised that oil yield appears to be determined by a complex pattern of quantitative change in individual or groups of compounds with some remaining constant, some increasing or decreasing with leaf age and that patterns can vary even between individuals of the one species at the same site and, in fact, appear to be dependent on the genetic constitution of individual trees (Simmons and Parsons 1987).

With respect to the qualitative variation in eucalypt essential oils it has been reported that oil components change rapidly in young actively growing leaves and then stabilize at the time when leaf expansion ceases (Willis *et al.* 1963). The greater change of essential oils in young compared to mature leaves may be due to the active physiological development of young leaves with the environment having a greater influence on young, rapidly-changing oil than in mature, stable oil. If sampling a whole tree, these rapid changes are only evident during growth flushes as it is then that a large amount of young material is present (Berry 1947). During the growth flush constituents are present which almost entirely disappear later, indicating that they are involved in some biogenetic relationship, being precursors to constituents which increase. Bryant (1950) reported that qualitative variation between old and young leaves of *E. radiata* in which young leaves contained high percentage contents of phellandrene which was absent in older leaves. An investigation of variation in the oil composition with leaf age of *E. camphora* and *E. ovata* indicated that while substantial quantitative changes may be taking place with age, the composition of leaf oils is invariable related to the biosynthetic production of components (Simmons 1974). A study of variation with leaf aging in the essential oil of *E. radiata var australiana* was undertaken by Burgess (1986) and involved the observation of sampled

leaves on labelled nodes of individual trees and branches which had all been exposed to the same environment at different developmental stages of leaf development. It was found that the essential oil was produced as a by-product of the physiological development of leaves and accumulated in young leaves and stabilised in mature leaves. There was both quantitative and qualitative variation with leaf age. However, individual major components of oils were related to the possible biosynthetic conversions between biogenetically related components during active periods of leaf development.

(3) Seasonal variation

A number of studies on seasonal influence in essential oils has been carried out for eucalypts, however like that for conifers, there is a lack of agreement in regard to the nature and trends in this variation. Thus, some workers found that oil yields were higher in summer than winter (e.g. Berry 1947; Guenther 1950; Penfold and Willis 1961; Dzhnashiya and Fogel 1973) but some found that the highest oil yield occurred in winter rather than in summer (e.g. Nour El-Din *et al.* 1976; Rajeskara *et al.* 1984, Yatagai and Takahashi 1984). Oil composition of eucalypts was also found to differ seasonally. Studies have found that the percent cineole content of leaf oils was highest in winter oil with the actual yield highest in summer (e.g. Berry 1947; Abou-Dahad and Abou-Zied 1974). Kapur *et al.* (1982) recorded that seasonal variation in essential oils of *E. citrodora* grown in India and sampled at monthly intervals over one year indicated that there was a pattern of consistent variation in oil yield in all observed trees. Thus, oil yield was minimal the during summer months but, after the monsoons, yields increased gradually through to early winter reaching maximum levels during the coldest months while the citronellal content was lowest in early summer but higher for rest of the year in all trees.

The observed fluctuations in oil yield and composition are almost entirely due to changes in the young growing leaves, with no marked variation in the older material. Moreover, almost all studies considered that seasonal variation in oil component levels are mainly due to changes in the young leaves during growth flushes but in the dormant period of winter, oil composition stabilises due to the non production of young, active material. A study on seasonal variation undertaken by Simmons and Parsons (1987) of the oils of *E. ovata* and *E. camphora* indicated that the oil yield in mature leaves of *E. ovata* remained relatively constant throughout the summer and winter months with only slight changes in *E. camphora.* They concluded that variation in oil composition was not related with seasonal influence but rather to leaf ageing effect. Seasonal variation in cineole production was studies by Brooker *et al.* (1988) for *E. kochii* and *E. plenissima.* There was a small seasonal effect with the highest production of 1,8-cineole occurring in January and February and the lowest in August. They found that the variation between individual trees

within the one site far outweighed the modest seasonal effect again pointing to the overriding importance of individual genetic makeup on volatile oils.

Studies on the seasonal variation in species of two myrtaceous genera closely related to *Eucalyptus* are of interest. In *Angophora costata* Leach and Whiffin (1989) found that the seasonal and diurnal variations in oil composition were much less than genotypic variation between individuals. Their results indicated that seasonal variation was weekly linked to monthly or seasonal temperatures and diurnal variation appears to be associated with humidity. In contrast, study in *Melaleuca alternifolia* (Murtagh 1988) indicated that the monthly pattern of variation in oil concentration was strongly correlated with water availability while the pattern between days showed oil concentration to be negatively correlated with daily minimum temperatures. Although the factors affecting seasonal variation may be inconsistent as found by above authors, they suggested an association between oil concentration and the metabolic activity of the plants. Oil yields reveal a significant annual cycle of change being at their lowest in late winter and early spring and highest during summer and autumn (Williams and Home 1988).

(4) Environmental variation

In contrast to conifer studies, there is little information available concerning the effects of environmental factors on eucalypt essential oils. A study for *E. citriodora* undertaken by Rajeskara *et al.* (1984) used the same tree strains at two different sites and thus had the same genetic material at each location which differed environmentally and as a result the essential oil also differed in yield. They chose Site A at 2200m altitude with an annual rainfall of 1600mm and Site B at 100m with an annual rainfall of 820mm. At site B, the highest oil yield (2.37%) was obtained, with no significant differences between any of the monthly values. The oil yield was lower at site A (1.43%) than site B and the yield varied significantly between months, with the highest level being obtained in winter. These findings indicated that the agronomic condition affected oil yield, however, there was no significant variation in oil composition to be observed in their studies. In contrast, Shieh (1978) studied *E. citriodora* at four localities in Taiwan and found that oil composition varied between sites. Thus, proportions of citronellal varied from 32.2% to 77.94%. However, Shieh (1978) did not mention the source of the trees used or their genetic relationships and therefore failed to describe whether this variation can be attributed to environment or genetic causes.

2.6. Leaf waxes - a background

A wax layer is usually deposited over cuticle surfaces, occurring particularly on leaves and fruits but also on the stems, petals, bark and root surfaces of a wide variety of higher plant species and also on some bryophytes, bacteria and fungi.

As an important component of the cuticle, plant epicuticular wax has been extensively examined by a variety of methods for its morphology, chemical composition, biosynthesis, physiological significance and usefulness in plant taxonomy. Most aspects of epicuticular waxes have been discussed by Martin and Juniper (1970) and Cutler *et al.* (1982). Reviews dealing with the chemistry and biochemistry of epicuticular waxes are contained in Tulloch (1976a) and Kolattukudy (1980). A review dealing with relationships between the fine structure and chemistry of epicuticular waxes has been made by Jeffree *et al.* (1976). More recently, biochemical and morphological genetics of wax formation have been discussed by Bianchi (1985; 1987) and Avato (1987). Important aspects relating to this study are briefly reviewed below based on these sources of information in conjunction with other published works.

2.6.1. Definition of plant waxes

Wax is an important component of the plant cuticle. The term wax is used to denote a class of substances which qualitatively have certain physical properties such as plasticity in common (Hatt and Lamberton 1956) rather than to define a precise chemical entity. Kolattukudy (1980) explained that wax is a complex mixture of relatively non-polar lipids with physical properties similar to those of the honeycomb. The wax is embedded within and sometimes exuded over the surface of cuticle.

2.6.2. Functions

The cuticle is an important structural element of plants as it holds the cellular tissues compact and firm and forms the boundary and site of interaction between the plant and its environment. It therefore plays an important role in the interaction between the two. Cuticular waxes, as a component of the cuticle, take part in the functions of the plant cuticle and can be viewed from various aspects.

The most common functions that have been attributed to cuticular waxes are in preserving a favourable water balance for the plant through cuticular and stomatal transpiration and its ability to repel water from the plant surface. It has been demonstrated that the waxes constitute the major diffusion barrier in the cuticle and that structural waxes have an important role limiting cuticular transpiration (Martin and Stott 1957; Hall and Jones 1961; Horrocks 1964; Danna 1970; Schoncherr 1976). The removal of waxes or damage

results in increased transpiration or a decrease in diffusion resistance (Clark and Leavitt 1956; Hall and Donaldson 1963; Espelie *et al.* 1980; Schoncherr and Ziegler 1980). Evidence has shown that the inhibition of wax synthesis, but not the formation of the major aliphatic components of polymers of cuticle, inhibited development of diffusion resistance (Soliday *et al.* , 1979). Some studies have shown certain wax components to be more effective in reducing transpiration than others (Baker and Bukovac 1971; Hadley 1981; Proksch *et al.* 1981). The important role of wax in minimizing cuticular transpiration was summarized by Martin and Juniper (1970) as:

"....the degree of impregnation of the cuticle with wax, and not the thickness of the cuticle, is the important factor in controlling cuticular transpiration. Furthermore, the chemical composition of the wax markedly affects its efficiency as a water barrier. No generalisations can be made on the role of the cuticle in preventing the loss of water from plants...."

Similarly, wax form, composition and the amount of wax present all play a role in water repellence. The ability of the plant surface to repel water has been suggested to be related to the roughness of the surface in the form of projecting crystals that form a dense covering. The wettability of leaf surfaces is often indicated by contact angle measurements which are used to determine comparative values for various isolated waxes. For example, wettability increases with increasing polarity, hence alkanes were the most, and triterpenoids the least hydrophobic compounds (Holloway 1969a). Wax removal tended to increase surface wettability although there was no apparent correlation between the contact angle measured on a leaf surface and its chemical composition (Holloway 1969b).

It has been suggested that wax deposits on leaves play a role in the prevention of damage by frost (Hall and Jones 1961; Martin and Juniper 1970, Single and Marcellos 1974; Kolattukudy 1980). Barber (1955) observed in Tasmanian eucalypts that clines in glaucousness were correlated with degree of frost activity with the more glaucous populations of *Eucalyptus* spp. occurring in the more frosty localities. A rodlet type of wax predominates on the leaves of *Eucalyptus urnigera* at high altitudes which becomes progressively less conspicuous at lower levels (Hall *et al.* 1965). Furthermore, the difference in supercooling temperatures of the glaucous leaves which remain dry and wettable green leaves of *E. urnigera* may be of ecological and selective significance (Thomas, 1961; Thomas and Barber 1974a,b).

It has been suggested that surface waxes may contribute to drought resistance, although these aspects have not been adequately investigated (Leyton and Juniper 1963; Tulloch 1976b; Richards *et al.* 1986; Dixon 1986). Leaf waxes may assist in controlling the temperature of leaves by reflection of incident radiation and protect the cellular tissues

from excessive ultraviolet radiation (Martin and Juniper 1970). For example, the reflectivity of the rodlet form of the wax of *E. urnigera* at the higher altitudes has probably been of selective significance in the origin and maintenance of the cline (Thomas 1961).

An important consequence of cuticular waxes is in relation to the deposition and penetration of chemicals present in various agricultural sprays. The water repellence of many plant surfaces has raised the problem of the deposition of chemicals used as pesticides or for other purposes (Martin and Juniper 1970).

Cuticular wax has been supposed to play an important role in the interaction between the plant and other organisms in its environment (Kolattukudy 1980) or to provide a potential barrier to the entry of fungi, bacteria and viruses (Martin and Juniper 1970). The possibility that the wax bloom on foliage prevents deposition of inoculum and the formation of the surface water film required by pathogens has been suggested (Dickinson 1960; Martin 1964), as, for example, the glaucous coating of *Eucalyptus bicostata* affects the deposition of conidia of *Phaeoseptoria eucalypti* (Heather 1967).

The leaf waxes have also been demonstrated to be involved in insect-plant relationships (Way and Murdie 1965; Lin *et al.* 1971; Bernays *et al.* 1985; Eumatsu and Sakanoshita 1989). The glaucous wax of plant leaves could provide physical protection from insect attack by preventing insect attachment to leaves (Stork 1980b, Edwards 1982) and such an effect has been observed for *Eucalyptus globulus* juvenile foliage preventing *C. bimaculata* adult attachment (Leon 1988).

Chemicals present in cuticular waxes are also known to be involved in host selection by insects (Martin and Juniper 1970; Jackson and Blomquist 1976). Leaf wax chemicals had been found to act as feeding and ovipositional attractants and stimulants, e.g. Thibout *et al.* (1982), Takabayashi and Takahashi (1985), Eumatsu and Sakanoshita (1989), Takahashi *et al.* (1990); or repellents and deterrents, e.g. Atkin and Hamilton (1982), Hubbell and Howard (1984); or antibiosis, e.g. Cook *et al.* (1987).

Moreover, some studies indicated that the host selection by some insects could affected by both wax morphology and chemicals. For example, dispersal and survival of neonate diamondback moth larvae, *Plutella xylostella,* were found to be affected by wax morphology of cabbage while the acceptance of cabbage for larvae was correlated to chemical differentiation of waxes (Eigenbrode and Shelton 1990, Eigenbrode *et al.* 1991).

2.6.3. Wax chemistry, morphology and their genetic control

Wax chemicals and their biosynthesis

The composition of plant surface waxes is complex and varies considerably in the numbers, relative abundance and distribution of chemical homologues and constituent classes (Baker 1982). Several major structural features which characterise surface waxes and add to their complexity are: chain length, degree of branching, positioning and number of functional groups and degree of unsaturation (Kolattukudy 1976, 1980).

Plant surface waxes consist of mainly lipid compounds, such as long-chain fatty acids, fatty alcohols, esters, hydrocarbons and triterpenoids in conjunction with some other components. Based on the reviews of Tulloch (1976a), Kolattukudy (1980) and Baker (1982), the constituents occurring in plant surface waxes are listed in Table 2.7 and briefly described below:

a) *Hydrocarbons* are widespread in plant waxes and n-alkanes are the most common hydrocarbons. They comprise a wide range of homologues (C_{17} - C_{37}) of predominantly straight chain with odd-carbon-numbers, usually with C_{29} and C_{31} prominent. Even straight chain, branched, cyclic and unsaturated hydrocarbons may show a restricted distribution pattern but occur generally only as very minor constituents.

b) *Free primary alcohols* are widespread and their straight chain compounds consist almost entirely of an even number of carbons ranging from C_{23}-C_{32}, yet only three homologues consistently constitute major wax components (C_{26}, C_{28}, and C_{30}). Branched primary alcohols occur generally as minor components.

c) *Secondary alcohols and ketones* occur in plant waxes with chain length generally corresponding to those of the alkanes present. Among the secondary alcohols, only nonacosan-10-ol (C_{29}) has been reported as a dominant wax constituent. Ketones usually exhibit similar homologue and isomer profiles as the secondary alcohols.

d) *ß-Diketones* and *hydroxy-ß-diketones*. ß-diketones are often a major component of waxes which are confined to a restricted range of plants, e.g. *Eucalyptus*, cereals and other graminaceous species. ß-Diketones often dominate with a single homologue, usually C_{29}, C_{31} or C_{33}. The chain length of the dominant ß-diketone homologue does not correspond to that of the hydrocarbons.

e) *Aldehydes* are generally only found in small quantities in plant waxes. They occur as straight chains with an even number of carbons and have a chain length distribution similar to that of primary alcohols.

f) *Alkyl esters* are formed almost exclusively through the esterification of even chain n-alcohols (C_{22}-C_{32}), frequently C_{24}, C_{26} and C_{28}, with even chain fatty acids of the short series (C_{12}-C_{18}).

g) *Esters of secondary alcohols* (alkanol-2-ols), are composed of odd chain length homologues (C_9-C_{17}). Esterified to even chain fatty acids have so far been identified only in the leaf waxes of *Eucalyptus* sp. (Horn *et al.* 1964) and *Hordeum* (von Wettstein-Knowles 1976).

h) *Cyclic constituents*. Triterpenoids of the ursane, oleanane and lupane series are widely distributed among surface waxes and form a significant proportion of the wax in some plant waxes.

i) *Phenolic constituents*. Flavonoids and their glycosides have been found from the surface waxes of certain plants and form a group of compounds which may be present as a minor wax constituent.

Table 2.7. Plant surface wax components*

A. Principal classes of constituents occurring in plant surface waxes

Component class	Homologue Range	Dominant carbon-number	Most common constituents
Hydrocarbons			
n-alkanes	C_{17} - C_{37}	odd	C_{29} and C_{31}
Alcohols			
n-alkanols	C_{22}-C_{32}	even	C_{26}, C_{28} and C_{30}
2-alkanals	C_{21}-C_{33}	odd	C_{29} and C_{31}
Ketones	C_{23}-C_{33}	odd	C_{29} and C_{31}
Aldehydes (alkanals)	C_{14}-C_{34}	even	C_{26}, C_{28} and C_{30}
Acids			
short series	C_{12}-C_{18}	even	C_{16} and C_{18}
long series	C_{20}-C_{32}	even	C_{26}, C_{28} and C_{30}
ß-Diketones	C_{29}-C_{35}	odd	C_{29}, C_{31} and C_{33}
Hydroxy- or oxo-ß-diketones	C_{29}-C_{35}	odd	derivative of ß-diketones
Alkyl esters	C_{36}-C_{72}	even	C_{26}-ol and C_{28}-ol esters of C_{16}, C_{18}, C_{20} and C_{22} acids
Triterpenoid			
acid			Ursolic acid and oleanolic acid
alcohols			ß- and α–amyrin, lupeol
esters			ß-amyrenyl acetate, tatraxeryl acetate and lupeyl acetate
ketones			taraxerone and lupene-3-one

B. Minor or unusual wax constituents.

Component class
Aliphatic constituents
Unsaturated and Branched-chain hydrocarbons
Branched-chain primary alcohols
Branched-chain acids
Branched-chain esters
Hydroxy ketones
α, ω–diols; α, ω–diol esters; γ–diols
Oxo-ß-diketones
Acetates
Polyestolides
Cyclic constituents
Sterols, Steryl esters
Uvaol
Erythrodiol
Dihydroxy triterpenoid acids
Phenolics
Flavonoids; Flavonoid glycosides

* Compiled from Tulloch 1976; Kolattukudy 1980; Baker 1982.

Studies dealing with the biosynthesis of plant waxes can be traced back to the work of Channon and Chibnall (1929) who proposed a head to head condensation mechanism for

the biosynthesis of hydrocarbons. Since that time several biosynthetic mechanisms for various lipid compounds have been proposed and modified but little advance was made until exogenous labelled precursors were applied in the early 1960s. These early works have been summarized by Eglinton and Hamilton (1967), Martin and Juniper (1970) and Caldicott and Eglinton (1973). Since Kolattukudy (1967) proposed an elongation-decarboxylation mechanism for biosynthesis of hydrocarbons, and this mechanism has been adopted up to now. Kolattukudy was the main contributor to research in this field, and has produced alone and in collaboration with other workers a series of papers and provided the most recent review of the available observations (Kolattukudy 1980). Here, the aspects related to study in this thesis, in conjunction with other recent reports, are briefly reviewed below according to Kolattukudy's 1980 review.

According to Kolattukudy, hydrocarbons are biosynthesized from palmitin acid, the end product of fatty acid, which is elongated by the addition of C_2 units until the main length reaches the appropriate size (e.g., C_{30}-C_{32}), the acid is decarboxylated, and hydrocarbon is released. The intermediates in this process could be channelled into reductive pathways, to generate fatty alcohols which would be subsequently esterified to give wax esters through an acyl transfer mechanism under physiological conditions (Kolattukudy 1967). The hydrocarbons could then be hydroxylated, and the resulting secondary alcohols could give rise to ketones by oxidation (Kolattukudy 1965, 1966, 1968a,b,c, 1971; Wettstein-Knowles 1985). Another important class, the ß-diketones, which are widely found in leaf waxes of eucalypt and other species are unlike the monocarbonyl compounds and do not correspond to the hydrocarbons in their chain length distribution (Tulloch, 1976a). Some studies have indicated the different pathways involved in the biosynthesis of ß-diketones and hydrocarbons (von Wettstein-Knowles 1976; Mikkelson and von Wettstein-Knowles 1978; Mikkelson 1978). The exact mechanism of the biosynthesis of ß-diketone is yet to be elucidated (Kolattukudy 1980). However, on the basis of the distribution of radioactive labels along the carbon chain of hentriacontan-14,16-dione derived from specifically labelled [^{14}C]acetate suggested that this ß-diketone was generated by an elongation process starting from the C_{31} end of the chain (Netting and von Wettstein-Knowles 1976) and that the two carbonyl groups in the ß-diketones are conserved during the elongation process (Mikkelson and von Wettstein-Knowles 1978, 1984). Some secondary alcohols (alkan-2-ols), that are found frequently in plants which contain ß-diketones, are probably derived by the decarboxylation of ß-keto acid followed by reduction (von Wettstein-Knowles 1976).

Wax morphology and their structural formation

Some of the earliest recorded observations characterising wax morphology are briefly reviewed by Martin and Juniper (1970) and Baker (1982). These include Bary (1871) who attempted the earliest classification of plant waxes and identified four main structural forms: needles, rods, granular layers and films as seen under a light microscope. Kreger (1949) confirmed many of these wax types using a micro X-ray diffraction technique. Since that time techniques for the study of plant waxes have advanced considerably with the development and continual refinement of the electron microscope.

The majority of the early studies employed the transmission electron microscope (TEM) and the studies of ultra-microscopic structures developed by Juniper and Bradley (1958). The greater resolution now obtained with the scanning electron microscope (SEM), coupled with its greater flexibility has indicated a diverse array of configurations ranging from thin films to complex three dimensional structures, e.g. Heslop-Harrison and Heslop-Harrison (1969), Baker and Holloway (1971) and Baker and Parsons (1971). These wax types and the structure of plant wax from the most recent findings have been reviewed and summarised by Baker (1982).

Baker (1982) reported that the aerial surface of all higher plants carry a partial or continuous coverage of amorphous wax and that the formation of crystalline wax is frequently superimposed upon these amorphous layers. He considered that the crystalline waxes on specific organs of individual plants consist predominantly of a single structural form but occasional deposits are composite. Baker (1982) broadly classified those crystalline waxes as plates, tubular waxes, ribbons, rodlets, filaments and dendrites and the plates and tubular waxes are the most widely reported wax types.

The cuticular or epicuticular waxes are known to be formed mainly by the epidermal cells and occur at an early stage in leaf development and synthesis continues at least throughout the period of leaf expansion (Kolattukudy 1968c, 1970; Martin and Juniper 1970; Cassagne and Lessire 1974, 1975; Lessire *et al.* 1982). However the mechanism by which wax is transported to the leaf surface and how it is deposited on the plant surface in a crystalline form remains unresolved, although many theories have been proposed, such as those reviewed by Martin and Juniper (1970), Jeffree *et al.* (1976), Baker (1982) and Hallam (1982).

These proposals deal mainly with wax transport and are dominated by the extrusion and exudation theories including the view that wax is released via either cuticular pores and channels (Mueller *et al.* 1954; Scott *et al.* 1948, 1958, Davis 1971; von Wettstein-Knowles 1974; Jeffree *et al.* 1976 and references therein; Miller 1982, 1983, 1985,

1986) or in a softened form under pressure (Mueller *et al.* 1954; Schieferstein and Loomis 1959). Alternatively it is proposed that wax is exuded to the surface via cuticular pores (De Bary 1871 and Wijnberg 1909 in Jeffree *et al.* 1976) or through the cuticle as a liquid (Weber 1942 in Jeffree *et al.* 1976; Juniper 1959, Juniper and Bradley 1958; Leigh and Matthews 1963; Hall 1967a,b). Some other workers have proposed that wax is transported to the cuticle surface by either molecular diffusion through intermolecular spaces in the cuticular membrane (Franke 1967; Jeffree *et al.* 1976) or via a protein or carbohydrate carrier (Hallam 1981, 1982) or secreted in a volatile solvent (Chambers *et al.* 1976). In addition, it also been suggested that wax lamellae may occur within the cuticle itself (Hallam 1964 in Martin and Juniper 1970). However, the extrusion and exudation theories of the mechanism for wax formation have been rejected by many workers for pores or canals could not be found in many cases (Jeffree *et al.* 1976 and references therein) while others claimed that conclusive experimental evidence is lacking.

A number of mechanisms by which the final wax structure is formed on the cuticle surface following transport of wax have been proposed. These mechanisms include (a) that pore number, density and orientation determine the final structure of the wax (Hall and Donaldson 1962; Hall 1967a,b), (b) that the rate of exudation of wax and it's chemical composition determines the structure of the wax (von Wettstein-Knowles 1974) or (c) evaporation of the solvent containing wax at rates determined by environmental conditions (Baker 1974). However, Hallam (1970) demonstrated that some eucalypt leaf waxes could be recrystallised from solution in a form related to the shape in which they naturally occur. Hallam and Chambers (1970) found that two types of eucalypt leaf waxes were determined by the presence or absence of ß-diketones, and evidence now exists that wax structure is primarily dependent on the chemical composition of the wax and that chemical composition is determined by specific genes. The different aspects of this theory have been discussed in detail by Jeffree *et al.* (1976) and supported by more recent studies, e.g. the principal aspects of the theory in conjunction with earlier and current research are summarized as follows:

The formation of wax structure could be affected by several factors and particularly environmental and developmental factors and the effects of the chemical composition on crystal formation:

1. Environmental factors, particularly temperature and light intensity, can markedly influence the structure of plant waxes, e.g. Juniper 1960a,b; Troughton and Hall 1967; Hallam 1970; Baker 1974. Changes in these environmental factors, may affect component distribution among the classes of compound of a wax (Giese 1975) and environmentally induced changes in wax structure may occur more readily in those waxes

which are composed of a complex mixture of chemical components rather than in those in which one class of compound is dominant.

2. Waxes sometimes undergo chemical and structural modification during development and ageing, e.g. Bain and McBean 1967; Mazlia 1963; Tulloch 1973. These modifications may also be related with seasonal variation, e.g. Schuck 1969; Schutt and Schuck 1973.

3. The wax structure may be concerned with the relationship between the crystalline behaviour of waxes and their chemical compositions, e.g. Piper *et al.* 1931; Fontana 1953; Birdwell and Jessen 1966; Edwards 1957. Kreger (1948) found that the arrangement of the molecules in the wax crystals on leaf surfaces was a function of their chemical composition. Furthermore, re-crystallisation and surface diffusion studies show that accurate representations of normal wax formations can be produced artificially (Jeffree 1974; Jeffree *et al.* 1975, 1976; Chambers *et al.* 1976).

While considering the above major effects on the final formation of a wax structure in conjunction with some other aspects, such the site of wax synthesis and transport, Jeffree *et al.* (1976) concluded that: ".....wax is capable, under appropriate conditions, of organizing itself into crystalline structures independently of underlying cells, and it is possible that subsequent to wax synthesis, the epidermal cells and cuticular membrane may play no further part in the development of ultrastructure of plant surface waxes."

Furthermore, Jeffree demonstrated, in a study of plant mutants that one of three effects of mutation could occur. The first type of effect is characterized by a severe reduction in wax production caused by a metabolic block in the synthesis of major components, such as a reduction in the quantities of elongated wax products resulting in the loss of the tube and dendritic structures present on normal glaucous plants, e.g. Hall *et al.* 1965; Macey and Barber 1970a,b; Baker 1972, 1974; Lundqvist *et al.* 1968. The second effect was a change in the chemical composition of the wax accompanied by the appearance of new crystalline structures, e.g. Macey and Barber 1970a,b; Baker 1972, 1974; Lundqvist *et al.* 1968. Finally, the third effect was that the quantity and chemical compositions of the wax was similar to the normal form but its ultrastructure dramatically altered, again giving a sub-glaucous appearance, e.g. Netting *et al.* 1972; Baker 1972, 1974.

Meanwhile, an interesting finding in this connection was the discovery of the genetic control of ß-diketone and hydroxy-ß-diketone synthesis in waxes of barley (von Wettstein-Knowles 1972) in which five eceriferum (cer) mutants were characterized. The results indicated that changes in proportions of ß-diketone and hydroxy-ß-diketone were determined by genes and correlated with changes in the morphology of epicuticular

waxes. Thus hydroxy-ß-diketones were shown to be unnecessary for the formation of wax tubes and that tube waxes lacked hydroxy-ß-diketone. In contrast, the proportions and amount of ß-diketone were positively related to the presence of a wax tube. This finding agrees with that found by Hallam (1970) in eucalypts in that the wax tubes or plates were dependent on the presence or absence of ß-diketones. Moreover, it was further indicated that specific genes determine the presence and proportion of ß-diketones in wax. Hallam (1970) reported that probably at least 50 gene loci (Blakely' classification, 1956) in *Eucalyptus* control the formation of epicuticular wax. It is also likely that the polymorphism in wax morphology observed, e.g. in *Eucalyptus camaldulensis* (Hallam 1970) was caused by the presence and segregation of various eceriferum alleles. Similarly genetical polymorphism may be involved in the observed species differences.

The evidence from these studies has confirmed that a relationships exists between wax chemistry and structure and suggest that specific genes determine the nature of the classes of wax compounds and govern the homologues composition within each class of substance (Jeffree *et al.* 1976; Bianchi *et al.* 1980).

The variability in leaf wax chemistry and/or structure has been linked to genetic control and reported from a number of studies dealing with different aspects. The variability may take the form of differences in the proportions of the component wax classes (Silva *et al.* 1964; Barber and Netting 1968; Tulloch 1973; Chang and Grunwald 1976; Bianchi and Corbellini 1977; Franich *et al.* 1978; Baas and Fidgor 1978; Hunt and Baker 1979; Baker and Hunt 1981; Woodhead *et al.* 1982; Avato *et al.* 1984; Freeman and Turner 1985). Meanwhile, some reports have indicated that changes in homologue distribution within an individual compound class may occur (Herbin and Robins 1969; Dyson and Herbin 1970; Tulloch 1973; Schuck 1976; Stocker and Wanner 1975; Baker *et al.* 1979; Gamou and Kawashima 1980; Faboya *et al.* 1980a,b; Baker and Hunt 1981; Salasoo 1981, 1983). In addition, some change only in the amount of wax deposited i.e. the same components are produced in the same proportion throughout development (Leon and Bulovac 1976; Baker and Hunt 1981).

2.6.4. Epicuticular waxes and plant taxonomy, and their use in eucalypt taxonomy

Epicuticular waxes as chemotaxonomic criteria

Two different approaches have been used to incorporate information from the study of epicuticular waxes into a taxonomic framework. Firstly there is the chemotaxonomic approach using the chemistry of selected constituents of epicuticular wax. Second, there

is the approach using the fine structure of the crystalline waxes themselves as a relevant taxonomic character.

Many studies have indicated that the chemical composition of waxes is an valuable aid to the botanical classification of plants (Douglas and Eglinton 1967; Herbin and Robins 1969; Harborne 1968; Martin and Juniper 1970; Harborne and Turner 1984). So far, the chemical composition of waxes as a chemotaxonomic character has mainly related to the species and generic level, but the possibility of using it at the family level was discarded (Eglinton and Hamilton 1963). There is evidence that indicates that the wax composition or compound pattern is usually distinctive, although not invariably so. Many studies has shown that the chemical composition of a wax could distinguish species and often be related to different taxonomic groups at the generic level (Horn *et al.* 1964; Hallam 1970; Evans *et al.* 1975a,b; Salatino and Salatino 1983; Guelz 1986; Avato *et al.* 1990). Moreover, some studies have indicated that the distribution of different compound homologues could indicate species specificity (Eglington *et al.* 1962; Herbin and Robin 1968a,b; Nordby and Nagy 1977; Sorensen *et al.* 1978; Guelz 1986; Salatino *et al.* 1989; Page *et al.* 1990; Avato *et al.* 1990) or related to taxonomy at the generic level, such as alkanes, although it is still not entirely clear in spite of the fact that a variety of genera have now been explored in some depth (Harborne and Turner 1984 and references therein).

In most plants cuticular wax is found on the surface and the crystalline structure of this wax is a rather unique characteristic of each species. The structures of plant epicuticular waxes are now commonly described as morphological characters (Martin and Juniper 1970). Wax morphology, as revealed by scanning electron microscopy (SEM), shows comparatively few types occurring throughout the family and indicates phenetic groups of genera which are supported from other lines of evidence. A number of genera may be uniform in wax morphology, and others variable with two or three types occurring. Thus at lower taxonomic levels (i.e. species, genus) wax morphology may not distinguish between taxa but indicate similarities between some genera and, at higher levels correlates with sectional grouping. Although only a limited number of studies have examined morphology from a taxonomic viewpoint and not all have been successful, those that have however, perceived differences at all levels of the taxonomic hierarchy. Distinction between species was achieved for apple (Gough and Shutak 1972) and barley (Marchylo and Laberge 1981) cultivars; banana varieties (Freeman and Turner 1985); eucalypt spp. (Hallam and Chamber 1970), *Prosopis* spp. (Hull and Bleckmann 1977), *Isocoman* spp. (Mayeaux *et al.* 1981), *Pinus* spp. (Yoshie and Sakai 1985) as well as characterizing genera within the Triticeae (Baum *et al.* 1980) and orders within monocotyledons (Barthlott and Froelich 1983).

Epicuticular waxes as taxonomic criteria for Eucalyptus species

The structure and morphological characters of plant cuticular waxes received attention as early last century. It is possible that *Eucalyptus* was one of the earliest investigated due to the fact leaves and stems of many *Eucalyptus* species are glaucous, particularly during the juvenile period of growth.

The earliest recorded observations on plant surface waxes were made by de Bary (1871) who published drawings of large wax crystals from a large number of species as seen under a light microscope. He recognised four groups. In de Bary's work, waxes of *Eucalyptus globulus* were investigated and classified as several layers of very small needles.

The leaf waxes of eucalypt species were used by taxonomists to separate species populations. It is of interest that the Tasmanian eucalypts were the first species group of *Eucalyptus* to be recorded. Barber (1955) observed the wax blooms present on Tasmanian eucalypts and classified them into either structural or non-structural types. He defined the structural type to possess a blue-gray colour of foliage which is stable to the touch. In contrast, the non-structural type is glaucous which is easily removed by rubbing. The wax which provides a glaucousness between these two types is the clinal form. In this report, the classification of the 24 Tasmanian species into the three groups as regards waxy glaucousness is based on a preliminary field survey: seven species are wholly green, six wholly glaucous while eight show clinal variation in the degree of glaucousness with the remaining three species also probably clinal (see Table 2.1). Furthermore, it was found that the melting point of wax is apparently correlated with Blakely's (1956) classification. Waxes from a group of species of section Macrantherae, belonging to the series *Globulares*, have melting points within the range 57-61^{0}C, but those from the section Renantherae fall into two groups - in the series Piperitales the melting points are much lower (45-48 ^{0}C), while in the series *Fraxinales* and *Longitudinales* the melting points range from 51-54 ^{0}C.

Since that time techniques for the study of plant ultrastructure have advanced considerably with the development and continual refinement of electron microscopy. In this area, a notable study of a wide range of leaf waxes was undertaken by Hallam and Chambers (1970). They investigated 315 species of the genus *Eucalyptus* by electron microscopy and demonstrated that the leaf wax structure of *Eucalyptus* can, in some instances, aid in the classification of this taxonomically complex genus. They reported three main wax types within *Eucalyptus*: plates, tubes, and a mixed wax of both plates and tubes. Referring to Barber's (1955) classification of eucalypt waxes, Hallam and Chambers defined tubes to represent "non-structural" waxes and plates the "structural"

waxes. Whether the mixed "tube and plate" waxes produce a "structural" or non-structural" wax depends on the relative amount of each wax type present on the leaves. Furthermore, they described the edges of plate waxes to be entire, sinuate, crenate, or digitate and concluded that the degree of ornamentation of the margins of the plates can frequently be correlated with taxonomic groupings or suggested evolutionary trends based on other lines of evidence. In addition tube waxes, depending on their branching pattern and arrangement on the leaf surface, also proved useful as indicators of natural groupings of species. In their study, the affinities of large groups of species within genus *Eucalyptus* were recognized by evidence derived from wax morphology.

Beside contributing to the classification of *Eucalyptus* spp. on the basis of wax morphological characters, the chemistry of eucalypt waxes shown by infrared and ultraviolet absorption spectroscopy also confirms many of the results of the taxonomic study based on wax morphology. Hallam and Chambers found that a marked correlation existed between the presence of ß-diketones in waxes and the formation of tubes, and conversely a tendency towards the absence of ß-diketones in waxes which form only plates. Their results also showed the common presence of flavonoids and esters in all species which had no correlation with different morphological types of waxes. Although their study of wax chemicals was limited to the presence or absence of a few classes of compounds by simple observation of infrared spectral analysis of crude wax extracts, they did indicate a relationship between wax chemistry and morphological structure and suggested that chemicals play an important role in the formation of the wax structure of *Eucalyptus* species.

Up to now, there have been no published works which deal with morphological and chemical characters of leaf waxes from the genus *Eucalyptus* in broad species groups to demonstrate their taxonomic significance.

In responding to Barber's (1955) report which noted differences in the physical properties of waxes from various eucalypt species groups, Horn *et al.* (1964) published their extensive study of the leaf wax components of the genus *Eucalyptus* (29 species) in which the occurrence of long chain ß-diketones (e.g. C_{33} n-tritriacontan-16,18-dione) as major components was reported. However, in a few species this type of compound was entirely lacking. Although waxes from most of the glaucous eucalypts showed a broad general resemblance, certain correlations between the chemical content of leaf cuticular wax and the sub-classification of genus *Eucalyptus* (according to Blakely's classification, 1955) were observed by Horn *et al.* , as for example the existence of shorter chain ß-diketones (C_{29}, n-nonacosan-12,14-dione) in *E. risdonii* and *E. coccifera,* the only species examined in the Series *Piperitales* and the absence of ß-diketones in four species from the Series *Corymbosaepeltatae.* Beside ß-diketones, some compound classes, such

as hydrocarbons, esters, free acids and alcohols, eucalyptin and triterpenoids also were identified from the wax samples but only a few species were examined in detail. Lamberton (1964) reported demethyl eucalyptin as a related flavonoid of eucalyptin from the leaf waxes of *E. torelliana* and *E. urnigera*. Herbin and Robins (1968) examined the hydrocarbons of leaf waxes from 19 species of eucalypts and described the alkane patterns of waxes. In agreement with Horn *et al.*, they found that the frequent dominance of alkane homologues were C_{27}, C_{29} and, in a few cases, C_{25}. However, for these hydrocarbon contents and patterns did not show any correlation with Blakely's (1956) classification.

Leaf waxes of *Eucalyptus*, as reviewed above, have contributed valuable information to the taxonomy of the genus. The results of Hallam and Chambers (1970) indicated that the morphological structure of leaf waxes may have considerable taxonomic value in *Eucalyptus* at the species group level of the taxonomic hierarchy, which correlated with morphological characters of Blakely's classification (1956), and related to the melting point and glaucousness of the wax determined by Barber (1955).

Some important aspects of the relationship between chemical composition and morphological structure in leaf waxes of *Eucalyptus* remains unresolved. The study by Hallam and Chamber (1970) has only shown the positive correlation of ß-diketones with tube wax structure but what compounds correlate to the plate wax remains unclear. Moreover, there are no detailed reports that deal with the composition of leaf wax for species which have plate wax structure.

2.7. Numerical methods for chemotaxonomic studies

Biochemical characteristics can be used as one of the bases for determining phenetic relationships between plants. These relationships can be evaluated at many taxonomic levels (eg. within or between populations, species and families). Numerical data analyses, including pattern analysis (Williams 1976), are commonly used to elucidate such relationships. A number of numerical methods capable of identifying patterns of phenotypic variation have been described by Sneath and Sokal (1973), Neff and Marcus (1980), Pielou (1984), Sokal and Rohlf (1987). The pattern analysis techniques which were used in this study can be divided into those where the variation of each variable was assessed independently of the rest (univariate analysis) and those where a number of variables were assessed simultaneously (multivariate analysis). For pattern analysis, multivariate analysis allows for the assessment of the overall variation between a set of Operational Taxonomic Units (OTUs) (Sneath and Sokal 1973; Neff and Marcus 1980;

Pielou 1984). Univariate analyses can be a valuable adjunct to multivariate analyses by providing further information about particular variables (Sokal and Rohlf 1987).

Univariate analysis techniques can usefully demonstrate population differentiation. Simmons (1974) and Whiffin (1978) have used multiple range tests to disclose population patterns for morphological and biochemical characteristics. Analysis of variance (ANOVA) has been employed in a number of studies as an indicator of population differentiation in leaf oils, e.g. Snajberk *et al.* (1979), Whiffin (1978, 1982a,b), Zavarin *et al.* (1989, 1991). In taxonomic and biological studies should often be appropriate to have samples, sub-samples and random sub-sub-samples for estimation of the numbers of organisms in heterogeneous taxa or test materials. Several layers of nesting may occur in biological assays where a random sample of plant populations are maintained under each of a series of treatments, e.g. subgenera of *Eucalyptus*, species within subgenera and localities within species. In such cases nested anova for data was suggested as appropriate by Sokal and Rohlf (1987) and the two or/and three level nested anova for both equal and unequal sample sizes was also provided.

Sokal and Rohlf (1987) stated that the distribution-free methods (non-parametric methods) could be used if none of the transformations managed to make observed data meet the assumptions of ANOVA. These techniques (e.g. Kruskal-Wallis and Mann-Whitney tests) are simple to compute and permit freedom from worry about the distributional assumptions of an ANOVA. Non parametric methods have become quite popular in recent years and have been used in studies of leaf oil characteristics, e.g. Zavarin *et al.* (1975, 1977, 1980) and Zavarin and Snajberk (1986).

Correlation and regression techniques (Sokal and Rohlf, 1987) can be useful for establishing the relationships between variable factors of populations. Juvonen (1966), Irving and Adams (1973) and Forrest (1980) used correlation analyses to establish biochemical relationships of terpenoids of essential oils.

Leaf oils often provide a much larger number of quantitative characters in comparison to morphological characteristics. Some works had used the sum of variances of individual oil components (ΣV) to characterise and summarise the overall variabilities of oil characteristics between samples within a given OTU while the variabilities of oil characteristics between OTUs could be compared by ΣV, e.g. Zavarin and Snajberk (1986) and Zavarin *et al.* (1991).

Multivariate analysis allows the relationships between population attributes to be assessed simultaneously. Many multivariate techniques operate on a resemblance matrix. Different resemblance measures and their properties have been described by Sneath and Sokal

(1973) and Neff and Marcus (1980), Pielou (1984). The nature of relationships within the matrix can be assessed using cluster analysis or ordination techniques.

Cluster analysis aims to identify groups of OTUs (clusters) which are more similar to one another than to OTUs outside the cluster and classify groups of OTUs of objects judged to be similar according to a distance or similarity measure. The OTUs (individuals, populations, species, chemical components, etc.) are frequently ordered into a hierarchical, non-overlapping system of clusters (Whiffin 1978, 1982a,b; Neff and Marcus 1980). Cluster analysis has been the primary method used in phenetic taxonomy, where many attributes are considered simultaneously and the objects (OTUs) are clustered according to their overall similarity (Sneath and Sokal 1973). However, clusters resulting from cluster analysis depend on both the similarity measure chosen and the algorithm used for clustering. Whiffin (1982a,b) suggested that Manhattan metric is a convenient and accurate distance measure for use with volatile oil data. Moreover, Digby and Kempton (1987) suggested that the clustering using the unweighted pair-group method using averages method (UPGMA) usually distributes the objects into a reasonable number of groups and this has become the conventional method in ecology and systematics. It calculates differences between clusters as the average of all the point-to-point distances between a point in one cluster and a point in the other (Sneath and Sokal 1973, Pielou 1984).

Standardisation and weighting of the oil data could help to find distinguishing groups where the aim of the investigation is to study the significant difference. Some works (Adams and Turner 1970, Adams 1975b; Whiffin 1982a) suggest weighting standardised data by the F-value which is computed from an analysis of variance as the variance among groups divided by variance within groups. Weighting of characters by their F-value thus assumes that the samples are, a priori, divided into groups. The use of weighting by the F-1 value could minimise the influence of characters showing random fluctuations and which may be affected by environmental factors or factors other than the genotype (Adams 1975b). The weighted pair-group method using averages (WPGMA) is the cluster analysis using standardised and weighted data for the measure of distance (Whiffin 1982b).

Ordination is a process which represents OTUs as coordinates on axes derived from those components of the original variables which account for the observed range of variation in a minimum number of dimensions. Ordination can indicate relationships between OTUs without assuming continuity or discontinuity of data. Three ordination techniques commonly used in taxonomic studies are Principal Component Analysis (PCA), Principal Coordinates Analysis (PCORD), and Canonical Variate Analysis (CVA) (Neff and Marcus 1980). PCA is usefully to describe a matrix of data consisting of

objects and attributes by reducing its dimensions, usually for graphical display and to find uncorrelated linear combination of the original variables (attributes) with maximal variance. This technique has been used by many workers to describe variation in plant oils, e.g. von Rudloff *et al.* (1988) and Zavarin *et al.* (1989). Although PCORD is a generalisation of PCA in which non-Euclidean distances may be used, PCORD cannot indicate combinations of variables (attributes) while CVA is only appropriate where data can be divided into a priori groups.

Chapter 3

Variation in Leaf Oils of Four *Eucalyptus* Species

3.1. Introduction

In forest trees, a number of hierarchical categories of variation exist that can be broadly grouped into species, geographic sources (provenances), stands, sites, individual trees, and the variability within individual trees (Zobel *et al.* 1960). Yield and composition of leaf oils produced by *Eucalyptus* populations for species, provenances, localities or individuals, may differ both within and between populations and this variation reflects four major variables: (i) individual genetic divergence, (ii) ontogenetic and phenological stage of leaf material, (iii) environment and (iv) techniques of extraction and analysis (Doran 1991). When comparing either qualitative or quantitative aspects of leaf oils of species or even different individuals of the same species, the above factors must be considered using appropriate methodologies and sampling methods.

Stebbins (1977) considered that each individual plant must possess the ability to respond to its environment, but this variation must remain within the limits set by the genetic make-up of the taxon. Phenotypic expression, therefore, is determined by both genotypic composition and reaction to a specific environment. However, it is emphasised that a study of variation in natural stands can give no proof of the intensity of genetic control of a characteristic because in such a study, one cannot separate the effects of environment, genetics and their interaction (Zobel and Talbert 1984). Zobel and Talbert (1984) considered that the way to partition phenotypic variation into causal components was through growing clones or families collected from a field source of populations in a common environment. To determine the magnitude of the genotype x environment interaction (GE), genetic material must be planted in trials established across a range of environments. Conversely, this means that observations made on the same genetic material grown in different trials established across a range of environments could also reveal the environmental effect.

In cooperation with the Tasmanian Forestry Commission, the CSIRO Division of Forestry (Tasmania) established an experiment (species/provenance trial) in 1983 to determine the seasonal growth and developmental patterns of four eucalypt species in plantations to explain variation in these patterns in terms of environmental factors. As the experiment was established on a four-site altitudinal sequence and received uniform treatments of fertilizer, weed and pest control, it presented a unique opportunity for

comparing the essential oils of leaves from trees under intensive management. This chapter reports on three major experiments comparing essential oils between species, between provenances within species, and between and within sites. Seasonal, ontogenetic and physiological age effects were exposed to the same environmental conditions.

The comparison of two provenances of four eucalypt species at four sites consisted of *E. globulus* and *E. nitens* from the subgenus *Symphyomyrtus* (the gums) and *E. delegatensis* and *E. regnans* from the subgenus *Monocalyptus* (the ashes). The comparison of leaf oils with seasonal, ontogenetic and physiological effects consisted of one provenance of the species *E. globulus*, *E. nitens* and *E. delegatensis*.

3.2. General Materials and Methods

3.2.1. Site location and plant material

Site characteristics and original lay out of plant material was described by Turnbull *et al.* (1988).

The study sites were located at altitudes 60, 240, 440, and 650 m (sites 1 to 4) in the Esperance Valley, southeastern Tasmania (Fig.3.1.A).

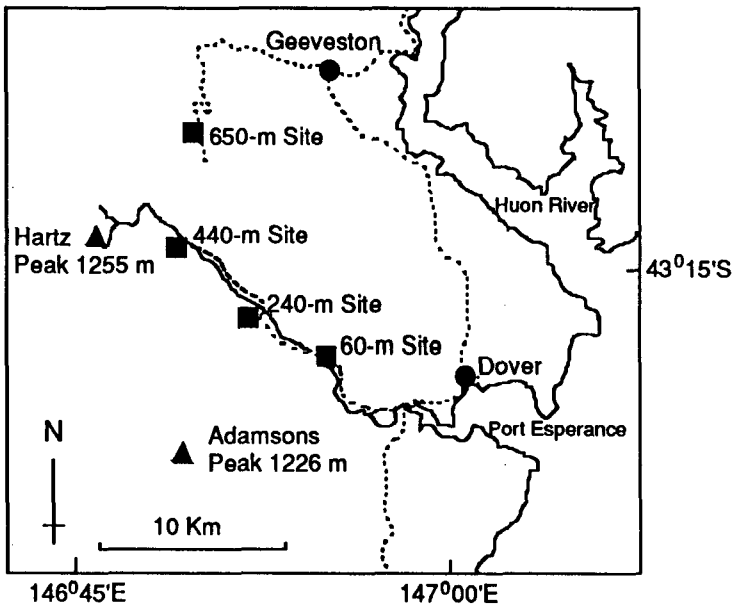


Fig. 3.1.A. Locations of the four experimental sites in the Esperance Valley, southern Tasmania. (following Hallam *et al.* 1989)

E. globulus B1	*E. regnans* A1
E. globulus B2	*E. regnans* A2
E. nitens D1	*E. delegatensis* C1
E. nitens D2*	*E. delegatensis* C2

Fig. 3.1.B. Arrangement of planting blocks at each of the four provenance trial sites in the Esperance Valley.

All sites were of similar topography and selected to average or exceed the average Site Index classification for the Southern Forests of Tasmania. Sites 1 to 4 had similar soil type. Each of the four study sites was 1.0 hectare in area and received uniform treatments of fertilizer, weed and pest control.

Table 3.1. Species and provenances in the trials in the Esperance Valley

Subgenus	Species	Code	Provenance	Lat. (E)	Long. (S)	Elevation (m)
Monocalyptus	*E. regnans* F. V. Muell.	A1	Moogara, Tas.	$42^{0}47'$	$146^{0}36'$	550
		A2	Traralgon Creek, Vic.	$38^{0}27'$	$146^{0}31'$	500
	E. delegatensis R. T. Bak	C1	Guildford, Tas.	$41^{0}20'$	$145^{0}40'$	580
		C2	Maydena, Tas.	$42^{0}33'$	$146^{0}34'$	650
Symphyomyrtus	*E. globulus* Labill.	B1	Geeveston, Tas.	$43^{0}10'$	$146^{0}16'$	200
		B2	Otways, Vic.	$38^{0}43'$	$143^{0}26'$	125
	E. nitens Maiden	D1	Penny Saddle, Vic.	$37^{0}48'$	$146^{0}16'$	900
		D2*	Bendoc, Vic.	$37^{0}12'$	$148^{0}52'$	1070

* This provenance of *E. nitens* was later classified into new species *E. denticulata* (Cook and Ladiges 1991). Detailed are given in chapter 11. In the chapter, this provenance was treated as a provenance of *E. nitens*.

Planting stock of two provenances of each of the four *Eucalyptus* species was raised from seed collected from different localities (Table 3.1) and seedlings planted at 2x2 m spacings in August 1983. Within each trial, each species was planted alongside a second provenance of that species in square measurement blocks of 120 trees with 60 trees of each provenance. The blocks were surrounded by four buffer rows of the same species on the outer perimeter of the plantation or two rows if adjacent to another species. The trial was maintained free of all other competing plant species and large animals and vermin were excluded. Insect feeding on the foliage was controlled, but not eliminated, by monthly applications of insecticide.

3.2.2. Leaf sampling and preparation

This chapter is divided into three experiments. Leaf sampling and preparation are described in individual experiments.

3.2.3. Oil analysis

Oil eaxtraction

Oil extraction was described in individual experiments.

GC-MS analysis

The leaf oils were analysed by combined GC-MS using a HP-5890 GC directly coupled to a VG 707F mass spectrometer with 2035 datasystem. The GC column was a 50m x 0.3mm BP20 (SGE Pty Ltd), and temperature was programmed from 50 to 220 ^{0}C at 6 ^{0}C per minute after a one minute hold, using helium as carrier gas. The oils were made up as solutions of 5 microlitres/ml of hexane prior to injection.

Identification of individual compounds was based on reference mass spectra (Stenhagen, Abrahamsson and McLafferty "Registry of Mass Spectral Data"; NIST Mass Spectral Database) and an 'in-house' library of reference spectra built up from standard compounds and oils of known composition. Gas chromatographic retention indices (Davies 1991) were also used to aid in the interpretation, as mass spectral information was often not sufficient to permit unambiguous assignment of identity. Some compounds which could not be identified by GC-MS analyses were separated and identified by using IR, UV, NMR and chemical reaction analyses.

The essential oils were run under identical conditions using a Flame Ionization Detector on a HP5890 Gas Chromatography to give relative peak areas for individual compounds.

3.2.4. Data analysis

The ANOVA and nested ANOVA for variation in oil components was estimated following Sokal and Rohlf's method (1981) and computed using the ANOVA program of the super ANOVA (Abacus Concepts, Inc.). Non-parametric comparison was computed using the non-parametric comparison program of Statview written by Daniel *et al.* (1988, Abacus Concepts, Inc.). Principal component analysis (PCA) was undertaken based on the total correlation matrix derived from the percentage component data of individual leaf oils. The orthogonal principal component axes and the loading of the populations on these axes were plotted in pairs. The amount of variation between samples which is accounted for by each ordinate was calculated. In this way, the effectiveness of each plot

in summarising the relationships between the populations can be found by calculating the percentage of the total variation accounted for by the axes. The major variation is normally contained in the first few axes although, in more complex situations, useful information may still be found on minor axes. PCA was undertaken using the factor analysis program of Statview based on Sneath and Sokal's (1973) method. All analyses were run on a Macintosh SE/30 computer.

3.3. Experiment 1: Variation between species and provenances within site

The purpose of this investigation was to examine differences in leaf oils between species, between provenances within species and between individual trees within provenances within a site. Although different species and provenances were not randomly mixed and were separated into different plots within a site, environmental conditions between plots were very similar due to the very small site area and uniform treatments of fertilizer, weed and pest control. In this experiment, all leaf samples were collected at the same time. Therefore, both qualitative and quantitative variation between phenotypes were thus examined using leaves considered to be of the same physiological age grown under the same environmental conditions. Phenotypic expression within site, therefore, is determined by genotypic composition.

3.3.1. Materials and methods

Sample collection and preparation

Samples of adult foliage of ten randomly selected trees from each provenance of 6 year old *E. globulus, E. nitens, E. delegatensis* and *E. regnans* were collected at the end of April 1989 from CSIRO Esperance site 1 when leaf expansion had ceased (the end of March). Fresh leaf samples (c.a. 500gm) of each sampled tree were sealed in plastic bags and transported to the University of Tasmania within 3-4 hours and stored at 4 ^{o}C until processed. Samples were processed within two weeks of collection.

Individual leaves were plucked from the upper 30-40 cm of the sample branches (last year's old leaves were excluded), cut into 1-2 cm^2 pieces and 100g lots taken for oil extraction. Another 100 g lot of chopped leaves was prepared for each sample, and dried to constant weight at 50 ^{0}C for moisture content determination. The fresh weigh (FW) vs dry weight (DW) parameter was obtained from the latter.

Oil extraction

Fresh leaf samples were steam distilled, using a glass distillation unit, for 6 hours. The oils were collected and weighed. Oil yield for each sample was calculated on a dry weight of leaf basis (g/g), calculated using the FW/DW rates.

3.3.2. Results

Oil yield and Composition

The oil yields and chemical compositions of adult leaves from different provenances of *E. regnans*, *E. delegatensis*, *E. nitens* and *E. globulus* grown at Site 1 are listed in Table 3.2.

The ANOVA indicated that oil yields were significantly different between subgenera ($p<0.001$), between species ($p<0.001$) within subgenera and between provenances within species ($p<0.001$) (Table 3.3). Table 3.4 indicated that *E. regnans* had the highest mean value of oil yields (5.12%) and *E. nitens* the lowest (0.73%) while the *E. delegatensis* (3.51%) had similar oil yield to *E. globulus* (3.89%).

The t-Test (Table 3.2) indicated that there were significant differences in oil yields between provenances within species, *E. regnans* ($\Delta_{A1-A2}=1.8$; $p<0.01$), *E. delegatensis* ($\Delta_{C1-C2}=0.9$; $p<0.05$) and *E. globulus* ($\Delta_{B1-B2}=-0.6$; $p<0.05$).

Forty three chemical components were detected from the freshly steam-distilled oils (Table 3.2). The chemical components identified from these leaf oils were dominated by monoterpenoids and sesquiterpenoids with the esters, isobutyl isobutanoate and isoamyl isovalerate, occurring in *E. nitens* and methyl cinnamate in *E. delegatensis*.

The percentage contents of most oil components were significantly different between subgenera, between species within subgenera and between provenances within species (Table 3.3).

With the exception of *p*-cymene, citronellol, and viridiflorol, the percentage contents of all oil components were significantly ($p<0.05$ or $p<0.001$) different between subgenera. Oil components, such as monoterpenoids, α–pinene (compound 1), α–thujene (2), 1,8-cineole (12), α– and β–phellandrene (7 and 11), limonene (10) and *cis*- and *trans*-*p*-menth-2-en-1-ol (20 and 25) and sesquiterpenoids elemol (36) and α–, β– and γ–eudesmol showed highly significant differences between subgenera, and had greater *F*-values (over 1000) than any other components (Table 3.3).

Table 3.2. The mean oil yields and chemical composition of adult leaf oils from different provenances and species and *t*-test for differences in the percentages of individual oil components between provenances within species.

t = trace = less than 0.5%; * p<0.05; ** 0.05<p<0.01, *** p<0.001, ns p>0.05

(a) *E. regnans* and *E. delegatensis*:

	Species	*E. regnans*						*E.delegatensis*					
	Provenances	A1		A2		*t*-Test A(1:2)		C1		C2		*t*-Test C(1:2)	
	No. of trees	10		10		d.f.=9		10		10		d.f.=9	
		Mean	S.D.	Mean	S.D.	A1-A2	Sign.	Mean	S.D.	Mean	S.D.	C1-C2	Sign.
	Oil yield	6.1	1.1	4.3	0.7	1.8	**	4.0	0.8	3.0	0.4	0.9	*
1	α–Pinene	t	t	t	t	0.0	n	-	-	-	-	-	-
2	α–Thujene	1.1	t	0.8	t	0.3	**	3.3	1	1.9	0.5	1.4	**
3	Camphene	t	t	t	t	0.0	n	-	-	-	-	-	-
4	β–Pinene	t	t	t	t	0.0	n	t	t	t	t	0.0	**
5	Isobutyl isobutanoate	t	t	-	-	0.1	n	-	-	-	-	-	*
6	Sabinene	t	t	t	t	-0.1	n	t	t	t	t	-0.2	n
7	α–Phellandrene	7.2	1.1	5.6	1.1	1.6	*	15.9	3.6	20.9	4.4	-1.0	*
8	α–Terpinene	1.4	0.3	1	0.4	0.4	*	3.1	0.8	3.7	0.6	-0.6	n
9	Isobutyl isopentanoate	-	-	-	-	-	n	-	-	-	-	-	n
10	Limonene	0.4	t	0.5	t	-0.1	n	0.5	t	0.5	t	0.0	n
11	β–Phellandrene	4.7	1	3.8	0.7	0.9	*	11.4	3	9.9	1.9	1.5	n
12	1,8-Cineole	t	t	t	t	0.0	n	t	t	t	t	0.0	n
13	*cis*-β–Ocimene	0.4	t	0.3	t	0.1	*	0.6	t	0.7	t	-0.2	n
14	γ–Terpinene	0.4	t	0.3	t	0.1	n	0.7	0.3	0.6	t	0.1	n
15	*trans*-β–ocimene	t	t	t	t	0.0	n	0.4	t	0.4	t	0.0	n
16	*p*-Cymene	3.8	1.5	4.4	2	-0.6	n	9.8	2.5	7.7	1.8	2.2	n
17	Terpinolene	0.5	0.4	0.5	t	0.0	n	1.1	0.3	1.4	0.4	-0.4	*
18	Isoamyl isovalerate	-	-	-	-	-	n	-	-	-	-	-	n
19	Linalool	t	t	0.5	t	-0.3	**	0.5	t	0.6	t	-0.1	n
20	*trans-p*-Menth-2-en-1-ol	5.7	1	3.9	0.9	1.8	**	10.5	2.3	8.5	2.5	2.0	n
21	Pinocarvone	-	t	-	-	0.0	n	-	-	-	-	-	-
22	*b*-Caryophyllene	t	t	t	t	-0.1	n	-	-	-	-	-	-
23	Aromadendrene	t	t	t	t	0.0	n	-	-	-	-	-	-
24	Terpinen-4-ol	1.5	0.3	1	t	0.5	**	2.4	1	2.8	0.7	-0.3	n
25	*cis-p*-Menth-2-en-1-ol	4	1	2.6	0.6	1.4	***	7.9	1.9	6.1	1.7	1.8	**
26	*Allo*-aromadendrene	-	-	-	-	-	n	-	-	-	-	-	-
27	*trans*-Pinocarveol	-	-	-	-	-	n	-	-	-	-	-	-
28	*cis*-Piperitol	1.5	0.5	1	0.4	0.5	*	2.7	0.9	2.2	0.9	0.5	*
29	Terpinyl acetate	t	t	t	t	0.0	n	-	-	-	-	-	-
30	α–Terpineol	0.6	0.3	0.5	t	0.1	n	1.2	0.5	1.6	0.5	-0.5	n
31	Piperitone	1.4	1.2	2.2	2.4	-0.9	n	1.4	0.6	2	1.2	-0.6	n
32	*trans*-Piperitol	3.4	0.8	1.9	0.6	1.5	***	4.7	1.2	3.9	1.3	0.8	n
33	Citronellol	t	t	-	-	0.0	n	-	-	-	-	-	-
34	*cis*-Sabinol	t	0.3	0.3	t	-0.1	n	0.3	t	0.4	t	-0.1	n
35	*p*-Cymene-8-ol	t	t	0.6	0.3	-0.4	*	0.5	t	0.5	t	0.0	n
36	Elemol	6.6	1.2	7	1	-0.4	n	-	-	-	-	-	-
37	Globulol	-	-	-	-	-	n	1.4	0.6	2.3	1.1	-0.9	n
38	Viridiflorol	0.5	t	0.6	t	-0.1	n	0.7	t	0.8	t	-0.2	n
39	Methyl cinnamate	t	t	t	t	-0.1	n	1.8	0.4	0.4	0.3	1.4	***
40	Spathulenol	2	0.6	1.4	0.3	0.6	*	3.2	0.9	2.5	0.5	0.7	n
41	α–Eudesmol	14.8	2.9	19.7	3	-4.9	**	3.6	1.2	5.2	1.5	-1.6	n
42	γ–Eudesmol	10.4	1.7	13	2	-2.6	*	3.3	1.4	3.9	1	-0.6	n
43	β–Eudesmol	12.5	1.3	16.2	2.1	-3.7	***	2.8	1	3.3	0.6	-0.6	n
44	Unidentified	8.3	2.6	1.9	1.1	6.3	***	-	-	-	-	0.0	-

Table 3.2. Continued.

t = trace = less than 0.5%; * $p<0.05$; ** $0.05<p<0.01$, *** $p<0.001$, ns $p>0.05$

(b) *E. nitens* and *E. globulus*:

	Species	*E. nitens*						*E.globulus*					
	Provenances	D1		D2		t-Test D(1:2)		B1		B2		t-Test B(1:2)	
	No. of trees	10		10		d.f.=9		10		10		d.f.=9	
		Mean	S.D.	Mean	S.D.	D1-D2	Sign.	Mean	S.D.	Mean	S.D.	B1-B2	Sign.
	Oil yield	0.7	0.2	0.8	0.2	-0.1	ns	3.6	0.6	4.2	0.7	-0.6	*
1	α–Pinene	12.8	3.5	1.6	1.4	11.2	***	21.2	5.1	16.1	4.4	5.1	n
2	α–Thujene	-	-	-	-	-	-	-	-	-	-	-	-
3	Camphene	t	t	-	-	0.1	*	t	t	t	t	0.1	n
4	β–Pinene	t	0.3	t	-	0.2	*	0.5	t	t	t	-0.1	n
5	Isobutyl isobutanoate	3	1.6	6.7	1.8	-0.8	***	-	t	-	-	0.0	n
6	Sabinene	t	t	t	t	0.2	n	t	t	t	t	0.0	n
7	α–Phellandrene	1.3	0.7	0.3	t	1.0	**	t	t	t	t	-0.1	n
8	α–Terpinene	t	t	t	t	0.1	n	t	t	t	t	-0.4	n
9	Isobutyl isopentanoate	3.8	1.4	5.1	0.8	-1.3	*	-	-	-	-	-	-
10	Limonene	1.3	0.3	0.9	0.3	0.4	*	4.7	1.2	4.2	1.3	0.5	n
11	β–Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-
12	1,8-Cineole	27.6	6.5	14.4	2.9	13.5	***	44.6	5.7	52.4	9.9	-7.8	n
13	*cis*-β–Ocimene	3.4	1.8	9.2	3.2	-5.5	***	0.4	t	t	t	0.2	n
14	γ–Terpinene	9.4	2.9	17.5	2.8	-8.0	***	0.8	0.7	0.9	0.8	-0.1	n
15	*trans*-β–ocimene	0.3	t	0.3	t	0.0	n	t	t	t	t	0.0	n
16	*p*-Cymene	10.1	2.7	20.9	4.6	-10.8	***	0.9	0.4	2.3	1.6	-1.4	n
17	Terpinolene	0.4	t	0.6	0.3	-0.1	n	t	t	t	t	-0.1	n
18	Isoamyl isovalerate	2.1	1.1	0.4	0.3	1.7	**	t	t	t	t	-	-
19	Linalool	0.3	t	t	t	0.1	n	t	t	t	t	0.0	n
20	*trans-p*-Menth-2-en-1-ol	t	t	t	t	0.0	n	t	t	t	t	0.0	n
21	Pinocarvone	1.4	0.6	0.3	t	1.2	***	1.5	0.6	1.9	0.9	-0.4	n
22	*b*-Caryophyllene	t	t	t	t	0.0	n	1.8	0.9	0.3	t	1.5	***
23	Aromadendrene	1.3	0.4	t	t	1.2	***	2.1	0.8	1.9	1	0.2	n
24	Terpinen-4-ol	1.1	0.4	2.5	1.1	-1.5	**	1.2	1.7	0.4	t	0.7	n
25	*cis-p*-Menth-2-en-1-ol	t	t	t	t	0.0	n	-	-	-	-	-	-
26	*Allo*-aromadendrene	0.5	t	t	t	-0.3	**	0.5	t	0.6	0.3	-0.1	n
27	*trans*-Pinocarveol	1.9	0.6	t	t	1.8	***	2.7	1.3	1.4	0.8	1.3	**
28	*cis*-Piperitol	t	t	t	t	0.0	n	t	t	t	t	0.1	*
29	Terpinyl acetate	0.6	0.4	0.5	0.3	0.1	n	1.1	0.7	0.9	0.4	0.3	n
30	α–Terpineol	3.3	1.4	3.7	1.2	-0.4	n	3.2	1.1	2.5	1.2	0.7	n
31	Piperitone	t	t	t	t	0.0	n	t	t	t	t	0.0	n
32	*trans*-Piperitol	t	t	t	t	0.0	n	t	t	t	t	0.0	n
33	Citronellal	-	-	-	-	-	-	-	-	t	t	0.0	n
34	*cis*-Sabinol	t	t	t	t	-0.2	**	t	t	t	t	0.0	n
35	*p*-Cymene-8-ol	t	t	0.5	t	-0.4	**	t	t	t	t	0.0	n
36	Elemol	-	-	-	-	-	-	-	-	-	-	-	-
37	Globulol	1.7	0.8	0.7	0.3	1.0	**	5.7	1.6	5.6	1.9	0.1	n
38	Viridiflorol	0.6	0.3	0.4	t	0.2	n	0.7	0.3	0.7	0.3	0.0	n
39	Methyl cinnamate	-	-	-	-	-	-	-	-	-	-	-	-
40	Spathulenol	3.5	1.4	2.7	1.1	0.8	n	0.6	t	0.7	0.t	-0.1	n
41	α–Eudesmol	0.6	0.3	0.5	t	0.1	n	t	0.3	t	t	0.0	n
42	γ–Eudesmol	0.5	t	0.6	t	0.0	n	t	t	0.5	0.3	-0.3	**
43	β–Eudesmol	0.6	t	0.4	0.4	0.1	n	0.3	0.3	0.4	0.3	-0.2	n
44	Unidentified	-	-	-	-	-	-	-	-	-	-	-	-

Table. 3.3. ANOVA for oil yields and percentage content of components of adult leaf oils from different provenances and species at site 1 based on log(√x) transformed data.

Significance: *** p<0.001; ** 0.001<p<0.01; * 0.01<p<0.05; ns: p>0.05; # Components had substantial amounts (mean percentage > 3% in leaf oils of individual provenances) and significant variation between speices or provenances within species (p<0.01)

		Source of variation	Between subgenera		Between species within sugenera		Between provenances within species		Residual
		Degree of freedom	1		2		4		72
			F-values	Signt.	*F*-values	Signt.	*F*-values	Signt.	
		Oil yield	334.41	***	271.00	***	11.94	***	
1	αP	α–Pinene	1167.00	***	111.00	***	43.73	***	#
2	αTh	α–Thujene	2210.70	***	125.81	***	18.38	***	#
3	Ca	Camphene	51.98	***	10.08	***	7.19	***	
4	βP	β–Pinene	17.83	***	40.52	***	4.49	**	
5	Iso 1	Isobutyl isobutanoate	422.00	***	436.92	***	20.81	***	#
6	Sa	Sabinene	7.01	*	2.57	ns	3.90	**	
7	αPh	α–Phellandrene	1562.00	***	169.00	***	9.94	***	#
8	αT	α–Terpinene	883.00	***	96.79	***	4.49	**	#
9	Iso2	Isobutyl isopentanoate	138.00	***	101.24	***	19.76	***	#
10	Lim	Limonene	1015.00	***	6.10	**	3.21	*	#
11	βPh	β–Phellandrene	2774.00	***	149.00	***	2.44	ns	#
12	Ci	1,8-Cineole	3030.00	***	185.00	***	17.39	***	#
13	cO	*cis*-β–Ocimene	177.00	***	279.00	***	26.38	***	#
14	γT	γ–Terpinene	489.00	***	423.00	***	18.73	***	#
15	tO	*trans*-β–ocimene	16.59	***	42.27	***	0.16	ns	
16	Cy	*p*-Cymene	1.13	ns	266.00	***	21.16	***	#
17	T	Terpinolene	71.75	***	32.64	***	1.32	ns	
18	Iso 3	Isoamyl isovalerate	135.00	***	100.00	***	19.58	***	
19	Lin	Linalool	41.72	***	7.62	***	3.29	*	
20	tM	*trans-p*-Menth-2-en-1-ol	1894.00	***	60.83	***	5.87	***	#
21	Pi	Pinocarvone	639.55	***	25.35	***	17.51	***	
22	bC	b-Caryophyllene	136.00	***	79.73	***	21.25	***	
23	Ar	Aromadendrene	476.00	***	59.26	***	23.87	***	
24	Ter	Terpinen-4-ol	21.36	***	27.36	***	6.66	***	
25	cM	*cis-p*-Menth-2-en-1-ol	1620.00	***	70.64	***	6.32	***	#
26	Al	*Allo*-aromadendrene	628.00	***	5.92	**	6.18	***	
27	tPin	*trans*-Pinocarveol	509.00	***	25.50	***	30.39	***	
28	cPi	*cis*-Piperitol	528.00	***	21.51	***	2.64	*	
29	Ta	Terpinyl acetate	206.00	***	10.57	***	0.69	ns	
30	αTer	α–Terpineol	145.00	***	13.20	***	1.71	ns	#
31	Pip	Piperitone	153.00	***	0.40	ns	0.92	ns	
32	tPi	*trans*-Piperitol	834.00	***	18.35	***	5.49	***	#
33	Cit	Citronellol	0.09	ns	1.00	ns	1.00	ns	
34	cSa	*cis*-Sabinol	88.72	***	9.97	***	5.33	***	
35	pCy	*p*-Cymene-8-ol	45.65	***	10.51	***	8.08	***	
36	El	Elemol	3021.00	***	3021.00	***	0.78	ns	#
37	Gl	Globulol	260.96	***	203.00	***	4.53	**	#
38	Ver	Viridiflorol	0.93	ns	7.78	***	1.54	ns	
39	Mc	Methyl cinnamate	278.00	***	156.00	***	34.63	***	
40	Sp	Spathulenol	13.75	***	76.65	***	2.66	*	#
41	αEu	α–Eudesmol	1538.00	***	275.00	***	8.38	***	#
42	γEu	γ–Eudesmol	1300.00	***	192.00	***	5.16	***	#
43	βEu	β–Eudesmol	1448.00	***	355.00	***	6.70	***	#
44	Un	Unidentified	460.66	***	189.56	***	61.17	***	#

Table 3.4. The mean oil yields and multi-range test (Fisher's PLSD) for four species.

Species	No. of trees	Mean values	S.D.	Significance of differences	
				0.05	0.001
E. nitens	20	0.73	0.19		
E. delegatensis	20	3.51	0.78		
E. globulus	20	3.89	0.68		
E. regnans	20	5.12	1.26		

With the exception of sabinene, piperitone and citronellol, the variabilities of percentage contents of all oil components were highly significant ($p<0.01$ or $p<0.001$) between species within subgenera. Most oil compounds also differed significantly ($p<0.05$, $p<0.01$ or $p<0.001$) between provenances within species.

The Principal Component Analysis (PCA) for variation between provenances of all four species

Variation in oil components among trees, provenances and species were summarised by principal component analysis (PCA). The monoterpenoids and sesquiterpenoids, which occurred in substantial amounts (mean percentage > 3% in leaf oils of individual provenances) and varied significantly between provenances within species ($p<0.01$) (Table 3.3), were selected and used for PCA. The principal components and the individual tree scores of different provenances and species on the first two principal component axes (PCs) are shown in Fig. 3.2 (for compound identity see Table 3.3).

The first two PCs accounted for 46.8% and 20.3% of the total variance of the components among trees respectively. The components, from these leaf oils, on the first two PCs (PC1 and PC2) are shown in Fig. 3.2a.

Plots of the individual tree scores of different provenances of each species (Fig. 3.2b) indicated that the first two principal components (PC1 and PC2) clearly separated trees into different groups that corresponded to the four species. The first principal component (PC1) differentiated species belonging to different subgenera and the leaf oils of the two 'ash' species had high levels of the monoterpenes α– and β–phellandrene, *cis*- and *trans*-*p*-menth-2-en-1-ol, α–terpinene, *trans*-piperitol and α–thujene or the sesquiterpenes α–, β– and γ–eudesmol and elemol. In contrast, the leaf oils of the two gum species were high in the monoterpenes 1,8-cineole, α–pinene, limonene, α–terpineol, *cis*–β–ocimene and γ–terpinene, the sesquiterpene globulol and the esters isobutyl isobutanoate and isobutyl isopentanoate.

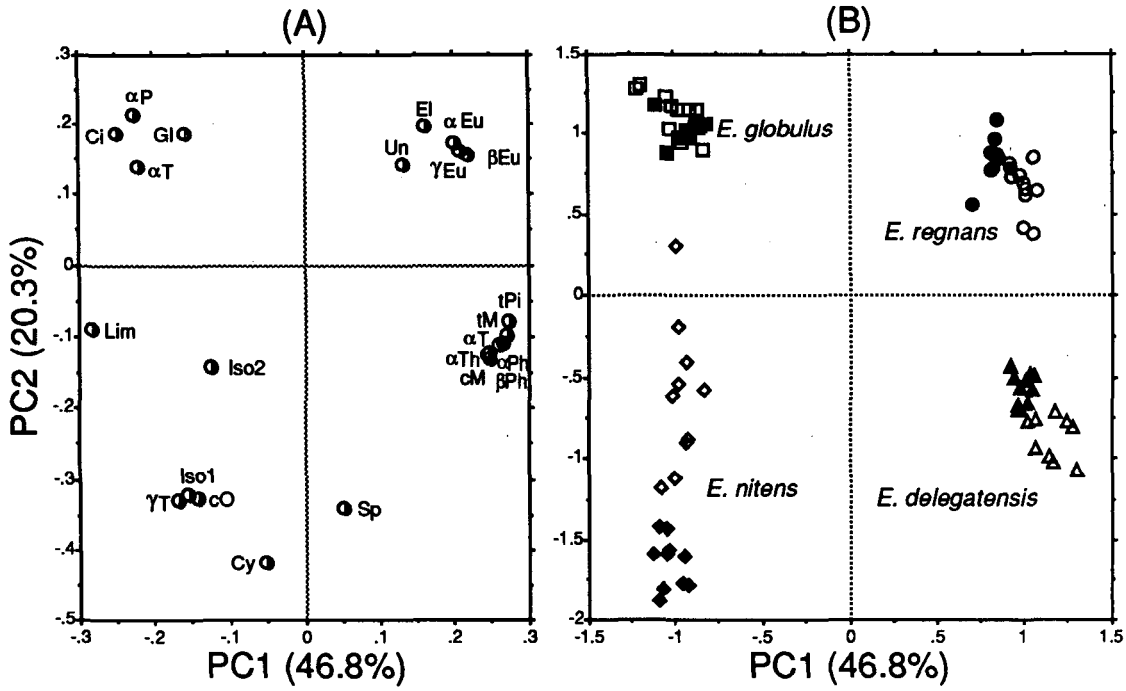


Fig. 3.2. Scatter plot of the oil parameters (A) and species/provenance populations (B) on the axes of the first two principal components (PC1 and PC2).

E. regnans:	○ Provenance A1 ● Provenance A2	*E. nitnes:*	◇ Provenance D1 ◆ Provenance D2
E. delegatensis:	△ Provenance C1 ▲ Provenance C2	*E. globulus:*	□ Provenance B1 ■ Provenance B2

The second principal component (PC2) simultaneously separated the two 'ash' species and also the two gum species. The leaf oils of leaves of *E. delegatensis* had higher levels of the monoterpenes α–, β– and γ–phellandrene, *cis*- and *trans-p*-menth-2-en-1-ol, α–terpinene, *trans*-piperitol and α–thujene than *E. regnans* but lower levels of the sesquiterpenes α–, β– and γ–eudesmol and elemol. The two ash species were clearly separated from each other and in this PC space, different provenances of both species could also be differentiated although there was overlap at the individual level.

PC2 also differentiated *E. globulus* and *E. nitens* samples due to high levels of the two monoterpenes, 1,8-cineole and α–pinene, and one sesquiterpenoid, globulol, in *E. globulus* and low levels of the three monoterpenes, γ–terpinene, *cis*–β–ocimene and *p*-cymene compared with *E. nitens*. There was no difference between the two provenances of E. globulus in this 2 dimensional space. However, the two provenances of *E. nitens* were differentiated along PC2, with continuous variation in trees of *E. nitens* along PC2, but no overlap at the provenance level. The trees of the Bendoc provenance (D2) had low levels of 1,8-cineole and α–pinene but high levels of *p*-cymene, γ–terpinene and *cis*–β–ocimene. Trees of the Penny Saddle provenance (D1) were intermediate between the Bendoc *E. nitens* and *E. globulus* on this axis and showed considerable variation

associated with increasing 1,8-cineole and α–pinene and decreasing amounts of *p*-cymene, γ–terpinene and *cis*–β–ocimene.

The PCA, as shown above, indicated that the trees of the four species were clearly differentiated by their leaf oil components when grown in a common environment (Fig. 3.2). Trees from different provenances of *E. regnans*, *E. delegatensis* and *E. nitens* could be differentiated, but there was very little difference between the *E. globulus* provenances.

3.3.3. Discussion

Each species in the trial in the Esperance Valley is presented by two different genetic sources (Turnbull *et al.* 1988). Therefore, with the same environment, soil and cultural treatments, the phenotype differences between provenances and species should be determined essentially by their genotype as environmental effects can be assumed to be minimal. Variation between trees within individual provenances can still be due to environmental and sampling differences within a trial. However, at the provenance level, these effects should have been largely removed, although not completely due to the non-random allocation of provenances within the trial.

The differences in leaf oil composition between species and between provenances within species are thus maintained when grown in a common environment (ten trees of each provenance at Site 1) and these differences should have a strong genetic basis.

There were large differences in the oil composition of species from the two subgenera. The monoterpenoids in the leaf oils of provenances of *E. regnans* and *E. delegatensis* were characterized by α– and β–phellandrene, *cis*- and *trans-p*-menth-2-en-1-ol, *cis*- and *trans*-piperitol and *p*-cymene. However, the monoterpenoids from provenances of *E. globulus* were characterized by 1,8-cineole and α–pinene and *E. nitens* by 1,8-cineole, γ–terpinene, *p*-cymene and *cis*–β–ocimene. The sesquiterpenoids of leaf oils from the two *Monocalyptus* species were characterized by α–, β– and γ–eudesmol whereas the two *Symphyomyrtus* species, contained globulol, spathulenol and aromadendrene.

The oil characteristics exhibited significant qualitative and quantitative differences between species within subgenera. In the *Monocalyptus* species, *E. regnans* had a lower ratio of monoterpenoids to sesquiterpenoids than *E. delegatensis*. The two *Symphyomyrtus* species also showed significant differences in oil composition. 1,8-cineole and α–pinene occurred in higher proportions in *E. globulus* oils than *E. nitens*. The leaf oil of *E. nitens* had high proportion of γ–terpinene, *p*-cymene and *cis*–β–ocimene but these components were either absent or in trace amounts in *E. globulus*.

Oil characteristics have indicated differences between provenances within all four species, especially within *E. nitens*. The Penny Saddle (D1) provenance of *E. nitens* produced leaf oils characterized by high 1,8-cineole and α–pinene. However, Bendoc (D2) provenance had high *p*-cymene, γ–terpinene and low 1,8-cineole with α–pinene only in trace amounts. The significant difference in leaf oils between the two *E. nitens* provenances was consistent with the morphological differences between these two provenances (Pederick 1979; Pederick and Lennox 1979). The, Penny Saddle provenance is the 'juvenile persistent form' and is consistent with the proposal of Cook and Ladiges (1991) who recognised the 'early adult form' as a new species *E. denticulata* (In this chapter, the species *E. denticulata* is still referred to as the 'early adult form' of *E. nitens*). Details will be discussed in chapter 11). However, there is a continuum in oil composition between these two forms (Fig. 3.2).

The oil yield varied significantly between species and between provenances within species in this common environment. In particular, the two *E. nitens* provenances had very low oil yields compared with provenances of the other three species.

3.4. Experiment 2: Variation in leaf oils between sites

This investigation aimed to document the effect of different sites on eucalypt leaf oil variation. The experimental design also permitted a comparison of species and provenances under different environmental conditions.

3.4.1. Materials and methods

In May 1991, adult leaves from 5 trees of each provenance of each species (tree age eight years) were collected at Sites 1, 2, 3 and 4. Fresh leaf samples of each provenance were mixed and sealed in plastic bags and then treated in the same manner as in experiment 1 (see sections 3.2.2. and 3.2.3). Thus a single sample was obtained for each provenance at each site.

3.4.2. Results

The oil yield and chemical composition of each provenance of the four eucalypt species is listed in Appendix 3.1 and the ANOVA for variation between sites, species and provenance within species are shown in Table 3.5.

Results of ANOVA (Table 3.5) indicated that the oil yields were significantly different between sites ($p<0.01$), species ($p<0.01$), provenances within species ($p<0.01$) and interaction of sites x species ($p<0.01$). Plots of oil yields of provenances with respect to

different sites indicated that site had a marked effect on the oil yields of the two 'ash' species, but little effect on the two gum species (Fig. 3.3A). While provenance differences within *E. regnans* and *E. globulus* were still maintained at each site, the two *E. delegatensis* provenances had very similar oil yields at high altitudinal sites.

The oil yields of provenances of *E. regnans* and *E. delegatensis* tended to decrease with increasing altitude. Provenances A1 and A2 of *E. regnans* had the highest oil yields at site 1 and linearly decreased with altitude. The two provenances C1 and C2 of *E. delegatensis* had higher oil yields at sites 1 and 2 than at sites 3 and 4. In contrast, only slight variations in oil yields between sites were recorded in the two gum species. The oil yield of provenance D1 of *E. nitens* appeared to increase with increasing altitude but provenance D2 remained constant. The oil yields of *E. globulus* were slightly higher at site 2 than at site 1 and then slightly decreased at sites 3 and site 4.

The percentage content of all oil components were significantly different between species (see Table 3.5 for significance levels). Moreover, most oil components also differed significantly between provenances within species but there were some exceptions. In contrast, site and the interaction of site x species did not significantly affect percentage contents of most oil compounds.

The percentage composition of four oil compounds, pinocarvone ($p<0.05$), *trans*-pinocarveol ($p<0.01$), piperitone ($p<0.001$) and methyl cinnamate ($p<0.001$) differed significantly between sites, and showed a significant site x species interaction. Pinocarvone and *trans*-pinocarveol occurred in the two 'gum' species and piperitone and methyl cinnamate occurred in the 'ash' species but rare or absent in the 'gum' species.

Plots of percentage of pinocarvone and *trans*-pinocarveol in leaf oils in provenances of the gum species indicated that these two compounds tended to have higher percentage content in leaf oils of *E. globulus* and provenance D1 of *E. nitens* at sites 1 and 2 and decreased with increasing altitude at sites 3 and 4. However, these two compounds did not vary between sites in provenance D2 of *E. nitens*.

The percentage of piperitone was high in leaf oils of both provenances of *E. regnans* at the high altitude site 4 and low at other sites but varied little between sites in *E. delegatensis*. However, in *E. delegatensis*, the percentage of methyl cinnamate was higher at the low altitude site 1 and high altitude site 4 than sites 2 and 3 in both provenances.

Table. 3.5. ANOVA for oil yields and percentage contents of components of adult leaf oils from different provenances and species at different sites based on log(√x) transformed data.

		Source of variation	Between sites		Between species		Between provenances within species		Sites*species		Residual
		Degree of freedom	3		3		4		9		12
			F-values	Signt.	*F*-values	Signt.	*F*-values	Signt.	*F*-values	Signt.	
		Oil yield	10.32	**	87.19	***	7.52	**	4.55	**	
1	αP	α–Pinene	1.71	ns	104.00	***	18.81	***	1.50	ns	#
2	αTh	α–Thujene	0.36	ns	63.26	***	4.26	*	0.71	ns	#
3	Ca	Camphene	0.53	ns	5.71	*	6.63	**	1.08	ns	
4	βP	β–Pinene	2.19	ns	93.88	***	1.72	ns	3.17	*	
5	Iso 1	Isobutyl isobutanoate	0.92	ns	181.00	***	61.96	***	0.97	ns	#
6	Sa	Sabinene	1.97	ns	9.08	**	6.78	**	0.96	ns	
7	αPh	α–Phellandrene	0.89	ns	274.00	***	8.61	**	2.08	ns	#
8	αT	α–Terpinene	1.42	ns	479.00	***	6.17	**	0.75	ns	#
9	Lim	Limonene@	1.54	ns	82.30	***	1.01	ns	1.41	ns	#
10	βPh	β–Phellandrene	0.63	ns	723.00	***	3.10	ns	0.41	ns	#
11	Ci	1,8-Cineole	1.90	ns	520.00	***	6.48	**	1.33	ns	#
12	cO	*cis*–β–Ocimene	1.90	ns	48.45	***	3.15	ns	1.83	ns	#
13	γT	γ–Terpinene	1.26	ns	71.34	***	12.14	***	0.81	ns	#
14	tO	*trans*–β–ocimene	0.95	ns	6.33	**	0.70	ns	0.53	ns	
15	Cy	*p*-Cymene	0.79	ns	67.76	***	5.93	**	2.13	ns	#
16	T	Terpinolene	2.48	ns	40.07	***	2.88	ns	1.49	ns	
17	Iso 3	Isoamyl isovalerate	1.02	ns	44.92	***	30.85	***	1.28	ns	
18	Lin	Linalool	0.52	ns	35.50	***	2.03	ns	1.72	ns	
19	tM	*trans-p*-Menth-2-en-1-ol	0.37	ns	187.00	***	4.21	*	1.44	ns	#
20	Pi	Pinocarvone	4.31	*	32.20	***	2.17	ns	3.35	*	
21	bC	b-Caryophyllene	2.88	ns	17.75	***	6.74	**	2.93	*	
22	Ar	Aromadendrene	0.20	ns	49.41	***	1.75	ns	0.71	ns	
23	Ter	Terpinen-4-ol	0.14	ns	33.66	***	3.02	ns	0.46	ns	
24	cM	*cis-p*-Menth-2-en-1-ol	0.23	ns	219.00	***	5.22	*	1.37	ns	#
25	Al	*Allo*-aromadendrene	0.22	ns	66.43	***	7.07	**	0.10	ns	
26	tPin	*trans*-Pinocarveol	6.22	**	39.45	***	7.18	**	4.28	*	
27	cPi	*cis*-Piperitol	0.65	ns	218.62	***	5.98	**	1.37	ns	
28	Ta	Terpinyl acetate	0.90	ns	7.66	**	8.84	**	0.97	ns	
29	αTer	α–Terpineol	0.51	ns	87.03	***	0.95	ns	0.37	ns	#
30	Pip	Piperitone	13.96	***	29.75	***	2.44	ns	13.02	***	
31	tPi	*trans*-Piperitol	0.21	ns	211.00	***	2.44	ns	1.49	ns	#
32	Cit	Citronellol	0.61	ns	4.01	*	1.19	ns	0.61	ns	
33	cSa	*cis*-Sabinol	0.19	ns	47.56	***	0.65	ns	1.44	ns	
34	pCy	*p*-Cymene-8-ol	0.35	ns	5.38	*	1.08	ns	1.00	ns	
35	El	Elemol	0.70	ns	120.00	***	1.82	ns	0.70	ns	#
36	Gl	Globulol	0.47	ns	126.00	***	9.95	***	0.59	ns	#
37	Vir	Viridiflorol	0.56	ns	5.52	*	6.96	**	1.04	ns	
38	Mc	Methyl cinnamate	16.87	***	372.00	***	108.00	***	15.95	***	
39	Sp	Spathulenol	0.30	ns	28.87	***	4.04	*	0.69	ns	#
40	αEu	α–Eudesmol	1.10	ns	236.00	***	7.85	**	0.42	ns	#
41	γEu	γ–Eudesmol	0.30	ns	207.00	***	5.65	**	0.23	ns	#
42	βEu	β–Eudesmol	1.03	ns	201.00	***	5.18	*	0.25	ns	#
43	Un	Unidentified	0.84	ns	45.57	***	18.45	***	0.84	ns	#

@ Limonene and isobutyl isopentanoate were unable separated in GC in this experiment. The percentage content of limonene therefore included isopentyl isopentanoate.

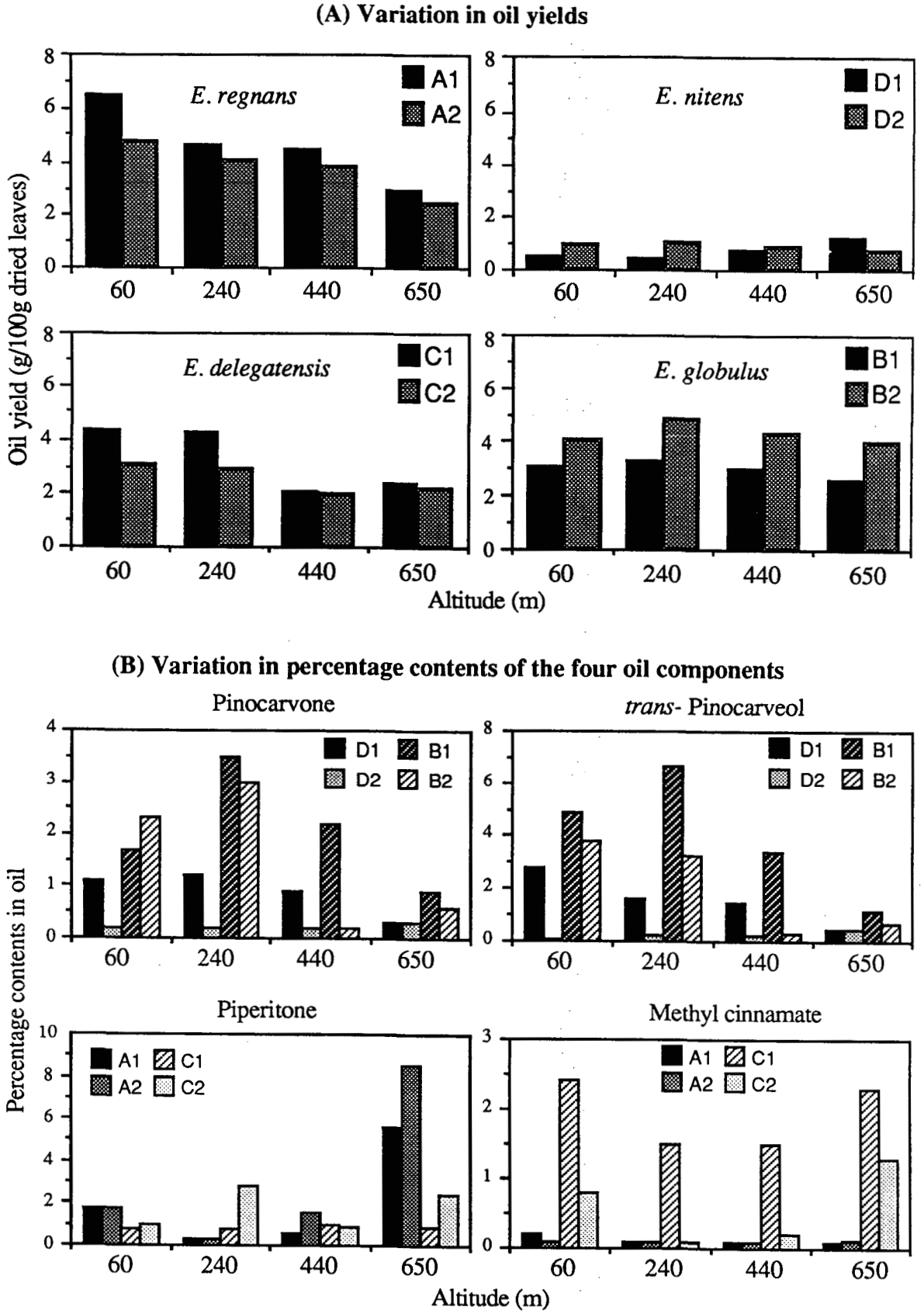


Fig. 3.3. Variations in oil yields and some oil components of the four eucalypt species with respect to altitude.

Variation in oil components among provenances, species and sites were summarised by PCA using important oil components which occurred in substantial amounts (mean percentage > 3% in leaf oils of individual provenances). Plots of the individual provenance scores of each species (Fig. 3.4) indicated that all samples for individual provenances formed distinct species groups in spite of the considerable variation between sites. For *E. nitens*, samples of the provenance D1 and D2 were readily distinguished without overlap.

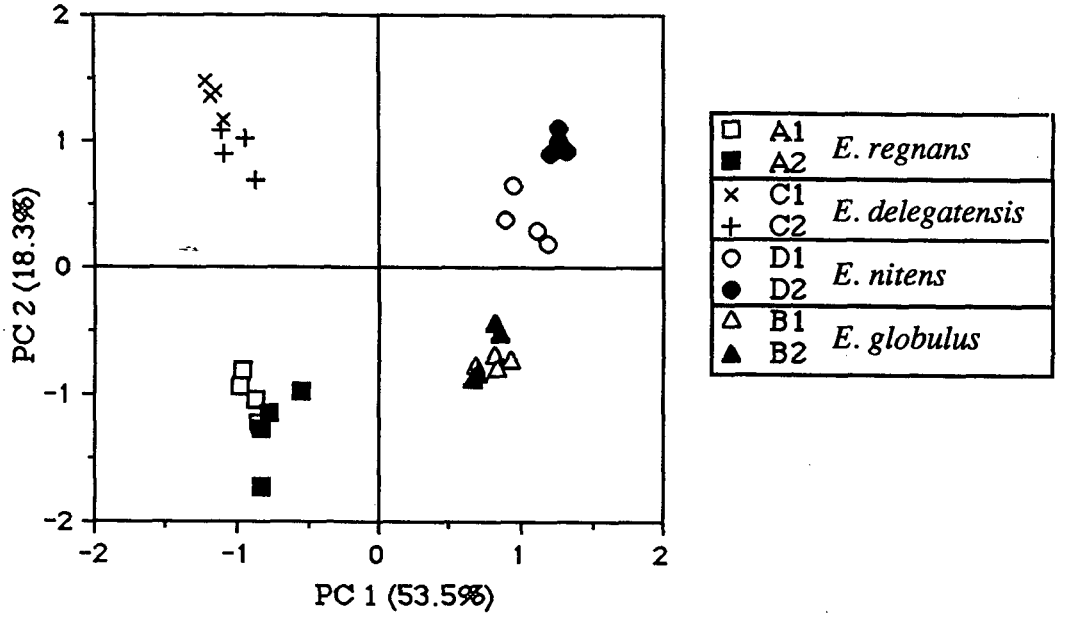


Figure 3.4. Plots of individual provenance scores of each species from different sites on the first and second principal components of leaf oils.

3.4.3. Discussion

Environmental variation in oil composition of leaf oils were examined across four sites along an altitudinal gradient in two provenances in each species of *E. regnans*. *E. delegatensis*, *E. globulus* and *E. nitens*.. However, the leaf sample of each provenance was a mixture of five trees, therefore, the data did not indicate the variability between trees within provenances with respect to altitude. With this limitation, the only conclusion that can be drawn are from a general rather than a statistical point of view.

The variation in leaf oils of each provenance of the four eucalypt species exhibited no marked qualitative differences between sites. Environment changed only the quantity of oil and some individual components. Thus for each species and provenance at different sites, the compositional leaf oil characters were comparatively stable suggested that the differences between species and provenances are under strong genetic control and little

modified by environmental influences such as altitude and rainfall and temperature related to altitude as described by Turnbull *et al.* (1988). However, significant differences in oil yields of provenances of 'ash' species could be attributed to the altitude of the different sites. This finding is consistent with the observations of Rajeskara *et al.* (1984) who found that oil yield of *E. citriodora* varied with altitude but there was no significant variation in oil composition. In summary, results of this study indicated that environmentally induced variation of leaf oil composition has been shown to be relatively insignificant in terms of taxonomic studies, although environmental factors were found to induce quantitative variation in leaf oils. Almost all qualitative and quantitative variation was considered to be under fairly strong genetic control.

The two 'ash' species are known to survive and grow best at low altitude (Sites 1 and 2) but perform poorly at higher altitudes. *E. nitens* grew well on all sites and *E. globulus* grew best at site 2 (200 m altitude) (see Turnbull *et al.* 1988). The oil yield and tree growth of individual provenances appeared to be related. Higher oil yields were recovered from the two 'ash' species at low altitudes in contrast to the low oil yields at higher altitudes where they were grew poorly. The *E. globulus* provenances had highest oil yields at site 2 where they were most successful. In contrast, *E. nitens* showed little difference in tree growth and oil yields between sites.

In the comparison of leaf oil yields between young and adult trees (see chapter 4), yields from leaves of seedling trees were significantly lower than from adult trees for all *Monocalyptus* species. In this study, the morphological characteristics of the two *Monocalyptus* species at sites 1 and 2 have now (1991) changed to adult leaves. However, the two 'ash' species at sites 3 and 4 still retain many of the phenological characters of the juvenile or intermediate leaves, especially trees of *E. regnans* at site 4. The leaf material of the two 'ash' species thus differed in the ontogenetic stage and it is considered that the low oil yields at high altitude could be attributed, in part, to the ontogenetic stage of tree development.

Similarly, comparison of the oil composition between juvenile and adult leaves in all Tasmanian localities of *E. regnans* (see chapter 4) showed a significantly higher percentage of piperitone in juvenile than in adult leaf oils. Therefore, the higher percentage of piperitone in the leaf oils of the two *E. regnans* provenances at high altitude sites in this study further indicates that variation in leaf oil of *E. regnans* with altitude was mainly related to the ontogenetic difference of leaf material. That is, the main effect of altitude may be through the delayed ontogenetic development of leaves at higher altitude.

Overall, despite sites encompassing covering a wide range of altitudes, the differences in oil composition due to environmental effects is insignificant when compared to the

differences between species. Provenance differences were also maintained across sites and, in particular, the provenances of *E. nitens* were readily distinguished without overlap. This indicates that a very large component of variation in oil composition between species and provenances has a genetic basis and this variation is maintained across a wide range of environments.

3.5. Experiment 3: Seasonal, ontogenetic and physiological age variation

This investigation was designed to examine the variation of leaf oils with (a) variation between seasons over one year and (b) time, ontogenetic and physiological leaf age within the active growing season.

3.5.1. Material and methods

Seasonal variation in leaf oil over a one year period

Variation with season was followed at two monthly intervals from November 1988 to November 1989 using one provenance from each of the three species *E. nitens* (D2), *E. globulus* (B2) and *E. delegatensis* (C2) at site 1. At each time interval, adult leaves was collected from each of the five trees of each provenance of each species (same five trees were sampled on each season). Each sample was sealed in plastic bags and stored in the cool room and then treated as in experiment 1 (see sections 3.3.1).

Examination of variability in leaf oils with time and leaf age

Experiment design

This experiment used a single provenance from each of the three species, *E. globulus* (B2), *E. nitens* (D2) and *E. delegatensis* (C2). Samples were collected from site 1.

Five trees from each provenance were selected on the basis of having good growth with well developed adult leaves. Tertiary branches on each of the five trees were selected from the partly closed crown and identified by numbered metal tags at the fourth internode from the apical tip (Figure 3.5).

Leaves were collected at twenty-eight day intervals on six (*E. nitens* and *E. delegatensis*) or seven (*E. globulus*) sampling dates. Leaf age of *E. nitens* and *E. delegatensis* leaves were coded as Age A (new leaves on the second leaf node position of a branch tip), Age B (28 days from first sampling), Age C (56 days), Age D (84 days), Age E (112 days)

and Age F (140 days). The age of *E. globulus* leaves included one more leaf age sample - Age G (168 days). As shown in Fig. 3.6 (a and b), this experimental design suited the measurement of the three sources of variation in the determination of the leaf oils.

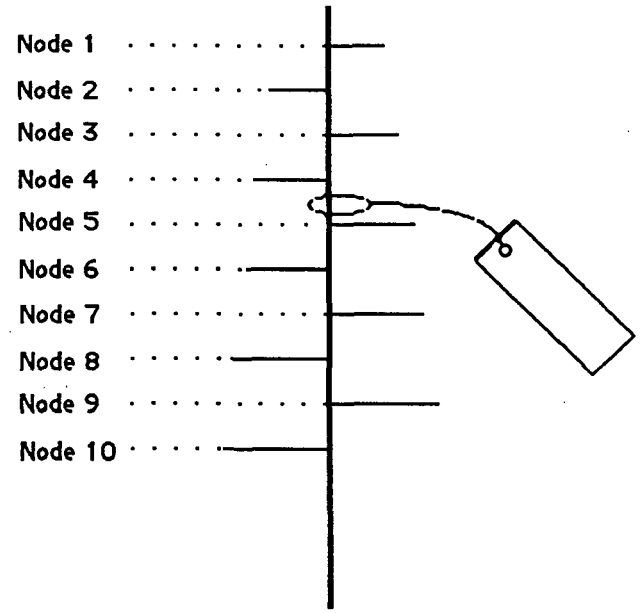
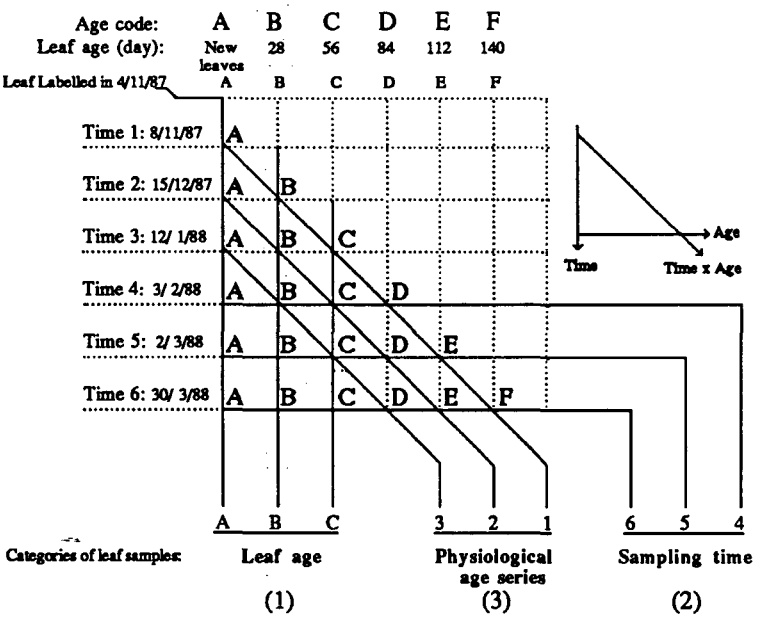


Fig. 3.5. Position of identification tag on shoot in relation to leaf node number.

(i) Variation with time in leaves of the same age (series 1)

Leaves of the same known physiological age (i.e. ages A–G), were sampled at each sampling time as indicated in Fig. 3.6. The new leaves with leaf age code A were thus sampled six times (time 1 to time 6) and leaves with leaf age code B (28 day old) were sampled five times (from time 2 to time 6) for *E. nitens* and *E. delegatensis*. As indicated by the ordination of 'time', this procedure allowed for the comparison of oil yield and components of leaf samples between sampling times for a given ***leaf age class*** [(1) in Fig. 3.6)]. For example, six sampling times could be compared (times 1 to 6) for Age A and five sampling times (times 2 to 6) compared for Age B on *E. nitens* (D2). This experimental design aimed mainly to compare variation with sampling time for the three leaf age classes of A, B and C (i.e. new, young leaves and immature leaves). The leaf samples within each age class with the same physiological leaf age, which initiated at different times, were sampled at different times throughout the study period. Therefore, variation between samples of each age class were affected by a sampling time factor. Thus, it compounded environment/seasonal effects with ontogenetic effects and time of initiation.

(a) *E. nitens* and *E. delegatensis*



(b) *E. globulus*

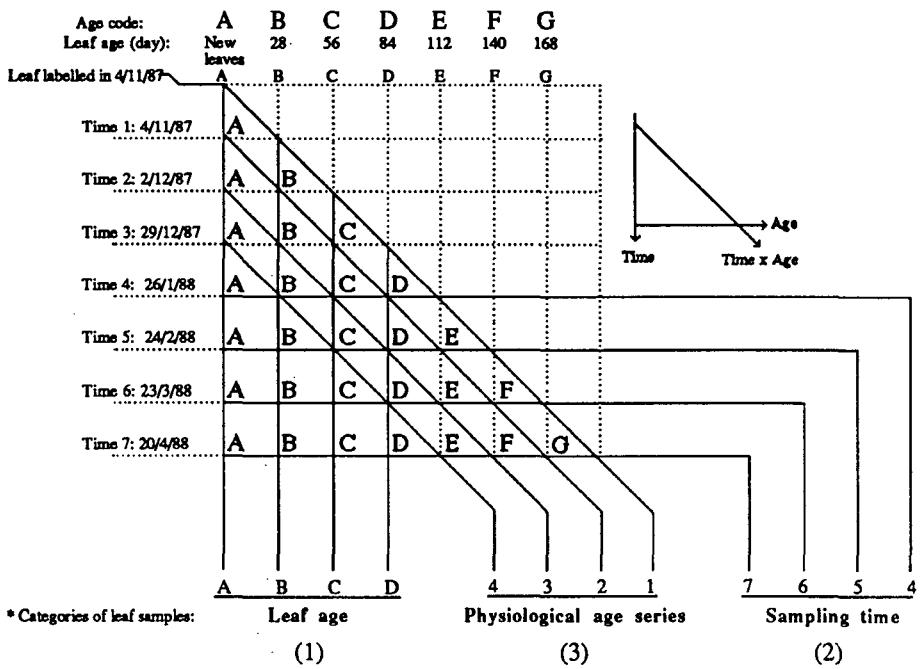


Figure 3.6. The experimental design used in the measurement of the three sources of variation in chemical constituent levels for each tree during leaf aging over the study period.

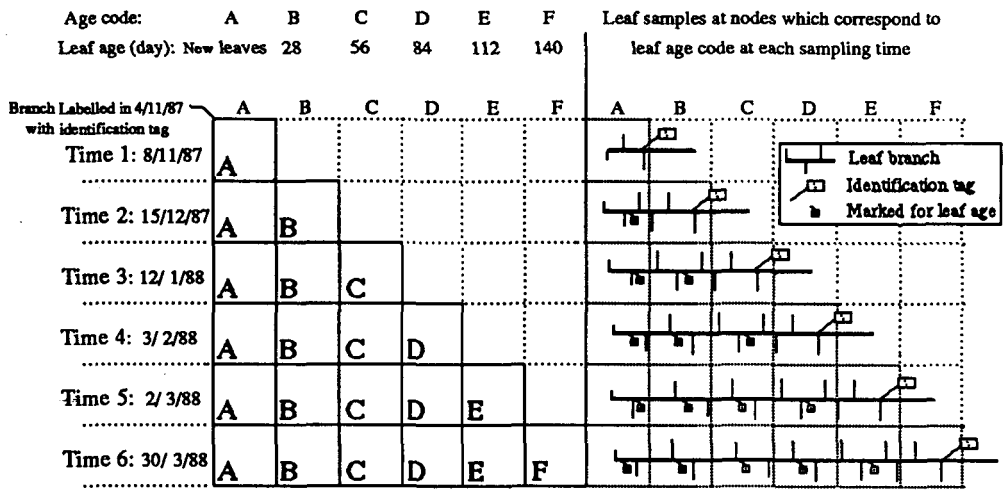
* Categories of leaf samples and sources of variation for each category of leaf sample:

(1) **Leaf age classes:** leaves initiated at different time, same physiological age sampled at different times throughout the study period. Variation between leaf samples within each age class are affected by sampling time factor and could compound environment/seasonal effects with ontogenetic effects.

(2) **Time series:** leaves initiated at different times but sampled at same time. Variation between leaf samples within each time series is affected by age factor and could compound seasonal/time effects on initiation of leaves, ontogenetic and physiological aging effects.

(3) **Physiological age series:** leaves initiated at same time but sampled at different time. Variation between leaves within each series are mainly affected by physiological age differences, but could compound differences due to environment at time of sampling.

(a) *E. nitens* and *E. delegatensis*



(b) *E. globulus*

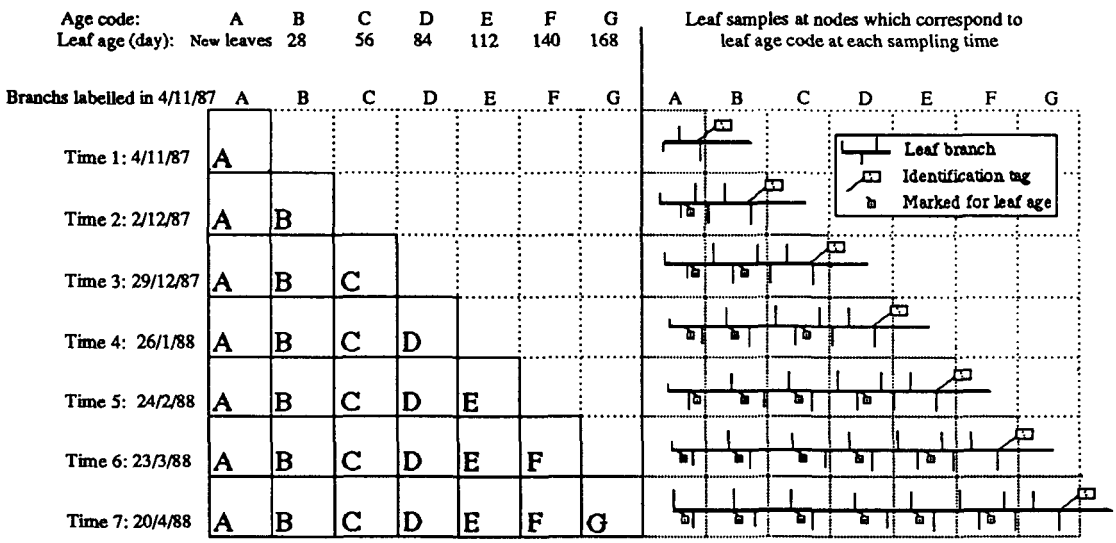


Figure 3.7. The leaves sampled from each tree of *E. nitens* (D2; Bendoc), *E. delegatensis* (C2; Maydena) and *E. globulus* (B2; Otways) during leaf aging over the study period.

(ii) Variation between leaf ages at the same sampling time (series 2)

The leaves of different physiological age, which initiated at different times, were also sampled at each sampling time. For example, while only new leaves with age code A were sampled at time 1, leaves of five different ages (age codes A, B, C, D and E) were sampled at time 5 and six ages at time 6. As indicated by the ordination of 'age', this design provided a number of ***time series*** [(2) in Fig. 3.6] of leaf samples (e.g. time 4, time 5 and time 6) for the comparison of oil yield and components between different leaf ages, which initiated at different times but were sampled at the same time. Therefore, variation between samples within each time series is composed of physiological age and ontogenetic effects and seasonal/time effects on initiation of leaves. This experiment compared differences in oil components and yield between leaf ages at the three sampling times (Times 4, 5 and 6) for *E. nitens* and *E. delegatensis* and the four sample times (Times 4, 5, 6 and 7) for *E. globulus*.

(iii) Variation with leaf age (physiological age effect)

The experimental design for the above two comparisons also allowed the comparison of oil yields and components between leaves initiated at the same time but sampled at different times. Thus, new leaves which expanded at the first sample time were labelled with age code A became age code B at sample time 2, age code C at time 3 to age code F at time 6. As indicated by the ordination of 'time x age', these leaves provided a number of ***physiological age series*** [(3) in Fig.3.6] of samples which initiated at time 1, time 2 and time 3 respectively with leaves aging from time 1 to time 6, time 2 to time 6 and time 3 to time 6. Therefore, variation between these leaves within each series would consist of mainly physiological aging and sampling time effects but not ontogenetic effects.

The leaves at the nodes which corresponded to leaf age codes were taken from the labelled branches of trees according to the leaves present at each sample time (Fig. 3.7). At each sampling date, a number of branches were randomly chosen and leaves from them destructively sampled according to the need of approximately 4-5g of leaves from each tree per age class and time (new leaves sometimes yielded only 2g/per time or age class).

Because the number of leaves that expanded at different sample times was variable, leaves of the same age could be sampled from different leaf nodes which corresponded to the leaf age codes.

For each tree, fresh leaf samples for each age and time sampling were mixed and sealed in a plastic bag and transported to the University of Tasmania. The leaf samples were stored at 4^0C until processed.

Sample preparation

Individual leaf samples were weighed and then cut into 2-3 cm pieces. Approximately 3-4 g of cut leaves were used for oil and wax extraction. Another 1-2g lot was used for moisture determination. The first lot of leaf samples were stored in a 4 ^{0}C cool room and extracted within a week.

Oil extraction and analysis

The fresh leaf sample was placed in a small flask and approximately 7-10 ml hexane added. The flask was then shaken at room temperature for 5 minutes. This extraction process was repeated twice. The hexane extracts were mixed and used for other experiments. After washing off the surface waxes, the leaf samples were steam distilled for six hours, using a small glass steam-distillation unit in which 1ml hexane had been added. The oils were collected in the hexane.

The leaf oil in hexane solution was subjected to the same qualitative analysis as for the experiment 1. The yield determination of these leaf oils was made possible by the use of n-tetradecane as an internal standard (see Appendix 3.3D). The internal standard chosen had a retention time which did not interfere with any of the peaks being measured.

Data analysis

The analysis of variance and significance tests for variation in oil compounds with time, leaf age and time x age during the active growth season and with season over one year based on untransformed data were computed using the non parametric comparison program of Statview written by Daniel *et al.* (1987) (Abacus Concepts.)

3.5.2. Results

Seasonal variation in leaf oils over one year

The mean oil yields and compositions of *E. nitens* (D2), *E. globulus* (B2) and *E. delegatensis* (C2) leaves over a one year period are listed in Appendix 3.2A, B and C. Results of analysis of variance for oil yields and percentage content of components of each species are also shown in these tables. It should be noted that the leaf samples for study of seasonal variation in this section consisted of new leaves which expanded during the study period (November 1987 to November 1988) and did not include the old leaves

of the previous year. Thus, the variation in leaf samples was not only affected by seasonal differences but also compounded the variation in physiological and ontogenetic leaf age.

Oil yield

Kruskal-Wallis *H*-test indicated that significant difference ($p<0.05$) in mean oil yield of five trees (based on dry leaf weight basis) between seasons was only occurring in *E. nitens* (Appendix 3.2). However, the mean percentage oil yield of *E. nitens* remained markedly lower (0.7-1.0%) than *E. delegatensis* (2.6-3.1%) and *E. globulus* (3.6-4.4%). Plots of mean oil yield for individual species indicated that oil yields of all three species increased gradually throughout the springs and summer seasons, at tainting a maximum value in March following a linear decline during the fall and winter (Fig. 3.8).

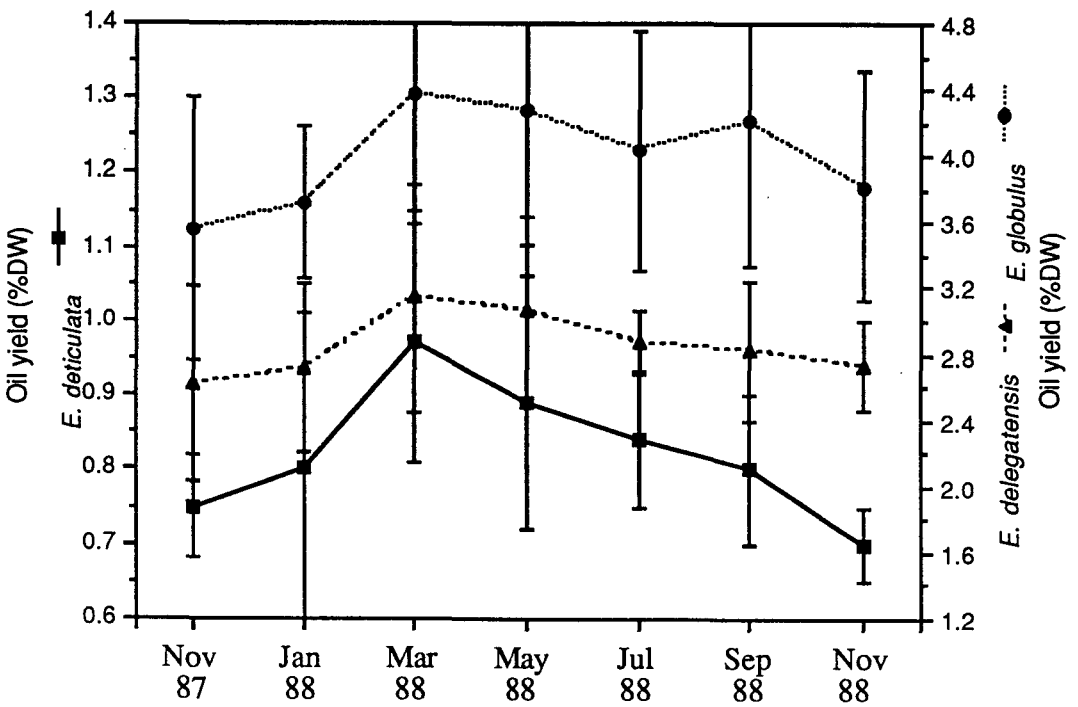
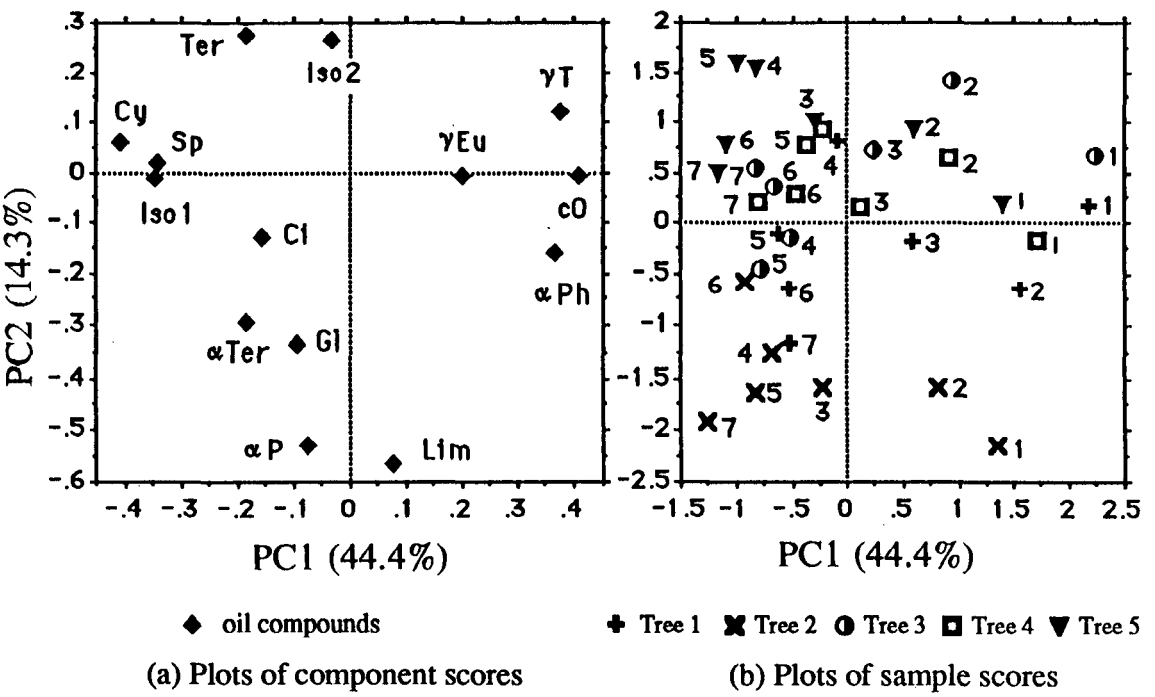


Fig. 3.8. Variation mean percentage content of oil yield (% DW) of *E. nitens* (D2), *E. delegatensis* and *E. globulus* at different sampling times over a year. (Error bar indicated S. D. of five trees).

Oil composition

(1) Oil composition of *E. nitens* (D2)

Overall variation in oil components, which occurred in substantial amounts (mean percentage > 0.3% in leaf oils of five trees at one sample time, see Appendix 3.2A) among trees of *E. nitens* (D2) over a one year period were summarised by PCA based on the percentage data of individual samples (Fig. 3.9). The first two PCs accounted for 44.1% and 14.3% respectively of the total variance of the components among trees. The ordination of compounds in the space defined by PC1 and PC2 indicated that positive variation along PC1 was mainly due to high levels of *cis*–β–ocimene, γ–terpinene and α–phellandrene and low levels of *p*-cymene, spathulenol and isobutyl isobutanoate. The positive variation along PC2 was mainly due to high levels of terpinen-4-ol and isobutyl isopentanoate and low levels of α–pinene, limonene, α–terpineol and globulol (Fig. 3.9a).



(a) Plots of component scores (b) Plots of sample scores

Fig. 3.9. Principal component analysis (PCA) for variation in oil components of all samples for individual trees of *E. nitens* (D2) sampled at different seasons over a year period. Code of Sampling time: Nov./87=1, Jan./88=2, Mar./88=3, May./88=4, Jul./88=5, Sep./88=6, Nov./88=7.

Plots of the individual tree scores over different seasons on the first two principal components (Fig. 3.9b) indicated that variation between samples from early sampling times to later sampling times over a year for individual trees was mainly due to variation along PC1 with increases in levels of *p*-cymene, spathulenol and isobutyl isobutanoate and decreases in *cis*–β–ocimene, γ–terpinene and α–phellandrene. Variation along PC2 mainly differentiated samples of tree 2 from samples of other trees due to high levels of α–pinene, limonene, α–terpineol and globulol and low levels of other components.

Principal component analysis thus indicated that the main axis (PC1) was due to seasonal variation (44.1%) and variation between trees contributed little (14.3%).

Moreover, variation between samples in the first four sampling times (1, 2, 3 and 4) contributed most variation over season along PC1 in contrast to little variation occur between later three sampling times (5, 6 and 7).

Again, samples from individual trees did not form clear groups, in spite of the considerable differentiation in variation pattern between trees, although the difference between tree 2 and others was maintained throughout the season.

The Kruskal-Wallis H test (Appendix 3.2A) for variation in percentage content of individual components between seasons further indicated that five monoterpene hydrocarbons, α–phellandrene ($p<0.001$), *cis*–β–ocimene ($p<0.001$), γ–terpinene ($p<0.01$) and *p*-cymene ($p<0.001$) varied significantly. The ester component isobutyl isobutanoate and the sesquiterpenoid, spathulenol, also varied significantly ($p<0.01$) with season but other components did not.

Fig. 3.10 indicates that variation in these oil components was mainly due to changes that occurred during the major growth period in summer. Little change occurred during the dormant winter stage when leaf expansion had ceased. For example, the mean percentage of *p*-cymene, *cis*–β–ocimene, γ–terpinene and α–phellandrene differed significantly between the November and May sample times but did not differ significantly between May and the following November. It can be seen that the percentage content of components of leaf oil in November 1987, when the sample mainly contained new leaves (old leaves of last season were excluded), differed markedly from that in November 1988, when the sample mainly contained mature leaves, therefore, leaf samples only contained leaves which expanded during and after November 1987). Therefore, this result compounded large differences in physiological leaf age in sampling.

The percentage content of one of the major components, 1,8-cineole, showed little variation over the sample periods and did not relate to variation in *cis*–β–ocimene, *p*-

cymene and γ–terpinene etc. In addition, α–pinene remained as a very minor component (0.5% to 0.7%) throughout the year (Appendix 3.2A).

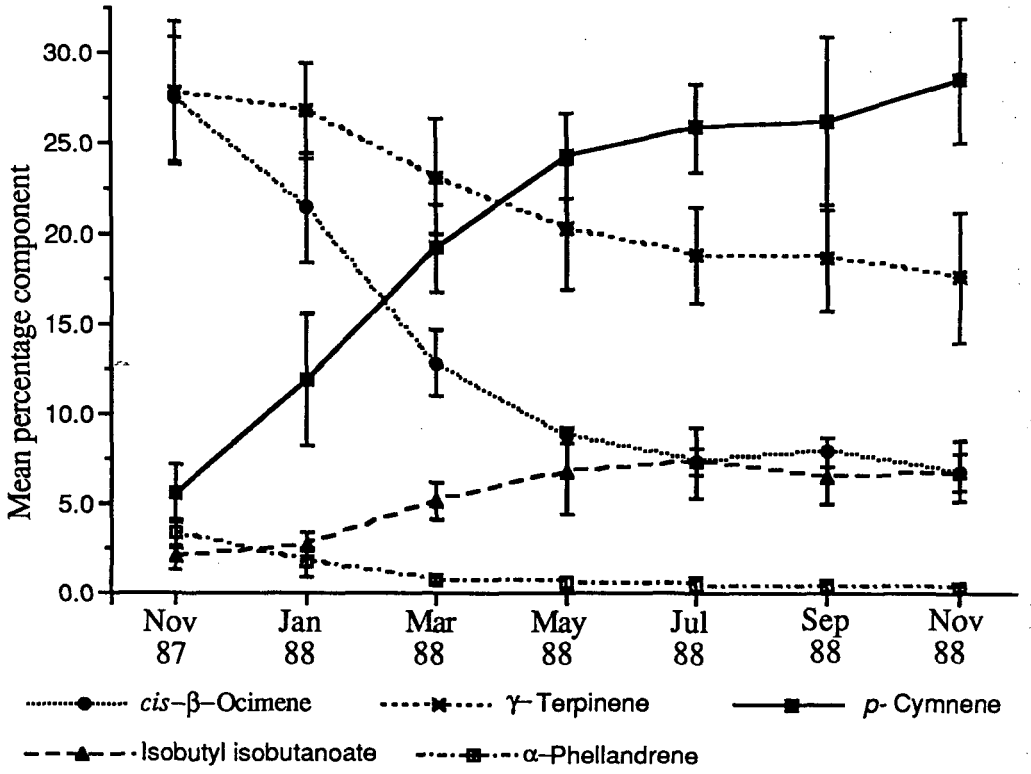


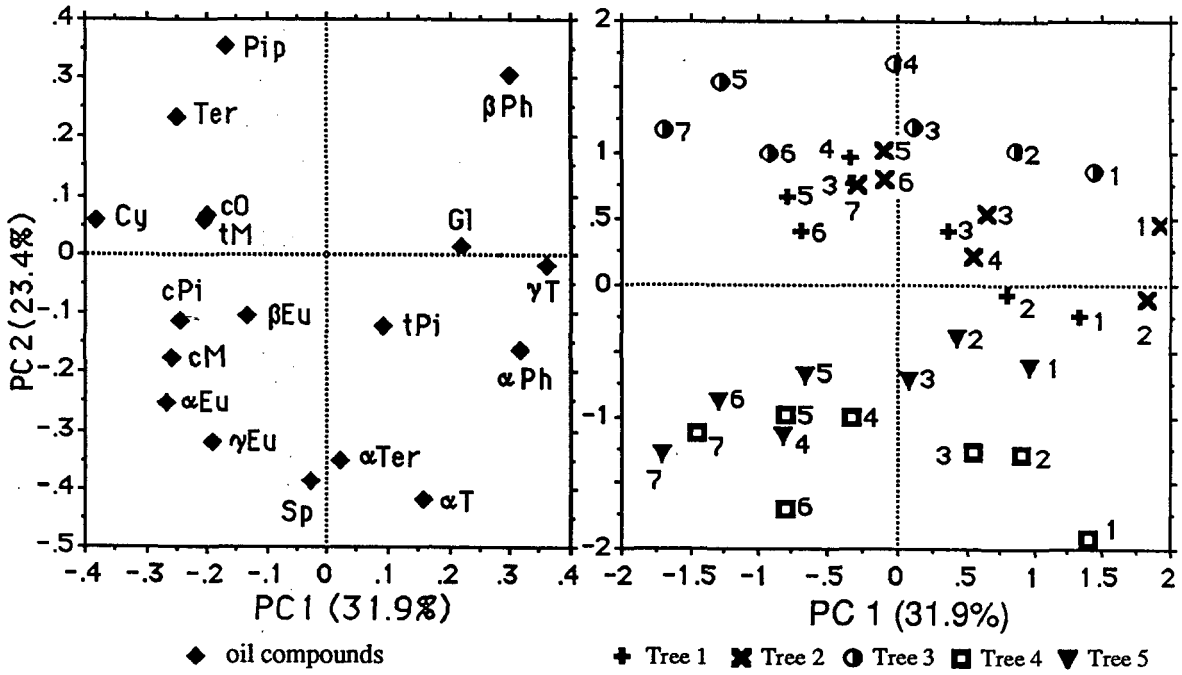
Fig. 3.10. Variation in mean percentage content of major monoterpenoids of *E. nitens* (D2) leaf oil at different sampling times over a year. Error bar indicate S.D.

(2) Oil composition of *E. delegatensis* (C2; Maydena)

In the principal component analysis (PCA) for variation in oil components of *E. delegatensis* (D2), the first two PCs accounted for 31.9% and 23.4% respectively of the total variance of the components among trees (Fig. 3.11). The PC1 mainly described variation between samples of different seasons for individual trees. In a negative direction along PC1 from early to later sample times, there was continuous variation with increased levels of *p*-cymene, piperitone, monoterpene alcohols (*cis*- and *trans*-menth-2-en-1-ol, *cis*-piperitol, terpinen-4-ol) and sesquiterpenoids (α–, β– and γ–eudesmol) and decrease levels of a number of monoterpene hydrocarbons, α– and β–phellandrene, α– and γ–terpinene, and the sesquiterpenoid, globulol.

PC2 differentiated samples of trees 4 and 5 from other tree samples due to higher levels of spathulenol, α–terpineol and γ–terpinene.

The PCA thus indicated that variation between trees is of similar magnitude to the variation between sample times. The differences between samples of individual trees sampled in different seasons had similar variation pattern, although the difference between the trees 4 and 5 and the other trees was maintained throughout the season.



(a) Plots of component scores (b) Plots of sample scores

Fig. 3.11. Principal component analysis (PCA) for variation in oil components of all samples for individual trees of *E. delegatensis* (C2) sampled at different seasons over a year period. Code of Sampling time: Nov./87=1, Jan./88=2, Mar./88=3, May./88=4, Jul./88=5, Sep./88=6, Nov./88=7.

The Kruskal-Wallis H test (Appendix 3.2B) indicated that there were significant variation ($p<0.05$, $p<0.01$ or $p<0.001$) in the monoterpenoids α–phellandrene, γ–terpinene, *p*-cymene, terpinolene, and terpinen-4-ol and the sesquiterpenoid, spathulenol, between samples over a one year period.

Fig. 3.12 shows that the percentage content of α–phellandrene decreased with sampling time. In contrast, the position of *p*-cymene and terpinen-4-ol were the reverse. The above monoterpenoids changed markedly between November and either May or Jul but changed only slightly from March or May to the next November. This indicated that variation in oil components of *E. delegatensis* (C2) also mainly occurred during the active growth period during summer.

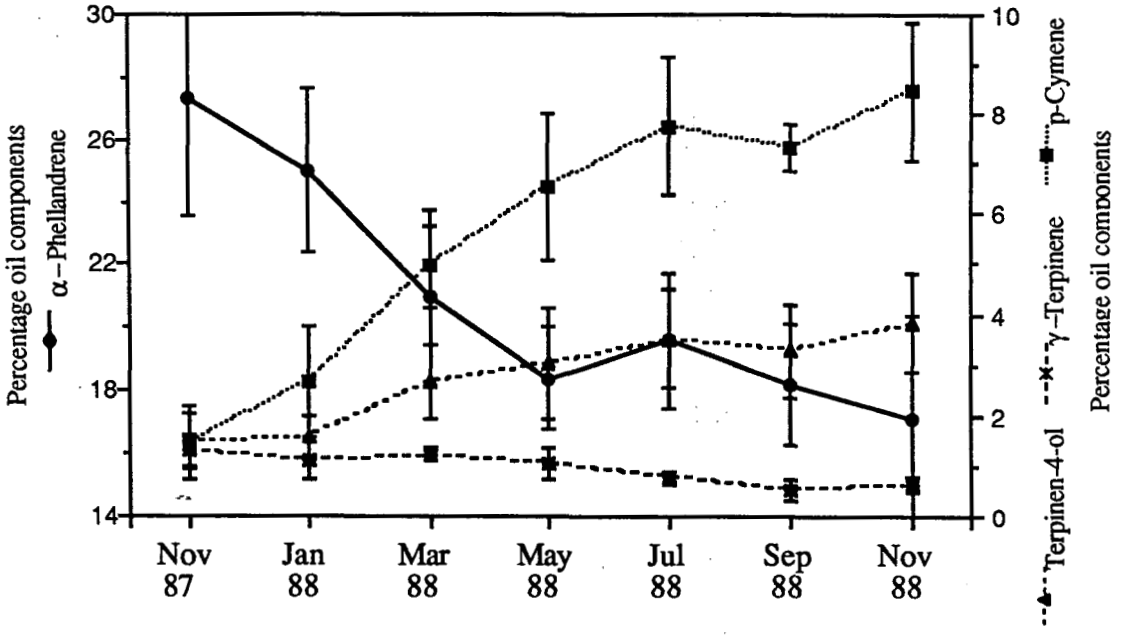
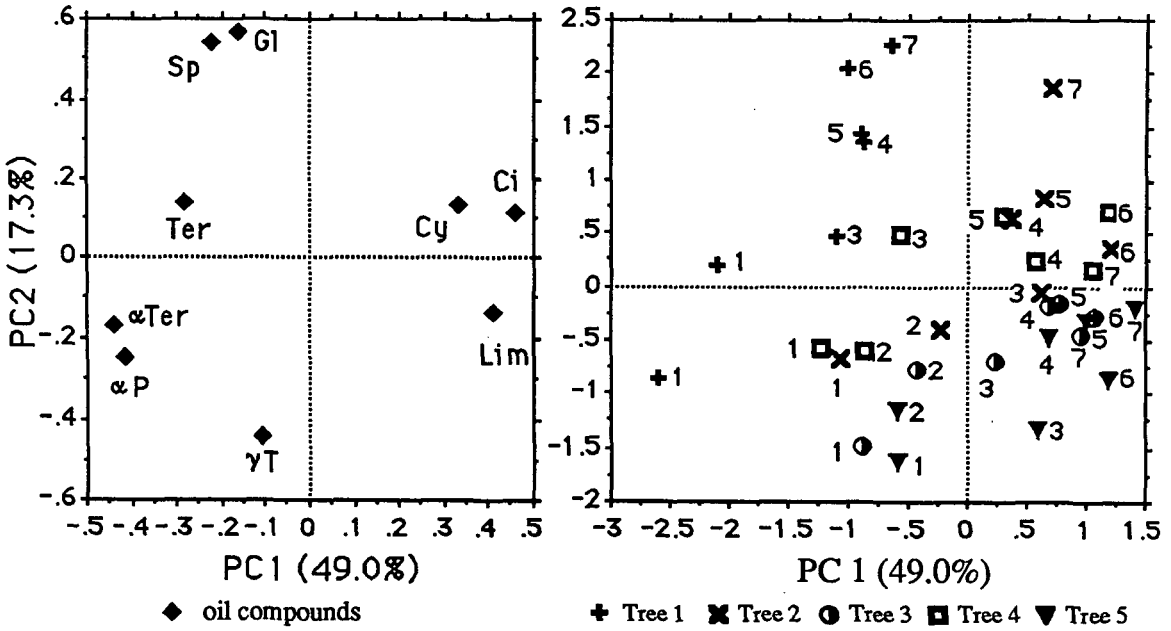


Fig. 3.12. Variation in mean percentage content of major monoterpenoids of *E. delegatensis* (C2) leaf oil at different sampling times over a year period (Error bar indicated S.D.).

(3) Oil composition of *E. globulus* (B2; Otways)

Variation in oil components for leaf samples in different seasons among *E. globulus* (B2) trees on the first two principal components of PCA accounted for 49.0% and 17.3% of the total variance (Fig. 3.13). The positive variation along PC1 was mainly due to high levels of 1,8-cineole, limonene and *p*-cymene and low levels of α–pinene, α–terpineol and terpinen-4-ol and the positive variation along PC2 was mainly due to high levels of the sesquiterpenoids, globulol and spathulenol, and low levels of γ–terpinene, α–pinene and α–terpineol.

Plots of sample scores on these two principal components indicated that both between and within tree variations were slightly oblique to PC1 and PC2. There was continuous variation between samples from early sample times to later sample times with increased levels of 1,8-cineole, limonene and *p*-cymene and decrease levels of α–pinene and α–terpineol in a positive direction along PC1 which was slightly oblique to PC2. However, there were differences between tree 1 and other trees in which samples of tree 1 had higher levels of the sesquiterpenoids, spathulenol and globulol.



(a) Plots of component scores (b) Plots of sample scores

Fig. 3.13. Principal component analysis (PCA) for variation in oil components of all samples for individual trees of *E. globulus* (B2) sampled at different seasons over a year period. Code of Sampling time: Nov./87=1, Jan./88=2, Mar./88=3, May./88=4, Jul./88=5, Sep./88=6, Nov./88=7.

Further analysis of variance of individual oil components (shown in Appendix 3.2C) indicates that α–pinene ($p<0.01$), 1,8-cineole ($p<0.01$), α–terpineol ($p<0.01$) and *p*-cymene ($p<0.05$) varied significantly ($p<0.01$) between seasons over the sample period.

Changes in mean percentage contents of the three major components, 1,8-cineole, α–pinene and α–terpineol, were significantly different between November and May but not between May and the following November (Fig. 3.14). Thus, variation in percentage content of oil components of *E. globulus* was also mainly due to changes in the summer season when leaves were active expanding.

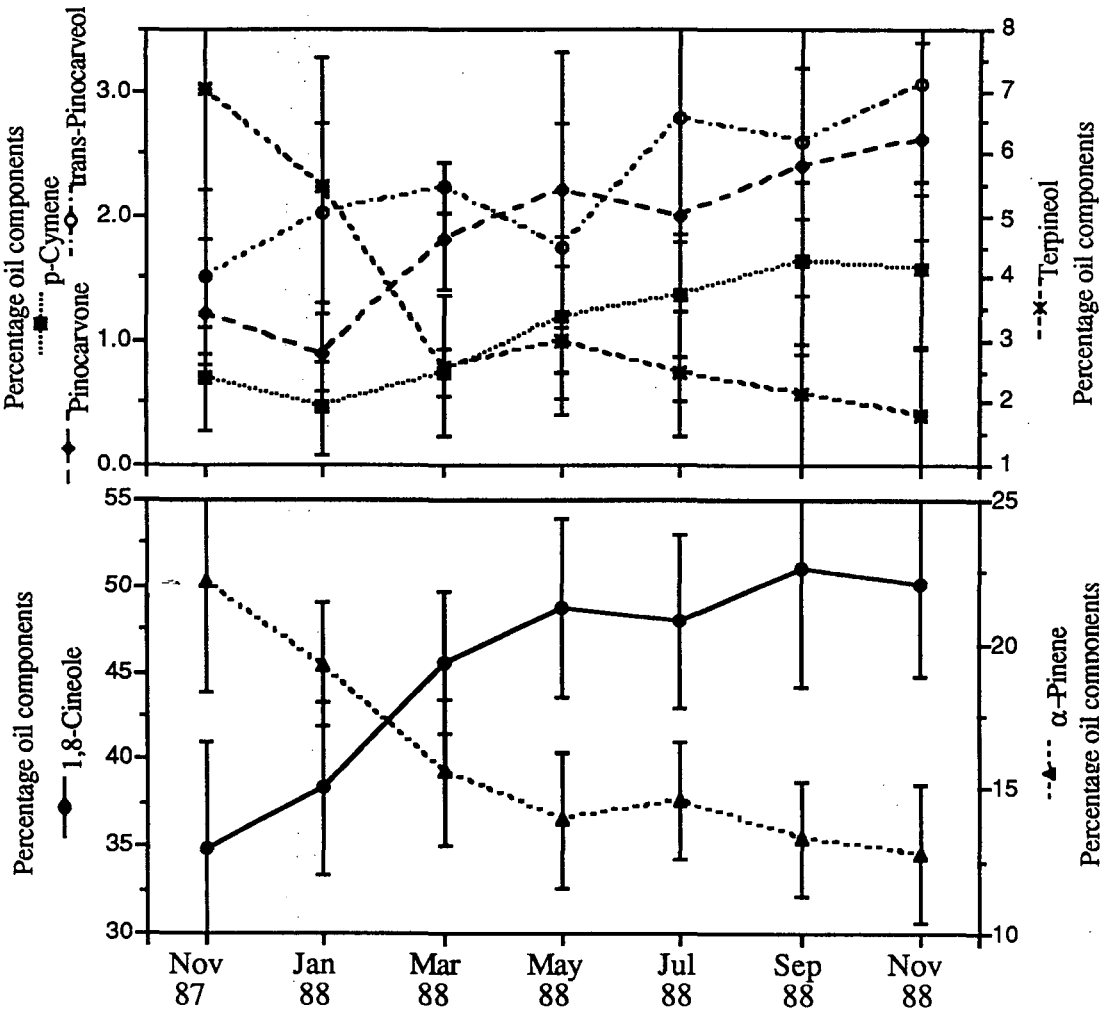


Fig. 3.14. Variation in mean percentage content of major monoterpenoids of *E. globulol* (B2) leaf oil at different sampling times over a year period (Error bar indicate S.D.).

Summary of variation in oil components among trees and species over a year

Overall variation in oil components of trees and species over different seasons of a year were summarised by the first two principal components of PCA as shown in Fig. 3.15. For each species, sample scores of all five trees over the study period formed a close group readily distinguished from groups of other species in spite of considerable seasonal variation observed over the sampling period. Thus, although the major compounds of individual species may vary with season, this seasonal variation is small when compared to the differences between species. It is considered that variation in oil components between samples of *E. globulus* (B2) trees was comparatively less than that for *E.*

delegatensis (C2) and *E. nitens* (D2), since samples of *E. globulus* (B2) trees formed a tighter group.

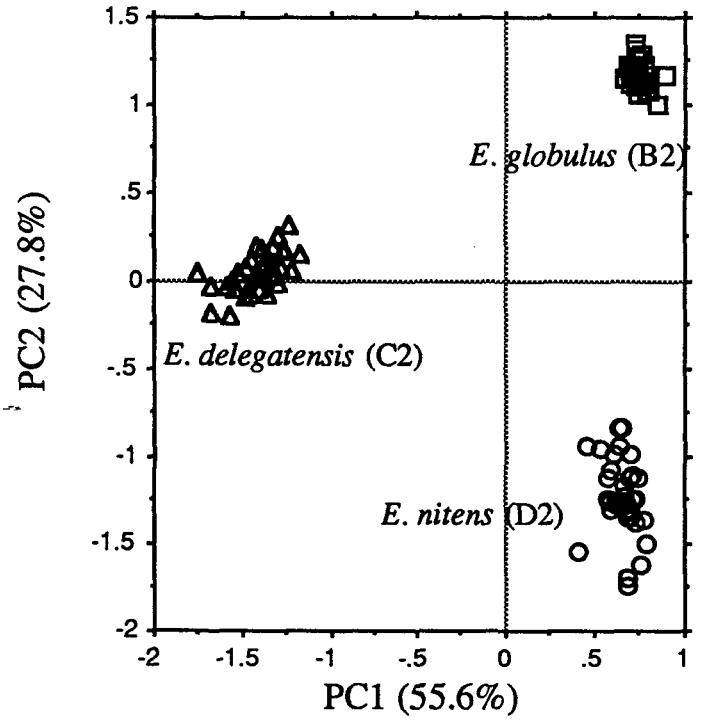


Fig. 3.15. Plots of samples of all trees of *E. nitens, E. delegatensis* and *E. globulus* over different seasons of a year on the first two principal components of PCA.

Variation in leaf oils with time and leaf age during the active growing season

The variation in the oil yields and major chemical compositions over time and leaf age for leaves of *E. nitens* (D2), *E. delegatensis* (C2) and *E. globulus* (B2) are shown in Appendixes 3.3A, B and C. Due to reasons of tree growth and insect damage, one *E. delegatensis* (C2) tree failed to provide enough leaf samples for inclusion in the analysis. Only the data of the remaining four trees were used for analysis and discussion.

Variation in leaf oil of *E. nitens*

The mean values of oil yields and percentage composition of individual oil components of leaf samples from different leaf age codes collected at different sample times are shown in Appendix 3.3A.

Oil yield

The Kruskal-Wallis *H*-test indicated that there was no significant variation in oil yield of *E. nitens* (D2) for all three sources of variation (Fig. 3.16). The mean percentage oil yield (0.46 to 0.70% leaf dry weight) of the five trees remained relatively constant throughout the study period although a slight increase was found with leaf ageing (Appendix 3.3A).

Variation in oil components

(1) Variation with time in leaves of the same age

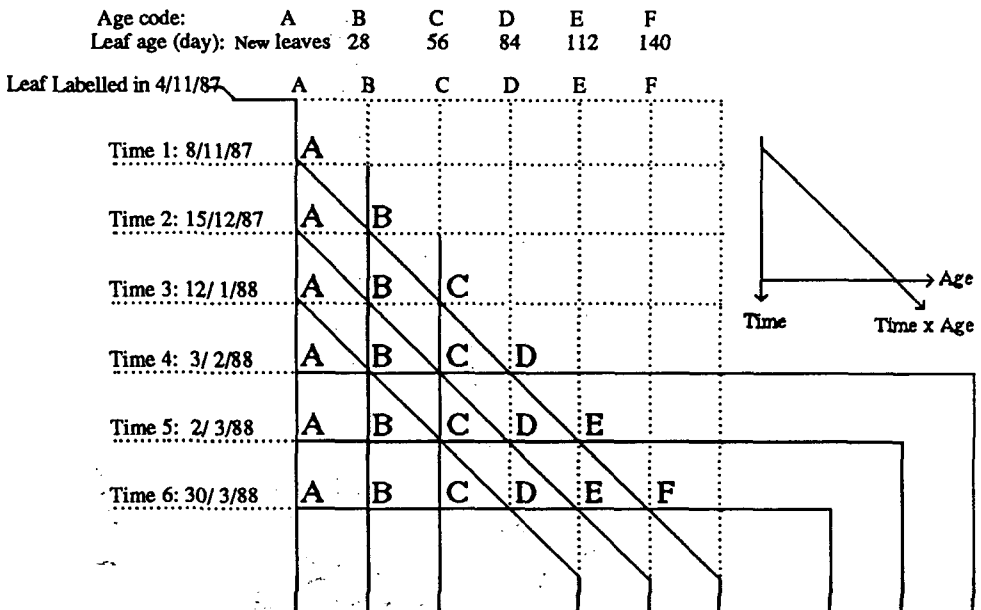
The Kruskal-Wallis H-test (Fig. 3.16-1) for individual leaf age classes indicated that the percentage contents of α–phellandrene ($p<0.05$), *cis*–β–ocimene($p<0.01$ or $p<0.05$), α–terpineol ($p<0.01$ or $p<0.05$) and spathulenol ($p<0.01$ or $p<0.05$), varied significantly with time in leaves of the same age (time series) for new leaves (leaf age code A), leaves of age 28 days (leaf age code B) and 56 days (leaf age code C). *p*-cymene varied significantly for leaf ages A ($p<0.05$) and B ($p<0.01$).

(2) Variation between leaf ages at the same sampling time

Only one oil component, the ester compound, isobutyl isopentyl, was found to vary significantly ($p<0.01$) between leaf ages at each of the three sample times (Time 4, Time 5 and Time 6) while the two oil components, *cis*–β–ocimene ($p<0.01$) and *p*-cymene ($p<0.05$), varied significantly at each of the two sample times, Time 5 and Time 6 (Fig. 3.16-2). In addition, α–phellandrene varied significantly ($p<0.05$) between leaf ages at the last sample time (Time 6). However, the low *H*-value (1.5 and 1.8 respectively) for variation in α–phellandrene between leaf ages at sample times 4 and 5 did not support a true time/age effect on this compound.

(3) Variation with leaf aging (physiological age effect)

Four oil components, α–phellandrene ($p<0.01$ or $p<0.001$), isobutyl isopentyl ($p<0.05$, $p<0.01$ or $p<0.001$), *cis*–β–ocimene ($p<0.001$ or $p<0.01$) and *p*-cymene ($p<0.01$ or $p<0.001$) varied significantly ($p<0.05$, $p<0.01$ and $p<0.001$ respectively) with leaf aged for three physiological age series (series 1, 2 and 3) and spathulenol varied significantly ($p<0.01$) for series 1 and 2 while α–terpineol varied significantly ($p<0.05$) for series 1.



	H-value	*H*-value	*H*-value#	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value
Oil Yield	3.4ns	0.8ns	7.0ns	2.3ns	1.5ns	5.3ns	2.5ns	1.4ns	1.5ns
α–Pinene	6.5 ns	0.4 ns	3.4 ns	1.7 ns	4.2 ns	5.3 ns	3.0 ns	1.1 ns	3.0 ns
Isobutyl isobutanoate	4.4 ns	4.8 ns	1.6 ns	3.3 ns	3.5 ns	9.2 ns	0.9 ns	5.5 ns	1.5 ns
α–Phellandrene	12.2 *	13.2 *	9.5 *	11.7 **	14.6**	21.8***	11.9 *	1.5 ns	1.8 ns
Limonene	3.1 ns	5.2 ns	2.6 ns	3.3 ns	2.2 ns	5.0 ns	4.2 ns	3.3 ns	1.6 ns
Isobutyl isopentanoate	4.0 ns	4.3 ns	7.6 ns	10.5 *	16.9***	20.3**	15.5**	11.7**	9.5**
1,8-Cineole	4.2	3.9 ns	2.4 ns	2.3 ns	4.1 ns	5.0 ns	0.3 ns	0.6 ns	0.5 ns
cis–β–Ocimene	16.9**	16.7**	10.5 *	14.1**	18.9***	23.4***	16.8**	11.4**	3.4 ns
γ–Terpinene	5.9 ns	6.5 ns	3.8 ns	4.5 ns	1.8 ns	10.2 ns	2.4 ns	2.5 ns	0.4 ns
p-Cymene	11.6 *	16.0**	9.8 ns	14.0**	19.5***	22.8***	14.8 *	14.9**	6.4 ns
Terpinen-4-ol	5.4 ns	1.9 ns	1.5 ns	2.9 ns	2.3 ns	1.5 ns	4.3 ns	0.7 ns	1.7 ns
α–Terpineol	20.6**	12.3**	11.8 *	4.6 ns	5.3 ns	11.6 *	7.4 ns	3.8 ns	2.4 ns
Globulol	6.5 ns	3.3 ns	5.0 ns	0.7 ns	3.6 ns	5.6 ns	4.4 ns	2.5 ns	0.2 ns
Spathulenol	16.9**	15.6**	11.5 *	7.6 ns	17.5**	16.1**	8.3 ns	5.4 ns	0.4 ns
Degrees of freedom (d.f.):	5	4	3	3	4	5	5	4	3
Number of different groups:	6	5	4	4	5	6	6	5	4
Total number of samples:	30	25	20	20	25	30	30	25	20
†Categories of leaf samples:	A B C Leaf Age			3 2 1 Physiological age series			6 5 4 Sampling time		
	(1)			(3)			(2)		

Figure 3.16. The Kruskal-Wallis (*H*) test for variation in major chemical components of *E. nitens* oils with time and leaf age during growth season (Nov. 1987 to Mar. 1988).

†Categories of leaf samples:

(1) **Leaf age classes:** Variation between leaf samples within each age class is affected by sampling time factor and could compound environmental/seasonal effects with ontogenetic effects.

(2) **Times series:** Variation between leaf samples within each time series is affected by age difference and could compound seasonal/time effects on initiation of leaves, physiological aging and ontogenetic effects.

(3) **Physiological age series:** Variation between leaves within each series are mainly affected by physiological age differences, but could compound differences due to environment at time of sampling.

*Singificant levels of probability of *H*-value: *** $p<0.001$; ** $0.001<p<0.01$; * $0.01<p<0.05$; ns $p>0.05$.

H-values and probabilities listed in here are Kruskal-Wallis *H* statistic and probability. If there are tied groups the *H*-values listed here are Kruskal-Wallis *H* corrected for ties and probability.

Summary of observations

Analysis of the three sources of variation in oil components between samples indicated that significant change in five oil components occurred during the growing season and their changes were affected by different factors.

i) The percentages of the two oil components, *cis*–β–ocimene and *p*-cymene, were found to vary significantly between sampling times of leaves of the same age (series 1), between leaf ages sampled at the same time (series 2) and between physiological ages of leaves (series 3). Thus, variation in these two compounds during the growth season was affected by all three sources of variation.

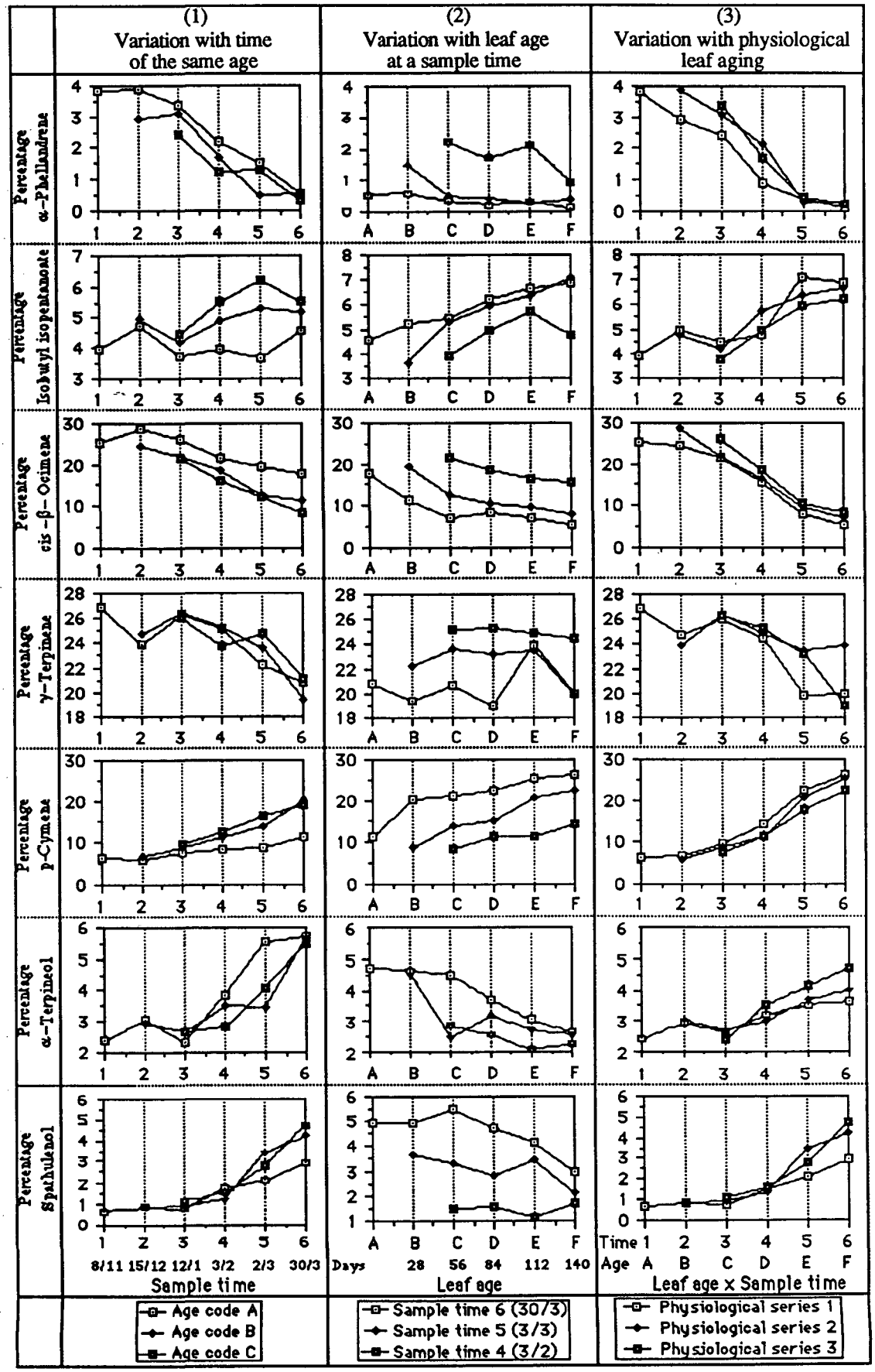
ii) Variation in percentages of the three oil components, α–phellandrene, α–terpineol and spathulenol, were significantly affected by time (series 1) and physiological age (series 3) factors but not age differences at each sampling time (series 2). This indicates that it is most likely a sampling time effect rather than a true maturation effect on these two compounds.

iii) The ester compound isobutyl isopentyl was significantly affected by physiological (series 3) aging through the study period and age difference at individual sampling times but not affected by sampling time difference with leaves of the same age (series 1). Therefore, variation in this compound was probably due to physiological effect.

The percentage contents of the two components, α–phellandrene and *cis*–β–ocimene significantly decreased with time of the same leaf age (Fig. 3.17.1) and leaf age through sampling times (Fig. 3.17.2) and between leaf ages at individual sampling times (Fig.3.17.3) while *p*-cymene increased with time, leaf age and between leaf ages. *cis*–β–Ocimene was the major component in new and young leaf oil in the early sample times and became a minor component in leaf oil of older leaves of later sample time (Appendix 3.3A). In contrast, the position of *p*-cymene was the reverse. Thus, the decrease of *cis*–β–ocimene was correlated with the increase of *p*-cymene and variation in these two oil components was the major variation in the percentage composition of *E. nitens* (D2) leaf oil during the major growth season.

Although, another major compound, γ–terpinene, did not change significantly, it also decreased with time and leaf age through sample times (Fig.3.17.1 and 2). The other two compounds, α–terpineol and spathulenol, significantly increased with sample time and leaf age through sampling times.

Fig. 3.17. Variation in mean percentage content of some important components in leaf oils of *E. nitens* with time and leaf age.



Variation in leaf oil of *E. delegatensis*

The mean values of oil yields and percentage composition of individual oil components of each leaf sample from different leaf age codes at different sample times are given in Appendix 3.3B. The results of Kruskal-Wallis H-test are shown in Fig. 3.18.

Oil yield

The oil yields only varied significantly ($p<0.05$) between sample times for leaves of leaf ages B (28 days old) (Fig. 3.18). There was no significant variation in oil yield as leaves aged over the study period and between leaf ages at sample times 4, 5 and 6.

Variation in oil components

(1) Variation with time of leaves of the same age

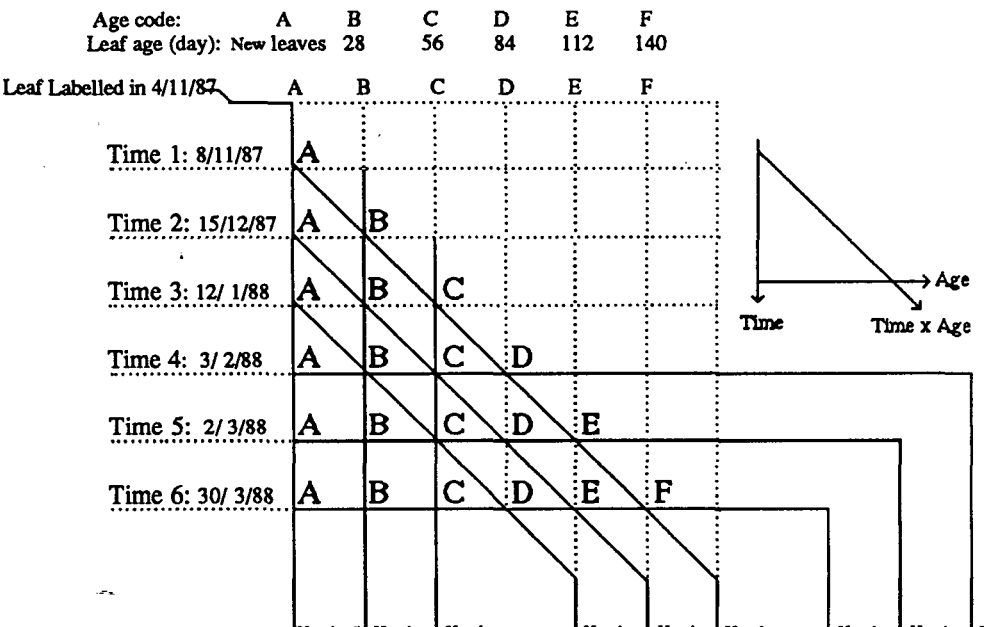
Only one oil component, α–phellandrene, varied significantly ($p<0.05$ and $p<0.01$) with time for all three age series (Ages A, B and C) and that α–terpinene and *p*-cymene changed significantly ($p<0.05$) for age B (the *H*-values for age A were also relatively greater, although not significant) (Fig.3.18). Other monoterpenoids showed no significant variation between sampling times for all leaf age classes. In addition, the sesquiterpenoids, α–, β– and γ–eudesmol, varied significantly ($p<0.05$ and $p<0.01$ respectively) for ages B and C.

(2) Variation between leaf ages at the same sampling time

Only percentages of the two monoterpenoids, α–phellandrene and *p*-cymene, were found to vary significantly ($p<0.05$ or $p<0.01$) between leaf ages for all three sample times tested (Times 4, 5 and 6). In addition, terpinen-4-ol varied significantly ($p<0.05$) between leaf ages at sample time 4 and γ–eudesmol ($p<0.05$) at sample time 5. There was no significant variation between leaf ages for any other monoterpenoids and sesquiterpenoids at the three sample times (Times 4, 5 and 6).

(3) Variation with leaf age through sample times (physiological age effect)

The monoterpenoids α–phellandrene and *p*-cymene varied significantly ($p<0.05$ or $p<0.01$) with leaf age through the sample times for physiological age series 1, 2 and 3. Three monoterpenoids, α– and γ–terpinene and *cis*-*p*-menth-2-en-1-ol and two sesquiterpenoids, α– and γ–eudesmol, varied significantly ($p<0.05$ or $p<0.01$) for series 1 and 2 and terpinolene and *cis*-piperitol significantly for series 1 ($p<0.05$ and $p<0.01$ respectively). In addition, the sesquiterpenoid, spathulenol, varied significantly ($p<0.05$) for series 2 and 3.



	H-value#	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value
Oil Yield	10.9 ns	11.4 *	4.9 ns	5.4 ns	6.9 ns	5.4 ns	4.8 ns	3.6 ns	2.1
α–Phellandrene	14.9 *	14.8 **	9.1 *	11.8 **	15.3 **	18.7 **	12.2 *	12.5 *	10.9 *
α–Terpinene	9.2 ns	10.6 *	5.4 ns	4.1	9.9 *	17.5 **	4.4 ns	8.4 ns	2.4 ns
β–Phellanene	7.1 ns	7.4 ns	3.0 ns	3.0 ns	5.5 ns	16.6 **	3.0 ns	5.5 ns	2.8 ns
γ–Terpinene	9.9 ns	6.0 ns	0.3 ns	1.7 ns	9.5 *	16.6 **	6.9 ns	2.7 ns	1.0 ns
p-Cymene	10.3 ns	12.5 *	7.1 ns	13.5 **	15.8 **	20.1 **	12.2 *	14.6 **	11.9 **
Terpinolene	7.2 ns	7.2 ns	2.0 ns	3.8 ns	6.7 ns	12.8 *	6.2 ns	4.5 ns	3.9 ns
trans-*p*-Menth-2-en-1-ol	3.2 ns	3.0 ns	6.0 ns	0.7 ns	5.2 ns	6.9 ns	6.7 ns	6.8 ns	6.0 ns
Terpinen-4-ol	2.3 ns	1.0 ns	1.1 ns	2.9 ns	4.8 ns	9.0 ns	13.3 *	3.9 ns	0.9 ns
cis-*p*-Menth-2-en-1-ol	0.8 ns	2.6 ns	6.4 ns	6.2 ns	11.2 *	15.1 *	8.7 ns	8.3 ns	7.7 ns
cis-Piperitol	5.2 ns	3.5 ns	1.1 ns	5.5 ns	7.3 ns	17.0 **	10.1 ns	5.1 ns	2.2 ns
Piperitone	3.6 ns	3.0 ns	1.7 ns	0.1 ns	2.3 ns	3.0 ns	5.7 ns	2.9 ns	0.3 ns
trans-Piperitol	3.4 ns	1.6 ns	0.7 ns	0.6 ns	2.4 ns	3.9 ns	1.7 ns	4.4 ns	0.7 ns
Methyl cinnmate	4.6 ns	6.9 ns	0.5 ns	1.0 ns	1.8 ns	7.2 ns	1.7 ns	0.7 ns	0.3 ns
Spathulenol	8.4 ns	1.1 ns	4.8 ns	9.3 *	11.5 *	6.7 ns	4.4 ns	3.4 ns	1.8 ns
α–Eudesmol	8.1 ns	10.2 *	8.7 *	0.5 ns	10.4 *	12.2 *	6.9 ns	1.6 ns	2.8 ns
γ–Eudesmol	8.9 ns	14.1 **	9.6 *	3.9 ns	12.3 *	19.0 **	7.7 ns	9.7 *	6.5 ns
β–Eudesmol	7.2 ns	10.1 *	7.9 *	2.9 ns	5.8 ns	14.7 *	5.9 ns	2.1 ns	4.3 ns
Degrees of freedom (d.f.):	5	4	3	3	4	5	5	4	3
Number of different groups:	6	5	4	4	5	6	6	5	4
Total number of samples:	24	20	16	24	20	16	24	20	16
†Categories of leaf samples:	A B C Leaf Age			3 2 1 Physiological age series			6 5 4 Sampling time		
	(1)			(3)			(2)		

Figure 3.18. The Kruskal-Wallis (*H*) test for variation in major chemical components of *E. delegatensis* oils with time and leaf age during growth season (Nov 1987 to Mar 1988).

† Categories of leaf samples:

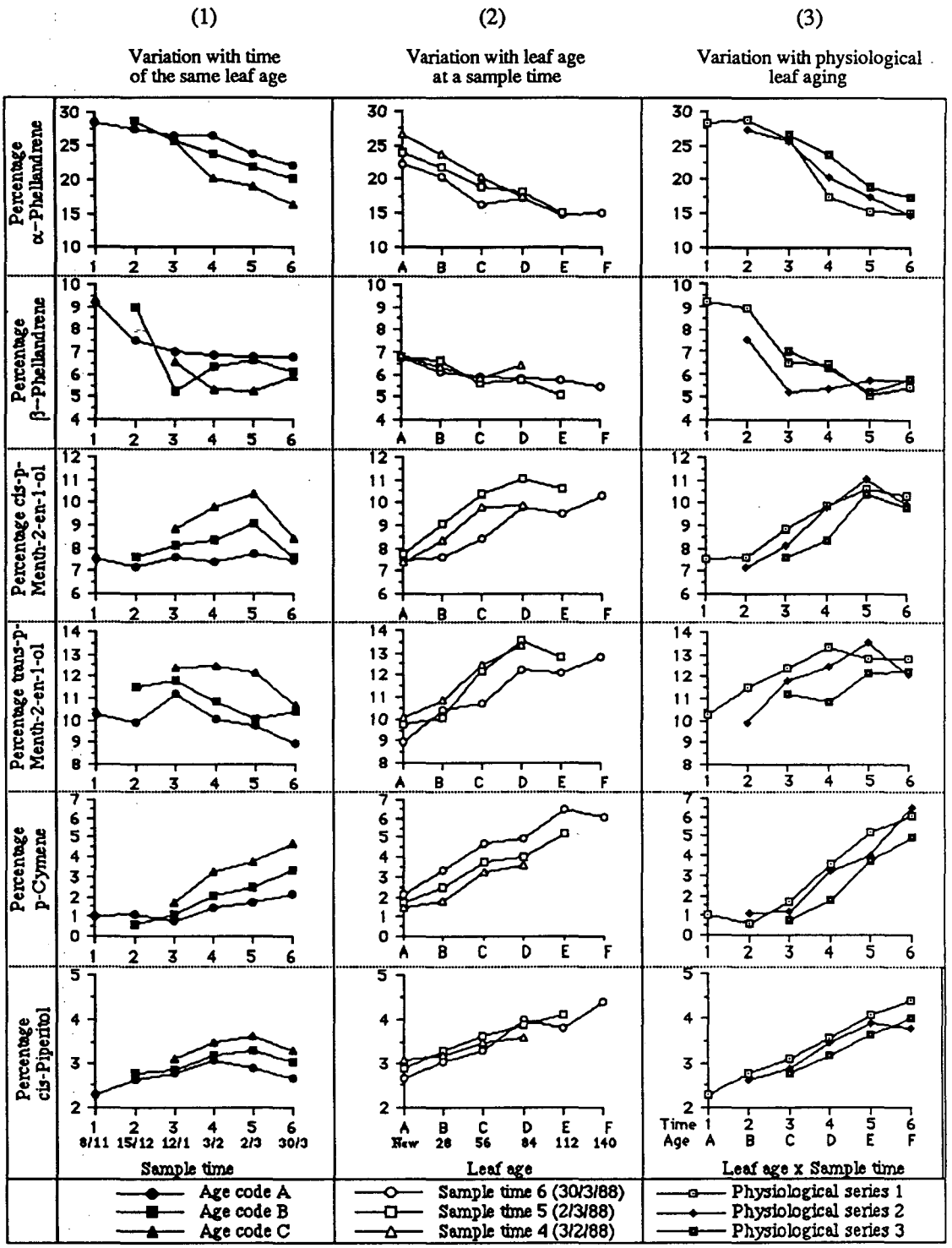
(1) **Leaf age classes:** Variation between leaf samples within each age class is affected by sampling time factor and could compound environmental/seasonal effects with ontogenetic effects.

(2) **Times series:** Variation between leaf samples within each time series is affected by age difference and could compound seasonal/time effects on initiation of leaves, physiological aging and ontogenetic effects.

(3) **Physiological age series:** Variation between leaves within each series are mainly affected by physiological age differences, but could compound differences due to environment at time of sampling.

*Singificant levels of probability of H-value: *** $p<0.001$; ** $0.001<p<0.01$; * $0.01<p<0.05$; ns $p>0.05$.
 # H-values and probabilities listed in here are Kruskal-Wallis H statistic and probability. If there are tied groups the H-values listed here are Kruskal-Wallis H corrected for ties and probability.

Fig. 3.19. Variation in mean percentage content of some important components in leaf oils of *E. delegatensis* (C1)with time and leaf age.



Summary of observations

The oil yield of *E. delegatensis* leaves was significantly affected by sampling time. Thus, oils of young leaves (Age B) were found to increase significantly from early to late sample times (Appendix 3.3B).

Results indicate that only the percentage contents of the two monoterpenoids, α–phellandrene and *p*-cymene, in *E. delegatensis* (C2) leaf oil were significantly affected by all three factors, (i) between times for leaves of the same age, (ii) between leaf ages at individual sample times and (iii) leaf age through sample times. A number of other monoterpenoids were significantly affected by the physiological age factor, they were: α– and γ–terpinene, β–phellandrene, terpinolene, *cis*-*p*-menth-2-en-1-ol and *cis*-piperitol. Variations in sesquiterpenoids, such as α–, β– and γ–eudesmol, were mainly due to the effect of sample times. It is considered that variation in monoterpenoids of *E. delegatensis* is mainly effected by physiological and ontogenetic leaf age factors and sesquiterpenoids by sampling times.

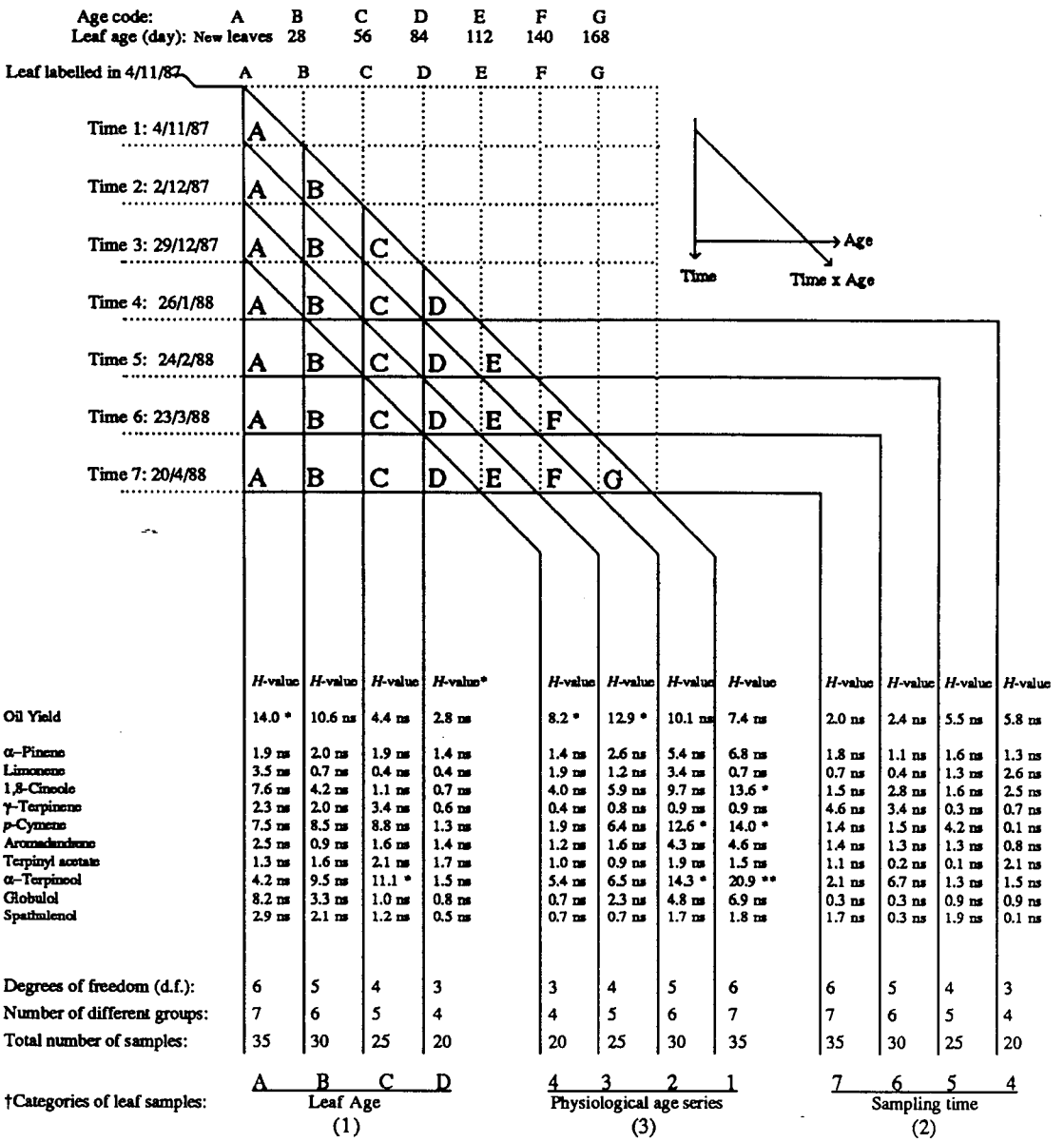
Fig. 3.19 indicates that the decrease in the monoterpene hydrocarbons α– and β–phellandrene corresponded to increases in the monoterpene hydrocarbon, *p*-cymene, and several monoterpene alcohols, such as *cis*- and *trans*-*p*-menth-2-en-1-ol and *cis*-piperitol, with increasing leaf age through sampling times and between leaf ages at individual sampling times.

Variation in leaf oil of *E. globulus* (B2)

The mean values of oil yields and percentage composition of individual oil components for leaf samples from different leaf ages at different sample times are given in Appendix 3.3C. Results of data analysis are shown in Fig. 3.20.

Oil yield

There was significant ($p<0.05$) variation in oil yield between sample times for new *E. globulus* leaves (Age A) and with leaf age through sample times for the physiological age series 3 and 4 (Fig. 3.20). However, there was no significant variation in oil yield between leaf ages at individual sample times.



	H-value	*H*-value	*H*-value	*H*-value*	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value	*H*-value
Oil Yield	14.0 *	10.6 ns	4.4 ns	2.8 ns	8.2 *	12.9 *	10.1 ns	7.4 ns	2.0 ns	2.4 ns	5.5 ns	5.8 ns
α–Pinene	1.9 ns	2.0 ns	1.9 ns	1.4 ns	1.4 ns	2.6 ns	5.4 ns	6.8 ns	1.8 ns	1.1 ns	1.6 ns	1.3 ns
Limonene	3.5 ns	0.7 ns	0.4 ns	0.4 ns	1.9 ns	1.2 ns	3.4 ns	0.7 ns	0.7 ns	0.4 ns	1.3 ns	2.6 ns
1,8-Cineole	7.6 ns	4.2 ns	1.1 ns	0.7 ns	4.0 ns	5.9 ns	9.7 ns	13.6 *	1.5 ns	2.8 ns	1.6 ns	2.5 ns
γ–Terpinene	2.3 ns	2.0 ns	3.4 ns	0.6 ns	0.4 ns	0.8 ns	0.9 ns	0.9 ns	4.6 ns	3.4 ns	0.3 ns	0.7 ns
p-Cymene	7.5 ns	8.5 ns	8.8 ns	1.3 ns	1.9 ns	6.4 ns	12.6 *	14.0 *	1.4 ns	1.5 ns	4.2 ns	0.1 ns
Aromadendrene	2.5 ns	0.9 ns	1.6 ns	1.4 ns	1.2 ns	1.6 ns	4.3 ns	4.6 ns	1.4 ns	1.3 ns	1.3 ns	0.8 ns
Terpinyl acetate	1.3 ns	1.6 ns	2.1 ns	1.7 ns	1.0 ns	0.9 ns	1.9 ns	1.5 ns	1.1 ns	0.2 ns	0.1 ns	2.1 ns
α–Terpineol	4.2 ns	9.5 ns	11.1 *	1.5 ns	5.4 ns	6.5 ns	14.3 *	20.9 **	2.1 ns	6.7 ns	1.3 ns	1.5 ns
Globulol	8.2 ns	3.3 ns	1.0 ns	0.8 ns	0.7 ns	2.3 ns	4.8 ns	6.9 ns	0.3 ns	0.3 ns	0.9 ns	0.9 ns
Spathulenol	2.9 ns	2.1 ns	1.2 ns	0.5 ns	0.7 ns	0.7 ns	1.7 ns	1.8 ns	1.7 ns	0.3 ns	1.9 ns	0.1 ns
Degrees of freedom (d.f.):	6	5	4	3	3	4	5	6	6	5	4	3
Number of different groups:	7	6	5	4	4	5	6	7	7	6	5	4
Total number of samples:	35	30	25	20	20	25	30	35	35	30	25	20
†Categories of leaf samples:	A B C D Leaf Age (1)				4 3 2 1 Physiological age series (3)				7 6 5 4 Sampling time (2)			

Figure 3.20. The Kruskal-Wallis (*H*) test for variation in major chemical components of *E. globulus* oils with time and leaf age during growth season (Nov. 1987 to April 1988).

†Categories of leaf samples:

(1) **Leaf age classes:** Variation between leaf samples within each age class is affected by sampling time factor and could compound environmental/seasonal effects with ontogenetic effects.

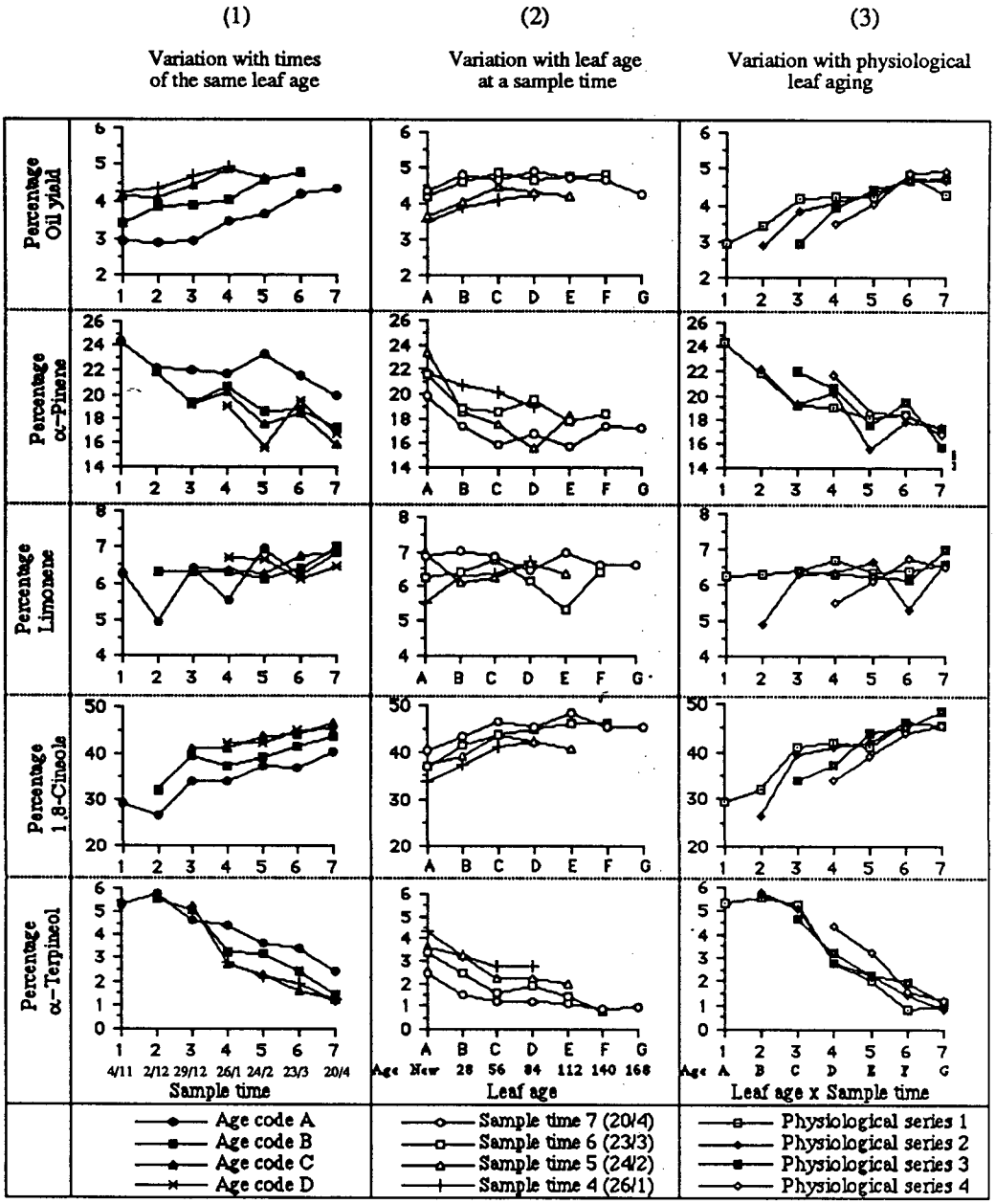
(2) **Times series:** Variation between leaf samples within each time series is affected by age difference and could compound seasonal/time effects on initiation of leaves, physiological aging and ontogenetic effects.

(3) **Physiological age series:** Variation between leaves within each series are mainly affected by physiological age differences, but could compound differences due to environment at time of sampling.

*Singificant levels of probability of H-value: *** $p<0.001$; ** $0.001<p<0.01$; * $0.01<p<0.05$; ns $p>0.05$.

H-values and probabilities listed in here are Kruskal-Wallis *H* statistic and probability. If there are tied groups the *H*-values listed here are Kruskal-Wallis *H* corrected for ties and probability.

Fig. 3.21. Variation in mean percentage content of some important components in leaf oils of *E. globulus* (B1)with time and leaf aging.



Variation in oil components

(1) Variation with time of leaves of the same age (time effect)

With the exception of α–terpineol, which varied significantly ($p<0.05$) between sample times for age C, no components varied significantly between sample times with leaves of the same age for all age series (Fig. 3.20).

(2) Variation between leaf ages within individual sample times

There was no significant variation detected between leaf ages for each sample time.

(3) Variation with leaf age through sample times (physiological age effect)

Only three oil components, 1,8-cineole, *p*-cymene and α–terpineol, varied significantly ($p<0.05$) with leaf age through sampling times for physiological age series 1 and 2.

Summary of observations

The chemical composition of *E. globulus* (B2) leaf oil remained relatively stable over different leaf ages and sample times. Only physiological age significantly affected the percentage contents of 1,8-cineole, *p*-cymene and α–terpineol. As indicated in Fig. 3.21 the percentage contents of 1,8-cineole significantly increased and α–terpineol significantly decreased with leaf age through sample times. Although there was no significant variation in α–pinene observed, the percentage of α–pinene tended to decrease with leaf age through sample times.

The oil yield of *E. globulus* (B2) increased significantly with leaf age from early to late sample times.

3.5.3. Discussion

Leaf oil composition is known to vary during the ontogenetic and physiological development of plant organs (Adams 1970a and von Rudloff 1972a). Results from studies on seasonal variation are contradictory. While some studies reported no significant seasonal variation in leaf oils (e.g. von Rudloff and Hefendehl 1966; von Rudloff 1972a), other workers found that seasonal variation in leaf oil composition did occur (Adams 1970a). Moreover, some studies suggested that seasonal variation in leaf oil was due to the effects of leaf maturation but not seasonal influences (von Rudloff 1967a,b,; Simmons and Parsons 1987). In contrast, Leach and Whiffin (1989) reported that there was no variation in leaf oil with season that was attributable to leaf maturation. In addition, studies have demonstrated that seasonal variation in leaf oils was due mainly

to changes occurring in young leaves during the major growth 'flush' period, e.g. Adams (1970a), von Rudloff (1972a), Simmons and Parsons (1987). However, the actual relevance of variation in leaf oils with time and physiological age remained unclear for studies did not include a detailed strategy for the sampling of leaf age. These studies only separated new leaves from mature leaves (Simmons and Parsons 1987) or made gross comparisons between immature and mature leaves (Leach and Whiffin 1989).

In agreement with von Rudloff (1972a) and Simmons and Parsons (1987), results of this study indicated that the variable stage in the leaf oils of these eucalypts was associated with the active growth of trees and the winter period can be regarded as a period of physiological and chemical stability. In Tasmania, eucalypts grow actively from late October to April while the period from April to September is regarded as a dormant stage (Turnbull *et. al.* 1988). The significant changes of oil components in leaves of all three species occurred mainly from November to March-May but were minor from March-May to the following November.

Although the variations in oil yields between seasons (see Appendix 3.2A, B and C), for individual provenances were not significant for each species, a similar pattern of variation could be seen. In all three species (*E. nitens* - D2, *E. delegatensis* - C2 and *E. globulus* - B2), the oil yields increased throughout the growing season and were highest during March-May (autumn). These results differ to reports for *E. citriodora* Hook (Kapur *et al.* 1982) and *E. camphora* (Simmons and Parsons 1987) where the highest yields were recorded in the winter months. It is generally considered that yields from young leaves are generally higher than from old leaves (Berry 1947; McKern *et al.* 1954; Kapur *et al.* 1982) and this may be simply due to differences in the water content of young and old leaves (Simmons and Parsons 1987). However, this study has shown that oil yield is higher in autumn when a large number of newly mature leaves are present compared with young leaves or old leaves. Oil yields are maximum in newly mature leaves.

According to the experimental design, the old leaves which formed before the study period were excluded and only leaves formed during the study period were sampled. The new leaves of these eucalypts were formed by October-November. The leaf samples during the growth season can be regarded as an even-aged crop in which the proportion of mature leaves increased with time. For example, leaves sampled early in the growing season were new young leaves but the March-May sample consisted of leaves with a large proportion of immature and mature leaves relative to new young leaves. The leaves sampled in late winter could be regarded as an even-aged crop of mature leaves. Hence it is considered that differences in pooled leaf samples at different sampling times could reflect differences due to change in leaf age and time effects. Therefore, a detailed study for variation during the active growth season, could reveal the actual cause of seasonal

variation during a year, since seasonal variation over a year mainly occurred during the active growth season.

Results of this study indicated that variation in oil composition was attributable to changes with sampling time, ontogenetic (leaf initiated at different times while plant is still undergoing ontogenetic change) and physiological aging (leaf initiated at same time) over the active growth season. However, the observed variation in the individual components of individual oil could be related to one, two or all three of these variables. Provenances responded differently to these effects in terms of oil compositions. Thus, variations in leaf oils with season appear to be determined by individual species and there was no general conclusion for variation in chemical composition of eucalypt leaf oils could be made.

A point of controversy is the actual cause of seasonal variation in leaf oil components: whether it is genetic or environmental and whether losses and gains are due to catabolism, biosynthesis or inter conversions.

Simmons and Parsons (1987) concluded that variation in leaf oils of a particular tree with respect to age and seasonal effects was dependent on the genetic background of that tree. Results of this study indicated that the genetic make-up of the individual species and provenances determines chemical make up and the variation patterns of oil composition. Thus, the chemical composition of leaf oils of the three species, *E. nitens, E. globulus* and *E. delegatensis*, differed as did the pattern of variation with respect to seasonal, ontogenetic and physiological aging effects. There was no uniformity of response. Each species retained it's identity and was readily distinguished from others throughout the study period.

Differences in seasonal variation of oil components between trees within individual provenances were also detected in this study. However, differences between trees within provenances were insignificant in comparison to the overall differences between provenances. This finding contrasts with the observations of Simmons and Parsons (1987) who found that there were likely to be considerable differences in patterns of variation between trees within a species where the difference is due to high tree to tree variability. It is considered that each provenance of the species used in this study was grown under the same environmental conditions. Whereas, leaf samples from Simmons and Parsons's study (1987) were collected from natural trees growing in different localities with a high level of environmental variation compounded in the data.

The results indicate that the major monoterpenoids of each leaf oil were closely related to the biochemical relationships proposed for terpenoid biosynthesis (see chapter 4).

Moreover, variation in these major monoterpenoids may be due to either biosynthesis or inter conversions of monoterpenoids

For variation in leaf oils of *E. nitens* (D2) during the growing season, the percentage content of *cis*–β–ocimene decreased with time, leaf age and leaf development to reach a very low proportion in the mature leaves by the end of growing season, whereas *p*-cymene increased to a high proportion in the mature leaves by the end of growth season. The percentage of γ–terpinene and α–phellandrene also decreased in the same way. Hence, the proposed sequence: precursor > γ–terpinene > *p*-cymene (Poulose and Croteau 1978) and precursor > *cis*–β–ocimene > *p*-cymene or α–phellandrene > *p*-cymene (von Rudloff 1975) appears to be occurring in the terpene biosynthesis of leaves of *E. nitens* over the growth season. Since *p*-cymene may be a product of aerial oxidation of *p*-menthadienes it has been suggested that it should not be used as a phylogenetic marker (Weston 1984). However, the fact that γ–terpinene, *cis*–β–ocimene and α–phellandrene decrease in relative amounts and that there was a corresponding increase in *p*-cymene associated with time, leaf maturity and phenological growth suggested that *p*-cymene as the end product in the leaf oil of *E. nitens* (D2). The combination of these biochemically related compounds is a useful marker to distinguish *E. nitens* from the other three species and it is also important in the differentiation of the two provenances (D1 and D2) of *E. nitens*.

The relative stability of 1,8-cineole in the leaf oil of *E. nitens* - D2 also suggested that this component is not correlated to variation in the above major components. α–Pinene occurred in trace amounts in the leaves of *E. nitens* (D2) over the growth season. A comparison of the oil composition of the two *E. nitens* provenances, D1 and D2, showed that there is an increase in the proportion of α–pinene and 1,8-cineole in provenance D1 which is correlated with a decrease in *cis*–β–ocimene, γ–terpinene and *p*-cymene. It is possible that β–ocimene may lie on the biosynthetic pathway between NPP and α– and β–pinene (Gleizes 1976 in Charlwood and Banthorpe 1978) (see Fig. 3.22). It would be in accord with the 'radical' route to the pinane skeleton and this 'radical' route appears to relate to the variation in the ratio of *cis*–β–ocimene and α–pinene between provenances of *E. nitens*. Thus, the biosynthesis toward *cis*–β–ocimene would reduce biosynthesis of α–pinene. Moreover, α–pinene and 1,8-cineole have been assumed to be raised from a common precursor (1-*p*-menthen-8-carbonium ion). If this 'radical' route is involved in the biosynthesis of monoterpenoids in *E. nitens*, it would be useful to explain relative variation in percentage contents between the two monoterpenoid component groups.

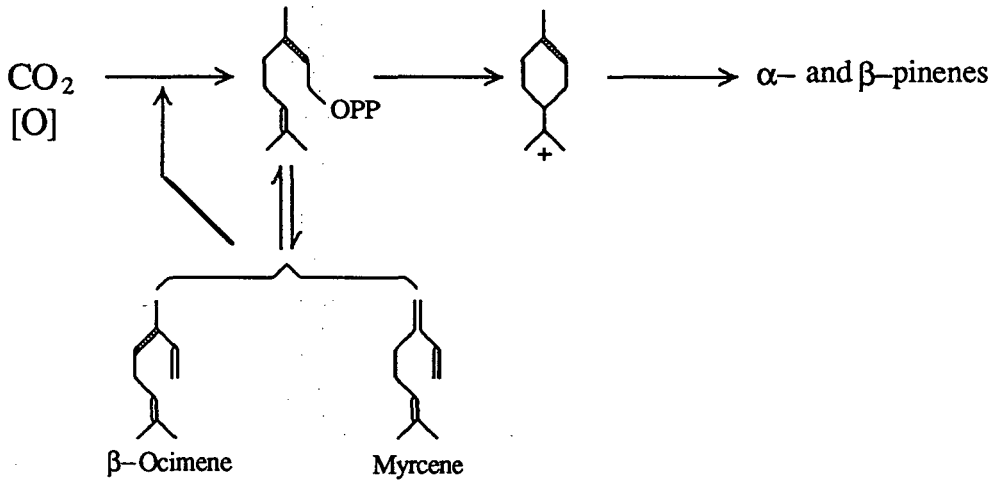


Fig. 3.22. Possible involvement of β–ocimene in α– and β–pinene biosynthesis (following Charlwood and Banthorpe 1978)

In contrast with *E. nitens* (D2), the oil composition of *E. globulus* (B2) showed little variation with all three variables during the active growth season. However, a trend of change in the percentage content of major components could be seen. Thus, the percentage of α–pinene decreases with all three variables and 1,8-cineole increased with time and leaf development over the study period (See Fig. 3.21).

The difference in variation patterns in oil composition between *E. nitens* - D2 and *E. globulus* -B2 over the active growth season further confirmed the genetic divergence in oil composition between these two species provenances.

The leaf oil of *E. delegatensis* has been reported to have significant ontogenetic variation in oil yield between juvenile and adult leaves (Boland *et al.* 1982). This study indicated that variation in the major components of this leaf oil was affected by physiological ageing of leaves during the growth season.

The *E. delegatensis* (C2) oil exhibited an entirely different composition and variation pattern compared to *E. globulus* and *E. nitens* in all leaves during the growing season. The observed pattern of variation in oil composition of *E. delegatensis* (C2) was mainly due to variation in percentage contents of α– and β–phellandrene, *cis*- and *trans*-*p*-menth-2-en-1-ol, *cis*-piperitol and *p*-cymene.

All major monoterpenoids of *E. delegatensis* oil were biochemically related to a series of methane monoterpenes, such as the two *p*-2-menthen-1-ol isomers, two piperitol isomers and two phellandrene isomers. It has been speculated that the two *p*-2-menthen-1-ol and two piperitol isomers arise in the plant by non-specific hydroxylation of the same allylic

carbonium ion (Lassak and Southwell 1982), hence they may be biochemically related. Weston (1984) suggested that the monoterpene hydrocarbons in steam distilled oils of *E. delegatensis* leaves were probably artefacts and not original compounds of leaves. However, the examination of the hexane extracted oils of *E. delegatensis* in the preliminary experiments for the selection of extraction methods showed that α– and β–phellandrene occurred in relatively large proportions without steam distillation, although proportions were lower in hexane extracts than in steam distilled oil. Moreover, α–phellandrene is the major component of volatile chemicals diffused from the fresh leaves of *E. delegatensis* without any treatment (see Appendix 7.6). This indicated that α– and β–phellandrene are the original products in the leaves of *E. delegatensis* rather than artefacts of steam distillation although some part of them could be an artefact from the dehydration of monoterpene alcohols. It has been suggested that piperitone is unchanged during steam distillation and is probably the end-product of methane biogenesis in the eucalypts, and therefore it might possibly be a better product to use as a genetic marker in *E. delegatensis* (Weston 1984). However, piperitone occurred in only moderate proportions (%) in all leaf oils of *E. delegatensis* in this study and other previous reports (Boland *et al.* 1990). Therefore, piperitone is not suitable for use as a major chemical marker for *E. delegatensis* leaf oil as its percentage content was low.

The major components of an essential oil are the most appropriate compounds to use as taxonomic characters since they are probably the end products of synthetic pathways (Banthorpe *et al.* 1972). However, it is difficult to judge which component is the end product when several components co-occur in similar proportions together as the major components of an essential oil. Nevertheless, the data obtained from a group of chemically or biosynthetically related compounds give more chemosystematically useful information than can be obtained from data concerned with single or unrelated compounds (von Rudloff 1968). Hence the major monoterpenoids of *E. delegatensis* oil can be used together as a monoterpenoid group as a chemotaxonomic mark, since they are chemically and biochemically related.

The variation in oil components of individual provenances and their possible biochemical relationships as described above further indicated that the differences between the three leaf oils are not only distinguished by chemical composition and variation pattern, but also by the biogenetic divergence of monoterpenoids (see Devon and Scott (1972) for explanation of biogenetic categories of monoterpenoids).

3.6. Concluding remarks

Many studies in eucalypt leaf oils have found that marked qualitative differences in oils are most common between species of eucalypts, whereas, quantitative variability is typically within species (Doran 1990). However, there has been a lack of comparison of the oil compositions of species and provenances grown under uniform cultural and environmental conditions. Therefore, earlier studies did not clearly distinguish the genetic difference between species or provenances of species and environmental effects. In addition, the effects of environmental factors are likely to involve a highly complex network of variables in eucalypt leaf oils, and very little information is available concerning their effects (Doran 1990).

This study demonstrates the differences in oil characters between species and provenances of a species from different subgenera of the genus *Eucalyptus* growing in extensive and carefully controlled species/provenance trial. At the same time, this study used the same genetic material from different species and provenances in sites of different altitude, similar soil type and uniform cultural management. Consequently, the effects of altitude and related environmental differences at the different sites were demonstrated. As a detailed strategy for sampling of leaf age during the active growth season was adopted, this study described not only variation between different seasons over a 12 mo period, but also variations with respect to sampling time and ontogenetic and physiological aging of leaves with time.

Results have indicated that variation in leaf oil characteristics of *Eucalyptus* species are intimately related to genetic control, leaf development and the environment.

The differences in leaf oils of the four eucalypt species were determined essentially by differences in genotypic composition, particularly at the species level and these differences had a strong genetic basis. Environmental (site), sampling time, leaf ontogeny/aging effects on oil composition appear to be relatively small when compared to the genetically based differences between species and, in cases, between provenances within a species. Seasonal, ontogenetic and physiological age variation in leaf oil of individual provenances also depended on the genetic background of each species and differences involved the biogenetic divergence in the biochemical relationships of monoterpenoid compounds. Environmental variation mainly induced only quantitative variation of leaf oil. Therefore, the leaf oil character of eucalypts could be useful in chemotaxonomic and genetic studies. In addition, the winter season is the most suitable time for collection of leaf samples for chemotaxonomic study when seasonal and leaf aging variation is at a minimum.

Chapter 4

Chemotaxonomy of Tasmanian *Eucalyptus* spp. based on Leaf Oils

4.1. Introduction

Chemotaxonomic studies were undertaken based on the analysis of leaf oils from populations of 29 eucalypt species that occur naturally in Tasmania. The populations investigated in this chapter were confined to Tasmania, although some species have extensive mainland distribution. The samples consisted of both juvenile and adult leaves from each natural stand and included the major clinal varieties of some species. The objective of this work is to describe the range of chemical variation which exists in the Tasmanian provenances and identify chemical characteristics of individual species. Comparisons between and within species are made to (a) describe the relationships that exist between chemical and morphological characteristics and (b) indicate the possible ecological function of these relationships with defoliating paropsine chrysomelid beetles.

The description of chemotaxonomic features are presented at a very general level as chemical variation occurs in all of the chemotaxonomic features discussed. To fully consider such chemical and biogenetic features is beyond the scope of this thesis.

4.2. Materials and methods

4.2.1. Survey

Foliage from 29 species of eucalypt was collected throughout the island of Tasmania (40^0 40' to 43^0 40' S, and 144^0 40' to 148^0 20' E) between May and December 1989. Sampling was conducted from sea level to the tree-line, across an altitude range of approximately 1,100 m. Access was mainly gained by the general public road network, and the only area not covered was the south-west region where there are few roads. Since this region supports only three species of eucalypt, *E. nitida, E. vernicosa* and *E. ovata* (Jackson 1965), and these species and their characteristic associations are well represented in other regions, the region was not considered a major omission.

The number of localities of each species sampled as mainly determined by their geographical distribution and the degree of morphological variation within species. The geographic distribution and morphologically forms of the taxa and species were determined by examination of existing distribution maps and botanical information (eg. Jackson 1965; Davidson *et al.* 1981) as described in chapter 2. To fully evaluate each species, collection sites were chosen to cover the geographic ranges of the taxa and localities to match the known variation patterns within certain species.

Each population sampled was assigned a seven-letter code for identification. The first three letters of the code were derived from the first three letters of the species name followed by four letters indicating respectively the locality and any specific feature of the collection site. Each populations sampled was also assigned a code on map for their identification in Fig. 4.1. Details of the locations of populations sampled and their assigned codes are listed in Appendix 4.1 and depicted in Fig. 4.1.

4.2.2. Leaf samples

At each locality, a species collection comprised a pooled sample of adult leaves collected from 15-20 reproductively mature trees and a pooled juvenile leaf sample collected from 15-20 seedlings from the same area. Fresh leaf samples were sealed in plastic bags, labelled and transported to the University of Tasmania as soon as possible. The samples were then stored at 4 ^{0}C until processed within two weeks of collection.

4.2.3. Extraction and analysis

Oil extraction and analysis was as described in chapter 3.

4.2.4. Standard procedure of numerical analysis

The numerical methods used in this chapter have been reviewed (see Chapter 2). In this chapter, the basic data matrix for all analyses was the matrix of individual populations of all species derived from the oil compounds of both adult and juvenile samples.

Significance tests

The basic data matrix was first subjected to frequency distribution analysis in order to determine patterns of variation among populations. This indicated the distribution of oil characters (percentage/proportions of components) in different subgenera. The non-parametric analysis was undertaken, including calculation of the significance of H (Kruskal-Wallis) values (as variance among species/variance within species). In this way, H values were computed for each character and used to determine which characters gave significant differences between species at any desired level, such as (1) between all

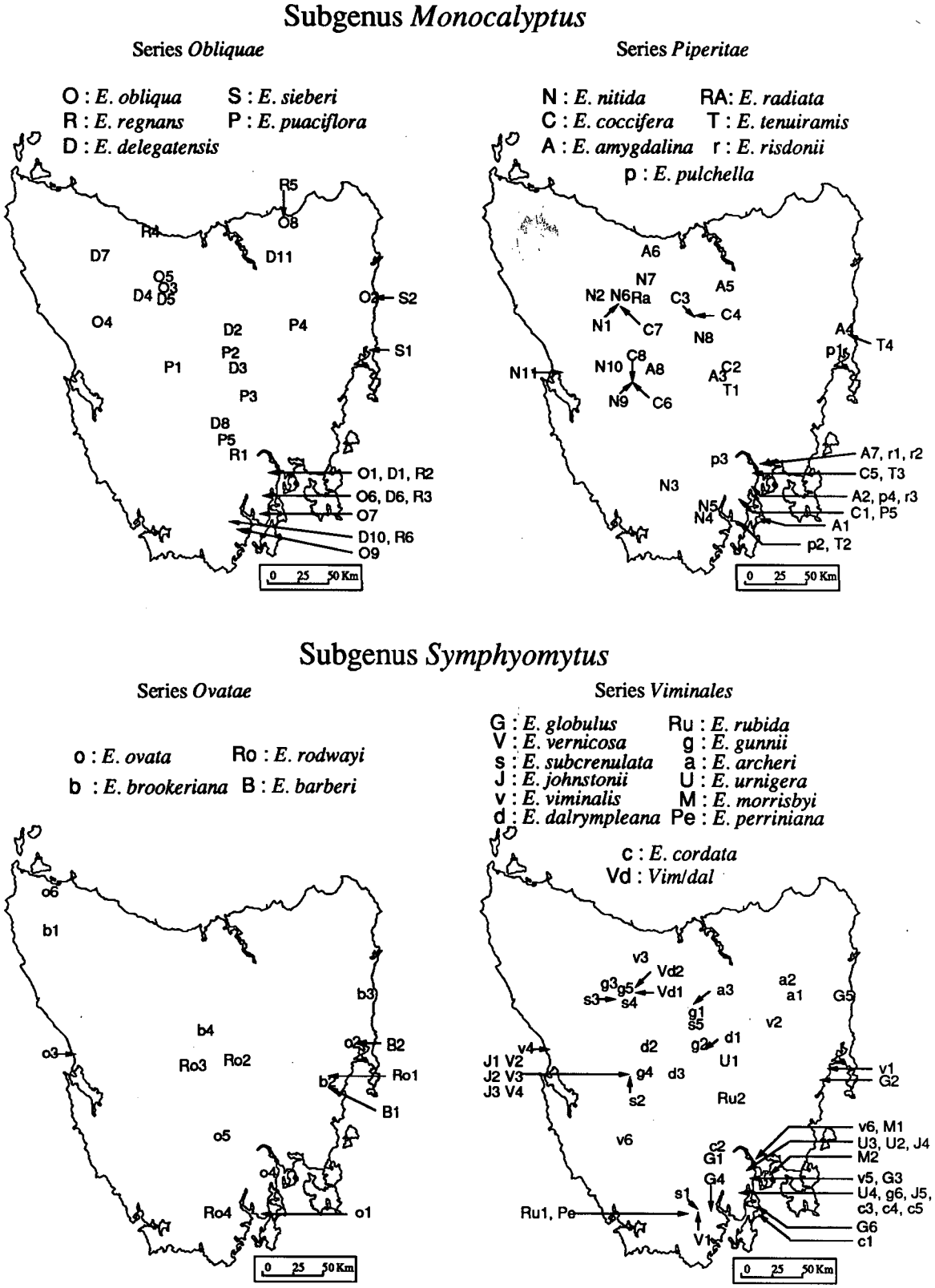


Fig. 4. 1. Collection sites of leaf samples of 29 *Eucalyptus* species in Tasmania. (see Appendix 4.1 for population codes on map)

species, (2) between species within subgenera and (3) between species within a morphological series. For each chemical component, the difference between adult and juvenile leaves was tested for each species using the *H*-test (Kruskal-Wallis).

Summary statistics were derived from the basic data matrix, including the mean values of the proportion of each chemical compound for each species. These data were neither standardised nor weighted. The resulting data was first submitted to the U-test (Mann-Whitney) to determine, for all characters in turn, which characters were significantly different between different OTUs at any desired level: such as between subgenera and between series within a subgenus. The U-test was also used to determine the difference in characters between adult and juvenile leaves at different desired levels, such as (1) all species, (2) subgenera and (3) series.

Correlation analyses

Pearson correlations were employed to determine the relationships of variation of individual chemical components between populations of individual species. The matrices of Pearson correlation coefficients were calculated based on the percentage content of individual components and using all species populations. The chemical components were also classified using the Average Linkage Clustering (SPSSx) based on the correlation coefficients.

Ordination, Distance matrices and Clustering

Principal component analysis (PCA) was undertaken based on the total correlation matrix derived from the basic data matrix of percentage components. The orthogonal principal component axes and the loading of the populations on these axes were plotted in pairs. The amount of the variation between samples which is accounted for by each ordinate was calculated. In this way, the effectiveness of each plot in summarising the relationships between the populations can be found by calculating the percentage of the total variation accounted by the axes. The major variation is normally contained in the first few axes although, in more complex situations, useful information may still be found on minor axes.

Next, the Manhattan metric distance was computed between populations based on the original percentage content of chemical components of individual populations. The distance between any two populations is the maximum absolute difference between the values of the clustering variables: $\text{Distance}(x,y) = \text{Maximum}_i|x_i-y_i|$. As a method of population classification, the average linkage dendrogram would summarise the overall variation patterns of the leaf oil and at different classification levels: (i) all populations of

the 29 species, (ii) populations within each subgenus and (iii) populations within each series within subgenera.

The Manhattan metric distance was also computed between all species based on species means or percentage proportion of each component found in adult and juvenile leaf samples. The distance or dissimilarity matrix produced was used as the input to the cluster analysis to produce a hierarchical classification of species. This is depicted as the familiar dendrogram, summarising the fusion of average linkages by the use of the unweighted and *F*-weighted pair-group method. For *F*-weight method, all oil characters were weighted by *F* - 1 (*F*-value: variance between species/variance within species) following Adams and Turner (1970) and Adams (1975b)

Computational details

The calculation of the H (Kruskal-Wallis) and U (Mann-Whitney) values, were computed using the non parametric comparison program of Statview written by Daniel *et al.* (Abacus Concepts, Inc., 1987). Correlations and regressions were calculated and Principal Component Analyses (PCA) undertaken using the factor analysis program of Statview based on Sneath and Sokal's (1973) method, and were run on a Macintosh SE/30 computer.

The Manhattan metric distance matrix and Average Linkage Clustering (UPGMA and WPGMA) analyses, which are based on Sneath and Sokal (1973) method, were computed and performed using the Cluster sub program of SPSSx (SPSS inc. 1986) and were run on the main computer at the Computing Centre, University of Tasmania.

4.3. Results and general survey

4.3.1. Oil yield

Population and Species variability

As shown in Table 4.1, the oil yield of the Tasmanian eucalypts varied widely. The mean values for oil yields of adult leaves from *Monocalyptus* species ranged from 1.3 to 6.3 percent dry weight and for the juvenile leaves from 0.1 to 3.1 percent. The adult leaves of *E. delegatensis* were amongst the highest mean (4.4%) oil yield but its juvenile leaves had the lowest yield (0.7%) within the *Obliquae* series. Among the *Piperitae* species, both adult and juvenile leaves of *E. radiata* had the highest oil yield (6.3% for adult and 2.1% for juvenile respectively). The mean value of oil yields of *E. coccifera* were the lowest for both adult (1.3%) and juvenile leaves (0.1%) within *Piperitae* and *Monocalyptus* species.

The mean values of adult *Symphyomyrtus* oil yields ranged from 0.8 to 5.3 percent and for juvenile leaves from 1.4 to 5.1 percent. *E. cordata* had the highest mean values for both adult (5.3%) and juvenile (5.1%) leaves among all species of this subgenus and *E. dalrympleana* the lowest (0.8 for adult and 1.4 percent for juvenile leaves).

The Kruskal-Wallis *H*-test (Table 4.2A) indicated significant ($p < 0.001$) variation in oil yields amongst the 29 species tested for both adult and juvenile leaves. The variation of oil yields between *Monocalyptus* species were significant for adult ($p < 0.01$) and juvenile ($p < 0.001$) leaves and the variance between *Symphyomyrtus* species was also significant for both adult ($p < 0.05$) and juvenile ($p < 0.01$) leaves. In the subgenus *Monocalyptus*, the variation in oil yields between *Piperitae* species was significant ($p < 0.01$) for both adult and juvenile leaves but variation between *Obliquae* species was only significant for juvenile leaves ($p < 0.01$). Within *Symphyomyrtus* species, there was significant variation ($p < 0.01$) in oil yields between *Viminales* species for both adult and juvenile leaves. However there was no significant variation amongst the *Ovatae* species in oil yield for both adult and juvenile leaves.

The difference in oil yields between subgenera and series within subgenera was tested by the Mann-Whitney *U*-test based on the mean values of individual species and the results are shown in Table 4.2B. The oil yields of adult leaves were on average significantly ($p < 0.05$) higher in the subgenus *Monocalyptus* than that in the *Symphyomyrtus*. In contrast, the oil yields of juvenile leaves were significantly higher ($p<0.001$) in subgenus *Symphyomyrtus* than that in *Monocalyptus*. However, there were no significant differences between series for either adult and juvenile leaves within either *Monocalyptus* or *Symphyomyrtus*.

Table 4.1. The oil yield (g/100g dry weight) of leaves of Tasmanian *Eucalyptus* species, the test for difference between adult and juvenile leaves (Mann-Whitney *U*) and the test for variation in oil yield (Kruskal-Wallis *H*).

Significance of difference: p<0.001 = ***; 0.001<p<0.01 = **; 0.01<p<0.05 = *; p>0.05 = ns
Mean is the average value of populations of each species; Mean difference: Δ(A-J) is difference of mean values of adult and juvenile leaves. The number of populations sampled per species is indicated.

		Adult leaves			Juvenile leaves			Differences between adult and juvenile		
Species	Species Code	No. Pop.	Mean	S.D.	No. Pop.	Mean	S.D.	Mean Δ(A-J)	*U*- test P-value	Sign.
Monocalyptus Series *Obliquae*										
E. delegatensis	DEL	10	4.4	1.2	10	0.7	0.5	3.7	0.0002	***
E. regnans	REG	6	4.4	1.4	6	3.1	1.3	1.3	0.0163	*
E. obliqua	OBL	9	3.8	1.6	9	1.9	0.4	1.9	0.0009	***
E. sieberi	SIE	3	3.6	0.3	2	1.8	0.3	1.8	0.0833	ns
E. pauciflora	PAU	5	2.6	0.8	5	1.9	0.5	0.7	0.1172	ns
Series *Piperitae*										
E. radiata	RAD	1	6.3	6.3	1	2.1	2.1	4.3	-	-
E. pulchella	PUL	5	4.9	0.7	5	1.4	0.3	3.5	0.0090	**
E. tenuiramis	TEN	4	4.4	1.3	4	1.6	1.7	2.8	0.0433	*
E. amygdalina	AMY	8	4.4	1.9	8	2.0	2.1	2.4	0.0117	*
E. nitida	NIT	10	3.9	1.7	10	0.7	0.4	3.2	0.0002	***
E. risdonii	RIS	3	1.8	0.5	2	0.4	0.4	1.4	0.0833	ns
E. coccifera	COC	6	1.3	0.7	6	0.1	0.1	1.2	0.0039	**
Symphyomyrtus Series *Ovatae*										
E. brookeriana	BRO	3	3.6	0.9	3	3.2	0.6	0.3	0.5127	ns
E. rodwayi	ROD	4	2.8	0.5	4	2.9	0.6	-0.1	1.0000	ns
E. barberi	BAR	2	2.3	0.0	2	2.5	1.0	-0.2	1.0000	ns
E. ovata	OVA	6	2.1	1.8	6	2.2	2.0	-0.1	0.8099	ns
Series *Viminales*										
E. cordata	COR	5	5.3	1.1	5	5.1	0.2	0.1	0.9168	ns
E. johnstonii	JOH	5	4.8	0.2	3	3.0	2.1	1.8	0.2938	ns
E. urnigera	URN	4	3.5	2.1	4	2.7	1.0	0.8	0.5637	ns
E. globulus	GLO	6	3.4	0.6	6	5.0	0.9	-1.7	0.0103	*
E. perriniana	PER	1	3.2	3.2	1	4.8	4.8	-1.6	-	-
E. morrisbyi	MOR	2	2.9	1.7	2	3.0	0.2	-0.1	1.0000	ns
E. viminalis	VIM	6	2.8	0.6	6	3.6	0.8	-0.9	0.0374	*
E. subcrenulata	SUB	5	2.6	1.5	4	2.4	1.2	0.2	0.8057	ns
E. rubida	RUB	2	2.5	1.4	2	4.5	1.0	-2.0	0.1213	ns
E. vernicosa	VER	4	2.3	0.2	3	2.5	0.3	-0.2	0.4795	ns
E. archeri	ARC	3	2.3	0.2	3	1.8	0.6	0.5	0.5127	ns
E. gunnii	GUN	6	2.1	1.3	6	1.8	1.3	0.2	0.6310	ns
E. dalrympleana	DAL	3	0.8	0.0	3	1.4	0.2	-0.6	0.0495	*

Table 4.2A. Kruskal-Wallis (*H*) analysis of variance of oil yields between/within species.

Significances of difference: p<0.001 = ***; 0.001<p<0.01 = **; 0.01<p<0.05 = *; p>0.05 = ns

	Adult leaf samples					Juvenile leaf samples				
	No. Species	No. Popn.	H-values	*p*-value	Sign	No. Species	No. Popn.	H-values	*p*-value	Sign
between/within all species	29	140	75.3	0.0001	***	29	132	91.2	0.0001	***
between/within *Monocalyptus* species	12	70	31.1	0.0011	**	12	68	44.8	0.0001	***
between/within *Obliquae* species	5	33	8.2	0.828	ns	5	32	21.3	0.003	**
between/within *Piperitae* species	7	37	20.2	0.0026	**	7	36	19.8	0.003	**
between/within *Symphyomyrtus* species	17	68	36.1	0.028	*	17	64	36.8	0.0022	***
between/within *Ovatae* species	4	15	2.8	0.4174	ns	4	15	1.4	0.7085	ns
between/within *Viminales* species	13	52	31.6	0.0016	**	13	48	32.8	0.0011	**

Table 4.2B. The difference in oil yields between subgenera and series based on the *U*-test using species mean values.

		Adult leaves	Juvenile leaves
Between subgenera *Monocalyptus* and *Symphyomyrtus*	N of species: 12/17		
	Difference (Mono.-Sym.)	0.91	-1.61
	p-Value	0.046	0.001
	Significant	*	***
Between series *Obliquae* and *Piperitae*	N of species: 5/7		
	Difference (Obl.-Pip.)	-0.10	0.69
	p-Value	0.563	0.254
	Significant	ns	ns
Between series *Ovatae* and *Viminales*	N of species: 4/13		
	Difference (Ova.-Vim.)	-0.26	-0.50
	p-Value	0.649	0.610
	Significant	ns	ns

Difference between adult and juvenile leaves

The differences in oil yields between leaf types among all species were examined by *U*-tests based on mean values of oil yields of individual species (Table 4.3). On average the mean values of adult leaves of all species were significantly higher than those for juvenile leaves ($\Delta_{(A-J)}$ = 0.9% for mean; $p<0.05$). However, the subgenera differed markedly in the difference between adult and juvenile leaf oil yields. The *U*-test indicated that on average oil yields were significantly greater in adult leaves of the *Monocalyptus* species ($p < 0.01$; $\Delta_{(A-J)}$ = 2.3%) but not for the *Symphyomyrtus* species ($p = 0.73$). The differences between adult and juvenile leaves at the series level were also significant ($p < 0.05$) for the two series *Obliquae* ($\Delta_{(A-J)}$ = 1.8 %) and *Piperitae* ($\Delta_{(A-J)}$ = 2.7%) of the subgenus *Monocalyptus* but were not significant for any series within the subgenus *Symphyomyrtus*.

As shown in Table 4.1, the mean values for oil yields of adult leaves of individual *Monocalyptus* species were consistently higher than those for juvenile leaves but results for individual *Symphyomyrtus* species were inconsistent.

In the majority of species of the subgenus *Monocalyptus* the oil yields of adult leaves were significantly higher than those for juvenile leaves, with the exception of *E. sieberi*, *E. pauciflora* and *E. risdonii*. *E. radiata,* which was sampled at only one locality, showed a marked difference in oil yield between adult and juvenile leaves ($\Delta_{(A-J)}$ = 4.3%) but this could not be tested statistically.

In contrast, there were no significant differences in oil yields between leaf types for most species of the subgenus *Symphyomyrtus* (Table 4.1). Only three species had significant differences ($p < 0.05$) in oil yields between adult and juvenile leaves with the juvenile oil yields greater than those from adult leaves. These three species were *E. globulus* (Mean $\Delta_{(A-J)}$ = - 1.7%), *E. viminalis* (Mean $\Delta_{(A-J)}$ = - 0.9 %) and *E. dalrympleana* (Mean $\Delta_{(A-J)}$ = - 0.6%).

Table 4.3. The differences in oil yields between leaf types based on the Mann-Whitney *U*-test using species mean values.

	Species	Adult		Juvenile		Difference between adult and juvenile					
	No.	Mean	Med.	Mean	Med.	Mean $\Delta_{(A-J)}$	P-value	Sign.	Med. $\Delta_{(A-J)}$	P-value	Sign.
All species	29	3.3	3.4	2.4	2.5	0.9	0.013	*	0.9	0.019	*
Sub. *Monocalyptus*	12	3.8	4.0	1.5	1.9	2.3	0.001	***	2.3	0.001	**
Obliquinae series	5	3.4	3.6	1.6	1.7	1.8	0.016	*	1.8	0.021	*
Piperitae series	7	3.9	4.0	1.2	1.5	2.7	0.018	*	2.5	0.018	*
Sub. *Symphyomyrtus*	17	2.9	3.1	3.1	3.3	-0.1	0.744	ns	-0.1	0.730	ns
Ovatae series	4	2.7	2.8	2.7	2.9	0.0	0.773	ns	-0.1	0.561	ns
Viminales series	13	3.0	3.0	3.2	3.2	-0.3	0.739	ns	-0.2	0.898	ns

4.3.2. Oil Compositions

Forty-six chemical components were identified from the leaf oils of 29 Tasmanian eucalypt species and their chemical names and codes are listed in Table 4.4A. The GLC separation of oil samples in selected species and representative peaks of oil compounds are shown in Appendix 4.2. The unidentified components were included in calculation of percentage composition and their total percentages were indicated as a unknown category (Un) in Table 4.5. Therefore, percentage proportion of the forty-six chemical components listed in Table 4.5 were the original percentage data of GLC.

The Tasmanian *Eucalyptus* leaf oils are mainly composed of complex mixtures of volatile terpenoid compounds of such chemical types as hydrocarbons, alcohols, ketones, and esters (Table 4.4B). These terpenoid compounds belonged to two major categories the monoterpenoids and the sesquiterpenoids. The monoterpenoids of eucalypt leaf oil were classified into three groups - acyclic, monocyclic and bicyclic while the sesquiterpenoids fell into three groups - acyclic, bicyclic and tricyclic according to their structures (Table 4.4B). Some aromatic components, methyl cinnamate, regnanone and 3,5-dimethyl conglomerone, and the tasmanone type compounds were identified from few species.

Table 4.4A. The chemical components identified from the leaf oils of Tasmanian *Eucalyptus* species.

Compounds	No. in retention time	Letter Code	Compounds	No. in retention time	Letter Code
α–Pinene	1	αP	*trans*-Pinocarveol	24	tPi
α–Thujene	2	αTh	*cis*-Piperitol	25	cPip
Camphene	3	Ca	Terpinyl acetate	26	TA
β–Pinene	4	βP	α–Terpineol	27	αTe
Sabinene	5	S	Piperitone	28	Pip
α–Phellandrene	6	αPh	*trans*-Piperitol	29	tPip
α–Terpinene	7	αT	Citronellal	30	Cit
Limonene	8	Lim	*cis*-Sabinol	31	cS
β–Phellandrene	9	βPh	*p*-Cymene-8-ol	32	Cy8
1,8-Cineole	10	Ci	Nerolidol	33	Ner
cis–β–Ocimene	11	cO	Elemol	34	El
γ–Terpinene	12	γT	Globulol	35	Gl
trans–β–Ocimene	13	tO	Viridiflorol	36	Vir
p-Cymene	14	Cy	Methyl cinnamate	37	MC
Terpinolene	15	Te	Spathulenol	38	Sp
Linalool	16	Lin	α–Eudesmol	39	αE
trans-p-Menth-2-en-1-ol	17	tM	γ–Eudesmol	40	γE
Pinocarvone	18	Pi	β–Eudesmol	41	βE
b-Caryophyllene	19	Ca	Regnanone	42	Ta1
Aromadendrene	20	Aro	3,5-Dimethyl conglomerone	43	Ta2
Terpinen-4-ol	21	T4	Tasmanone	44	Ta3
cis-p-Menth-2-en-1-ol	22	cM	Un 1(Tasmanone Type)	45	Ta4
Allo-aromadendrene	23	All	Un 2 (Tasmanone Type)	46	Ta5

Table 4.4B. The classification of terpene compounds found in the leaf oils of *Eucalyptus* according to their type and chemical structure.

Monoterpenes		
Acyclic	Monocyclic	Bicyclic
Hydrocarbons *cis*–β–Ocimene *trans*–β–Ocimene Alcohols Linalool Aldehydes Citronellal	Hydrocarbons α–Phellandrene β–Phellandrene Limonene α–Terpinene γ–Terpinene Terpinolene *p*-Cymene Alcohols *cis*-Piperitol *trans*-Piperitol *trans*-*p*-Menth-2-en-1-ol *cis*-*p*-Menth-2-en-1-ol Terpinen-4-ol α–Terpineol *p*-Cymene-8-ol Ketones Piperitone Esters Terpinyl acetate Ethers 1,8-Cineole	Hydrocarbons α–Pinene β–Pinene Camphene α–Thujene Sabinene Alcohols *trans*-Pinocarveol *cis*-Sabinol Ketones Pinocarvone
Sesquiterpenes		
Acyclic	Bicyclic	Tricyclic
Alcohols Nerolidol	Alcohols Elemol α–Eudesmol γ–Eudesmol β–Eudesmol	Hydrocarbons *b*-Caryophyllene Aromadendrene Alloaromadendrene Alcohols Globulol Viridiflorol Spathulenol

The mean percentage composition of the chemical components of leaf oils from the 29 eucalypts species are listed in Table 4.5. The mean percentage values of most chemical components in leaf oils were similar to the median values. In the following section, the components are expressed as mean percentage values except where indicated otherwise.

As shown in Table 4.5, most *Symphyomyrtus* species have a similar leaf oil composition with the exception of *E. ovata* and *E. dalrympleana*. The leaf oil consists generally of the monoterpene ether, 1,8-cineole (component 10, about 40-70%) as the main constituent, with the monocyclic hydrocarbon α–pinene (component 1, 2-20%) and the monoterpene hydrocarbon limonene (component 8, 3-7%) in lesser, but substantial amounts. The monoterpene alcohol α–terpineol (component 27), monoterpene ester terpinyl acetate (component 26), monoterpene hydrocarbon *p*-cymene (component 14) and the sesquiterpene alcohol globulol (component 35) were minor constituents (less than five

Table 4.5. The percentage composition (%) of leaf oil components from leaves of Tasmanian *Eucalyptus* species.

t = trace; - = components absent

(A) Subgenus *Monocalyptus*: Part 1- components 1 to 24

Species	Leaf type	No. Pop.	Statistics	1 αP	2 αTh	3 Ca	4 βP	5 S	6 αPh	7 αT	8 Lim	9 βPh	10 Cl	11 eO	12 γT	13 tO	14 Cy	15 Te	16 Lin	17 tM	18 Pl	19 Ca	20 Aro	21 T4	22 cM	23 All	24 tPi
Series *Obliquae*																											
E. obliqua	A	9	Mean	0.4	0.9	t	t	0.2	4.5	1.6	0.4	7.4	1.5	0.6	0.8	0.1	22.4	0.3	0.8	9.1	-	-	-	3.7	6.9	-	-
			S.D	0.1	0.8	-	-	0.1	1.8	0.5	0.1	2.8	1.5	0.2	0.4	0.1	3.0	0.1	0.3	3.3	-	-	-	1.9	3.0	-	-
	J	9	Mean	0.4	0.5	-	t	0.2	2.9	1.3	0.3	4.4	2.4	1.0	0.8	0.1	24.9	0.3	0.6	5.1	-	-	-	4.6	4.1	-	-
			S.D	0.2	0.2	-	-	0.1	1.3	0.5	0.1	1.2	2.9	0.8	0.6	0.1	3.0	0.2	0.4	1.6	-	-	-	2.7	1.3	-	-
E. delegatensis	A	10	Mean	0.2	2.2	t	t	0.1	16.4	2.9	0.3	10.5	0.3	0.3	0.7	0.3	11.3	1.1	0.7	11.2	-	-	-	3.2	8.4	-	-
			S.D	0.2	0.6	-	-	0.1	3.6	0.8	0.1	3.7	0.3	0.1	0.5	0.3	4.0	0.3	0.2	2.6	-	-	-	0.6	2.1	-	-
	J	10	Mean	0.2	1.6	-	t	0.2	15.1	3.1	0.5	10.7	0.7	0.7	0.8	0.9	12.0	1.2	0.9	8.4	-	-	-	3.7	6.0	-	-
			S.D	0.2	0.6	-	0.1	0.3	6.3	0.7	0.1	1.8	1.0	0.6	0.4	0.7	4.5	0.3	0.3	2.7	-	-	-	1.1	1.8	-	-
E. regnans	A	6	Mean	0.2	0.8	t	0.1	0.2	5.6	1.3	0.4	3.9	0.2	0.2	0.3	t	4.9	0.4	0.6	4.3	-	-	-	1.5	3.5	-	-
			S.D	0.1	0.4	-	0.2	0.2	2.3	0.6	0.2	2.4	0.1	0.2	0.2	0.1	3.8	0.1	0.5	2.5	-	-	-	0.8	1.6	-	-
	J	6	Mean	1.3	0.9	t	0.1	0.8	4.9	1.0	1.6	2.8	0.2	0.2	0.3	0.2	3.9	0.5	0.3	2.5	-	-	-	1.6	2.1	-	-
			S.D	0.8	0.3	-	0.2	1.8	1.0	0.3	1.4	1.2	0.2	0.1	0.1	0.2	2.3	0.1	0.3	1.3	-	-	-	1.0	0.9	-	-
E. sieberi	A	3	Mean	0.6	0.9	-	t	t	7.4	1.5	1.6	5.9	8.1	0.3	0.3	t	5.9	0.5	0.2	3.5	-	-	t	1.6	2.8	-	-
			S.D	0.2	0.3	-	0.1	0.1	2.7	0.7	1.1	3.5	3.4	0.2	0.1	0.1	3.8	0.3	0.2	1.6	-	-	-	0.6	1.2	-	-
	J	2	Mean	0.8	0.9	-	t	-	6.7	1.0	1.4	6.6	8.1	0.5	0.4	-	7.7	0.5	0.4	1.4	-	-	-	2.2	1.2	-	-
			S.D	0.3	0.1	-	0.1	-	0.7	-	0.7	4.9	0.7	0.1	-	-	0.6	0.1	0.1	0.2	-	-	-	0.5	0.1	-	-
E. pauciflora	A	5	Mean	10.9	0.7	t	0.3	t	5.8	0.8	1.2	2.1	9.0	0.2	0.2	1.1	1.4	0.7	0.2	2.4	t	-	-	0.8	2.3	-	0.1
			S.D	13.0	0.7	-	0.4	0.1	7.2	0.7	0.7	2.8	3.9	0.1	0.2	2.0	1.3	0.5	0.3	2.5	0.1	-	-	0.5	1.9	-	0.2
	J	5	Mean	10.7	0.2	t	1.3	t	4.4	0.5	2.3	4.5	13.9	0.2	0.2	0.6	2.1	0.6	0.4	1.6	0.2	-	t	1.0	1.5	t	0.1
			S.D	9.4	0.3	0.1	2.4	0.1	3.2	0.3	0.9	7.8	8.2	0.1	0.1	0.8	1.0	0.3	0.3	0.9	0.2	-	-	0.2	0.5	0.1	0.1
Series *Piperitae*																											
E. risdonii	A	3	Mean	2.5	-	t	0.3	-	3.3	0.5	6.1	0.9	60.4	0.3	0.6	0.4	0.4	0.3	0.3	1.1	-	-	-	1.6	0.8	-	-
			S.D	0.3	-	-	0.2	-	1.6	0.1	0.1	0.8	4.5	0.1	0.1	0.2	0.3	0.1	0.1	0.6	-	-	-	0.2	0.4	-	-
	J	2	Mean	2.6	t	-	0.6	0.3	2.7	0.4	6.8	-	60.9	0.2	0.7	0.4	0.2	0.3	0.2	0.2	-	-	-	1.8	0.2	-	-
			S.D	0.1	-	-	-	0.1	1.3	0.1	0.7	-	0.7	0.3	0.2	0.2	0.1	-	-	0.2	-	-	-	0.1	0.1	-	-
E. tenuiramis	A	4	Mean	1.6	1.0	t	0.3	0.2	10.7	1.1	2.6	4.9	34.8	0.3	0.4	0.6	3.1	0.9	0.5	5.5	-	-	t	2.2	4.2	t	-
			S.D	0.9	0.9	-	0.3	0.2	6.8	1.0	1.9	3.6	20.1	0.2	0.2	0.3	2.9	0.4	0.2	3.5	-	-	0.1	0.8	2.7	0.1	-
	J	4	Mean	2.2	0.9	t	0.1	0.3	11.7	1.3	5.0	3.6	38.3	0.7	0.6	1.2	4.1	1.0	0.4	1.4	-	-	-	1.6	1.1	-	-
			S.D	0.7	0.8	-	0.2	0.1	12.8	1.7	2.3	5.9	20.5	0.2	0.4	0.8	5.6	0.9	0.1	0.6	-	-	-	0.3	0.5	-	-
E. pulchella	A	5	Mean	1.7	1.5	t	0.2	0.3	5.6	1.0	4.6	3.2	46.5	0.3	0.5	0.9	3.0	0.4	0.3	2.1	-	-	-	1.8	1.6	-	-
			S.D	0.8	1.7	-	0.3	0.2	6.8	1.1	2.0	4.5	19.9	-	0.3	1.1	3.5	0.3	0.6	2.1	-	-	-	0.6	1.7	-	-
	J	5	Mean	1.6	0.8	t	0.2	0.3	6.0	0.8	4.9	1.8	42.8	0.6	0.6	2.4	2.2	0.5	0.1	1.8	-	-	-	1.7	1.3	-	-
			S.D	0.8	0.3	-	0.3	0.2	3.4	0.5	2.2	2.1	18.6	0.3	0.1	2.5	2.4	0.2	0.1	1.4	-	-	-	0.4	1.1	-	-
E. amygdalina	A	8	Mean	1.0	1.7	-	0.1	t	16.0	1.9	1.7	7.8	14.3	0.3	0.6	0.8	7.5	1.2	0.7	4.7	-	-	0.1	3.0	3.5	0.3	-
			S.D	0.9	0.7	-	0.2	0.1	6.8	1.1	1.9	5.1	17.4	0.1	0.2	0.6	5.0	0.5	0.5	3.6	-	-	0.1	0.6	2.6	0.3	-
	J	8	Mean	1.1	1.6	t	0.2	t	16.6	1.8	2.2	5.6	15.1	0.6	0.8	1.4	5.8	1.4	0.5	3.5	-	-	0.2	3.1	3.7	0.1	-
			S.D	0.9	0.7	-	0.2	0.1	5.9	1.0	2.3	4.1	14.4	0.3	0.2	0.9	3.5	0.5	0.3	4.3	-	-	0.5	0.9	2.9	0.1	-
E. nitida	A	10	Mean	0.6	1.9	-	t	t	16.1	3.3	0.5	13.6	0.9	0.4	0.5	0.8	12.8	0.8	1.0	5.2	-	-	t	2.8	3.8	t	-
			S.D	0.5	0.4	-	-	-	6.2	1.4	0.4	5.9	0.9	0.3	0.2	0.5	3.3	0.3	0.4	2.4	-	-	0.1	1.0	2.0	0.1	-
	J	10	Mean	1.8	1.0	t	t	t	7.6	1.5	1.6	7.7	10.6	0.8	0.5	1.4	13.6	0.6	1.0	4.7	-	-	t	2.9	4.0	t	-
			S.D	3.0	0.5	-	0.1	-	5.6	1.4	1.4	3.9	15.4	0.7	0.3	2.2	6.1	0.5	0.4	2.8	-	-	0.1	1.3	2.4	0.1	-
E. radiata	A	1	Mean	0.4	3.0	-	-	t	26.2	4.0	0.4	12.1	0.4	0.3	1.5	0.4	7.8	2.0	0.8	6.8	-	-	-	3.9	5.3	-	-
	J	1	Mean	0.5	2.7	t	t	t	19.9	2.0	0.9	5.7	4.6	0.8	0.9	0.8	8.1	1.8	0.8	2.3	-	-	-	3.1	1.9	-	-
E. coccifera	A	6	Mean	1.0	1.1	t	t	t	8.1	1.4	0.8	6.1	5.0	0.4	0.3	1.3	8.9	0.5	1.2	5.2	-	-	t	1.7	3.9	t	-
			S.D	0.8	0.7	-	-	0.1	4.8	0.9	0.6	3.0	6.0	0.4	0.2	1.0	4.0	0.3	0.4	1.4	-	-	0.1	0.9	0.8	0.1	-
	J	6	Mean	2.2	1.0	t	0.1	t	9.6	0.8	2.5	4.8	11.6	1.3	0.5	5.3	7.0	1.2	1.0	2.5	-	-	t	1.9	1.9	0.2	-
			S.D	3.0	0.5	-	0.1	0.1	6.2	0.7	1.5	3.8	11.7	1.1	0.3	3.2	7.7	0.8	0.4	1.8	-	-	0.1	1.0	1.3	0.1	-

Table 4.5. The percentage composition (%) of leaf oil components from leaves of Tasmanian *Eucalyptus* species.

*Un = Total precentage of unidentified components.

$\sum V$ = Sum of variances = $\nu_1+\nu_2+\nu_3+\nu_4+\nu_5.....+\nu_{45}+\nu_{46}$

(ν= variance of percentage contents of individual components among localities within each species.)

for example, ν_1 is the variance of percentage of α–Pinene (αP) among localites within a species.

(A) Subgenus *Monocalyptus*: Part 2- components 25 to 46

			25 cPip	26 TA	27 αTe	28 Pip	29 tPip	30 Clt	31 eS	32 Cyδ	33 Ner	34 El	35 Gl	36 Vir	37 MC	38 Sp	39 αE	40 γE	41 βEu	42 Ta1	43 Ta2	44 Ta3	45 Ta4	46 Ta5	*Un	∑V
Series *Obliquae*																										
E. obliqua	A	Mean	2.4	2.2	1.4	16.7	4.6	-	0.1	1.9	-	-	0.4	-	-	2.4	0.2	0.7	0.3	-	-	-	-	-	5.2	
		S.D	1.2	1.3	0.2	4.4	2.0	-	0.1	0.5	-	-	0.2	-	-	0.8	0.2	0.8	0.3	-	-	-	-	-		74.2
	J	Mean	1.8	1.8	1.2	28.9	3.9	-	0.1	2.3	-	-	0.2	t	-	1.8	0.3	1.0	0.3	-	-	-	-	-	2.4	
		S.D	1.0	0.9	0.2	8.3	1.7	-	0.1	0.5	-	-	0.2	0.1	-	0.7	0.3	0.6	0.3	-	-	-	-	-		108.1
E. delegatensis	A	Mean	2.8	0.2	1.0	1.0	5.2	-	t	0.3	-	-	1.2	0.4	1.6	1.8	3.5	2.9	2.8	-	-	-	-	-	5.2	
		S.D	0.6	0.1	0.5	0.5	1.3	-	-	0.1	-	-	0.8	0.2	1.1	1.1	2.2	1.6	1.9	-	-	-	-	-		72.3
	J	Mean	2.1	0.3	1.4	2.8	3.8	-	t	0.3	-	-	1.0	0.3	1.8	2.1	4.8	3.3	3.6	-	-	-	-	-	5.8	
		S.D	0.7	0.2	0.6	2.6	1.0	-	-	0.2	-	-	0.5	0.3	0.9	1.1	2.2	1.3	1.5	-	-	-	-	-		98.9
E. regnans	A	Mean	1.2	0.1	0.5	1.5	2.3	-	t	0.2	-	4.4	-	-	0.2	1.7	15.6	12.5	15.0	7.1	-	-	-	-	9.5	
		S.D	0.5	0.1	0.4	2.2	1.1	-	0.1	0.2	-	1.7	-	-	0.2	0.5	5.7	2.9	4.1	4.0	-	-	-	-		119.2
	J	Mean	0.8	0.2	0.5	9.0	1.4	-	t	0.1	-	2.8	-	t	0.2	1.6	18.7	12.9	13.8	6.1	-	-	-	-	7.3	
		S.D	0.2	0.1	0.4	4.8	0.5	-	0.1	0.1	-	1.1	-	-	0.1	0.8	3.3	1.3	1.5	3.0	-	-	-	-		67.3
E. sieberi	A	Mean	1.8	0.3	1.8	0.6	2.4	-	t	0.2	-	-	3.7	1.4	t	2.7	14.4	10.4	10.9	t	-	-	-	-	8.0	
		S.D	1.1	0.5	0.5	0.6	0.8	-	-	0.1	-	-	2.4	1.6	0.1	0.8	2.2	3.7	5.6	0.2	-	-	-	-		113.9
	J	Mean	0.6	0.2	2.1	18.2	0.6	-	-	0.3	-	-	1.6	0.8	t	2.9	11.2	8.5	9.2	-	-	-	-	-	4.3	
		S.D	0.2	0.3	0.9	1.3	0.3	-	-	0.1	-	-	0.3	0.3	-	0.2	1.6	1.7	2.6	-	-	-	-	-		41.7
E. pauciflora	A	Mean	0.9	0.2	1.7	2.1	0.9	-	t	0.2	-	-	2.6	1.0	t	2.8	10.1	12.9	15.7	-	-	-	-	-	7.3	
		S.D	0.7	0.2	1.3	1.1	1.0	-	-	0.2	-	-	1.2	0.7	-	1.8	4.3	8.1	11.8	-	-	-	-	-		496.3
	J	Mean	0.5	0.2	2.7	0.8	1.8	-	t	0.2	-	-	2.2	1.4	-	2.9	9.6	11.8	12.4	-	-	-	-	-	7.4	
		S.D	0.4	0.5	1.3	1.1	1.5	-	0.1	0.2	-	-	1.1	0.6	-	1.0	3.5	5.3	6.1	-	-	-	-	-		322.1
Series *Piperitae*																										
E. risdonii	A	Mean	0.3	6.1	5.8	0.3	1.2	-	-	-	-	-	0.3	0.1	-	0.2	0.3	0.4	0.4	-	-	-	-	-	5.1	
		S.D	0.1	5.2	5.3	0.1	0.5	-	-	-	-	-	0.1	0.1	-	0.2	0.2	0.3	0.3	-	-	-	-	-		80.1
	J	Mean	0.1	11.4	2.4	1.2	1.6	-	t	-	-	-	0.2	0.1	-	0.1	0.3	0.3	0.3	-	-	-	-	-	3.4	
		S.D	0.1	2.3	0.1	1.7	0.7	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-		11.7
E. tenuiramis	A	Mean	1.4	2.1	4.0	1.0	2.7	0.2	t	t	-	-	1.0	0.3	t	0.6	1.7	1.8	2.0	-	0.8	-	0.2	t	5.2	
		S.D	0.8	3.4	3.1	0.4	1.4	0.2	-	0.1	-	-	0.3	0.1	-	0.3	0.7	0.7	0.8	-	0.8	-	0.1	0.1		527.5
	J	Mean	0.5	1.9	5.0	3.5	1.5	0.2	0.2	0.2	-	-	0.6	0.3	-	0.4	1.2	0.8	0.9	-	1.1	-	0.2	0.2	6.1	
		S.D	0.2	3.1	3.4	4.6	0.5	0.3	0.1	0.5	-	-	0.3	0.2	-	0.3	0.8	0.7	0.6	-	1.1	-	0.1	0.1		707.6
E. pulchella	A	Mean	0.5	2.2	1.9	1.6	1.0	0.2	0.1	t	-	-	0.5	0.3	-	0.2	0.9	0.8	1.0	-	5.6	1.5	0.3	0.2	5.9	
		S.D	0.6	3.2	0.4	2.6	1.2	0.2	0.1	-	-	-	0.3	0.2	-	0.2	0.9	0.6	0.9	-	3.5	1.6	0.2	0.2		529.7
	J	Mean	0.5	2.4	1.8	2.9	0.9	0.3	t	t	-	-	0.4	0.3	-	0.3	0.5	0.4	0.4	-	11.6	1.9	0.7	0.8	3.9	
		S.D	0.4	3.3	0.4	4.8	0.7	0.4	0.1	0.1	-	-	0.2	0.2	-	0.2	0.3	0.3	0.3	-	7.8	2.6	0.4	0.7		485.1
E. amygdalina	A	Mean	1.1	0.6	2.4	12.6	2.1	0.1	t	0.2	-	-	1.4	0.8	-	1.0	2.3	1.7	1.8	-	0.1	-	0.1	-	4.6	
		S.D	0.8	0.4	3.0	18.8	1.4	0.2	-	0.2	-	-	0.9	0.5	-	0.7	1.2	0.9	1.0	-	0.2	-	0.2	-		796.9
	J	Mean	1.2	0.5	2.7	15.0	2.6	0.2	t	0.2	-	-	1.3	0.8	-	0.9	1.7	1.2	1.4	-	0.3	-	0.2	-	4.8	
		S.D	0.9	0.3	2.7	17.9	1.5	0.3	-	0.1	-	-	1.2	0.7	-	0.9	1.0	0.9	1.2	-	0.5	-	0.3	-		646.4
E. nitida	A	Mean	1.3	0.4	1.3	2.1	3.3	t	t	0.5	-	-	2.2	0.7	-	2.2	6.8	5.1	5.7	-	-	-	-	-	3.3	
		S.D	0.6	0.4	0.6	3.2	4.7	0.1	-	0.2	-	-	1.4	0.6	-	1.9	3.5	3.3	3.7	-	-	-	-	-		176.3
	J	Mean	1.3	0.9	2.2	5.5	2.8	-	t	0.3	-	-	2.0	0.9	-	2.5	6.7	4.7	5.4	-	-	-	-	-	3.4	
		S.D	0.7	1.1	0.7	4.2	1.9	-	0.1	0.3	-	-	1.7	1.1	-	2.2	5.0	3.7	4.2	-	-	-	-	-		445.4
E. radiata	A	Mean	1.7	0.3	1.1	10.1	3.3	-	t	0.4	-	-	1.2	0.6	-	1.0	1.7	1.7	1.3	-	-	-	-	-	0.4	
	J	Mean	0.7	0.5	1.7	33.3	1.3	-	t	0.6	-	-	1.5	0.8	-	0.7	0.2	0.9	0.3	-	-	-	-	-	0.8	
E. coccifera	A	Mean	1.5	0.4	1.3	0.7	2.8	t	t	0.4	-	1.5	3.2	1.6	t	3.0	12.7	10.0	11.7	-	-	-	-	-	2.1	
		S.D	0.5	0.2	0.8	0.5	0.4	0.1	0.1	0.3	-	1.7	1.4	1.3	-	3.2	7.1	4.8	5.8	-	-	-	-	-		216.8
	J	Mean	0.9	1.4	3.8	2.7	2.2	0.2	t	0.2	-	0.4	4.4	2.5	-	8.9	6.0	5.0	4.7	-	-	-	-	-	1.2	
		S.D	0.5	1.4	2.2	2.4	2.0	0.2	-	0.2	-	1.0	3.3	2.4	-	15.4	6.0	3.8	4.9	-	-	-	-	-		625.1

Table 4.5. (B) Subgenus *Symphyomyrtus*: Part 1- components 1 to 24

Species	Leaf type	No. Pop.	Statistics	1 αP	2 αTh	3 Ca	4 βP	5 S	6 αPh	7 αT	8 Lim	9 βPh	10 Ci	11 eO	12 γT	13 tO	14 Cy	15 Te	16 Lin	17 tM	18 Pi	19 Ca	20 Aro	21 T4	22 cM	23 All	24 tPi
Series *Ovatae*																											
E. barberi	A	2	Mean	2.4	-	t	t	-	0.2	0.2	7.1	-	72.4	0.6	0.6	t	0.7	0.2	0.1	-	-	-	0.2	0.3	-	0.1	-
			S.D	0.1	-	-	-	-	0.1	0.1	0.7	-	1.1	0.1	0.2	0.1	0.2	0.1	0.1	-	-	-	0.2	0.2	-	-	-
	J	2	Mean	2.7	-	-	-	-	0.2	-	6.5	-	70.6	0.9	0.7	-	0.8	0.3	0.9	t	-	-	t	0.2	-	t	-
			S.D	0.5	-	-	-	-	0.2	-	0.2	-	4.9	0.4	0.1	-	-	0.1	1.3	-	-	-	-	0.1	-	-	-
E. brookeriana	A	3	Mean	18.3	-	t	0.1	t	5.7	0.4	4.7	0.3	52.8	0.3	0.9	t	4.2	0.3	t	0.1	0.5	-	0.1	0.4	0.1	0.2	0.3
			S.D	5.5	-	0.1	-	0.1	9.9	0.3	0.4	0.6	15.3	0.1	0.7	0.1	5.3	0.2	0.1	0.2	0.4	-	-	0.3	0.2	0.1	0.1
	J	3	Mean	19.1	-	t	0.2	t	3.6	0.1	5.2	0.3	51.9	0.4	1.2	-	5.5	0.2	t	t	0.2	t	t	0.5	t	-	t
			S.D	6.2	-	-	-	-	6.0	0.2	0.8	0.5	12.2	0.1	0.2	-	6.2	0.1	0.1	0.1	0.1	0.1	-	0.2	-	-	-
E. ovata	A	6	Mean	11.3	0.1	t	0.2	-	1.3	0.1	3.0	0.9	23.2	0.9	1.9	0.3	6.1	0.3	13.1	t	0.2	t	1.4	1.1	0.2	0.3	0.5
			S.D	13.3	0.3	0.1	0.1	-	1.9	0.1	2.3	2.1	26.5	0.8	2.4	0.3	6.1	0.1	15.4	0.1	0.3	0.1	1.8	1.4	0.3	0.3	1.0
	J	6	Mean	9.9	0.1	t	0.1	-	1.0	0.2	2.6	0.3	22.5	0.8	2.1	0.4	5.8	0.3	17.5	t	0.2	t	0.9	1.2	0.2	0.5	0.5
			S.D	12.7	0.3	-	0.1	-	1.3	0.3	2.0	0.7	26.9	0.7	2.4	0.3	5.9	0.2	20.2	-	0.3	0.1	1.3	2.0	0.3	0.5	0.7
E. rodwayi	A	4	Mean	17.9	-	0.1	0.2	-	1.7	0.3	5.3	0.8	59.6	0.2	0.2	t	1.3	0.3	t	0.7	0.2	t	-	0.5	0.5	t	0.6
			S.D	4.1	-	-	-	-	3.0	0.6	0.8	1.6	7.6	0.1	0.3	0.1	1.6	0.2	0.1	1.2	0.1	-	-	0.5	0.9	0.1	0.3
	J	4	Mean	15.6	-	t	0.2	-	0.2	-	5.9	-	66.1	0.2	0.5	t	0.5	0.2	t	t	0.2	t	t	0.3	-	t	0.5
			S.D	3.2	-	0.1	0.1	-	0.2	-	0.6	-	3.2	0.1	0.1	0.1	0.1	-	-	0.1	0.1	-	-	-	-	-	0.3
Series *Viminales*																											
E. globulus	A	6	Mean	17.7	-	0.1	0.4	t	0.2	t	3.9	t	54.2	0.3	0.2	0.1	0.8	0.1	t	-	1.2	0.9	1.4	0.2	t	0.6	2.5
			S.D	6.7	-	0.1	0.1	-	0.2	-	1.3	-	4.4	0.2	0.2	0.1	0.4	-	-	-	0.9	1.2	0.8	0.1	0.1	0.4	1.5
	J	6	Mean	19.9	-	t	0.5	-	0.4	t	5.4	t	56.4	0.3	0.2	t	0.4	0.2	t	-	0.2	0.3	3.0	0.3	t	0.6	0.4
			S.D	0.9	-	0.1	0.1	-	0.3	-	0.7	-	6.2	0.2	0.2	0.1	0.3	0.1	0.1	-	0.1	0.2	3.0	0.1	0.2	0.2	0.2
E. vernicosa	A	4	Mean	18.0	-	0.2	0.4	-	-	-	5.6	-	62.2	0.2	2.0	t	1.8	0.2	0.1	-	0.3	0.1	0.1	0.4	t	t	0.6
			S.D	2.2	-	0.1	0.1	-	-	-	1.6	-	1.6	0.3	0.3	0.1	0.3	0.1	-	-	0.1	0.2	0.2	-	-	-	0.2
	J	3	Mean	18.6	-	0.3	0.4	t	-	-	7.2	t	58.1	t	1.9	t	2.4	t	0.2	t	0.2	t	t	0.5	t	-	0.7
			S.D	4.0	-	-	0.1	0.1	-	-	0.3	-	3.0	0.1	0.7	0.1	0.4	-	0.1	-	0.1	-	-	0.1	-	-	0.2
E. subcrenulata	A	5	Mean	15.4	-	0.1	0.3	t	1.4	t	4.6	0.2	47.6	0.3	2.9	t	4.3	0.3	0.2	t	0.4	t	0.1	0.5	0.2	0.2	0.8
			S.D	1.7	-	-	-	-	1.4	0.1	1.3	0.4	15.4	0.2	2.9	-	3.0	0.2	0.1	0.1	0.2	-	0.1	0.1	0.2	0.2	0.3
	J	4	Mean	15.9	-	t	0.3	-	1.5	t	5.3	0.2	52.6	0.2	1.6	t	2.7	0.2	0.1	t	0.4	t	0.2	0.5	t	0.2	0.9
			S.D	0.7	-	-	0.1	-	1.5	0.1	1.4	0.2	12.6	0.1	0.9	-	2.6	0.1	-	0.1	0.1	0.1	0.2	-	0.1	0.3	0.3
E. johnstonii	A	5	Mean	19.3	-	0.1	0.3	t	0.2	-	5.8	-	61.8	0.1	0.7	t	0.6	0.1	t	t	0.4	t	0.1	0.4	t	t	0.9
			S.D	2.9	-	0.1	0.1	-	0.2	-	0.4	-	1.9	0.2	0.2	-	0.2	-	-	-	0.2	-	0.2	0.1	-	0.1	0.3
	J	3	Mean	18.0	-	0.2	0.3	-	0.3	-	6.5	-	59.9	0.4	1.4	-	0.7	0.2	t	-	0.3	t	0.1	0.4	t	t	0.6
			S.D	2.3	-	0.1	-	-	0.3	-	0.1	-	0.6	0.2	0.6	-	0.5	-	0.1	-	0.1	-	0.2	0.2	0.1	-	0.2
E. viminalis	A	6	Mean	8.6	-	t	0.2	t	4.2	0.2	5.2	t	49.6	0.6	1.3	t	4.1	0.2	0.2	0.2	t	0.3	1.3	1.3	0.3	0.5	0.1
			S.D	3.3	-	-	0.1	0.1	8.3	0.3	1.1	0.1	12.3	0.6	1.0	0.1	2.8	0.1	0.1	0.2	0.1	0.2	1.3	1.2	0.3	0.3	0.1
	J	6	Mean	9.3	-	t	0.3	-	3.5	0.1	5.9	0.2	55.5	0.4	0.7	t	3.0	0.2	0.1	t	0.2	t	0.9	0.8	0.2	0.3	0.3
			S.D	5.2	-	-	0.1	-	7.6	0.3	0.9	0.4	14.1	0.3	0.7	0.1	2.3	0.1	0.1	0.1	0.2	0.1	1.5	0.9	0.1	0.3	0.2
E. dalrympleana	A	3	Mean	5.1	1.4	t	0.2	t	5.4	0.1	0.9	0.8	3.1	0.5	0.3	0.2	20.0	0.3	0.3	0.2	t	0.2	0.6	0.9	0.2	1.0	-
			S.D	1.7	0.3	-	-	0.1	1.6	-	0.3	0.2	2.3	0.4	0.3	0.4	1.5	-	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	-
	J	3	Mean	9.2	0.2	t	0.8	t	9.1	0.2	2.8	3.9	17.7	0.6	0.5	t	14.5	0.5	0.2	0.2	-	t	0.4	1.0	0.2	0.4	-
			S.D	3.0	0.4	0.1	0.9	-	3.8	-	1.0	5.4	15.2	0.1	0.2	-	2.5	-	0.1	0.1	-	0.1	0.2	0.2	0.1	0.3	-
E. rubida	A	2	Mean	11.1	-	t	0.2	-	1.7	-	4.4	-	45.7	0.4	0.9	t	5.0	0.2	0.1	t	0.2	0.1	t	0.6	t	0.2	0.7
			S.D	6.5	-	-	-	-	1.8	-	1.0	-	13.7	0.1	1.0	0.1	4.4	-	-	-	0.2	0.2	0.1	0.4	-	0.2	0.3
	J	2	Mean	13.3	-	t	0.3	-	1.6	t	6.0	-	51.0	0.2	1.8	-	1.8	0.2	0.2	-	0.4	t	0.3	1.0	t	t	0.7
			S.D	4.6	-	0.1	0.1	-	0.7	-	0.6	-	2.7	0.2	2.3	-	0.7	0.1	0.2	-	0.3	0.1	0.4	0.9	-	0.1	0.4
E. gunnii	A	6	Mean	16.1	0.2	t	0.3	t	1.9	t	3.9	0.7	38.1	1.0	0.7	t	7.4	0.2	0.1	t	0.2	0.1	0.2	0.6	0.1	0.3	0.7
			S.D	4.3	0.4	-	0.1	0.1	2.1	0.1	1.9	1.1	20.9	1.5	0.9	-	6.8	0.1	0.1	-	0.2	0.1	0.2	0.4	0.1	0.3	0.5
	J	6	Mean	16.2	t	t	0.2	t	3.7	0.1	3.7	0.4	30.9	1.0	0.5	t	9.2	0.3	0.1	t	0.1	t	0.2	0.6	t	0.4	0.3
			S.D	6.6	0.2	-	0.1	0.1	3.9	0.1	2.5	0.7	25.9	1.6	0.2	-	9.2	0.1	0.1	0.1	0.2	0.1	0.1	0.5	0.2	0.5	0.3
E. archeri	A	3	Mean	18.6	-	0.2	0.3	t	t	-	4.2	-	60.3	0.3	0.2	t	0.8	0.1	t	-	0.9	0.2	t	0.2	t	t	2.0
			S.D	0.6	-	0.2	0.2	-	0.1	-	1.0	-	2.1	0.1	0.2	-	0.1	-	-	-	0.4	0.1	0.1	0.1	-	-	1.1
	J	3	Mean	19.7	-	0.1	0.4	-	0.1	t	6.1	-	59.9	0.2	0.6	-	0.9	0.2	0.1	-	0.5	0.1	t	0.3	-	-	1.1
			S.D	1.8	-	0.1	0.1	-	0.1	-	0.7	-	2.9	0.2	0.5	-	0.3	0.1	0.1	-	0.3	0.1	0.1	0.1	-	-	0.7
E. morrisbyi	A	2	Mean	8.0	-	t	0.1	-	0.2	-	5.7	-	66.6	0.5	1.0	t	1.8	0.1	t	-	0.3	-	t	0.5	t	t	0.8
			S.D	1.0	-	0.1	-	-	-	-	0.5	-	2.6	0.1	0.3	-	0.8	-	-	-	0.1	-	-	-	-	-	0.1
	J	2	Mean	10.3	-	t	0.2	-	0.9	-	6.8	-	61.5	0.5	1.9	-	1.3	0.2	0.1	-	0.2	-	t	0.6	t	0.1	0.5
			S.D	2.0	-	-	0.1	-	0.7	-	0.2	-	6.2	0.1	0.2	-	0.5	-	0.1	-	0.1	-	0.1	0.2	-	0.2	0.2
E. urnigera	A	4	Mean	16.1	-	0.1	0.2	t	0.4	t	5.1	0.9	55.8	0.5	0.5	0.3	1.7	0.3	0.2	t	0.3	0.1	t	0.4	t	t	0.7
			S.D	3.9	-	0.1	0.1	0.1	0.4	0.1	1.9	1.9	7.6	0.4	0.5	0.4	2.1	0.1	-	-	0.1	0.1	0.1	-	-	-	0.3
	J	4	Mean	17.4	-	t	0.2	t	0.4	t	5.0	0.7	57.2	0.5	0.6	0.2	1.3	0.2	0.1	t	0.4	t	t	0.4	t	t	1.0
			S.D	2.9	-	-	-	0.1	0.6	-	0.8	1.4	5.3	0.2	0.2	0.3	1.1	0.1	-	-	0.1	0.1	0.1	0.1	-	-	0.3
E. perriniana	A	1	Mean	11.3	-	t	0.4	0.2	0.2	-	4.8	t	65.5	0.2	0.1	0.1	0.6	t	-	-	0.4	0.1	-	0.2	t	0.2	1.9
	J	1	Mean	11.9	-	0.4	0.4	0.4	-	-	6.8	t	68.5	0.2	0.3	-	0.5	-	-	-	0.1	-	-	0.2	t	-	0.9
E. cordata	A	5	Mean	15.5	-	t	0.2	-	0.2	-	6.0	-	63.5	0.3	0.5	0.1	0.6	0.2	t	-	0.5	-	t	0.3	t	t	1.0
			S.D	3.8	-	-	0.1	-	0.1	-	0.6	-	4.4	0.2	0.2	0.1	0.3	-	-	-	0.2	-	-	0.1	-	-	0.2
	J	5	Mean	10.2	-	t	0.2	t	0.6	t	6.9	-	63.7	0.3	0.7	t	0.5	0.2	0.1	-	0.3	-	-	0.7	t	-	0.8
			S.D	6.0	-	0.1	0.2	0.1	0.7	0.2	0.9	-	3.9	0.2	0.3	0.1	0.3	-	0.1	-	0.2	-	-	0.6	0.1	-	0.3

Table 4.5. (B) Subgenus *Symphyomyrtus*: Part 2- components 25 to 46

			25 cPip	26 TA	27 αTe	28 Pip	29 tPip	30 Cit	31 eS	32 Cyd	33 Ner	34 El	35 Gl	36 Vir	37 MC	38 Sp	39 αE	40 γE	41 βE	42 Ta1	43 Ta2	44 Ta3	45 Ta4	46 Ta5	Un	ΣV
Series *Ovatae*																										
E. barberi	A	Mean	t	7.5	2.9	t	0.3	-	-	-	-	-	0.5	t	-	0.1	t	t	t	-	-	-	-	-	3.3	
		S.D	0.1	0.2	0.5	0.1	0.1	-	-	-	-	-	0.1	-	-	0.1	0.1	0.1	0.1	-	-	-	-	-		2.2
	J	Mean	t	7.1	2.7	-	0.2	-	-	-	-	-	0.8	0.2	-	0.1	0.1	-	0.1	-	-	-	-	-	4.8	
		S.D	0.1	1.0	0.2	-	0.2	-	-	-	-	-	0.7	0.2	-	0.2	0.1	-	0.2	-	-	-	-	-		27.8
E. brookeriana	A	Mean	t	0.2	3.2	t	0.2	-	t	t	-	-	0.2	t	-	0.2	0.2	0.1	0.2	-	-	-	-	-	6.3	
		S.D	0.1	0.2	0.5	-	0.1	-	-	0.1	-	-	0.2	0.1	-	0.2	0.1	0.1	0.1	-	-	-	-	-		393.2
	J	Mean	0.1	0.9	3.7	t	0.2	-	-	0.3	-	-	0.2	0.2	-	t	0.2	0.2	0.2	-	-	-	-	-	5.4	
		S.D	0.1	0.6	0.4	-	-	-	-	0.3	-	-	0.2	0.1	-	0.1	0.1	0.1	0.1	-	-	-	-	-		264.1
E. ovata	A	Mean	0.2	1.2	3.0	0.3	0.6	0.2	-	t	6.4	-	5.4	1.5	-	5.2	0.4	1.2	0.7	-	-	-	-	-	7.9	
		S.D	0.4	1.7	1.7	0.3	0.8	0.2	-	0.1	7.7	-	6.9	1.8	-	9.6	0.4	1.4	0.7	-	-	-	-	-		1390.8
	J	Mean	t	0.8	2.7	0.3	0.5	0.1	t	0.1	7.8	-	4.8	1.2	-	5.1	0.6	1.0	1.2	-	-	-	-	-	7.8	
		S.D	0.1	0.9	1.5	0.3	0.8	0.1	-	0.2	7.5	-	3.8	1.3	-	10.3	0.7	1.4	1.6	-	-	-	-	-		1531.0
E. rodwayi	A	Mean	0.2	0.6	2.7	0.3	0.3	-	-	-	-	-	0.6	0.2	-	0.2	0.1	0.1	0.2	-	-	-	-	-	5.0	
		S.D	0.3	0.4	0.2	0.2	0.4	-	-	-	-	-	0.4	0.1	-	0.2	0.1	-	-	-	-	-	-	-		93.4
	J	Mean	t	0.9	3.4	0.1	t	-	-	-	-	-	0.6	0.1	-	0.3	t	0.1	0.2	-	-	-	-	-	4.5	
		S.D	0.1	0.8	0.9	0.1	0.1	-	-	-	-	-	0.3	0.1	-	0.2	0.1	0.1	0.1	-	-	-	-	-		22.4
Series *Viminales*																										
E. globulus	A	Mean	t	1.4	1.4	0.1	0.2	-	-	t	-	-	4.6	0.7	-	0.4	0.3	0.3	0.8	-	-	-	-	-	8.1	
		S.D	0.1	1.3	0.6	0.1	0.1	-	-	-	-	-	1.8	0.2	-	0.2	0.4	0.2	1.4	-	-	-	-	-		78.1
	J	Mean	0.1	1.7	2.6	0.2	0.2	-	-	t	-	-	2.9	0.5	-	0.1	0.1	0.1	0.2	-	-	-	-	-	5.9	
		S.D	0.1	1.2	1.0	0.4	0.2	-	-	-	-	-	1.7	0.3	-	0.1	0.1	0.1	0.2	-	-	-	-	-		55.3
E. vernicosa	A	Mean	0.1	0.2	2.5	t	0.1	-	-	-	-	-	0.6	t	-	0.1	0.1	t	0.2	-	-	-	-	-	5.0	
		S.D	0.1	0.1	0.2	0.1	-	-	-	-	-	-	0.2	0.1	-	0.1	0.1	-	-	-	-	-	-	-		10.5
	J	Mean	t	0.4	2.5	0.1	0.2	-	t	-	-	-	0.5	0.1	-	0.1	0.2	0.1	0.3	-	-	-	-	-	5.8	
		S.D	0.1	0.4	0.8	-	0.1	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	-	-	-	-	-		26.4
E. subcrenulata	A	Mean	0.3	0.4	2.5	0.1	0.2	-	-	-	-	-	3.3	1.3	-	6.1	0.3	0.5	0.4	-	-	-	-	-	6.1	
		S.D	0.3	0.4	2.6	0.1	0.2	-	-	-	-	-	2.3	1.1	-	6.2	0.3	0.7	0.4	-	-	-	-	-		315.0
	J	Mean	0.1	0.3	3.7	0.1	0.2	-	-	-	-	-	2.6	0.9	-	2.9	0.3	0.5	0.6	-	-	-	-	-	5.9	
		S.D	0.1	0.3	2.0	0.1	0.1	-	-	-	-	-	2.4	1.0	-	3.8	0.4	0.5	0.9	-	-	-	-	-		197.7
E. johnstonii	A	Mean	0.2	0.8	2.5	0.1	0.1	-	-	-	-	-	1.0	0.2	-	t	t	t	t	-	-	-	-	-	5.2	
		S.D	-	1.1	0.6	0.1	0.1	-	-	-	-	-	0.9	0.1	-	0.1	-	-	-	-	-	-	-	-		14.8
	J	Mean	0.2	1.8	2.9	0.1	0.2	-	-	-	-	-	1.4	0.2	-	-	0.1	t	0.1	-	-	-	-	-	4.9	
		S.D	-	2.4	0.7	-	0.1	-	-	-	-	-	0.8	0.1	-	-	-	0.1	0.1	-	-	-	-	-		13.8
E. viminalis	A	Mean	0.2	1.3	2.7	0.1	0.2	-	t	-	-	-	6.6	1.0	-	0.9	0.4	0.6	0.7	-	-	-	-	-	3.5	
		S.D	0.1	1.4	1.0	0.1	0.1	-	0.1	-	-	-	3.8	0.5	-	0.4	0.5	0.7	0.8	-	-	-	-	-		264.2
	J	Mean	0.1	2.1	2.5	0.1	0.1	-	-	-	-	-	5.9	1.0	-	0.6	0.3	0.5	0.6	-	-	-	-	-	5.6	
		S.D	0.1	2.2	0.8	0.1	0.1	-	-	-	-	-	4.0	0.6	-	0.4	0.2	0.3	0.5	-	-	-	-	-		318.1
E. dalrympleana	A	Mean	-	0.3	0.9	0.2	0.2	-	t	-	-	-	10.7	7.8	-	31.6	1.1	5.1	0.9	-	-	-	-	-	5.6	
		S.D	-	-	0.3	-	0.1	-	-	-	-	-	0.8	1.0	-	7.1	0.3	0.4	0.1	-	-	-	-	-		66.8
	J	Mean	0.1	1.1	2.3	2.4	0.2	-	-	-	-	-	6.2	3.8	-	16.3	0.3	2.8	1.2	-	-	-	-	-	6.9	
		S.D	0.1	1.1	0.5	3.7	0.1	-	-	-	-	-	0.4	0.5	-	2.6	0.1	1.3	1.1	-	-	-	-	-		318.9
E. rubida	A	Mean	0.1	3.2	5.6	2.9	t	-	-	-	-	-	2.2	1.5	-	5.7	0.1	1.0	0.3	-	-	-	-	-	6.7	
		S.D	0.1	3.3	2.4	4.0	0.1	-	-	-	-	-	2.1	1.5	-	4.8	-	1.0	0.1	-	-	-	-	-		318.2
	J	Mean	-	4.0	7.1	1.3	t	-	-	-	-	-	1.5	0.8	-	1.9	0.1	0.5	0.3	-	-	-	-	-	5.1	
		S.D	-	4.5	3.3	1.6	0.1	-	-	-	-	-	0.9	0.5	-	0.3	0.1	0.1	0.1	-	-	-	-	-		71.4
E. gunnii	A	Mean	0.2	1.0	3.0	0.3	t	-	t	-	-	-	3.6	2.2	-	9.7	0.4	1.1	1.1	-	-	-	-	-	5.8	
		S.D	0.1	0.9	1.5	0.4	0.1	-	0.1	-	-	-	4.0	2.8	-	10.7	0.4	0.9	1.6	-	-	-	-	-		656.3
	J	Mean	0.2	1.5	2.2	0.4	0.2	-	t	-	-	-	4.6	2.7	-	12.4	0.6	1.5	1.5	-	-	-	-	-	4.6	
		S.D	0.2	1.1	2.2	0.6	0.3	-	-	-	-	-	4.0	2.6	-	12.6	0.5	1.4	1.9	-	-	-	-	-		1019.6
E. archeri	A	Mean	-	0.9	3.0	0.2	0.1	-	-	-	-	-	1.2	0.3	-	0.7	0.1	t	0.2	-	-	-	-	-	8.0	
		S.D	-	0.6	1.1	0.1	0.1	-	-	-	-	-	0.7	0.1	-	0.3	0.1	-	0.2	-	-	-	-	-		9.4
	J	Mean	0.1	1.4	3.6	0.2	0.1	-	-	-	-	-	0.9	0.3	-	0.6	0.1	0.1	0.2	-	-	-	-	-	4.0	
		S.D	0.1	1.0	1.0	0.1	0.1	-	-	-	-	-	0.3	0.1	-	0.2	-	-	0.1	-	-	-	-	-		15.1
E. morrisbyi	A	Mean	t	3.5	5.2	0.1	0.3	-	-	-	-	-	0.5	0.2	-	0.6	0.1	0.2	0.2	-	-	-	-	-	4.4	
		S.D	0.1	4.8	3.4	-	0.2	-	-	-	-	-	0.1	0.1	-	0.6	0.1	0.1	0.1	-	-	-	-	-		43.5
	J	Mean	0.1	3.8	5.9	t	0.3	-	-	-	-	-	0.7	0.3	-	0.7	0.1	0.3	0.2	-	-	-	-	-	3.1	
		S.D	0.2	4.9	3.9	0.1	0.3	-	-	-	-	-	0.4	0.2	-	0.4	-	0.2	-	-	-	-	-	-		83.3
E. urnigera	A	Mean	0.1	4.2	3.4	0.1	0.1	-	t	-	-	-	1.1	0.4	-	1.3	0.1	0.2	0.2	-	-	-	-	-	6.3	
		S.D	0.1	1.6	0.2	-	-	-	-	-	-	-	0.8	0.4	-	1.8	0.1	0.2	0.1	-	-	-	-	-		92.3
	J	Mean	0.3	2.1	3.9	t	0.1	-	t	-	-	-	1.3	0.3	-	1.0	0.1	0.1	0.2	-	-	-	-	-	6.0	
		S.D	0.4	0.8	1.0	-	0.1	-	-	-	-	-	0.4	0.3	-	1.3	0.1	0.1	0.2	-	-	-	-	-		44.6
E. perriniana	A	Mean	-	1.9	1.6	0.2	0.1	-	t	-	-	-	1.7	0.4	-	t	0.1	0.1	0.2	-	-	-	-	-	9.7	
	J	Mean	-	0.9	1.2	0.1	-	-	-	-	-	-	1.1	0.3	-	t	0.1	0.3	0.5	-	-	-	-	-	6.4	
E. cordata	A	Mean	0.1	5.0	3.0	t	0.2	-	-	-	-	-	0.3	t	-	-	t	t	0.1	-	-	-	-	-	3.7	
		S.D	0.1	1.5	0.4	0.1	-	-	-	-	-	-	0.3	0.1	-	-	-	-	0.1	-	-	-	-	-		37.6
	J	Mean	0.2	7.8	3.4	t	0.6	-	t	-	-	-	0.2	t	-	t	0.1	0.1	0.2	-	-	-	-	-	2.9	
		S.D	0.1	3.4	0.6	0.1	0.9	-	0.1	-	-	-	0.1	0.1	-	0.1	0.2	0.1	-	-	-	-	-	-		66.0

percent occurring in variable amounts). However, the leaf oils of *E. ovata* consist of similar percentages of 1,8-cineole (22-23%) and linalool (13-18%) as the main constituents. The juvenile leaf oils of *E. dalrympleana* contained spathulenol (32%) and *p*-cymene (20%) and its adult leaf oils contained similar percentages (15-17%) of 1,8-cineole, *p*-cymene and spathulenol as major components.

In contrast, the chemical composition of the leaf oils from *Monocalyptus* species are characterized by a wide range of components with notable differences between species. Within the *Obliquae series*, *E. regnans, E. sieberi* and *E. pauciflora* produced leaf oils containing high contents of the sesquiterpene alcohols α–, β– and γ–eudesmol (components 39, 40, and 41) and variable amounts of monoterpenoids. The leaf oils of *E. obliqua* were characterized by high contents of the monoterpene hydrocarbon *p*-cymene (component 14) and the monoterpene ketone piperitone (component 28) and variable amounts of over ten monoterpenoids. *E. delegatensis* was characterized by similar proportions of the monoterpene hydrocarbon α– and β–phellandrene (components 6 and 9) and *p*-cymene, the monoterpene alcohols *trans*-, *cis-p*-menth-2-en-1-ol (components 17 and 22) and *trans*-, *cis*-piperitol (components 25 and 29). In the *Piperitae* series, the leaf oils of *E. risdonii*, *E. tenuiramis* and *E. pulchella* contained 1,8-cineole (component 10) as the principal component and variable contents of over ten components but the leaf oils of *E. amygdalina, E. nitida, E. radiata* and *E. coccifera* were characterized by variable amounts of over ten components which were similar to those of the *Obliquae species.*

There were some chemical components that only occurred within a number of *Monocalyptus* species. The aromatic components were found in *E. regnans, E. sieberi, E. tenuiramis, E. pulchella* and *E. amygdalina.* Regnanone (component 42) only occurred in the leaf oils of *E. regnans* (7.1% in adult leaf and 6.1% in juvenile leaf oils) and in trace amounts in *E. sieberi* . The leaf oils of *E. pulchella* have higher contents of 3,5-dimethyl conglomerone (component 43) (5.6% and 11.6% in adult and juvenile leaf oils respectively) and tasmanone (component 44) (1.5% and 1.9% in adult and juvenile leaf oils respectively). The leaf oils of *E. tenuiramis* and *E. amygdalina* had low (less than 1.1 percent) proportions of 3,5-dimethyl conglomerone and tasmanone.

The sesquiterpene alcohol elemol (component 34) was only found in leaf oils of *E. regnans* (4.4 % and 2.8 % in adult and juvenile leaf oils) and *E. coccifera* (1.5 % and 0.4 %). Pinocarvone and *trans*-pinocarveol (components 18 and 24) mainly occur in *Symphyomyrtus* species (trace to 2.5% in leaf oils) but were also found in one *Monocalyptus* species, *E. pauciflora* (trace to 0.2 %).

A long chain sesquiterpene, nerolidol, was found in adult and juvenile leaf oils of *E. ovata* at levels from 6.4 to 7.8 percent.

The distribution of oil components

The frequency distributions of the percentage representation of the main oil components were compared between subgenera and between juvenile and adult leaves (Fig. 4.2). Each individual sample (population) was treated as a separate entity. As indicated by Fig.4.2, the percentage content of most chemical components in both juvenile and adult leaf oils possess a wide range of variation but there was no major difference in frequency distributions of chemical components between juvenile and adult leaf oils within each subgenus. However, some chemical components exhibited markedly different frequency distributions in both percentage content and distribution pattern between the two subgenera.

The frequency distributions of most monoterpene hydrocarbon and alcohol compounds, such as α and β–phellandrene, α–terpinene, *p*-cymene, terpinolene, terpinen-4-ol (Fig.4.2-1), *trans*- and *cis*-menth-2-en-1-ol, *cis*- and *trans*-piperitol (Fig.4.2-2), were positively skewed and their percentage occurrence was significantly higher in the subgenus *Monocalyptus* compared to the *Symphyomyrtus* except for the two monoterpene hydrocarbons, γ–terpinene and limonene (Fig.4.2-1), and a monoterpene alcohol α–terpineol (Fig.4.2-2). The frequency distribution of the monoterpene ketone, piperitone (Fig. 4.2-3), and acyclic monoterpene hydrocarbons, *cis*- and *trans*–β–ocimene, and alcohol linalool (Fig.4.2-5) were also positively skewed and higher in *Monocalyptus* leaf oils. All sesquiterpene alcohols were positively skewed in both subgenera with the percentage distributions of α–, β– and γ–eudesmols being higher in *Monocalyptus* species and globulol, viridiflorol and spathulenol in *Symphyomyrtus* species being higher or similar to *Monocalyptus* species (Fig.4.2-6). In addition, one of the bicyclic monoterpene hydrocarbon, α–thujene, was also positively skewed with higher percentage contents in *Monocalyptus* species (Fig.4.2-4).

In contrast, some compounds had multimodal or Gaussian type distribution and their percentage occurrence was significantly higher in the subgenera *Symphyomyrtus* compared to the *Monocalyptus*. This was particularly evident in the frequency distributions of monoterpene ether 1,8-cineole (Fig.4.2-3), bicyclic monoterpene hydrocarbon α–pinene (Fig.4.2-4), monoterpene hydrocarbon limonene and alcohol α–terpineol. In the *Symphyomyrtus*, 1,8-cineole exhibited trimodality with modes at approximately 1-6%, 18-36%, 42-76% with the 42-76% mode approximating a Gaussian distribution in both adult and juvenile leaf oils. α–Pinene exhibited bimodality with modes at approximately 0.5-5% and in the range of 5-33%. Limonene also showed

(1) Monoterpene hydrocarbons

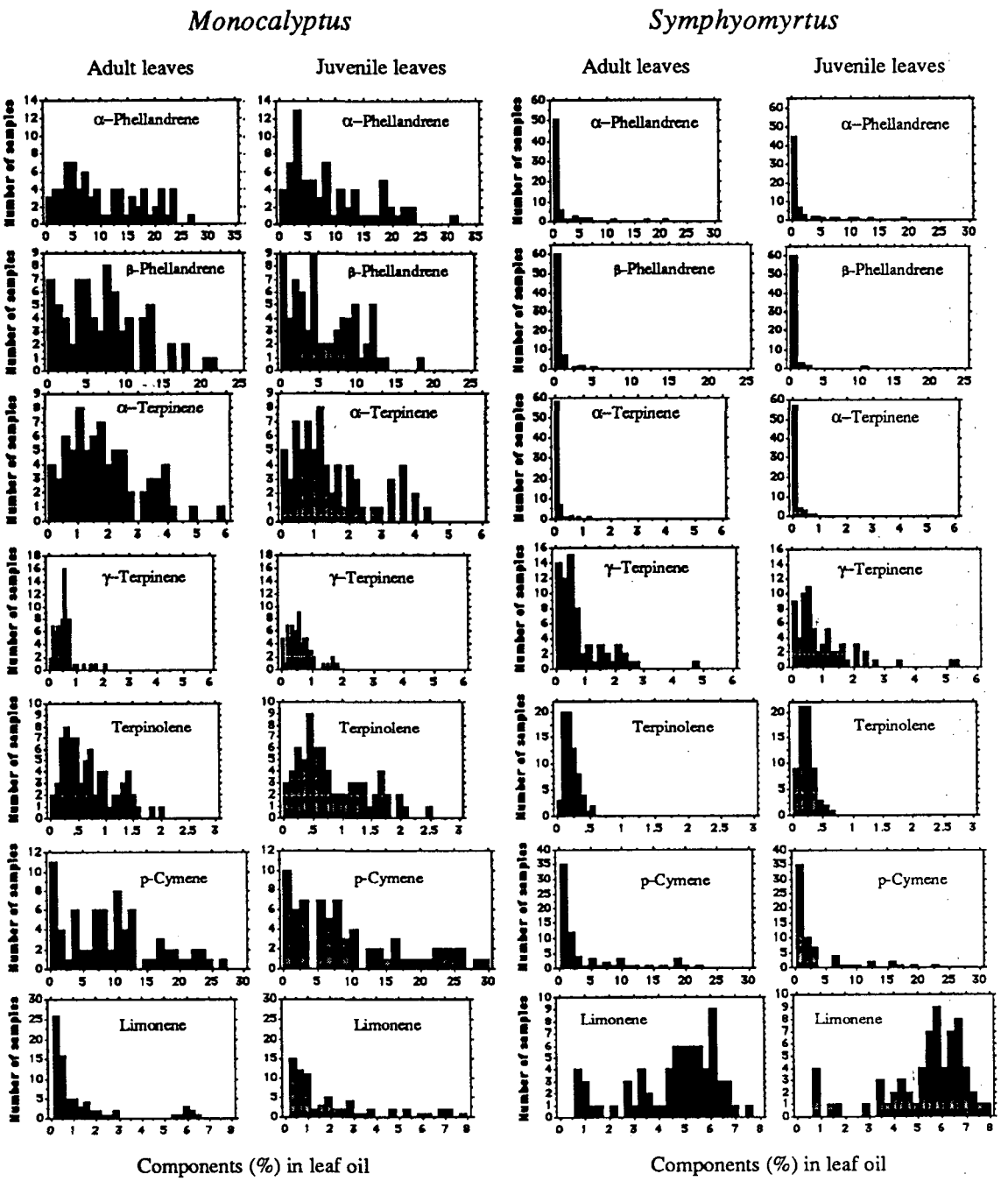


Fig.4.2. Frequency distributions of chemical components based on percentage composition of adult and juvenile leaf oils for *Monocalyptus* and *Symphyomyrtus* species. Vertical axis = Number of samples ; Horizontal axis = Content (%) in leaf oils.

(2) Monoterpene alcohols

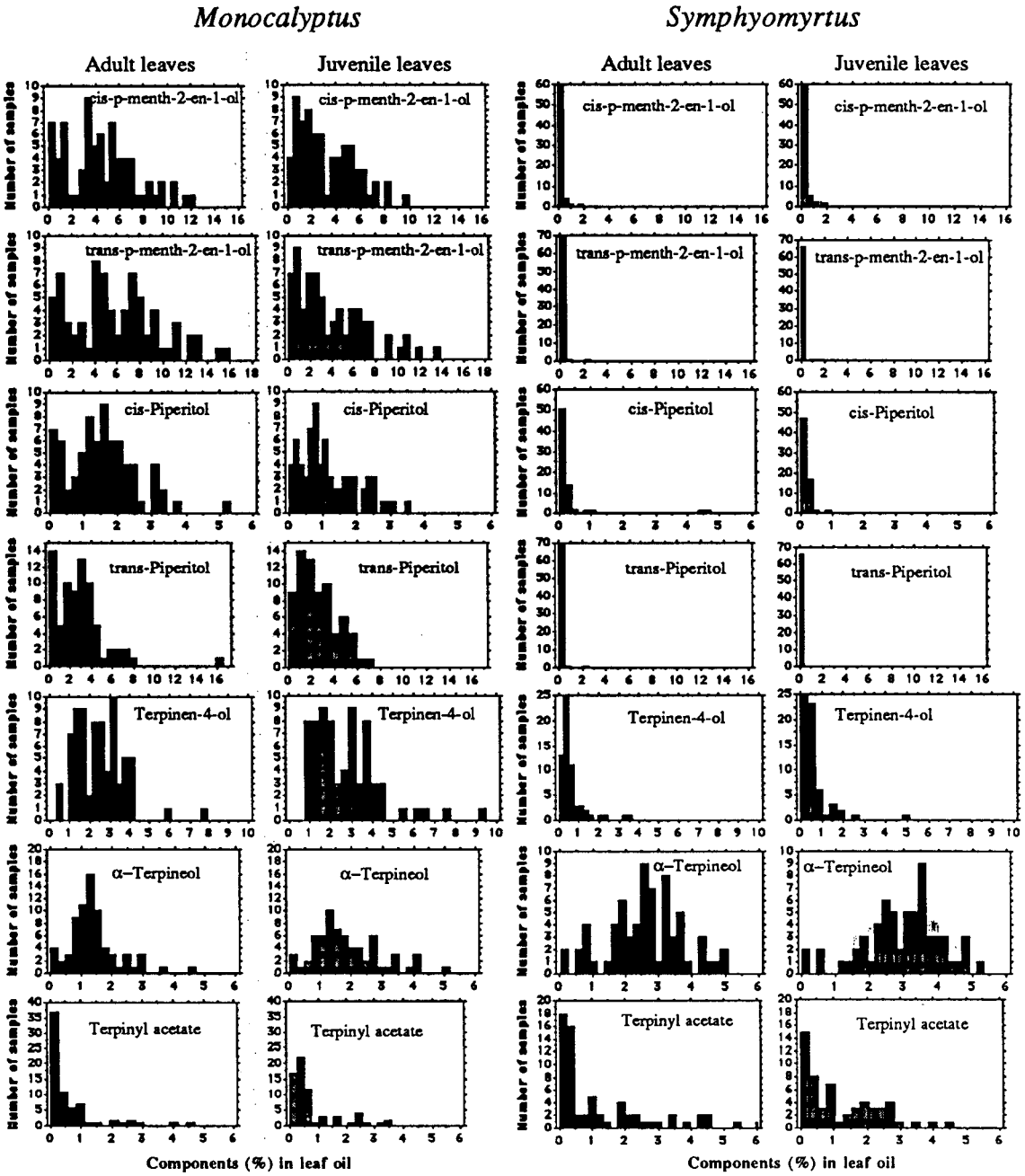
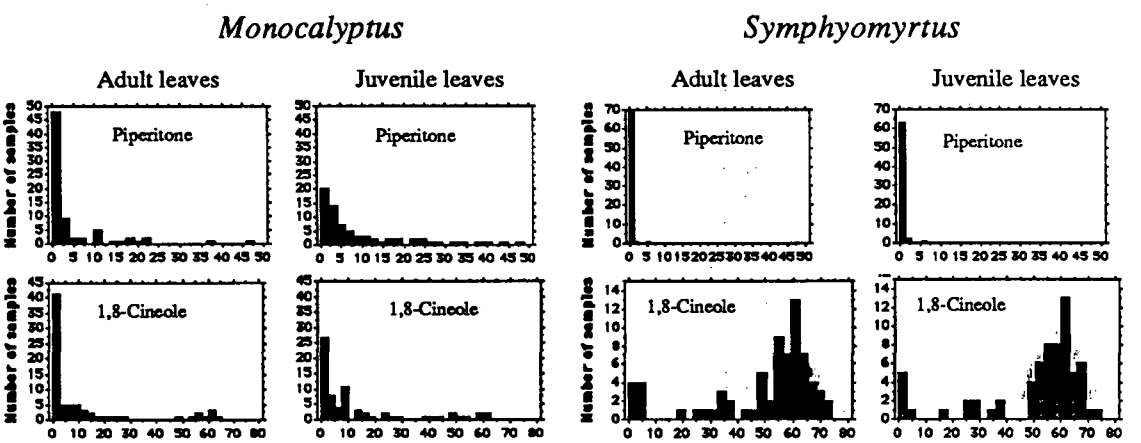
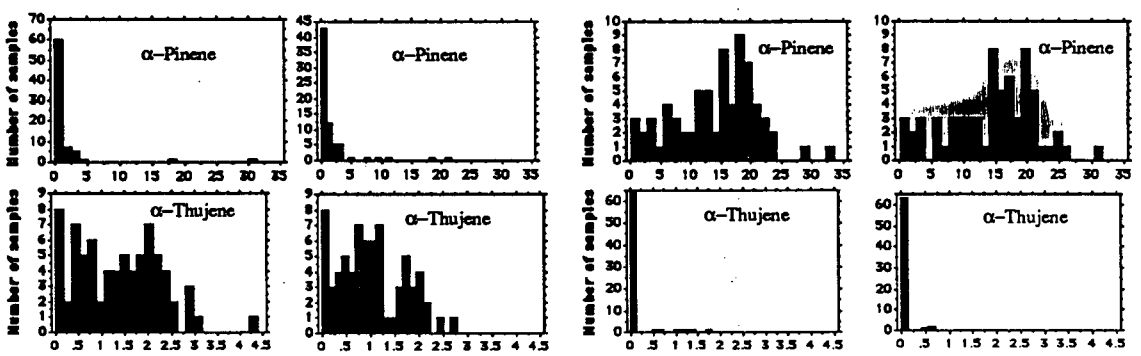


Fig.4.2. Frequency distributions of chemical components based on percentage composition of adult and juvenile leaf oils for *Monocalyptus* and *Symphyomyrtus* species. Vertical axis = Number of samples ; Horizontal axis = Content (%) in leaf oils.

(3) Monoterpene ketones and ether



(4) Bicyclic monoterpene hydrocarbons



(5) Acyclic monoterpene hydrocarbons and alcohols

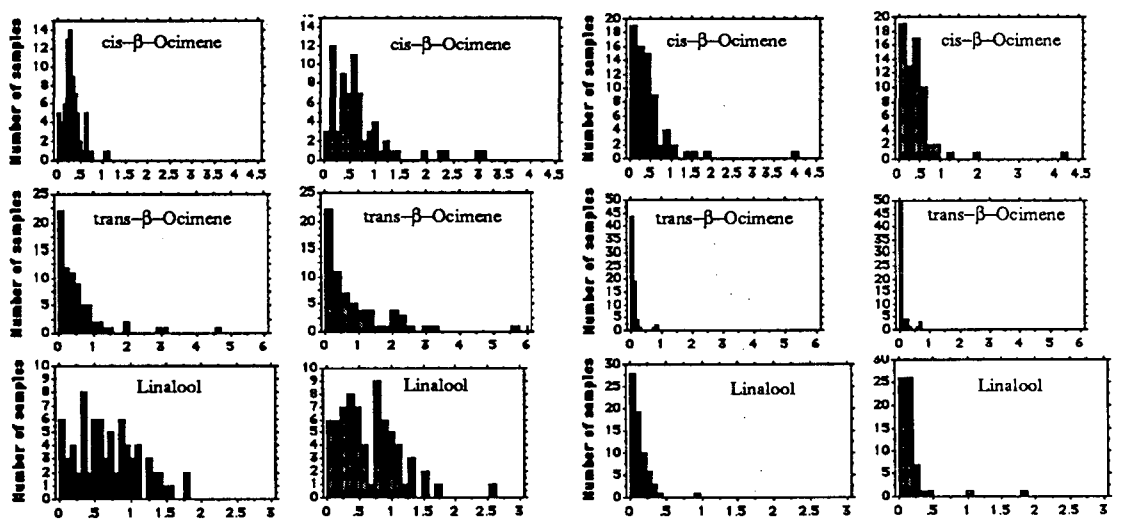


Fig.4.2. Frequency distributions of chemical components based on percentage composition of adult and juvenile leaf oils for *Monocalyptus* and *Symphyomyrtus* species. Vertical axis = Number of samples ; Horizontal axis = Content (%) in leaf oils.

(6) Sesquiterpene alcohols

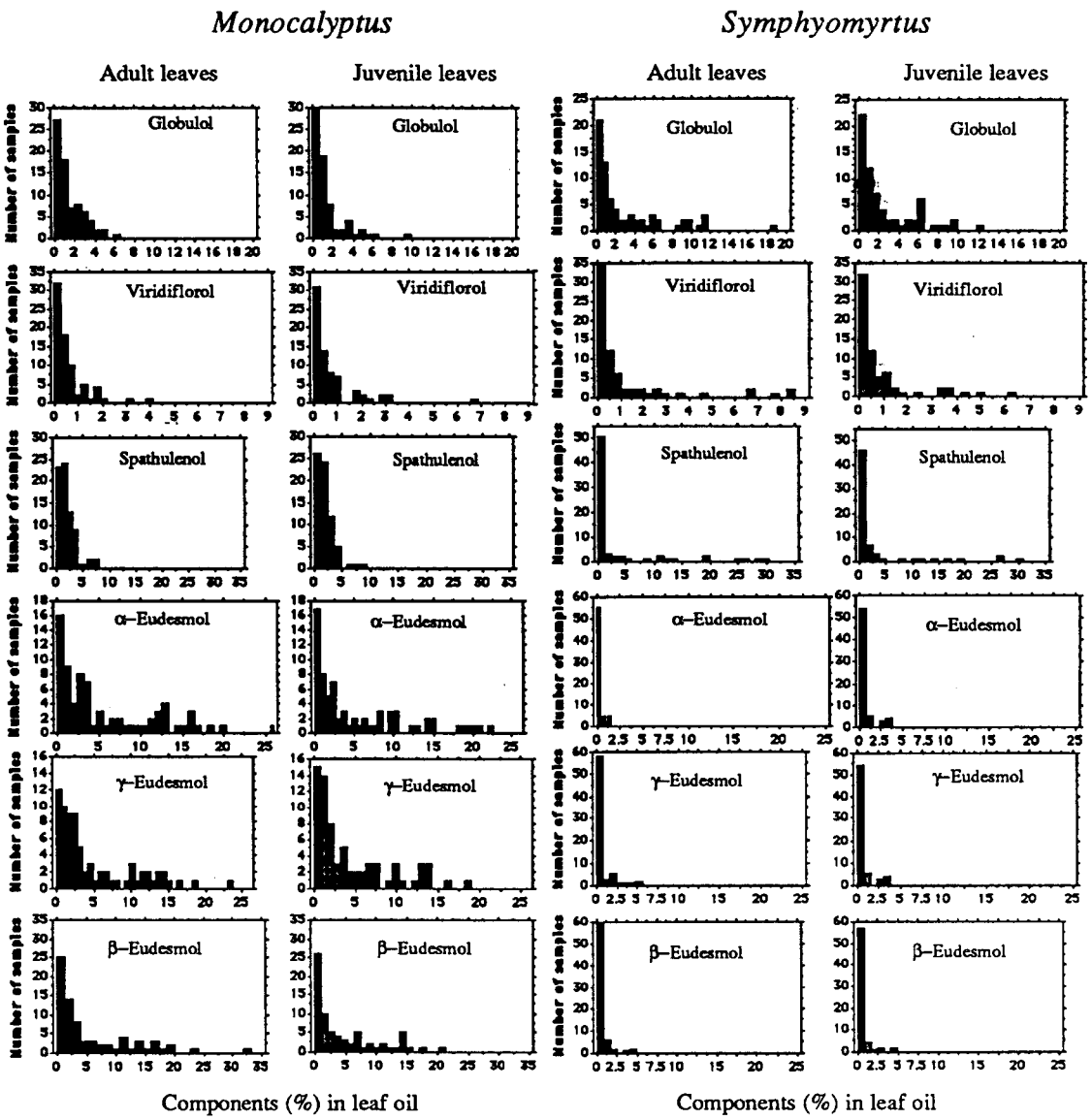


Fig.4.2. Frequency distributions of chemical components based on percentage composition of adult and juvenile leaf oils for *Monocalyptus* and *Symphyomyrtus* species. Vertical axis = Number of samples ; Horizontal axis = Content (%) in leaf oils.

trimodality with modes at approximately ranges 0.5-2%, 2-4% and 4-8% which also displayed an approximate Gaussian distribution in both leaf types. α–Terpineol exhibited a Gaussian distribution in the range from 0-5.5% with a peak at about 2.6%. However, in *Monocalyptus*, the distribution of these components was positively skewed with low contents in both juvenile and adult leaf oils. Only one compound, α–terpineol, had Gaussian distribution in leaf oils of *Monocalyptus* species.

Overall, the frequency distribution analysis indicated that the chemical composition of leaf oils of *Monocalyptus* populations were dominated by monoterpene hydrocarbons and alcohols and *Symphyomyrtus* species by the monoterpene ether, 1,8-cineole, and the bicyclic monoterpene hydrocarbon, α–pinene. The frequency distributions of most major components in leaf oils of *Monocalyptus* populations were positively skewed and frequency distributions of major components in *Symphyomyrtus* populations approximated a Gaussian distribution.

Tests for significance (*U*-test) for differences between subgenera and between series within a subgenus based on mean percentage values of oil components of species (Table 4.6) indicated that the proportions of most components were significantly different between the subgenera *Monocalyptus* and *Symphyomyrtus*.

All pinane skeleton bicyclic monoterpenoids, α– and β–pinene, camphene, pinocarvone and *trans*-pinocarveol were significantly higher in *Symphyomyrtus* species In contrast, all thujane skeleton bicyclic monoterpenoids of α–thujene, sabinene and sabinol were significantly higher in *Monocalyptus* species (see Table 4.6 for significance levels).

The mean percentage values of monoterpenoids 1,8-cineole, limonene, linalool and α–terpineol were significantly higher in *Symphyomyrtus* species while all other monocyclic monoterpenoids were significantly higher in *Monocalyptus*, with the exception of *cis*–β–ocimene, γ–terpinene and terpinyl acetate which did not differ between subgenera.

The tricyclic sesquiterpenoids, *b*-caryophyllene (component 19), aromadendrene (20) and *allo*-aromadendrene (23) had significantly higher mean percentage values in *Symphyomyrtus* species. In contrast, the bicyclic sesquiterpenoids α–, β– and γ–eudesmol (components 39-41) were significantly higher in *Monocalyptus* species. However, other sesquiterpenoids did not differ significantly between subgenera, although globulol, viridiflorol and spathulenol had relatively higher percentages in *Symphyomyrtus* and elemol was higher in *Monocalyptus*.

Table 4.6. Mann-Whitney*U*-test for significance of the difference in percentage contents of oil compounds between subgenera and series based on mean percentage values for individual species.
Significance of difference: p<0.001 = ***; 0.001<p<0.01 = **; 0.01<p<0.05 = *; 0.05<p =ns
- = Component absent.

	Difference between	*Monocalyptus/ Symphyomyrtus*		Series *Obliquae/Piperitae*		Series *Ovatae/Viminales*	
	Leaf types	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
	Number of species	12/17	12/17	5/7	5/7	4/13	4/13
		Δ(M-S) Sign.	Δ(M-S) Sign.	Δ(O-P) Sign.	Δ(O-P) Sign.	Δ(O-V) Sign.	Δ(O-V) Sign.
1	α–Pinene	-11.81 ***	-11.82 ***	1.20 ns	0.95 ns	-1.44 ns	-2.77 ns
2	α–Thujene	1.20 ***	0.98 ***	-0.35 ns	-0.30 ns	-0.09 ns	- ns
3	Camphene	-0.07 ***	-0.08 ***	- ns	- ns	-0.02 ns	-0.06 ns
4	β–Pinene	-0.12 *	-0.06 *	-0.01 ns	0.12 ns	-0.13 *	-0.23 **
5	Sabinene	0.09 **	0.15 **	0.03 ns	0.11 ns	-0.02 ns	-0.04 ns
6	α–Phellandrene	9.02 ***	7.43 ***	-4.32 ns	-3.78 ns	0.99 ns	-0.48 ns
7	α–Terpinene	1.67 ***	1.25 ***	-0.27 ns	0.15 ns	0.20 **	0.02 ns
8	Limonene	-2.99 ***	-3.06 ***	-1.61 ns	-2.19 ns	0.44 ns	-0.67 ns
9	β–Phellandrene	6.23 ***	4.49 ***	-0.97 ns	1.61 ns	0.29 ns	-0.27 ns
10	1,8-Cineole	-36.75 ***	-35.76 ***	-19.32 ns	-21.23 *	0.15 -	-0.51 ns
11	*cis*-β–Ocimene	-0.11 ns	0.22 ns	-0.01 ns	-0.18 ns	0.10 ns	0.24 ns
12	γ–Terpinene	-0.31 ns	-0.42 ns	-0.18 ns	-0.14 ns	0.02 ns	0.12 ns
13	*trans*-β–Ocimene	0.48 ***	1.17 ***	-0.40 ns	-1.50 *	0.03 ns	0.07 ns
14	*p*-Cymene	3.82 *	4.58 *	2.96 ns	4.27 ns	-0.75 ns	0.13 ns
15	Terpinolene	0.55 ***	0.63 ***	-0.28 ns	-0.35 ns	0.06 ns	0.02 ns
16	Linalool	-0.27 ***	-0.63 **	-0.15 ns	-0.07 ns	3.23 ns	4.50 ns
17	*trans-p*-Menth-2-en-1-ol	5.01 ***	2.92 ***	1.75 ns	1.47 ns	0.18 ns	- ns
18	Pinocarvone	-0.34 ***	-0.22 ***	0.02 ns	0.03 ns	-0.18 ns	-0.10 ns
19	b-Caryophellene	-0.13 ***	-0.06 ***	- -	- -	-0.16 *	-0.02 ns
20	Aromadendrene	-0.31 **	-0.32 **	-0.03 ns	-0.04 ns	0.12 ns	-0.16 ns
21	Terpinen-4-ol	1.81 ***	1.87 ***	-0.27 ns	0.32 ns	0.07 ns	0.01 ns
22	*cis-p*-Menth-2-en-1-ol	3.81 ***	2.35 ***	1.48 ns	0.94 ns	0.11 ns	-0.01 ns
23	*Allo*-aromadendrene	-0.19 **	-0.12 *	-0.06 ns	-0.04 ns	-0.08 ns	-0.04 ns
24	*trans*-Pinocarveol	-0.81 ***	-0.53 ***	0.02 ns	0.02 ns	-0.64 *	-0.36 ns
25	*cis*-Piperitol	1.28 ***	0.80 ***	0.72 ns	0.42 ns	0.02 ns	-0.04 ns
26	Terpinyl acetate	-0.69 ns	-0.46 ns	-1.13 ns	-2.19 *	0.51 ns	0.18 ns
27	α–Terpineol	-0.86 *	-1.03 *	-1.25 ns	-1.24 ns	0.06 ns	-0.26 ns
28	Piperitone	3.90 ***	9.99 ***	0.32 ns	2.78 ns	-0.19 ns	-0.30 ns
29	*trans*-Piperitol	2.45 ***	1.83 ***	0.76 ns	0.47 ns	0.20 **	0.03 ns
30	Citronellal	0.04 *	0.06 *	-0.09 *	-0.12 ns	0.04 ns	0.03 ns
31	*cis*-Sabinol	0.04 **	0.05 ***	0.01 ns	-0.01 ns	-0.01 ns	- ns
32	*p*-Cymene-8-ol	0.34 ***	0.36 ***	0.31 ns	0.40 ns	0.04 *	0.09 *
33	Neriodiol	-0.38 ns	-0.46 ns	- -	- -	1.60 ns	1.95 ns
34	Elemol	0.49 ns	0.27 ns	0.66 ns	0.50 ns	- -	- -
35	Globulol	-1.12 ns	-0.83 ns	0.20 ns	-0.49 ns	-1.19 ns	-0.69 ns
36	Viridiflorol	-0.45 ns	-0.06 ns	-0.09 ns	-0.30 ns	-0.79 ns	-0.47 ns
37	Methyl cinnamate	0.16 **	0.17 *	0.38 *	0.41 **	- -	- -
38	Spathulenol	-2.06 ns	-0.39 ns	1.12 ns	0.29 ns	-2.98 ns	-1.43 ns
39	α–Eudesmol	5.61 ***	4.88 ***	4.97 ns	6.55 ns	-0.08 ns	0.04 ns
40	γ–Eudesmol	4.45 ***	3.73 ***	4.82 ns	5.60 *	-0.34 ns	-0.23 ns
41	β–Eudesmol	5.33 ***	3.91 **	5.53 ns	5.96 ns	-0.11 ns	-0.06 ns
42	Regnanone	0.60 ns	0.50 ns	1.44 ns	1.21 ns	- -	- -
43	3,5-Dimethyl conglomerone	0.54 *	1.08 *	-0.92 ns	-1.85 ns	- -	- -
44	Tasmanone	0.13 ns	0.16 ns	-0.22 ns	-0.27 ns	- -	- -
45	Un 1 (tasmanone type)	0.05 *	0.08 *	-0.08 ns	-0.14 ns	- -	- -
46	Un 2 (tasmanone type)	0.02 *	0.08 ns	-0.04 ns	-0.14 ns	- -	- -

No significant differences were detected for most oil components between series within either the subgenus *Monocalyptus* or *Symphyomyrtus*. The *U*-test indicated that only the mean percentage values of β–pinene in juvenile leaf oils and α–terpinene and *trans*-piperitol in adult leaf oils were significantly different ($p < 0.01$) between the series *Ovatae* and *Viminales*.

Analysis of difference in oil composition between leaf types

Tests for differences in the percentages of oil components between adult and juvenile leaves at different levels based on mean percentage oil components of species are shown in Table 4.7. No components were significantly different between adult and juvenile leaves across all species tested. Further tests showed that the percentages of most oil components did not differ significantly between juvenile and adult leaves for individual subgenera and series, with the exception of few components.

The *U*-test for significance of differences in the proportions of oil components between adult and juvenile leaves of each species based on population data are shown in Table 4.8A and Table 4.8B.

As indicated in Table 4.8A, there were significant differences in some oil components between adult and juvenile leaves in a few *Monocalyptus* species but not for any components in adult and juvenile leaves of *E. sieberi, E. risdonii* and *E. pulchella.* The most important difference is that the mean percentage content of piperitone was significantly higher in juvenile leaf oil of *E. obliqua, E. delegatensis, E. regnans, E. tenuiramis, E. nitida and E. coccifera* and relatively higher in juvenile leaf oils of all other *Monocalyptus* species, with the exception of *E. pauciflora* (see Table 4.8 for detail). In contrast, the adult leaf oils of *E. obliqua, E. delegatensis, E. regnans, E. tenuiramis, and E. coccifera* contained significantly or relatively higher mean percentages of α and β–phellandrene or/and *trans*- and *cis*-*p*-menth-2-en-1-ol (see Table 4.8). In *E. obliqua* and *E. nitida*, the contents of α and β–phellandrene of adult leaves were significantly higher ($p<0.01$ or $p<0.05$) than that in juvenile leaves. Juvenile leaf oils of *E. nitida* also had significantly ($p<0.05$ or $p<0.01$) higher percentage of 1,8-cineole, limonene and α–terpineol in comparison to adult leaf oils which contained significantly higher ($p<0.01$ or $p<0.05$) proportions of α–thujene, α–terpinene and terpinyl acetate.

As indicated in Table 4.8B, there were no significant differences in most of the oil components between adult and juvenile leaves of *Symphyomyrtus* species. Only one species, *E. dalrympleana*, showed a wide difference in the proportions of oil components between adult and juvenile leaves. The juvenile leaf oils of this species contained significantly ($p<0.05$) higher mean percentages of β–pinene, α–terpinene, limonene,

Table 4.7. Mann-Whitney (*U*) test for significance of the difference in percentage contents of oil compounds between adult and juvenile leaves at different taxnomic levels.
Significance of variance: p<0.001 = ***; 0.001<p<0.01 = **; 0.01<p<0.05 = *; 0.05<p = ns; - = Component absent.

	Categories of classification	All Species	*Monocalyptus*	*Symphyomyrtus*	Series *Obliquae*	Series *Piperitae*	Series *Ovatae*	Series *Viminales*
	No. of species	29	12	5	7	17	4	13
		Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.
1	α–Pinene	-0.37 ns	-0.37 ns	-0.38 ns	-0.22 ns	-0.47 ns	0.64 ns	-0.69 ns
2	α–Thujene	0.16 ns	0.30 ns	0.07 ns	0.26 ns	0.32 ns	0.00 ns	0.10 ns
3	Camphene	-0.01 ns	0.00 ns	-0.01 ns	0.00 ns	0.00 ns	0.03 ns	-0.02 ns
4	β–Pinene	-0.08 ns	-0.12 ns	-0.06 ns	-0.20 ns	-0.06 ns	0.02 ns	-0.08 ns
5	Sabinene	-0.03 ns	-0.06 ns	-0.01 ns	-0.11 ns	-0.03 ns	0.01 ns	-0.01 ns
6	α–Phellandrene	0.54 ns	1.47 ns	-0.12 ns	1.15 ns	1.69 ns	1.01 ns	-0.46 ns
7	α–Terpinene	0.20 ns	0.45 ns	0.03 ns	0.21 ns	0.62 ns	0.16 ns	-0.01 ns
8	Limonene	-0.81 ns	-0.77 ns	-0.84 *	-0.43 ns	-1.01 ns	0.00 ns	-1.10 *
9	β–Phellandrene	0.65 ns	1.67 ns	-0.07 ns	0.16 ns	2.75 ns	0.36 ns	-0.20 ns
10	1,8-Cineole	-1.72 ns	-2.31 ns	-1.31 ns	-1.20 ns	-3.10 ns	-0.80 ns	-1.46 ns
11	*cis*–β–Ocimene	-0.12 ns	-0.32 *	0.02 ns	-0.22 ns	-0.39 *	-0.09 ns	0.05 ns
12	γ–Terpinene	-0.09 ns	-0.02 ns	-0.14 ns	-0.04 ns	0.00 ns	-0.21 ns	-0.11 ns
13	*trans*-β–Ocimene	-0.24 ns	-0.65 ns	0.05 **	0.00 ns	-1.11 ns	0.01 ns	0.06 *
14	*p*-Cymene	0.26 ns	-0.19 ns	0.58 ns	-0.95 ns	0.36 ns	-0.09 ns	0.79 ns
15	Terpinolene	-0.05 ns	-0.10 ns	-0.02 ns	-0.05 ns	-0.13 ns	0.02 ns	-0.03 ns
16	Linalool	-0.17 ns	0.05 ns	-0.32 ns	0.00 ns	0.08 ns	-1.28 ns	-0.02 ns
17	*trans-p*-Menth-2-en-1-ol	0.91 ns	2.14 *	0.05 ns	2.31 ns	2.02 ns	0.19 ns	0.01 ns
18	Pinocarvone	0.07 ns	-0.01 ns	0.12 ns	-0.01 ns	0.00 ns	0.06 ns	0.14 ns
19	*b*-Caryophellene	0.04 ns	0.00 ns	0.08 ns	0.00 ns	0.00 ns	-0.03 ns	0.11 ns
20	Aromadendrene	-0.02 ns	-0.01 ns	-0.02 ns	-0.01 ns	-0.02 ns	0.19 ns	-0.09 ns
21	Terpinen-4-ol	-0.06 ns	-0.09 ns	-0.03 ns	-0.43 ns	0.15 ns	0.01 ns	-0.05 ns
22	*cis-p*-Menth-2-en-1-ol	0.65 ns	1.51 ns	0.05 ns	1.82 ns	1.28 ns	0.15 ns	0.02 ns
23	*Allo*-aromadendrene	0.04 ns	0.00 ns	0.07 ns	-0.01 ns	0.01 ns	0.04 ns	0.07 ns
24	*trans*-Pinocarveol	0.16 ns	0.00 ns	0.28 ns	0.00 ns	0.00 ns	0.07 ns	0.35 ns
25	*cis*-Piperitol	0.20 ns	0.49 ns	0.01 ns	0.66 ns	0.36 ns	0.06 ns	-0.01 ns
26	Terpinyl acetate	-0.40 ns	-0.54 ns	-0.30 ns	0.08 ns	-0.98 ns	-0.06 ns	-0.38 ns
27	α–Terpineol	-0.36 ns	-0.26 ns	-0.43 ns	-0.26 ns	-0.25 ns	-0.20 ns	-0.51 ns
28	Piperitone	-2.54 ns	-6.11 *	-0.02 ns	-7.55 ns	-5.09 ns	0.06 ns	-0.05 ns
29	*trans*-Piperitol	0.26 ns	0.62 ns	0.00 ns	0.79 ns	0.50 ns	0.13 ns	-0.04 ns
30	Citronellal	0.00 ns	-0.02 ns	0.00 ns	0.00 ns	-0.03 ns	0.01 ns	0.00 ns
31	*cis*-Sabinol	0.00 ns	-0.01 ns	0.01 ns	0.00 ns	-0.01 ns	0.00 ns	0.01 ns
32	*p*-Cymene-8-ol	-0.02 ns	-0.03 ns	-0.01 ns	-0.08 ns	0.00 ns	-0.05 ns	0.00 ns
33	Nerolidol	-0.05 ns	0.00 ns	-0.08 ns	0.00 ns	0.00 ns	-0.35 ns	0.00 ns
34	Elemol	0.09 ns	0.22 ns	0.00 ns	0.31 ns	0.16 ns	0.00 ns	0.00 ns
35	Globulol	0.35 ns	0.18 ns	0.47 ns	0.59 ns	-0.10 ns	0.08 ns	0.59 ns
36	Viridiflorol	0.13 ns	-0.10 ns	0.29 ns	0.02 ns	-0.19 ns	0.04 ns	0.36 ns
37	Methyl cinnamate	0.00 ns	-0.01 ns	0.00 ns	-0.03 ns	0.00 ns	0.00 ns	0.00 ns
38	Spathulenol	0.53 ns	-0.46 ns	1.22 ns	0.03 ns	-0.80 ns	0.03 ns	1.59 ns
39	α–Eudesmol	0.32 ns	0.76 ns	0.02 ns	-0.16 ns	1.41 ns	-0.08 ns	0.05 ns
40	γ–Eudesmol	0.45 ns	0.87 ns	0.15 ns	0.41 ns	1.19 ns	0.07 ns	0.18 ns
41	β–Eudesmol	0.51 ns	1.34 ns	-0.08 ns	1.09 ns	1.52 ns	-0.11 ns	-0.07 ns
42	Regnanone	0.04 ns	0.10 ns	0.00 ns	0.23 ns	0.00 ns	- -	- -
43	3,5-Dimethyl conglomerone	-0.22 ns	-0.54 ns	- -	- -	-0.93 ns	- -	- -
44	Tasmanone	-0.01 ns	-0.03 ns	- -	- -	-0.05 ns	- -	- -
45	Un 1 (tasmanone type)	-0.01 ns	-0.04 ns	- -	- -	-0.06 ns	- -	- -
46	Un 2 (tasmanone type)	-0.03 ns	-0.06 ns	- -	- -	-0.11 ns	- -	- -

Table 4.8A. Mann-Whitney (*U*) test for significance of differences in the percentage contents of oil compounds between adult and juvenile leaves of *Eucalyptus* species

(*Monocalyptus* species)

Significance of difference: $p < 0.001$ = *** $0.001 < p < 0.01$ = ** $0.01 < p < 0.05$ = * $p > 0.05$ ns Δ(A-J) is the mean differences between adult and juvenile leaves; A = adult; J = juvenile; - = Component absent.

	Subgenus	*Monocalyptus*												*Symphyomyrtus*	
	Series	*Obliquae*					*Piperitae*							*Ovatae*	
	Code of species	Obl	Del	Reg	Sie	Pau	Ris	Ten	Pul	Amy	Nit	Rad	Coc	Bar	Bro
	No. of populations (A/J)	9/9	10/10	6/6	3/2	5/4	3/3	4/4	5/5	8/8	10/10	1/1	6/6	2/2	3/3
		Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.	Δ(A-J) Sign.
1	α–Pinene	0.05 ns	-0.04 ns	-1.05 **	-0.2 ns	0.13 ns	-0.13 ns	-0.67 ns	0.06 ns	-0.03 ns	-1.17 ns	-0.1	-1.23 ns	-0.34 ns	-0.8 ns
2	α–Thujene	0.39 ns	0.57 *	-0.12 ns	-0.01 ns	0.49 ns	-0.04 ns	0.1 ns	0.72 ns	0.08 ns	0.88 **	0.3	0.18 ns	- -	- -
3	Camphene	0.01 ns	0.01 ns	0.01 ns	0.003 ns	-0.03 ns	0.01 ns	0.02 ns	0.01 ns	-0.006 ns	-0.017 ns	-0.05	0.01 ns	0.01 ns	-0.01 ns
4	β–Pinene	- -	-0.03 ns	0.02 ns	- -	-0.97 ns	-0.33 ns	0.16 ns	0.02 ns	-0.04 ns	-0.03 ns	-0.08	-0.11 *	0.06 ns	-0.04 ns
5	Sabinene	0.05 ns	-0.03 ns	-0.59 ns	0.05 ns	-0.03 ns	-0.28 ns	-0.04 ns	0.04 ns	- -	0.03 ns	0.03	0.01 ns	- -	0.05 ns
6	α–Phellandrene	1.61 *	1.28 ns	0.66 ns	0.79 ns	1.41 ns	0.62 ns	-0.93 ns	-0.44 ns	-0.63 ns	8.47 *	6.28	-1.52 ns	0.06 ns	2.17 ns
7	α–Terpinene	0.29 ns	-0.19 ns	0.21 *	0.41 ns	0.31 ns	0.06 ns	-0.24 ns	0.2 ns	0.1 ns	1.79 *	1.91	0.51 ns	0.17 ns	0.26 ns
8	Limonene	0.06 ns	-0.14 *	-1.21 ns	0.22 ns	-1.1 *	-0.72 ns	-2.39 ns	-0.31 ns	-0.41 ns	-1.11 **	-0.45	-1.7 *	0.63 ns	-0.47 ns
9	β–Phellandrene	2.94 **	-0.19 ns	1.17 ns	-0.7 ns	-2.42 ns	0.87 ns	1.27 ns	1.35 ns	2.19 ns	5.93 *	6.4	1.23 ns	- -	0.06 ns
10	1,8-Cineole	-0.92 ns	-0.32 ns	0.05 ns	0.05 ns	-4.84 ns	-0.54 ns	-3.51 ns	3.68 ns	-0.8 ns	-9.77 **	-4.17	-6.59 ns	1.75 ns	0.88 ns
11	*cis*-β–Ocimene	-0.45 ns	-0.42 ns	-0.03 ns	-0.21 ns	0.03 ns	0.08 ns	-0.4 *	-0.28 ns	-0.26 *	-0.46 ns	-0.5	-0.88 ns	-0.31 ns	-0.08 ns
12	γ–Terpinene	-0.04 ns	-0.09 ns	-0.04 ns	-0.02 ns	- -	-0.11 ns	-0.12 ns	-0.11 ns	-0.14 ns	0.08 ns	0.59	-0.22 ns	-0.08 ns	-0.27 ns
13	*trans*-β–Ocimene	0.03 ns	-0.56 *	-0.07 ns	0.05 ns	0.53 ns	0.02 ns	-0.56 ns	-1.56 ns	-0.57 ns	-0.68 ns	-0.41	-4 ns	0.07 ns	0.06 ns
14	*p*-Cymene	-2.53 ns	-0.68 ns	0.97 ns	-1.8 ns	-0.71 ns	0.21 ns	-1.07 ns	0.8 ns	1.71 ns	-0.78 ns	-0.27	1.91 ns	-0.15 ns	-1.3 ns
15	Terpinolene	-0.02 ns	-0.16 ns	-0.09 ns	-0.06 ns	0.07 ns	-0.01 ns	-0.15 ns	-0.14 ns	-0.25 ns	0.19 ns	0.22	-0.75 ns	-0.07 ns	0.04 ns
16	Linalool	0.18 ns	-0.14 ns	0.3 ns	-0.13 ns	-0.2 ns	0.03 ns	0.07 ns	0.19 ns	0.12 ns	0.01 ns	-	0.17 ns	-0.82 ns	- -
17	*trans-p*-Menth-2-en-1-ol	4.03 *	2.74 *	1.87 ns	2.02 ns	0.87 ns	0.9 ns	4.06 *	0.31 ns	1.21 ns	0.54 ns	4.45	2.69 *	-0.01 ns	0.04 ns
18	Pinocarvone	- -	- -	- -	- -	-0.06 ns	- -	- -	- -	- -	- -	-	- -	- -	0.25 ns
19	*b*-Caryophyllene	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	-	- -	- -	-0.04 ns
20	Aromadendrene	- -	- -	- -	0.01 ns	-0.04 ns	- -	0.03 ns	- -	-0.1 ns	-0.01 ns	-	-0.04 ns	0.17 ns	0.08 ns
21	Terpinen-4-ol	-0.84 ns	-0.47 ns	-0.03 ns	-0.64 ns	-0.19 ns	-0.13 ns	0.62 ns	0.19 ns	-0.16 ns	-0.07 ns	0.8	-0.18 ns	0.04 ns	-0.08 ns
22	*cis-p*-Menth-2-en-1-ol	2.82 *	2.37 *	1.46 *	1.65 ns	0.82 ns	0.61 ns	3.08 *	0.25 ns	-0.23 ns	-0.18 ns	3.44	1.99 *	- -	0.11 ns
23	*Allo*-aromadendrene	- -	- -	- -	- ns	-0.03 ns	- -	0.05 ns	- -	0.14 ns	-0.03 ns	-	-0.1 ns	0.07 ns	0.19 *
24	*trans*-Pinocarveol	- -	- -	- -	- ns	-0.01 ns	- -	- -	- -	- -	- -	-	- -	- -	0.17 *
25	*cis*-Piperitol	0.61 ns	0.68 *	0.41 ns	1.27 ns	0.35 ns	0.13 ns	0.91 ns	-0.04 ns	-0.08 ns	-0.08 ns	1.03	0.64 *	- -	-0.05 ns
26	Terpinyl acetate	0.42 ns	-0.08 ns	-0.07 ns	0.1 ns	0.01 ns	-5.27 ns	0.21 ns	-0.23 ns	0.07 ns	-0.51 *	-0.14	-1 ns	0.4 ns	-0.74 ns
27	α–Terpineol	0.22 ns	-0.44 ns	0.1 ns	-0.25 ns	-0.95 ns	3.45 ns	-1.03 ns	0.16 ns	-0.37 ns	-0.9 **	-0.59	-2.47 ns	0.2 ns	-0.54 ns
28	Piperitone	-12.2 ***	-1.81 *	-7.49 *	-17.6 ns	1.36 *	-0.91 ns	-2.5 ns	-1.26 ns	-2.4 ns	-3.39 *	-23.13	-2.03 ns	0.05 ns	- -
29	*trans*-Piperitol	0.65 ns	1.35 *	0.92 ns	1.89 ns	-0.86 ns	-0.42 ns	1.23 ns	0.1 ns	-0.52 ns	0.56 ns	1.98	0.55 ns	0.13 ns	- -
30	Citronellal	- -	- -	- -	- ns	- -	- -	-0.06 ns	-0.07 ns	-0.03 ns	0.07 ns	-	-0.1 ns	- -	- -
31	*cis*-Sabinol	- -	- -	-0.01 ns	0.02 ns	-0.02 ns	-0.04 ns	-0.08 ns	0.02 ns	- -	-0.02 ns	-	0.02 ns	- -	0.01 ns
32	*p*-Cymene-8-ol	-0.39 ns	0.01 ns	0.1 ns	-0.12 ns	-0.02 ns	- -	-0.15 ns	-0.02 ns	0.01 ns	0.14 ns	-0.2	0.24 ns	- -	-0.18 ns
33	Nerolidol	- -	- -	- ns	- ns	- -	- -	- -	- -	- -	- -	-	- -	- -	- -
34	Elemol	- -	- -	1.57 ns	- ns	- -	- -	- -	- -	- -	- -	-	1.1 ns	- -	- -
35	Globulol	0.14 ns	0.25 ns	- ns	2.15 ns	0.39 ns	0.02 ns	0.45 ns	0.1 ns	0.07 ns	0.21 ns	-0.34	-1.23 ns	-0.3 ns	0.02 ns
36	Viridiflorol	-0.04 ns	0.06 ns	-0.02 ns	0.55 ns	-0.43 ns	-0.02 ns	0.06 ns	0.04 ns	-0.01 ns	-0.23 ns	-0.27	-0.91 ns	-0.12 ns	-0.06 ns
37	Methyl cinnamate	- -	-0.24 ns	0.02 ns	0.03 ns	0.036 *	- -	0.01 ns	- -	- -	- -	-	0.01 ns	- -	- -
38	Spathulenol	0.58 ns	-0.28 ns	0.07 ns	-0.17 ns	-0.07 ns	0.04 ns	0.22 ns	-0.02 ns	0.07 ns	-0.32 ns	0.34	-5.93 ns	-0.03 ns	0.11 ns
39	α–Eudesmol	-0.12 ns	-1.24 ns	-3.13 ns	3.24 ns	0.45 ns	-0.04 ns	0.49 ns	0.47 ns	0.65 ns	0.1 ns	1.51	6.71 *	-0.04 ns	-0.01 ns
40	γ–Eudesmol	-0.27 ns	-0.41 ns	-0.4 ns	1.97 ns	1.16 ns	0.09 ns	0.99 ns	0.4 ns	0.53 ns	0.43 ns	0.82	5.08 *	0.04 ns	-0.03 ns
41	β–Eudesmol	-0.04 ns	-0.8 ns	1.19 ns	1.77 ns	3.34 ns	0.07 ns	1.11 ns	0.63 ns	0.46 ns	0.32 ns	1.01	7.04 *	-0.05 ns	0.04 ns
42	Regnanone	- -	- -	1.054 ns	0.089 ns	- -	- -	- -	- -	- -	- -	-	- -	- -	- -
43	3,5-Dimethyl conglomerone	- -	- -	- -	- -	- -	- -	-0.354 ns	-6.026 ns	-0.136 ns	- -	-	- -	- -	- -
44	Tasmanone	- -	- -	- -	- -	- -	- -	- -	-0.363 ns	0.003 ns	- -	-	- -	- -	- -
45	Un 1 (tasmanone type)	- -	- -	- -	- -	- -	- -	-0.01 ns	-0.399 ns	-0.024 ns	- -	-	- -	- -	- -
46	Un 2 (tasmanone type)	- -	- -	- -	- -	- -	- -	- -	- ns	- ns	- -	-	- -	- -	- -

Table 4.8B. Mann-Whitney (*U*) test for significance of differences in the percentage contents of oil compounds between adult and juvenile leaves of *Eucalyptus* species

(*Symphyomyrtus* species)

Significance of difference: $p < 0.001$ = *** $0.001 < p < 0.01$ = ** $0.01 < p < 0.05$ = * $p > 0.05$ ns Δ(A-J) is the mean differences between adult and juvenile leaves; A = adult; J = juvenile; - = Component absent.

Subgenus	*Symphyomyrtus*															
Series	*Ovatae*		*Viminales*													
Code of species	Ova		Rod		Glo		Ver		Sub		Joh		Vim		Dal	
No. of populations (A/J)	6/6		4/4		6/6		4/4		5/5		5/5		6/5		3/5	
	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.
α–Pinene	1.35	ns	2.35	ns	-2.17	ns	-0.62	ns	-0.44	ns	1.3	ns	-0.71	ns	-4.05	ns
α–Thujene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.12	*
Camphene	0.06	ns	0.04	ns	0.05	ns	-0.05	ns	0.04	ns	-0.04	ns	0.01	ns	-0.02	ns
β–Pinene	0.04	ns	-	-	-0.09	ns	-0.07	ns	-	-	-0.03	ns	-0.09	ns	-0.6	*
Sabinene	-0.01	ns	-	-	0.02	ns	-0.08	ns	0.01	ns	0.01	ns	0.04	ns	0.03	ns
α–Phellandrene	0.3	ns	1.51	ns	-0.18	ns	-	-	-0.10	ns	-0.12	ns	0.74	ns	-3.7	ns
α–Terpinene	-0.07	ns	0.29	ns	-0.01	ns	-	-	-0.02	ns	0.01	ns	0.1	*	-0.08	*
Limonene	0.37	ns	-0.52	ns	-1.50	ns	-1.64	*	-0.74	ns	-0.72	ns	-0.62	ns	-1.94	*
β–Phellandrene	0.60	ns	0.78	ns	-	-	-0.02	ns	0.04	ns	-	-	-0.17	ns	-3.1	ns
1,8-Cineole	0.69	ns	-6.53	ns	-2.17	ns	4.06	ns	-5.01	ns	1.91	ns	-5.99	ns	-14.6	ns
cis-β–Ocimene	0.07	ns	-0.02	ns	0.06	ns	0.14	ns	0.09	ns	-0.26	ns	0.19	ns	-0.08	ns
γ–Terpinene	-0.26	ns	-0.24	ns	-	-	0.06	ns	1.23	ns	-0.65	ns	0.58	ns	-0.25	ns
trans-β–Ocimene	-0.15	ns	0.06	ns	0.05	ns	0.04	ns	-0.01	ns	0.02	ns	0.02	ns	0.21	ns
p-Cymene	0.34	ns	0.74	ns	0.42	ns	-0.59	ns	1.56	ns	-0.05	ns	1.08	ns	5.48	*
Terpinolene	0.01	ns	0.10	ns	-0.11	ns	0.10	*	0.04	ns	-0.03	ns	0.06	ns	-0.26	*
Linalool	-4.37	ns	0.05	ns	-0.05	ns	-0.06	ns	0.11	ns	-0.04	ns	0.04	ns	0.08	ns
trans-*p*-Menth-2-en-1-ol	0.04	ns	0.67	ns	-	-	-0.02	ns	-0.01	ns	0.01	ns	0.17	ns	-0.05	ns
Pinocarvone	-	-	-	-	1.09	**	0.04	ns	-0.04	ns	0.09	ns	-0.12	ns	0.04	ns
b-Caryophyllene	-0.05	ns	-0.02	ns	0.6	ns	0.13	ns	-	-	-	-	0.25	*	0.14	ns
Aromadendrene	0.54	ns	-0.04	*	-1.51	ns	0.07	ns	-0.05	ns	-0.02	ns	0.43	ns	0.2	ns
Terpinen-4-ol	-0.12	ns	0.19	ns	-0.14	ns	-0.05	ns	0.03	ns	0.05	ns	0.48	ns	-0.04	ns
cis-*p*-Menth-2-en-1-ol	-0.01	ns	0.49	ns	-0.01	ns	-0.01	ns	0.07	ns	-0.01	ns	0.07	ns	-0.02	ns
Allo-aromadendrene	-0.13	ns	0.02	ns	0.08	ns	0.02	ns	-0.01	ns	0.07	ns	0.18	ns	0.58	*
trans-Pinocarveol	0.03	ns	0.07	ns	2.13	**	-0.12	ns	-0.11	ns	0.27	ns	-0.15	ns	-	-
cis-Piperitol	0.14	ns	0.13	ns	-0.03	ns	0.06	ns	0.16	ns	-0.02	ns	0.07	ns	-0.1	ns
Terpinyl acetate	0.41	ns	-0.29	ns	-0.29	ns	-0.22	ns	0.05	ns	-0.97	ns	-0.75	ns	-0.78	*
α–Terpineol	0.25	ns	-0.69	ns	-1.21	ns	-0.02	ns	-1.24	ns	-0.41	ns	0.23	ns	-1.39	*
Piperitone	0.02	ns	0.17	ns	-0.09	ns	-0.03	ns	-	-	-0.04	ns	0.01	ns	-2.14	ns
trans-Piperitol	0.12	ns	0.28	ns	-0.05	ns	-0.06	ns	0.05	ns	-0.05	ns	0.04	ns	0.07	ns
Citronellal	0.05	ns	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cis-Sabinol	-0.02	ns	-	-	0.003	ns	-0.02	ns	-	-	-	-	0.07	ns	0.01	ns
p-Cymene-8-ol	-0.03	ns	-	-	0.01	ns	-	-	-	-	-	-	-	-	-	-
Nerolidol	-1.41	ns	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Elemol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Globulol	0.53	ns	0.08	ns	1.74	ns	0.09	ns	0.66	ns	-0.36	ns	0.74	ns	4.49	*
Viridiflorol	0.29	ns	0.04	ns	0.18	ns	-0.01	ns	0.4	ns	-0.06	ns	-0.01	ns	4	*
Methyl cinnamate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spathulenol	0.07	ns	-0.03	ns	0.29	*	-0.01	ns	3.16	ns	0.08	ns	0.29	ns	15.31	*
α–Eudesmol	-0.29	ns	0.04	ns	0.2	ns	-0.07	ns	-0.06	ns	-0.03	ns	0.14	ns	0.77	*
γ–Eudesmol	0.24	ns	0.03	ns	0.1	ns	-0.08	ns	0.01	ns	0.01	ns	0.12	ns	2.32	*
β–Eudesmol	-0.48	ns	0.04	ns	0.59	ns	-0.12	ns	-0.18	ns	-0.04	ns	0.13	ns	-0.36	ns
Regnanone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3,5-Dimethyl conglomerone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tasmanone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Un 1 (tasmanone type)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Un 2 (tasmanone type)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Subgenus	*Symphyomyrtus*															
Series	*Viminales*															
Code of species	Rub		Gun		Arc		Mor		Urn		Per		Cor			
No. of populations (A/J)	2/2		6/6		3/3		2/2		4/4		1/1		5/5			
	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.	Δ(A-J)	Sign.		
α–Pinene	-2.25	ns	-0.09	ns	-1.15	ns	-2.28	ns	-1.3	ns	-0.55		5.34	ns		
α–Thujene	-	-	0.11	ns	-	-	-	-	0.01	ns	-		-	-		
Camphene	-0.02	ns	0.04	ns	0.04	ns	-	-	0.08	ns	-0.32		-0.05	ns		
β–Pinene	-0.04	ns	0.03	ns	-0.09	ns	-0.07	ns	0.01	ns	0.05		-0.07	ns		
Sabinene	-	-	0.03	ns	0.02	ns	-	-	-0.03	ns	-0.19		-0.04	ns		
α–Phellandrene	0.12	ns	-1.77	ns	-0.08	ns	-0.7	ns	0.03	ns	0.15		-0.4	ns		
α–Terpinene	-0.02	ns	-0.06	ns	-0.02	ns	-	-	0.01	ns	-		-0.07	ns		
Limonene	-1.59	ns	0.14	ns	-1.87	*	-1.1	ns	0.09	ns	-2		-0.86	ns		
β–Phellandrene	-	-	0.3	ns	-	-	-	-	0.24	ns	0.06		-	-		
1,8-Cineole	-5.28	ns	7.22	ns	0.39	ns	5.1	ns	-1.41	ns	-2.97		-0.28	ns		
cis-β–Ocimene	0.22	ns	-0.01	ns	0.14	ns	0.09	ns	-	-	-0.02		0.05	ns		
γ–Terpinene	-0.87	ns	0.24	ns	-0.33	ns	-0.97	ns	-0.11	ns	-0.16		-0.23	ns		
trans-β–Ocimene	0.05	ns	-0.01	ns	0.07	*	0.03	ns	0.13	ns	0.1		0.03	ns		
p-Cymene	3.17	ns	-1.86	ns	-0.11	ns	0.48	ns	0.36	ns	0.15		0.15	ns		
Terpinolene	-0.02	ns	-0.13	ns	-0.04	ns	-0.1	ns	0.06	ns	0.09		-0.01	ns		
Linalool	-0.12	ns	-0.03	ns	-0.03	ns	-0.1	ns	0.01	ns	-		-0.07	ns		
trans-*p*-Menth-2-en-1-ol	0.03	ns	-0.05	ns	-	-	-	-	-	-	-		-	-		
Pinocarvone	0.04	ns	0.05	ns	0.41	ns	0.07	ns	-0.11	ns	0.28		0.15	ns		
b-Caryophyllene	0.06	ns	0.06	ns	0.01	ns	-	-	0.04	ns	0.1		-	-		
Aromadendrene	-0.22	ns	-	-	-	-	-0.03	ns	-0.02	ns	-		0.01	ns		
Terpinen-4-ol	-0.37	ns	0.01	ns	-0.11	ns	-0.13	ns	0.02	ns	-0.03		-0.34	ns		
cis-*p*-Menth-2-en-1-ol	0.07	ns	0.04	ns	0.09	*	-	-	0.01	ns	0.03		-0.03	ns		
Allo-aromadendrene	0.1	ns	-0.15	ns	0.02	ns	-0.11	ns	-0.05	ns	0.23		0.01	ns		
trans-Pinocarveol	-0.05	ns	0.43	ns	0.88	ns	0.28	ns	-0.27	ns	1.04		0.16	ns		
cis-Piperitol	0.13	ns	-0.01	ns	-0.13	ns	-0.03	ns	-0.2	ns	-		-0.02	ns		
Terpinyl acetate	-0.84	ns	-0.58	ns	-0.56	ns	-0.33	ns	2.14	*	1.04		-2.85	ns		
α–Terpineol	1.63	ns	-0.07	ns	0.02	ns	0.02	ns	0.03	ns	0.02		-	-		
Piperitone	0.02	ns	-0.08	ns	-0.02	ns	-0.07	ns	-0.02	ns	0.11		-0.4	ns		
trans-Piperitol	-	-	-	-	-	-	-	-	-	-	-		-	-		
Citronellal	-	-	-	-	-	-	-	-	-	-	-		-	-		
cis-Sabinol	-	-	0.02	ns	-	-	-	-	-	-	0.05		-0.02	ns		
p-Cymene-8-ol	-	-	-	-	-	-	-	-	-	-	-		-	-		
Nerolidol	-	-	-	-	-	-	-	-	-	-	-		-	-		
Elemol	-	-	-	-	-	-	-	-	-	-	-		-	-		
Globulol	0.73	ns	-0.95	ns	0.29	ns	-0.22	ns	-0.19	ns	0.56		0.06	ns		
Viridiflorol	0.78	ns	-0.54	ns	-0.02	ns	-0.13	ns	0.05	ns	0.07		0.01	ns		
Methyl cinnamate	-	-	-	-	-	-	-	-	-	-	-		-	-		
Spathulenol	3.84	ns	-2.66	ns	0.04	ns	-0.04	ns	0.33	ns	0.02		-0.04	ns		
α–Eudesmol	-0.02	ns	-0.2	ns	-0.01	ns	-	-	-0.01	ns	-0.02		-0.09	ns		
γ–Eudesmol	0.49	ns	-0.43	ns	-0.04	ns	-0.05	ns	0.1	ns	-0.17		-0.1	*		
β–Eudesmol	0.01	ns	-0.44	ns	0.05	ns	-0.01	ns	-0.09	ns	-0.25		-0.14	**		
Regnanone	-	-	-	-	-	-	-	-	-	-	-		-	-		
3,5-Dimethyl conglomerone	-	-	-	-	-	-	-	-	-	-	-		-	-		
Tasmanone	-	-	-	-	-	-	-	-	-	-	-		-	-		
Un 1 (tasmanone type)	-	-	-	-	-	-	-	-	-	-	-		-	-		
Un 2 (tasmanone type)	-	-	-	-	-	-	-	-	-	-	-		-	-		

terpinyl acetate and α–terpineol and relatively higher 1,8-cineole and α–pinene. In contrast its adult leaf oils contained significantly ($p<0.05$) higher proportions of sesquiterpenoids, *allo*-aromadendrene, globulol, viridiflorol, spathulenol, and α and γ–eudesmol and monoterpenoids, *p*-cymene and α–thujene.

The adult and juvenile leaves of *E. globulus* were significantly different in proportions of three components: pinocarvone ($p<0.01$), *trans*-pinocarveol ($p<0.01$) and spathulenol ($p < 0.05$). Leaf oils of only few other *Symphyomyrtus* species had significant differences in a few oil compounds between leaf types.

Variation between species

In the Kruskal-Wallis *H*-test for adult and juvenile leaf samples of the 29 species, all oil components showed significant ($p < 0.001$) variation between species except for *cis*–β–ocimene which was not significant for adult leaves (Table 4.9). Furthermore, the *H*-test for individual subgenera indicated that variation in oil components between species of the subgenus *Monocalyptus* were more significant than in *Symphyomyrtus*. The variation of most components was highly significant ($p < 0.001$ for most components) between *Monocalyptus* species, with the exception of few minor components.

The variation in most oil components between species within the subgenus *Symphyomyrtus* was lower than that for the *Monocalyptus*. Moreover, the variation of oil components in adult leaves of the *Symphyomyrtus* species tended to be more significant than for juvenile leaves. The monoterpenoids, 1,8-cineole, limonene, γ–terpinene, *p*-cymene, terpinolene, pinocarvone, *trans*-pinocarveol, terpinyl acetate and citronellal and the sesquiterpenoids, aromadendrene, *allo*-aromadendrene, nerolidol, globulol, viridiflorol, spathulenol and γ–eudesmol showed significant variation at levels of $p < 0.001$ to < 0.05 between species for both adult and juvenile leaf oils respectively. However, the components α–pinene, α–thujene, α–terpinene, linalool, *b*-caryophyllene, terpinen-4-ol, α–terpineol, *p*-cymene-8-ol, α and β–eudesmol showed significant variation ($p < 0.001$ to $p < 0.05$) for adult leaves but not for juvenile leaves.

The Kruskal-Wallis *H*-test for significance of the variation between species within series indicated that most significant variation was among *Obliquae* species of the subgenus *Monocalyptus*. Within this series, most components showed significant variation between species (see Table 4.9 for significant levels) for both adult and juvenile leaf oils. Among *Piperitae* species, the variation of all sesquiterpenoids of both adult and juvenile leaf oils was significant but not so for many monoterpenoids. Variation in monoterpenoids, such as β–pinene, α–phellandrene, limonene, 1,8-cineole, *p*-cymene, terpinolene, and linalool was significant ($p < 0.001$ to $p < 0.05$) for both adult and juvenile leaves.

Table 4.9. Kruskal-Wallis test for significance of variation in chemical components of leaf oils among species (between species/between populations).
Significance of variance: p<0.001 = ***; 0.001<p<0.01 = **; 0.01<p<0.05 = *; 0.05<p = ns; - = Component absent.

		Total Variance		Variance within subgenera				Variance within series in *Monocalyptus*				Variance within series in *Symphyomyrtus*			
	Categories of classification	All Species		*Monocalyptus*		*Symphyomyrtus*		Series *Obliquae*		Series *Piperitae*		Series *Ovatae*		Series *Viminales*	
	Leaf types	A	J	A	J	A	J	A	J	A	J	A	J	A	J
	d.f	28	28	11	11	16	16	4	4	6	6	3	3	12	12
	No. of species	29	29	12	12	17	17	5	5	7	7	4	4	13	13
	No. of populations	140	136	72	68	68	64	33	32	37	36	15	15	52	48
1	α–Pinene	***	***	***	***	**	ns	**	***	*	ns	ns	ns	**	**
2	α–Thujene	***	***	***	***	**	ns	**	***	*	*	ns	ns	***	*
3	Camphene	***	***	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns
4	β–Pinene	***	***	*	ns	**	**	ns	ns	ns	ns	ns	ns	**	*
5	Sabinene	***	***	**	*	ns	ns	*	ns	*	**	ns	ns	ns	ns
6	α–Phellandrene	***	***	***	***	*	ns	***	**	**	*	ns	ns	**	*
7	α–Terpinene	***	***	***	***	***	ns	***	***	**	ns	ns	ns	**	ns
8	Limonene	***	***	***	***	*	***	**	***	***	*	ns	*	*	**
9	β–Phellandrene	***	***	***	**	ns	ns	***	**	**	ns	ns	ns	*	ns
10	1,8-Cineole	***	***	***	***	***	**	***	***	***	**	*	*	***	**
11	*cis*-β–Ocimene	ns	***	ns	**	ns	ns	**	**	ns	ns	ns	ns	ns	ns
12	γ–Terpinene	***	**	**	*	*	*	**	*	ns	ns	ns	ns	**	*
13	*trans*-β–Ocimene	***	***	***	***	ns	ns	ns	**	ns	ns	ns	*	ns	ns
14	*p*-Cymene	***	***	***	***	**	**	***	***	**	**	ns	ns	***	**
15	Terpinolene	***	***	***	***	*	*	**	***	**	*	ns	ns	*	*
16	Linalool	***	***	***	***	**	ns	**	**	**	***	ns	*	***	ns
17	*trans-p*-Menth-2-en-1-ol	***	***	***	***	ns	ns	***	***	ns	ns	ns	ns	*	ns
18	Pinocarvone	***	***	**	***	**	*	*	**	***	-	ns	ns	**	*
19	b-Caryophellene	***	***	-	-	**	ns	-	-	***	-	ns	ns	**	*
20	Aromadendrene	***	***	**	***	***	**	***	**	ns	*	ns	ns	***	**
21	Terpinen-4-ol	***	***	***	***	*	ns	***	***	*	ns	ns	ns	**	ns
22	*cis-p*-Menth-2-en-1-ol	***	***	***	***	ns	ns	***	***	ns	ns	ns	ns	ns	ns
23	*Allo*-aromadendrene	***	***	**	***	***	**	-	**	ns	*	ns	ns	**	**
24	*trans*-Pinocarveol	***	***	**	***	***	*	*	**	***	-	ns	ns	**	**
25	*cis*-Piperitol	***	***	***	***	ns	ns	**	***	ns	ns	ns	ns	ns	ns
26	Terpinyl acetate	***	***	**	***	**	*	*	**	ns	ns	ns	ns	**	*
27	α–Terpineol	***	***	**	**	*	ns	**	**	ns	ns	ns	ns	*	ns
28	Piperitone	***	***	***	***	ns	ns	***	***	ns	ns	ns	ns	ns	ns
29	*trans*-Piperitol	***	***	***	**	ns	ns	***	***	ns	ns	ns	ns	ns	ns
30	Citronellal	***	***	***	***	*	*	-	-	ns	*	ns	ns	-	-
31	*cis*-Sabinol	***	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
32	*p*-Cymene-8-ol	***	***	***	***	*	ns	***	***	***	ns	ns	ns	ns	ns
33	Nerolidol	***	***	-	-	***	***	-	-	***	-	ns	ns	-	-
34	Elemol	***	***	***	***	-	-	***	***	**	ns	-	-	-	-
35	Globulol	***	***	***	***	***	**	***	***	**	**	ns	ns	***	**
36	Viridiflorol	***	***	***	***	***	**	***	***	*	*	ns	ns	***	**
37	Methyl cinnamate	***	***	***	***	-	-	***	***	***	-	-	-	-	-
38	Spathulenol	***	***	***	***	***	***	ns	ns	**	**	ns	ns	***	***
39	α–Eudesmol	***	***	***	***	*	ns	***	***	***	**	ns	ns	**	ns
40	γ–Eudesmol	***	***	***	***	***	*	***	***	***	**	ns	ns	**	*
41	β–Eudesmol	***	***	***	***	*	ns	***	***	***	**	ns	ns	*	ns
42	Regnanone	***	***	***	***	-	-	***	***	***	-	-	-	-	-
43	3,5-Dimethyl conglomerone	***	***	***	***	-	-	-	-	***	***	-	-	-	-
44	Tasmanone	***	***	***	***	-	-	-	-	***	***	-	-	-	-
45	Un 1 (tasmanone type)	***	***	***	***	-	-	-	-	***	***	-	-	-	-
46	Un 2 (tasmanone type)	***	***	***	***	-	-	-	-	***	***	-	-	-	-

In addition, α–pinene, α–terpinene, β–phellandrene, terpinen-4-ol, *p*-cymene-8-ol and elemol were significant ($p < 0.001$ to $p < 0.05$) for adult leaf oils but not for juvenile oils while for aromadendrene, *allo*-aromadendrene and citronellal the reverse applied.

In the subgenus *Symphyomyrtus*, variation in most oil components between *Ovatae* species was not significant. Only 1,8-cineole was significant ($p < 0.05$) in both adult and juvenile leaves while camphene, limonene, *trans*–β–ocimene and linalool were significant only ($p < 0.05$) for juvenile leaves. The *H*-test indicated that most variation in oil components among *Symphyomyrtus* species was contributed by species within *Viminales* series. The significance of the variation in oil components between *Viminales* species was similar to levels reported above for variation among all species of the subgenus *Symphyomyrtus*.

Variation between localities within species

Following Zavarin and Snajberk (1985), the component variability between localities within species was characterized by: ΣV = sum of variances of individual components between localities within species (see Table 4.5A - part 2 and B - part 2) and the mean ΣV of the different subgenera and series as listed in Table 4.10.

Table 4.10. The mean ΣV* of oil components within species according to subgenera and series

Categories of classification	No. of species	Mean ΣV	
		Adult leaves	Juvenile leaves
Subgenus *Monocalyptus*	12	291.2	323.6
Series *Obliquae*	5	175.2	127.6
Series *Piperitae*	7	387.9	486.9
Subgenus *Symphyomyrtus*	17	236.6	254.7
Series *Ovatae*	4	469.9	461.3
Series *Viminales*	13	158.9	185.8

* mean ΣV= the average value of ΣV of all species within a taxa. For example, the mean ΣV of the subgenus *Monocalyptus* = $(\Sigma V_{Obl}+\Sigma V_{Reg}+\Sigma V_{Del}+.....\Sigma V_{Rad}+\Sigma V_{Coc})/12$

As indicated in Table 4.10, there were no notable differences between adult and juvenile leaf samples in the mean ΣV of different subgenera and series. There was also no notable difference in mean ΣV between the subgenera *Monocalyptus* (291.2 for adult and 323.6 for juvenile leaves) and *Symphyomyrtus* (236.6 and 254.7). However, there were marked differences between the different series of each subgenus. The mean ΣV's of both adult and juvenile leaf samples of the *Piperitae* series (387.9 and 486.9) of the subgenus *Monocalyptus* were higher than that of the *Obliquae* series (175.2 and 127.6). In the subgenus *Symphyomyrtus*, the mean ΣV's of both adult and juvenile leaf samples of the *Ovatae* series (469.9 and 461.3) were higher than in the *Viminales* series (158.9

and 185.8). These indicated that the component variability between localities within species in the *Piperitae* and *Ovatae* series were greater than that in *Obliquae* and *Viminales* series.

Within the subgenus *Monocalyptus*, the ΣV's (see Table 4.5) of the species *E. obliqua, E. delegatensis, E. regnans, E. sieberi* of the *Obliquae* series were low (72.3 to 119.2 for adult and 41.7 to 108.1 for juvenile leaves) and only the ΣV for *E. pauciflora* was high (493.9 for adult and 322.1 for juvenile leaves). In *Piperitae* species, the ΣV was high in *E. amygdalina* (796.9 for adult and 646.4 for juvenile), *E. tenuiramis* (527.5 and 707.6), *E. pulchella* (529.7 and 485.1) but very low in *E. risdonii* (80.1 and 11.7). The ΣV's of juvenile leaves of *E. coccifera* (625.1) and *E. nitida* (445.4) were notably higher than their adult leaves (216.0 and 176.3 respectively).

Within *Symphyomyrtus* species, ΣV was very high in *E. ovata* (1390.8 and 1531.0) and *E. gunnii* (656.3 and 1019.6), and low in *E. barberi, E. rodwayi, E. globulus, E. vernicosa, E. johnstonii, E. archeri, E. morrisbyi, E. urnigera, E. perriniana* and *E. cordata* (2.2 to 93.4 for adult and 15.1 to 95.0 for juvenile leaves respectively) while the ΣV of *E. brookeriana, E. subcrenulata* and *E. viminalis* were intermediate (197.7 to 393.2). However, the ΣV's of the two remaining species, *E. dalrympleana* and *E. rubida* were notably different between leaf type. The ΣV of juvenile leaves (318.9) of *E. dalrympleana* was higher than adult leaves (66.8) but the ΣV of adult leaves (318.2) of *E. rubida* was higher than the estimate for juvenile leaves (71.4).

4.4. Biochemical relationships of terpenoid components of Tasmanian eucalypt leaf oils

In order to investigate the biochemical relationships of terpenoid compounds and to construct a biogenetic model for the terpenoid compounds of leaf oils of Tasmanian eucalypt species, correlation analyses were employed to examine the relationship of terpenoid compounds (compounds which were not terpenoids were excluded from this analysis, i.e. tasmanone type phenolics) of the leaf oils of all populations (Appendix 4.3). Following Irving and Adams (1973), the resulting correlation matrix was further subjected to cluster analysis in order to reveal groups such as highly correlated compounds. A schematic model was derived which included the biosynthetic interrelationships of the more important monoterpenoids and sesquiterpenoids of eucalypt leaf oils. The model was constructed on the basis of the known stereochemistry of monoterpenoids and sesquiterpenoids, and reports for terpenoid compounds (see chapter 2) and the statistical results from this study.

4.4.1. The correlation of terpenoid compounds of eucalypt leaf oils

The average linkage dendrogram derived from analysis of adult and juvenile data sets are shown in Fig. 4.3 A and B respectively.

The major dichotomy (I) in the dendrogram of adult samples (Fig. 4.3 A) divided all terpenoids into two negatively correlated fractions I and II (correlation coefficient between fractions = - 0.20). With the exception of monoterpene ester, terpinyl acetate, components were further grouped into five different highly correlated terpenoid groups (combined correlation coefficients between compounds within group > 0.162). There were two major groups, groups A and B, within fraction I and the three groups C, D and E in fraction II .

With the exception of γ–terpinene, all cyclic monoterpenoid components fused into two clusters (groups A and C) and all cyclic sesquiterpenoid components into clusters B and D. The acyclic monoterpenoid and sesquiterpenoid components were grouped into cluster E, with the exception of *cis*- and *trans*-ß-ocimene. Thus, this dendrogram revealed the monoterpenoids in group A to be negatively correlated with those in group C and the sesquiterpenoids in group B to be negatively correlated with those in group D. In addition, this dendrogram also indicated that the acyclic monoterpenoids and sesquiterpenoids of group E were more associated with the sesquiterpenoids of group D than to other groups.

Monoterpenoid group A was dominated by menthane class monoterpenoids. Within this group, the two pairs of monoterpene alcohols, *cis*- and *trans*-*p*-menth-2-en-1-ol and *cis*- and *trans*-piperitol formed a typical monoterpene alcohol cluster. The α– and β–phellandrene, α–terpinene, terpinolene and α–thujene formed a typical monoterpene hydrocarbon cluster while the alcohol, terpinen-4-ol, was closely associated with these hydrocarbons. For the other remaining monoterpenoids, two structural related component pairs were formed respectively. They are the *p*-cymene and *p*-cymene-8-ol compound pair and the sabinene and *cis*-sabinol pair. The only monoterpene ketone, piperitone, was more closely associated with the *p*-cymene and *p*-cymene-8-ol.

Group C consisted of three menthane class monoterpenoids, the monoterpene ether 1,8-cineole, the hydrocarbon, limonene, and the alcohol, α–terpineol, and all five bicyclic monoterpenoids of the pinane class were found in this group. Within this group, the three bicyclic monoterpenoids α– and β–pinene and camphene were more closely related to 1,8-cineole and limonene than were the other two bicyclic monoterpenoids pinocarvone and *trans*-pinocarveol.

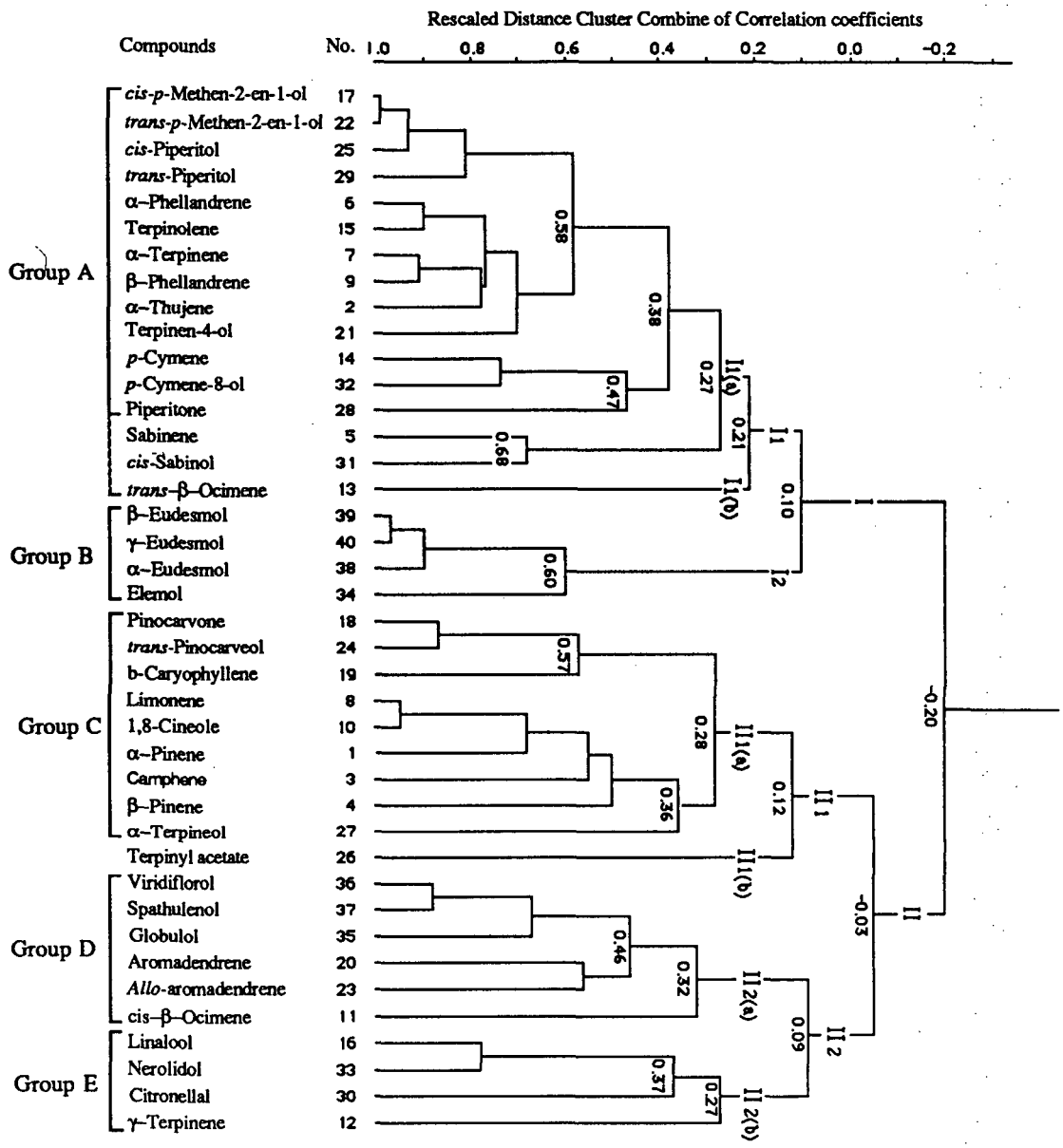


Fig.4.3 (A). Average linkage clustering of terpenoid compounds of *Eucalyptus* adult leaf oil using correlation coefficients.

*Correlation matrix of terpenoid compounds using percentage contents of individual components in adult leaf oils of all populations of 29 eucalypt species.

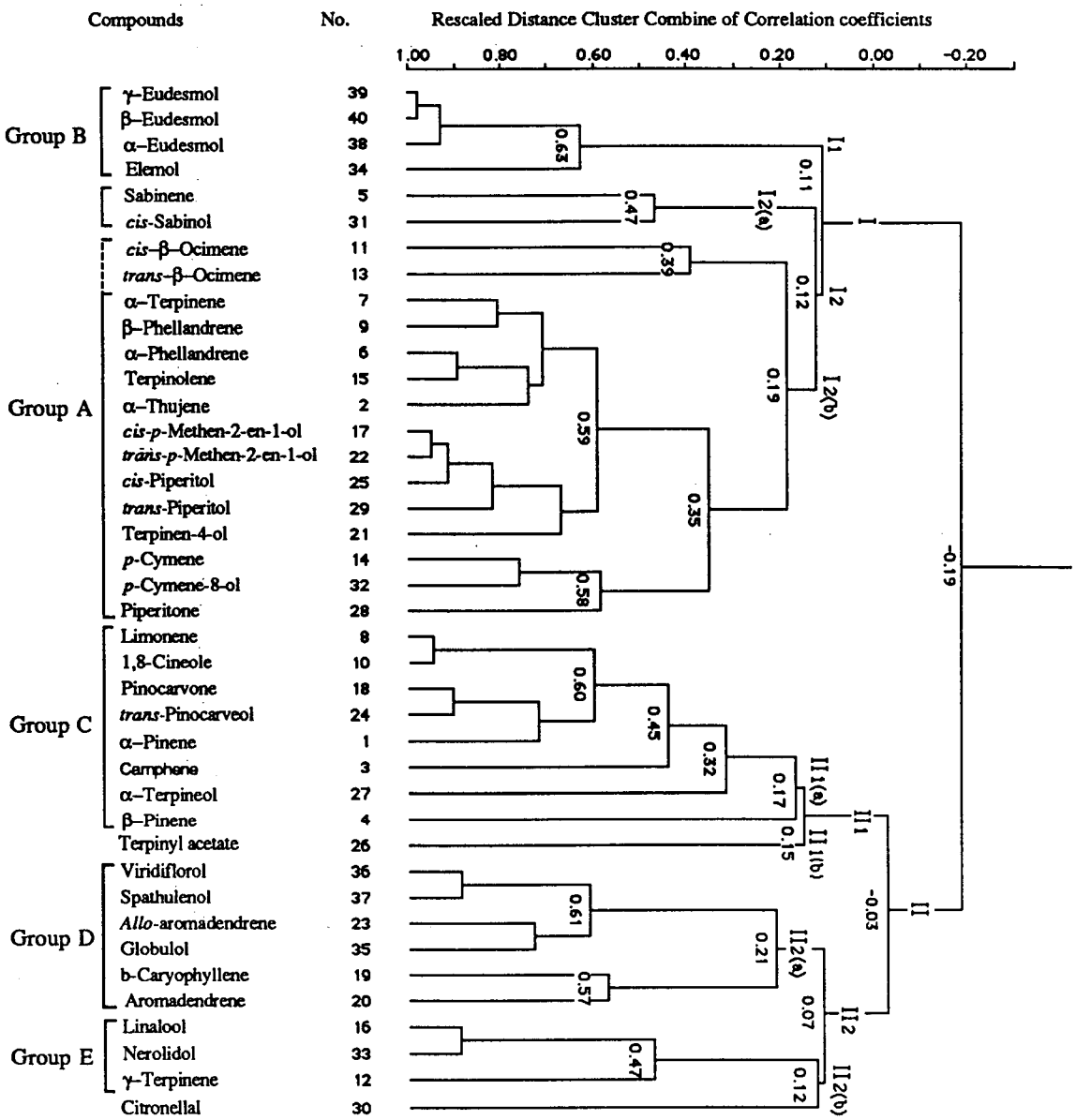


Fig.4.3B. Average linkage clustering of terpenoid compounds of *Eucalyptus* juvenile leaf oil using correlation coefficients.

*Correlation matrix of terpenoid compounds using percentage contents of individual components in juvenile leaf oils of all populations of 29 eucalypt species.

The two different sesquiterpenoid groups were clearly distinguished by their carbon skeletons. All sesquiterpenoids in group B were built on the eudesmane skeleton and those in group D on the aromadendrane skeleton.

The dendrogram of terpenoid compounds of the juvenile leaf oils (Fig. 4.3B) showed a similar clustering of components to that derived from adult leaf oils, although a few terpenoids were rearranged to independently cluster. For example, the sabinene and sabinol pair was separated, however, it still showed a considerable correlation with group A. The two isomer, *cis*- and *trans*–β–ocimene, formed a single ocimene cluster which was linked to the monoterpenoids of group A.

The correlation matrix and the derived average linkage clustering dendrogram have indicated the important relationships among terpenoid components of Tasmanian eucalypts leaf oils. The average linkage clustering has divided these terpenoid components into different groups on the basis of structures. First, the monoterpenoids and sesquiterpenoids were separated into different groups. Second, the sesquiterpenoids were separated into the eudesmane and aromadendrane compound groups (i.e. group B and D). Thirdly, the menthane class cyclic monoterpenoids were separated into two different groups, group A and C. Finally, the thujene class of bicyclic monoterpenoids were closely associated with menthane class cyclic monoterpenoids of grouped A and pinane class compounds were grouped into group C. The correlation matrix indicated that the menthane class monoterpenoid groups, A and C, were negatively correlated. This suggests that the biosynthesis of these two component groups in eucalypts leaf oils could involve different biochemical pathways.

4.4.2. Proposed biosynthesis and biogenesis of terpenoid components in eucalypt leaf oils

The statistical results for the distribution of terpenoids in eucalypt leaf oils suggested tentative biosynthetic pathways of the compounds under study. The interrelationships of terpenoid components and the highly correlated groups of terpenoid compounds correspond to the main biogenetic categories of monoterpenoids and sesquiterpenoids (Devon and Scott 1972) and the principal proposals of biosynthesis of terpenoid components (Ruzicka *et al.* 1953; Banthorpe *et al.* 1972; Croteau 1987). These relationships could help to now propose a model of biogenetic relationships of terpenoids in eucalypt leaf oils and in particular for the monoterpenoids.

The proposed model of the biosynthetic interrelationships of the monoterpenoids and sesquiterpenoids of eucalypts species is given in Fig.4.4 and Fig.4.5.

Monoterpenoids

Fig.4.4 is a schematic model of the biosynthetic interrelationships of the monoterpenoids in the leaf oil of eucalypt species. It was constructed on the basis of the gross proposals of Ruzicka and his coworkers (Ruzicka *et al.* 1953), known stereochemistry of monoterpenoids (Banthorpe *et al.* 1972; Dev *et al.* 1982; Croteau 1987), and the statistical results of this investigation and the ideas of von Rudloff (1975). This model describes the formation of different biogenetic series of monoterpenoids from possible parents, 1-*p*-menthene-8-carbonium ion CI, 1-*p*-menthene-4-carbonium CII, carbonium ions CIII and CIV, with the exception of the acyclic series of monoterpenoids.

According to the proposals of Ruzicka *et al.* (1953), bicyclic skeletons (CIII) of the pinane series are derived by internal additions of positive centres to double bonds with monocyclic frameworks in a direction governed either by electronic factors (Markovnikov addition) or by a steric factor from carbonium ion CI. Hydride shift within the ion CI followed by cyclization of CII gives rise to the thujane skeleton (CIV). Using a labelled specific site precursor, generally [2-^{14}C]-mevalonic acid, the biosynthesis of (+)-isothujone, camphor and α–pinene etc. has been investigated and the results essentially confirmed Ruzicka's proposals (Dev *et al.* 1982). The schemes from the most recent biosynthetic work and review have both arranged the pinane and thujane skeletons into different biosynthetic pathways from Carbonium ion CI and CII (Croteau, 1987). In the leaf oils of Tasmanian eucalypts, the bicyclic monoterpenoids have only the pinane and thujane skeletons. All pinane skeleton monoterpenoids were highly positively correlated to each another and were negatively correlated with thujane skeleton monoterpenoids. This agrees with the separation in the postulated biogenetic relationship, that the pinane series monoterpenoids α–pinene, β–pinene, camphene, pinocarvone and *trans*-pinocarveol are involved in the biogenetic pathway from CIII and the thujane skeleton monoterpenoid α–thujene to the biogenetic pathway from CIV. It should be noted that of the three thujane skeleton monoterpenoids found in this study only α–thujene occurred in substantial amounts while the other two thujane skeleton monoterpenoids, sabinene and *cis*-sabinol, were present in only very low proportions so that the data obtained in this study for these two components could be unreliable. Therefore, this proposal mainly considers the statistical results for α–thujene. The sabinene and *cis*-sabinol were included in the same biogenetic pathway with α–thujene from CIV according to skeleton category but this was not substantiated by the statistical results.

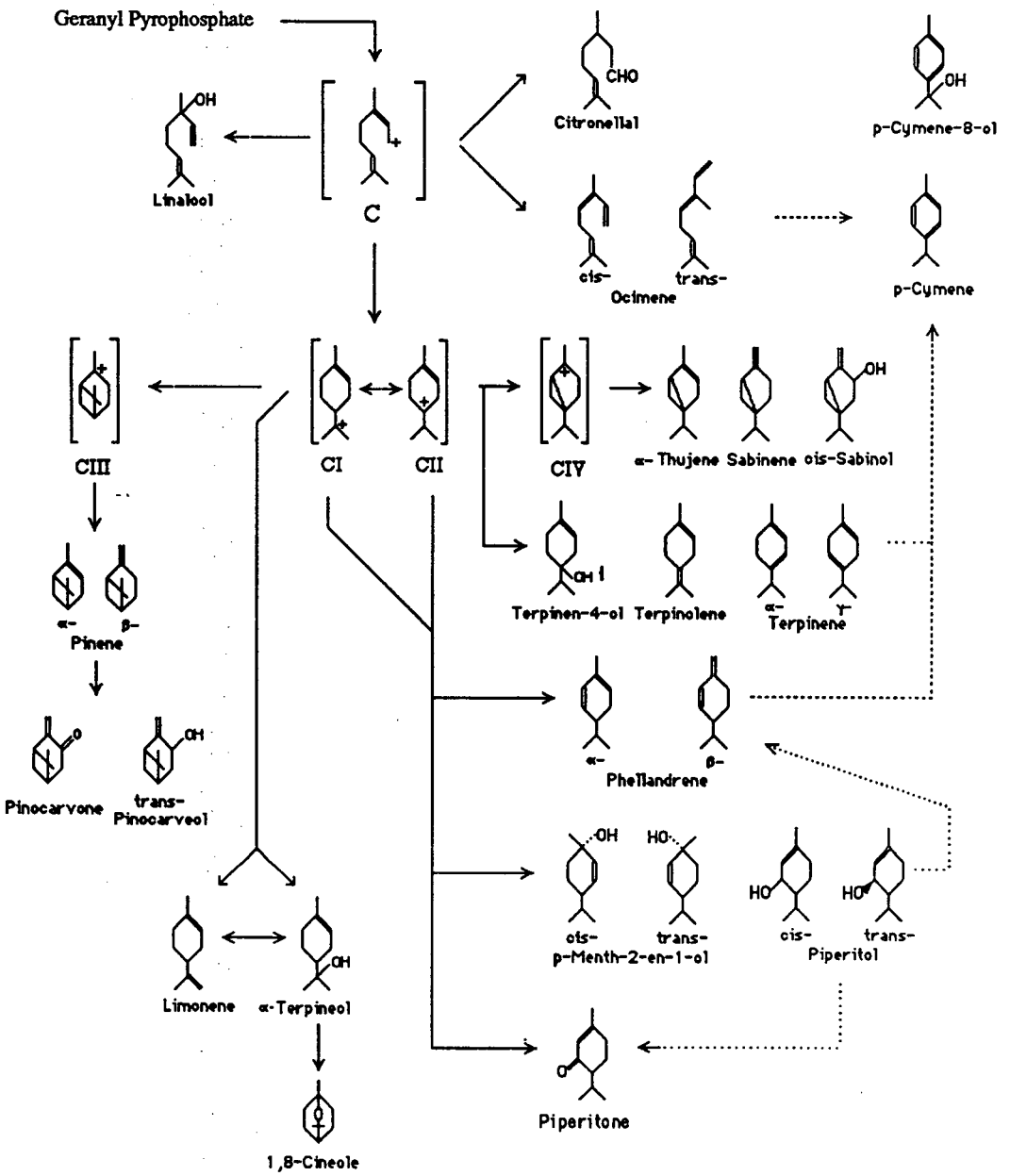


Fig. 4.4. Some chemical and biochemical interrelationships of monoterpenoids found in the essential oils of leaves of the Tasmanian *Eucalyptus* species.

The monocyclic monoterpenoids, which predominate in eucalypt leaf oils are separately involved in several biogenetic series. According to the proposals of Ruzicka, the monocyclization of the carbonium ion CI gives α–terpineol appears to be chemically feasible. Incorporation of [2-^{14}C]-MVA into 1,8-cineole by *Eucalyptus globulus* (Birch *et al.* 1959), 1-^{14}C-geraniol into 1,8-cineole by *Rosmarinus officinalis* (Achilladelis and Hanson 1963) and the formation of limonene from [2-^{14}C]-MVA in *Pinus pinea* has been investigated (Sandermann and Bruns 1962). Moreover, a cell-free system from *Salvia officinalis* has established the synthesis of 1,8-cineole, limonene and α–terpineol from NPP (Croteau and Karp 1976). Nevertheless, the biochemical details that the 1,8-cineole, limonene, and α–terpineol are formed either directly by independent routes from the precursor CI or in the expected sequence α–terpineol > 1,8-cineole, or limonene > α–terpineol is still conjectural. Most early or recently published schemes have consistently presumed these compounds to be derived from carbonium ion CI but not from the later carbonium ion CII (Banthorpe *et al.* 1972; von Rudloff 1975; Charlwood and Banthorpe 1978; Dev *et al.* 1982; Zavarin and Snajberk 1985).

The high correlation between 1,8-cineole and α–pinene also supports the proposed model for it suggests that they are derived from the same carbonium ion CI while 1,8-cineole, limonene and α–terpineol combine with all pinane class bicyclic monoterpenoids to form a highly correlated component group (see Fig.4.3). A comprehensive survey of the chemical composition of eucalypt leaf oils from species through out Australia indicated that 1,8-cineole and α–pinene always co-occur in leaf oils as major components for many species (Boland *et al.* 1991). This suggests that there is a common biosynthetic mechanism controlling the production of the monoterpenoids which results in the production of 1,8-cineole, limonene and α–terpineol and pinane class monoterpenoids.

Ruzicka (1953) suggested that the hydride shift within the ion CI followed by cyclization of CII to form terpinen-4-ol also appears chemically feasible. In many plant species, the co-occurrence of the aromatic monoterpenes with the structurally related cyclohexadienes and terpinen-4-ol suggest the possibility that these compounds may be biogenetically related. In some *Pinus* species, γ–terpinene, terpinolene, *p*-cymene and thujane skeleton monoterpoids from wood have been known to arise from the common precursor 1-*p*-menthene-4-carbonium ion CII (Zavarin and Snajberk 1985). The chemosystematic study of North American conifers based on leaf oils has also indicated that the α– and γ–terpinene, terpinolene, terpinen-4-ol and *p*-cymene are biosynthetically related to the precursor ion CII (von Rudloff 1975). The incorporation of $^{14}CO_2$ into the volatile terpenoids of *Thyme* (Poulose and Croteau 1978) has provided strong evidence bearing on the precursor-product relationship that *p*-cymene is biosynthesized by the aromatisation of γ–terpinene. No biochemical reports were located that any biochemical

relationship exists between α and β–phellandrene and the above cyclohexadienes but von Rudloff (1975) suggested that they could be precursors of further aromatisation and production of *p*-cymene in conifer leaf oils. The high correlations between α–terpinene, terpinolene, terpinen-4-ol and α– and β–phellandrene found in the present study strongly support the view that they could arise from a closely related common precursor, 1-*p*-menthene-4-carbonium (CII).

The two *cis*- and *trans*-*p*-menth-2-en-1-ol isomers share certain similar properties with *cis*- and *trans*-piperitol in that they contain the axial hydroxyl factor. These isomers were highly correlated with *cis*- and *trans*-piperitol (Fig. 4.3A and B), because presumably these two pairs of isomers are biochemically related by a simple rearrangement, possibly by way of a non-concerted carbonium ion rearrangement. These unusual monoterpenoid alcohols have been found only in relatively large amounts in *Eucalyptus* leaf oils within the family Myrtaceae (Lassak and Southwell 1982). According to the results of the analysis of leaf oils from some species of *Eucalyptus* subseries *Strictinae,* Lassak and Southwell (1982) speculated that these monoterpenoid alcohols arise in the plant by non-specific hydroxylation of the same allylic carbonium ion. Weston (1984) also suggested that they might be biochemically related to occur together in the oil glands of the leaves of *E. delegatensis*. Furthermore, these monoterpenoid alcohols have been found to co-occur with α– and β–phellandrene as major components in the leaf oils from a large number of eucalypt species (Boland *et al.* 1991). The results of the present study demonstrated that their frequent co-occurrence in the leaf oils of most *Monocalyptus* species in Tasmania could be utilised as a genetic marker for these species, although at this level, there are no results to indicate which precursor to which they might be related. However, the high correlations found in the present study suggest that these cyclohexenene alcohols might be biogenetically related to the cyclohexadienes hydrocarbons. In addition, piperitone is probably the end-product of menthane biogenesis in some *Eucalyptus* species (Weston 1984) and it was also found to co-occur with the cyclohexane alcohols and cylohexadienes in most *Monocalyptus* species in Tasmania although the correlation coefficients were not highly significant. It is also possible that piperitone might also be biochemically related to these menthane monoterpenoids or that it arises independently from the precursor, 1-*p*-menthene-4-carbonium or 1-*p*-menthene-8-carbonium.

Sesquiterpenoids

Interrelationships amongst sesquiterpenoids are still poorly understood which is due, in part, to many of them not having been adequately or correctly identified. However, the sesquiterpenoids found in leaf oils of Tasmanian eucalypt species consisted of only two major classes, the eudesmane and aromadendrane skeleton sesquiterpenoids, which have been well identified from leaf oils of a large number of eucalypt species (Boland *et al.* 1991). The statistical results in this study have shown these sesquiterpenoids to be highly correlated to certain chemical compound groups and therefore provide reasonable evidence for the proposed biogenetic model.

Fig. 4.5 is a schematic model of the biosynthetic interrelationships of the sesquiterpenoids in the leaf oil of Tasmanian eucalypt species. It was constructed on the basis of the general findings of Ruzicka *et al.* (1953) and Richards and Hendrickson (1964) and the classification of naturally occurring compounds by Devon and Scott (1972).

As indicated by the correlation analysis, the sesquiterpenoids, spathulenol, viridiflorol, globulol, *allo*-aromadendrene and aromadendrene, were significantly and positively correlated to make up a closely related group. Similarly, α–, β– and γ–eudesmol and elemol were also significantly and positively correlated to form another sesquiterpenoid group. According to the proposed biogenetic interrelationships of sesquiterpene skeletons of Ruzicka *et al.* (1953) and the stereochemical aspects of this problem as discussed by Hendrickson (1959), it is assumed that the sesquiterpenes originate by appropriate cyclization of farnesyl pyrophosphate. The compounds *cis*- and *trans*-farnesol are suitable precursors for cyclization to all the cyclic sesquiterpenes. Hendrickson (1959) suggested that the biogenesis of sesquiterpenes from *cis*- and *trans*-farnesol would involve ionisation of the allylic hydroxyl and cyclization of one of the other double bonds to the cation thus forming CI and CII. According to this proposal and the classification of Devon and Scott (1972), the eudesmane and elemane skeleton sesquiterpenoids arise from *trans*-farnesol via the precursor germacrane cation (CIII) and the aromadendrane skeleton sesquiterpenoids from *trans*-farnesol via the bicyclogermacrene skeleton (CIV). In addition, the caryophellane skeleton sesquiterpenoids arise from *cis*-farnesol via the *cis*-humulane cation (CV). The proposed biogenetic formation of the sesquiterpenoids found in the leaf oils of Tasmanian eucalypts would support their development from *trans*-farnesol and only one sesquiterpenoid, *b*-caryophyllene, from *cis*-farnesol and the linear skeleton sesquiterpenoid nerolidol from farnesyl pyrophosphate without cyclization (nerolidol might be an allylic isomeric intermediate for *cis-trans* isomerization (Richards and Hendrickson 1964)). In this study, the three eudesmane skeleton sesquiterpenoids, α–, β– and γ–eudesmol, were the most highly correlated among sesquiterpenoids and

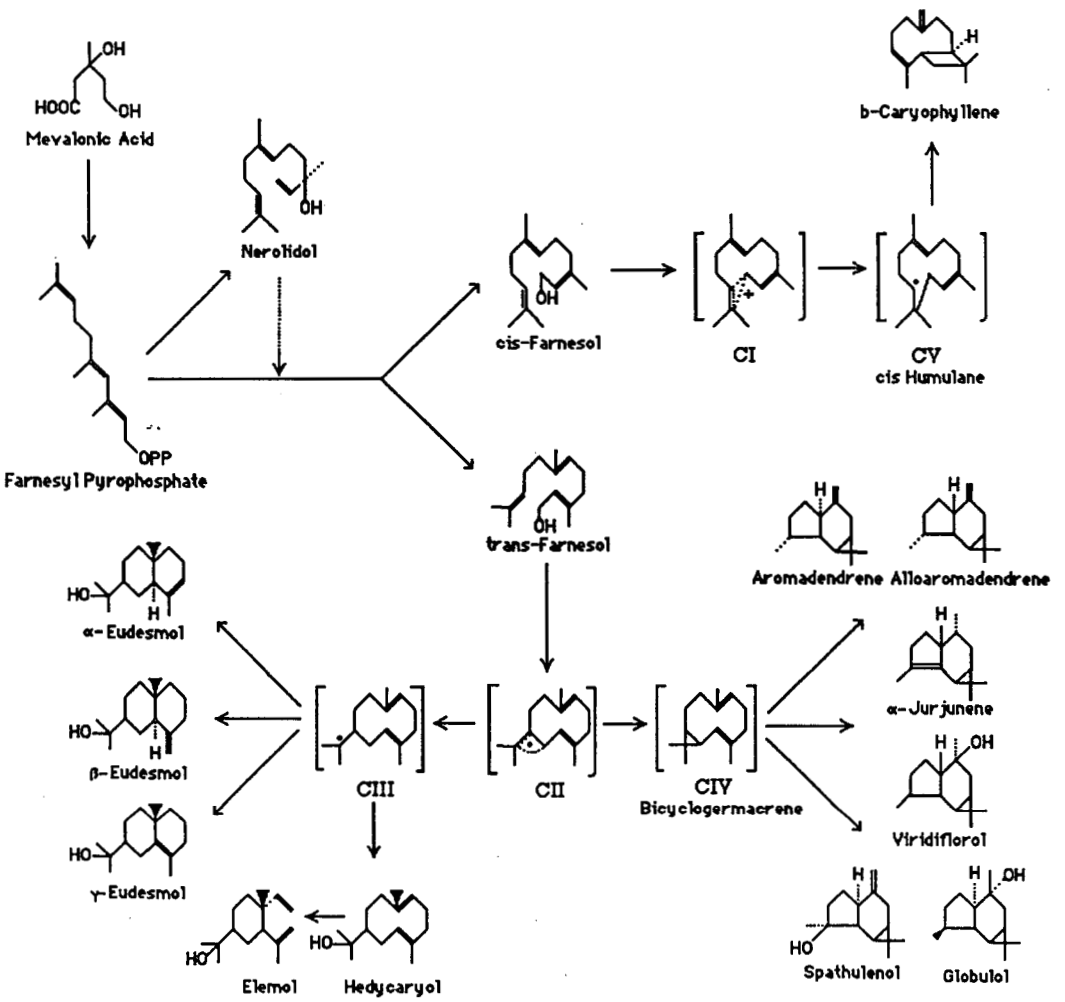


Fig. 4.5. Some chemical and biochemical interrelationships of sesquiterpenoids found in the essential oils of leaves of the Tasmanian *Eucalyptus* species.

were also highly correlated with the elemane skeleton sesquiterpenoid, hedycaryol and elemol. Their synthesis was obviously from *trans*-farnesol via the precursor germacrane cation (CIII). Most aromadendrane skeleton sesquiterpenoids, such as spathulenol, viridiflorol, globulol, and allaromedendrene were also correlated. Although aromadendrene had a lower correlation coefficient it was more closely related to all the above aromadendrane sesquiterpenoids than to any others with the exception of *b*-caryophyllene. These aromadendrane skeleton sesquiterpenoids are synthesised from *trans*-farnesol via the precursor bicyclogermacrane cation (CIV) (Richards and Hendrickson 1964). However, *b*-caryophyllene arose from *cis*-farnesol via the precursor cation CI and the *cis*-humulane skeleton cation (CV) (Devon and Scott 1972).

4.4.3. Summary

The above analysis and discussion suggested that the terpenoid compounds of Tasmanian eucalypt leaf oils mainly involved biogenetic divergent of terpenoid biosynthesis. Thus, the monoterpenoid group A (Fig. 4.3A and B) is biogenetically correlated to the 1-*p*-menthene-8-carbonium ion CI (Fig. 4.4) and monoterpenoid group C to 1-*p*-menthene-4-carbonium CII while the sesquiterpenoid group B were biogenetically related to the precursor germacrane cation (CIII) (Fig. 4.5) and the sesquiterpenoid group D to bicyclogermacrene (CIV).

The biogenetic relationships of monoterpenoids (Fig. 4.4) indicated that the relationship between 1-*p*-menthene-8-carbonium ion CI and 1-*p*-menthene-4-carbonium CII is reversible. Thus, a tendency toward synthesis of CI biogenetic related compounds is negatively correlated to the synthesis of CII biogenetic related compounds. The negative correlation between the monoterpenoid group C and group A (Fig. 4.3A and B) was in agreement with this biochemical mechanism. These results indicated that increase in monoterpenoids of group C in eucalypt leaf oil is generally balanced by the decrease in those of group A and *vice versa*.

4.5. The chemotaxonomy of Tasmanian *Eucalyptus* species based on essential oil data

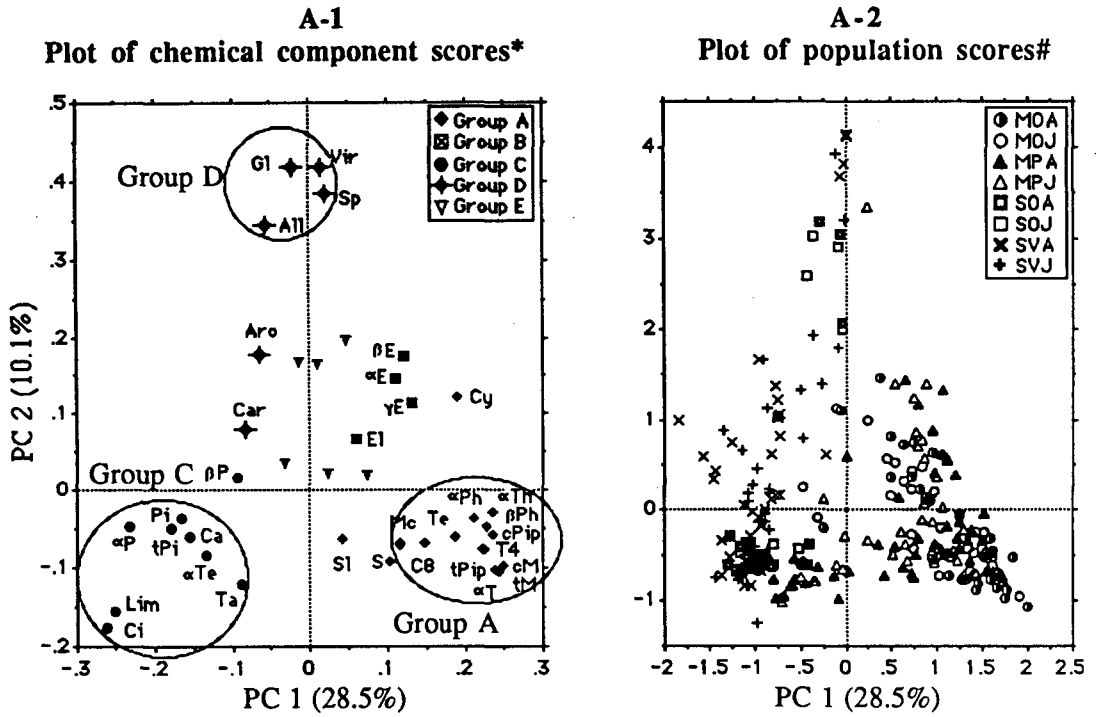
4.5.1. The principal biochemical trends and population classification

Trends in principal chemical variation among all species

The variation of all terpenoid compounds of the leaf oils among populations and species of eucalypts was summarised by Principal Component Analysis (PCA). The eigenvalues and the population scores on the main principal component axes are shown in Fig. 4.6A and B. The main variation in oil components among all populations could be summarised by the variation in the biosynthetically related groups of chemical components discussed in the previous section.

The eigenvalues of the PCA indicated that the two first Principal Components (PCs) accounted for 28.5% and 10.1% of the total variance of terpenoid components among populations (Fig. 4.6A). The terpenoid components, from both juvenile and adult leaves, located on the axes of the first two PCs (PC 1 and PC 2) are shown in the scatter plot of Fig. 4.6A-1. This figure indicated that the major variance of terpenoid components was associated with variation in the major components of three biosynthetically related monoterpenoid compound groups, A and C, and the sesquiterpenoid compound group D (i.e. groups A, C and D characterized in Fig. 4.3A and B). Positive variation along PC 1 is associated with increasing levels of group A components and decreasing levels of group C components whereas variation along PC 2 is associated with variation in group D components. Three monoterpenoids, 1,8-cineole, limonene and α–pinene of group C were most highly and negatively weighted along the PC 1 axis while some monoterpenoids of group A, such as *cis*- and *trans*-menth-2-en-1-ol, α– and β–phellandrene, *cis*- and *trans*-piperitol, terpinen-4-ol, α–terpinene and α–thujene were most highly positively weighted.

Most sesquiterpenoid compounds of group D were highly positively weighted along the PC 2 axis with the major compounds, globulol, viridiflorol, spathulenol and aromadendrene, grouped together. These compounds contributed little to variation along PC 1 axis. The compounds of group B and E contributed little to variation in the space defined by PC 1 and 2.



A-3: Plots of population scores of individual series on the first two principal components

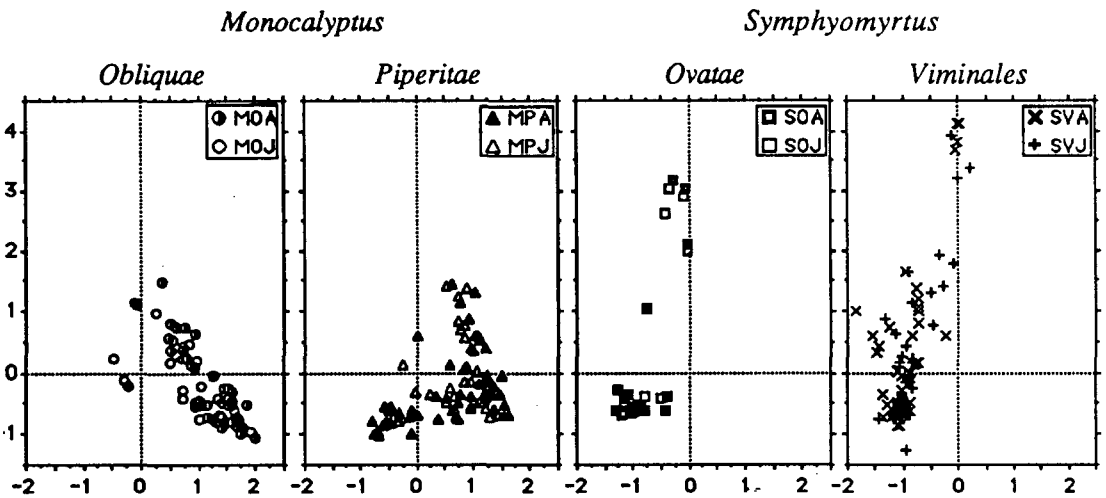


Fig.4.6.A. Scatter plot of the terpenoid parameters on the first two principal components (PC 1 and PC 2) and scatter plot of juvenile and adult samples of all populations of 29 eucalypt species on these two principal components.

* Chemical components were indicated in the plot of component scores using compound code, for chemical names see Table 4.5. Component groups indicated here are the biochemical related terpeneoid compound groups as showed in Fig. 4.3.

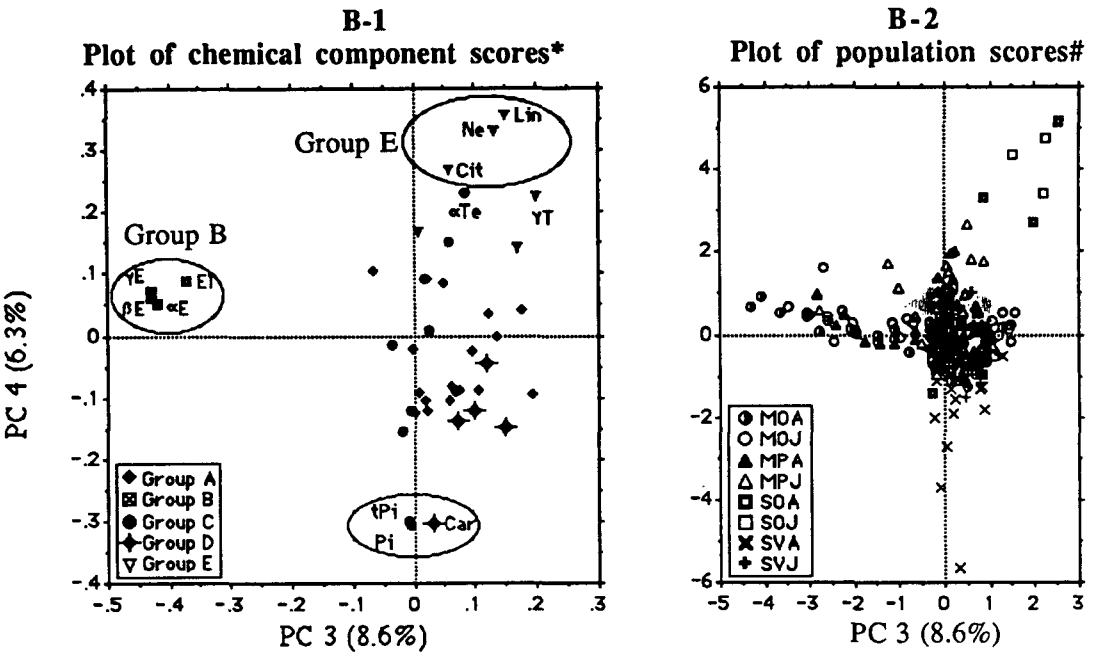
Populations scores were indicated by both juvenile and adult leaf samples of individual series as follow:

MOA=*Monocalyptus-Obliquae*-Adult leaves; MOJ=*Monocalyptus-Obliquae*-Juvenile leaves

MPA=*Monocalyptus-Piperitae*-Adult leaves; MPJ=*Monocalyptus-Piperitae*-Juvenile leaves

SOA=*Symphyomyrtus-Ovatae*-Adult leaves; SOJ=*Symphyomyrtus-Ovatae*-Juvenile leaves

SVA=*Symphyomyrtus-Viminales*-Adult leaves; SVJ=*Symphyomyrtus-Viminales*-Juvenile leaves



B-3: population scores of individual series on the PC3 and PC4

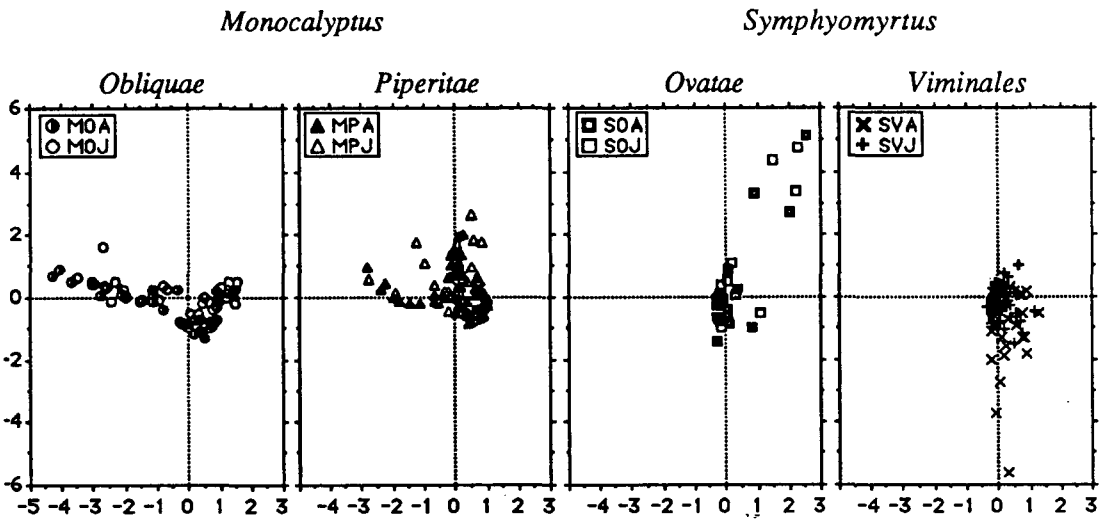


Fig.4.6.B. Scatter plot of the terpenoid parameters on the first two principal components (PC 3 and PC 4) and scatter plot of juvenile and adult samples of all populations of 29 eucalypt species on these two principal components.

* Chemical components were indicated in the plot of component scores using compound code, for chemical names see Table 4.5. Component groups indicated here are the biochemical related terpeneoid compound groups as showed in Fig. 4.3.

Populations scores were indicated by both juvenile and adult leaf samples of individual series as follow:

MOA=*Monocalyptus-Obliquae*-Adult leaves; MOJ=*Monocalyptus-Obliquae*-Juvenile leaves

MPA=*Monocalyptus-Piperitae*-Adult leaves; MPJ=*Monocalyptus-Piperitae*-Juvenile leaves

SOA=*Symphyomyrtus-Ovatae*-Adult leaves; SOJ=*Symphyomyrtus-Ovatae*-Juvenile leaves

SVA=*Symphyomyrtus-Viminales*-Adult leaves; SVJ=*Symphyomyrtus-Viminales*-Juvenile leaves

The *Monocalyptus* and *Symphyomyrtus* samples are basically separated along PC 1 (Fig. 4.6A-2). Most populations of *Symphyomyrtus* species have higher levels of group C compounds and are located on the lower left quadrant and most populations of *Monocalyptus* species had higher levels of group A compounds and are located on the lower right quadrant. PC 2 was mainly associated with variation amongst *Symphyomyrtus* populations which had a continuous variation with increasing levels of sesquiterpenoids of compound group D and decreasing levels of monoterpenoids of group C in a direction slightly oblique to PC 2. Variation amongst the *Monocalyptus* samples had two directions: (i) continuous variation occurred along the horizontal axis, which was mainly associated with taxa of *Piperitae* (see population scores in Fig. 4.5A-3), with decreasing levels of group A compounds and increasing levels of group C compounds and, (ii) continuous variation associated with all *Obliquae* species and the majority of *Piperitae* species, with decreasing levels of monoterpenoids of group A in a direction oblique to PC 2. However, variation within both subgenera did not follow the series classification. There was no tendency for juvenile and adult leaves to be separated in this ordination.

Plots of chemical components on the third and fourth PCs (Fig. 4.6B-1) indicated that the major variation of terpenoid components along PC 3 (8.6%) was associated with variation in levels of sesquiterpenoid compounds of groups B and variation along PC4 (6.3%) was mainly associated with increasing levels of acyclic mono- and sesquiterpenoid compounds of group E and decreasing levels of pinocarvone, *trans*-pinocarveol and caryophyllene. Negative variation along PC3 was mainly associated with *Obliquae* species and some *Piperitae* species, with increasing levels of sesquiterpenoids of group B and decreasing levels other components (Fig. 4.6B-2 and 3). The majority of *Symphyomyrtus* samples had little variation in the space defined by the PC 3 and PC4. Only a few samples of *Ovatae* species were separated from the main group due to high levels of group E compounds (Fig.4.6B-2 and 3) and a few samples of *Viminales* species had high levels of pinocarvone, *trans*-pinocarveol and caryophyllene.

Overall, the main trends in oil chemical variation amongst samples can be summarised as:

i) variation in oil components is continuous both within and between subgenera;

ii) subgenera were basically separated by PC 1 and

iii) the main directions of variation can be summarised by variation in A, B, C and D groups of compounds indicated in Fig. 4.3A and B. Thus, the majority of *Symphyomyrtus* samples had higher levels of group C compounds and the majority of *Monocalyptus* samples had higher levels of group A compounds. Within *Monocalyptus*

species, some samples of *Piperitae* species had higher levels of group C compounds and tended toward *Symphyomyrtus* species while some populations of both *Obliquae* and *Piperitae* species tended to display increased levels of sesquiterpenoids of group B and decreased levels of monoterpenoids. Within *Symphyomyrtus* species, there was a continuous variation with decreased levels of monoterpenoids of group C and increased levels of sesquiterpenoids of group D.

Population classification for all species

The overall variation amongst the populations of the 29 eucalypt species and intermediates as estimated from the percentage composition data of both adult and juvenile leaf oils, is summarised in the dendrogram in Fig. 4.7. This dendrogram, derived by average linkage clustering using the Manhattan matrix distance as discussed previously, confirms the division of most populations of the two subgenera although some populations of a few species of each subgenus occur as outliers. Thus, most populations of *Monocalyptus* species were grouped into the major cluster of *Monocalyptus* species and most populations of *Symphyomyrtus* species were in the major cluster of *Symphyomyrtus.* All populations of *E. dalrympleana*, one *Vim/dal* population, one *E. ovata* population (NaHi) and two *E. gunnii* populations (ShLa and MaPl), which had high levels of sesquiterpenoids of compound group D, formed a single cluster with a population of *E. coccifera* (PrBl). Three of the *E. ovata* populations (CtHi, HaCr and LeaK), which had high levels of acyclic terpenoids of group E, formed another single cluster separated from the main cluster of *Symphyomyrtus* populations. The two *E. risdonii* populations, two of *E. tenuiramis* , three of *E. pulchella*, one of *E. amygdalina* and *E. pauciflora* had high levels of monoterpenoids of group C. They were separated from the other *Monocalyptus* populations and grouped into the main cluster of *Symphyomyrtus* populations. Clusters within each subgenus did not follow the series divisions of Pryor and Johnson's classification (1971).

In addition, this dendrograms (Fig. 4.7) indicated that the variability in leaf oil components amongst the majority of *Symphyomyrtus* species was less than that in *Monocalyptus* species, with the exception of a number of populations of *E. ovata, E. dalrympleana* and *E. gunnii*. In fact, the fusion level of the majority of *Symphyomyrtus* species (typical 1,8-cineole populations) was lower than fusion levels of populations within individual species of some *Monocalyptus* species, such as *E. obliqua* and *E. delegatensis*.

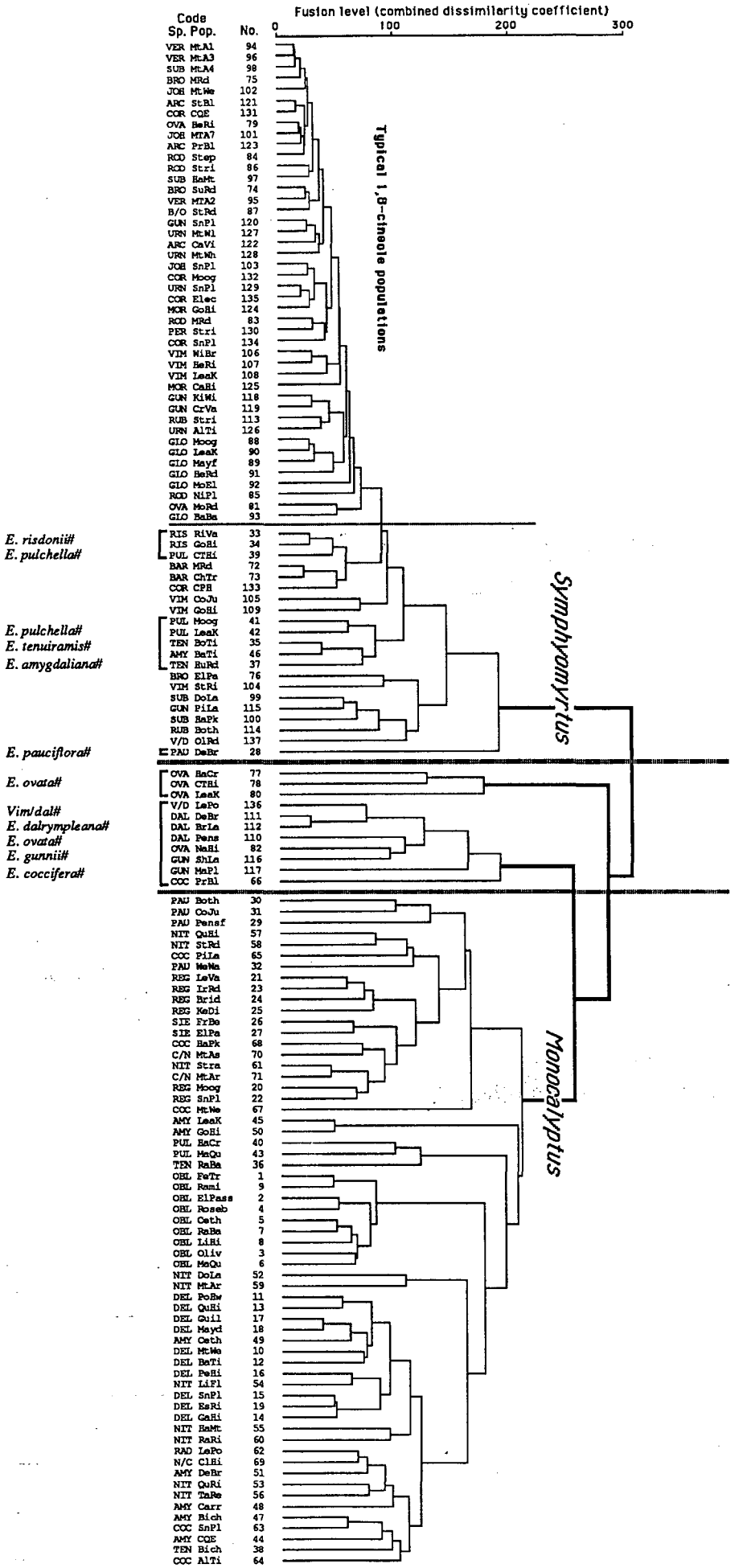


Fig. 4.7. Chemotaxonomic representation of average linkage clustering of 137 locality populations of 29 eucalypt species, using percentage composition data of all components of both adult and juvenile leaf oils of individual populations. #: Species populations lying outside the subgenus cluster.

The classification of populations using adult and juvenile leaf samples separately are shown in Appendix 4.4A and B respectively. The two dendrograms give a similar classification structure to that using both adult and juvenile leaf samples combined. However, in using only juvenile leaf samples, two of the *E. dalrympleana* populations (DeBr and BrLa) were grouped into the main cluster of *Symphyomyrtus* populations and only one population (Pens) was an outlier. Using adult leaves, all these three populations of *E. dalrympleana* were outliers from the main cluster of *Symphyomyrtus* samples.

The principal biochemical variation trends and population classification within the subgenus Monocalyptus

Variation in all terpenoid components of the leaf oils of *Monocalyptus* species populations was summarized by PCA. The first two PCs accounted for 20.8% and 13.0% of the total variance of terpenoid components among *Monocalyptus* species populations (Fig. 4.8). This analysis showed that the main variation within *Monocalyptus* was due to variation in levels of the terpenoids of compound groups A, B and C.

The variation along the horizontal axis of PC 1 was predominantly due to variation of the monoterpenoids of group A contrasted against variation in the monoterpenoids and sesquiterpenoids of groups C and B. Variation along the vertical axis (PC 2) was mainly due to the variation between the sesquiterpenoids (α–, β– and γ–eudesmol) of group B contrasted against the four monoterpenoids, 1,8-cineole, limonene, α–terpinene and terpinyl acetate, of group C (left figure).

The main variation amongst *Monocalyptus* species populations is oblique to both axes (variation between groups I and II and between groups I and III). The main variation amongst *Obliquae* populations involved continuous variation with increasing levels of group B and decreasing levels of group A compounds. All *E. obliqua* and *E. delegatensis* populations had high levels of group A compounds and were grouped into population group I and all populations of *E. regnans*, *E. sieberi* and *E. pauciflora* had high levels of group B sesquiterpenoids and grouped into population group II, with the exception of some *E. pauciflora* samples which formed a group IV. However, the *Piperitae* populations showed continuous variation in the two directions just described. Populations of *E. pulchella*, *E. tenuiramis* and *E. amygdalina* were separated into population groups I and III. Populations of *E. nitida* and *E. coccifera* were separated into population groups I and II. The *E. risdonii* populations had higher levels of 1,8-cineole, limonene, α–terpineol and terpinyl acetate and were grouped in population group III while the *E. radiata* population had higher levels of group A compounds and was located in population group I.

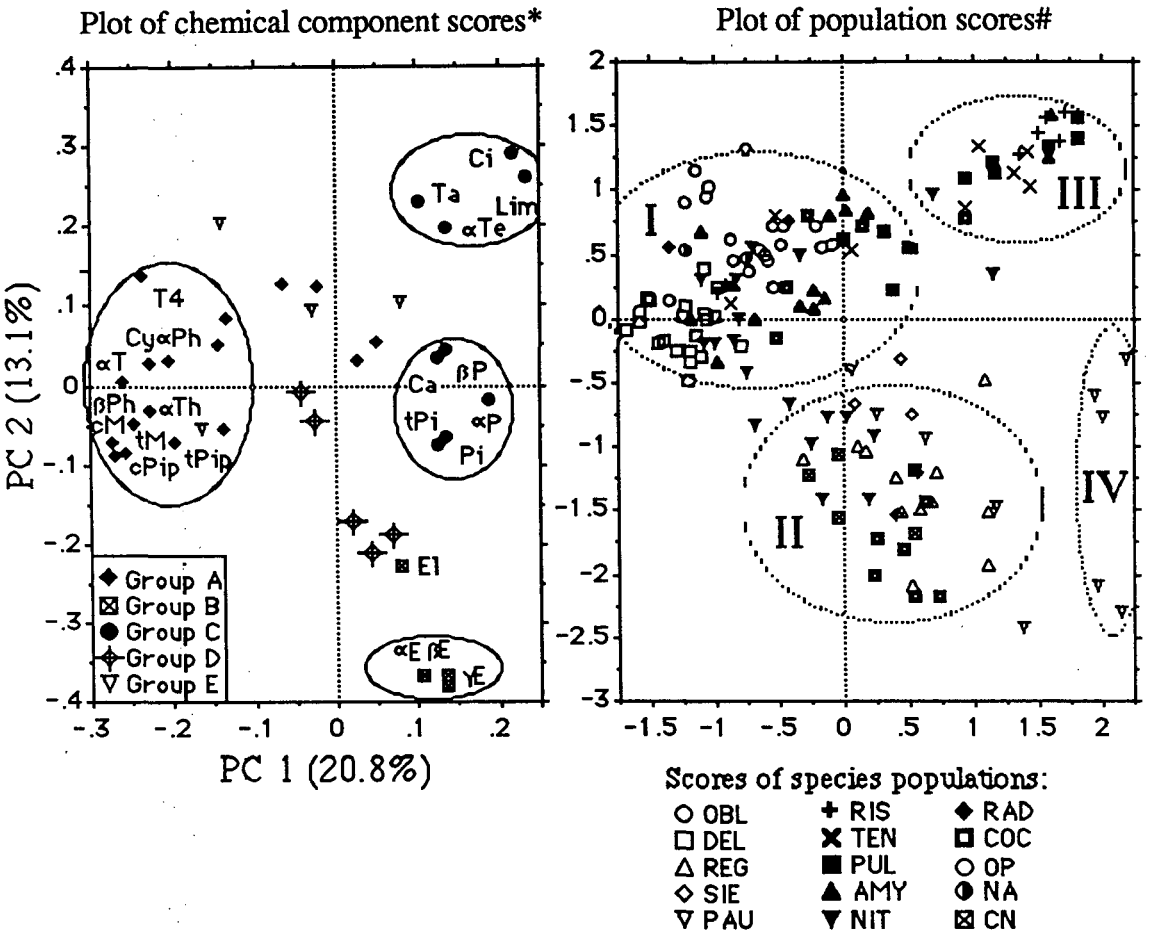


Fig. 4.8. Scatter plot of the terpenoid components (leaf) and samples of the 12 *Monocalyptus* species (right) on the two first principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf oils.

* Chemical compounds are indicated in the plot using compound codes, for chemical names see Table 4.4. Component groups indicated here are the biochemically related terpenoid compound groups as shown in Fig. 4.3.
# Population scores include juvenile and adult leaf samples

The average linkage clustering of all populations within *Monocalyptus* species (Fig. 4.9), as estimated from the percentage composition data of both adult and juvenile leaf oils, does not follow the series division, but separated the populations into three major clusters which corresponded to the population groups I, II and III in the principal component analysis shown in Fig. 4.8. The population group IV in Fig. 4.8 was included into population group II in Fig. 4.9. All populations of *E. risdonii*, *E. pulchella* and three of the *E. tenuiramis* and one of the *E. amygdalina* populations, which had high levels of 1,8-cineole, limonene, α–terpineol and terpinyl acetate, were grouped into cluster and corresponded to populations group III in PCA (Fig. 4.8). The second dichotomy separated the remaining populations into groups I and II. All populations of *E. obliqua* and *E. delegatensis* and five of *E. amygdalina*, seven of *E. nitida*, two of *E. coccifera* and

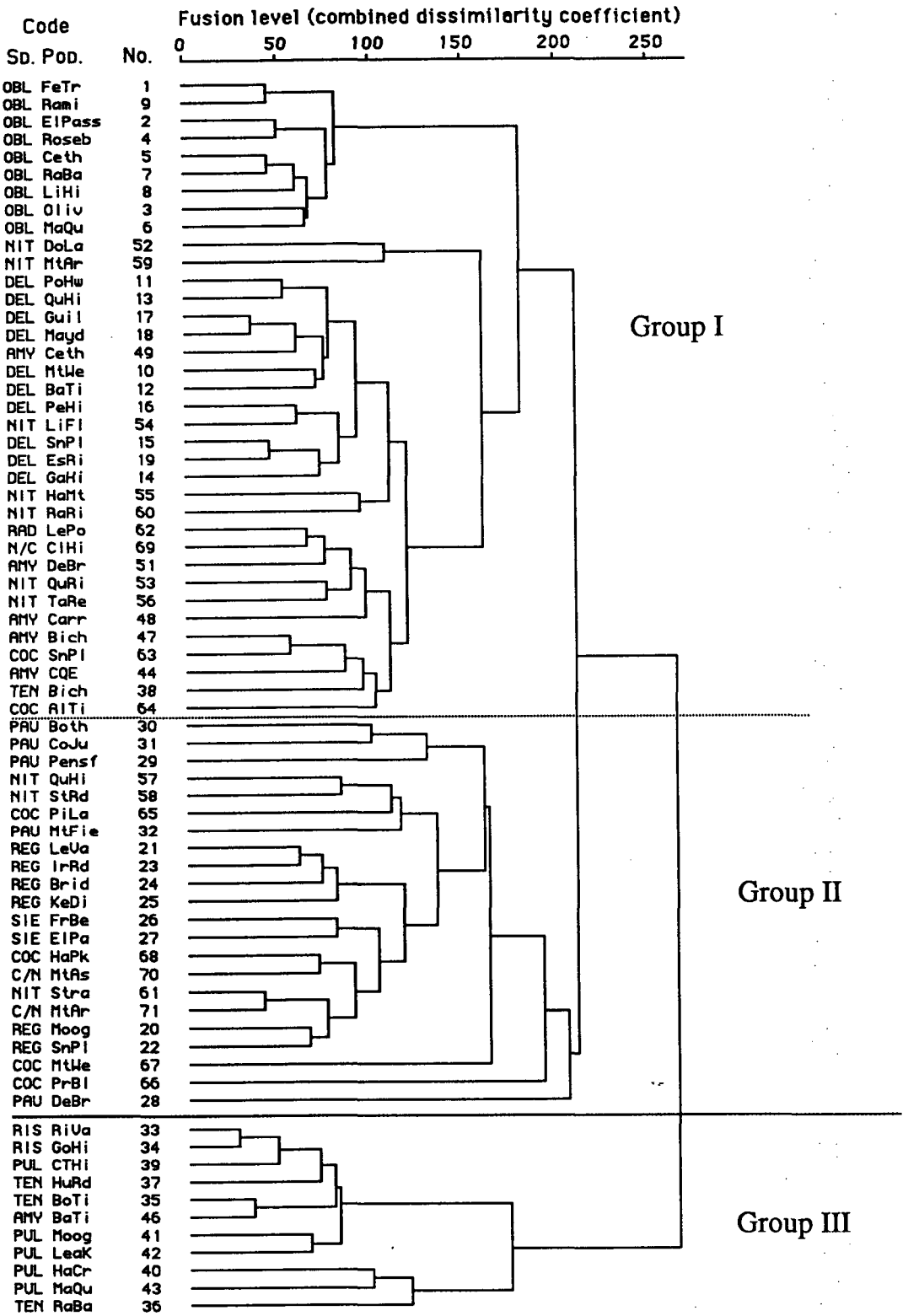


Fig.4.9. Average linkage clustering of populations of *Monocalyptus* species, using percentage data of all components of both adult and juvenile leaf oils of individual populations.

one of *E. tenuiramis* as well as the single population of *E. radiata* of the *Piperitae* series, which had higher levels of group A compounds, were grouped into cluster I. All populations of the *Obliquae* species, *E. regnans*, *E. sieberi* and *E. pauciflora*, which had high levels of sesquiterpenoids of group B, were grouped together with the remaining populations of *E. nitida* and *E. coccifera* of the *Piperitae* into cluster II which corresponded to population groups II and IV in PCA. This dendrogram indicated that the *Obliquae* populations had, with the exception of *E. pauciflora*, less variation within a species compared to populations of the *Piperitae* where variation within species was large and species differentiation was poor.

Population classification within the series *Obliquae*

The ordinations from PCA of variation in terpenoid components between populations within the series *Obliquae* indicated that all populations were grouped into individual species groups in the space defined by the first three PCs (Appendix 4.5 and discussion therein). The chemical distance between populations of *Obliquae* species, estimated by average linkage using percentage composition data of both adult and juvenile leaf samples, is shown in Fig. 4.10.

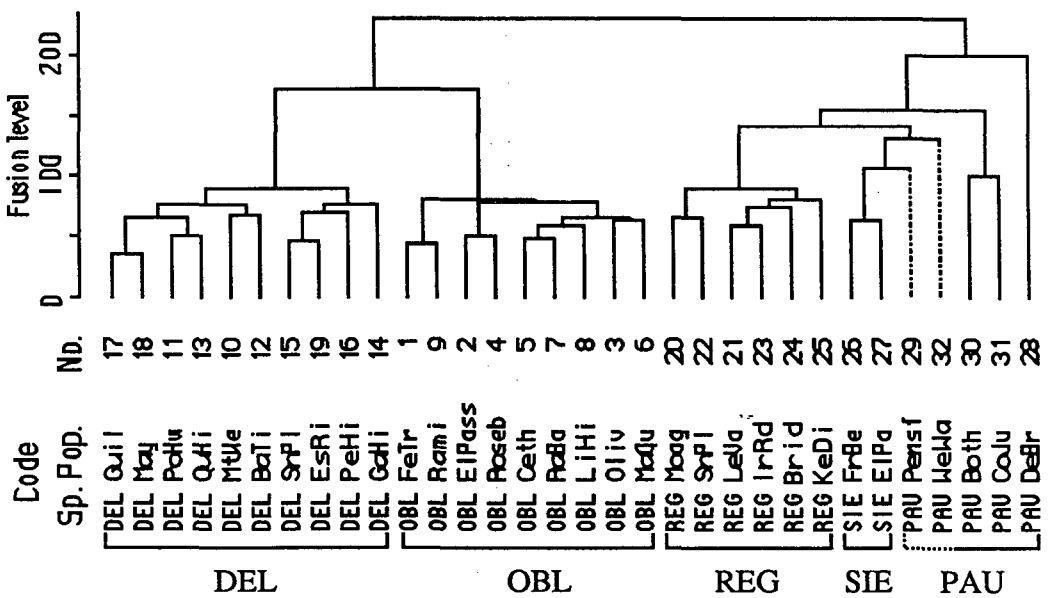


Fig. 4.10. Average linkage clustering of populations of the series *Obliquae* using percentage data of all components of both adult and juvenile leaf oils of individual populations.

This dendrogram indicated that the *Obliquae* populations, with the exception of *E. sieberi* and *E. pauciflora*, grouped well into species (i.e. *E. regnans*, *E. obliqua* and *E. delegatensis*). The major dichotomy is between the populations of *E. delegatensis* and *E.*

obliqua and those of *E. sieberi*, *E. regnans* and *E. pauciflora*. The leaf oils of *E. delegatensis* and *E. obliqua* are, therefore, markedly different from the other *Obliquae* species. The dendrogram indicated that populations of *E. pauciflora* were fused at much higher levels than those of *E. delegatensis*, *E. obliqua*, *E. regnans* and *E. sieberi*, indicating greater variation within *E. pauciflora*.

Variation within the series *Piperitae*

The ordinations from PCA of variation in terpenoid compounds between populations of the series Piperitae indicated that all leaf samples could be separated into three population groups on the space defined by the first two PCs (Appendix 4.6 and discussion therein). The population group I had higher levels of monoterpenoids of group C components and group II had higher levels of sesquiterpenoids of group B and D while populations had higher levels of monoterpenoids of group A were grouped into population group III.

The classification of populations of the *Piperitae* species, as indicated in the dendrogram in Fig. 4.11, showed marked intraspecific and interspecific variation. The first dichotomy separated all the populations of *E. risdonii*, three of *E. pulchella*, two of *E. tenuiramis* and one of *E. amygdalina*, with high levels of monoterpenoids of compound group C, from other populations. The second and third dichotomy then separated one *E. coccifera* and two *E. amygdalina* populations from the other populations. The remaining populations were separated into groups II and III which corresponded to population groups II and II in PCA (Appendix Fig. 4.6). The five populations of *E. nitida*, three of *E. coccifera* and two populations of the intermediate form between *E. nitida* and *E. coccifera* all had high levels of sesquiterpenoids of compound groups B and D and formed the cluster group II. However, cluster group III is a mix of populations from all *Piperitae* species with the exception of *E. risdonii*.

In summary, *Piperitae* species are less well defined on the basis of leaf oils compared with *Obliquae* species.

The principal trends in biochemical variation and population classification within the subgenus Symphyomyrtus

Variation in all terpenoid compounds of the leaf oils from *Symphyomyrtus* species populations was summarized by PCA and the population scores on the first two principal component axes are shown in Fig. 4.12. Population scores on these two axes indicated that the majority of populations had high levels of 1,8-cineole, limonene and α–pinene and related monoterpenoids of compound group C. These populations occurred together and were located on the right hand side of the horizontal axis. There were two major

directions of continuous variations which separated outlying populations of the *Symphyomyrtus*. One was due to increasing levels of acyclic monoterpenoids of group E and a decrease in levels of monoterpenoids of group C. It is notable that this variation was mainly due to populations of *E. ovata*. Another was the variation associated with increasing levels of sesquiterpenoids of group B and D and a decrease in levels of monoterpenoids of group C. All populations of *E. dalrympleana* and some of the *E. gunnii*, *E. vernicosa* and *E. ovata* populations varied in this direction from the main cluster of *Symphyomyrtus* populations.

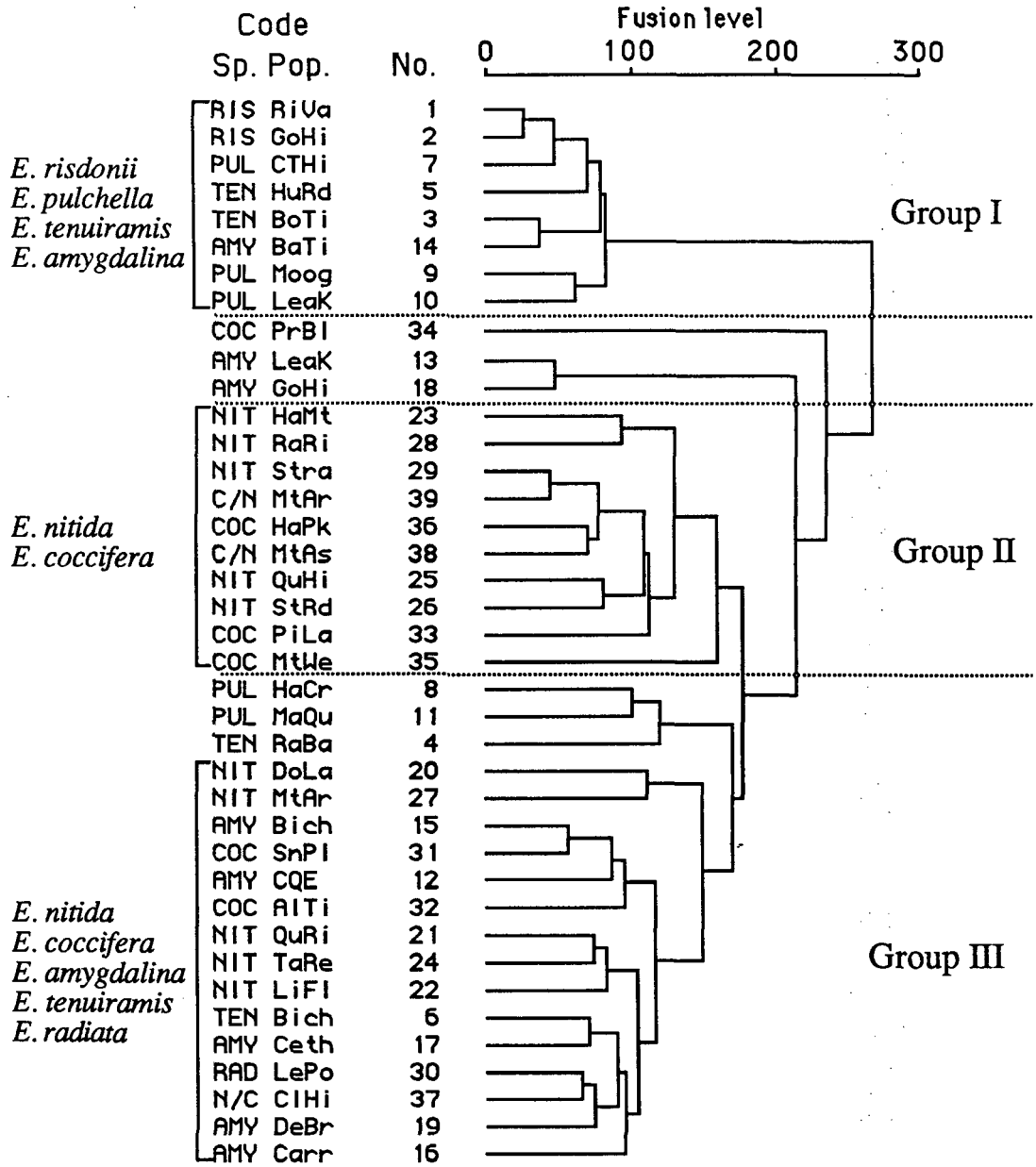


Fig. 4.11. Average linkage clustering of populations of the series *Piperitae*, using percentage data of all components of both adult and juvenile leaf oils of individual populations.

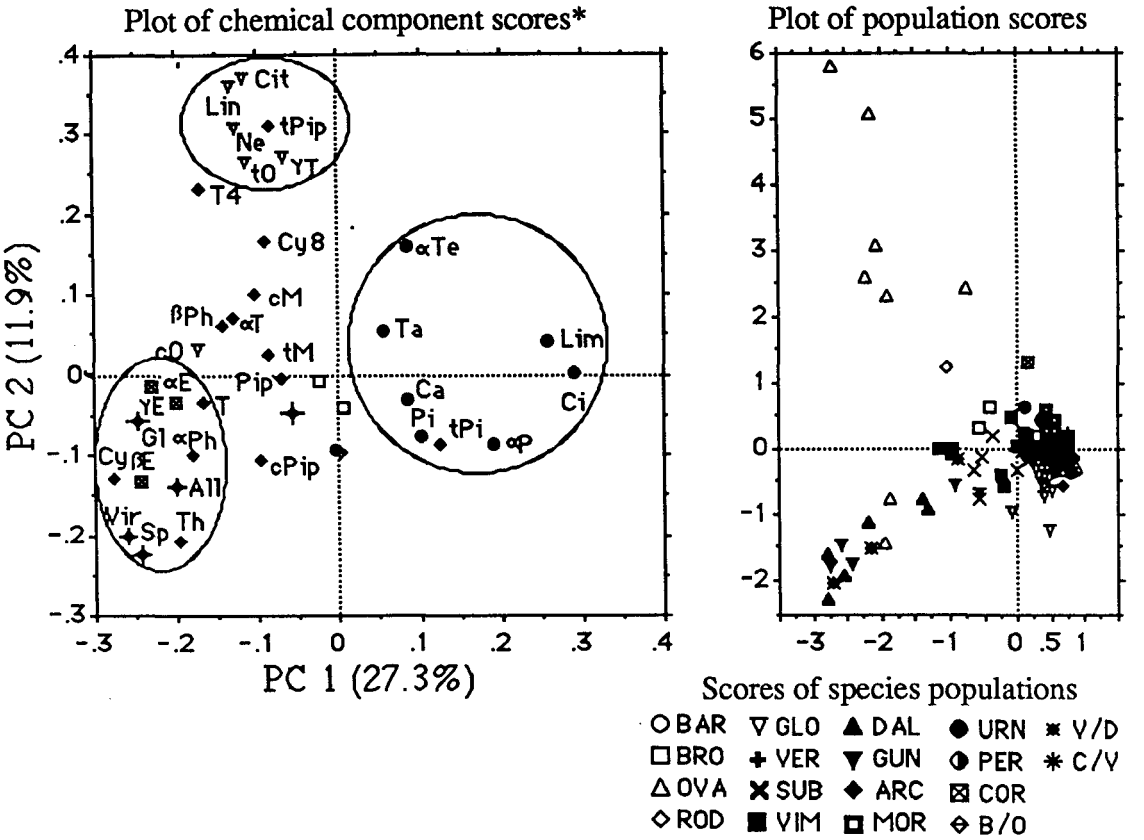


Fig. 4.12. Scatter plots of the terpenoid components (leaf) and samples of the 12 Symphyomyrtus species (right) on the first two principale components (PC1 and PC2) derived from analysis of juvenile and adult leaf oils.

The overall variation in the leaf oils between the *Symphyomyrtus* populations, is summarized in the average linkage dendrogram in Fig. 4.13. This dendrogram does not correspond to the separation of the species into the two series of Pryor and Johnson (1971) and other species groups, such as yellow gum etc. The first dichotomy separated all populations of *E. dalrympleana*, four of *E. ovata* and two of *E. gunnii* and one of the *Vim/dal* populations which had low levels of monoterpenoids of compound group C and high levels of sesquiterpenoids of groups B and D or monoterpenoids of group E. The second dichotomy then separated one of the *E. brookeriana*, *E. viminalis*, *E. rubida* and *Vim/dal* populations and two of the *E. subcrenulata* populations from the main population cluster. However, the remaining populations in the main cluster exhibited very little variation. Variation between these populations was even less than that between populations of *E. dalrympleana*.

This dendrogram indicated little consistent variation between the *Symphyomyrtus* species in leaf oil composition.

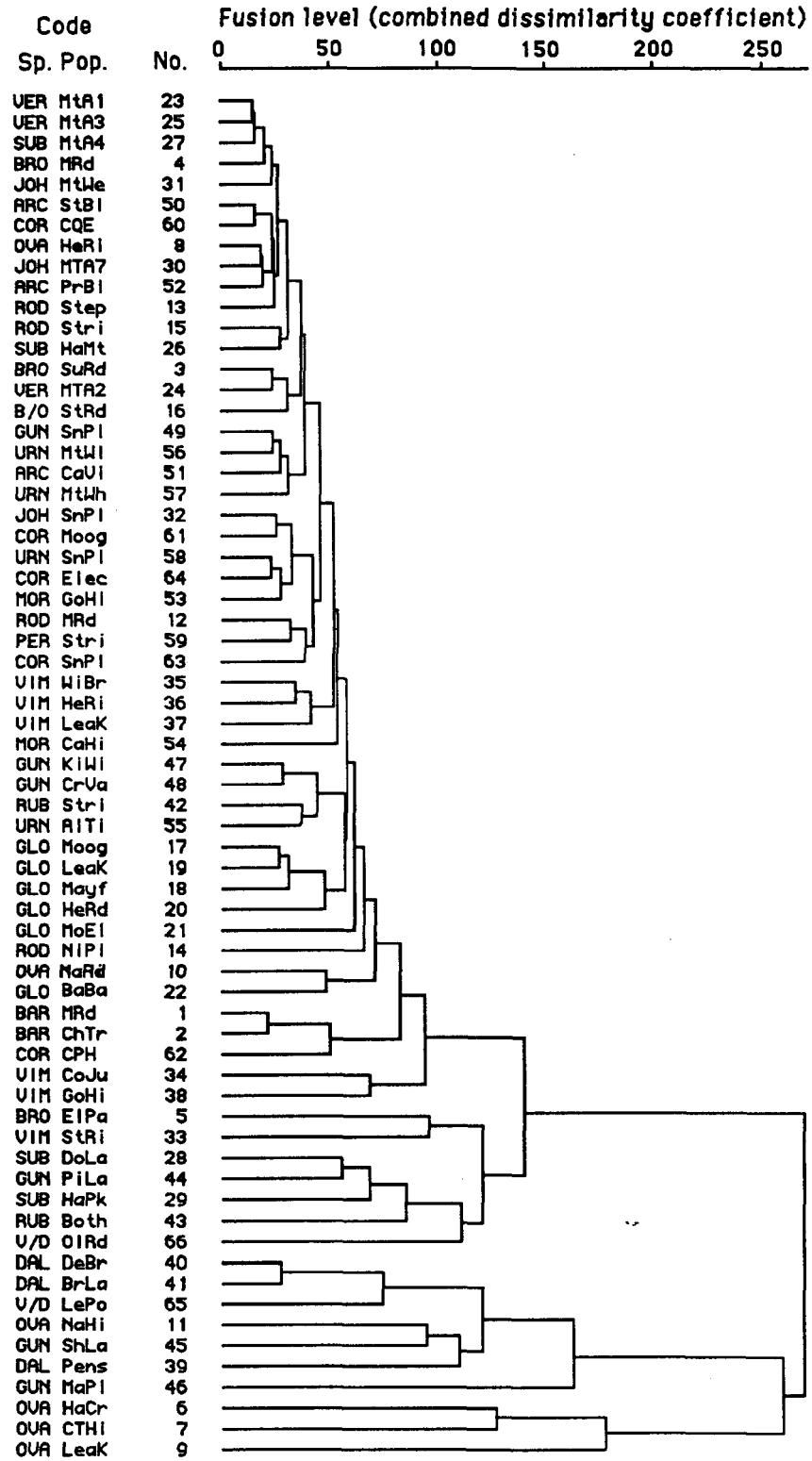


Fig. 4.13. Average linkage clustering of all populations of *Symphyomyrtus* species, using percentage data of all components of both adult and juvenile leaf oils of individual populations.

Variation within the series *Ovatae*

The average linkage clustering classification of populations from *Ovatae* species (Pryor and Johnson 1971) is shown in Fig. 4.14. There is a large difference between populations within *E. ovata*. The two populations HeRi and MoRd classified as *E. ovata* on morphological criteria differed markedly from the main group of *E. ovata* populations. One of the west coast population, HeRi, of *E. ovata* had affinities to the western population SuRd of *E. brookeriana* and the intermediate population, StRd, between *E. ovata* and *E. brookeriana*. The ElPa population of *E. brookeriana* also differs markedly from other *E. brookeriana* populations. There was relatively little variation within *E. barberi* and the two populations of this species differed markedly from other species.

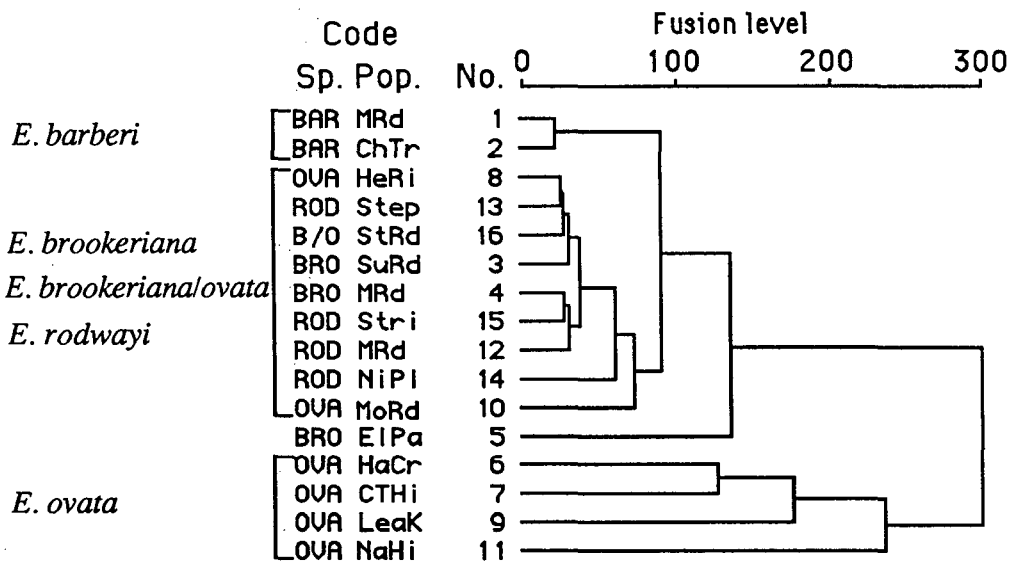


Fig. 4.14. Chemotaxonomic representation of average linkage clustering of populations of the series *Ovatae*, using percentage data of all components of both adult and juvenile leaf oils of individual populations.

Variation within the series *Viminales*

As indicated in Fig. 4.15, there is little variation amongst the majority of *Viminales* populations and species are poor defined. This dendrogram indicated that only populations of *E. dalrympleana*, one of *Vim/dal* and two of *E. gunnii* populations (ShLa, MaPl) differed significantly from other populations. There was very little variation between the Mt Arrowsmith populations of yellow gums (*E. johnstonii, E. subcrenulata* and *E. vernicosa*) despite the large morphological variability on this mountain (see Jackson 1960). All yellow gum populations from Mt Arrowsmith were grouped into a

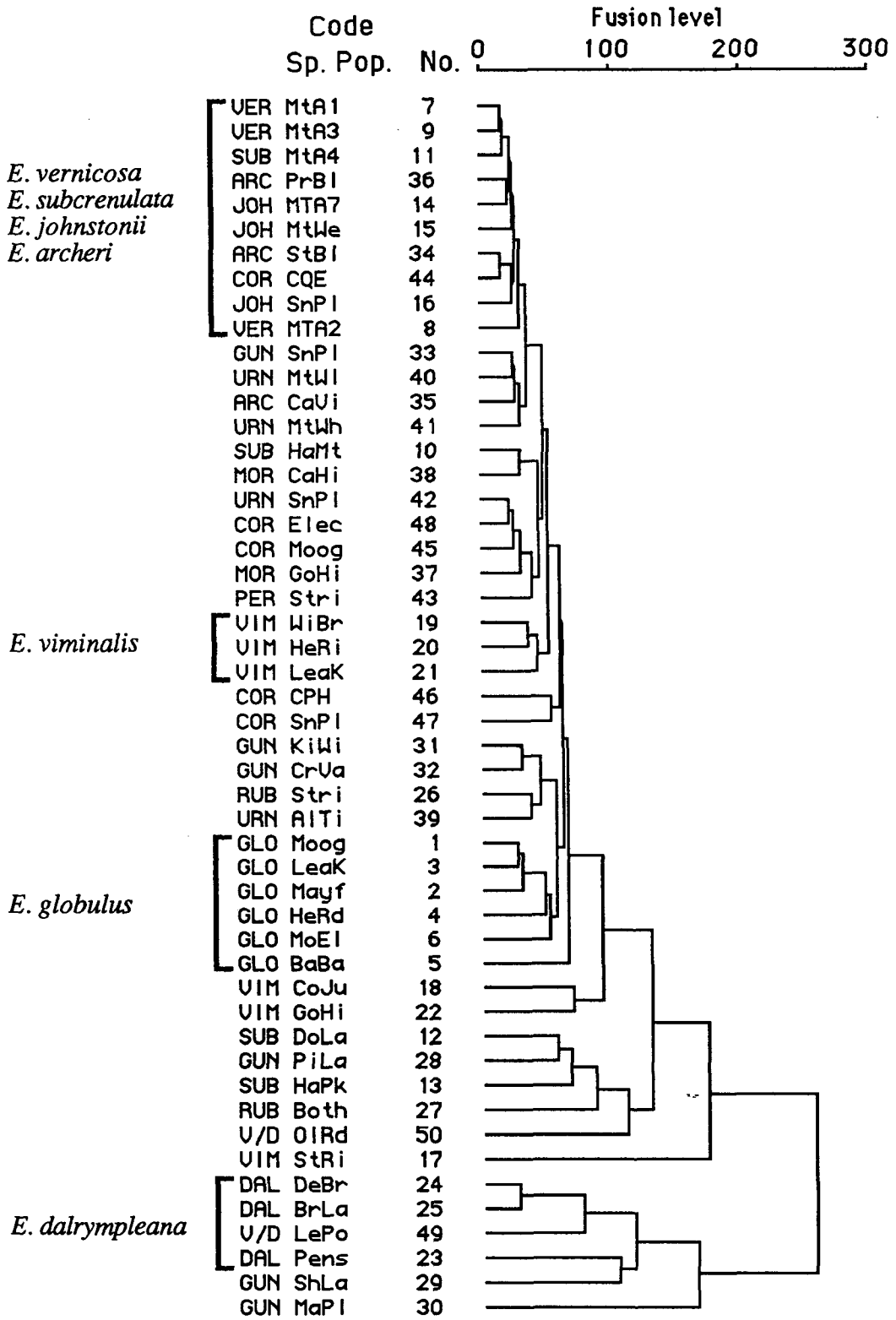


Fig. 4.15. Chemotaxonomic representation of average linkage clustering of populations of the series *Viminales*, using percentage data of all components of both adult and juvenile leaf oils of individual populations.

relatively fused cluster with other yellow gum populations. However, the populations of *E. subcrenulata* from Cradle Mountain (HaPk and DoLa) differed from the Mt Arrowsmith populations and were separated from the main cluster of populations. As indicated by the dendrogram, the leaf oils of the two *E. archeri* populations, PrBl and StBl, were similar to the main group of the yellow gum populations.

4.5.2. The species classification of Tasmanian *Eucalyptus*

The dendrogram resulting from the average-linkage clustering of the mean percentage composition of all 46 volatile compounds of both adult and juvenile leaves of individual species (unstandardised and unweighted data), is shown in Fig.4.16. For the Tasmanian *Eucalyptus* species, the association of these volatile chemical compounds support the major morphological division at the subgenera level of the genus, but with several exceptions. *E. dalrympleana*, *E. ovata* and *E. gunnii* were separated from the major *Symphyomyrtus* species cluster group. All other *Symphyomyrtus* species clustered closely together with little variation. In contrast, the *Monocalyptus* species fused at a much higher level with three species, *E. tenuiramis*, *E. pulchella* and *E. risdonii*, lying outside the *Monocalyptus* cluster and clustering with *Symphyomyrtus* species.

As discussed above, the leaf oil composition of the three *Piperitae* species, *E. risdonii*, *E. tenuiramis* and *E. pulchella* had a higher percentage of 1,8-cineole and other monoterpenoids of compound group C than the other *Monocalyptus* species. The other two species groups had high percentages of sesquiterpenoids of group B or higher percentage of monoterpenoids of group A. The main species cluster of the subgenus *Monocalyptus* was further divided into two groups: one comprised of *E. sieberi*, *E. pauciflora*, *E. regnans* and *E. coccifera* and the other by *E. delegatensis*, *E. nitida*, *E. amygdalina*, *E. radiata* and *E. obliqua*. Thus, there was greater differentiation among most *Monocalyptus* species in leaf oil composition than among the majority of *Symphyomyrtus* species.

The outlying species of the subgenus *Symphyomyrtus* appear to be species with low 1,8-cineole and monoterpenoids of group C and are low in oil yields. *E. dalrympleana* had the lowest oil yield among *Symphyomyrtus* species and oil composition was not related to any specific chemotaxonomic group. *E. ovata* and *E. gunnii* had a large variation in oil yield and composition between populations. Many populations of these two species had very low levels of 1,8-cineole and compounds of group C.

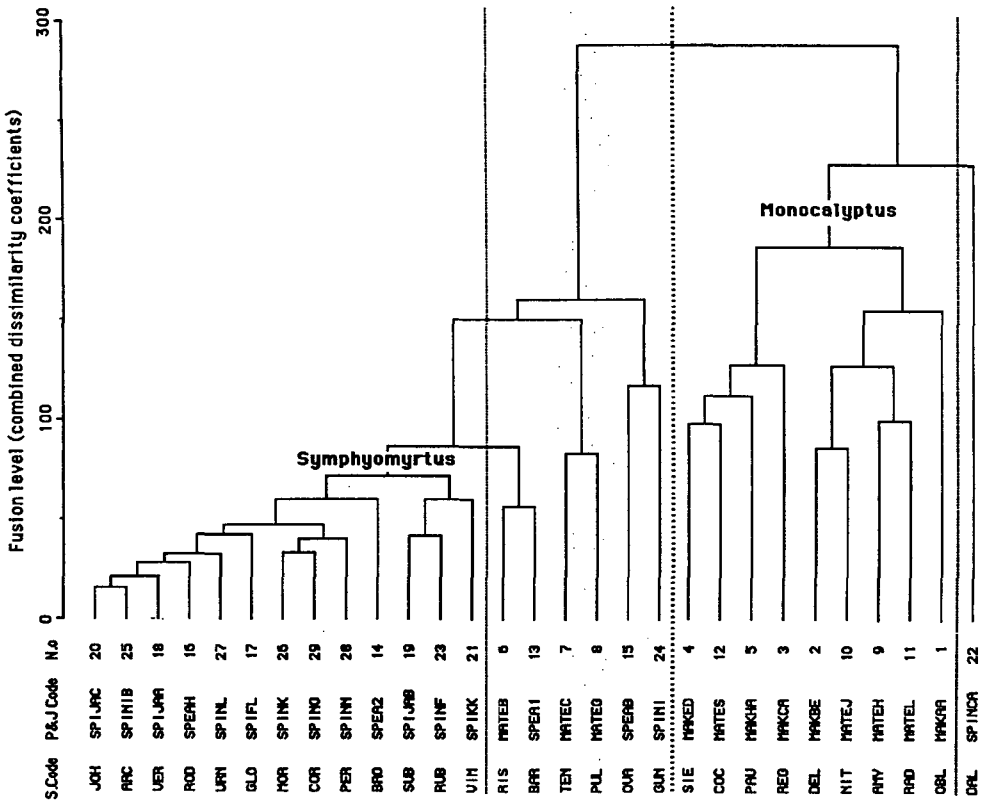


Fig. 4.16. Average linkage clustering of 29 *Eucalyptus* species, using the mean percentage of all oil compounds of both adult and juvenile leaf oils of individual species (unweighted data).

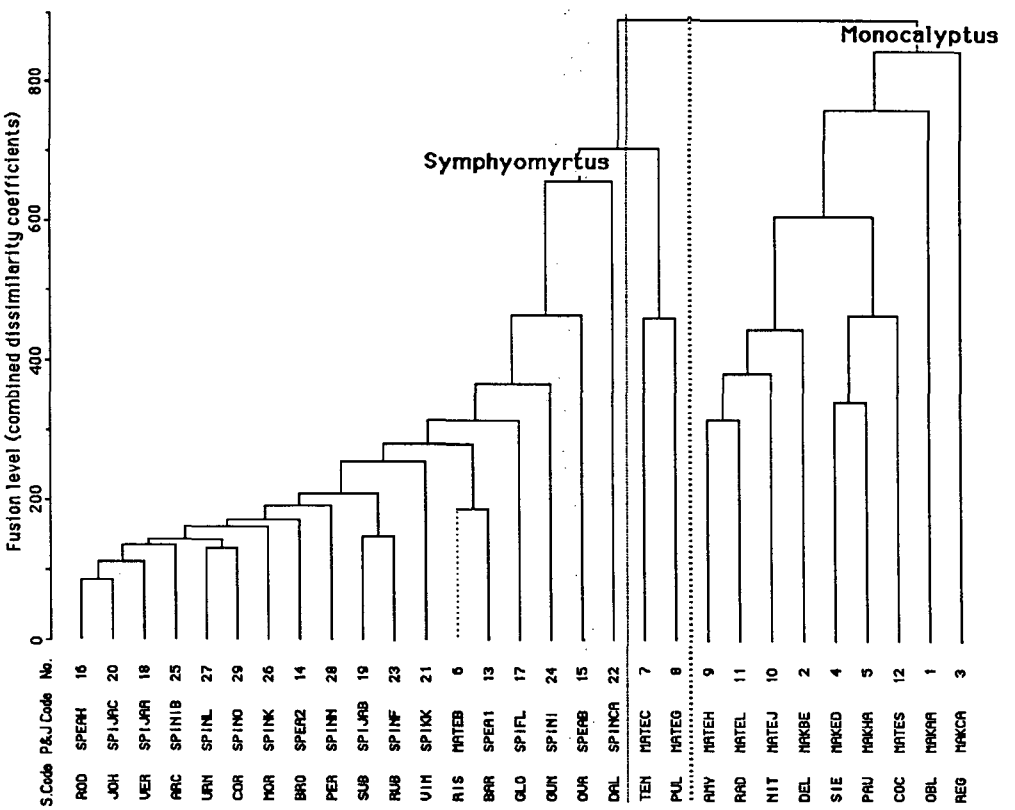


Fig. 4.17. Average linkage clustering of 29 *Eucalyptus* species, using the mean percentage of all oil compounds of both adult and juvenile leaf oils of individual species (standarized and *F*-weighted).

The average-linkage clustering using the standardised and *F*-weighted data of both adult and juvenile leaf samples is shown in Fig. 4.17. This analysis weights characters in the manner which emphasise characters that show little intraspecific variation but display large differences between species. The dendrogram (Fig. 4.17) for both adult and juvenile leaf samples supported the major subgeneric split but with *E. risdonii* having closer affinities to the *Symphyomyrtus* species. In this dendrogram, all *Symphyomyrtus* species cluster close together and there is clearly an order to their similarity that is indicated by *E. dalrympleana* being the most dissimilar from the core of the cluster followed by *E. ovata* and then *E. gunnii*. Although the *Monocalyptus* species, *E. tenuiramis* and *E. pulchella,* lay outside the *Monocalyptus* species cluster they were not associated with any of the species clusters of the subgenus *Symphyomyrtus*. However, the trend toward the *Symphyomyrtus* leaf oils has occurred independently in populations of several *Piperitae* species - *E. risdonii*, *E. tenuiramis* and *E. pulchella.*

For both subgenera, series differences are not maintained in those average-linkage clustering using the standardised and *F*-weighted data of oil composition.

The dendrogram for adult (Fig. 4.18) and juvenile (Fig. 4.19) leaf samples gave similar results. The dendrogram of adult leaf samples indicates that only *E. pulchella* and *E. risdonii* lay outside the species cluster of the subgenus *Monocalyptus* and that of juvenile leaf samples indicated that only *E. risdonii* lay outside the *Monocalyptus* species cluster. Comparison of the dendrogram of adult and juvenile leaf samples also indicated that it is mainly in adult leaf oil composition that *E. dalrympleana* is divergent from the main *Symphyomyrtus* species cluster. In contrast, *E. risdonii* and *E. ovata* are atypical for their respective subgenera in both adult and juvenile leaf oil composition.

4.6. Discussion

The leaf oils of twenty five of the 29 Tasmanian eucalypt species have been studied by other works (see Lassak *et al.* 1991). However, many of these species were examined by early studies (Baker and Smith 1920) when the classification of *Eucalyptus* was poorly defined. Only eighteen species were examined after publication of Blakely's (1956) and Pryor and Johnson's (1971) classification and some of these reports had poorly identified oil compounds, (e.g. Yatagai and Takahashi 1983). The leaf oils of *E. tenuiramis* (including the old name of *E. tasmanica*), *E. barberi* and *E. archeri* had not been analysed previously. This report on leaf oils of the 29 Tasmanian eucalypt species is the first study in which species identification and sampling were under strict control and comparisons were made for both adult and juvenile leaves of range of provenances of each species.

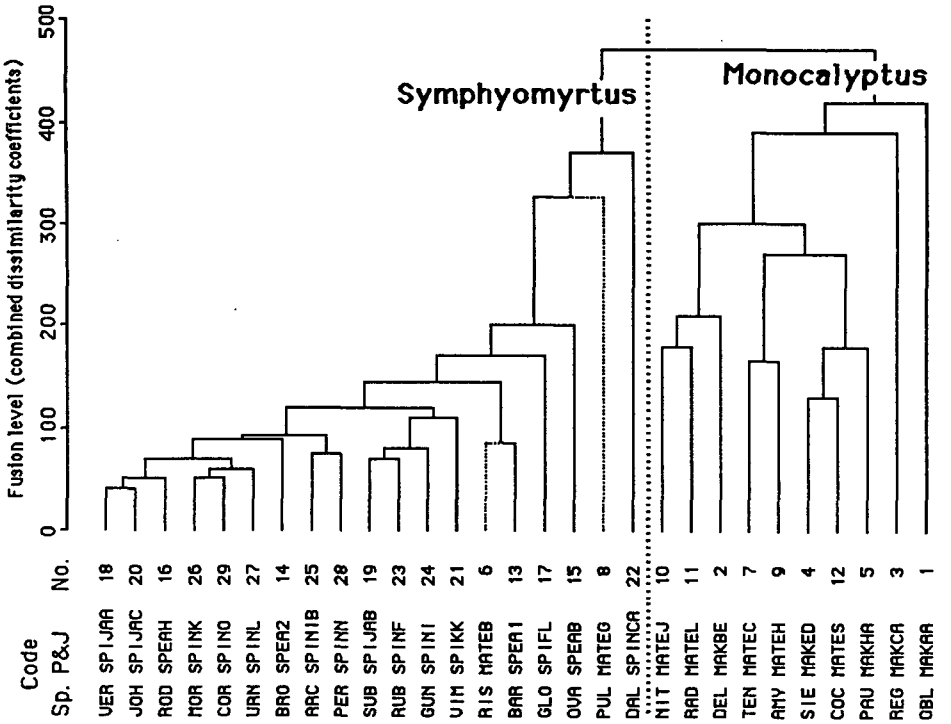


Fig. 4.18. Average linkage clustering of 29 *Eucalyptus* species, using the mean percentage contents of all oil compounds of adult leaf oils of individual species (standarized and *F*- weighted).

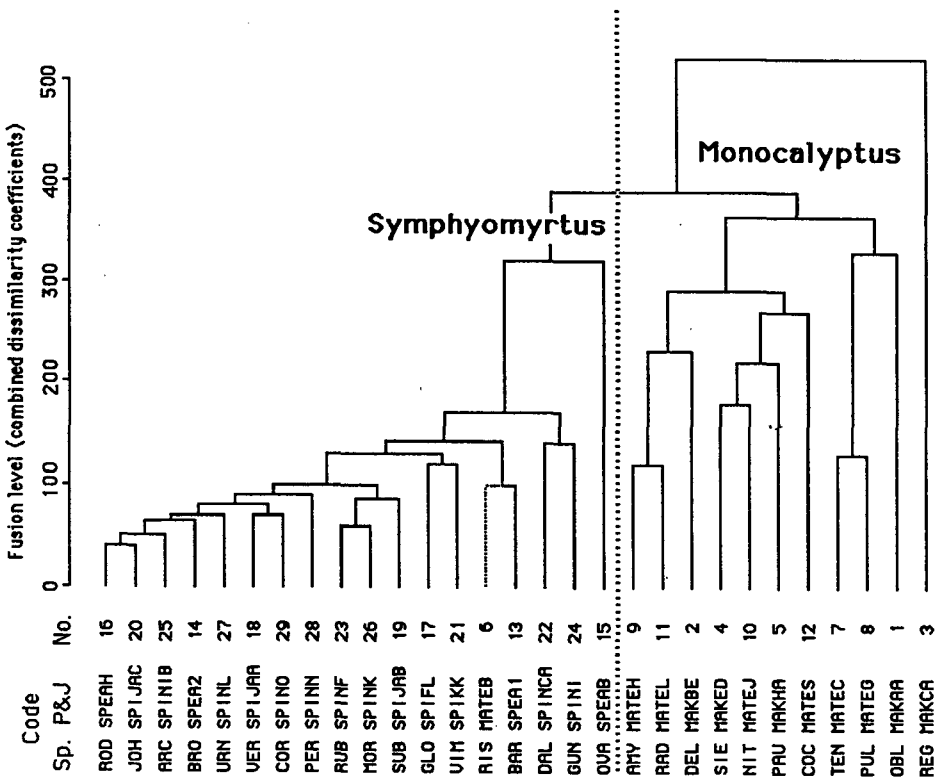


Fig. 4.19. Average linkage clustering of 29 *Eucalyptus* species, using the mean percentage of all oil compounds of juvenile leaf oils of individual species (standarized and *F*- weighted).

Results indicated that the chemical compositions of many leaf oils identified were similar to those summarised by Lassak *et al.* (1991). However, the leaf oils from some species were found to have markedly different chemical compositions to that identified by other works, particularly in *E. ovata, E. dalrympleana, E. rubida* and *E. gunnii.* For example, no nerolidol was identified from the leaf oils of a number of populations (including an east coast locality) of *E. ovata* in Tasmania by Brooker and Lassak (1981). However, nerolidol was found to be one of the major components of *E. ovata* oil in this study, particularly in the case of unambiguous *E. ovata* populations. 1,8-Cineole and α–pinene were identified to be the principal compounds of *E. rubida* in this study in contrast to the report of Boland *et al.* (in Lassak *et al.* 1991) who found that the adult trees of *E. rubida* in NSW produced a leaf oil characterised by spathulenol and *p*-cymene but 1,8-cineole only occurred in trace amounts.

Results indicated that the composition of the volatile terpenoids and oil yields varied widely among Tasmanian eucalypts. Variation in oil composition occurs at all levels of the taxonomic hierarchy but the major differences in oil composition occur at the subgeneric level. Thus, in agreement with the published data of Lassak *et al.* (1990), most *Symphyomyrtus* species were characterized by 1,8-cineole and α–pinene as principal components and most *Monocalyptus* species by α– and β–phellandrene, *trans*- and *cis*-*p*-menth-2-en-1-ol, *trans*- and *cis*-piperitol, *p*-cymene and piperitone. This major qualitative difference between subgenera was supported by evidence of divergence of biosynthetic pathways in the formation of specific monoterpenoid compounds. The biosynthetic pathways proposed are consistent with the principle of current hypothesis and theories on the subject.

Samples examined in this study tend to occupy different ecological habitats. However, there is strong evidence to suggest that differences in the oil chemistry of Tasmanian eucalypts are mainly determined by the genetic makeup of the taxa. Differences are maintain between species collected from the same locality, (e.g. species from Snug Plains mixed and other mixed forest) while species similarities and differences were maintained in many cases amongst different populations of a species which are geographically widely distributed (e.g. *E. delegatensis, E. regnans and E. globulus*). In addition in the previous chapter (chapter 3), species differences are maintained when genetically similar material is grown under different environmental conditions. Therefore, it is highly likely that both composition and biosynthesis of compounds of leaf oils of these species are under strict genetic control.

The results indicated that differences between species in the subgenus *Monocalyptus* were much greater than that in *Symphyomyrtus*. Within the subgenus *Monocalyptus, Obliquae*

species are well differentiated whereas in the *Piperitae* there was poor species differentiation for most species.

The chemical compositions of leaf oils from species in the subgenus *Monocalyptus* exhibited a complex variation among all major components. First, the major oil components of leaf oils from *Obliquae* species varied from monoterpenoids to sesquiterpenoids. The leaf oils of *E. regnans, E. sieberi* and *E. pauciflora* contained high proportions of sesquiterpenoids, such as α–, β– and γ–eudesmol, as major components but *E. obliqua* and *E. delegatensis* contained mainly monoterpenoids and less sesquiterpenoids. Second, the chemical compositions of leaf oils from *Piperitae* species showed wide variation among all components. In this series, the leaf oil of *E. coccifera* was characterized by higher proportions of sesquiterpenoids as major components while other species were characterised by monoterpenoids. The monoterpenoid, 1,8-cineole, predominated in the leaf oils of *E. risdonii, E. pulchella* and *E. tenuiramis* but in other species, such as *E. nitida, E. radiata* and *E. amygdalina,* leaf oils were predominated by α– and β–phellandrene, piperitone or *p*-cymene respectively. Finally, the variation in the chemical composition of leaf oils from *Piperitae* species intergraded in virtually all possible combinations.

The variation in chemical composition of leaf oils within the *Monocalyptus* species was related to the level of morphological divergence within species and intergradation between species as described by Davidson *et al.* (1981) (see Fig. 2.1 in Chapter 2). With the exception of *E. risdonii* and a single locality of *E. radiata*, the *Piperitae* species exhibited very high variation (ΣV, see table 2.1) in oil composition between populations within species. This result is in agreement with findings of other studies in which the leaf oil some *Piperitae* species had different chemical forms, such as *E. radiata* and *E. dives* (Johnstone 1984). In contrast, some species, such as *E. delegatensis, E. obliqua, E. regnans* and *E. globulus*, which occur over an extensive geographic range in Tasmania, exhibited only low variation (ΣV) in oil composition over the wide range of localities sampled. These findings were consistent with the morphological patterns as these species exhibited less clinal variation and intergradation between species when compared with the extreme intergradation of *Piperitae* species.

Results indicated that variation in leaf oil between juvenile and adult leaves among Tasmanian eucalypt species was mainly quantitative rather than qualitative. With the exception of only one species, *E. dalrympleana*, which had significant differences in the percentage contents of all major compound between leaf types, most species showed no significant differences in major compounds between leaf types. The adult and juvenile leaves of *E. obliqua, E. nitida* and *E. delegatensis* had significant differences in the amounts of the individual components, e.g. piperitone. However, the chemical

composition of the leaf oils from adult and juvenile leaves was qualitatively similar without change in nature. This result is similar to some reports (e.g. in *E. dives* Penfold and Willis 1961, in *E. nitens* Franich 1986 and in *E. polybractea* Brooker *et al.* 1988), which suggested there was none or only limited qualitative differences between juvenile and adult leaves of eucalypt species. Similarly, a wide geographic study of *E. delegatensis* found that only small qualitative differences existed between juvenile and adult phases (Weston 1984).

However, the oil yields showed a subgeneric difference in ontogenetic change. The juvenile leaves of *Monocalyptus* species produced significantly lower oil yields than adult leaves in spite of any specific and habitat differences. The most significant differences in oil yields between the juvenile and adult leaves were found for *E. delegatensis*. This is consistent with the finding of Boland *et al.* (1982) who found that the yield of oil from juvenile (seedling) *E. delegatensis* leaves was invariably much lower than that of adult (Boland *et al.* 1982). As stated by Doran (1991), there are very few definitive studies on the effects of ontogeny on leaf oils, and in a number of studies it is not always obvious whether comparisons were free of the confounding influence of physiological leaf age. However, the results indicated that ontogenetic differences in leaf oils could be genetically based and be characteristic of *Monocalyptus* species in Tasmania.

In contrast, there was no consistent variation in oil yields between the adult and juvenile leaves of *Symphyomyrtus* species and, with the exception of *E. globulus*, *E. viminalis* and *E. dalrympleana*, there was no significant difference in the oil yield between adult and juvenile leaves. This indicated that ontogenetic differences in oil yield may be an important subgeneric difference for *Eucalyptus* species in Tasmania. Moreover, this suggests that there may be other phytochemical changes which also occur with ontogeny, in particular vegetative (and possibly reproductive) phase change. They may related to changes in faces of physiological behaviour and changes in pest and decease resistance.

Chapter 5
Leaf Waxes

5.1. Introduction

This chapter reports on the chemistry and morphology of leaf waxes of 29 eucalypt species in Tasmania. All samples examined in this study were similar to those used for leaf oil analysis as listed in Chapter 4, Table 4.1. Part A of this chapter describes the distribution of leaf wax chemicals among species and taxa and their chemotaxonomic structure. Part B considers the morphology of the waxes from selected species so as to determine if wax structure can be related to chemical composition and assess the taxonomic value of wax chemicals and their morphology.

PART A

5.2. Wax chemicals

5.2.1. Materials and methods

All leaf samples were similar to those used for oil analysis as listed in Appendix 4.1.

Individual leaf samples (25g) were placed in a glass flask, then 30 ml of pure hexane added and shaken at room temperature for 15 min. This extracting process was repeated 3 times. The hexane extract solutions were filtered, the solvent removed in a rotary evaporator at 25 ^{0}C and reduced to dryness in pre-weighed and labelled vials. After washing with hexane, the leaves were extracted with chloroform using the same process as above. The hexane and chloroform soluble waxes were stored as dry samples at room temperature prior to analysis. Wax yield was expressed as a percentage of dry leaf weight as for leaf oils. In this thesis only the hexane soluble wax is considered and chloroform soluble wax is to be discussed separately after further study.

The crude hexane soluble wax (as a 1% hexane solution) was analysed by combined GC-MS using a HP-5890 GC directly coupled to a VG 707F mass spectrometer with 2035 *data system*. The GC column was a 12m x 0.32mm x 0.17 µm film thickness Hewlett Packard (HP1) cross linked methyl-silica capillary column (SGE Pty Ltd). A SGE on

column injector was used. The oven temperature was programmed from 30 to 240 ^{0}C at 30 ^{0}C/minute, 240 to 300 ^{0}C at 6^{0}C/minute and held at 300 ^{0}C for 3 minutes. Helium was used as the carrier gas at 70KPa and a flow rate of approximately 2ml/minute.

Identification of individual compounds was based on reference mass spectra (Stenhagen, Abrahamsson and McLafferty "Registry of Mass Spectral Data"; NIST Mass Spectral Database).

Quantitative measurements of the components were determined by GLC using automatic sample injection on an identical capillary column and a HP-5880 GC linked to a HP integration system. They are based upon integrated peak areas using octadecane as internal standard.

All data analysis methods used in this chapter are similar to those described in Chapter 4.

5.2.2. Results and general survey

Hexane soluble wax yields and the mean percentage content of individual classes of compounds are shown in Table 5.1 and Fig. 5.1. The mean percentage content of each compound class listed in this table is the species mean of total percentage of each compound class. A category of unknown/unidentified components is also listed which usually represents low amounts of a large number of components whose spectra were too weak for positive identification. Some long chain esters and phenol esters identified with carbon number over 39 were also included in the unidentified category. The mean percentage and standard deviation of individual compounds of major compound classes resolved by GLC for all species analysed are shown in Table 5.2. The mean percentage distribution of homologues within each compound class for all species are shown in Fig. 5.2.

The GC analysis of the untreated crude wax was limited by several factors. Perhaps the most important has been the elution of different components at the same time resulting in mixed peaks of two and, occasionally, three different components. This occurred particularly with the triterpenoids that form the largest proportion of the wax in some species, such as *E. regnans* and *E. obliqua*. In many cases, peaks are sometimes a mixture of the triterpenoid and long chain phenolic or benzonic esters and in some instances a mixture of long chain diketones and phenolic or benzonic esters. This may result in some inaccuracy in the quantitative results as it is difficult to obtain absolute values for individual components. In such cases, if the peak is a mixture of two triterpenoids, the percentage composition represents the total of the peak area and it is listed under the dominant component (which could usually be determined) in Table 5.2. Because the long chain phenolic or benzonic esters often occur in low amounts and some

of their peaks were often covered by other major components in mature leaves of *Eucalyptus*, all components of these two compound types were included in the category of unidentified compounds in Table 5.1. At the same time, if the peak consists of a mixture of triterpenoids or long chain diketones with these esters, the percentage composition represents the total of the peak area and is listed under the dominant triterpenoid or long chain ß–diketone components, but the ester components present are not indicated. The method by which the integration is performed may also distort the true proportions of the components present by artificially inflating one component over the other when double peaks (indicating the presence of two components) occur. Therefore, the quantitative results for such peaks are an indication of the components present and their relative dominance rather than absolute measures.

These problems could be eliminated by the separation of the wax and derivatization to enhance the separation of individual components. However this would significantly increase the preparation and analysis time for each sample and for such a large number of samples could not be finished for this study. Therefore, a balance had to be struck between two alternatives - i.e. whether absolute quantitative measurements were necessary for every component thereby reducing the number of samples which could be examined in a given time or, whether the time taken in obtaining this information would be better employed in analysis of the untreated wax to better sample the taxonomic groups and use the same number of samples as used for the study of leaf oils. As this is a biological and taxonomically based study rather than a chemical study, the second option was taken as any inaccuracies were considered minor in the context of the study. The chemistry of some species was studied in detail and this information provided for identification of compounds in this study (The full details of these chemical studies involved those compounds which were separated and identified by using IR, UV, NMR, Mss and chemical reaction analyses, are not included in this thesis but will be published later).

The GLC separation of hexane soluble wax components in selected species and representative mass spectra of the major compound types are shown in Appendix 5.1. Any components listed in Table 5.2. A and B could be found from the named peaks of the GLC picture. The triterpenoids represent one class of compounds in which many of the components remain unnamed. All triterpenoids have been given an arbitrary alphabetical name and the mass spectra of these triterpenoids are showed on Appendix 5.1.

Table. 5.1. Mean percentage contents of compounds and yield of hexane soluble waxes of leaves from 29 eucalypt species

Species	Ch. Series †	Spp. Group¥	L.T *	Pop. No.	Yield**	n-Alkanes	Aldehydes	Alcohols	Diketones	Flavonoids	Triterpenoids	Ester1	Ester2+Unid
Subgenus Monocalyptus#													
Series Obliquae													
E. regnans	*Regnantes*	Ash	J	6	1.3	5.1	11.5	2.1	10.9	15.0	52.4	-	2.0
			A	6	0.9	6.2	11.5	1.6	9.9	13.1	49.6	-	7.0
E. obliqua	*Eucalyptus*	Ash	J	9	1.4	8.8	5.8	1.0	12.2	7.7	55.7	-	7.8
			A	9	1.3	8.4	6.4	1.3	14.4	8.8	50.3	-	9.5
E. delegatensis	*Eucalyptus*	Ash	J	10	1.2	3.7	26.4	19.2	6.0	13.7	25.5	-	4.5
			A	10	1.3	4.0	25.4	6.4	7.2	14.5	30.5	-	11.0
E. sieberi	*Psathyroxyla*	Ash	J	2	0.3	5.8	41.8	17.7	6.1	7.7	20.3	-	0.1
			A	3	0.4	5.9	17.6	4.1	18.5	6.8	41.5	-	4.7
E. pauciflora	*Eucalyptus*	Ash	J	5	0.6	2.8	21.7	3.3	2.9	15.2	48.6	-	4.5
			A	5	0.6	4.2	14.4	3.1	4.2	10.9	53.9	-	8.3
Series Piperitae													
E. radiata	*Radiatae*	Peppermint	J	1	0.3	8.1	58.6	4.1	0.4	16.3	8.4	0.4	3.8
			A	1	0.3	9.1	50.4	2.1	1.0	21.4	11.1	0.5	3.5
E. amygdalina	*Radiatae*	Peppermint	J	8	0.5	6.4	39.0	10.6	2.9	11.0	24.2	0.3	4.6
			A	8	0.5	6.7	31.0	8.5	3.0	14.4	31.4	0.5	3.5
E. puchella	*Radiatae*	Peppermint	J	5	0.6	5.3	23.1	10.1	17.4	15.5	14.4	1.6	11.6
			A	5	0.9	9.0	21.4	7.2	19.9	14.5	14.6	1.5	11.1
E. nitida	*Radiatae*	Peppermint	J	10	0.6	5.6	19.7	5.6	1.3	9.1	58.0	0.1	0.6
			A	10	0.8	4.7	17.2	5.1	1.1	8.9	61.3	0.3	1.4
E. coccifera	*Radiatae*	Peppermint	J	6	0.8	3.8	34.4	5.9	3.3	9.8	30.8	0.8	10.1
			A	6	0.6	5.3	24.7	4.6	15.0	8.7	29.1	4.9	6.7
E. tenuiramis	*Radiatae*	Peppermint	J	4	0.9	4.1	14.1	2.5	55.2	8.9	1.7	7.6	4.9
			A	4	0.9	8.1	7.5	0.6	57.9	9.3	2.3	8.5	4.9
E. risdonii	*Radiatae*	Peppermint	J	2	1.0	2.4	6.7	-	61.0	6.5	1.4	12.8	8.1
			A	3	1.1	7.8	2.5	0.1	66.0	4.7	0.5	11.5	6.0
Subgenus Symphyomyrtus													
Series Ovatae													
E. ovata	*Foveolatae*	Black Gum	J	6	0.4	18.3	44.9	12.9	9.2	3.4	0.3	-	10.0
			A	6	0.4	20.0	42.9	15.1	7.7	4.2	0.4	-	8.6
E. brookeriana	*Foveolatae*	Black Gum	J	4	0.4	29.0	14.0	4.3	27.6	8.4	1.7	-	14.0
			A	4	0.5	26.6	13.7	6.6	21.7	5.0	5.6	-	19.7
E. rodwayi	*Foveolatae*	Black Gum	J	4	0.3	27.1	7.8	1.0	36.2	13.1	4.7	-	9.1
			A	4	0.4	22.9	8.7	0.8	39.4	12.0	2.4	0.5	12.2
E. barberi	*Foveolatae*	Black Gum	J	2	0.2	74.3	5.9	0.2	5.4	4.7	0.3	1.2	7.0
			A	2	0.3	61.6	7.7	0.3	11.4	5.2	1.9	0.8	10.1
Series Viminales													
E. viminalis	*Viminales*	White Gum	J	6	0.6	8.5	6.5	0.2	61.0	7.0	0.7	0.1	14.9
			A	6	0.5	9.2	7.3	0.6	51.7	10.9	4.3	0.5	14.5
E. dalrympleana	*Viminales*	White Gum	J	3	0.8	3.7	5.6	2.6	54.2	17.3	3.5	-	12.0
			A	3	0.4	4.2	7.2	5.5	48.5	17.3	4.7	0.5	11.0
E. rubida	*Viminales*	White Gum	J	2	0.8	4.8	3.5	-	68.0	9.1	0.9	0.8	11.8
			A	2	0.8	4.8	5.7	-	71.0	7.1	1.7	1.5	7.1
E. johnstonii	*Viminales*	White Gum	J	5	0.7	11.4	4.2	0.1	22.8	6.0	46.3	-	8.2
			A	5	0.5	7.4	5.6	0.4	34.3	8.1	35.0	-	8.2
E. subcrenulata	*Viminales*	White Gum	J	4	0.6	10.7	4.1	0.2	23.1	6.7	46.3	-	7.9
			A	5	0.5	9.7	4.6	0.2	25.6	7.9	44.1	0.1	6.9
E. vernicosa	*Viminales*	White Gum	J	3	0.2	9.2	7.0	0.7	26.7	5.7	47.9	-	1.8
			A	4	0.2	11.2	5.7	0.7	26.8	7.7	40.2	-	6.7
E. globulus	*Viminales*	Blue Gum	J	6	1.5	3.9	2.7	0.1	65.1	8.8	2.1	-	16.4
			A	6	0.7	4.0	3.4	-	73.2	6.6	2.1	-	9.8
E. cordata	*Viminales*	Blue Gum	J	5	1.0	23.7	8.3	2.1	41.1	7.8	5.5	1.0	9.5
			A	5	1.0	8.9	15.0	0.9	52.4	6.9	6.7	1.4	6.8
E. gunnii	*Viminales*	Alpine White Gum	J	6	0.6	2.5	8.9	0.4	57.3	10.2	9.3	0.2	10.4
			A	6	0.5	4.3	11.4	0.5	50.5	8.3	11.5	-	12.6
E. archeri	*Viminales*	Alpine White Gum	J	3	0.3	10.8	10.3	6.2	39.1	10.8	5.9	-	15.9
			A	3	0.3	10.7	11.6	6.6	31.0	13.5	6.0	-	19.7
E. urnigera	*Viminales*	Alpine White Gum	J	4	0.5	15.1	5.2	0.8	56.0	7.7	6.5	1.2	6.5
			A	4	0.4	24.8	7.4	0.8	39.1	8.2	5.5	1.3	11.8
E. perrinana	*Viminales*	Alpine White Gum	J	1	1.5	0.6	1.2	-	69.3	5.7	7.8	0.2	14.2
			A	1	0.9	0.7	1.6	-	59.2	13.0	8.3	0.3	15.9
E. morrisbyi	*Viminales*	Alpine White Gum	J	2	1.0	2.9	6.1	-	65.2	4.2	2.3	1.3	17.0
			A	2	1.0	2.6	8.0	-	67.4	5.3	2.3	1.8	11.6

The mean percentage of the compounds were caculated as the mean percentage of each wax compound class across population samples of each species

† Series proposed by Chippendale (1988) ¥ Species groups proposed by Jackson (1965) * Leaf type, i.e. adult and juvenile leaf types

** Yield = g/100g dried leaves - : Zero

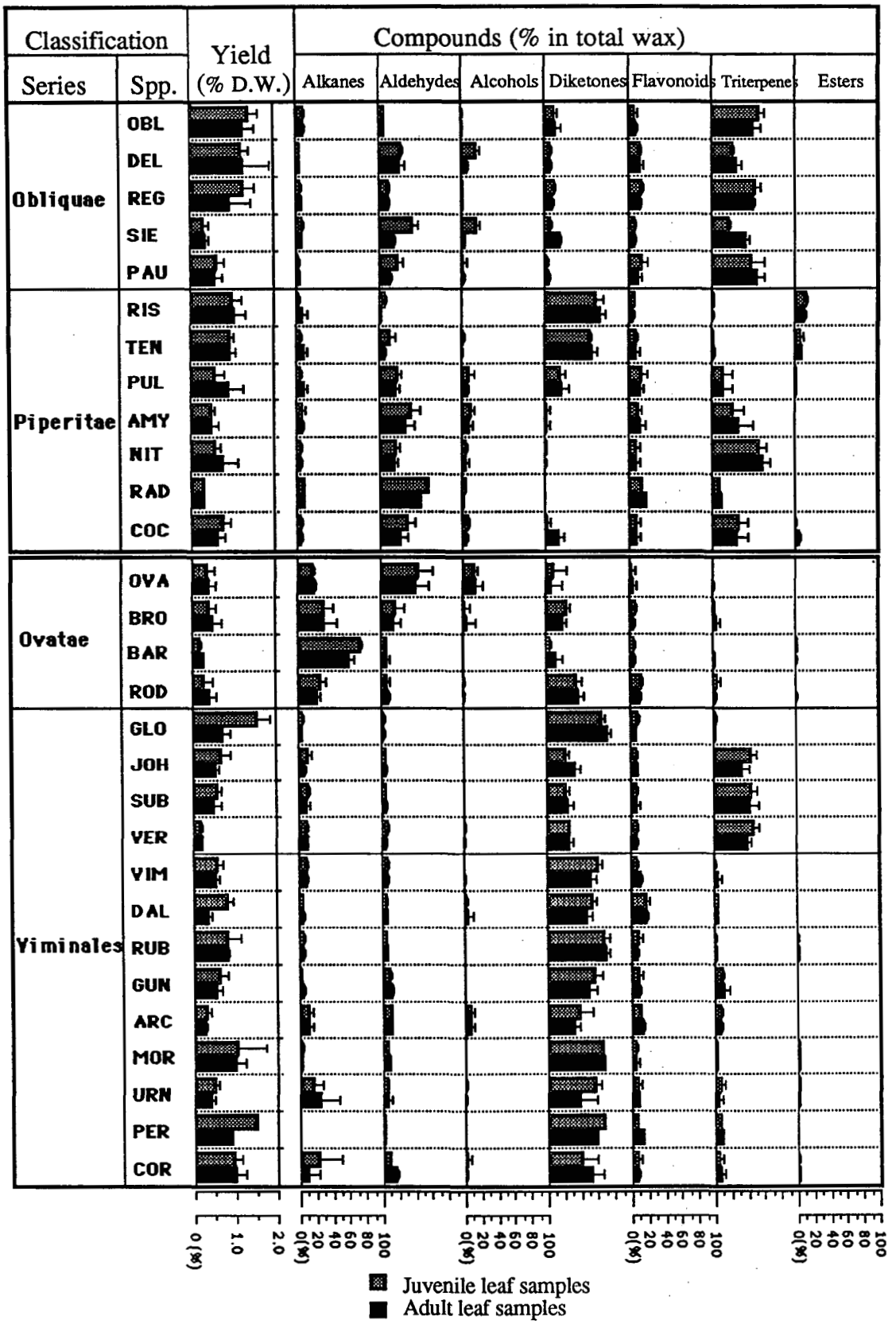


Fig. 5.1. Mean percentage content (main bar) and standard deviation of (error bar) of compounds and yields of hexane soluble waxes of leaves from 29 eucalypt species. Species mean data from Table 5.1. In this figure, species (Spp.) are indicated by codes as listed in Table 4.1.

Table 5.2.A. Percentage composition of major compound classes in the hexane soluble waxes of leaves of 29 *Eucalyptus* species.

Alkanes (HY), aldehydes (AL) and alcohols (OL). t = trace amount < 0.5% - = Zero
Subgenus *Monocalyptus* * leaf types: J = juvenile, A = adult; # number of populations sampled

Compound class				n-Alkanes (HY)									Alkanals (AL)								n-Alkanols (OL)				
(Code)																									
Homologues				C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}
Compound code				H23	H24	H25	H26	H27	H28	H29	H30	H31	A22	A23	A24	A25	A26	A27	A28	A29	A30	O20	O22	O24	O26	O28
E. regnans	J*	6#	Mean	t	-	0.7	-	1.7	t	2.2	-	-	-	-	1.1	t	6.0	t	3.9	-	t	-	t	1.4	t	-
			S.D.	t	-	t	t	t	t	t	-	-	-	-	t	t	1.5	t	0.9	-	t	-	t	t	t	-
	A	6	Mean	t	-	0.9	-	2.2	t	2.6	-	-	-	-	0.9	-	6.2	t	4.0	-	t	-	t	1.0	t	-
			S.D.	t	-	t	-	t	t	t	-	-	-	-	0.5	-	0.9	t	1.0	-	t	-	t	t	t	-
E. obliqua	J	9	Mean	t	-	1.4	t	4.2	t	2.8	-	-	t	-	t	-	1.6	t	3.0	-	0.6	-	t	0.5	t	-
			S.D.	t	-	1.0	t	1.0	t	1.4	-	-	t	-	t	-	0.9	t	1.1	-	t	-	t	t	t	-
	A	9	Mean	t	-	1.0	t	4.3	t	2.7	-	-	t	-	t	-	1.9	t	3.1	-	0.8	-	t	0.7	t	-
			S.D.	t	-	0.5	t	1.3	t	1.2	-	-	t	-	t	t	0.7	t	0.8	-	0.5	-	t	0.6	t	-
E. delegatensis	J	10	Mean	0.5	-	1.1	t	0.9	t	1.0	-	-	t	-	17.7	0.6	6.1	t	1.1	-	t	-	0.6	18.0	0.5	-
			S.D.	t	-	0.6	t	t	t	0.5	-	-	t	t	3.0	t	1.7	t	t	-	t	-	t	3.9	t	t
	A	10	Mean	0.5	-	1.1	t	1.2	t	1.0	-	-	t	-	16.0	t	5.9	t	1.6	-	0.8	-	0.5	5.3	0.5	-
			S.D.	t	t	0.6	t	0.5	t	t	-	t	t	-	5.4	t	2.0	t	0.7	-	0.6	-	0.5	1.7	t	-
E. sieberi	J	2	Mean	t	-	2.4	-	1.0	t	1.7	-	-	t	-	4.1	0.9	30.5	0.7	5.1	-	-	-	-	4.1	12.8	0.8
			S.D.	t	-	1.8	-	0.5	t	0.9	-	-	t	-	0.6	0.8	6.9	t	3.1	-	-	-	-	0.6	3.6	t
	A	3	Mean	t	-	1.8	-	1.6	-	2.3	-	-	0.6	-	1.2	0.7	12.5	t	2.3	-	-	-	-	1.4	2.7	-
			S.D.	-	-	t	-	t	-	t	-	-	t	-	t	t	1.2	t	1.7	-	-	-	-	0.6	t	-
E. pauciflora	J	5	Mean	t	-	0.8	t	1.3	-	0.5	-	-	-	-	6.7	t	11.5	t	2.4	-	0.6	-	t	2.6	0.5	-
			S.D.	t	-	t	t	1.0	t	t	-	-	-	-	3.8	t	4.6	t	1.4	-	0.6	-	t	2.5	t	-
	A	5	Mean	t	t	1.3	t	1.6	t	0.8	-	-	-	-	3.6	0.5	7.5	t	1.7	-	0.9	-	t	2.4	t	-
			S.D.	t	t	0.6	t	0.6	t	t	-	-	-	-	1.2	t	1.9	t	0.6	t	0.7	-	t	1.5	t	-
E. radiata	J	1	Mean	t	-	1.3	-	2.4	-	4.2	-	-	t	-	11.5	0.6	36.2	t	8.9	-	0.9	-	t	2.7	1.2	-
			S.D.	t	-	1.3	-	2.4	-	4.2	-	-	t	-	11.5	0.6	36.2	t	8.9	-	0.9	-	t	2.7	1.2	-
	A	1	Mean	t	-	1.0	-	2.6	-	5.3	-	-	t	-	7.3	t	33.2	t	7.9	-	1.4	-	t	1.3	0.7	-
			S.D.	t	-	1.0	-	2.6	-	5.3	-	-	t	-	7.3	t	33.2	t	7.9	-	1.4	-	t	1.3	0.7	-
E. amygdalina	J	8	Mean	0.8	-	1.5	-	2.3	t	1.7	-	-	t	-	24.0	0.6	11.3	t	2.4	-	t	-	t	9.7	0.7	-
			S.D.	0.8	-	2.0	t	2.6	t	1.3	-	-	t	t	8.4	t	3.3	t	0.8	-	t	-	t	6.6	0.7	-
	A	8	Mean	1.5	t	1.4	t	1.8	t	1.6	-	-	t	-	18.7	0.5	7.6	t	3.3	-	t	-	t	6.7	1.4	-
			S.D.	1.3	t	0.9	t	1.2	t	1.1	-	-	t	t	7.7	t	2.0	t	5.4	-	t	-	t	3.9	1.4	-
E. puchella	J	5	Mean	0.8	-	1.1	-	2.0	-	1.3	-	-	t	-	11.4	0.6	6.4	t	3.8	-	t	-	-	9.2	0.9	-
			S.D.	t	-	0.7	-	0.8	-	t	-	-	t	t	3.8	0.7	1.4	t	0.6	-	0.5	-	t	4.7	t	-
	A	5	Mean	1.1	t	1.5	t	3.5	t	2.6	-	-	t	-	10.0	0.6	6.4	t	3.3	-	t	-	t	6.2	0.7	-
			S.D.	0.8	t	0.9	t	1.7	t	0.6	-	-	t	-	2.5	t	1.1	t	0.7	-	0.6	-	t	2.5	0.5	t
E. nitida	J	10	Mean	0.7	t	2.3	t	1.7	t	0.7	-	-	t	-	8.8	0.7	7.8	t	1.7	-	t	-	t	4.4	0.9	t
			S.D.	t	t	1.1	t	0.7	t	0.5	-	-	t	-	4.0	0.5	2.5	t	0.9	-	t	-	t	1.5	0.6	0.5
	A	10	Mean	0.8	t	1.9	t	1.2	-	0.5	-	-	t	-	7.3	0.6	7.1	t	1.4	-	t	-	t	3.6	1.0	t
			S.D.	t	t	1.0	t	0.6	t	t	-	-	t	t	3.3	0.5	2.0	t	t	-	t	-	t	2.5	0.7	0.5
E. coccifera	J	6	Mean	0.8	t	1.1	t	0.7	t	0.8	-	-	t	-	17.7	0.8	12.2	t	2.4	-	0.7	-	t	4.3	1.2	-
			S.D.	t	t	0.8	t	0.6	t	0.8	-	-	t	-	7.8	0.5	6.1	t	1.2	-	t	t	t	2.0	t	-
	A	6	Mean	1.6	t	1.7	t	0.9	t	0.8	-	-	t	-	10.9	0.6	9.0	t	2.8	-	0.7	-	t	3.0	1.1	t
			S.D.	1.1	t	0.7	t	0.5	t	0.5	-	-	t	-	3.5	0.5	4.6	t	1.2	-	t	t	t	1.6	0.7	t
E. tenuiramis	J	4	Mean	t	-	0.6	t	1.7	t	1.3	-	-	-	-	2.7	t	7.8	t	2.5	-	0.5	-	-	1.7	0.8	-
			S.D.	t	-	t	t	1.4	t	0.8	-	t	t	-	2.6	t	3.2	t	0.6	-	0.6	-	-	1.9	0.7	-
	A	4	Mean	0.5	t	1.8	t	3.1	t	2.0	-	-	-	-	0.8	t	4.0	t	1.9	-	0.5	-	-	t	t	-
			S.D.	t	t	0.8	t	2.2	t	1.6	-	t	-	-	t	t	1.6	t	0.5	-	0.6	-	-	t	t	t
E. risdonii	J	2	Mean	-	-	0.8	-	0.6	t	0.9	-	-	-	-	0.5	t	3.2	t	1.9	-	0.8	-	-	-	-	-
			S.D.	t	-	-	-	0.7	t	t	-	-	-	-	t	t	1.3	t	0.6	-	1.2	-	-	-	-	-
	A	3	Mean	t	-	2.6	-	4.5	t	2.7	-	-	t	-	t	t	1.0	-	0.8	-	t	-	t	-	-	-
			S.D.	-	-	t	-	2.1	t	1.1	-	-	-	-	t	t	-	-	t	-	t	-	t	-	-	-

Continuous for Table 5.2.A. Alkanes, aldehyde and alcohol.
Subegenus *Symphyomyrtus*

Compound class (Code)				n-Alkanes (HY)									Alkanals (AL)								n-Alkanols (OL)				
Homologues				C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}
Compound code				H23	H24	H25	H26	H27	H28	H29	H30	H31	A22	A23	A24	A25	A26	A27	A28	A29	A30	O20	O22	O24	O26	O28
E. ovata	J	6	Mean	0.7	0.5	2.6	0.6	7.1	1.1	4.6	-	0.5	0.6	-	3.4	1.2	16.1	0.9	16.8	t	4.2	9.5	1.0	1.0	0.6	t
			S.D.	t	0.5	0.6	0.5	0.7	0.7	1.8	-	0.7	t	t	2.0	1.3	7.5	1.5	5.7	0.5	2.8	3.9	0.5	1.0	0.6	0.7
	A	6	Mean	0.8	t	4.0	0.7	7.5	0.7	4.5	-	0.5	0.5	t	2.8	1.0	17.0	1.6	14.4	0.8	2.7	8.6	4.4	0.7	0.7	t
			S.D.	t	t	0.9	0.7	1.2	0.8	1.6	-	0.6	t	0.5	1.5	1.2	8.6	1.1	4.2	0.7	1.7	4.4	7.8	t	0.9	t
E. brookeriana	J	4	Mean	t	-	3.4	t	9.5	1.5	8.8	-	t	t	-	1.0	t	3.4	t	5.6	-	1.2	-	1.2	1.0	0.7	0.8
			S.D.	t	-	2.8	t	4.5	1.1	3.6	-	t	t	t	t	t	2.1	t	5.2	-	2.0	-	2.1	1.6	1.1	1.3
	A	4	Mean	t	-	2.9	t	12.7	1.0	6.7	-	t	t	t	0.8	-	3.2	t	5.5	0.5	1.6	-	2.0	1.9	1.5	0.6
			S.D.	-	-	2.2	t	9.1	0.7	4.1	t	t	t	t	0.5	-	1.5	t	4.6	0.8	2.8	-	3.4	3.3	2.6	1.1
E. rodwayi	J	4	Mean	t	t	2.6	0.8	11.5	0.9	9.5	-	t	t	t	0.5	t	1.3	t	3.4	-	1.5	-	t	t	t	t
			S.D.	t	t	3.2	0.8	1.1	t	1.8	-	0.5	t	t	t	t	t	t	1.7	-	0.9	-	t	0.6	t	t
	A	4	Mean	t	-	4.1	t	9.3	t	6.6	-	t	t	-	0.6	t	1.9	t	3.4	-	1.0	-	t	t	t	t
			S.D.	t	-	2.0	t	1.7	t	1.9	-	t	t	-	t	t	0.5	t	1.0	t	t	-	t	t	t	t
E. barberi	J	2	Mean	t	t	7.7	0.6	42.5	1.0	21.5	-	t	t	-	0.6	-	1.4	t	3.0	-	0.6	-	t	-	t	-
			S.D.	t	t	3.8	0.9	2.4	1.4	5.5	-	0.6	t	-	0.5	-	t	t	1.0	-	t	-	t	-	t	-
	A	2	Mean	1.1	-	5.8	0.8	38.9	0.7	14.1	-	t	0.5	t	0.6	t	2.3	t	2.5	t	1.0	t	t	-	-	-
			S.D.	1.3	-	8.0	1.1	1.0	1.0	3.7	-	t	t	t	t	t	1.5	t	0.5	t	t	t	t	-	-	-
E. viminalis	J	6	Mean	-	-	0.6	t	1.8	t	5.3	-	t	-	-	0.5	t	2.2	t	2.1	-	0.9	-	-	-	t	-
			S.D.	t	-	t	t	0.9	t	2.1	-	t	t	-	t	t	1.2	t	1.0	-	0.7	-	-	-	t	-
	A	6	Mean	t	-	1.5	t	3.0	t	3.7	-	t	t	-	0.8	0.5	3.1	t	2.0	-	0.5	-	t	-	0.5	-
			S.D.	t	t	0.5	t	0.9	t	0.9	-	t	t	-	t	t	1.0	t	0.5	-	t	-	t	t	1.0	-
E. dalrympleana	J	3	Mean	-	-	0.6	-	1.3	t	1.7	-	-	-	-	0.7	t	2.4	-	1.9	-	0.6	-	-	1.1	1.5	-
			S.D.	t	-	t	t	0.5	t	0.6	-	-	-	-	t	t	0.6	-	t	-	t	-	-	t	0.6	-
	A	3	Mean	t	-	0.8	-	1.8	t	1.3	-	-	-	-	1.0	t	3.2	-	2.3	-	0.7	-	-	1.5	4.0	-
			S.D.	t	-	t	t	t	t	1.1	-	-	t	-	t	t	t	-	0.5	-	t	-	t	0.9	4.2	-
E. rubida	J	2	Mean	-	-	0.5	-	1.0	-	3.2	-	-	-	-	t	t	0.6	t	1.9	t	0.6	-	-	-	-	-
			S.D.	-	-	t	-	t	-	0.6	-	-	-	-	t	t	t	t	0.7	t	t	-	-	-	-	-
	A	2	Mean	-	-	0.8	-	1.6	-	2.4	-	-	-	-	0.6	t	1.9	-	2.6	-	0.5	-	-	-	-	-
			S.D.	-	-	t	-	0.5	-	t	-	-	-	-	t	t	0.5	-	t	-	t	-	-	-	-	-
E. johnstonii	J	3	Mean	t	-	0.6	t	3.5	0.5	6.6	-	t	-	-	t	-	0.6	-	1.1	t	2.3	-	-	-	t	-
			S.D.	t	-	t	t	1.6	t	4.0	-	t	-	-	t	-	t	t	t	t	0.9	-	-	-	t	-
	A	5	Mean	t	-	1.1	-	3.0	0.5	2.6	-	-	-	-	t	t	1.4	t	1.3	t	2.0	t	-	-	t	t
			S.D.	t	-	t	t	1.5	t	1.1	-	-	t	-	t	t	t	t	t	t	1.0	t	t	-	t	t
E. subcrenulata	J	4	Mean	t	-	1.1	-	3.7	t	4.4	t	t	-	-	t	t	0.9	t	1.4	-	0.7	-	t	-	t	t
			S.D.	t	-	t	t	1.0	t	1.0	t	0.5	t	-	-	t	t	t	t	-	0.9	-	t	-	t	t
	A	5	Mean	t	-	1.5	t	3.9	t	3.2	-	t	t	-	t	t	1.3	t	1.3	-	0.6	-	t	-	t	-
			S.D.	t	-	0.7	t	1.3	0.5	1.2	-	t	t	-	t	t	t	t	t	t	0.9	-	t	t	t	-
E. vernicosa	J	3	Mean	t	-	3.2	t	2.4	t	3.1	-	-	t	-	0.5	t	2.0	t	1.7	-	2.3	t	t	t	t	-
			S.D.	t	-	0.9	t	0.7	t	1.8	-	-	t	t	t	t	0.5	t	0.5	t	t	t	t	t	t	-
	A	4	Mean	0.5	t	4.8	t	2.8	t	2.3	-	t	t	-	0.5	t	1.9	t	0.9	-	1.6	t	t	-	t	t
			S.D.	t	t	1.4	t	0.7	t	0.8	-	t	t	-	t	t	t	t	t	-	t	t	t	-	t	t
E. globulus	J	6	Mean	t	-	0.5	t	0.8	t	1.7	-	t	-	-	0.6	-	1.0	t	0.5	-	0.5	-	-	-	-	t
			S.D.	t	-	t	t	0.7	t	0.6	-	t	-	t	1.0	-	1.3	t	t	-	0.5	0.0	-	-	-	-
	A	6	Mean	t	t	0.6	t	1.0	t	1.5	-	t	-	-	t	-	1.0	t	1.2	t	0.6	-	-	-	-	-
			S.D.	t	t	t	t	t	t	t	-	t	-	-	t	-	t	t	0.6	t	0.5	-	-	-	-	-
E. cordata	J	5	Mean	t	-	4.7	t	12.2	t	5.8	-	t	t	-	t	t	3.1	t	2.7	t	1.3	-	t	-	1.7	t
			S.D.	t	-	4.2	0.7	16.1	0.6	4.3	-	t	t	-	t	t	1.5	t	1.2	t	0.5	-	t	t	3.8	t
	A	5	Mean	t	t	2.0	0.5	2.9	t	2.8	-	t	t	t	t	t	6.6	0.8	3.9	0.5	2.5	-	t	t	t	t
			S.D.	t	t	2.9	0.9	5.4	0.5	3.8	-	0.5	t	t	t	-	3.0	0.7	1.1	t	1.2	-	t	0.6	0.6	t
E. gunnii	J	6	Mean	t	-	t	t	0.6	t	1.2	-	-	-	-	1.3	t	2.0	t	3.6	t	1.5	-	-	t	t	t
			S.D.	t	-	t	t	t	t	t	-	-	-	-	2.0	t	1.4	t	1.5	t	0.9	-	-	t	t	t
	A	6	Mean	0.5	t	0.8	t	1.3	t	1.2	-	-	t	t	1.4	t	2.8	t	4.5	t	1.3	-	t	-	t	t
			S.D.	t	t	0.5	t	1.0	t	0.8	-	-	t	t	0.6	t	0.9	t	1.5	t	0.8	-	t	t	t	t
E. archeri	J	3	Mean	0.8	t	1.6	t	2.9	t	4.1	-	1.1	t	-	1.3	0.9	2.3	t	3.5	t	1.6	0.8	0.9	2.9	1.3	t
			S.D.	1.0	t	1.0	t	2.2	t	2.4	-	1.2	t	-	0.5	0.9	0.6	t	0.6	t	t	0.7	0.8	3.6	1.4	0.7
	A	3	Mean	0.9	t	2.1	t	2.6	t	2.6	-	0.7	0.5	-	1.7	1.4	2.6	t	3.6	-	0.6	1.4	1.1	2.0	1.2	t
			S.D.	t	t	0.6	t	1.2	t	2.2	-	1.2	t	-	t	1.2	0.5	t	0.9	-	0.5	1.6	1.1	1.8	1.0	0.5
E. urnigera	J	4	Mean	-	-	2.7	t	5.3	0.6	5.9	-	0.5	t	t	t	t	1.0	t	2.1	t	1.3	-	t	t	0.5	t
			S.D.	t	-	2.2	t	5.6	0.5	5.5	-	0.6	t	t	t	t	0.8	t	0.9	t	0.9	-	t	t	1.0	t
	A	4	Mean	t	-	4.5	0.5	10.5	1.0	7.3	-	0.6	-	t	t	t	1.4	t	3.4	t	1.6	-	-	t	t	t
			S.D.	t	-	3.9	t	10.4	0.8	6.9	-	0.6	t	t	t	t	0.8	t	1.8	t	1.1	-	-	t	0.9	0.5
E. perriniana	J	1	Mean	-	-	-	-	t	t	t	-	-	-	-	-	-	t	-	t	-	0.6	-	-	-	-	-
			S.D.	-	-	-	-	t	t	t	-	-	-	-	-	-	t	-	t	-	0.6	-	-	-	-	-
	A	1	Mean	t	-	t	-	t	-	t	-	-	-	-	t	t	t	-	0.5	-	0.7	-	-	-	-	-
			S.D.	t	-	t	-	t	-	t	-	-	-	-	t	t	t	-	0.5	-	0.7	-	-	-	-	-
E. morrisbyi	J	2	Mean	t	-	t	-	1.2	-	1.3	-	-	-	t	t	-	3.0	t	1.7	-	0.8	-	-	-	-	-
			S.D.	t	-	t	-	1.1	-	t	-	-	-	t	t	-	2.0	t	0.7	-	1.2	-	-	-	-	-
	A	2	Mean	-	-	t	-	1.1	-	1.1	-	-	-	t	t	-	4.5	t	1.7	t	1.2	-	-	-	-	-
			S.D.	-	-	t	-	0.6	-	-	-	-	-	t	t	-	1.1	t	-	t	1.7	-	-	-	-	-

Table 5.2B. Mean percentage composition of chemical compounds in the hexane soluble waxes of leaves of 29 *Eucalyptus* species. (The mean percentage of compounds was caculated as the mean percentage of each wax compound across population samples of each species)

$\Sigma V = V_1 + V_2 + V_3 + V_4 + ...V_n$, is the sum of variances in percentage content of individual compounds (included all homologues of all compound classes in Table 5.2A and B) among localities within species

ß-diketones, flavonoids, triterpenoids and esters t = trace amount < 0.5% - = Zero

Subgenus *Monocalyptus*

Compound classes (Code)			β–Diketone (DI)								Flavonoids (FL)		Triterpenoids (TR)												*Esters (ES)			
Homologues			C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{37}	F1	F2	A	B	C	D	E	F	G	H	I	J	K	L	M	E1	E2	E3	ΣV
Compound code			D29	D30	D31	D32	D33	D34	D35	D37	EU	D8	TA	TB	TC	TD	TE	TF	TG	TH	TI	TJ	TK	TL	TM	E1	E2	E3	
E. regnans	J	Mean	-	-	2.2	-	8.2	-	0.5	-	6.1	9.0	-	-	-	6.5	4.6	9.9	6.9	1.7	-	-	13.7	9.1	-	-	-	-	32.83
		S.D	t	-	1.2	-	1.1	-	t	-	1.5	1.2	-	-	-	1.1	1.4	3.0	2.4	1.2	-	-	1.4	0.8	-	-	-	-	
	A	Mean	t	-	1.2	-	8.4	-	t	-	5.4	7.7	-	-	-	6.6	3.7	8.8	6.4	-	-	-	12.6	11.5	-	-	-	-	34.54
		S.D	t	-	0.6	-	1.2	-	t	-	1.6	1.2	-	-	-	1.3	1.0	0.9	2.8	-	-	-	1.8	3.1	-	-	-	-	
E. obliqua	J	Mean	t	-	1.6	-	9.6	-	0.8	-	2.5	5.2	-	-	-	9.2	7.7	4.7	10.3	1.0	2.1	-	15.3	5.4	-	-	-	-	112.2
		S.D	t	-	t	-	3.4	-	t	-	1.1	1.9	-	-	-	3.3	3.4	3.3	5.7	0.9	1.7	-	3.6	2.7	-	-	-	-	
	A	Mean	t	-	1.9	-	11.7	-	0.7	-	2.7	6.2	-	-	-	8.6	6.1	5.2	10.7	-	-	-	15.4	4.3	-	-	-	-	109.1
		S.D	t	-	0.5	-	4.7	-	0.6	-	1.0	2.3	-	-	-	2.6	2.1	2.6	4.8	-	-	-	4.2	4.0	-	-	-	-	
E. delegatensis	J	Mean	t	-	4.4	-	1.2	-	-	-	4.0	9.6	-	-	-	1.1	1.2	1.8	2.4	-	-	-	14.1	4.9	-	-	-	-	101.9
		S.D	t	t	2.5	-	0.7	-	t	-	1.3	2.1	-	-	-	t	0.9	1.0	1.0	-	-	-	6.0	4.7	-	-	-	-	
	A	Mean	t	t	5.3	-	1.4	-	t	-	4.6	10	-	-	-	1.1	1.8	1.3	2.3	-	-	-	21.1	2.8	-	-	-	-	139.1
		S.D	t	t	2.5	-	1.1	-	t	-	1.5	1.7	-	-	-	t	1.0	0.7	0.8	-	-	-	6.0	2.1	-	-	-	-	
E. sieberi	J	Mean	-	-	1.1	-	4.8	-	t	-	2.2	5.5	-	-	-	0.5	0.5	3.0	1.2	-	-	-	13.4	1.6	-	-	-	-	108.3
		S.D	-	-	t	-	2.3	-	t	-	0.6	1.2	-	-	-	0.7	t	0.9	1.2	-	-	-	4.6	0.9	-	-	-	-	
	A	Mean	0.7	-	2.0	-	15.3	-	t	-	2.8	4.0	-	-	-	1.1	0.7	t	-	-	-	-	33.7	5.7	-	-	-	-	31.41
		S.D	0.5	-	t	-	1.7	-	0.5	-	1.3	2.4	-	-	-	0.7	t	t	-	-	-	-	3.7	0.6	-	-	-	-	
E. pauciflora	J	Mean	1.0	-	1.4	-	1.4	-	t	-	4.0	11.2	-	-	-	1.2	3.5	1.9	0.5	-	-	-	39.4	2.0	-	-	-	-	326.8
		S.D	2.0	-	1.0	-	0.5	-	t	-	1.8	6.0	-	-	-	0.7	1.2	1	0.5	-	-	-	15.4	0.9	-	-	-	-	
	A	Mean	t	-	1.3	-	2.7	-	t	-	3.2	7.7	-	-	-	1.7	4.3	1.3	0.6	-	-	-	42.3	3.7	-	-	-	-	176.0
		S.D	t	-	0.6	-	1.8	-	t	-	1.9	3.5	-	-	-	0.9	1.4	t	t	-	-	-	11.8	2.0	-	-	-	-	
E. radiata	J	Mean	t	-	t	-	-	-	-	-	5.8	10.5	-	-	-	0.9	0.6	t	-	-	-	-	5.9	0.5	-	-	t	t	-
		S.D	t	-	t	-	-	-	-	-	5.8	10.5	-	-	-	0.9	0.6	t	-	-	-	-	5.9	0.5	-	-	t	t	
	A	Mean	t	-	t	-	0.7	-	-	-	5.1	16.3	-	-	-	1.4	t	t	-	-	-	-	8.4	1.0	-	-	t	t	-
		S.D	t	-	t	-	0.7	-	-	-	5.1	16.3	-	-	-	1.4	t	t	-	-	-	-	8.4	1.0	-	-	t	t	
E. amygdalina	J	Mean	0.8	t	0.8	-	1.1	-	t	-	3.9	7.1	-	-	-	t	t	t	-	-	-	-	20.9	2.4	-	-	t	t	506.0
		S.D	0.6	t	0.6	-	2.3	-	t	-	1.4	2.8	-	-	-	t	t	t	t	-	-	-	13.5	2.4	-	-	t	t	
	A	Mean	1.4	-	1	-	0.6	-	-	-	4.8	9.7	-	-	-	0.5	0.6	t	t	-	-	-	27.7	2.4	-	-	t	t	498.5
		S.D	1.8	t	1	-	0.9	-	t	-	1.7	3.7	-	-	-	t	t	t	t	-	-	-	18.8	3.0	-	-	0.5	t	
E. pulchella	J	Mean	5.0	-	4.0	t	8.0	-	t	-	5.3	10.2	-	-	-	0.6	t	t	t	-	-	-	8.7	4.2	-	-	1.0	0.6	302.3
		S.D	6.4	-	1.9	t	7.6	-	t	-	1.7	5.1	-	-	-	0.6	0.5	0.7	t	-	-	-	6.0	4.1	-	-	0.8	t	
	A	Mean	5.4	-	4.3	-	9.8	-	t	-	4.4	10.1	-	-	-	0.6	t	t	t	-	-	-	7.4	5.8	-	-	0.6	0.9	257.2
		S.D	8.2	-	3.0	-	9.9	-	0.6	-	0.9	3.9	-	-	-	0.5	0.6	t	t	-	-	-	6.4	3.8	-	t	0.6	0.9	
E. nitida	J	Mean	t	-	t	-	0.5	-	-	-	3.7	5.4	-	-	-	0.9	1	0.9	t	-	-	-	51.9	3.1	-	-	-	-	114.3
		S.D	0.5	-	t	-	t	-	t	-	1.7	2.1	-	-	-	t	0.5	1.1	t	-	-	-	6.1	1.8	-	-	t	-	
	A	Mean	0.5	-	t	-	t	-	-	-	2.9	6.0	-	-	-	1.1	0.8	0.9	t	t	-	-	56.0	2.3	-	-	t	t	78.9
		S.D	t	-	t	-	t	-	-	-	1.5	2.9	-	-	-	t	t	1	t	t	-	-	6.1	2.5	-	-	t	t	
E. coccifera	J	Mean	1.1	-	2.0	-	t	-	-	-	3.3	6.5	-	-	-	0.5	1.2	0.9	t	-	-	-	19.3	8.9	-	-	0.5	t	308.7
		S.D	1.2	-	3.1	-	t	-	-	-	1.6	2.5	-	-	-	0.6	1.5	0.7	t	-	-	-	9.4	5.2	-	-	0.6	0.5	
	A	Mean	9.1	-	5.0	-	0.9	-	-	-	3.6	5.1	-	-	-	0.6	1.0	0.9	t	-	-	-	18.9	7.5	-	-	2.4	2.4	227.0
		S.D	5.2	-	3.6	-	t	-	-	-	1.8	3.3	-	-	-	t	1.0	0.7	t	-	-	-	9.2	6.6	t	t	1.1	1.1	
E. tenuiramis	J	Mean	37.0	t	15.4	t	2.5	-	-	-	1.6	7.3	-	-	-	-	t	t	-	-	-	-	1.1	t	-	t	3.6	3.6	59.6
		S.D	5.1	t	1.3	t	2.2	-	-	-	t	2.6	-	-	-	-	0.6	t	-	-	-	-	t	t	-	t	1.5	2.0	
	A	Mean	36.3	t	18.1	t	2.9	-	-	-	1.4	7.9	-	-	-	-	-	-	-	-	-	-	1.3	0.8	t	t	3.8	4.4	63.4
		S.D	4.7	0.6	2.5	t	1.5	-	-	-	0.9	3.8	-	-	-	-	-	-	-	-	-	-	1.7	1.4	t	t	0.5	0.7	
E. risdonii	J	Mean	42.3	t	17.0	-	1.3	-	-	-	3.0	3.6	-	-	-	t	t	t	-	-	-	-	0.7	t	-	4.5	5.4	2.9	107.2
		S.D	8.0	0.6	1.9	-	t	-	-	-	2.2	2.9	-	-	-	t	t	t	-	-	-	-	0.9	t	-	0.8	t	2.5	
	A	Mean	45.0	t	19.0	-	1.6	-	-	-	3.8	0.9	-	-	-	t	t	-	t	-	-	-	t	-	-	3.7	4.1	3.7	67.6
		S.D	7.1	t	1.8	-	0.7	-	-	-	0.9	t	-	-	-	t	t	t	t	-	-	-	t	-	-	1.3	0.9	0.9	

*Long chain ester compounds (esters of 2-alcohol):

E1: Nonanoyl hexadecanoate (C_{25}); E2: Undecanoyl hexadecanoate (C_{27}); E3: Undecanoyl eicosanoate (C_{29})

Continued for Table 5.2.B. β-Diketones, flavonoids, triterpenoids and esters
Subgenus *Symphyomyrtus*

Compound classes (Code)			β–Diketone (DI)							Flavonoids (FL)		Triterpenoids (TR)											Esters (ES)			ΣV
Homologues			C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{37}	F1	F2	A	B	C	D	E	F	G	H	I	J	K	L	M	E1	E2	E3	
Compound code			D29	D30	D31	D32	D33	D34	D35	D37	EU	D8	TA	TB	TC	TD	TE	TF	TG	TH	TI	TJ	TK	TL	TM	E1	E2	E3	
E. ovata	J	Mean	-	-	t	-	8.2	-	0.6	-	1.1	2.3	-	-	-	-	-	-	-	t	-	-	t	t	-	-	-	-	1531.0
		S.D	-	-	0.6	-	13.8	-	0.9	-	1.2	2.5	-	-	-	-	-	-	-	t	-	-	t	t	-	-	-	-	
	A	Mean	-	-	0.5	-	6.9	-	t	-	1.7	2.5	-	-	-	-	-	-	-	-	-	-	t	t	-	-	-	-	1391.0
		S.D	t	-	0.9	-	11.5	-	0.6	-	2.4	3.0	t	-	t	-	-	-	-	-	-	-	0.5	t	-	-	-	-	
E. brookeriana	J	Mean	-	-	1.1	-	22.9	-	3.3	t	2.3	6.1	-	-	-	-	-	-	-	-	-	t	t	t	1.2	-	-	-	264.1
		S.D	-	-	0.7	-	2.7	-	1.3	0.7	0.9	1.1	-	-	-	-	-	-	-	-	-	t	t	t	1.9	-	-	-	
	A	Mean	-	-	1.4	-	17.9	-	2.4	-	1.5	3.6	-	-	-	-	-	-	-	-	-	-	t	t	5.2	-	-	-	393.2
		S.D	-	-	1.2	-	3.3	-	t	-	1.0	1.6	-	-	-	-	-	-	-	-	-	-	t	t	2.8	-	-	-	
E. rodwayi	J	Mean	t	-	1.5	-	28.2	-	5.7	0.7	5.0	8.1	0.6	t	-	-	-	-	t	-	t	-	t	t	3.4	-	-	-	22.5
		S.D	t	-	t	-	5.1	-	1.5	1.4	0.9	2.3	1.3	t	-	-	-	-	t	-	t	-	t	t	2.8	-	-	-	
	A	Mean	t	t	2.1	t	31	-	5.5	t	4.9	7.1	-	-	-	-	-	-	-	-	-	-	t	t	1.9	t	t	-	93.4
		S.D	t	t	t	t	5.9	-	1.2	0.7	1.5	0.8	-	-	-	-	-	-	-	-	-	-	0.6	t	2.8	t	0.7	t	
E. barberi	J	Mean	t	-	0.9	-	1.9	-	1.8	0.5	1.7	3.0	-	-	-	-	-	-	-	-	-	-	t	t	t	0.6	t	t	27.8
		S.D	-	-	t	-	1.4	-	0.6	0.7	t	1.0	-	-	-	-	-	-	-	-	-	-	t	t	t	0.9	t	t	
	A	Mean	t	-	t	-	8.6	-	1.6	0.9	2.3	3.0	-	-	-	-	-	-	-	-	-	-	t	-	1.5	t	0.5	t	2.2
		S.D	t	-	-	-	9.5	-	t	1.3	0.8	1.2	-	-	-	-	-	-	-	-	-	-	t	-	1.1	t	0.7	t	
E. viminalis	J	Mean	-	-	3.2	t	54.7	-	2.9	t	2.4	4.6	t	-	-	-	-	-	-	t	-	-	t	t	t	-	t	-	318.1
		S.D	t	-	0.9	t	4.8	-	t	t	1.0	0.6	t	t	-	-	-	-	-	t	-	-	t	t	t	-	t	-	
	A	Mean	t	t	2.2	t	45.6	-	3.3	t	3.9	7.0	t	t	t	-	-	-	t	t	-	-	2.0	0.7	t	-	t	t	264.1
		S.D	t	0.6	0.8	t	6.5	-	1.9	t	0.9	2.6	0.6	0.6	0.6	-	-	-	0.5	t	-	-	3.7	0.5	t	t	t	t	
E. dalrympleana	J	Mean	t	-	4.2	-	47.4	-	2.3	-	7.5	9.8	t	t	t	-	-	-	t	-	-	-	0.8	t	1.6	-	-	-	318.9
		S.D	t	-	1.4	-	4.9	-	1.3	-	1.3	3.3	t	t	t	-	-	-	t	-	-	-	t	t	0.7	-	-	-	
	A	Mean	t	-	4.0	-	40.6	-	3.6	-	7.3	10	t	t	t	-	-	-	-	-	-	-	1.2	1	2.0	-	t	t	66.8
		S.D	t	-	1.9	-	5.0	-	2.7	-	1.8	2.1	t	t	t	-	-	-	-	-	-	-	t	t	t	-	t	t	
E. rubida	J	Mean	0.7	-	5.8	-	59.7	-	1.8	-	7.2	1.9	-	-	-	-	-	-	-	-	-	-	t	t	0.5	t	0.5	t	71.4
		S.D	t	-	0.5	-	7.2	-	t	-	3.1	0.6	-	-	-	-	-	-	-	-	-	-	t	-	t	t	t	t	
	A	Mean	0.5	-	6.4	-	62.8	-	1.3	-	3.5	3.6	-	-	-	-	-	-	-	-	-	-	0.6	t	0.6	t	0.8	t	318.2
		S.D	t	-	t	-	4.3	-	t	-	t	2.3	-	-	-	-	-	-	-	-	-	-	t	t	t	t	0.8	t	
E. johnstonii	J	Mean	t	-	1.3	-	20.4	-	0.8	-	2.0	4	9.2	5.7	t	-	-	-	2.2	4.7	17.2	0.5	3.5	2.9	-	-	-	-	13.8
		S.D	t	-	t	-	3.6	t	0.5	-	t	2.2	5.3	1.1	0.5	-	-	-	0.9	6.2	10.8	t	3.8	2.5	-	-	-	-	
	A	Mean	t	-	2.5	-	30.0	t	1.3	-	2.0	6.1	8.4	2.6	1.4	-	-	-	0.8	2.2	9.5	0.5	7.3	2.3	-	-	-	-	14.8
		S.D	t	-	t	-	6.8	t	0.9	-	0.6	1.5	3.4	1.3	1.4	-	-	-	0.6	1.9	2.8	t	5.9	1.9	-	-	-	-	
E. subcrenulata	J	Mean	t	-	1.7	-	19.8	-	1.3	-	2.1	4.6	10.4	7.6	1.4	-	-	-	0.6	3.9	7.7	0.5	11.3	3.0	-	-	-	-	197.7
		S.D	t	-	0.9	-	4.1	-	0.5	-	0.8	2.7	6.8	5.0	1.0	-	-	-	0.8	4.8	11.2	t	7.7	2.2	t	-	-	-	
	A	Mean	t	-	2.1	-	21.5	-	1.7	-	2.4	5.5	8.1	3.7	2.3	-	-	-	0.8	4.2	8.5	0.9	12.3	2.8	t	-	-	t	315
		S.D	t	-	1.3	-	7.1	-	0.6	-	0.6	2.2	4.9	2.8	1.8	-	-	-	0.5	4.1	10.5	0.5	7.5	4.6	0.9	-	-	t	
E. vernicosa	J	Mean	t	-	t	-	24.7	-	1.2	-	3.3	2.5	11	4.2	2.7	-	-	-	t	10.8	5.1	0.7	7.5	5.4	-	-	-	-	26.4
		S.D	t	-	0.5	t	0.7	-	-	-	1.3	1.7	1.6	1.1	0.8	-	-	-	t	2.0	1.0	0.6	0.6	1.7	-	-	-	-	
	A	Mean	0.5	-	1.3	-	23.8	-	1.2	t	1.9	5.9	9.2	4.9	2.3	-	-	-	t	6.7	3.9	0.5	9.6	3.0	-	-	-	-	10.48
		S.D	t	-	0.9	-	4.8	-	t	t	0.6	1.8	1.8	3.0	0.8	-	-	-	t	2.5	2.9	t	1.4	0.8	-	-	-	-	
E. globulus	J	Mean	t	-	5.3	-	56.4	-	3.1	t	4.9	3.9	-	-	-	-	-	-	-	t	-	-	-	-	1.9	-	-	-	55.2
		S.D	t	-	1.2	-	5.5	-	1.7	t	1.5	1.5	-	-	-	-	-	-	t	t	-	-	-	-	1.6	-	-	-	
	A	Mean	t	-	4.3	-	66.2	-	2.4	-	2.3	4.2	-	-	-	-	-	-	-	-	-	-	-	-	2.1	-	-	-	78.1
		S.D	t	-	1.3	-	2.9	-	0.8	t	1.0	0.8	-	-	-	-	-	-	-	-	-	-	-	-	1.1	-	-	-	
E. cordata	J	Mean	t	-	2.1	-	36.7	-	2.2	-	4.3	3.6	0.8	t	-	-	-	-	t	t	t	-	0.7	0.7	3.0	t	0.6	t	66.3
		S.D	t	-	1.4	-	17.1	-	1.1	-	2.5	2.7	1	t	t	-	-	-	t	t	t	-	0.8	0.7	2.2	t	t	t	
	A	Mean	t	-	2.9	-	47.6	-	1.8	-	3.7	3.2	0.5	t	t	-	-	-	t	-	-	-	t	0.7	4.4	t	0.9	t	37.6
		S.D	t	-	1.6	-	14.2	-	1.3	-	1.7	2.2	1.1	t	t	-	-	-	t	-	-	-	0.6	0.8	2.6	t	0.8	0.5	
E. gunnii	J	Mean	0.6	-	3.7	-	51.4	-	1.6	-	7.7	2.5	1.3	-	t	-	-	-	-	-	t	-	1	2.8	4.0	-	t	t	1020
		S.D	t	-	0.7	-	9.6	-	t	-	3.7	0.9	1.5	-	t	-	-	-	t	t	t	-	0.6	0.9	1.4	-	t	t	
	A	Mean	1.3	-	4.3	-	43.1	-	1.9	-	5.9	2.4	1.1	t	t	-	-	-	t	1.7	t	-	0.8	2.3	4.4	-	-	-	656.3
		S.D	0.7	-	2.0	-	9.6	-	0.5	-	3.4	0.7	1.4	0.6	0.5	-	-	-	t	3.9	0.8	-	0.6	1.4	1.2	-	-	-	
E. archeri	J	Mean	0.5	-	5.5	t	30.9	t	1.6	t	4.8	6.1	-	-	-	-	-	-	-	t	-	-	0.9	1.6	3.3	-	-	-	15.1
		S.D	0.5	-	2.7	0.7	15.4	t	1.6	t	1.1	1	-	-	-	-	-	-	-	t	-	t	1	1.2	1.7	-	-	-	
	A	Mean	1.4	-	6.3	t	20.6	t	1.7	0.5	5.7	7.8	-	-	-	-	-	-	-	-	-	-	t	1.6	4.0	-	-	-	9.4
		S.D	1.4	-	1.8	0.5	5.5	t	1.1	0.6	1.1	1.2	-	-	-	-	-	-	-	-	-	-	t	0.8	1.7	-	-	-	
E. urnigera	J	Mean	0.5	t	3.2	0.5	49.9	t	1.1	t	5.6	2.1	0.7	t	t	-	-	-	-	-	t	-	t	0.9	4.1	t	0.8	t	44.5
		S.D	t	t	2.1	0.7	7.8	t	0.8	t	3.8	t	0.9	t	t	-	-	-	-	-	t	-	0.5	1.0	1.9	t	0.6	t	
	A	Mean	0.9	t	2.8	t	32.7	t	1.6	t	4.4	3.8	0.7	t	t	-	-	-	t	-	-	-	0.5	0.5	3.2	t	1	t	92.3
		S.D	0.7	t	2.1	t	17.9	t	0.5	t	1.4	0.9	1.0	0.5	t	-	-	-	0.5	-	-	-	0.6	t	1.2	t	0.9	t	
E. perriniana	J	Mean	t	-	4.1	-	63.7	-	1.4	-	3.7	2.0	0.6	4.6	t	-	-	-	t	-	-	-	0.9	t	1.1	-	t	-	-
		S.D	t	-	4.1	-	63.7	-	1.4	-	3.7	2.0	0.6	4.6	t	-	-	-	t	-	-	-	0.9	t	1.1	-	t	-	
	A	Mean	t	t	2.7	-	55.0	-	1.3	-	6.8	6.2	0.7	t	-	-	-	-	1.2	-	-	-	0.5	t	5.4	t	t	t	-
		S.D	t	t	2.7	-	55.0	-	1.3	-	6.8	6.2	0.7	t	-	-	-	-	1.2	-	-	-	0.5	t	5.4	t	t	t	
E. morrisbyi	J	Mean	0.5	-	3	-	61.2	-	0.5	-	1.6	2.6	-	-	-	-	-	-	-	-	-	-	-	-	2.3	t	0.6	t	83.3
		S.D	0.7	-	0.9	-	0.9	-	0.7	-	1.5	t	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	t	
	A	Mean	t	-	2.8	-	64.1	-	t	-	2.4	3.0	-	-	-	-	-	-	-	t	-	-	-	-	2.2	t	1.0	0.5	43.5
		S.D	t	-	1.2	-	1.2	-	t	-	1.7	1.9	-	-	-	-	-	-	-	t	-	-	-	-	0.7	t	1.0	0.7	

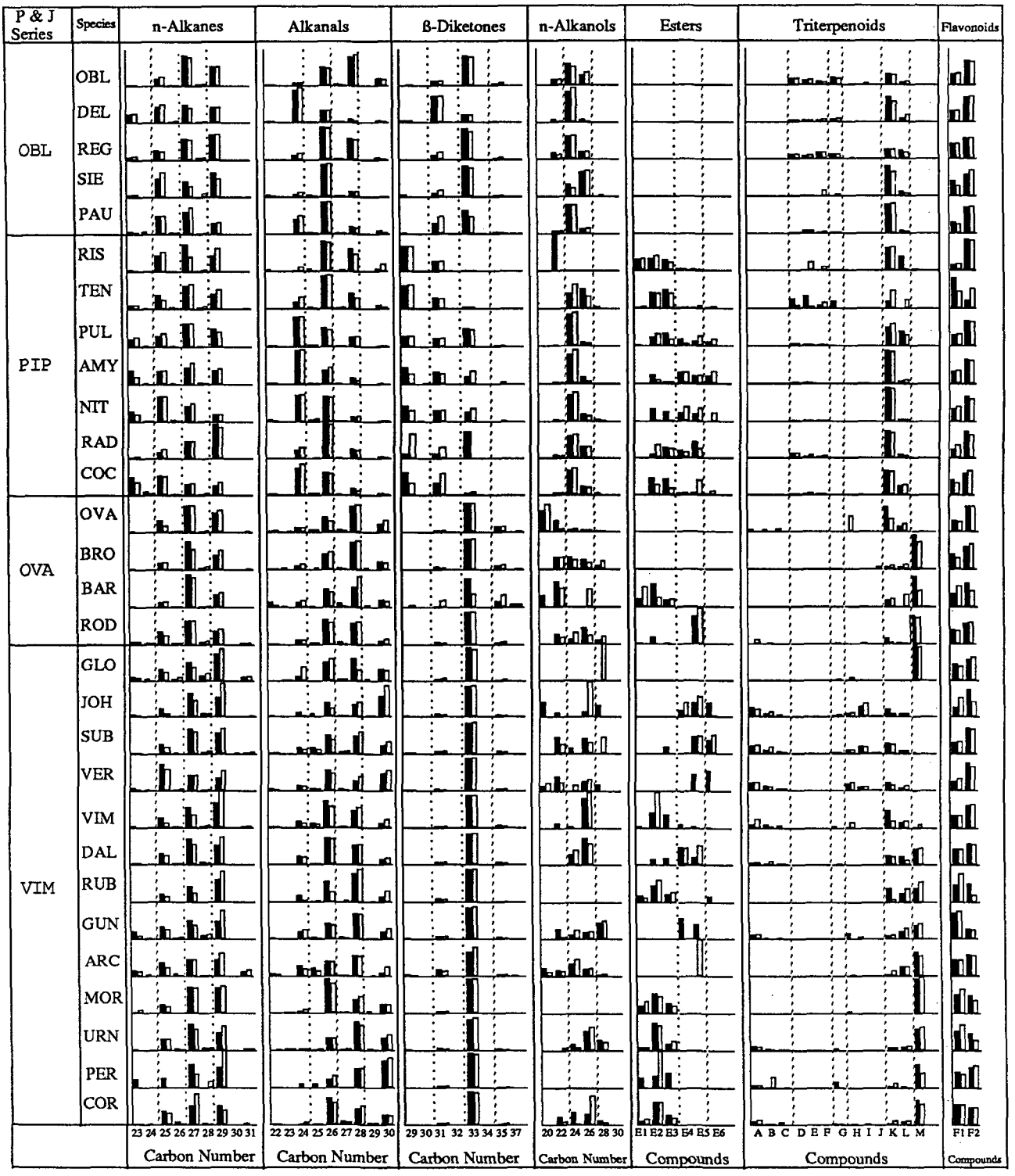


Fig. 5.2. Distribution of homologues of individual compound classes in the hexane soluble waxes of 29 *Eucalyptus* species.

(Individual homologues are expressed as percentage composition within each compound class)

■ Adult leaves
□ Juvenile leaves

An aspect which requires mention is that the isolated peaks included in the unidentified category were not used for data analysis. The components involved in any data analysis were limited to those components listed in Table 5.2. The percentage of each component in samples represents the original observed data from GLC quantitative analysis without treatment (except where stated).

GLC of individual wax samples showed that the major classes of compounds of hexane soluble leaf waxes of Tasmanian eucalypts were n-alkanes (hydrocarbon), alkanals (aldehydes), n-alkanols (primary alcohols), long chain β–diketones, flavonoids, triterpenoids and long chain esters.

(1) n-Alkanes (hydrocarbons) $H_3C\text{-}(CH_2)_n\text{-}CH_3$

Chain lengths from C_{23} to C_{31}, in which the odd carbon-numbered homologues were dominant. n-Heptacosane (C_{27}), n-pentacosane (C_{25}) and n-nonacosane (C_{29}) were the major constituent of n-alkanes in most wax samples.

(2) n-Alkanals (aldehydes) $H_3C\text{-}(CH_2)_n\text{-}CH_2O$

The n-alkanal consisted of saturated n-alkenals with chain lengths ranging between C_{22} to C_{30}, in which the even carbon-numbered homologues were dominant. n-Tetracosanal (C_{24}), n-hexacosanal (C_{26}) and n-octacosanl (C_{28}) were the major constituent of n-alkanal in wax samples.

(3) n-Alkanols (Free primary alcohols) $H_3C\text{-}(CH_2)_n\text{-}CH_3OH$

The n-alkanols consisted almost entirely of even chain length homologues (C_{20} to C_{28}).

(4) Long chain β–diketones

Long chain β–diketones were composed essentially of chain lengths from C_{29} to C_{37}, in which the odd carbon numbered homologues were dominant. However no β–diketones with a C_{36} chain length were identified. The major β–diketones were the homologues, C_{29}, C_{31} and C_{33}:

$n\text{-}C_{15}H_{31}\text{-}CO\text{-}CH_2CO\text{-}C_{15}H_{31}$	n-tritriacontane-16,18-dione (C_{33})
$n\text{-}C_{15}H_{31}\text{-}CO\text{-}CH_2CO\text{-}C_{13}H_{27}$	n-hentriacontane-14,16-dione (C_{31})
$n\text{-}C_{15}H_{31}\text{-}CO\text{-}CH_2CO\text{-}C_{11}H_{23}$	n-nonacosane-12,14-dione (C_{29})

(5) Long chain esters

The leaf waxes of the two subgenera contained five series of long chain esters:

i) esters of 2-alkanols (secondary alcohols) with a general formula $H_3C\text{-}(CH_2)_n\text{-}CH(CH_3)\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3$ consisted of a mixture of acids of even carbon number ranging from 14 to 24 and 2-alkanols of odd carbon number ranging from 9 to 15,

ii) esters of n-alkanols with a general formula $H_3C\text{-}(CH_2)_n\text{-}CH_2\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3$ consisted of a mixture of acids of even carbon number ranging from 14 to 22 and n-alkanols of even carbon number ranging from 22 to 28 and,

iii) three series of long chain aromatic esters: benzyl esters [$C_6H_5CH_2\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3$] and phenyl ethyl ester [$C_6H_5CH_2CH_2\text{-}O\text{-}CO\text{-}(CH_2)_n\text{-}CH_3$], in which acids in the esters had even carbon numbers from 20 to 30, and benzoic esters ([$C_6H_5\text{-}CO\text{-}O\text{-}(CH_2)_n\text{-}CH_3$] while n-alkanols in the esters had even carbon numbers from 20 to 30.

In this study, the esters of n-alkanols and three series of long aromatic esters were included in the category of unidentified compounds and were not used for data analysis. Only the major esters of n-alkanols were used (see Table 5.2B).

(6). Flavonoids

Eucalyptin (5-hydroxy-4'7-dimethoxy-6,8-dimethylflavone) and demethyl eucalyptin (5-hydroxy-4'7-dimethoxy-6-methylflavone).

(7). Triterpenoids

Many of the triterpenoid components remain unidentified (individual compounds were separated in other studies and structure have to be confirmed by detailed study). However, typical fragments of those unidentified components have indicated that the triterpenoids in wax samples belong to the oleanane and ursane series.

Triterpenoids	Mss Basic peak (m/e)		Identified
A	410	204	Unknown
B	410	204	Unknown
C	424	218	Unknown
D	410	163	28-nor-Δ^{17}-Oleanene
E	412	204	Unknown
F	412	204	Unknown
G	426	218	ß-Amyrin
H	438	203	Unknown (ursane series triterpenoid)
I	438	203	Unknown (ursane series triterpenoid)
J	440	203	Unknown (ursane series triterpenoid)
K	468	189	Methyl moronate
L	468	189	isomer of Methyl moronate
M	496	203	11,12,-Dehydroursolic lactone acetate

5.2.3. Data analysis and discussion

The composition of the leaf wax of 29 species from two subgenera of *Eucalyptus* were determined from 135 localities in Tasmania. In agreement with the published data (Horn *et al.* 1964; Herbin and Robins 1968a,b; Hallam and Chambers 1970), ß-diketones, alkanes, alkanols, esters, flavonoids and triterpenoids were the common compounds in eucalypt leaf waxes. The long chain n-alkanal and a wide range of triterpenoids, which had not been reported by the above workers, were identified in many species examined in this study. It is not surprising that the long chain n-alkanal are generally only found in small quantities in plant waxes (Tulloch 1976a,b; Kolattukudy 1980 and Baker 1982). Eucalypt waxes, which have high contents of long chain n-alkanal or triterpenoids, have not been analysed in detail in previous studies.

Waxes consisted mainly of ß-diketone, triterpenoid and aldehyde compounds (Table 5.1, Fig. 5.1). The wax composition of most *Monocalyptus* species was dominated by aldehyde and triterpenoid compounds, with a low aldehyde content usually balanced by a high triterpenoid content and *vice versa* (Fig. 5.1). In *Symphyomyrtus* species, the ß-diketones usually dominated and where this did not occur there was a concomitant increase in triterpenoids, alkanes or n-alkanols (Fig. 5.1). Alkanes and flavonoids were generally minor components, although alkanes dominated in individual species (e.g. *E. barberi*). Esters and n-alkanols occurred only rarely in many species, although exceptions did occur and the level of n-alkanols was generally higher in the *Monocalyptus* species.

The homologue range and the dominant homologues of the alkane (hydrocarbon) and ß-diketone compound classes concur with those previously reported in eucalypts (Horn *et al.* 1964; Herbin and Robins 1968a,b) and other plant waxes (Tulloch 1976a,b; Kolattukudy 1980 and Baker 1982). C_{25}, C_{27} or C_{29} are usually the dominant alkane homologues and C_{29} or C_{33} are the dominant ß-diketone homologues (Table 5.2, Fig. 5.2). The free n-alkanols found in plant waxes generally had a chain length from C_{22}-C_{34}, yet only three homologues consistently constitute major wax components, C_{26}, C_{28} and C_{30} (Kolattukudy 1980). However, the free n-alkanols identified in most eucalypt leaf waxes, were uncommon in this study with C_{24} and C_{26} being dominant. In some species, such as *E. ovata*, the free n-alkanols were dominated by shorter chain lengths, C_{20} and C_{22}.

Alkanals from plant waxes are generally related to free n-alkanols with an even number of carbons and have a chain length distribution quite similar to that of n-alkanols (e.g. Kolattukudy 1970; Baker *et al.* 1975). While n-alkanal of most species identified here have a similar chain length distribution (frequently C_{24}, C_{26} and C_{28}) as the n-alkanols, the chain length distribution of alkanals (frequently C_{26} and C_{28}) in *E. ovata* differed

markedly from that of their n-alkanols (C_{20} and C_{22} dominant). Such differentiation in chain length distribution between alkanals and free n-alkanols is uncommon for plant waxes (Kolattukudy 1980).

The esters of 2-alkanols (secondary alcohols) have been reported in some *Eucalyptus* and barley waxes but they are uncommon in cuticular waxes where their presence might be expected in waxes which contain ß-diketones (von Wettstein-Knowles and Netting 1976; Kolattukudy 1980). In this study, these esters have been commonly detected from many waxes and the chain length distribution was similar to that identified by Horn *et al.* 1964). Although they were not detected from untreated wax samples of some species which contained high ß-diketones (e.g. *E. globulus*, Fig. 5.1), they were obtained after separation of fraction on sephadex (see Chapter 11). However, the percentage distribution of these esters did not commonly correlate with variation in ß-diketones and only did so within the series of *Piperitae* where their percentage varied with ß-diketone contents (Fig. 5.1).

The phenyl ethyl, benzyl esters and benzoate esters have not been reported from eucalypt waxes. However, the chain length distribution of long chain acids identified in phenyl ethyl and benzyl esters and long chain n-alkanols in benzoate ester components (see previous section) agreed with those identified by Horn *et al.* (1964) following saponification of esters, although original structures of esters were not identified in their work. Preliminary work with several species (*E. globulus, E. delegatensis* and *E. regnans*) showed that wax composition of newly expanded adult leaves was variable due to variation in these aromatic esters which often comprised from 20 to 30 percentage of the total waxes. However, their contents declined rapidly with leaf expansion and occurred in only small amounts in mature leaves where the wax composition was very stable and variation was not significant. Because peaks of these aromatic esters were often covered by other major components in GC analysis, they were included in the category of unidentified compounds and were not used for data analysis.

A wide range of triterpenoids was detected from waxes in this study whereas only one of the triterpenoids, 11,12,-dehydroursolic lactone acetate (triterpenoid M, Table 5.2B) has been reported from eucalypts (Horn *et al.* 1964). The methyl moronate (K), which was first detected from eucalypts in this study, was the dominant triterpenoid and often comprised a large proportion of many waxes in conjunction with it's isomer (L). Nevertheless, the structures of many other triterpenoids were not identified, therefore it was not possible to group these triterpenoids into different series. To simplify description, individual compounds of triterpenoids were called triterpenoid homologues, irrespective of what series they may be belong (e.g. ursane and oleanane series).

Variation within and between species

Table 5.1 and Fig. 5.1 indicated that the mean percentage composition of wax compound classes was extremely variable between species ranging from high proportions of either n-alkanes (61.6% in adult and 74% in juvenile leaves of *E. barberi*) or ß-diketones (73% in adult and 65.1% in juvenile leaves of *E. globulus*) to a mixture of six compound classes whose dominance also varied between species. The range in dominance of all compound classes was as follows: n-alkanes 0.7-61.6% for adult and 0.6-74.3% for juvenile leaves; alkanals 1.6-42.9% for adult and 1.2-44.9% for juvenile leaves; n-alkanols 0-15% for adult and 0-19.2% for juvenile leaves; ß-diketones 1.0-73.2% for adult and 0.4-69.3% for juvenile leaves; flavonoids 5.3-21.4% for adult and 4.2-17.3% for juvenile leaves; triterpenoids 0.4-61.3% for adult and 0.3-58.0% for juvenile leaves and esters 0-11.5% for adult and 0-12.8% for juvenile leaves.

Table 5.3. H-test (Kruskal-Wallis) for significance of variation in compound types between species.

Sign.= Significance of variance; $p < 0.001$ = ***; $0.001<p<0.01$ = ** ; $0.01<p<0.05$ = * ; $p > 0.05$ = ns

	Among all species				Within *Monocalyptus*				Within *Symphyomyrtus*			
d.f.	28		28		11		11		17		17	
Cases	138		132		72		70		68		67	
Leaf types	A		J		A		J		A		J	
	H-value	Sign.	H-value	Sign.	H-value	Sign.	H-value	Sign.	H-value	Sign.	H-value	Sign.
Yield	93	***	107	***	36	***	55	***	45	***	49	***
n-Alkanes	87	***	91	***	27	**	21	**	48	***	48	***
Alkanals	120	***	113	***	55	***	55	***	54	***	44	***
n-Alkanols	101	***	102	***	47	***	54	***	34	**	31	*
Diketones	124	***	118	***	58	***	53	***	56	***	53	***
Flavonoids	75	***	74	***	32	***	32	***	36	**	33	**
Triterpenoids	123	***	120	***	54	***	54	***	53	***	52	***
Esters	104	***	102	***	54	***	52	***	49	***	50	***

The Kruskal-Wallis *H*-test (Table 5.3) based on population sample data, indicated that variability (between species/between populations) in wax yields and total percentage of each compound classes was significant ($p<0.001$) for the 29 species in both juvenile and adult leaf samples. The variation between species within each subgenus for each compound class was also significant ($p<0.001$, $p<0.01$ and $p<0.05$) in all cases for both the *Monocalyptus* and *Symphyomyrtus* subgenera.

Moreover, the Kruskal-Wallis *H*-test (Table 5.4) of the population data indicated that variation between the 29 species in the percentage composition of most compound homologues was significant for both adult and juvenile leaf samples ($p<0.001$, $p<0.01$ and $p<0.05$). However, the variability between species of the dominant homologues of long chain n-alkanes, alkanals, n-alkanols and ß-diketones, was generally greater than that of minor homologues. For example, the *H*-values for variations in the odd carbon numbered homologues of ß-diketones ($H = 92$, 101, 124 and 114 for C_{29}, C_{31}, C_{33} and C_{35} in adult leaf samples) were higher than in the minor, even carbon numbered homologues ($H = 63$, 41 and 46 for C_{30}, C_{32} and C_{34} homologues in adult leaf samples) (Table 5.4). Within each subgenus the most dominant homologues of long chain n-alkanes, alkanals, n-alkanols and ß-diketones also varied significantly ($p<0.001$, $p<0.01$ and $p<0.05$) but not many of the minor homologues were significant. Nevertheless, the *H*-values for variation in triterpenoid homologues were similar and virtually all highly significant, although this compound class consists of 13 compounds (Table 5.4).

The results of the above analysis thus indicated that the distributions of different compound classes and homologues of individual compound classes between species was distinctive and varied significantly. In general, the variation between species in the dominant homologues of long chain compound classes was significantly greater than that for minor homologues.

Following Zavarin and Snajberk (1985), the component variability between localities within species was characterised by the sum of variances (ΣV) of individual compounds of all compound classes (see Table 5.2. part 2).

The ΣV values of both adult and juvenile leaf samples of *E. ovata* (1391 and 1531) were the highest amongst all species, followed by *E. gunnii* (656 and 1020). Thus, the chemical composition of leaf waxes of these two species was the most variable between localities within species. In contrast, some species had low ΣV for both adult and juvenile leaves, notably *E. globulus, E. vernicosa and E. johnstonii.* Some of the *Symphyomyrtus* species showed a marked difference in ΣV values between adult and juvenile leaf samples, such as *E. subcrenulata* (315 for adult and 197 for juvenile), *E. dalrympleana* (67 for adult and 319 for juvenile) and *E. rubida* (318 and 71 for adult and juvenile).

Within the subgenus *Monocalyptus*, the ΣVs of adult and juvenile leaf samples of *E. amygdalina* were the highest (506 and 498) followed by *E. coccifera*, *E. pulchella* and *E. pauciflora* (227, 257 and 176 for adult leaves and 306, 302 and 326 for juvenile leaves respectively) but values for other species were low (less than 140).

Table 5.4. Test (Kruskal-Wallis) of significance of variation in percentage contents of leaf oil components among speci (between species/between populations).

Significance of variance: $p < 0.001$ = *** ; $0.001 < p < 0.01$ = ** ; $0.01 < p < 0.05$ = * ; $p > 0.05$ = ns. - : no statistics

	ALL species		Subgenus Moncalyptus		Subgenus Symphyomyrtus	
Leaf types	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
d.f.	28	28	11	11	16	16
Cases	140	134	72	68	68	64
H-values	H S	H S	H S	H S	H S	H S
Compounds						
n-Alkanes						
23	89 ***	92 ***	46 ***	44 ***	35 **	30 *
24	46 *	36 ***	22 *	9 ns	22 ns	25 ns
25	72 ***	76 ***	16 ns	21 ns	43 ***	46 ***
26	50 **	42 *	19 ns	13 ns	26 *	23 ns
27	92 ***	85 ***	38 ***	36 ***	46 ***	44 ***
28	54 **	63 ***	18 ns	16 ns	22 ns	31 *
29	99 ***	101 ***	43 ***	35 ***	44 ***	46 ***
30	34 ns	33 ns	- -	- -	16 ns	15 ns
31	68 ***	73 ***	10 ns	16 ns	27 *	24 ns
Alkanals						
22	75 ***	79 ***	38 ***	39 ***	33 **	35 **
23	40 ns	29 ns	6 ns	9 ns	28 *	12 ns
24	119 ***	116 ***	60 ***	58 ***	41 ***	40 ***
25	73 ***	73 ***	35 ***	35 ns	35 **	28 *
26	115 ***	112 ***	44 ***	49 ***	53 ***	41 ***
27	48 *	36 ns	18 ns	20 ***	29 *	11 ns
28	98 ***	84 ***	37 ***	37 ***	57 ***	46 ***
29	59 ***	54 **	4 ns	6 ns	30 *	24 ns
30	64 ***	61 ***	27 **	19 ***	27 *	24 ns
n-Alkanols						
20	92 ***	103 ***	11 ns	10 ns	50 ***	54 ***
22	65 ***	72 ***	20 *	27 **	34 **	34 **
24	114 ***	113 ***	49 ***	53 ***	38 **	29 *
26	71 ***	65 ***	28 **	27 **	23 ns	18 ns
28	43 *	39 ns	24 *	30 **	18 ns	12 ns
ß-Diketones						
29	92 ***	83 ***	52 ***	49 ***	36 **	28 *
30	63 ***	42 *	34 ***	17 ns	28 *	30 *
31	101 ***	95 ***	56 ***	44 ***	43 ***	50 ***
32	41 *	38 ns	17 ns	13 ns	20 ns	20 ns
33	124 ***	117 ***	57 ***	51 ***	55 ***	51 ***
34	46 *	53 **	- -	- -	21 ns	24 ns
35	114 ***	102 ***	42 **	39 ***	37 **	35 **
37	51 **	38 ns	- -	- -	22 ns	15 ns
Flavonoids						
F1	72 ***	76 ***	28 **	29 **	41 ***	43 ***
F2	84 ***	84 ***	31 ***	28 **	41 ***	34 **
Triterpenoids						
A	105 ***	97 ***	- -	- -	49 ***	43 ***
B	104 ***	106 ***	- -	- -	48 ***	50 ***
C	95 ***	84 ***	- -	- -	43 ***	38 **
D	129 ***	116 ***	53 ***	49 ***	- -	- -
E	127 ***	117 ***	55 ***	49 ***	- -	- -
F	119 ***	109 ***	53 ***	42 ***	- -	- -
G	101 ***	104 ***	57 ***	57 ***	35 **	37 **
H	99 ***	87 ***	6 ns	51 ***	49 ***	37 **
I	117 ***	101 ***	- -	58 ***	57 ***	44 ***
J	139 ***	119 ***	- -	- -	66 ***	55 ***
K	119 ***	116 ***	55 ***	45 ***	38 **	41 ***
L	83 ***	91 ***	34 ***	35 ***	42 ***	42 ***
M	114 ***	115 ***	13 ns	- -	49 ***	47 ***
Esters						
E1	88 ***	106 ***	48 ***	67 ***	39 **	42 ***
E2	100 ***	103 ***	53 ***	52 ***	45 ***	50 ***
E3	95 ***	87 ***	54 ***	49 ***	38 **	33 **

These results indicated that the wax composition often exhibited greater variation in those species which showed greater intra–specific variation in other morphological characters. This was particularly evident in a number of *Piperitae* species, *E. amygdalina, E. pulchella and E. coccifera* and *Symphyomyrtus* species *E. ovata* and *E. gunnii*. In contrast, in *E. delegatensis, E. obliqua* and *E. globulus* with localities distributed over a wide geographical range within individual species, variation was low in morphological characters (Davidson *et al.* 1981) and also low in wax composition. Nevertheless, one of the *Piperitae* species, *E. nitida*, had a greater variation in morphological characters but showed little variation in wax composition. Hence, the variability in wax composition was not always in parallel with variability of morphological characters within species.

Comparison between adult and juvenile leaves

The *U*-tests for comparing the yield and percentage composition of compound classes between leaf types (juvenile and adult) of each species based on population data are shown in Table 5.5.

The only two species, *E. globulus* and *E. dalrympleana,* exhibited significant difference in wax yields between adult and juvenile leaves. In these two species the juvenile wax yields were significantly higher (Δ_{A-J}=-0.8%, $p<0.001$ and Δ_{A-J}=-0.4%, $p<0.05$ respectively) than adult yields.

There were no significant differences in the percentage composition of each compound class between juvenile and adult leaves for most species. Within the *Monocalyptus* species, the juvenile leaf waxes of the two "blue ashes", *E. sieberi and E. pauciflora*, had significantly higher alkanals (Δ_{A-J}=-24.0, $p<0.05$ and Δ_{A-J}=-7.3, $p<0.05$ respectively) and the other "blue ash", *E. delegatensis,* had significantly higher alcohol (Δ_{A-J}=-12.8, $p<0.001$) but significantly lower triterpenoid (Δ_{A-J}=5.0, $p<0.05$) contents compared to adult leaf waxes. The adult leaf waxes of one peppermint species, *E. coccifera,* had significantly higher ß-diketone (Δ_{A-J}=11.7, $p<0.05$) and ester (Δ_{A-J}=4.1, $p<0.01$) contents than juvenile leaf waxes.

In the *Symphyomyrtus* species, percentage contents of ß-diketones were significantly higher in adult leaf waxes than in juvenile leaf waxes for *E. globulus* (Δ_{A-J}=8.1, $p<0.01$) and *E. johnstonii* (Δ_{A-J}=11.5, $p<0.05$). However, ß-diketones were significantly higher (Δ_{A-J}=-9.3, $p<0.05$) in juvenile leaf waxes than in adult leaf waxes for *E. viminalis*. Flavonoids were significantly lower (Δ_{A-J}=-2.2, $p<0.05$) in adult leaf waxes for *E. globulus* and significantly higher (Δ_{A-J}=3.9, $p<0.05$) in juvenile leaf waxes for *E. viminalis*. In addition, the alkanals were significantly higher in adult leaf waxes than in

Table 5.5. Significance (Mann-Whitney U test) of the difference in the wax yield and major compo types between adult and juvenile leaves of individual species.

Significance of difference: $p < 0.001$ = ***; $0.001 < p < 0.01$ = **; $0.01 < p < 0.05$ = *; $p > 0.05$ = ns

(-) = no statistics Δ A-J = Difference between adult and juvenile leaves

Species	Pop. No A/J	Yield ΔA-J	Sign.	Alkanes ΔA-J	Sign.	Aldehydes ΔA-J	Sign.	Alcohols ΔA-J	Sign.	Diketones ΔA-J	Sign.	Flavonoids ΔA-J	Sign.	Triterpenoid ΔA-J	Sign.	Esters ΔA-J	Sign.
E. regnans	6/6	-0.4	ns	1.1	ns	0	ns	-0.5	ns	-1	ns	-1.9	ns	-2.8	ns	0	ns
E. obliqua	9/9	-0.1	ns	-0.4	ns	0.6	ns	0.3	ns	2.2	ns	1.1	ns	-5.4	ns	0	ns
E. delegatensis	10/10	0.1	ns	0.3	ns	-1	ns	-13	***	1.2	ns	0.8	ns	5	*	0	ns
E. sieberi	3/2	0.1	ns	0.1	ns	-24	ns	-14	ns	12.4	ns	-0.9	ns	21.2	ns	0	ns
E. pauciflora	5/5	0	ns	1.4	ns	-7.3	*	-0.2	ns	1.3	ns	-4.3	ns	5.3	ns	0	ns
E. radiata	1/1	0	-	1	-	-8.2	-	-2	-	0.6	-	5.1	-	2.7	-	0.1	-
E. amygdalina	8/8	0	ns	0.3	ns	-8	ns	-2.1	ns	0.1	ns	3.4	ns	7.2	ns	0.2	ns
E. puchella	5/5	0.3	ns	3.7	ns	-1.7	ns	-2.9	ns	2.5	ns	-1	ns	0.2	ns	-0.1	ns
E. nitida	10/10	0.2	ns	-0.9	ns	-2.5	ns	-0.5	ns	-0.2	ns	-0.2	ns	3.3	ns	0.2	ns
E. coccifera	6/6	-0.2	ns	1.5	ns	-9.7	ns	-1.3	ns	11.7	*	-1.1	ns	-1.7	ns	4.1	**
E. tenuiramis	4/4	0	ns	4	ns	-6.6	ns	-1.9	ns	2.7	ns	0.4	ns	0.6	ns	0.9	ns
E. risdonii	3/2	0.1	ns	5.4	ns	-4.2	ns	0	ns	5	ns	-1.8	ns	-0.9	ns	-1.3	ns
E. ovata	6/6	0	ns	1.7	ns	-2	ns	2.2	ns	-1.5	ns	0.8	ns	0.1	ns	0	ns
E. brookeriana	4/4	0.1	ns	-2.4	ns	-0.3	ns	2.3	ns	-5.9	ns	-3.4	ns	3.9	ns	0	ns
E. rodwayi	4/4	0.1	ns	-4.2	ns	0.9	ns	-0.2	ns	3.2	ns	-1.1	ns	-2.3	ns	0.5	ns
E. barberi	2/2	0.1	ns	-13	ns	1.8	ns	0.1	ns	6	ns	0.5	ns	1.6	ns	-0.4	ns
E. viminalis	6/6	-0.1	ns	0.7	ns	0.8	ns	0.4	ns	-9.3	*	3.9	*	3.6	**	0.4	ns
E. dalrympleana	3/3	-0.4	*	0.5	ns	1.6	*	2.9	ns	-5.7	ns	0	ns	1.2	ns	0.5	*
E. rubida	2/2	0	ns	0	ns	2.2	ns	0	ns	3	ns	-2	ns	0.8	ns	0.7	ns
E. johnstonii	5/5	-0.2	ns	-4	ns	1.4	ns	0.3	ns	11.5	*	2.1	ns	-11.3	ns	0	ns
E. subcrenulata	5/4	-0.1	ns	-1	ns	0.5	ns	0	ns	2.5	ns	1.2	ns	-2.2	ns	0.1	ns
E. vernicosa	4/3	0	ns	2	ns	-1.3	ns	0	ns	0.1	ns	2	ns	-7.7	ns	0	ns
E. globulus	6/6	-0.8	**	0.1	ns	0.7	ns	0	ns	8.1	**	-2.2	*	0	ns	0	ns
E. cordata	5/5	0	ns	-15	ns	6.7	**	-1.2	ns	11.3	ns	-0.9	ns	1.2	ns	0.4	ns
E. gunnii	6/6	-0.1	ns	1.8	ns	2.5	ns	0.1	ns	-6.8	ns	-1.9	ns	2.2	ns	-0.2	ns
E. archeri	3/3	0	ns	-0.1	ns	1.3	ns	0.4	ns	-8.1	ns	2.7	ns	0.1	ns	0	ns
E. urnigera	4/4	-0.1	ns	9.7	ns	2.2	ns	0	ns	-17	ns	0.5	ns	-1	ns	0.1	ns
E. perriniana	1/1	-0.6	-	0.1	-	0.4	-	0	-	-10	-	7.3	-	0.5	-	0.1	-
E. morrisbyi	2/2	0	ns	-0.3	ns	1.9	ns	0	ns	2.2	ns	1.1	ns	0	ns	0.5	ns

juvenile leaf waxes for *E. dalrympleana* (Δ_{A-J}=1.6, p<0.05) and *E. cordata* (Δ_{A-J}=6.7, p<0.01) while alkanals were significantly higher (Δ_{A-J}=3.9, p<0.05) in adult leaf waxes of *E. viminalis*.

Comparison of the homologue distributions within most compound classes (Fig. 5.2) showed no marked difference between juvenile and adult leaf samples. Difference in the distribution of major alkane (hydrocarbon) homologues between foliage types were only noted for the waxes of *Symphyomyrtus* species. The alkanes of juvenile waxes contained higher proportions of longer chain length homologues than those of adult waxes (alkanes of juvenile waxes had a higher proportion of C_{29} homologue but lower C_{27} and C_{25} than adult waxes), with the exception of *E. cordata* (Fig. 5.2).

Comparison of wax chemicals between subgenera and series within subgenera

Significance tests

The *U*-test (Table 5.6) based on mean percentage chemical contents of species indicated that the distribution of alkanals (Δ_{M-S}=9.3, p<0.5 for adult leaves and Δ_{M-S}=16.6, p<0.001 for juvenile leaves), n-alkanols (Δ_{M-S}=0.7, p<0.5 for adult and Δ_{M-S}=5.2, p<0.01 for juvenile leaves) and triterpenoids (Δ_{M-S}=20.6, p<0.5 for adult and Δ_{M-S}=17.2, p<0.05 for juvenile leaves) in both adult and juvenile leaf waxes of the subgenus *Monocalyptus* were significantly higher than that in the subgenus *Symphyomyrtus*. The flavonoids were also significantly higher (Δ_{M-S}=3.3, p<0.05) in juvenile leaf waxes for *Monocalyptus*.

In contrast, the percentage of ß-diketone was significantly higher in the subgenus *Symphyomyrtus* for both adult and juvenile leaves (Δ_{M-S}=-23.6, p<0.01 for adult and Δ_{M-S}=-27.8, p<0.01 for juvenile leaves). However, there was no significant difference in the percentage contents of alkanes and long chain esters between the subgenera.

The results of the *U*-test, comparing the percentage distribution of individual homologues of different compound classes between subgenera or between series within a subgenus are shown in Table 5.7. The percentage contents of the major homologues, C_{24}, C_{26} and C_{30}, of alkanals and C_{24} of n-alkanols were significantly higher in *Monocalyptus* species. The percentages of C_{33} and C_{35} of ß-diketones were significantly higher (p<0.01) in *Symphyomyrtus* species. However, the distribution of individual triterpenoid compounds differed between subgenera. The five triterpenoid homologues, D, E, F, K and L were significantly higher (p<0.001) in the *Monocalyptus* species and the four homologues A, B, C (p<0.01) and M (p<0.001) were significantly higher in *Symphyomyrtus* species. The individual alkane homologues, C_{27}, C_{29} and C_{31}, were

significantly ($p<0.01$ or $p<0.05$) higher in *Symphyomyrtus* species, although the majority of alkane homologues did not differ significantly between subgenera.

Table 5.6. Significance test (*U*-test) for differences in percentage composition of the compound types between subgenera and series based on mean percentage values for individual species.

Sign.=Significance of variance: $p<0.001$ = ***; $0.001<p<0.01$ = **; $0.01<p<0.05$ = * ; $p > 0.05$ = ns

	Between subgenera *Monocalyptus/Symphyomyrtus*				Between series *Obliquae/Piperitae*				Between series *Ovatae/Viminales*			
Species No.	12/17				5/7				4/13			
Leaf types	A		J		A		J		A		J	
	Δ_{M-S}	Sign.	Δ_{M-S}	Sign.	Δ_{O-P}	Sign.	Δ_{O-P}	Sign.	Δ_{O-V}	Sign.	Δ_{O-V}	Sign.
Yield	0.3	*	0.1	ns	0.2	ns	0.3	ns	-0.2	ns	-0.5	*
n-Alkanes	-7.1	ns	-9.9	ns	-1.5	ns	0.1	ns	24.9	**	28.9	**
Alkanals	9.3	*	16.6	***	-7.0	ns	-6.5	ns	11.0	*	12.5	ns
Alkanols	0.7	*	5.2	**	-0.7	ns	2.2	ns	3.9	ns	3.3	ns
Diketones	-23.6	**	-27.8	**	-12.6	ns	-12.6	ns	-28.5	*	-30.3	*
Flavonoids	2.7	ns	3.3	*	-0.9	ns	0.8	ns	-2.7	ns	-0.8	ns
Triterpenoids	20.6	*	17.2	*	23.7	ns	20.7	ns	-10.7	ns	-12.6	*
Esters	1.8	ns	1.6	ns	-4.0	**	-3.4	**	-0.2	ns	-0.1	ns

Within the subgenus *Monocalyptus*, there was only one compound class, the long chain esters, that was significantly higher (Δ_{O-P}=-4.0, $p<0.01$ for adult and Δ_{O-P}=-3.4, $p<0.01$ for juvenile leaves) in the series *Piperitae* when compared to the series *Obliquae* for both adult and juvenile leaves (Table 5.6). However, the percentage content of individual homologues of some compound classes, in which total percentage of compound classes did not differ significantly, did differ significantly between series (Table 5.7). For example, the C_{29} homologue of ß-diketones ($p<0.05$) and long chain esters E2 and E3 ($p<0.01$) were significantly higher in the *Piperitae* species whereas the C_{35} homologues were significantly higher ($p<0.05$) in the *Obliquae* species. Furthermore, compounds D, E, F and G of triterpenoids were also significantly higher ($p<0.05$) in the *Obliquae* species.

In *Symphyomyrtus*, the waxes of both adult and juvenile leaves of the *Ovatae* series had significantly higher percentage contents of alkanes (Δ_{O-V}=24.9, $p<0.01$ for adult and Δ_{O-V}=28.9, $p<0.01$ for juvenile leaves) than that in the series *Viminales*. In contrast, the series *Viminales* had significantly higher percentage contents of ß-diketones (Δ_{O-P}=-28.5, $p<0.05$ for adult and Δ_{O-P}=-30.3, $p<0.05$ for juvenile leaves) for both adult and juvenile leaves. In addition, the triterpenoids were significantly higher (Δ_{O-P}=-12.6, $p<0.05$) in the series *Viminales* for juvenile leaves and alkanals for adult leaves (Δ_{O-P}=11.0, $p<0.05$).

Table 5.7. U-test for the significance of the difference in the individual homologues of each compound type between subgenera and between series within each subgenus based on mean percentage values of individual species

Significance of difference: $p < 0.001$ = *** ; $0.001 < p < 0.01$ = ** ; $0.01 < p < 0.05$ = * ; $p > 0.05$ = ns

	Between subgenera Monocalyptus and Symphyomyrtus		Subgenus Moncalyptus Between Obliquae and Piperitae		Subgenus Symphyomyrtus Between Ovatae and Viminales	
Leaf types	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
No. of species	12/17	12/17	5/7	5/7	4/13	4/13
	Δ(M-S) Sig.	Δ(M-S) Sig.	Δ(O-P) Sig.	Δ(O-P) Sig.	Δ(O-V) Sig.	Δ(O-V) Sig.
Compounds						
n-Alkanes						
23	0.25 ns	0.22 ns	-0.57 ns	-0.25 ns	0.39 *	0.14 ns
24	0.00 ns	-0.03 ns	-0.04 ns	-0.02 ns	0.07 ns	0.16 **
25	-0.85 ns	-0.74 ns	-0.38 ns	0.05 ns	2.78 *	2.53 *
26	-0.17 ns	-0.18 ns	-0.03 ns	0.02 ns	0.37 *	0.51 **
27	-4.03 **	-4.78 **	-0.22 ns	0.18 ns	14.94 **	12.39 **
28	-0.28 ns	-0.36 ns	0.00 ns	0.10 ns	0.44 *	0.78 **
29	-1.88 *	-3.81 **	-0.25 ns	0.08 ns	5.82 **	6.47 *
30	0.00 ns	-0.01 ns	0.00 ns	0.00 ns	0.01 ns	-0.01 ns
31	-0.18 **	-0.26 **	0.00 ns	-0.01 ns	0.07 ns	0.23 *
Alkanals						
22	0.05 ns	0.09 ns	-0.03 ns	-0.06 ns	0.23 *	0.19 *
23	-0.05 ns	-0.02 ns	-0.01 ns	0.00 ns	0.14 *	0.01 ns
24	5.63 *	8.17 ***	-3.47 ns	-4.97 ns	0.66 ns	0.75 ns
25	0.05 ns	0.20 ns	-0.10 ns	-0.14 ns	0.07 ns	0.23 ns
26	5.13 **	9.07 ***	-2.98 ns	-0.98 ns	3.92 ns	4.41 ns
27	-0.09 ns	0.09 *	0.06 ns	0.07 ns	0.55 **	0.30 ns
28	-0.51 ns	0.01 ns	-0.56 ns	-0.25 ns	4.54 *	5.01 *
29	-0.16 ns	-0.06 *	0.01 ns	0.00 ns	0.30 *	0.10 ns
30	-0.73 **	-0.88 **	0.04 ns	-0.16 ns	0.57 ns	0.89 ns
n-Alkanols						
20	-0.64 ns	-0.64 ns	0.00 ns	0.00 ns	2.12 ns	1.92 ns
22	-0.33 ns	-0.06 ns	0.08 ns	0.11 ns	1.60 *	0.50 *
24	2.24 **	4.48 ***	-0.83 ns	0.74 ns	0.43 ns	0.23 ns
26	0.22 ns	1.25 *	0.11 ns	2.11 ns	0.16 ns	-0.05 ns
28	-0.09 ns	-0.06 ns	-0.07 ns	0.14 ns	0.16 ns	0.23 ns
ß-Diketones						
29	7.87 ns	7.02 ns	-13.72 **	-12.30 *	-0.39 *	-0.24 *
30	0.04 ns	0.05 ns	-0.11 ns	-0.11 ns	-0.03 ns	-0.02 ns
31	2.08 ns	1.40 ns	-4.51 ns	-3.54 ns	-2.38 **	-2.00 *
32	-0.05 ns	-0.04 ns	-0.01 ns	-0.03 ns	-0.04 ns	-0.08 ns
33	-31.67 ***	-34.31 ***	5.51 ns	3.13 ns	-26.50 *	-22.62 *
34	-0.03 ns	-0.02 ns	0.00 ns	0.00 ns	-0.03 ns	-0.03 ns
35	-1.77 ***	-1.78 ***	0.27 *	0.23 *	0.67 ns	0.98 ns
37	-0.14 *	-0.13 *	0.00 ns	0.00 ns	0.24 ns	0.26 *
Flavonoids						
F1	0.04 ns	-0.18 ns	0.01 ns	-0.02 ns	-1.42 ns	-1.62 ns
F2	2.64 *	3.51 **	-0.90 ns	0.87 ns	-1.24 ns	0.71 ns
Triterpenoids						
A	-1.74 **	-2.06 **	0.00 ns	0.00 ns	-2.26 ns	-2.41 ns
B	-0.73 **	-1.34 **	0.00 ns	0.00 ns	-0.95 ns	-1.71 ns
C	-0.43 **	-0.29 **	0.00 ns	0.00 ns	-0.55 ns	-0.30 ns
D	1.96 ***	1.82 ***	3.22 *	3.22 *	0.00 ns	0.00 ns
E	1.64 ***	1.81 ***	2.87 *	2.92 *	0.00 ns	0.00 ns
F	1.60 ***	2.02 ***	3.02 **	3.84 *	0.00 ns	0.00 ns
G	1.49 ns	1.59 ns	3.93 **	4.21 *	-0.29 ns	-0.22 ns
H	-0.88 ns	-0.95 ns	-0.02 ns	0.55 ns	-1.16 ns	-1.51 ns
I	-1.31 ns	-1.61 ns	0.00 ns	0.42 ns	-1.71 ns	-2.29 ns
J	-0.11 ns	-0.10 ns	0.00 ns	0.00 ns	-0.14 ns	-0.11 ns
K	18.25 ***	15.41 ***	7.91 ns	3.68 ns	-2.40 ns	-1.90 ns
L	3.03 **	2.42 *	2.78 ns	1.82 ns	-1.12 ns	-1.19 ns
M	-2.21 ***	-1.58 ***	-0.01 ns	0.00 ns	-0.07 ns	0.15 ns
Long chain esters						
E1	0.26 ns	0.32 ns	-0.57 ns	-0.70 ns	-0.02 ns	0.09 ns
E2	0.66 ns	0.73 ns	-1.66 **	-1.57 **	-0.11 ns	0.01 ns
E3	0.85 ns	0.56 ns	-1.70 **	-1.10 **	-0.12 ns	0.04 ns

The two series, *Ovatae* and *Viminales*, of *Symphyomyrtus* also differed significantly in the major homologues of n-alkanes, alkanals, 2-alkanols and ß-diketones but not the triterpenoids and the long chain esters. Most homologues of the n-alkanes, C_{22} and C_{28} alkanals and C_{24} n-alkanols were significantly higher (either $p<0.05$ or $p<0.01$) in *Ovatae* species for either adult and/or juvenile leaf waxes. C_{29}, C_{31} and C_{33} ß-diketones significantly higher ($p<0.05$ or $p<0.01$) in *Viminales* species for both adult and juvenile leaf samples.

Discussion

The above data analysis indicated that the distribution of wax composition and major homologues of individual compound classes was related to the current taxonomic division at the subgeneric level, while some wax compounds differentiated series within subgenera. The compound classes of most taxonomic significance were ß-diketones, triterpenoids, n-alkanols and alkanals.

ß-Diketones are often the major component of the wax and confined to a restricted range of plant. They are particularly predominant in the leaf and leaf sheath waxes of many graminaceous species and in the leaf waxes of *Eucalyptus* and *Acacia* (Baker 1982). Among eucalypt species, ß-diketones were characterised in some species groups and had taxonomic significance (Horn *et al.* 1964; Hallam and Chambers 1970). In this study, this compound class was detected in all species examined. However, a high percentage of ß-diketones in leaf waxes was mainly restricted to *Symphyomyrtus* species, particularly of the series *Viminales*. With the exception of a few species, ß-diketone contents were general low in *Monocalyptus* species.

The variation in the percentage content of the ß-diketones among *Symphyomyrtus* species (Fig. 5.1) is associated with morphological differences in the colour and glaucousness of leaf surfaces. The percentage of ß-diketones was higher in waxes in which leaves are glaucous or subglaucous, but were lower in black gum and yellow gum species, which have leaves that are green or non-glaucous. The alpine white gum species, *E. archeri*, which consists of only green and subglaucous populations, also had lower percentage of ß-diketones than other alpine white gum species. However, leaf waxes of the green leaved species, *E. viminalis*, had higher percentage contents of ß-diketone in contrast with other green leaved species.

Results of this study indicated that the ß-diketones in *Symphyomyrtus* species were dominated by n-tritriacontane-16,18-dione (C_{33}) in comparison to variable chain length distributions in *Monocalyptus species*, which are usually shorter (Fig. 5.2). The two series within *Monocalyptus* were also distinguished by their ß-diketone homologues with

C_{29} or relatively similar proportions of C_{29}, C_{31} and C_{33} dominating in *Piperitae* species in contrast to C_{31} or C_{33} dominance in *Obliquae* species. In agreement with Horn *et al.* (1964), the chain length distribution of ß-diketones in the leaf waxes of *E. risdonii* and *E. coccifera* (C_{29} dominate) is shorter than that in *Symphyomyrtus* species (Fig.5.2).

The taxonomic value of triterpenoids from eucalypt waxes have not yet been evaluated. Results of this study indicated that a wide range of triterpenoids occurred in many species and these often formed a major proportion of the leaf waxes and appear to be of taxonomic value. For example, a group of triterpenoid compounds, D, E and F, was only detected in *Monocalyptus* species but another group of triterpenoids, A, B, C, I, J and M was only detected in *Symphyomyrtus* species (Table 5.2B). The distribution of triterpenoid homologues also distinguished species groups within subgenera. Within *Monocalyptus*, the percentages of four triterpenoids, D, E, F and G were significantly higher in *Obliquae* species (Tables 5.2 and 5.7) than in *Piperitae* species. An initial split may be made in the subgenus *Symphyomyrtus* on the distribution of triterpenoids, isolating yellow gum species which have a high (>35%) triterpenoid content, represented by 9 compounds (A, B, C, G, H, I, J, K and L), in comparison to the low contents (<11.5%) and lesser compounds present in other *Symphyomyrtus* species. In addition, 11,12-dehydroursolic lactone acetate (M) occurred only rarely in the yellow gums but dominated in triterpenoids of many other *Symphyomyrtus* species. Methyl moronate (K), which was first identified in eucalypts in this study, is apparently restricted to *Monocalyptus* and the yellow gum species where they are the dominant triterpenoids. This compound is either a very minor compound or absent in other *Symphyomyrtus* species.

While data analysis indicated that the percentage of alkanals was significantly higher in *Monocalyptus* species, the significant taxonomic differences between subgenera for these compounds could also be related to their homologue distribution. The dominant chain length of alkanals in *Symphyomyrtus* species are C_{26} and C_{28} and less commonly similar amounts of C_{26}, C_{28} and C_{30}. The dominant alkanals in the *Monocalyptus* were C_{24} and C_{26} and less commonly similar amounts of C_{24}, C_{26} and C_{28} homologues with few exceptions (Fig. 5.2). It is of interest that the chain length distribution of alkanals was often correlated with that of the ß-diketones for where these waxes had a short chain length distribution in ß-diketones then the chain length of alkanals tended to be relatively shorter (Fig. 5.2). For example, *E. obliqua, E. regnans, E. sieberi* and *E. pauciflora* had ß-diketones dominated by C_{33} homologues and alkanals dominated by C_{25} or C_{28} but in *E. delegatensis* and other *Piperitae* species, with the exception of *E. risdonii* and *E. tenuiramis,* ß-diketones were dominated by C_{29} and alkanals by C_{24}.

Although n-alkanols were a minor compound class, their percentage distribution was significantly higher in *Monocalyptus* than in *Symphyomyrtus* species and the homologue distribution appeared of more taxonomic significance in differentiating subgenera. With the exception of *E. risdonii* and *E. sieberi* (where n-alkanols were absent or dominated by C_{26} chain lengths), n-alkanols in waxes of all *Monocalyptus* species had an identical pattern of C_{24} homologue dominating in comparison to the irregular alcohol patterns in *Symphyomyrtus* species where the C_{24} pattern was absent (Fig. 5.2). However, the variation in chain length distribution of n-alkanols did not correlate to variation in chain length of other long chain compounds.

The total percentage distribution of alkanes in waxes did not discriminate subgenera, however, the distribution of individual homologues did. The chain length distribution in some *Monocalyptus* species tended to be shorter than in *Symphyomyrtus* species (Fig. 5.2). Thus, in agreement with the finding of Horn *et al.* (1964) and Herbin and Robin (1968a,b), alkanes of *Monocalyptus* species had a maximum chain length of C_{29}, whereas, chain length may extend to a maximum of C_{31} in some of the *Symphyomyrtus* species. In addition, the dominant chain length in *Symphyomyrtus* is C_{27} and C_{29} followed by C_{25}, whereas the dominant alkanes in some *Monocalyptus* ranged from C_{23} to C_{29} (e.g. *E. coccifera, E. amygdalina* and *E. nitida*) (Fig.5.2).

Although there were no significant differences in both total alkane or individual homologues between series within *Monocalyptus*, the C_{23} homologues were often higher in *Piperitae* species in comparison to their rare occurrence in *Obliquae* species. However, the total percentage of alkanes clearly distinguished the two series within *Symphyomyrtus*, where *Ovatae* species had significantly higher contents of alkanes, although the distribution of alkane chain lengths did not differ markedly between the series. Alkane contents ranged from 18.3% to 61.6% in waxes of *Ovatae* species and 0.6% to 24.8% in *Viminales* species. The alkanes from *Monocalyptus* species did differ in the distribution of homologues between species. For example, alkanes of *E. radiata* were dominated by C_{29}, *E. obliqua* by C_{27} and *E. nitida* by C_{25} and *E. amygdalina* by similar proportion of C_{23}, C_{25}, C_{27} and C_{29} homologues.

Hydrocarbons are widespread in plant waxes and their complex pattern has provided a potentially valuable taxonomic fingerprint (e.g. Eglington *et al.* 1962; Martin and Juniper 1970; Scora *et al.* 1975) to distinguish species (e.g. Herbin and Robins 1968a; Nordby and Nagy 1977; Faboya *et al.* 1980a,b; Gamou and Kawashima 1981). In many of the studies in which the alkane pattern proved useful either in distinguishing taxa or forming species groupings, they only occasionally correlated with current or accepted botanical classification. In some studies, such alkane data has not been able to discriminate taxa (Salasoo 1983) or differentiate species (Herbin and Robin 1968b; Dyson and Herbin

1970; Corrigan *et al.* 1978). Results of this study indicate that the n-alkane pattern of eucalypt waxes has taxonomic value with the distribution of some individual homologues differentiating between subgenera. In addition, quantitative variation of n-alkanes in waxes permitted discrimination within some species groups in the subgenus *Symphyomyrtus* but not between *Monocalyptus* species.

Some studies have compared wax composition occurring during tissue development. Results often indicated changes in wax composition and homologue profiles of individual compound classes with leaf expansion or maturation, whilst the homologue distribution of individual compound classes usually shifts towards constituents of longer chain length during leaf expansion (e.g. Tulloch 1973; Schutt and Schuck 1973; Schuck 1976).

However, truly ontogenetic variation of leaf waxes has not yet been demonstrated by other studies. Although in *Picea abies* the waxes of 4 and 1 year-old leaves were compared by Schuck (1976), his specimens compounded large differences in physiological age. The study described in this chapter is the first comparison of ontogenetic differences for a large number of species within a genus, since all juvenile and adult leaf samples were collected during the same period and had similar physiological ages. Seasonal and physiological differences were minimised, although obviously differences in growing environments within a locality could not be removed. Results of this study indicate that the changes in homologue distribution of individual compound classes between juvenile and adult waxes occur in *Viminalis* species. Juvenile leaves of many *Viminalis* species are often very glaucous in contrast to adult leaves which have shiny and non-glaucous surfaces.

It is of interest that the change in distribution of alkane homologues in *Viminalis* species with increasing ontogenetic age is a shift towards shorter chain length. This is in contrast to the common change with physiological age which is towards longer chain length (Schuck 1976; Freeman 1979; Baker 1981). This suggests that the alkane chain length might be a factor contributing to contrast in glaucousness between juvenile and adult leaves. The shorter chain alkanes had lower melting point than the longer chain alkanes. This resulted in a concomitant decline in melting point of the adult wax. Therefore, loss of leaf glaucousness in adult leaves may take place in hot weather. In addition, the juvenile leaves of *Viminalis* species tended to have higher wax yields than adult leaves (although not significant), this might also correlate to the contrast in glaucousness.

The significant ontogenetic change in wax composition was restricted to the "blue ashes", *E. delegatensis,* etc. In these species, alkanals or/and n-alkanols were higher in juvenile leaf waxes but lower in adult leaf waxes. It was considered that these compounds were associated with the glaucousness of "blue ashes" species.

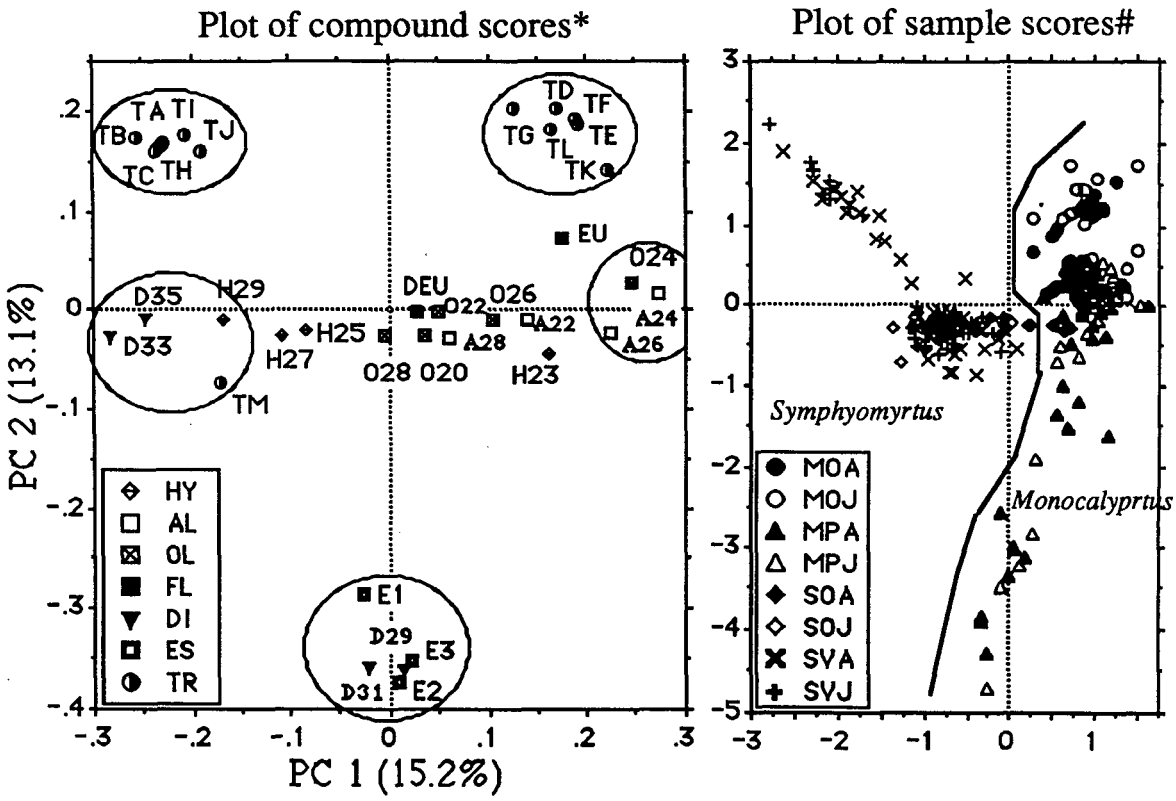
5.2.4. The chemotaxonomy of Tasmanian Eucalyptus species based on the leaf wax data

The principal trends of variation in wax chemicals and classification

The principal trend in chemical variation of both adult and juvenile leaf waxes among populations of the 29 eucalypt species is summarised by PCA using dominant homologues (homologues with even carbon number in alkanals and n-alkanols and homologues with odd carbon number in n-alkanes and ß-diketones) of individual long chain compound classes and all homologues of triterpenoids, flavonoids and esters as shown in Fig. 5.3A and B.

The eigenvalues of the PCA indicated that the first two PCs accounted for 15.2% and 13.1% of the total variance of wax components among samples (Fig. 5.3A. left figure). The variation along PC1 is associated with increasing levels of major alkanals (A24 and A26), n-alkanols (O24) and a group of triterpenoids (TD, TE, TF, TG, TK and TL) and decreasing levels of ß-diketones D33 and D35, major n-alkanes (H29, H27 and H25) and other triterpenoids (TA, TB, TC, TH, TI, TJ and TM). The alkanals A24 and A26 and alcohol O24 were most highly and positively weighted along PC 1 axis while the ß-diketone D33 was most negatively weighted. Variation along PC 2 was associated with increasing levels of triterpenoids and decreasing levels of ester compounds (E1, E2 and E3) and the two ß-diketones, D29 and D31.

The *Monocalyptus* and *Symphyomyrtus* samples are separated in the space defined by the first two PCs (Fig. 5.3A). Most samples of *Monocalyptus* species had higher levels of alkanals, n-alkanols, and a group of triterpenoids, TD, TE, TF, TG, TK and TL, and most samples of *Symphyomyrtus* species had higher levels of ß-diketones D33 and D35 and alkanes and triterpenoids, TA, TB, TC, TH, TI, TJ and TM. There was continuous variation among *Monocalyptus* samples along PC 2 with some samples of *Piperitae* species separated from the main *Monocalyptus* sample group due to increased levels of esters and ß-diketones, D29 and D31. Some samples of *Obliquae* species also separated from the main *Monocalyptus* sample group due to increased levels of triterpenoids. Within *Symphyomyrtus* samples, there was continuous variation among the *Viminales* species with increasing levels of specific triterpenoids along a direction oblique to PC 2. Variation among *Ovatae* species along PC 1 tended toward *Monocalyptus* species. However, variation within both subgenera did not follow the series classification. There was no tendency for juvenile and adult leaves to be separated in this ordination.



Extractions of population scores of individual series on the first two principal components

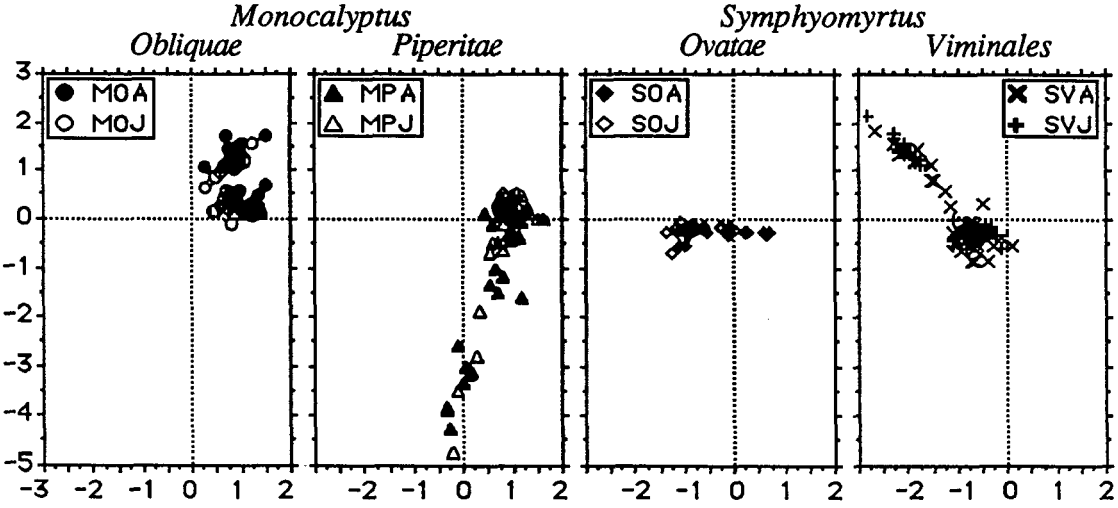
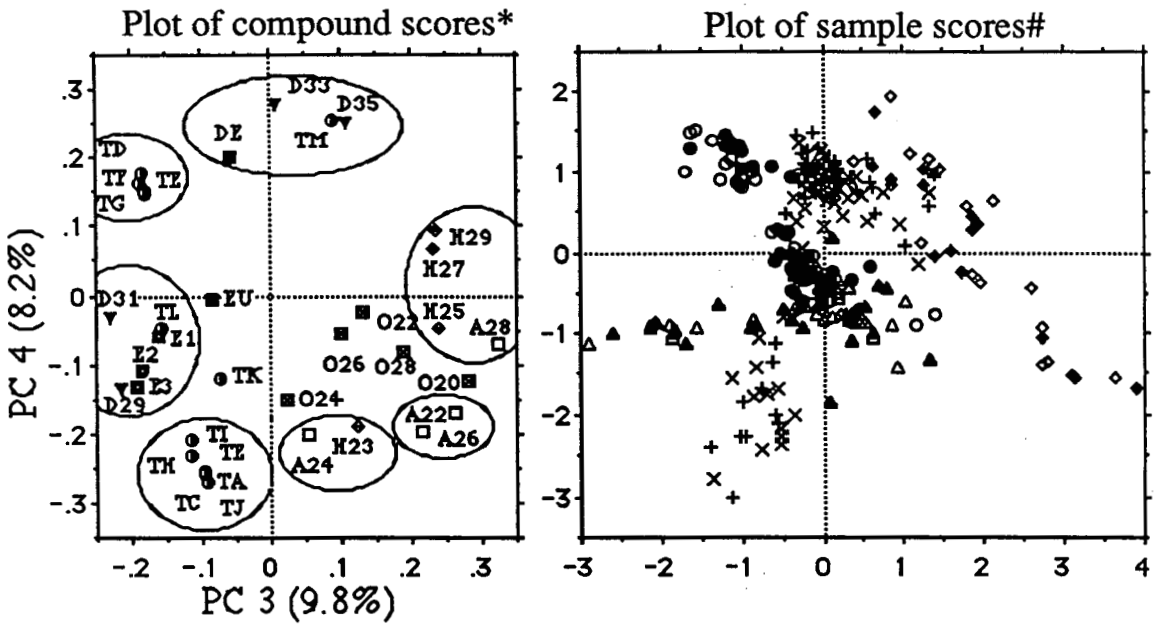


Fig.5.3A. Scatter plot of the wax chemical components on the first two principal components (PC1 and PC2) and scatter plot of juvenile and adult samples of all populations of the 29 eucalypt species on these two principal components.

* Compound scores are indicated by legends of individual compound classes and named by the code of compound class. Individual homologues of each compound class are indicated by using first letter of compound code and carbon-numbers of homologues: HY = hydrocarbon AL = aldehyde OL = alcohol FL = flavonoid ES = ester TR = triterpenoid. For example, hydrocarbon compound n-heptacosanal (C27) is named as 'H27' and aldehyde compound n-hexacosanal as 'A26'. Triterpenoids are named using letters as listed in Table 5.2. For example, TA is triterpenoid A and TB triterpenoid B. Flavonoid compound EU is eucalyptin and DE is demethyl eucalyptin.

Populations scores were indicated by both juvenile and adult leaf samples of individual series as follow:

MOA= *Monocalyptus -Obliquae* -Adult leaves; MOJ= *Monocalyptus -Obliquae* -Juvenile leaves
MPA= *Monocalyptus-Piperitae* -Adult leaves; MPJ= *Monocalyptus-Piperitae-* Juvenile leaves
SOA= *Symphyomyrtus-Ovatae-* Adult leaves; SOJ= *Symphyomyrtus-Ovatae-* Juvenile leaves
SVA= *Symphyomyrtus-Viminales* -Adult leaves; SVJ= *Symphyomyrtus-Viminales-* Juvenile leaves



Extractions of population scores of individual series on the first two principal components

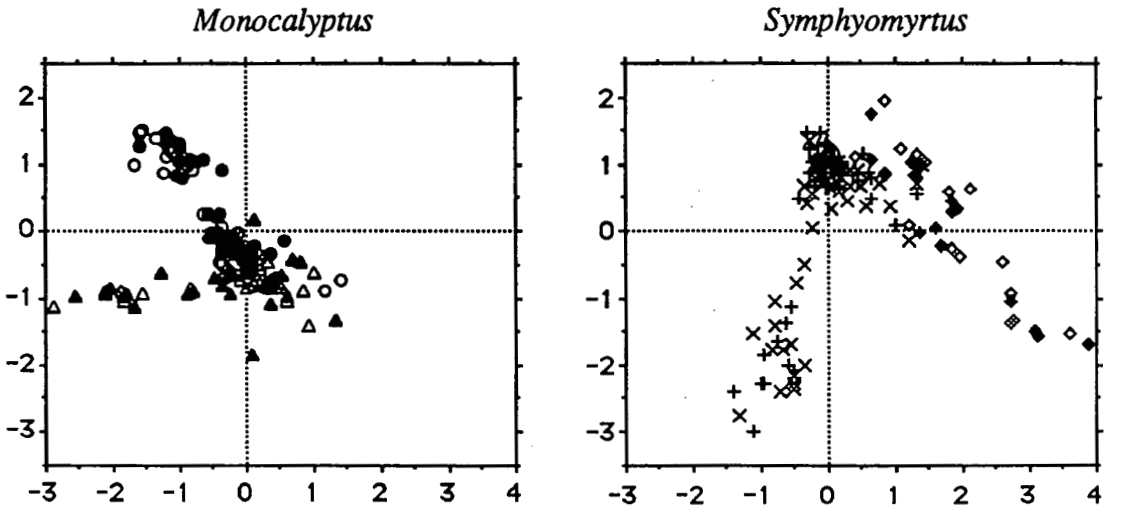


Fig.5.3.B. Scatter plot of the wax chemical components on the third and fourth principal components (PC3 and PC4) and scatter plot of juvenile and adult samples of all populations of 29 eucalypt species on these two principal components.

The majority of *Monocalyptus* and *Symphyomyrtus* species were also partly discriminated in the space defined by third and fourth PCs, which accounted for 9.8% and 8.2% of the total variation (Fig. 5.3B). Plots of populations scores of individual subgenera indicated that there were two independent directions of variation among both *Monocalyptus* and *Symphyomyrtus* populations, which resulted in the complex variation in wax chemicals as indicated in Fig. 5.3B.

In summary, the main trends in wax chemical variation amongst samples are:

i) subgenera can be separated with a combination of the first two PCs, but the variation within subgenera is multidimensional (the first four PCs only accounted for 46% of the total variation);

ii) the variation between the main taxonomic groups appears continuous;

iii) the main direction of variation between subgenera can be summarised by both variation in levels of compound classes and levels of individual compounds within compound classes;

iv) variation in levels of compound classes indicated that:

the majority of *Monocalyptus* samples had higher levels of alkanals and n-alkanols and the majority of *Symphyomyrtus* samples had higher levels of alkanes;

v) variation between compounds within individual compound classes indicated that:

the majority of *Symphyomyrtus* samples had higher levels of ß-diketone compounds with long chain length of C_{33} and C_{35} (D33 and D35) and some samples of the *Piperitae* species had higher levels of ß-diketones with shorter chain length of C_{29} and C_{31} (D29 and D31). In contrast the majority of *Monocalyptus* samples had low levels of ß-diketone compounds. *Symphyomyrtus* species had higher levels of triterpenoids TA, TB, TC, TH, TI, TJ and TM and *Monocalyptus* species had higher levels of triterpenoids TD, TE, TF, TG, TK and TL.

A classification of all populations based on the percentage data of both adult and juvenile leaf samples, is summarised in the dendrogram in Fig. 5.4. This dendrogram, confirms the division between subgenera with the four *E. ovata* populations as outliers. However, clusters within each subgenus did not correspond to the division of species into the series of Pryor and Johnson's classification. Within the *Symphyomyrtus*, those *Vernicosinae* species (yellow gum) and remaining *Ovatae* species (excluding pure *E. ovata* populations), are separated from the majority of other *Viminales* populations, except the green populations of *E. urnigera* and *E. archeri*. Furthermore, *E. viminalis, E. dalrympleana* and *Vim/Dal* populations were grouped into a single cluster and separated from glaucous *Viminales* populations. In contrast, some *Monocalyptus* populations, particularly in the *Obliquae*, showed a stronger clustering of species and greater species differences compared with *Symphyomyrtus* species, although clustering of the *Piperitae* species was poor.

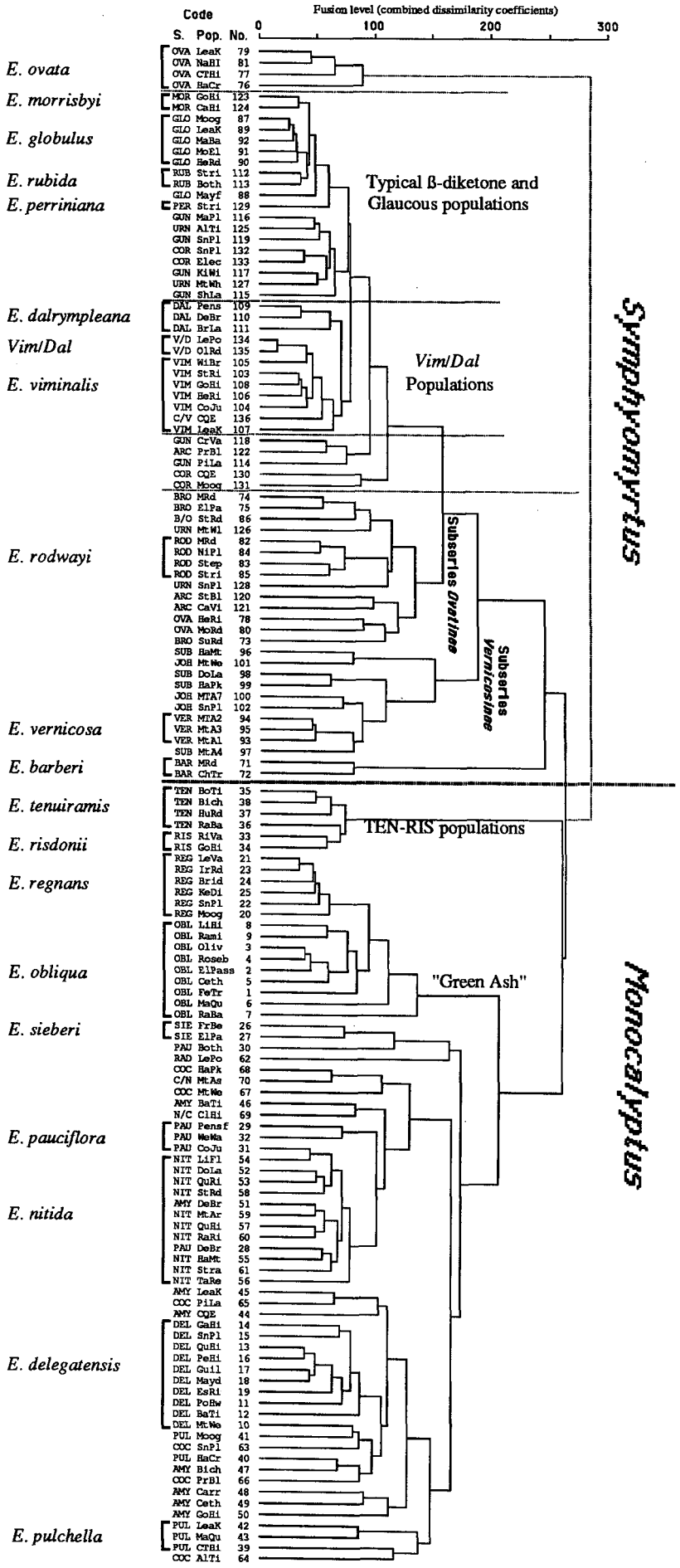


Fig. 5.4. Average linkage clustering of 135 populations of 29 eucalypt species, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

The principal trends of variation in wax chemicals and classification of populations within the subgenus Monocalyptus

Variation in wax chemicals amongst populations of *Monocalyptus* species samples was summarised by PCA. The first two PCs amounted for 21.1% and 17.0% of the total variance among juvenile and adult samples (Fig. 5.5).

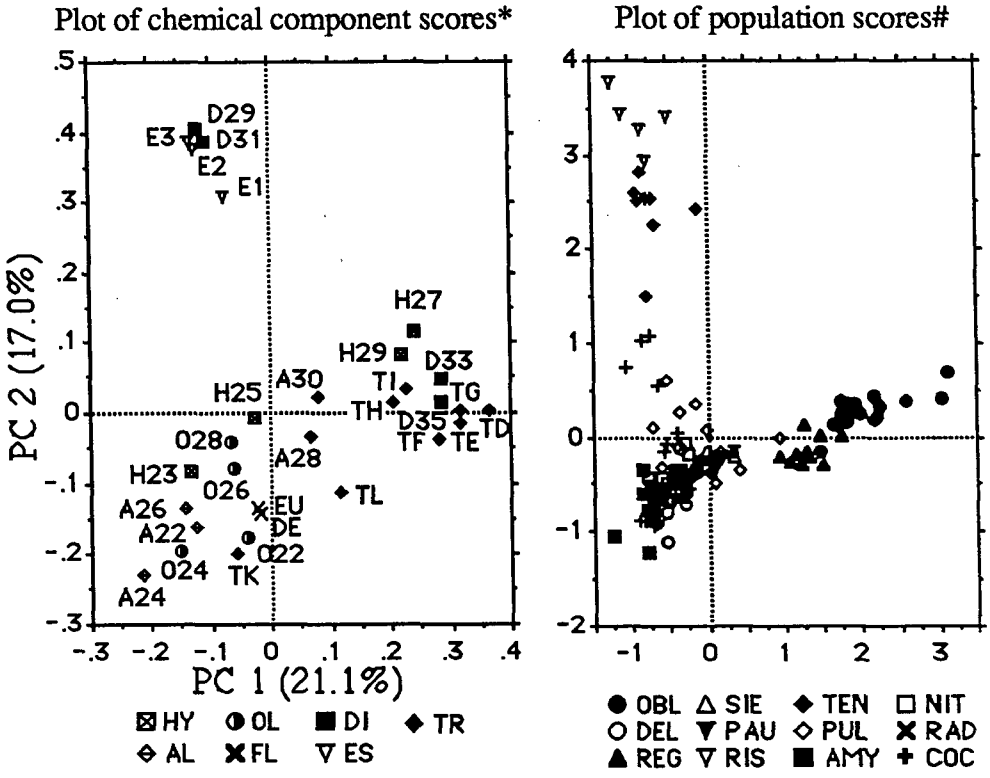


Fig. 5.5. Scatter plot of the wax chemical parameters (left) and samples of the 12 *Monocalyptus* species (right) on the two first principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf waxes.

This analysis indicated that the trends of variation in wax chemicals had two main directions both of which are slightly oblique to PC 1 and PC2 axes respectively (left figure of Fig. 5.5). Variation oblique to PC1 is associated with increasing levels of ß-diketones, D33 and D35, and triterpenoid compounds, TD, TE, TF, TG, TH and TI and decreasing levels of n-alkanols and triterpenoid TK. At the same time, alkanes and alkanals also contributed to variation along this direction with increasing levels of homologues of long chain lengths (H29, H27, A30 and A28) and decreasing levels of homologues of shorter chain lengths. Variation oblique to PC2 is associated with increasing levels of esters (E1, E2 and E3) and ß-diketones, D29 and D31.

Plots of sample scores on the two PCs indicated that variation along the first direction, as described above, was mainly associated with the *Obliquae* samples and variation along the second direction was associated with the *Piperitae* samples (right figure of Fig. 5.5). All *E. obliqua* and *E. regnans* samples were clearly separated from the main sample group due mainly to high levels of ß-diketones, D33 and D35, triterpenoids, TD to TI, and n-alkanes, H27 and H29, along the first direction. All *E. tenuiramis* and *E. risdonii* samples were separated from the main sample group due to high levels of ester compounds and ß-diketones, D29 and D31, along the second direction. However, there was no tendency for the majority of *Obliquae* and *Piperitae* samples to be separated in this ordination.

The classification of all populations within *Monocalyptus* species based on both juvenile and adult wax data shown in Fig. 5.6 indicated that the major dichotomy between chemical clusters was not between groups that could be assigned to the series *Obliquae* and *Piperitae,* but the division of the populations of the two species, *E. tenuiramis* and *E. risdonii,* in which leaf waxes were characterised by high ß-diketone and long chain ester values, from all other populations. The second dichotomy separated the populations of *E. regnans* and *E. obliqua* and the third dichotomy further separated the populations of *E. sieberi* and *E. radiata* and one of the *E. pauciflora* populations from the other clusters. The remaining two clusters did not correspond to the assignment of populations to series and the remaining populations of *E. pauciflora* and *E. delegatensis* were associated with different groups of populations of *Piperitae* species.

The *Obliquae* populations were, with the exception of *E. pauciflora,* one population of *E. delegatensis* (MtWe) and two populations of *E. obliqua* (MaQu and RaBa), grouped well into species clusters (i.e. *E. regnans, E. obliqua, E. sieberi* and *E. delegatensis*). However, apart from the *E. tenuiramis/risdonii* cluster, the grouping of *Piperitae* populations was not consistent with their species assignment. This dendrogram thus indicated that the leaf waxes of the *Obliquae* species had less variation between populations within a species and greater differentiation of the species compared to populations of the *Piperitae* where variation within species was large and species differentiation was poor, except for *E. tenuiramis/risdonii.*

Population ordination and classification with *Obliquae* species

The PCA of *Obliquae* species is given in Appendix 5.2.and the chemical distance between populations of *Obliquae* species is summarised in Fig. 5.7. With the exception of the populations RaBa and MaQu of *E. obliqua,* all populations were clustered in five clear species groups. This partitioning indicates that the leaf waxes of the *Obliquae*

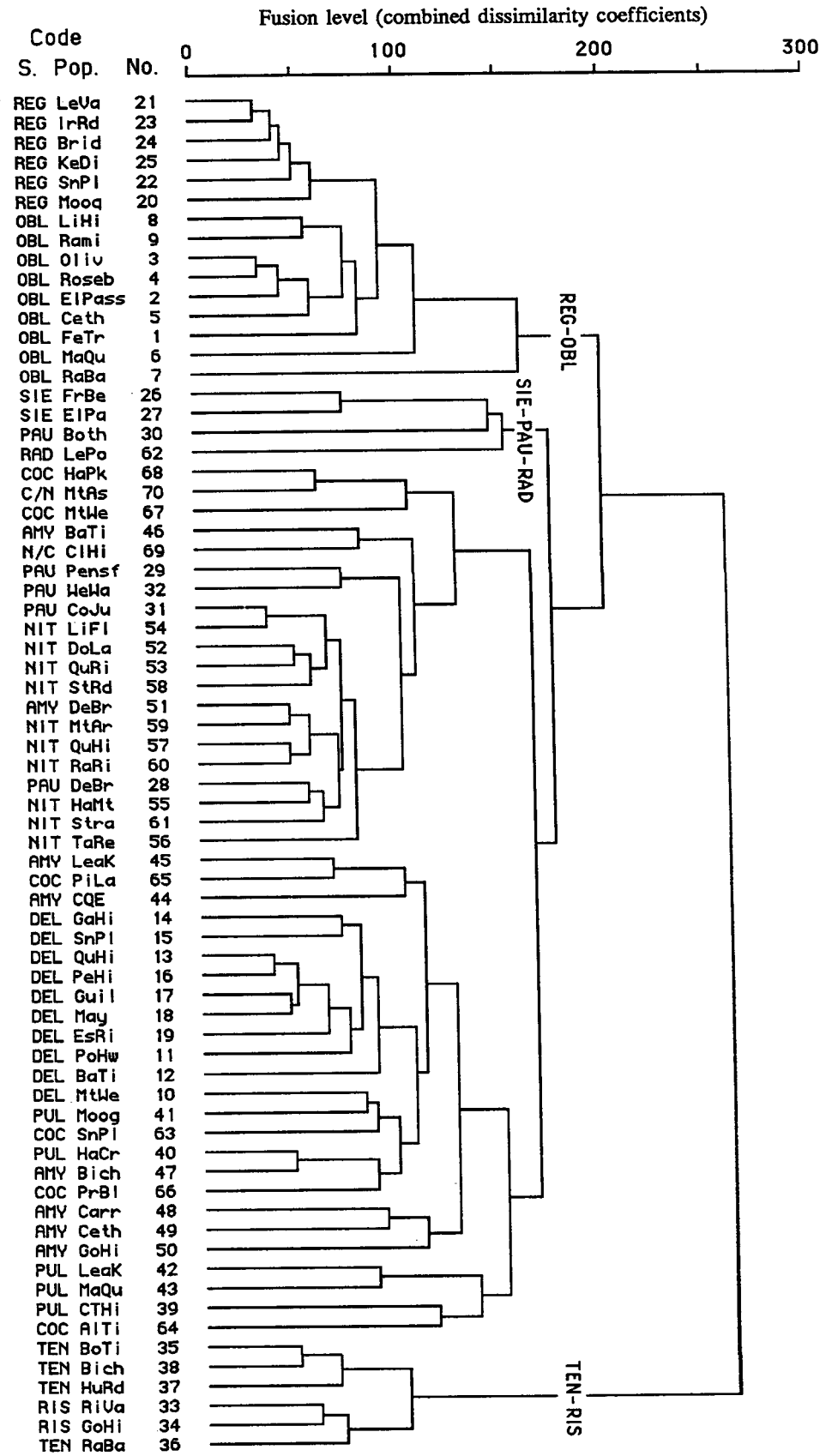


Fig. 5.6. Average linkage clustering of all populations of *Monocalyptus* species, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

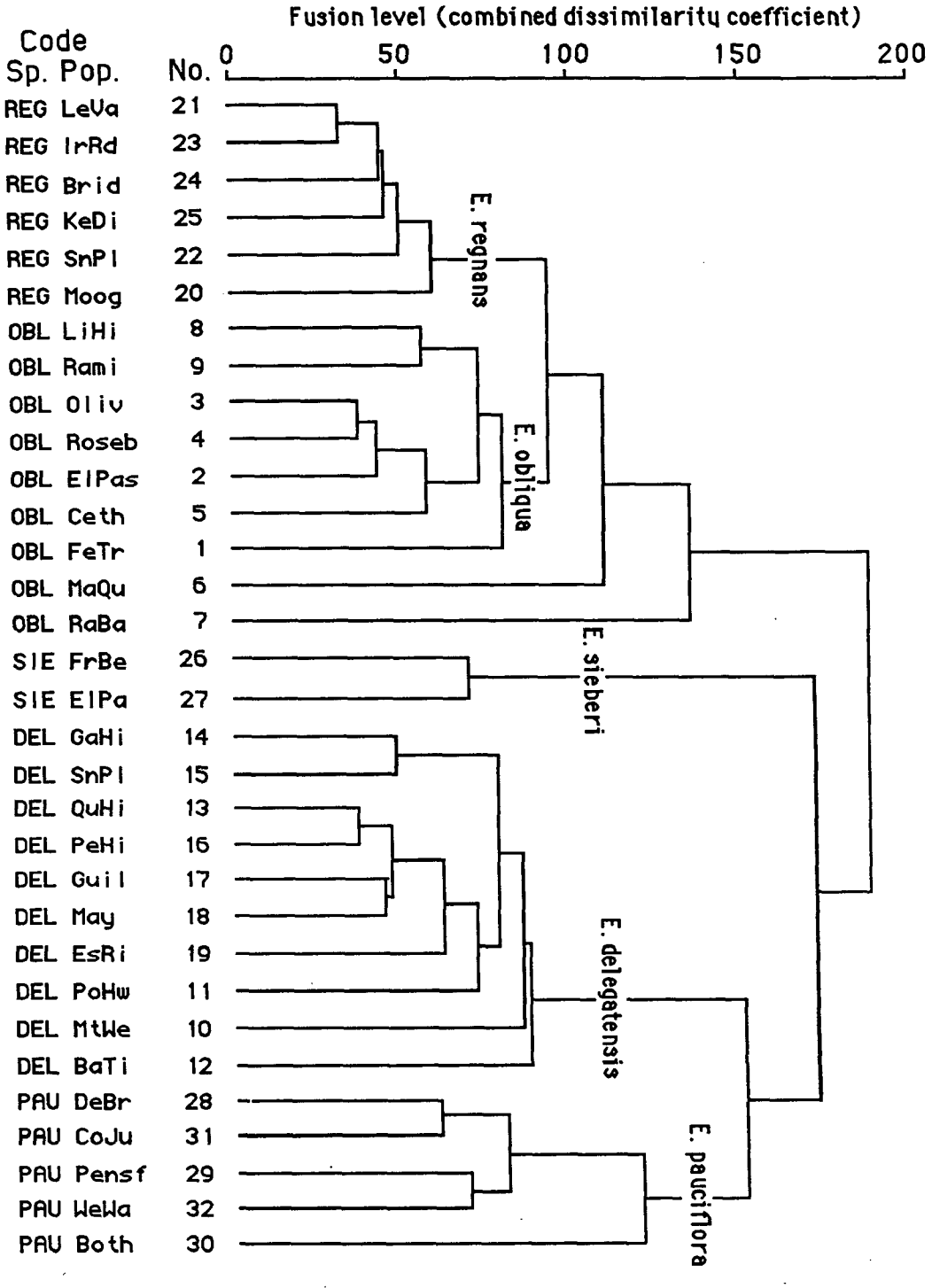


Fig. 5.7. Average linkage clustering of all populations of the series *Obliqua*, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

species were markedly different in composition between species with little variation between populations within species. The major dichotomy occur between populations of "green ashes", *E. regnans* and *E. obliqua*, and those of "blue ashes", *E. sieberi*, *E.*

delegatensis and *E. pauciflora*. The leaf waxes of *E. regnans* and *E. obliqua* are markedly different from the other *Obliquae* species. The fusion level of the clustering of populations also indicated that the difference between these two species was less than the distance between other species. Using this clustering schedule, *E. regnans* and *E. obliqua* have close affinities and of *E. pauciflora* appears to have closer affinities to *E. delegatensis* than to *E. sieberi*.

Population classification within the *Piperitae* series

The main trends of variation in wax chemicals among *Piperitae* species samples on the first two PCs (24.5% and 14.3%) of PCA is shown in Appendix 5.3. The classification of populations of the *Piperitae* species, as indicated in the dendrogram in Fig. 5.8, showed marked intraspecific and interspecific variation. With the exception of the single population of *E. radiata*, the dendrogram indicated three major clusters of populations. These are the *E. tenuiramis* - *E. risdonii*, the *E. coccifera* - *E. amygdalina* - *E. pulchella* and *E. nitida* - *E. amygdalina* - *E. coccifera* population clusters. The major dichotomy (Fig. 5.8) separated the *E. tenuiramis* - *E. risdonii* population cluster and the second dichotomy separated the single population of *E. radiata* from populations of the other species. Thus, populations of these three species differed markedly from other species. Populations of *E. nitida* and *E. pulchella* also differed markedly and no population of these two species transgressed the third major dichotomy. However, populations classified as *E. amygdalina* and *E. coccifera* on morphological criteria were separated into two different specific population clusters. The populations of these two species varied toward either *E. nitida* or *E. pulchella*. All populations of *E. nitida* occurred within a single cluster with a relatively low level of fusion. Hence, the species *E. nitida* has less intra-specific variation in wax composition than the other three species.

E. nitida is mainly confined to south-western and western Tasmania, whereas *E. amygdalina* has a predominantly eastern distribution (Shaw *et al.* 1984). The populations classified as *E. amygdalina* from Derwent Bridge (DeBr) is in the geographical transition zone between *E. amygdalina* and *E. nitida* and the classification in Fig. 5.8 suggests that this population has closer affinities to *E. nitida*. Similarly the population classified as *E. amygdalina* from Bakers Tier (BaTi) is near the centre of Tasmania and appears to deviate toward *E. nitida* in its wax chemistry. The populations (COC HaPk, C/N MtAs, N/C ClHi) with morphological affinities toward *E. coccifera* which classify closer to the *E. nitida* cluster are associated with altitudinal transitions between *E. nitida* and *E. coccifera* (Shaw *et al.* 1984). These populations were the most 'coccifera-like' phenotypes found at the culmination of altitudinal cline in *E. nitida* in the vicinity of Cradle Mountain (HaPk) and Mt. Arrowsmith (MtAs).

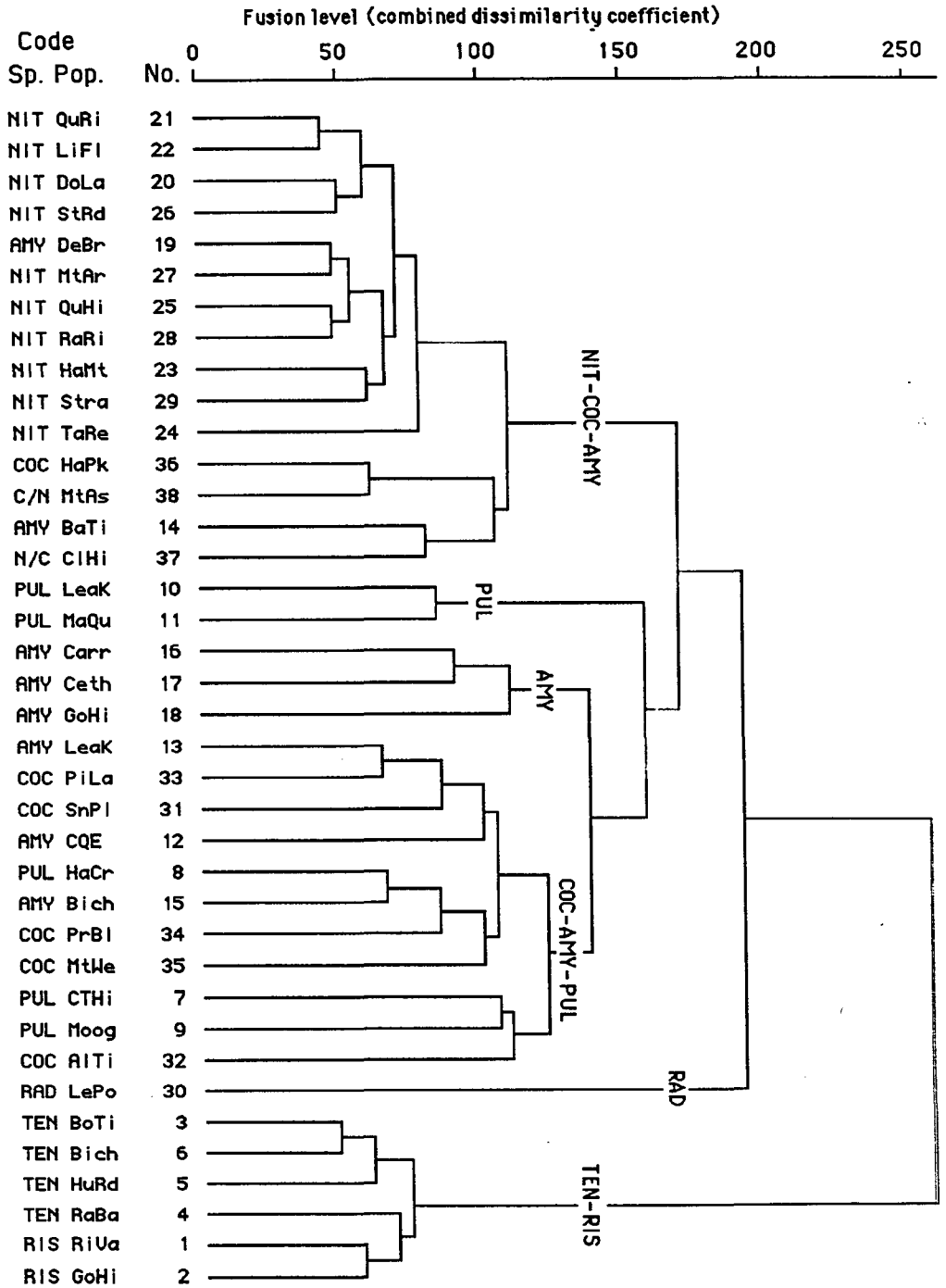


Fig. 5.8. Average linkage clustering of all populations of *Piperitae* species, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

This dendrogram (Fig. 5.8) indicated that wax chemical affinities of *Piperitae* populations were related to morphological variation within and between species, and particularly between species *E. amygdalina, E. pulchella, E. nitida* and *E. coccifera* (see Fig. 2.1 in Chapter 2). There was clinal intergradation between *E. amygdalina* and *E. pulchella. E. amygdalina* also intergrades into a specialised form of *E. nitida* while another cline occurs between *E. nitida* and *E. coccifera.* However, there was no clinal intergradation or hybridisation between *E. nitida* and *E. pulchella.* In addition, clinal intergradation also exists between *E. tenuiramis* and *E. risdonii.* These two species and *E. radiata* have very similar wax chemistry quite distinct from other *Piperitae* species in Tasmania. The chemical variation in leaf waxes between populations of these two species was less than that within any other species in the series *Piperitae* with the exception of *E. nitida.* The close chemical affinity between *E. tenuiramis* and *E. risdonii* waxes thus supports the suggestion of Wiltshire *et al.* (1991) who considered that these two species could be classified as a single species.

The atypical population from Alma Tier (COC AlTi) which appears morphologically intermediate between *E. coccifera* and *E. tenuiramis* (Wiltshire *et al.* 1991), clearly does not have close affinities to *E. tenuiramis* on wax chemistry and clusters with the *E. coccifera/pulchella/amygdalina* group. *E. pulchella* and *E. amygdalina* tend to converge in morphology toward the east coast of Tasmania, but these two species were poorly differentiated and highly variable in wax chemistry making the detection of any such trends difficult.

The principal trends of variation in wax chemicals and classification of populations within the subgenus Symphyomyrtus

The main trends in variation in wax chemicals among both juvenile and adult samples of all 29 eucalypt species was summarised by PCA and are shown in Fig. 5.9A. The eigenvalues of the PCA indicated that the first two PCs accounted for 20.1% and 17.6% of the total variance of wax compounds among populations (Fig. 5.9A). There were two major trends of variation in the space defined by the first two PCs, but in directions which were oblique to either axis (see top figure of Fig. 5.9A). One trend was along PC 2 in a direction positively oblique to PC 1 with increasing levels of compounds of hydrocarbon, aldehyde and n-alkanols and decreasing levels of ß-diketone, flavonoid and ester compounds. The two major ß-diketones, D33 and D35, were most negatively weighted along this direction and the alkanals and an alcohol, O20, were most positively weighted. The second trend was along PC 2 in a direction negatively oblique to PC 1 with increasing levels of triterpenoids and decreasing levels of ß-diketones.

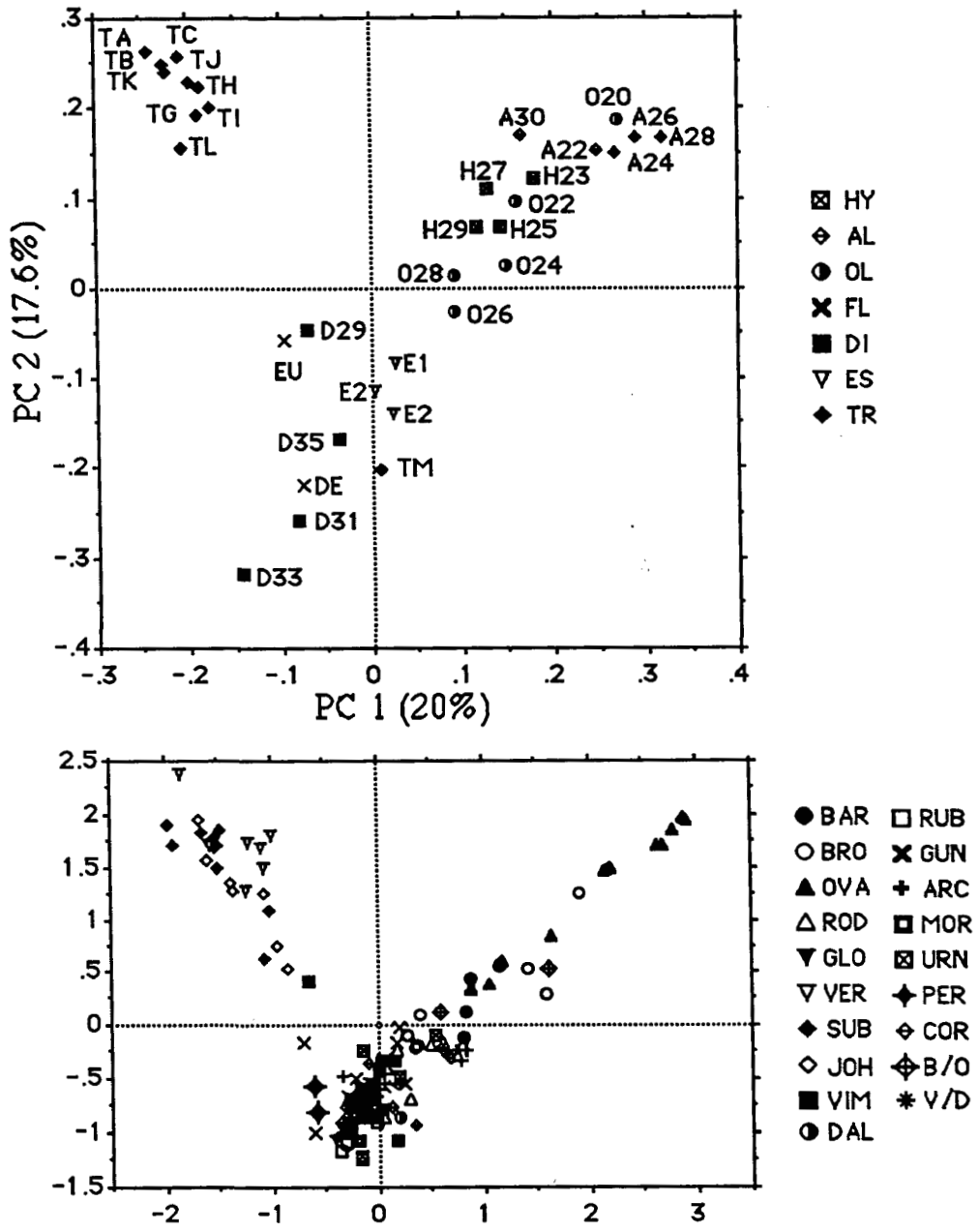


Fig. 5.9.A. Scatter plot of the wax chemical components (top) and samples of the 17 *Symphyomyrtus* species (bottom) on the two first principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf waxes.

* Compound scores are indicated as same with that in Fig. 5.3.

Juvenile and adult leaf sample scores of individual species were indicated by species code as listed in Table 4.1.

Variation along the first direction among samples was continuous and mainly associated with samples of *Ovatae* series (black gum group) and some of the *Viminales* samples with increasing levels of alkanals, n-alkanes and n-alkanols and decreasing levels of ß-diketones (bottom figure of Fig. 5.9A). The majority of *E. ovata* samples had the highest positive values along this direction. Variation along the second direction was relatively discontinuous and all samples of yellow gum were clearly separated from samples of

other species due to the high levels of triterpenoids and low levels of ß-diketones. However, the majority of *Symphyomyrtus* species samples had high levels of ß-diketones and grouped together, with the exception of one *E. viminalis* sample.

Plots of wax compound scores on the first and third (9.9%) PCs of PCA (Fig. 5.9.B) indicated that the variation along PC 3 was mainly associated with increasing levels of major n-alkanes and decreasing levels of other compounds. There was continuous variation among leaf samples along PC 3 and the sample of *E. barberi* had the highest levels of n-alkanes. Some samples of *E. rodwayi, E. brookeriana, E. urnigera* and *E. cordata* varied toward *E. barberi.*

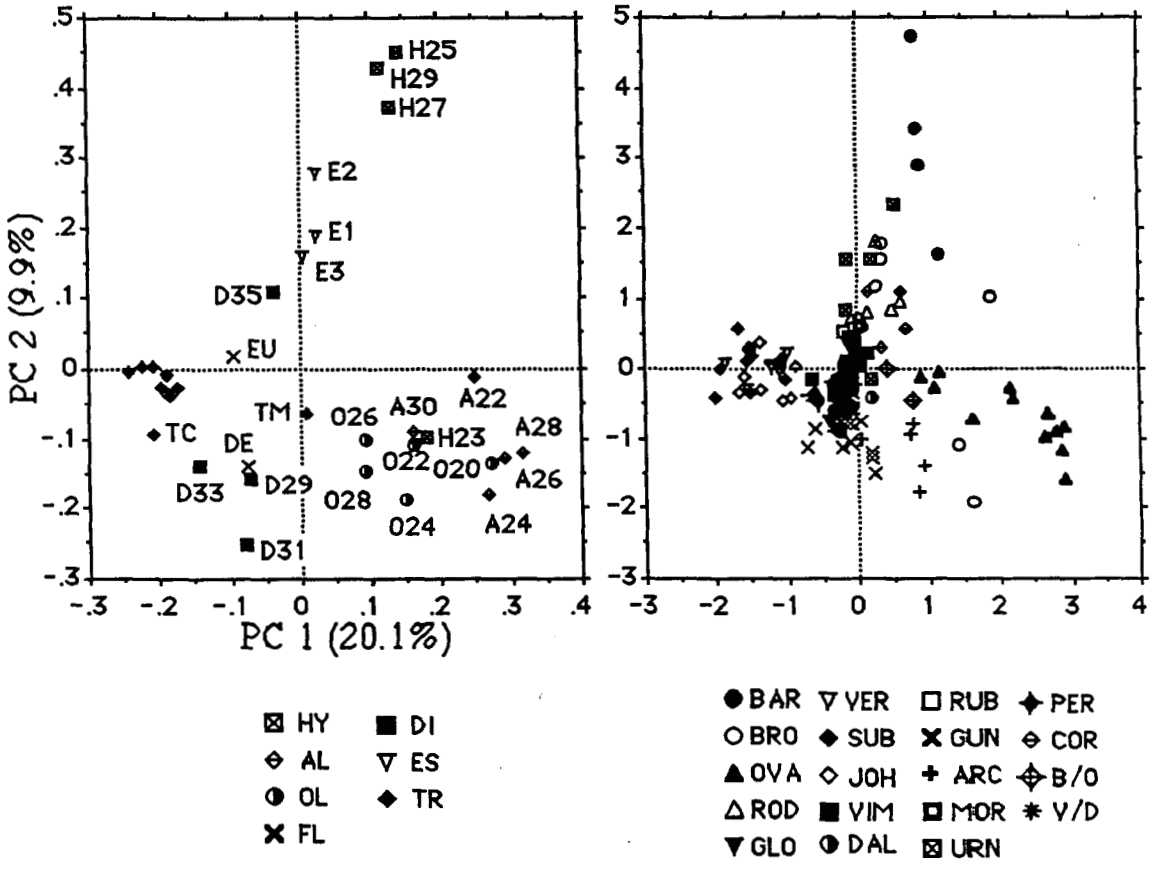


Fig. 5.9B. Scatter plot of the wax chemical components (left) and samples of the 17 *Symphyomyrtus* species (right) on the first and third principal components (PC1 and PC2) derived from the analysis of juvenile and adult leaf waxes.

The chemical distance between populations of all *Symphyomyrtus* species is summarised in Figure 5.10. This dendrogram does not correspond to the separation of the species

into the two series of Pryor and Johnson (1971) but relates to glaucousness levels of species. This classification separated all *Ovatae*, which had higher levels of long chain n-alkanes, alkanals or n-alkanols, and *Vernicosinae* species, which had high levels of triterpenoids, from the main cluster of *Viminales* species. The two green populations of *E. urnigera* and the two populations of *E. archeri*, an alpine white gum species, separated from glaucous populations of alpine white gums and fell into the cluster of black gum populations, which was mainly composed of *E. rodwayi, E. brookeriana* and west coast *E. ovata.*

Within the main cluster of white, alpine white and blue gum populations, the two glaucous populations of *E. cordata* (CQE and Moog) and the subglaucous populations of *E. gunnii* (CrVa and PiLa) and E. archeri (PrBl) were separated by the first and second dichotomies. The remaining populations were further separated into two clusters: one is green and green/subglaucous cluster, which includes all populations of *E. viminalis, E. dalrympleana* and clinal forms between these two species, Vim/*Dal*; another is a pure glaucous population cluster, which included all glaucous populations of white, alpine white and blue gum populations, with the exception of two glaucous populations of *E. cordata* (CQE and Moog). Furthermore, all three populations of *E. dalrympleana,* which are green/subglaucous, were discriminated from green populations of *E. viminalis, Vim/Dal* and a hybrid between *E. cordata* and *E. viminalis* (C/V CQE). This dendrogram thus indicated that the two *Vim/Dal* populations had a closer chemical affinity to *E. viminalis* than to *E. dalrympleana.*

This dendrogram also indicated that the variation between species and between localities within species for the *Ovatae* (black gum) species is much greater than that for *Viminales* species, since the fusion levels between localities classified as within *E. ovata* is greater than fusion levels of all other species groups.

Overall, variation in leaf wax chemicals of *Symphyomyrtus* species can be summarised as:

i) Variation in wax components did not follow the division of species series but did correspond to the glaucousness level.

ii) The majority of white, alpine white and blue gum populations can be described as a typical ß-diketone group. Within this group, the glaucous, subglaucous, green and green/subglaucous population could be further separated by wax chemicals.

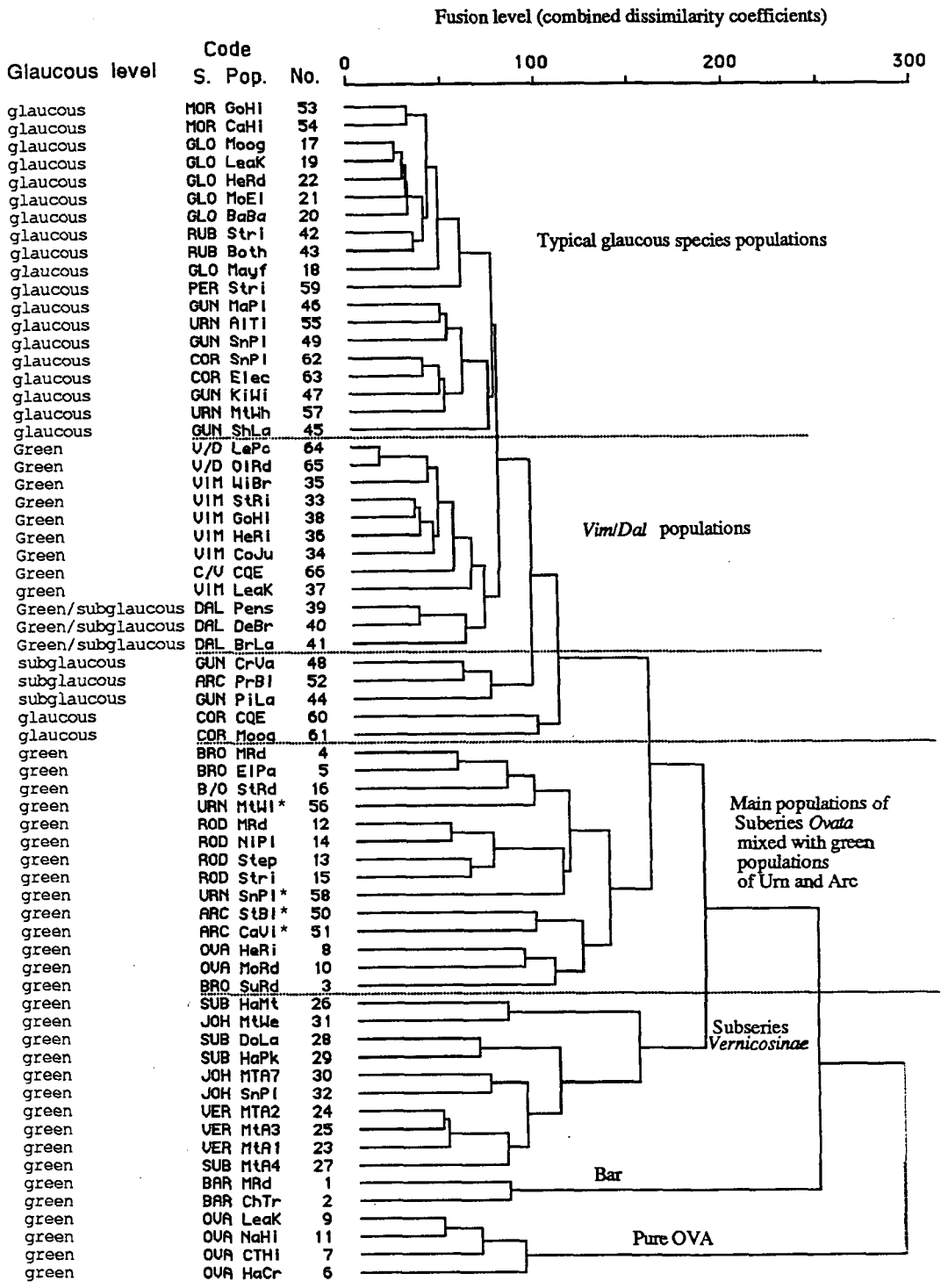


Fig. 5.10. Average linkage clustering of all populations of *Symphyomyrtus* species, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

iii) All populations of yellow gum can be described as a typical triterpenoid green group which is well differentiated from other *Viminales* populations.

iv) All populations of black gum and some green populations of alpine white gum can be described as an atypical wax green group. Within this group, the waxes of *E. barberi* populations have the highest levels of alkanes and the majority of *E. ovata* populations have high levels of alkanals. Variation between populations of this group and the typical ß-diketone group is continuous.

v) The green populations among *Symphyomyrtus* species could be described as of three types: ß-diketone wax type of the *E. viminalis* and *Vim/Dal* populations, triterpenoid wax type of the yellow gums and, an atypical wax type of *E. ovata* and *E. barberi*.

vi) green variates of normally glaucous *Viminales* species, *E. urnigera and E. archeri*, have wax composition closer to green *Ovatae* species, *E. brookeriana* and *E. rodwayi*.

Population classification within *Ovatae* series

A PCA analysis of variation within black gum (series *Ovatae*) species is given in Appendix 5.4. The classification of *Ovatae* populations is shown in Figure 5.11. The average linkage clustering of these populations confirmed a large difference between a group of eastern *E. ovata* populations, *E. barberi* populations and the remaining populations. There was relatively little variation within *E. barberi* and *E. rodwayi* whereas *E. ovata* was extremely variable. Two populations HeRi and MoRd, classified as *E. ovata,* differed markedly from the main group of *E. ovata* populations and had affinities to the population SuRd classified as *E. brookeriana*. The dendrogram also indicated that the morphologically intermediate population between *E. brookeriana* and *E. ovata* (B/O StRd) was closer to *E. brookeriana* than to *E. ovata* on wax composition.

There is some confusion as to whether some populations on the west coast are a 'west coast' variant of *E. ovata* (Jackson 1960) or *E. brookeriana*. This analysis indicates that there is a marked difference between west coast *E. ovata* (e.g. HeRi) and east coast forms and that the populations on the west coast (SuRd and MoRd), classified in the field as *E. ovata*, have closer affinities to *E. brookeriana and E. rodwayi*.

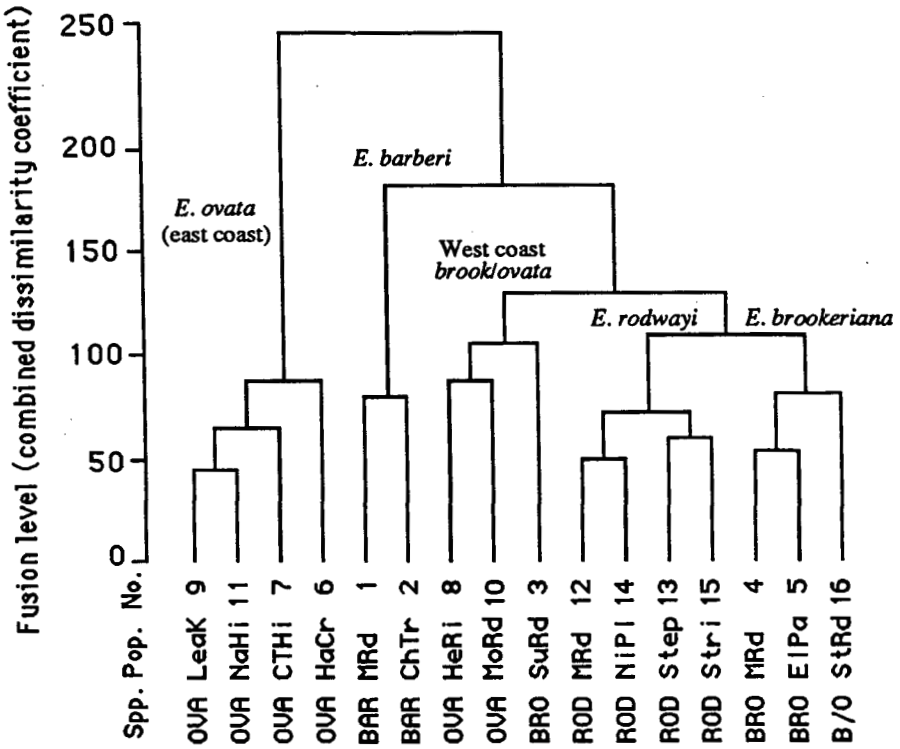


Fig. 5.11. Average linkage clustering of all populations of *Ovatae* species, using percentage data of all components of both adult and juvenile leaf waxes of individual populations.

Interspecific chemical distance

The chemical distance among the 29 species, as estimated from the leaf waxes, are summarised in Figure 5.12. This dendrogram is derived from the average-linkage clustering of the mean percentage composition of all wax components of both adult and juvenile leaves of individual species using unstandardised and unweighted data. The association of leaf wax compounds supports the major morphological division of the genus. With the exception of the two species *E. risdonii* and *E. tenuiramis* which were outliers, the close affinities that exist between certain species reflects the separation of *Monocalyptus* and *Symphyomyrtus* species. However, the interspecific structure within individual subgenera does not follow the division into series. Both *E. ovata* and *E. barberi* of the *Ovatae* series were very different from species of the *Viminales* series but the other two *Ovatae* species, *E. brookeriana* and *E. rodwayi*, were grouped with the two *Viminales* species, *E. urnigera* and *E. archeri*, and formed a single cluster which was intermediate between the main cluster of *Viminales* and the *E. ovata-E. barberi* cluster. The dendrogram also indicated less variation among yellow gum species and their

marked difference from other *Viminales* species. *Monocalyptus* species did not show any interspecific pattern associated with the separation of species into series.

The average-linkage clustering for the standardised and weighted data of both adult and juvenile leaf samples is shown in Figure 5.13. This analysis weights characters in a manner which emphasises characters which show little intraspecific variation and large differences between species. This dendrogram indicated that the two cluster pairs of the *Monocalyptus* species, *E. risdonii* and *E. tenuiramis* of the *Piperitae* and *E. obliqua* and *E. regnans* of the *Obliquae*, and the two *Symphyomyrtus* species (*E. ovata* and *E. barberi* of *Ovatae*) are outliers from all other species. The remaining species were clearly divided into the two subgenera. The interspecific relation of the remaining *Monocalyptus* species does not follow any morphological taxonomic affinities, however, the interspecific distance amongst *Symphyomyrtus* species did relate to glaucousness of species. Within the main cluster of *Symphyomyrtus* species, all glaucous, subglaucous, green/subglaucous and one green species, *E. viminalis*, which are typical ß-diketone species, formed the cluster core. The other species gradually became increasing dissimilar from the core with decreasing ß-diketone content through the species *E. urnigera* and *E. archeri*, which contain glaucous, subglaucous and green localities, then to *Ovatae* and *Vernicosinae* species, which are almost green. *E. archeri*, which was placed in a series *Subbexertae* with the black gum species by Blakely (1956) but was subsequently placed in the series *Viminales* (Pryor and Johnson 1972, Chippendale 1988), shows an intermediate position between black gum and other *Viminales* species. However, eastern samples of this species have wax composition closer to *E. rodwayi* and *E. brookeriana* (Fig. 5.11).

A dendrogram was also constructed using Ward's clustering method with standardised and weighted data (Fig. 5.14). This technique is particularly powerful in partitioning continuous variation into equitable units (Sneath and Sokal 1973). The classification from this analysis most closely followed the current taxonomy. The *Monocalyptus* and *Symphyomyrtus* species were clearly separated at the first dichotomy. With the *Symphyomyrtus* species, the black gum species were all grouped together as were the yellow gum species while the white, blue and alpine white gums formed another cluster in which ß-diketones dominated. White, blue and alpine white gum species were further separated into two subgroups: a pure glaucous species group and mixed group of glaucous, subglaucous, green/subglaucous and green species. However, *Monocalyptus* species did not show such interspecific relationships which relate to the series classification.

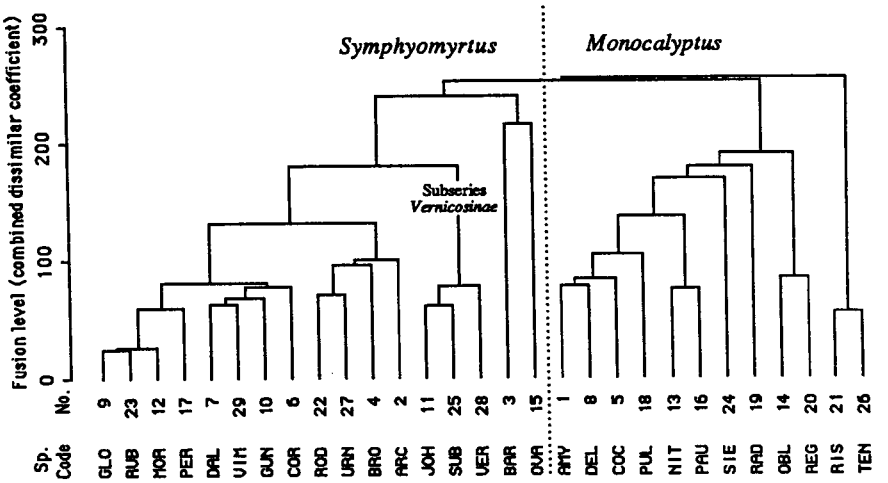


Fig. 5.12. Chemotaxonomic representation of average linkage clustering 29 *Eucalyptus* species, using mean percentage of all wax components of both adult and juvenile leaf waxes of individual species (unstandarized and unweighted).

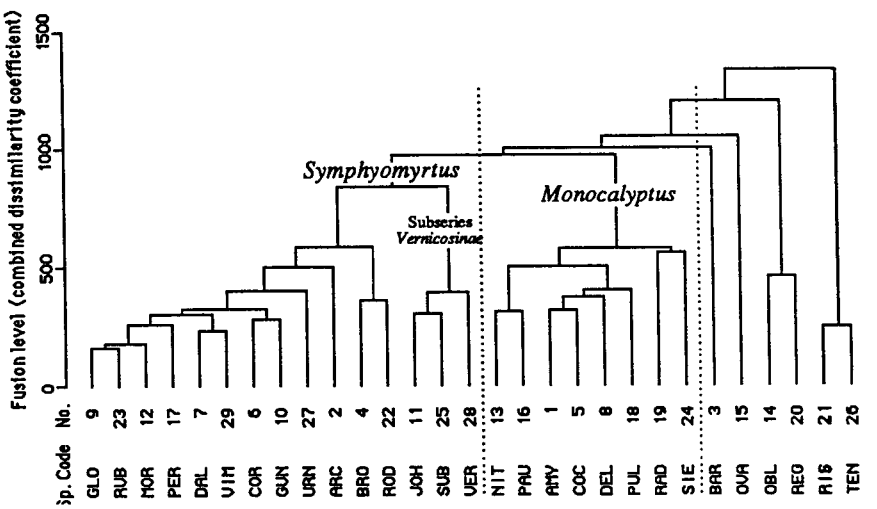


Fig. 5.13. Chemotaxonomic representation of average linkage clustering 29 *Eucalyptus* species, using mean percentage of all wax components of both adult and juvenile leaf waxes of individual species (standarized and *F*-weighted).

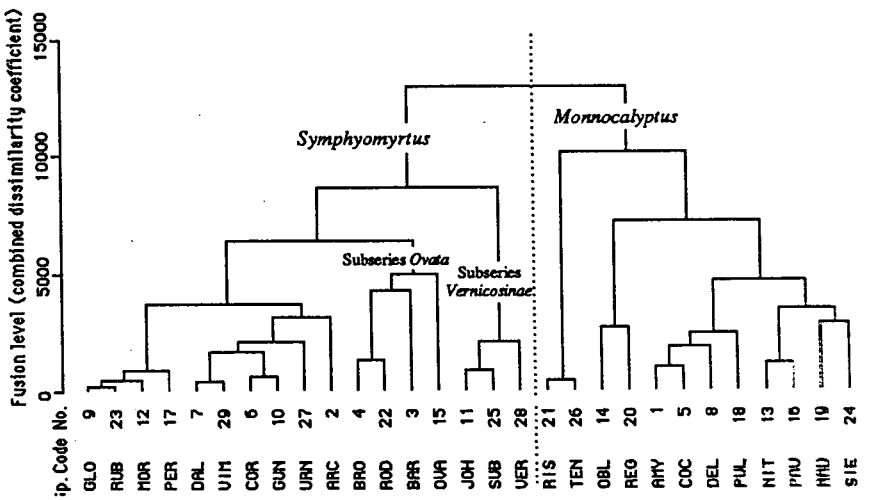


Fig. 5.14. Chemotaxonomic representation of clustering using the Ward Method for 29 *Eucalyptus* species, based on the mean percentage of all wax components of both adult and juvenile leaf waxes of individual species (standarized and *F*-weighted).

In agreement with the classification based on populations, the dendrograms for the above species further indicated that wax composition differs between subgenera while the affinity between wax chemicals within *Symphyomyrtus* species is correlated to leaf colour. Wax composition did not separate *Monocalyptus* species into taxonomic species groups (except *E. risdonii/tenuiramis* and *E. obliqua/E. regnans*), however, important affinities between some species have been indicated. The close affinity between *E. tenuiramis* and *E. risdonii* was further confirmed, since the difference between these two species was less than differences between any other *Monocalyptus* species in all three species dendrograms. In addition, these dendrograms also indicated that wax chemicals of *E. obliqua* were most similar to *E. regnans*. This is different to the chemical affinity of leaf oils where among *Obliquae* species, *E. obliqua* was closest to *E. delegatensis* (Fig. 4.10 in chapter 4).

Part B

5.3. Wax morphology

5.3.1. Materials and methods

Wax morphology of the fresh leaves used for chemical analysis was examined. Leaf pieces, which were stored at 4 ^{0}C before use, were fixed to 1 cm diameter aluminium stubs using double-sided adhesive tape. The specimens were sputter-coated with 25-30nm of gold in an argon atmosphere using a Balzers Union sputtering device. The coated leaf piece was examined directly in a Philips 505 scanning electron microscope at 80 KV and photographed using Ilford FP4 film.

Primary experiments indicated that the wax type remains the same in young and mature leaves, although the distribution and density of wax may vary. Tube wax was present in large amounts on young and newly mature leaves and plate wax had high density in newly mature and mature leaves. Wax crystals generally attained their mature form and had highest density at about the same time as leaves reached maturity, when wax structure was most identifiable. In this experiment, therefore, wherever possible, newly mature leaves were chosen for examination.

Comparison between the adaxial and abaxial surfaces of leaf petioles indicated no difference in wax type although its distribution and density was variable in some cases. Hence, the wax type of individual samples could be characterised by a single surface. In this study, the wax distribution and densities are those of abaxial surfaces unless otherwise indicated.

Populations (approximately one-third of the total populations sampled) selected for wax morphological examination are indicated in Table 4.1. Although the specimens of major localities of each species have been examined and photographed, relatively few of them have been reproduced in this thesis to avoid unnecessary repetition and volume.

5.3.2. Results

Two distinct types of wax were observed on the adaxial and abaxial surface of leaves:

(1) Structured wax, in which the wax projected above the cuticle surface in well-defined forms. Two basic types of structured wax patterns were recognised following the description of Hallam and Chambers (1970):

i) tube wax (15 species within both subgenera) was often branched and horizontally oriented on the cuticle surface. Most tube waxes had well-defined roundly tubular form (typical tube wax) (Fig.5.15A) with measurable diameter and length. In some cases, tube wax had a flat thread-like tube or had variable diameter and with complicated branches and knots (Fig.5.15B).

ii) plate wax (11 species within both subgenera) consisted of erect structures which varied in size and form of edge, such as sinuate, crenate or digitate (Fig.5.15C and D), and

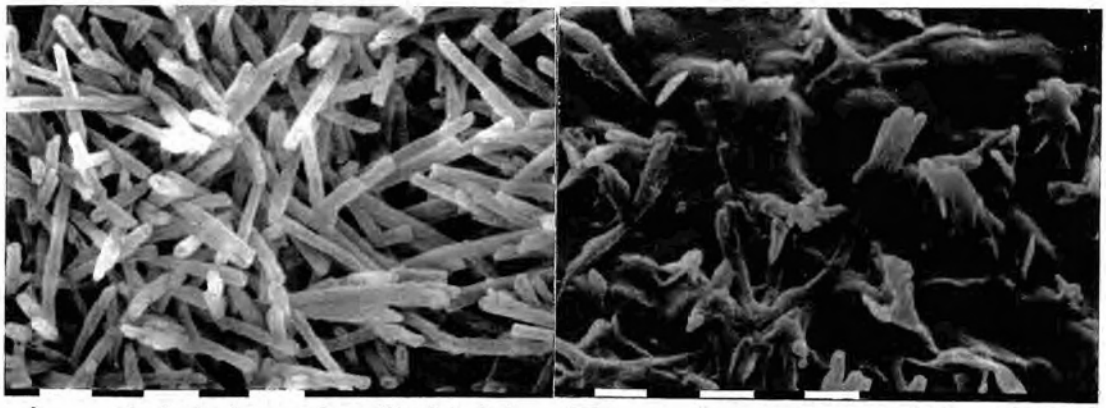
iii) an intermediate form of both tube and plate waxes was found in some locality populations of one species (Fig. 5.15E).

(2) Amorphous wax, a less uniform, featureless wax film deposited over the cuticle or as irregular-shaped blocks of wax laying on the leaf surface (Fig. 5.16D and 5.17N).

Wax distribution over the leaf surface is variable. With few exceptions, leaves of most species had a well-developed structured wax present, at varying density, on the adaxial and abaxial surfaces. The wax may completely cover the leaf surface to obscure the underlying cuticle, or be present as an open cover with the cuticle showing between the wax tubes or plates (Fig.5.15F), or sparse where the wax is present as solitary plates or tubes. In some species, the plate or tube wax was confined to particular areas of the guard cells or the lid cell of oil glands (Fig.5.15G and H).

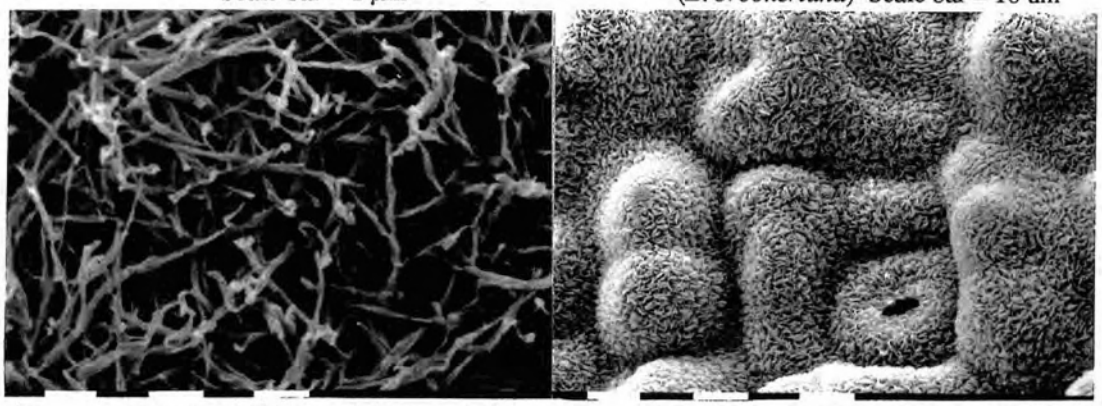
The principal features relating to the occurrence and distribution of wax types in the Tasmanian eucalypt species are shown in Table 5.8. The arbitrary measure of wax types and density of cover was made following the terms used by Hallam and Chambers (1970). Figures 5.16 - 5.17 contain representative micrographs showing the varieties of wax patterns present in each series of both subgenera.

Figure 5.15. Wax types and their distributions in the *Eucalyptus*.



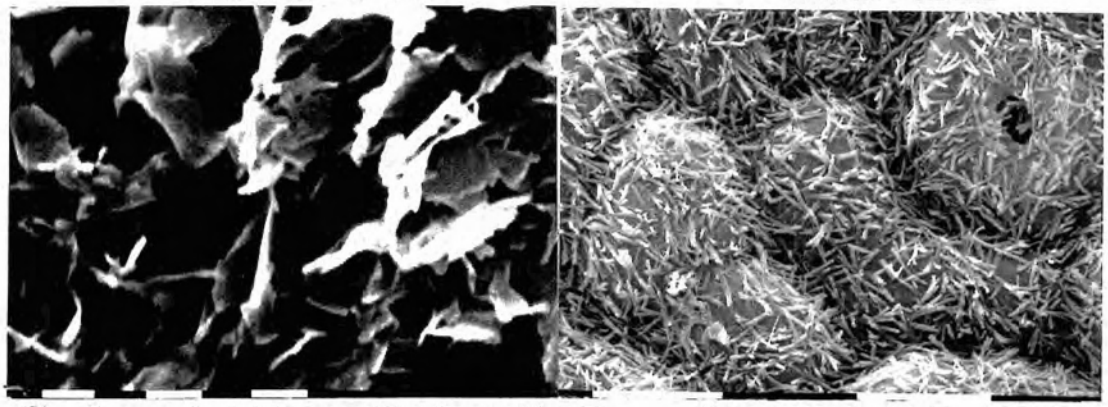
A Typical tube wax form (*E. globulus*) Scale bar = 1 μm

E Intermediate form of tube and plate (*E. brookeriana*) Scale bar = 10 um



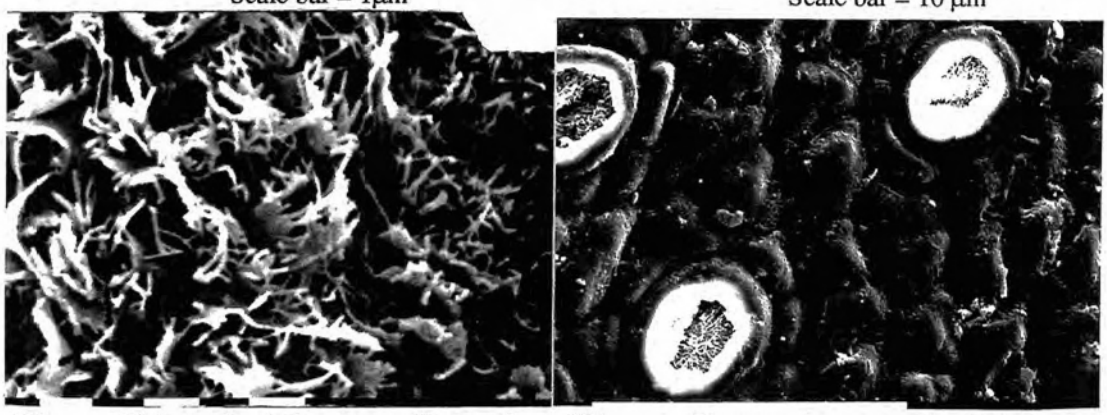
B Flat-thread liked tubes (*E. rodwayi*) Scale bar =10 mm

F Complete cover of plates (*E. coccifera*) Scale bar = 0.1 mm



C Crenate edged plate waxes (*E. delegatensis*) Scale bar = 1μm

G Open cover of tubes (*E. viminalis*) Scale bar = 10 μm



D Digitate edged plate waxes (*E. ovata*) Scale bar = 1 μm

H Plate wax in guard cells (*E. obliqua*) Scale bar = 10 μm

Irrespective of which basic botanical classification is favoured, the wax morphological data of Table 5.8 indicates that there is considerable diversity within Tasmanian eucalypts concerning the type and distribution of leaf surface waxes. Two of the basic structural wax types recognised are represented in both *Monocalyptus* and *Symphyomyrtus* species. Plate waxes were the dominant forms in *Monocalyptus* species and tube waxes dominant in the *Symphyomyrtus*.

Table 5.8. Wax type and distribution in 29 species of Tasmanian *Eucalyptus*.
(a) Subgenus *Monocalyptus*

	Leaf type	Structural wax form	Cover density	Comment
Series *Obliquae*				
E. regnans	juvenile adult	sinuate to crenate edged plates same as juvenile	very low very low	a thick amorphous wax film covered the leaf surface and plate waxes were mainly associated with guard cells
E. obliqua	juvenile adult	sinuate to crenate edged plates same as juvenile	very low very low	a thick amorphous wax film covered the leaf surface and plate waxes were mainly associated with guard cells
E. delegatensis	juvenile adult	sinuate to crenate edged plates same as juvenile	high open to low	
E. sieberi	juvenile adult	sinuate to crenate edged plates same as juvenile	high open to low	
E. pauciflora	juvenile adult	sinuate edged plates same as juvenile	high open to low	
Series *Piperitae*				
E. radiata	juvenile adult	sinuate to crenate edged plates same as juvenile	high high	
E. amygdalina	juvenile adult	sinuate to crenate edged plate same as juvenile	high open	
E. pulchella	Juvenile adult	sinuate to crenate edged plates or mixture of plate and tube	low or high low or high	populations with only plates had a thin amorphous wax film covered the leaf surface and plate waxes were associated with guard cells; populations with a mixture of wax had high cover density
E. nitida	juvenile adult	sinuate to crenate edged plate same as juvenile	high open to low	Adult leaves often had a thick amorphous wax film and plate waxes were mainly associated with guard cells
E. coccifera	juvenile adult	sinuate to crenate edged plate sinuate to crenate edged plate or tube	high high to open	Wax types of adult leaves varied between localities
E. risdonii	juvenile adult*	long and simple tubes same as juvenile	high high	tube overlapping the leaf surface
E. tenuiramis	juvenile adult	long tubes with rarely branching same as juvenile	high high	tube overlapping the leaf surface

* Normally homoplastic species, adult refers to the ontogenetically juvenile leaf type expressed at reproductive maturity (Wiltshire *et al.* 1991).

(b) Subgenus *Symphyomyrtus*

	Leaf type	Structural wax form	Cover density	Comment
Series *Ovatae*				
E. ovata	juvenile adult	crenate to digitate edged plates same as juvenile	high low	
E. brookeriana	juvenile adult	tube or intermediate form of tube/plate same as juvenile	high open to low	a thick amorphous wax film covered the leaf surface; sparse structured waxes occurred a thick amorphous wax film covered the leaf surface; structured waxes
E. rodwayi	juvenile adult	flat-tread like and typical tubes same as juvenile	high low	flat-tread like tubes dominate
E. barberi	juvenile adult	no structure waxes present same as juvenile		a thick amorphous wax film covered the leaf surface and amorphous granular waxes speared
Series Viminales				
E. viminalis	juvenile adult	tubes with acute angled branching same as juvenile	high or open low	A thick amorphous wax film covered the adult leaf surface and tubes mainly associated with guard cells
E. darympleana	juvenile adult	tubes with acute angled branching same as juvenile	high to open open to low	A thick amorphous wax film covered the adult leaf surface and sparse tubes or tubes associated with guard cells
E. rubida	juvenile adult	tubes with acute angled branching same as juvenile	high high or open	
E. johnstonii	juvenile adult	tubes with acute angled branching same as juvenile	low low	A thick amorphous wax film covered the leaf surface and tubes mainly associated with guard cells
E. subcrenulata	juvenile adult	tubes with acute angled branching same as juvenile	low low	A thick amorphous wax film covered the leaf surface and tubes mainly associated with guard cells
E. vernicosa	juvenile adult	tubes with acute angled branching same as juvenile	low low	A thick amorphous wax film covered the leaf surface and tubes mainly associated with guard cells
E. globulus	juvenile adult	tubes with acute angled branching same as juvenile	high low	A thick amorphous wax film covered the adult leaf surface and tubes mainly associated with guard cells
E. cordata	juvenile adult*	tubes with acute angled branching same as juvenile	high high	
E. gunnii	juvenile adult	tubes with acute angled branching same as juvenile	high high to low	
E. archeri	juvenile adult	flat-thread like and typical tubes same as juvenile	high to open low	flat-thread like tubes dominate in green phenotype and typical tube dominate in subglaucous phenotype
E. urnigera	juvenile adult	tubes with acute angled branching same as juvenile	high to low open to low	leaves of green phenotype were mainly covered by an amorphous wax film and rarely tubes
E. perriniana	juvenile adult†	tubes with acute angled branching same as juvenile	high high	
E. morrisbyi	juvenile adult	tubes with acute angled branching same as juvenile	high high	

* normally homoplastic species, adult refers to the ontogenetically juvenile leaf type expressed at reproductive maturity (Potts 1989).
† normally homoplastic species, however, truly adult leaves were found and sampled in this study.

Wax morphology within Monocalyptus

In the subgenus *Monocalyptus*, the series *Obliquae* is consistent in its wax type and may be divided into two groups. The first group, which includes the three "blue ashes" *E. delegatensis, E. sieberi* and *E. pauciflora,* has sinuate to entire-edged plates forming an almost complete cover over the juvenile leaf surface. The becomes an open cover or sparse on adult leaves (Fig. 5.16C; 5.16A, B and C). The second group, which includes the two "green ashes", *E. regnans and E. obliqua*, possessed a thick amorphous wax layer covering the leaf surface with a few plates on the amorphous wax layer or associated with stomata or guard cells of both young and adult leaves (Fig. 5.15H; Fig.5.16D and E). The above groups correlated well with groups based on wax chemical data and indicate a relationship between wax composition and morphology.

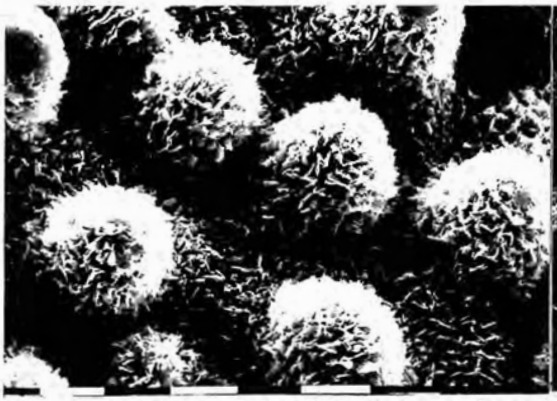
No tube waxes were found on juvenile and adult leaves of *E. regnans* of different localities, even though leaves of different ages were examined. This finding is inconsistent to that of Hallam and Chambers (1970) who identified both plate and tube waxes from *E. regnans*. The morphological characteristics of waxes among localities of each species within *Obliquae* series were very stable. Samples from several ecologically different locations varying in altitude (see Table 4.1) showed no significant change in wax type and distribution. This agrees with the findings of Hallam and Chambers (1970) who found that there was no change in wax type of *E. pauciflora* sampled from different localities.

Leaves of *Piperitae* species may be classified into three groups according to wax type: a tube wax group including only *E. risdonii* and *E. tenuiramis*, a plate wax group including *E. radiata, E. amygdalina* and *E. nitida* and a variable tube/plate wax group including only two species, *E. coccifera* and *E. pulchella.*

Both juvenile and adult leaves of *E. risdonii* and *E. tenuiramis,* which were clearly separated from other *Piperitae* species by wax chemicals due to their high ß-diketone contents, have overlapping simple and long tube waxes covering the surface (5.16F, G and H). The tubes of these two species were comparatively longer (>3 μm) than those in *E. globulus* (2-3 μm), particularly those of *E. risdonii* (usually over 10 μm).

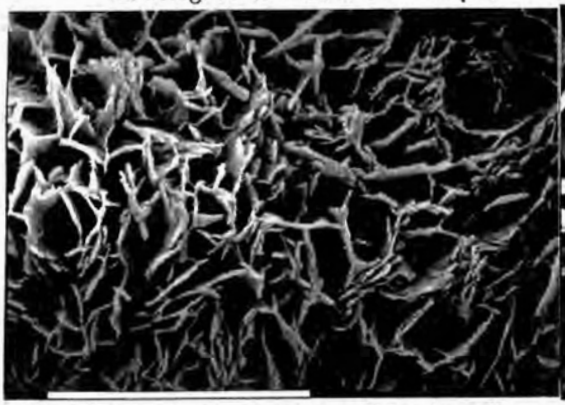
Within the plate wax group, the species *E. radiata, E. amygdalina* and *E. nitida* have identical sinuate to crenate edged plate waxes, which are presented at an angle to the leaf surface forming an almost complete cover on both juvenile and adult leaves (Fig.5.16I, J and K). However, in some localities of *E. nitida*, the adult leaves have either a low density of plate waxes or plate waxes associated with guard cells.

Figure 5.16A-H. Wax types and their distributions in the subgenus *Monocalyptus*. Part 1.

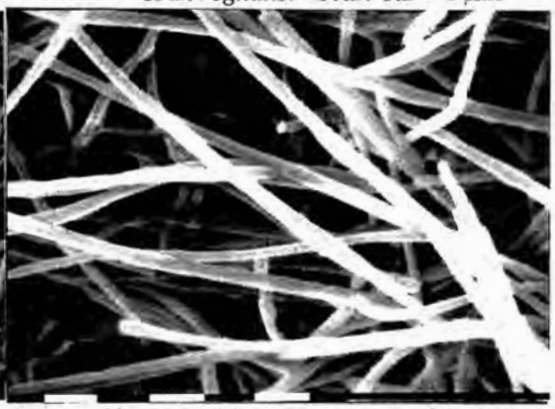


A Plate waxes covering juvenile leaf surface of *E. delegatensis*. Scale bar = 10 μm

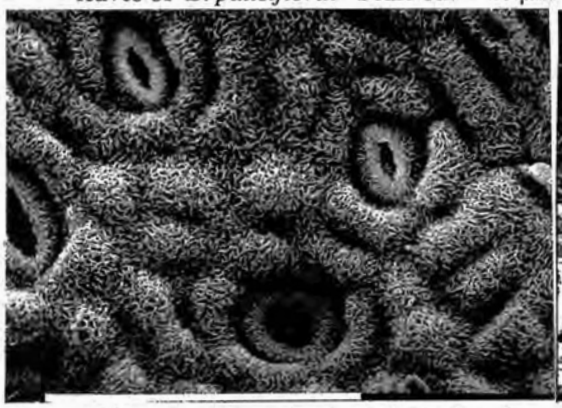
E Sparse plate waxes on amorphous wax film of *E. regnans*. Scale bar = 1 μm



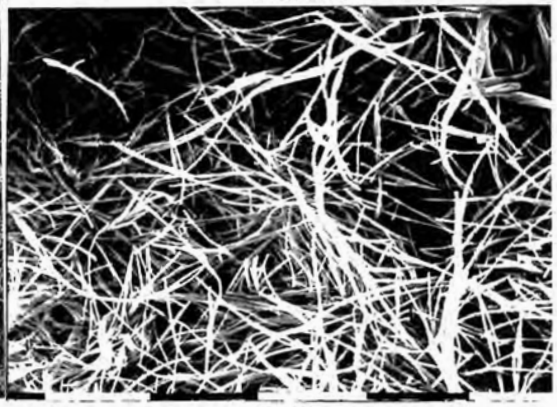
B Crenate edged plate waxes on juvenile leaves of *E. pauciflora*. Scale bar = 10 μm



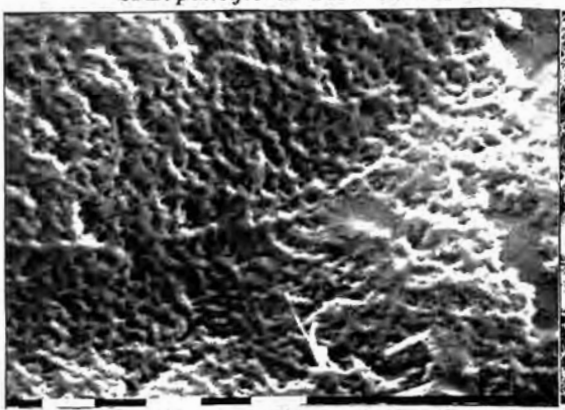
F Simple and long tube waxes of *E. risdonii* Scale bar = 1um



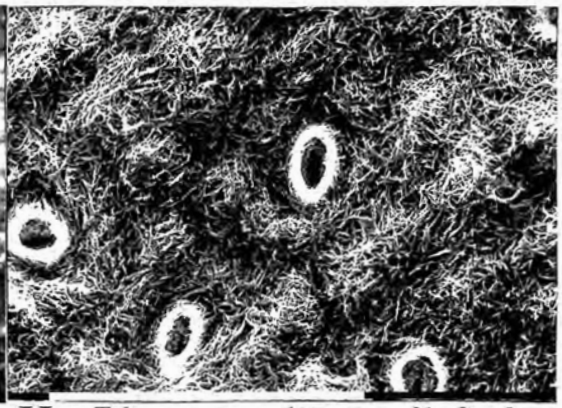
C Plate waxes covering juvenile leaf surface of *E. pauciflora*. Scale bar = 0.1mm



G Overlapping tuble waxes covering leaf surface of *E. risdonii*. Scale bar = 10 um

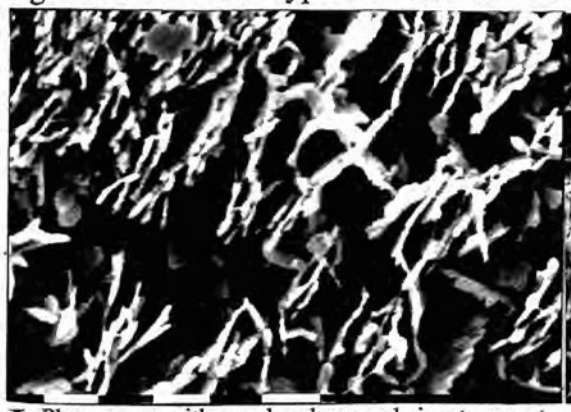


D Amorphous wax film on leaf surface of *E. regnans*. Scale bar = 1μm

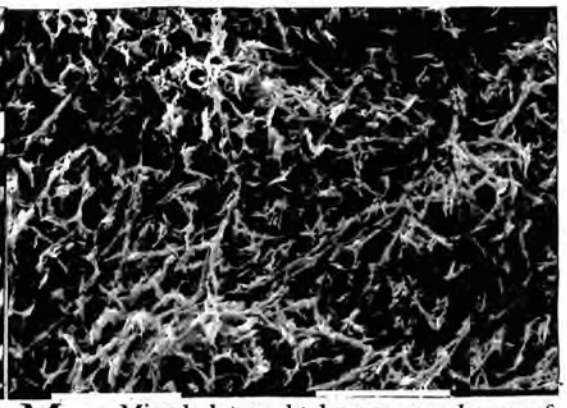


H Tube waxes complete cover of leaf surface of *E. tenuiramis*. Scale bar = 10 μm

Figure 5.16I-P. Wax types and their distributions in the subgenus *Monocalyptus*. Part 2.

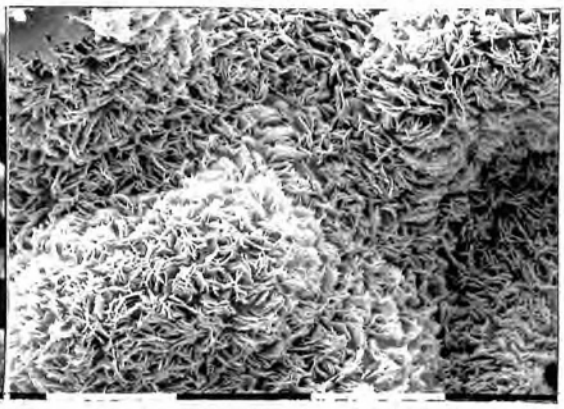


I Plate waxes with angular shape and sinuate-crenate edged on leaves of *E. radiata*. Scale bar=1 µm

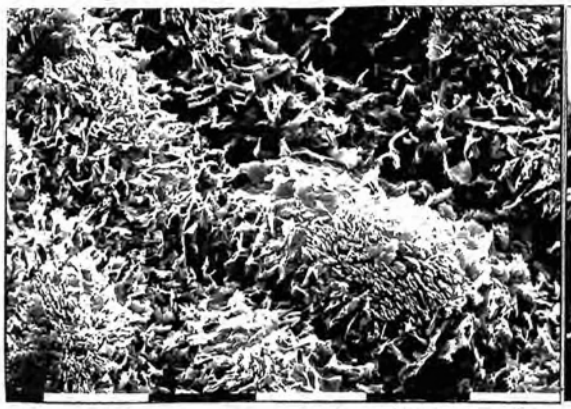


M Mixed plate and tube waxes on leaves of *E. pulchella*. Scale bar = 10 µm

J Plate waxes with angular shape and sinuate-crenate edged on leaves of *E. nitida*. Scale bar=1 µm

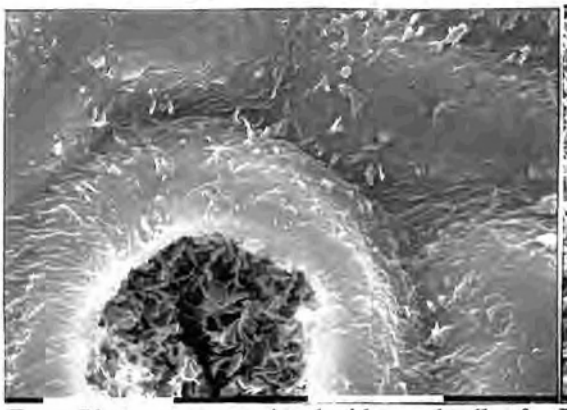


N Plate waxes with sinuate edged on juvenile leaves of *E. coccifera* (MtWe). Scale bar =10 µm

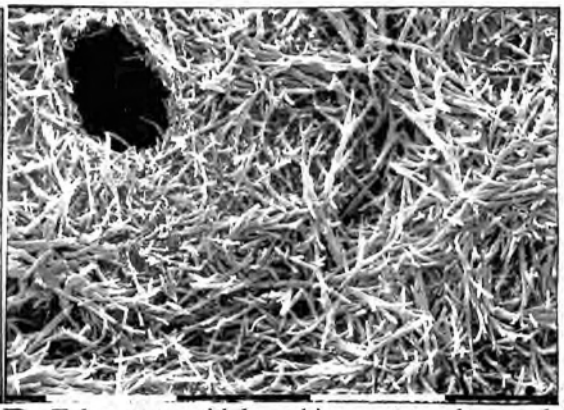


K Density of plates on the juvenile leaf surface of *E. amygdalina* . Scale bar = 10 µm

O Angular and crenate edged plate waxes on adult leaves of *E. coccifera* . Scale bar = 1um



L Plate waxes associated with guard cells of juvenile leaf of *E. pulchella*. Scale bar = 1um



P Tube waxes with branching acute angles on adult leaves of *E. coccifera* (MtWe). Scale bar = 10 µm

In the variable plate/tube wax group, the variation in wax types showed different variation patterns in *E. pulchella* and *E. coccifera*. *E. pulchella* had a parallel change of wax type in both juvenile and adult leaves between localities. However, wax type was changed between juvenile and adult leaves within some populations of *E. coccifera*.

Both juvenile and adult leaf surfaces of some populations (Leak, MaQu) of *E. pulchella* were mainly covered by an amorphous wax film with few plates which are most often associated with stomata or guard cells (Fig.5.16L). However, the east coast populations (e.g. CTHi) had a mixture of tube and plate waxes with high cover density on both juvenile and adult leaf surfaces (Fig. 5.16M). The juvenile leaves of all *E. coccifera* populations had identical plate waxes but adult leaves varied from plates to tubes depending on locality.

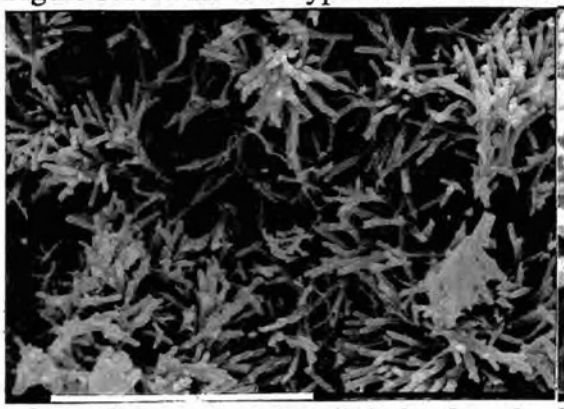
All juvenile leaves of *E. coccifera* examined had plate waxes with sinuate to entire-edges forming an almost complete cover (Fig. 5.15F; Fig. 5.16 N). Most adult leaves of *E. coccifera* populations had plate waxes similar with that of *E. amygdalina* (Fig. 5.16O) However, adult leaves of *E. coccifera* from Mt Wellington (MtWe) had tube waxes with rare branching forming an almost complete cover over the leaf surface (Fig. 5.16P), while Hansens' Peak (HaPk) population had irregular tube waxes with complex branching forming an open cover. The presence of tube waxes in adult leaves from these two localities correlated with their high contents of ß-diketones. In contrast, adult leaves of other localities rarely contained ß-diketones and had plate waxes similar to those on juvenile leaves.

Wax morphology within Symphyomyrtus

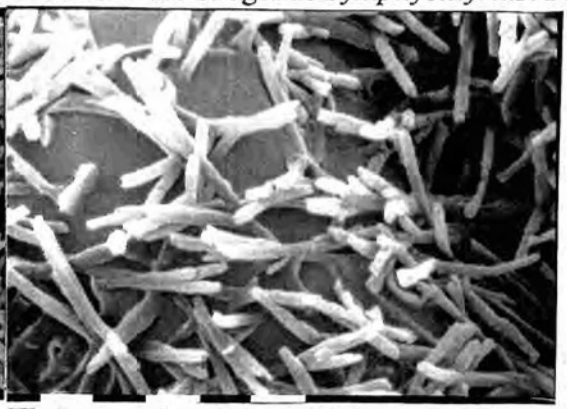
In *Symphyomyrtus*, the series *Viminales* formed a natural group with tube waxes, whereas wax type varied between species of the series *Ovatae*.

Within the series *Viminales*, all glaucous and subglaucous juvenile leaf samples from blue, white or alpine white gum species populations had tube waxes with acute angled branching forming an almost complete cover (Fig.5.15A; Fig. 17A, B and C). The adult leaves of *E. perriniana, E. cordata, E. rubida* and *E. morrisbyi* had cover densities similar to those on juvenile leaves. Those species having glaucous juvenile leaves and non-glaucous adult leaves showed a marked difference between juvenile and adult leaf samples. The adult leaf surfaces of *E. globulus* were mainly covered by a thick wax film, with tube waxes sparse or associated with guard cells, in contrast to the high cover densities of tube waxes on juvenile leaves (Fig.5.17D).

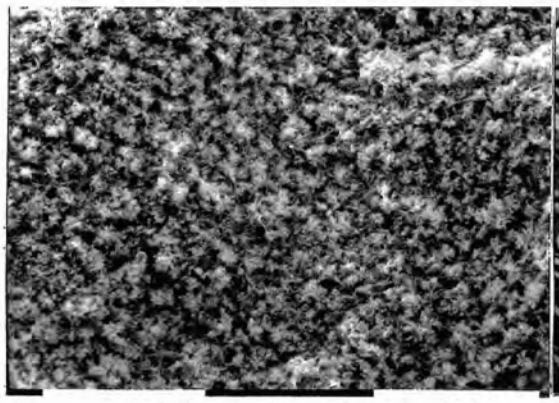
Figure 5.17A-H. Wax types and their distributions in the subgenus *Symphyomyrtus*. Part 1



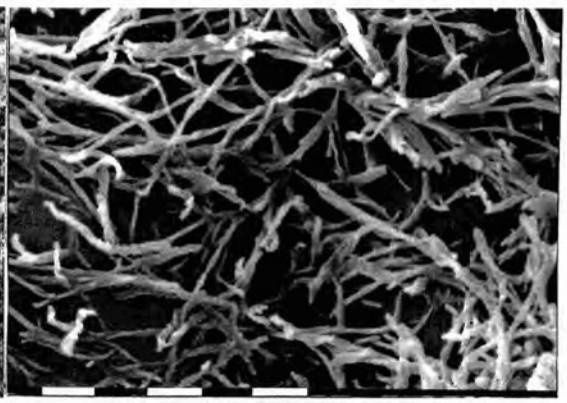
A Tube waxes on juvenile leaf surface of *E. cordata* Scale bar = 10 µm



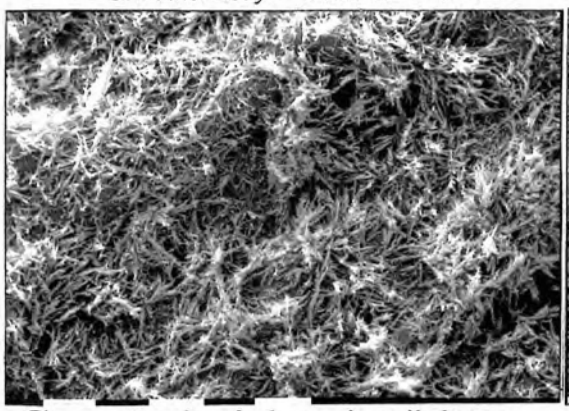
E Acute angle and branched tube waxes on juvenile leaves of *E. viminalis*. Scale bar = 1 um



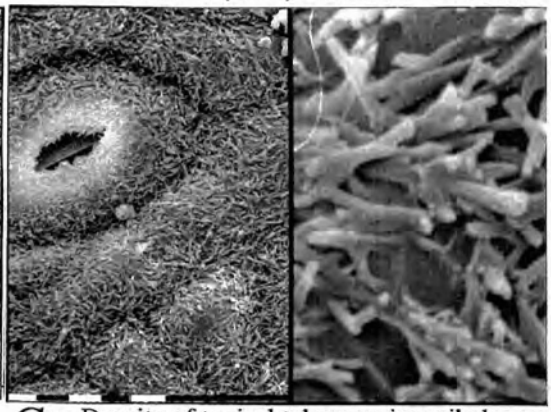
B Density of tubes on juvenile leaves of *E. morrisbyi* Scale bar = 0.1mm



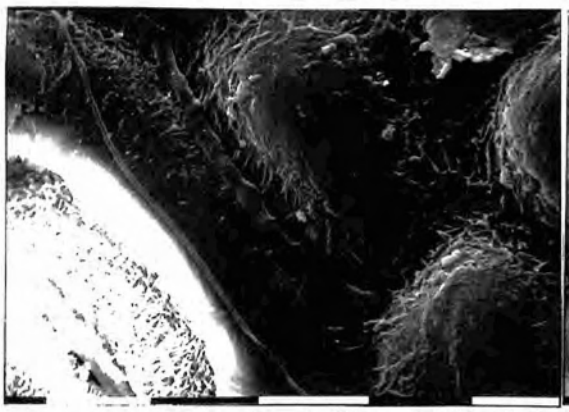
F Flat-thread like tubes on juvenile leaves of *E. archeri* (CaVi). Scale bar = 0.1 mm



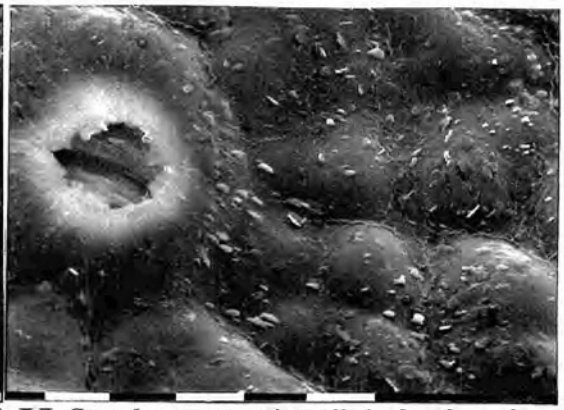
C Density of tubes on juvenile leaves of *E. globulus*. Scale bar = 10 µm



G Density of typical tubers on juvenile leaves of *E. archeri* (PrBl). Scale bar = 10 µm

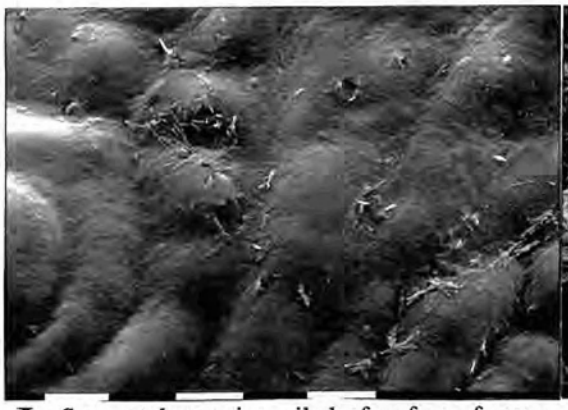


D Tube waxes associated with guard cells of adult leaves of *E. globulus*. Scale bar = 1 µm

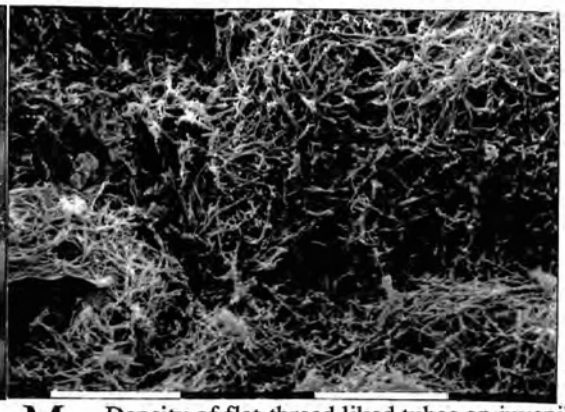


H Granular waxes on juvenile leaf surface of green *E. urnigera* (MtWe-600m). Scale bar = 10 µm

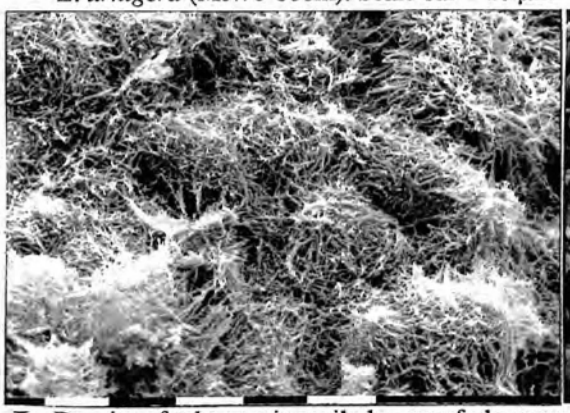
Figure 5.17I-P. Wax types and their distributions in the subgenus *Symphyomyrtus*. Part 2



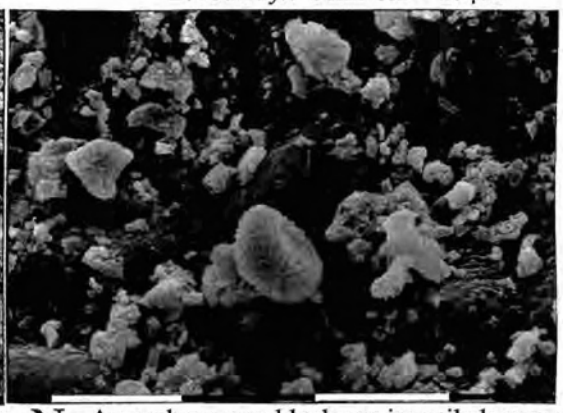
I Sparse tubes on juvenile leaf surface of green *E. urnigera* (MtWe-600m). Scale bar = 10 μm



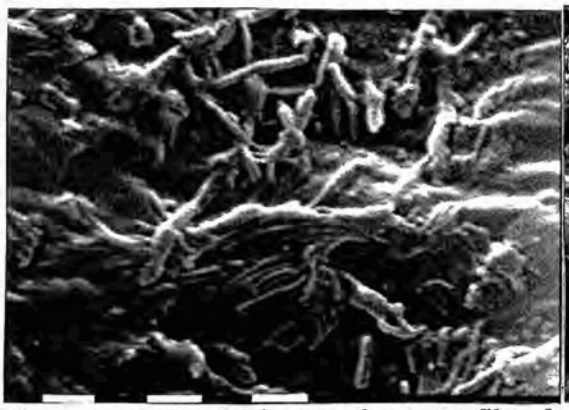
M Density of flat-thread liked tubes on juvenile *E. rodwayi*. Scale bar = 10 μm



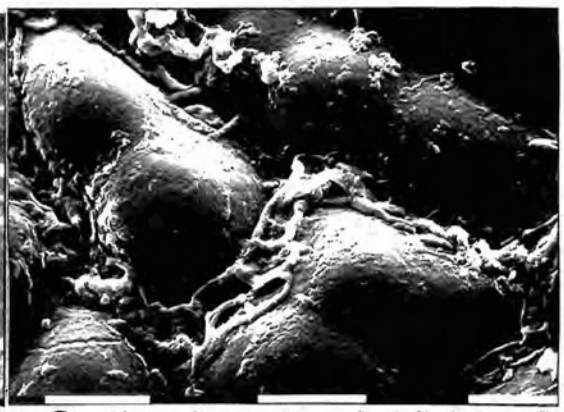
J Density of tubes on juvenile leaves of glaucous *E. urnigera* (MtWe-1100m) Scale bar = 0.1mm



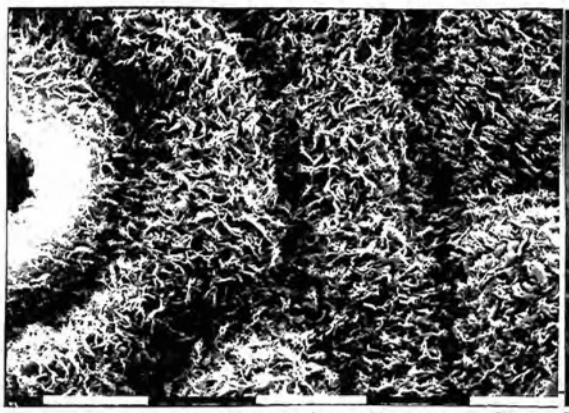
N Amorphous wax blocks on juvenile leaves of *E. barberi*. Scale bar = 10 μm



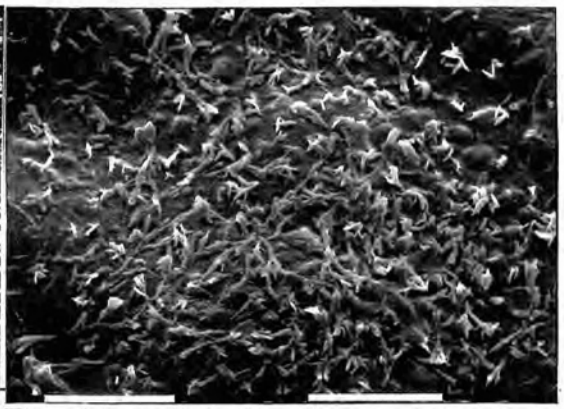
K Tube waxes on the amorphous wax film of juvenile leaves of *E. vernicosa*. Scale bar = 1 μm



O Amorphous waxes on juvenile leaves of *E. brookeriana* (MRd) Scale bar = 10 μm



L Density of plates on juvenile leaves of *E. ovata*. Scale bar = 10 um



P Intermediate tubes and sparse plates waxes on leaves of *E. brookeriana* (SuRd). Scale bar = 10 μm

The presence of identical tube waxes in glaucous and subglaucous populations was correlated with their wax chemicals, which were characterised by high contents of ß-diketones. Those species, that had marked differences in the distribution of tube waxes between juvenile and adult leaves showed no significant difference in wax chemical composition. Nevertheless, the wax yields of juvenile leaves for such species were generally higher than from adult leaves, in particular the wax yields of juvenile *E. globulus* which were significantly higher than from adult leaves. It is considered that the different density of tube waxes between juvenile and adult leaves correlates to the quantitative difference in wax yield rather than wax composition.

The green and green/subglaucous species populations within the series *Viminales* were different in both wax type and distribution and could be divided into different groups:

i) The *E. viminalis, E. dalrympleana* and *Vim/Dal* groups, which were classified into the typical ß-diketones group based on wax chemical data, had typical tube waxes similar to that of glaucous species and had complete or open density cover on juvenile leaves (Fig.20E). Adult leaves were mainly covered by an amorphous wax film with some sparse tube waxes which were generally associated with stomata or guard cells. Thus, the wax morphology of this group showed a lower density cover for juvenile leaves in comparison to glaucous species but did not differ in wax form.

ii) The green population Carr Villa (CaVi) of *E. archeri*, has flat-thread like tubes forming an open cover on juvenile leaves (Fig. 17F) similar to *E. rodwayi*. This contrasts to the typical tube wax of the subglaucous population Projection Bluff (PrBl) (Fig. 17G), which had higher ß-diketone contents.

iii) The green populations of *E. urnigera* from Mt Wellington 600m (MtWe-600m) and Snug Tiers (SnTi), which were separated from the glaucous population at Mt Wellington 1100m (MtWe-1100m) and Alma Tiers (AlTi-1010m) due to high percentage contents of alkanes and low ß-diketones in their wax composition, have an amorphous wax film with fewer tube waxes on juvenile leaves (Fig. 17H, I). No tubes were found in specimens of adult leaves from the green population MtWe-600m where granular waxes were sparse on an amorphous wax film. This contrasted to the dense cover of tube waxes for both juvenile and adult leaves of glaucous populations (MtWe-1100m; AlTi 1010m) (Fig.20J), which grew at higher altitude and had higher ß-diketone contents and rarely n-alkanes.

iv) The yellow gum species group, which had high triterpenoid contents and relatively low ß-diketone contents, are notably different from the blue, white and alpine white gums, which had high ß-diketone contents. Leaves of the three yellow gum species were

mainly covered by an amorphous wax film where few tube waxes lie on the amorphous wax layer or are associated with stomata or guard cells (Fig.5.20K). The wax morphology of *E. johnstonii* identified here is inconsistent with that identified by Hallam and Chambers (1970), who found high densities of typical tube waxes in this species.

The wax morphology of *Ovatae* species varied widely with typical wax types of individual species mainly identified from juvenile foliage. *E. ovata* has digitate plate wax with complete or open cover (Fig. 15D; Fig.5. 17L) whereas *E. rodwayi* has flat-thread like tube waxes ranging from open to sparse cover (Fig. 5.17M). *E. barberi* has only amorphous waxes over the leaf surface (Fig.20N). Wax type varied between populations of called *E. brookeriana*. The juvenile leaves of MRd and ElPa localities and the intermediate population (B/O StRd) between *E. brookeriana* and *E. ovata*, have only amorphous waxes (Fig. 17O) but the SuRd locality had an intermediate wax form of tube and plate waxes on an amorphous wax film (Fig. 15E; Fig. 5.17P). The west coast populations collected as *E. ovata* also had only amorphous waxes.

Variation in wax types and their distributions among *Ovatae* species clearly corresponded to variation in wax chemicals. The plate wax in *E. ovata* had high proportions of alkanals and n-alkanols and the tube wax in *E. rodwayi* relatively higher proportions of ß-diketones while the amorphous waxes in *E. barberi* and some *E. brookeriana* contained high proportions of alkanes.

5.3.3. Discussion

Morphological characteristics of plant waxes ranging from thin films to complex three dimensional structures have been described (Martin and Juniper 1970; Tulloch 1976b; Baker 1982). The aerial surfaces of all higher plants carry a partial or continuous coverage of amorphous wax. A range of structured wax types has also been defined, such as plates, tubes, ribbons, rods, filaments and dendrites of which plates, tubes and ribbons are most widely reported. In agreement with the general wax morphology of eucalypts described in the literature (Hallam and Chambers 1970), this study indicated that only plates, tubes, and their intermediate types occur in eucalypts.

Three distinctive common tubular wax forms have been found in plant waxes (Baker 1982) and tubular wax forms found in eucalypts were long and thick as distinct from stubby and thin-walled slightly truncated tubes. Comparison with published micrographs showed a strong resemblance between the tubes of the *Symphyomyrtus* species and tubular waxes of *Podocarpus macrophyllum* var. *angustifolius* (Morvan 1987) and epacrid species (Mihaich 1989) and the tubes of *E. risdonii* and *E. tenuiramis* are very similar to the tubular waxes on the lemma surface of *Hordeum vulgare* (in Baker 1982).

The plate wax shows some resemblance to the plates of *Trifolum repens* (Rentschler 1979; Baker and Hunt 1981), *Acacia pychntha* (in Baker 1982) and *Pisum sativum* (Hunt and Baker 1982).

Hallam and Chambers (1970) examined 20 of the 29 species included in this investigation. The identification of the structured wax types of these 20 species was in agreement with the observations of Hallam and Chambers (1970). Although, results of this study did not add much additional general information on wax morphology, they have provided a general view of wax morphology for Tasmanian *Eucalyptus* and furthered our understanding of the variation within and between species and the relationships between wax morphology and chemical status. As a first comparison between juvenile and adult leaf wax morphological characteristics of a large number of species, results of this study have also provided a general view of ontogenetic variation.

As the findings of Hallam and Chambers (1970) indicated, the plate and tube waxes occurred in different species groups with the plate type dominant in Blakely's (1956) section Renantherae Normales and those species of series *Globulares* in Section Macrantherae Normales form a natural tube wax group. This study further indicated that wax morphology shows two major structured types occurring throughout the Tasmanian eucalypts which, as supported from other lines of evidence, define the subgenera. Although exceptions occurred, the majority of *Monocalyptus* possess plate waxes and *Symphyomyrtus* tubes.

At the lower taxonomic level (i.e. series, species), tubes were unique in series *Viminales* and plates in *Obliquae*. Within series *Viminales*, the distribution of tube waxes correlates with levels of leaf glaucousness and indicates similarities between some species with similar leaf colour characteristics and differences between species groups, e.g. between yellow gum and other gum species groups. Wax morphology also indicated a close affinity between *E. regnans* and *E. obliqua* and differentiated these two species from other *Obliquae* species.

Wax type was not unique within the series *Piperitae* or *Ovatae*, but did indicate similarities between some species and differences with sectional groupings. In series *Piperitae*, the wax morphology of the two peppermints, *E. risdonii* and *E. tenuiramis*, was unique, in that they had typical tube waxes in contrast to the typical plate wax structure of a homologous group, *E. radiata, E. amygdalina* and *E. nitida*. This supports the findings in wax chemicals and other lines of taxonomic research on peppermints (e.g. Wiltshire *et al.* 1991). Moreover, wax morphology also indicates the variation between localities within *E. coccifera* which essentially related to the variation in wax chemicals for the higher the ß-diketone content the greater the presence of tube waxes.

It is obvious that, at the species level, wax morphology was most variable in the series *Ovatae* where all wax types found in the Tasmanian eucalypts occurred. While *E. barberi* was the species with an typical amorphous wax type among Tasmanian eucalypts, *E. ovata* was the only species characterised by plate waxes in the subgenus *Symphyomyrtus*. *E. rodwayi* was characterised by tube waxes and waxes of *E. brookeriana* varied from amorphous to a mixture of tubes and plates. This indicated a close affinity in wax morphology between *E. barberi* and *E. brookeriana* with *E. brookeriana* tending toward *E. ovata* while the amorphous waxes of *E. barberi* also showed difference with that of *E. brookeriana*.

Wax morphology of some plants has been examined from a taxonomic viewpoint but not all results were found to be of taxonomic significance. However, there are perceived differences at all levels of the taxonomic hierarchy. Thus there are differences between taxonomic groups within the genus *Prosopis* (Hull and Bleckmann 1977), *Isocoma* (Mayeaux *et al.* 1981) and *Pibus* (Yoshie and Sakai 1985) and between genera within the family Triticeae (Baum *et al.* 1980) and Epacridaceae (Mihaich 1989). Differences between apple (Gough and Shutak 1972) and barley cultivars (Marchylo and Laberge 1981) and banana varieties (Freeman and Turner 1985) also have been observed. Although few studies show wax morphology to be useful in distinguishing a limited number of species (e.g. Baum and Haland 1974), it has usually failed to discriminate large representations of species in those plant families and genera examined. *Eucalyptus* is possibly the first plant genus in which wax morphology has been examined in detail (Hallam and Chambers 1970), and results have shown a comparative taxonomic significance at low taxonomic levels compared to other plant taxa. However, results of this study and the previous investigation indicated that wax morphology was not able to discriminate between most species within different taxa (e.g. series or some other species groups), although wax morphology could discriminate sectional species groups within some subgenera and individual species within a few series (e.g. *Ovatae* species in Tasmania). Thus, wax morphology mainly indicated differences at higher taxonomic levels but was poor in the discrimination of individual species. For example, many blue gum and alpine white gum species had similar tube waxes.

In addition, some subtypes (e.g. edge type) with a structured wax type, which were identified by Hallam and Chambers (1970), showed much variation within individual species. For example, the size and edges of plate waxes of *Obliquae* species, *E. delegatensis, E. sieberi* and *E. pauciflora,* were very similar to those in *Piperitae* species, *E. amygdalina, E. nitida* and *E. radiata,* where a range of edge patterns could be found from the plates of individual species. Variation may occur on leaves of different trees or

on different branches of a tree within a species. Hence, those wax subtypes characterised by edge could not be used in discriminating species. Moreover, the tube waxes may also vary with leaf age. For example, the tube waxes of young juvenile leaves of *E. globulus* had tubes with different diameters and random arrangements before differentiating to that of fused tubes of similar diameter and length on mature juvenile leaves.

With the exception of a few populations of *E. coccifera*, most species examined had the same structured wax types on juvenile and adult leaves, although wax size, distribution or cover density can be variable, in particular in those species which have glaucous juvenile leaves and morphologically different non-glaucous mature leaves. Results of this study indicated that ontogenetic variation in structured waxes is not common in *Eucalyptus* species.

5.4. General discussion and concluding remarks

5.4.1. Wax chemistry and morphology

Both studies of wax chemistry and morphology of leaf epicuticular waxes used the same specimens. Thus, results of this study provided a direct comparison between the chemical composition and morphology of *Eucalyptus* waxes and highlighted a number of interesting phenomena.

The relationship between wax chemistry and morphology has long received attention and it has become increasingly evident that the type and distribution of structured wax deposits are determined principally by the composition of the wax exudates (Baker 1982). Results of this study further indicated a correlation between wax morphology and chemical composition among Tasmanian eucalypts.

The most obvious association is that which exists between the presence of ß-diketones in waxes and the formation of tubes which has been found to occur in cereals (Lundqvist *et al.* 1968), wheat (Barber and Netting 1968), barley (Wettstein-Knowles 1974; Simpson and Wettstein-Knowles 1980),*Vaccinium ashei* (Freeman *et al.* 1979) and eucalypts (Hallam and Chambers 1970). Results from the present study indicated that a similar association exists between Tasmanian eucalypts (Table 5.9). For example, the glaucous populations of *Symphyomyrtus* species, which had waxes with high ß-diketone contents, had typical tube waxes. The green populations of *E. rodwayi* and *E. archeri*, had

Table 5.9. The presence of wax types and dominant chemical compounds in *Eucalyptus*.

Classification reference: Pryor and Johnson (1971), Chippendale (1988), Jackson (1965) and Barber (1956).

Pryor and Johnson's classification			Chippendale's classification	Jackson's classification	Barber's Glaucousness	Species	Wax† type	Dominant compounds
Series	Subseries	Superspecies	Series	Group	Phenotype			
Obliquae	*Regnantinae*	*Regnans*	*Regnantes*	Ash	green	*E. regnans*	A, P	triterpenoids
Obliquae	*Obliquinae*		*Eucalyptus*	Ash	green	*E. obliqua*	A, P	triterpenoids
Obliquae	*Delegatensinae*		*Eucalyptus*	Ash	clinal	*E. delegatensis*	P	aldehydes, moronate acetate
Obliquae	*Consideninaninae*	*Consideniana*	*Psathyroxyla*	Ash	clinal	*E. sieberi*	P	aldehydes, moronate acetate
Obliquae	*Pauciflorinae*	*Pauciflora*	*Eucalyptus*	Ash	clinal	*E. pauciflora*	P	moronate acetate, aldehydes
Piperitae	*Amygdalininae*	*Amygdalina*	*Radiatae*	Peppermint	clinal	*E. radiata*	P	aldehydes
Piperitae	*Amygdalininae*	*Amygdalina*	*Radiatae*	Peppermint	clinal	*E. amygdalina*	P	aldehydes, moronate acetate
Piperitae	*Amygdalininae*	*Amygdalina*	*Radiatae*	Peppermint	clinal	*E. pulchella*	P T/P	aldehydes, ß-diketones, triterpenoids ß-diketones, aldehydes, triterpenoids
Piperitae	*Amygdalininae*	*Amygdalina*	*Radiatae*	Peppermint	clinal	*E. nitida*	P	moronate acetate
Piperitae	*Amygdalininae*	*Amygdalina*	*Radiatae*	Peppermint	clinal	*E. coccifera*	P T	aldehydes, moronate acetate ß-diketones
Piperitae	*Amygdalininae*	*Risdonii*	*Radiatae*	Peppermint	glaucous	*E. risdonii*	T	ß-diketones
Piperitae	*Amygdalininae*	*Risdonii*	*Radiatae*	Peppermint	glaucous	*E. tenuiramis*	T	ß-diketones
Ovata	*Oavatinae*	*Ovata*	*Foveolatae*	Black gum	Green	*E. ovata*	P	aldehydes
Ovata	*Oavatinae*	*Ovata*	*Foveolatae*	Black gum	Green	*E. brookeriana*	A-(T/P)	alkanes, ß-diketones, aldehydes,
Ovata	*Oavatinae*	*Aggregata*	*Foveolatae*	Black gum	Green	*E. rodwayi*	T (atypical)	ß-diketones, alkanes, aldehydes
Ovata	*Oavatinae*	*Ovata*	*Foveolatae*	Black gum	Green	*E. barberi*	A	alkanes
Viminales	*Viminalinae*		*Viminales*	White gum	Green	*E. viminalis*	T	ß-diketones
Viminales	*Cordatinae*	*Rubida*	*Viminales*	White gum	Subglaucous	*E. darympleana*	T	ß-diketones
Viminales	*Cordatinae*	*Rubida*	*Viminales*	White gum	Glaucous	*E. rubida*	T	ß-diketones
Viminales	*Vernicosinae*		*Viminales*	Yellow gum	Green	*E. johnstonii (vernicosa)*	A,T	triterpenoids, ß-diketones
Viminales	*Vernicosinae*		*Viminales*	Yellow gum	Green	*E. subcrenulata (vernicosa)*	A,T	triterpenoids, ß-diketones
Viminales	*Vernicosinae*		*Viminales*	Yellow gum	Green	*E. vernicosa (vernicosa)*	A,T	triterpenoids, ß-diketones
Viminales	*Globulinae*	*Globulus*	*Viminales*	Blue gum	Glaucous	*E. globulus*	T	ß-diketones
Viminales	*Cordatinae*		*Viminales*	Blue gum	Glaucous	*E. cordata*	T	ß-diketones
Viminales	*Cordatinae*	*Gunnii*	*Viminales*	Alpine white gum	Glaucous Subglaucous	*E. gunnii*	T T	ß-diketones ß-diketones
Viminales	*Cordatinae*	*Gunnii*	*Viminales*	Alpine white gum	Subglaucous Green	*E. archeri*	T T (atypical)	ß-diketones(35-53%), aldehydes(6-12%) ß-diketones(22-23%), alkanes (16-17%), aldehydes (10-12%)
Viminales	*Cordatinae*	*Gunnii*	*Viminales*	Alpine white gum	Glaucous green	*E. urnigera*	T A	ß-diketones alkanes
Viminales	*Cordatinae*		*Viminales*	Alpine white gum	Glaucous	*E. perriniana*	T	ß-diketones
Viminales	*Cordatinae*	*Gunnii*	*Viminales*	Alpine white gum	Glaucous	*E. morrisbyi*	T	ß-diketones

† Wax type: A = amorphyous; T = Tabe; P = Plate.

relatively low ß-diketone contents and had tubes with an flat thread-like appearance while some other green populations had low to trace amounts of ß-diketones and fewer or no tube waxes. In contrast, ß-diketones rarely occurred in the waxes of most *Monocalyptus* species and there was a concomitant formation of other wax types. However, the two *Monocalyptus* species, *E. risdonii and E. tenuiramis,* which had waxes with high ß-diketone contents, had typical tube waxes. However, the tube waxes of these two species were much longer than these on *Symphyomyrtus*.

The relationship between ß-diketones and tube waxes also occurred between localities within a species (e.g. *E. pulchella*), between juvenile and adult leaves of individual localities within a species (e.g. *E. coccifera*) and between different phenotypes within a species locality, (e.g. *E. urnigera*). The differences between glaucous (high altitude) and green (low altitude) phenotypes of *E. urnigera* within the Mt Wellington (Mt We) locality were investigated by Hall *et al.* (1965) and Hallam and Chambers (1970). However, their results for the green phenotype were inconsistent for Hallam and Chambers (1970) found an open cover of tube waxes in contrast to findings of Hall *et al.* (1965) who found that plate waxes predominated. The current study indicated that there were sparse tube waxes on the amorphous wax film of juvenile leaves and no tubes on adult leaves where only sparse wax granules on an amorphous film occurred. This correlates with the differences in wax chemical composition as juvenile leaves had relatively high ß-diketone content in contrast to the higher alkane content of adult leaves. In addition, some of the wax granules in adult leaves showed a somewhat plate-like appearance which was similar to that defined by Hall *et al.* (1965). Unfortunately both Hall *et al.* (1965) and Hallam and Chambers (1970) make no reference to juvenile and adult characters, which makes it impossible to check the ontogenetic identity of their samples. It is possible that the different observations made by the two groups may be due to differences in the ontogenetic stage of samples examined.

Some *Viminales* species (e.g. *E. globulus*) showed marked differences in the distribution of tube waxes between juvenile and adult leaves but wax composition was similar. In addition, the chemical composition of chloroform extracted waxes also showed no significant difference between juvenile and adult leaves for those species (data not shown). Therefore, this contrasts with the suggestion of Baker (1982) who considered that the change of wax morphology between juvenile and adult leaves of *E. globulus* was due to a change of chemical composition. It is considered that the differences in some species appeared to correlate to a quantitative difference in amounts of wax yield rather than wax composition, since the wax yields of juvenile leaves of *E. globulus* and *E. darympleana* were significantly higher than that of adult leaves. In addition, it is possible that although wax composition is an important and often an major factor in the formation

of structured wax and is under precise genetic control, variation in wax characters between juvenile and adult stages may be influenced by additional ontogenetic systems.

In contrast to the marked correlation between ß-diketone and tubes, plate waxes were more difficult to characterise. What appears to be the same basic plate wax may be composed of different compound classes in different species. The only generally consistent feature was that plate producing species often had their wax composition dominated by alkanals and triterpenoids. It was obvious that alkanals correlated closely with the production of plate structures for *E. radiata* and *E. ovata* species from different subgenera, as wax chemicals were characterised by alkanals and plates were formed. In contrast, waxes of *E. nitida and E. pauciflora* were characterised by triterpenoids and plates were formed. The waxes of all other plate producing species were characterised by similar amounts of alkanals and triterpenoids.

However, not all triterpenoids, and probably only particular triterpenoids, form plate waxes. For example, on the foliage of *E. regnans* and *E. obliqua*, which had high triterpenoid contents (> 49%), plate waxes occurred only in the region of stomata or guard cells and an amorphous film covered the remainder of the leaves. In contrast, foliage of *E. pauciflora* and *E. nitida*, which also had high triterpenoid contents (> 48%), had a high density of plate wax cover. Comparison of wax yields between these species indicated that the wax yields of *E. regnans* and *E. obliqua* were much higher than that of *E. pauciflora* and *E. nitida*. These findings indicated that the decreased plate wax density in *E. regnans* and *E. obliqua* was not due to quantitative differences in the amounts of waxes. The triterpenoids of *E. regnans* and *E. obliqua* were composed of a wider range of compounds, the triterpenoids, D, E, F, G, K and L (7-30% of the total triterpenoids respectively), but the triterpenoids in *E. pauciflora* and *E. nitida* were dominated by a single triterpenoid, methyl moronate and it's isomer (>78%). Triterpenoids in other plate producing species were also dominated by methyl moronate (>50%). The results strongly suggest that the multi-component mixtures of triterpenoids have a consequent effect on crystallisation behaviour, so that mainly amorphous waxes are formed on the surfaces of *E. regnans* and *E. obliqua*, and probably only a particular triterpenoid component, such as methyl moronate, forms plate waxes. In addition, the waxes composed of a wide range of triterpenoid compounds with high proportion on the foliage of the yellow gum species were concomitant with a amorphous wax but not the formation of plates.

A number of species, which are rich in alkanols, have been found to be covered by plate waxes and this finding has been used in other studies to indicate a relationship with plate wax production, e.g. barley (Lundqvist *et al.* 1968), wheat (Netting and Wettstein-Knowles 1973) and *Eucalyptus* (Baker 1982). However, results of this study indicated

that alkanols were only minor constituents in the waxes of Tasmanian eucalypts and there was no evidence to suggest that alkanols played a major role in plate formation. For example, only rarely did alkanols occur in waxes of *E. pauciflora,* which was one of the species used as an example for the relationship between alkanols and plates in Baker's review (1982).

Nevertheless, the presence of n-alkanols may aid the formation of plate waxes. For example, the juvenile leaves of the two *Obliquae* species, *E. delegatensis* and *E. sieberi,* had a much heavier glaucous coating and higher density of plate waxes than adult leaves but there was no difference in wax yield. This is at odds with the suggestion that the differences were due to a quantitative difference in the amount of waxes. However, juvenile leaves contained significantly higher contents of n-alkanols (19.2% and 17.7% respectively) than did adult leaves (6.4% and 4.1% respectively). The only plate wax producing species, *E. ovata,* in *Symphyomyrtus* also contained substantial amounts of n-alkanols (15.1% and 12.9% for juvenile and adult waxes respectively). It seems more likely that the plate structures are formed more readily when the wax mixture contains alkanals in addition to n-alkanols.

There was an association between alkane (hydrocarbon) content and amorphous wax (Table 5.9). For example, *E. barberi* is the only species among Tasmanian eucalypts carrying only amorphous wax and has a chemical composition dominated by alkanes (>61%). Leaves of other *Ovatae* species, which have waxes composed of substantial proportions of alkanes (18% to 29%), were mainly covered by amorphous wax, although structured waxes were also present. Moreover, the green phenotype of *E. urnigera* with amorphous wax had a chemical composition dominated by alkanes and, conversely, the glaucous phenotype with tubes rarely contained alkanes. Similar relationships have also been found among the Epacridaceae, where species with amorphous wax have a chemical composition invariably dominated by the alkanes (Mihaich 1989). Thus, alkanes influence the formation of amorphous wax.

In summary, results of this study agree with the findings of Hallam and Chambers (1970) that tube waxes in the genus *Eucalyptus* appear to be good indicators of the presence of ß-diketones. Results of this study also revealed a correlation between plate waxes and alkanals and a particular triterpenoid, methyl moronate, among Tasmanian eucalypts. Although, there was no correlation between amorphous wax and chemical composition, the presence of high contents of alkanes or a combination of multi-triterpenoids appeared to be indicative of a positive role. Thus, the overall variation observed reflects a strong link between wax structure and chemistry while genetic effects may alter this relationship (e.g. possible ontogenetic effects in *E. globulus*). These

findings concur with the proposal of Jeffree *et al.* (1976) and experimental evidence from other workers.

Wax formation is a function primarily of chemical composition. The morphology of waxes, which comprise a single dominant constituent, is remarkably uniform in view of the correlation made here. This would appear to negate the need for cuticular pores or channels as a means of structural formation (Hall and Donaldson 1962; Hall 1967). The findings of this study support the crystallisation theory (Kreger 1948) which has received considerable attention during recent years (Baker 1974, 1982; Jeffree *et al.* 1975). Additional investigations involving the growth and formation of waxes from young juvenile to mature juvenile leaves of *E. globulus* in this study indicated that the tubes continuously form on the leaf surface creating new branches and generating crystalline clusters on the young juvenile leaf surface. New tubes which grow on top of crystalline clusters may be longer (< 5 μm) and then break into shorter tubes (2-3μm) with increased physiological age. This suggested that the formation of tube structures could take place above the leaf surface without cuticular pores or channels, although tubes may originate from the cuticle, as demonstrated by Hallam (1970), or may form under a polymeric material layer as suggested by Wettstein-Knowles (1974).

The results and observations of this study also provided an understanding of the relationship between the appearance of the leaf surface of eucalypts and their wax morphology and chemistry and indicated that the glaucousness of a leaf surface is principally determined by the micro morphology and chemistry of leaf waxes. In conjunction with the general descriptions of eucalypt leaf surface appearance, these relationships can be described as:

i) the glaucous leaf surface with a characteristic white "bloom" has a high density of tube waxes and a high ß-diketone content - e.g. *E. risdonii, E. morrisbyi,* and juvenile *E. globulus*;

ii) distinctly dull matt surfaces have either plate waxes with high levels of alkanals or the triterpenoid, methyl moronate - e.g. juvenile leaves of *E. delegatensis, E. radiata, E. nitida* and *E. ovata* (these plates are difficult to remove completely by rubbing) or tube waxes in relatively low density but high ß-diketone content - e.g. juvenile leaves of *E. viminalis* and *E. dalrympleana* (these tubes are easily removed by rubbing and lose their dull matt appearance), and

iii) the shiny surface of a smooth cuticle has mainly an amorphous wax coating and structured waxes were rare or absent. Waxes of such leaves have a composition lacking ß-diketones, alkanals or methyl moronate and high levels of alkanes or multi-

triterpenoids (in general), with the exception of adult leaves of some *Viminales* species (e.g. *E. globulus, E. viminalis* and *E. dalrympleana*).

5.4.2. A taxonomic evaluation

The examination of leaf waxes indicated that they contribute valuable information to the taxonomy of Tasmanian eucalypts. Wax chemicals and their morphology support the phenetic groupings obtained from other lines of evidence. While leaf wax showed a distinct difference between subgenera, a number of species groups were uniform in both wax chemistry and morphology and some were variable with two or three wax types occurring. Thus, at low taxonomic levels (series or subseries as defined by Pryor and Johnson, 1971) waxes are not unique enough to distinguish taxa but do indicate similarities between some species and species groups, such as variation between species within series *Piperitae* and *Ovatae* and between species groups within series *Viminales*.

Although exceptions occurred, morphological variation was correlated with chemical composition and some components could be attributed to the formation of related structures, for example, the correlation between ß-diketones and tube waxes. Thus, variation in wax morphology among Tasmanian eucalypts generally parallels variation in wax chemicals.

It is obvious that wax chemicals generally have greater taxonomic significance than does wax morphology at all taxonomic levels, particularly at the subseries, superspecies, species and subspecies levels. For example, all glaucous and subglaucous populations of the blue, white and alpine-white gum species possessed a unique profile of tube waxes which failed to indicate species differences. However, wax chemical data clearly separated many of these populations into identifiable species groups and indicated relationships between species populations, e.g. the relationship between populations within the *Vim/Dal* group and its relationship to other species.

At the species level some species possessed a unique chemical composition such that the proportions and the relative distribution of homologues of individual compound classes could be used with some confidence as a taxonomic "fingerprint" of the species. This is particularly evident in the series *Obliquae* where all samples can be grouped into species-specific population groups following the complex arrangement in chemical composition.

Comparison of classifications based on the leaf wax data and that based on the leaf oil data indicated that wax characteristics better reflect the genetic makeup of species and species groups at lower taxonomic levels than does the leaf oil. This is particularly evident in wax data which successfully defined *Viminales* and *Piperitae* species (e.g. *E. viminalis* and *E. nitida*) and some related species groups in which oil data was unable to

indicate, for example the species group of *E. viminalis - Vim/Dal - E. dalrympleana* and the yellow gums.

The relationship of *Obliquae* species based on wax chemicals (Fig. 5.7) and morphology (Fig. 16) suggested that the *Obliquae* species can be divided into two groups. *E. regnans* and *E. obliqua* had a close wax affinity and formed a "green ashes" group and the other three species formed a "blue ashes" group in which *E. delegatensis* was closely related to *E. pauciflora*. This is in agreement with the suggestion of Ladiges *et al.* (1987) that the "ash" group, informal series *Obliquae* Pryor and Johnson (1971), was not monophyletic and supports their revised informal classification (Ladiges *et al.* 1989) in which "green ashes" species made up a superspecies *Eucalyptus* from which "blue ashes" were excluded. However, the wax relationships are in disagreement with Chippendale's (1988) classification which grouped *E. obliqua, E. delegatensis* and *E. pauciflora* into series *Eucalyptus* and placed *E. regnans* in series *Regnantes*. Again, the wax affinity among these "ash" species differed from that of leaf oil which classified *E. delegatensis* and *E. obliqua* into a high monoterpenoid group and *E. regnans, E. pauciflora* and *E. sieberi* into a high sesquiterpenoid group (see Chapter 4).

Pryor and Johnson's (1971) classification grouped the peppermint eucalypts in a subseries, *Amygdaliniae*, of series *Piperitae* and classified the two species, *E. risdonii* and *E. tenuiramis*, as a superspecies *Risdonii* and the three species, *E. amygdalina, E. nitida* and *E. radiata*, as a superspecies *Amygdalina*. In the revised classification of Ladiges *et al.* (1983), *E. risdonii* and *E. tenuiramis* were retained as sister species in superspecies *Risdonii* but grouped into a subseries *Cocciferinae* with *E. nitida* and *E. coccifera* while *E. amygdalina, E. pulchella* and *E. radiata* were arranged into different subseries.

The classification based on wax analysis here is in agreement with the treatments of Pryor and Johnson (1971) and Ladiges *et al.* (1983) who linked *E. risdonii* and *E. tenuiramis* into a superspecies, *Risdonii*. However, it does not agree with the speciation hypotheses of Ladiges *et al.* (1983) which considered *E. nitida* and *E. coccifera* to be sister species to *E. risdonii* and *E. tenuiramis* and grouped them into a subseries *Cocciferinae*, since *E. risdonii* and *E. tenuiramis* lay outside the majority of peppermints due to their completely different wax chemistry and morphology. The three species, *E. amygdalina, E. nitida* and *E. radiata,* were grouped into the superspecies *Amygdalina* in Pryor and Johnson's classification (1971), however, they did not constitute a natural group based on wax affinities.

It is possible that *E. risdonii* and *E. tenuiramis* are monospecific, since variation in waxes between these two species was continuous and smaller than variation between

populations within other peppermints. Moreover, the variation in wax chemicals between these two species was much smaller than that within *E. tenuiramis*. This is in agreement with the suggestion of Wiltshire *et al.* (1991) that *E. risdonii* may be the product of relatively recent changes in developmental timing (heterochrony) from *E. tenuiramis*. It is of interest that, among Tasmanian eucalypts, only adult waxes of *E. coccifera* had the same ß-diketone pattern (C_{29} homologue dominate) as *E. risdonii* and *E. tenuiramis* while some *E. coccifera* had tube waxes. Thus, the characters of the adult wax supported the suggestion of Wiltshire et al (1991) that there was close morphological affinity between *E. coccifera* and *E. tenuiramis*. However, juvenile waxes did not. The juvenile leaves of *E. coccifera* had lower amounts of ß-diketone and the pattern of ß-diketone was also different (C_{33} homologue dominate).

E. nitida is a species that has the most specific wax chemicals among peppermints. Although *E. nitida* populations occurred over a wide geographic range, their wax composition exhibited less variation then any other Piperitae species, with the exception of *E. risdonii* and *E. tenuiramis*. All intermediate phenotypes between *E. nitida* and other species (e.g. *E. amygdalina* - *E. nitida* and *E. coccifera* - *E. nitida*) tended to converge in wax chemicals toward *E. nitida* but not the other species. This suggested that these interspecific varieties may have arisen from the genetic pool of *E. nitida*.

The variation in leaf wax within *Symphyomyrtus* species showed some taxonomic implication in corresponding to some of the species groups in lower taxa as proposed by Pryor and Johnson's classification (1972) but did not follow the division into series. The variation among *Ovatae* species did not assign them as a series, since variation in wax chemistry between *Ovatae* species was greater than variation amongst *Viminales*. Although the majority of *Viminales* species populations were closely linked by wax chemistry, the yellow gum group was not related to any species group within this series. Thus, the results strongly suggested that species within series *Ovatae* or *Viminales* are not monophyletic and need taxonomic revision.

Amongst *Ovatae* species, the leaf waxes of *E. rodwayi* were most unlike *E. ovata*, which is in agreement with Pryor and Johnson (1971) who separated them into different superspecies, *Ovata* and *Aggregata*. *E. brookeriana* and *E. barberi* are closely associated with one another in morphological characteristics and suggested to be monophyletic, implying a sister species relationship (Gray 1979, Ladiges 1984). However, these two species exhibited marked differences in wax characteristics while the majority of *E. brookeriana* (east coat populations) were close to *E. rodwayi*. Thus, relationships between *Ovatae* species did not correlate with interspecific relationships based on seedling morphological characteristics as suggested by Ladiges (1984).

In Tasmania, *E. rodwayi* occurs in eastern and centre/west and *E. barberi* occurs only in eastern Tasmania while *E. ovata* and *E. brookeriana* occurred in both eastern and western Tasmania (Chippendale and Wolf 1981). Thus, these *Ovatae* species overlap in their biogeographical distribution in Tasmania. The highly specific difference among the eastern Tasmanian populations confirmed that these species are individual monophyletic and were not subjected to interspecific convergence in this area. However, *E. ovata, E. brookeriana* and *E. rodwayi* tended to converge in western Tasmania in which the wax characteristics of the west coast population classified as *E. ovata* was intermediate between east coast *E. ovata* and *E. brookeriana* and formed a group different from either east coast *E. ovata* or *E. brookeriana.* This findings corresponds to the probable convergence in morphological characteristics among west coast populations which has made identification difficult (Jackson 1960). Results of wax analysis thus suggested that the west coast populations classified as *E. ovata* (HeRi and MoRd) or *E. brookeriana* (SuRd) could be separated from the east coast populations and recognised as a single group, 'west coast ovata/brookeriana'. The 'west coast ovata/brookeriana' has closer affinities to *E. brookeriana* and *E. rodwayi.* The differentiation in wax chemicals between 'west coast ovata' and either east coast *E. ovata* or *E. brookeriana* is greater than that between species *E. rodwayi* and east coast *E. brookeriana.*

In addition, the wax analysis suggested that the west coast *E. brookeriana* has closer affinities to *E. rodwayi* than to *E. barberi.* This is inconsistent to the observation of Ladiges *et al.* (1984) that *E. brookeriana* is a sister species to *E. barberi.*

Results of this study indicated that, with the exception of yellow gum species, the diversity in leaf wax among other *Viminales* species did not agree with the subseries grouping of Pryor and Johnson (1972) and the division of white, blue and alpine white gum groups as proposed by Jackson (1965). For example, white gum species *E. viminalis* and *E. dalrympleana* were placed into two different subseries, *Viminalinae* and *Cordatinae,* in Pryor and Johnson's classification (1971), however, both had close affinities in wax characteristics and appeared to be monophyletic with their intermediate form *Vim/Dal.* Another white gum species, *E. rubida,* which was placed into superspecies *Rubida* with *E. dalrympleana,* had close wax affinities with blue gum species, *E. globulus,* and the alpine white gum species, *E. morrisbyi* and *E. perriniana,* while another blue gum, *E. cordata,* was close to the other alpine-white gums, *E. gunnii* and *E. urnigera.*

The variations in wax chemistry and morphology among *Viminales* species were reasonably correlated to the types of glaucousness proposed by Barber (1956). Results of this study have successfully shown that these glaucousness types can be characterized

chemically and thus have significant taxonomic implication, while the green types could be assessed into different chemotypes.

Similar to the peppermints, some of the interspecific clines of *Viminales* species were also linked by wax chemicals and the relative positions of intermediates were indicated, e.g. *E. viminalis* - *Vim/Dal* - *E. dalrympleana* and the cline between the yellow gum species. Results indicated that the intermediate form, *Vim/Dal*, had a close wax chemical affinity with the green species *E. viminalis*. This is in agreement with the finding of Phillips and Reid (1980) who indentified *Vim/Dal* as a green phenotype. In addition, the wax chemistry of hybrid C/V CQE between *E. viminalis* and *E. cordata*, which was indentified as a green phenotype (Potts 1988, 1989), was closer to that of *E. viminalis* than to the glaucous species *E. cordata*. These findings indicated that the variation in wax chemicals paralleled the change of glaucousness in intermediate forms or hybrids between glaucous and green phenotypes. The morphologically intermediate form between glaucous and non-glaucous species had an intermediate wax chemistry and generally tended toward the non-glaucous species.

Overall, wax characteristics are often correlated with other morphological taxonomic characteristics and are valuable in distinguishing species. The marked specific differences within individual localities showed that wax differences represent genetic differences, as found in species from the Snug Plains mixed forest. Although there appears to be ecological correlations in many cases of interspecific clinal change in wax characteristics, comparisons made between the intermediates within clines and the genetically different parent species indicated that the composition of wax chemicals of the intermediate forms were generally intermediate between genetically different species. It is assumed that the synthesis of wax chemicals is most probably genetically inherited and that change of wax chemistry in clines may represent changes in allelic frequencies, although these should be identified by genetic study.

Although waxes can sometimes be influenced by environmental factors or undergo chemical and structural modification during their development and ageing, many studies have indicated that the variability in wax chemistry and morphology was genetically linked (e.g. Baker 1972, 1974; Wettstein-Knowles 1972; Jeffree 1976; Baker and Hunt 1981; Woodhead *et al.* 1982; Freeman and Turner 1985). Some studies have provided evidence to confirm that a relationship exists between wax chemistry and structure and suggest that specific genes determine the nature of wax compounds and govern the homologous composition of homologues within each class of substance (Giese 1976; Bianchi *et al.* 1980).

Although wax morphology of some species may be significantly affected by ontogenetic factors (e.g. *E. globulus*), wax chemical composition showed a strong consistency during ontogenetic development from juvenile to adult eucalypt trees. This finding indicated that leaf waxes not only have taxonomic value in the *Eucalyptus* but that wax chemicals can also provide a useful approach for taxonomic study in the early stages of tree growth. This early analysis would save time and expense of research in both tree selection and hybrid studies. It is possible that further study in the wax chemistry of seedling eucalypt trees could add interesting information for taxonomic study and breeding programs.

5.4.3. Concluding remarks

Results of this study have shown that leaf waxes may have considerable taxonomic value in the Tasmanian eucalypts at all levels of the taxonomic hierarchy. Wax chemical composition appears to be linked to wax morphology and leaf glaucousness appearance. Their variation among Tasmanian *Eucalyptus* shows a complex pattern in many species which is predominantly a function of genetic make-up, with limited environmental modification and leaf age effects. Both wax chemistry and morphology are stable in the mature leaf under natural conditions and in particular in the qualitative expression of the chemical composition.

It is considered that wax chemistry is of more taxonomic importance than wax morphology and glaucousness because of the greater variability between taxa it expresses. When wax chemical characters are examined from mature leaves, valid comparisons may be made between species and varieties within species and these features do make wax chemicals a valuable diagnostic taxonomic character in *Eucalyptus*. In addition, the genetic nature and species differences make wax characteristics a valuable diagnostic approach in the identification of hybrids.

The most important result from the chemical analysis is that in many cases, the combination of all compound classes and the distribution of homologues of individual compound classes combine to form a unique "fingerprint" for individual species. Those species, which had marked infraspecific or interspecific variation in phenetic characteristics (e.g. clinals), were shown to vary in their "fingerprint", however, with few exceptions these variations indicated the species varieties and the interspecific relationship between species with few exceptions.

Analysis of the whole, untreated hexane-soluble wax was employed here and gave good separation of the components in most of the species examined. It has been a successful short-cut in obtaining an overall view of wax composition which has provided useful

information about the Tasmanian eucalypts. However, the results of untreated hexane-soluble wax do not provide complete information about the whole range of homologues and the essential distribution of individual compound classes. The homologues of alkane were identified to range from C_{12} to C_{31} in *E. globulus* wax after the wax was separated fractionally (see Appendix 11.3 for details). However, only the homologues from C_{23} to C_{31} could be recorded from the analysis of untreated hexane-soluble waxes in this study. In some species, and particularly in certain Viminales species which have a similar wax composition, the taxonomic implications are considerable and the distribution of homologues of particular compound classes are potentially important and warrant further and more detailed examination to provide a more exact "fingerprint" for individual species.

Chapter 6

General Classification and Discussion of Eucalypt Leaf Oils and Waxes

6.1. The chemotaxonomic structure of Tasmanian *Eucalyptus* spp. based on leaf oils and waxes

6.1.1. Population classification

Classification based on samples of juvenile and adult leaves

The overall pattern of chemical variation amongst Tasmanian *Eucalyptus* species, as estimated from percentage composition of oil and wax components of both adult and juvenile leaf samples, is summarised in the dendrogram in Figure 6.1. As expected, this dendrogram is somewhat intermediate between that using only leaf oils and that using only leaf wax data. Nevertheless, this dendrogram further confirms that overall chemical variation in leaf waxes and oils in Tasmanian eucalypt species corresponds to the major morphological classification.

With the exception of the outlying species, *E. tenuiramis* and *E. risdonii,* and some populations of *E. ovata* and *E. pulchella*, the populations were divided into the two major clusters that corresponded to the subgenera *Monocalyptus* and *Symphyomyrtus.* Moreover, many populations were fused into approximate species clusters, often despite the populations occupying a wide geographic range. Overall, twenty of the twenty nine species demonstrated identifiable clusters. Thus, this dendrogram showed a more distinct specific relationship among populations than the dendrograms that used either oil (4.6 in Chapter 4) or wax data (5.4 in Chapter 5) separately. Therefore, it indicated that in combination the oil and wax chemicals tended to identify phenetic groups corresponding to species.

Furthermore, this dendrogram indicated that the leaf oil and wax chemicals of *Monocalyptus* populations had more specific affinities and greater differences between species than that of *Symphyomyrtus* populations. It is obvious that the variation between *E. regnans* and *E. obliqua* is greater than that between all *Symphyomyrtus* species and, in particular, differences between the majority of *Viminales* species were very small.

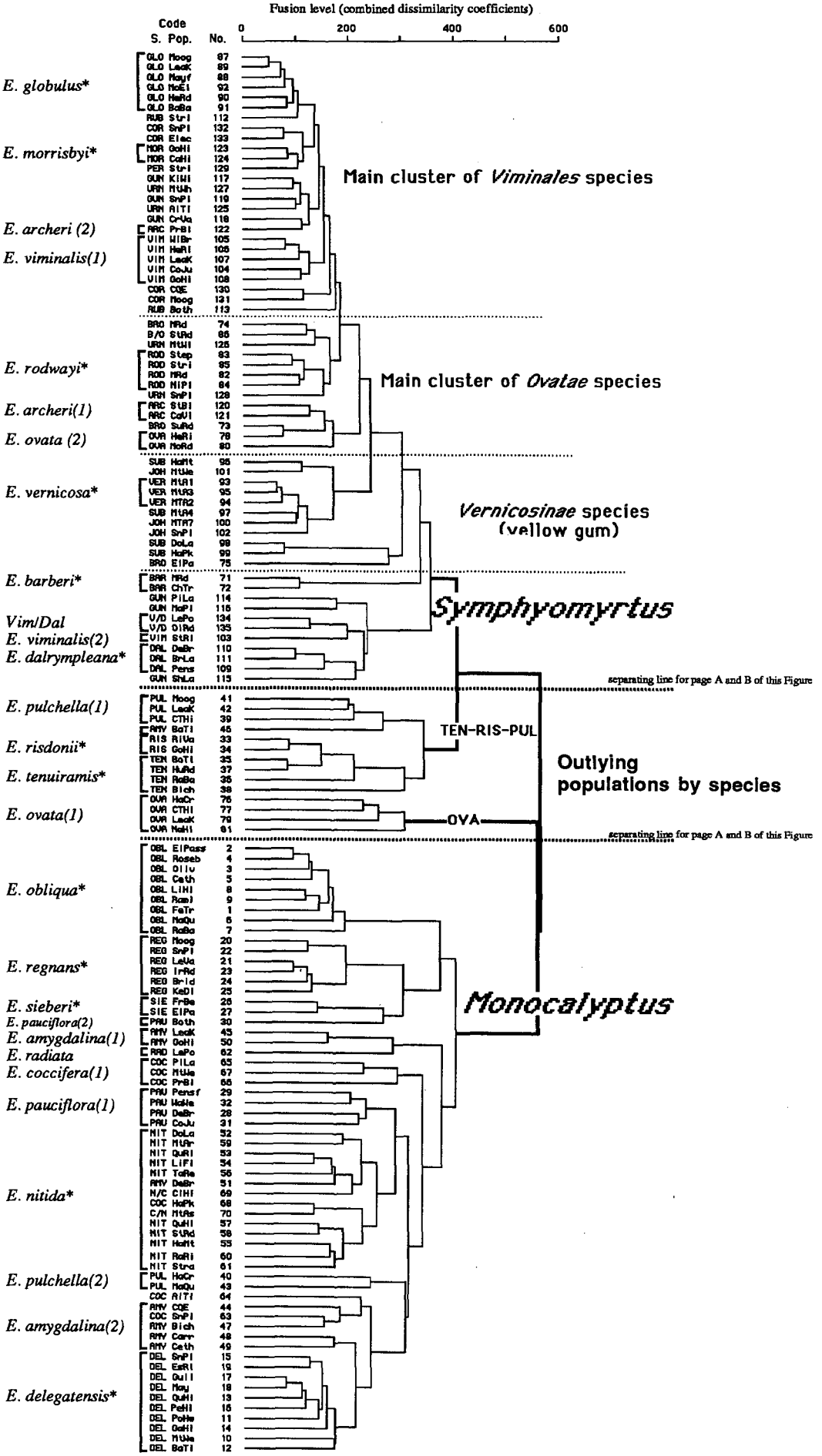


Fig. 6.1. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percentage of wax and oil components of both adult and juvenile leaves of individual populations. * All populations of a species fused in a single specific cluster.

In order to describe this dendrogram in detail, Figure 6.1. was separated into two. Fig. 6.1A includes the main cluster of *Monocalyptus* species and outlying species while Fig. 6.1B includes the main cluster of *Symphyomyrtus* species and outlying species. The chemical relationships among different taxa are discussed separately.

Monocalyptus species

All populations of *E. risdonii* and *E. tenuiramis* and three of the *E. pulchella* populations are differentiated clearly from all other *Monocalyptus* species and lay outside the main *Monocalyptus* cluster. Within the *Monocalyptus* population cluster (Fig. 6.1A), the major dichotomy between population clusters was not between groups that could be assigned to the series *Obliquae* and *Piperitae*, but the division of all populations of *E. obliqua*, *E. regnans* and *E. sieberi* from all other populations. The second dichotomy separated the single population of *E. radiata* and two of the *E. amygdalina* populations and the third dichotomy separated three of the *E. coccifera* populations from the remaining populations. The remaining populations of *E. pauciflora*, *E. delegatensis* and other *Piperitae* species did not separate according to morphological similarity and series. The populations of *E. delegatensis* and *E. pauciflora* were separated into two different clusters and associated with different groups of populations of *Piperitae* species.

Species from the series *Obliquae* in the subgenus *Monocalyptus* are markedly differentiated with respect to their chemotaxonomy, since all populations of the species in this series form five identifiable clusters with the exception of a single population of *E. pauciflora*. Populations of wet sclerophyll species, *E. obliqua*, *E. regnans* and *E. delegatensis*, encompassing a wide geographic range, are discrete and species fuse at a very high level. It is considered that the distinct separation of *Obliquae* species was in agreement with the taxonomic treatment of Pryor and Johnson (1971) who classified these five species into five different subseries. However, this is in disagreement with the proposal of Chippendale (1988). In contrast to the population classification using only wax data which separated species into 'green ash' and 'blue ash' groups, this dendrogram grouped one of the 'blue ash' species, *E. sieberi*, together with the 'green ash', *E. regnans*.

The species of the *Piperitae* show marked intraspecific variation in contrast to the low infraspecific variation of the *Obliqua* species. In particular there was greater genetic variation in wax and oil components within *E. amygdalina*, *E. pulchella* and *E. coccifera*.

Fig. 6.1A. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percentage of wax and oil components of both adult and juvenile leaves of individual populations.This figure shows the main cluster of *Monocalyptus* species and outliers. * All populations of a species fused in a signle specific cluster.

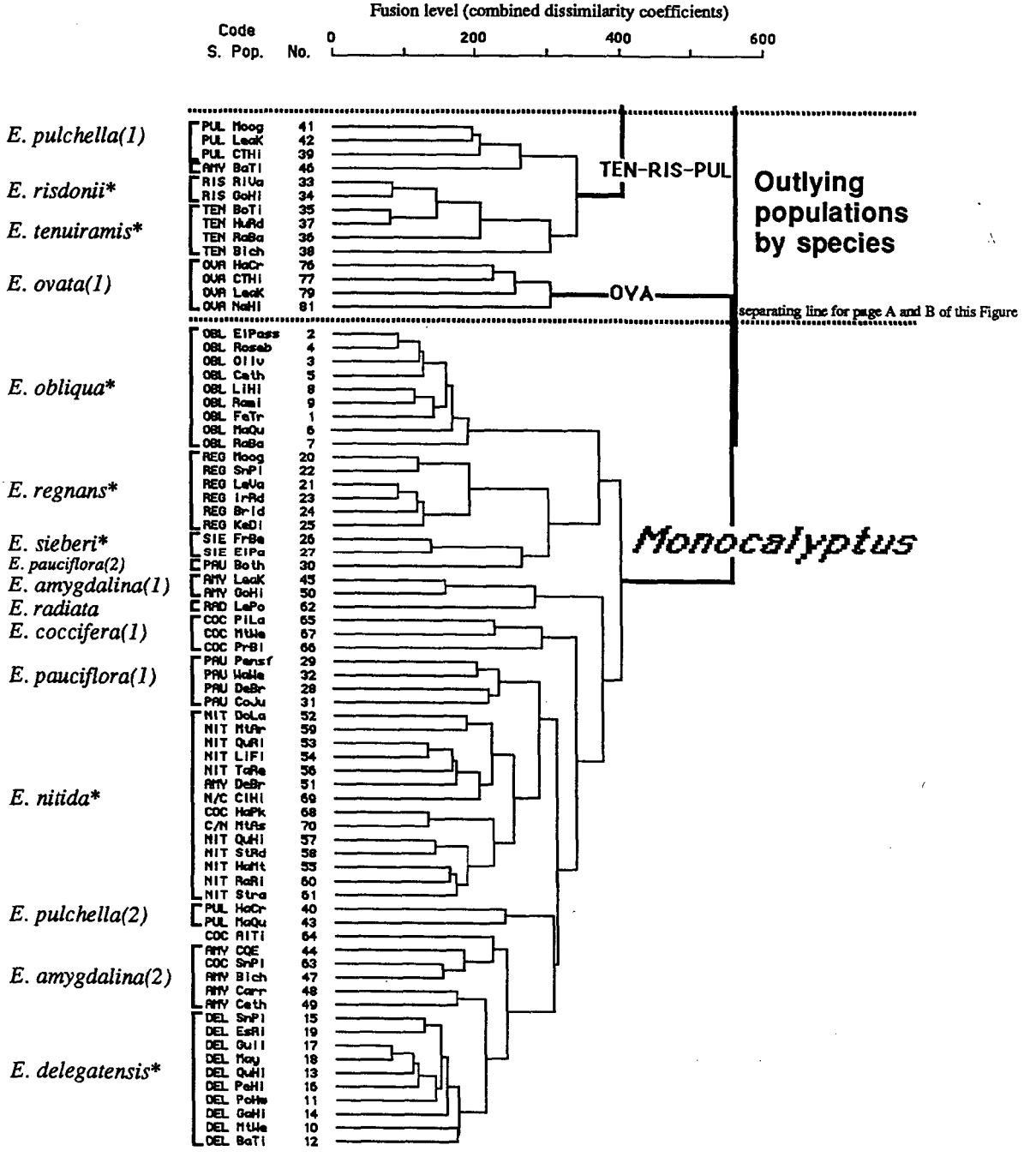


Fig. 6.1A. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percentage of wax and oil components of both adult and juvenile leaves of individual populations.This figure shows the main cluster of *Monocalyptus* species and outliers. * All populations of a species fused in a signle specific cluster.

While populations of *E. nitida*, were distributed over a wide geographic range, they fused together into a single cluster at a relatively low fusion level. *E. risdonii* and *E. tenuiramis* were maintained within a single cluster and the chemical variation between the populations of these two species was less than that within any other species in the series *Piperitae* with the exception of *E. nitida*. These results support the findings of Wiltshire *et al.* (1991) that there was less variation between populations classified as *E. risdonii* and *E. tenuiramis* than there was between populations within *E. tenuiramis*. In particular, the *E. tenuiramis* population from Bicheno (TEN Bich) is the most divergent on chemical criteria and it was also differentiated from most southern populations on morphological criteria (Wiltshire *et al.* 1991).

The populations of *E. pulchella* are classified into two groups, one [*E. pulchella (1)* in Fig. 6.1A] having a close affinity to *E. risdonii/tenuiramis*, the other [*E. pulchella (2)*] close to some *E. amygdalina* populations. The first group includes populations widely distributed from south of Hobart (LeaK) to the east coast (CTHi) whereas the two populations that comprise the other group occur mainly south of Hobart (HaCr and MaQu). There is a marked difference between *E. pulchella* populations sampled in close geographic proximity just south of Hobart from the Lea (Kingston) and at Margate (MaQu).

Chemically, populations of *E. amygdalina* tended toward all other species of the series *Piperitae* and fell into two main groups, a mainly southern locality group (1- LeaK and GoHi) with affinities to *E. radiata* and a predominantly northern group (2- Car, AMY Ceth, AMY Bich; an exception to this was the placement of the southern populations CQE into the northern group). The population from Bakers Tier (BaTi) was classified as *E. amygdalina* on morphological criteria, but clearly has close chemical affinities to *E. pulchella*. This population occurs in an area where intermediates between *E. pulchella* and *E. amygdalina* are widespread (Kirkpatrick and Potts 1986). The populations on Bakers Tier have persistent fibrous bark like *E. amygdalina*, but their leaves are narrower and tend toward *E. pulchella* and on chemical criteria clearly have closer affinities to *E. pulchella*, although on waxes they are closer to *E. nitida*. Similarly a geographical cline occurs between *E. nitida* (west) and *E. amygdalina* (east) (Davidson *et al.* 1986) and the population classified as *E. amygdalina* from Derwent Bridge (DeBr) is in a broad transitional zone. While having some morphological similarities to *E. amygdalina*, the analysis in Fig. 6.1A suggests that this population has closer chemical affinities to *E. nitida*.

The *E. coccifera* populations of group (1) are core populations of pure, high altitude *E. coccifera* encompassing a wide geographical range. The other populations are atypical. While some populations have morphological and chemicals affinities with *E. nitida* (COC

HaPk, N/C CiHi, C/N MtAr; see Shaw *et al.* 1984), the population SnPl which appears to be hybridising with *E. pulchella* (SnPl; see Davidson *et al.* 1981) has chemical affinities with *E. amygdalina*. The population classified as *E. coccifera* from Alma Tier (AlTi) is clearly distinct from the typical *E. coccifera* and has close chemical affinities to some *E. amygdalina* populations. This large stabilised population has been recorded as atypical *E. coccifera* (Shaw *et al.* 1984) and intermediate in morphology between *E. tenuiramis* and *E. coccifera* (Wiltshire *et al.* 1991) and its taxonomic status requires to be re-assessed.

Symphyomyrtus species

The eastern *E. ovata* populations are outliers from the main cluster of *Symphyomyrtus* species. Within the *Symphyomyrtus* cluster (Fig. 6.1B), the major dichotomy separated populations of *E. dalrympleana* and *Vim/dal* and one of *E. viminalis* (StRi) and three of *E. gunnii* (ShLa, PiLa and MaPl) from other populations. The second dichotomy separated the populations of *E. barberi* and the third separated two populations of the *E. subcrenulata* (DoLa and HaPk) and one of *E. brookeriana* from other species populations. The remaining populations were grouped into three clusters. All remaining populations of the yellow gums formed a single cluster and the remaining populations of the *Ovatae* species and two *E. archeri* populations formed another single cluster that was separated from the cluster of the remaining *Viminales* species populations.

Many populations of *Symphyomyrtus* species were fused into species specific clusters (e.g. *E. globulus, E. rodwayi, E. morrisbyi, E. vernicosa, E. barberi and E. dalrympleana*) (Fig. 6.1.B), or at least into groups which could be assigned to the series of *Ovatae* species and series *Viminales*, subseries *Vernicosinae* (yellow gums) and a mixture of subseries *Globulinae*, *Viminalinae* and *Cordatinae*. All these populations within the mixed group had very similar chemicals and the populations fused at a lower level than even some populations within the *Monocalyptus* species, e.g. *E. regnans* and *E. obliqua*. In contrast, *E. ovata*, *E. gunnii* and *E. urnigera* were extremely variable.

The dendrogram indicated that all yellow gum populations were well grouped into a cluster with high fusion level with the exception of the two populations of *E. subcrenulata* from Cradle Mountain (DoLa and HaPk). Thus, the chemical composition of leaf oils and waxes from these yellow gum populations was very similar while only two northern populations of *E. subcrenulata* (DoLa and HaPk) differed markedly from the core yellow gum populations to have close chemical affinities with *E. brookeriana* population (ElPa). Moreover, a similarity in chemical composition existed in the *E. vernicosa* complex on Mt Arrowsmith despite the marked morphological cline from tall forest (columnaris) to the short form on this mountain (see Jackson 1960).

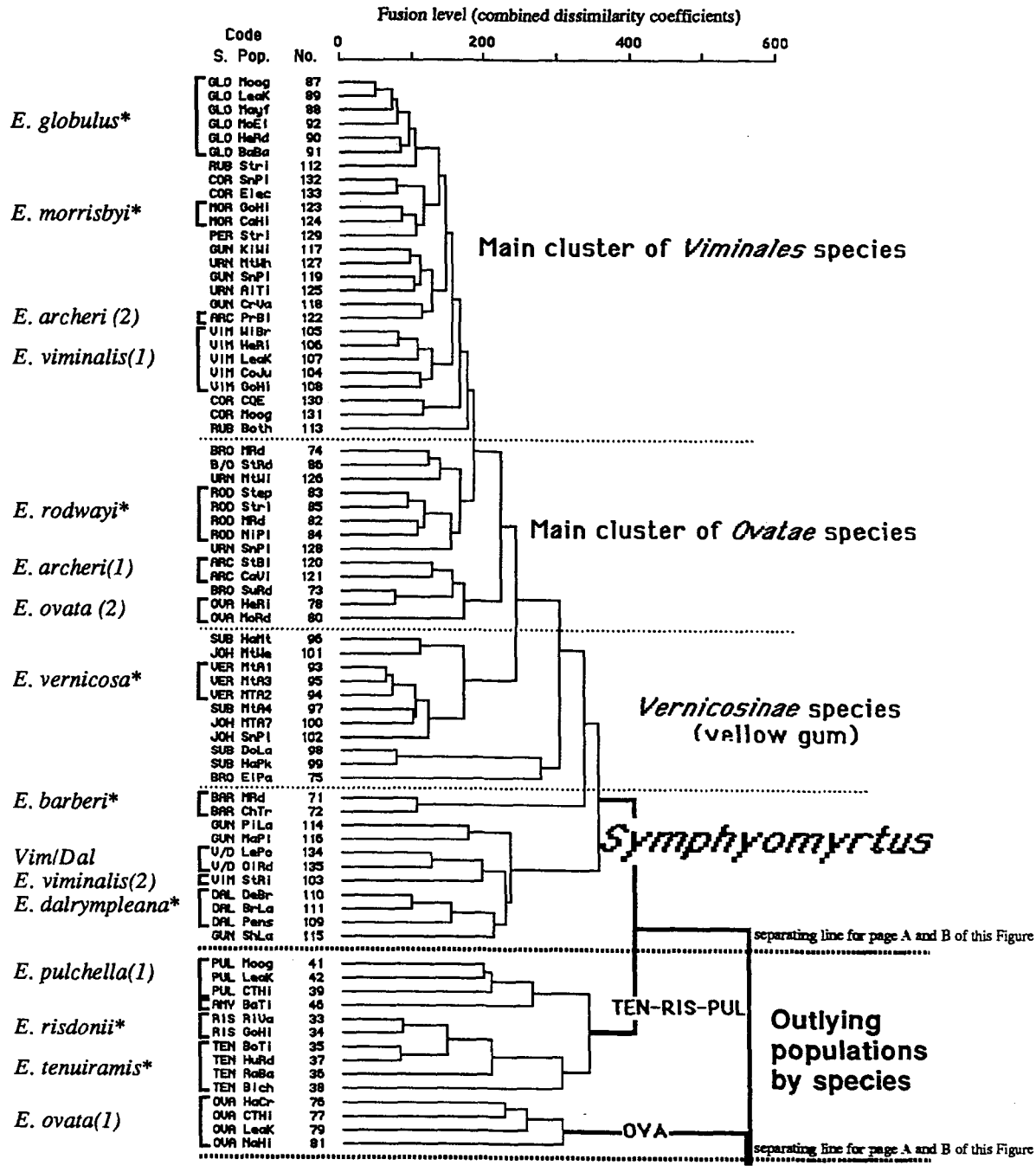


Fig. 6.1B. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percentage of wax and oil components of both adult and juvenile leaves of individual populations. This figure shows the main cluster of *Symphyomyrtus* species and outliers.

* All populations of a species fused in a signle specific cluster.

But, in contrast to the close affinities between species of yellow gums, the chemical composition of the *Ovatae* varied markedly between species and populations within species. There was relatively little variation within the species *E. barberi* and *E. rodwayi* whereas *E. ovata* was extremely variable. The eastern populations (1- HaCr, CTHi, LeaK and NaHi) did form a single fairly discrete cluster, but this was an outlier to the main series name cluster (and most other *Symphyomyrtus* species). The two western populations (2- HeRi and MoRd) classified on morphological criteria as *E. ovata* differed markedly from the eastern group of *E. ovata* populations and have close chemical affinities to the population SuRd which is classified as *E. brookeriana*. The variation between populations of *E. brookeriana* was also marked. While an *E. brookeriana* population (MRd) was close to *E. rodwayi* and another population (SuRd) to the western group of *E. ovata* populations, the eastern population (ElPa) of *E. brookeriana* differed markedly from other populations. In addition the dendrogram further confirmed that the intermediate (B/O StRd) between *E. brookeriana* and *E. ovata* has closer affinity to *E. brookeriana* than to *E. ovata*.

The results also indicated that within *Symphyomyrtus*, both *E. gunnii* and *E. urnigera* vary markedly as their populations were dispersed over the different population clusters within the subgenus. The two glaucous populations (AlTi and MtWh) of *E. urnigera* had close affinity with three of the *E. gunnii* populations (KiWi, SnPl and CrVa) and the subglaucous populations of *E. archeri* (PrBl) while the two green populations of *E. urnigera* (MtWl and SnPl) had closer affinities to the two green populations of *E. archeri* (StBl and CaVi) and *Ovatae* species. Populations of *E. gunnii* also were spread across separate clusters. The three populations, KiWi, SnPl and CrVa, were grouped with the glaucous populations of *E. urnigera* and the other three populations, PiLa, MaPl and ShLa, were grouped into the cluster of *E. dalrympleana* and Vim/dal populations. Populations of *E. cordata*, *E. archeri* and *E. rubida* were also widely separated in the dendrogram.

E. archeri is often treated as a green form of *E. gunnii* (e.g. Potts and Reid 1985). The green populations of *E. archeri* (*E. archeri* 1) from Ben Lomond (StBl and CaVi) are geographically isolated from other populations of *E. gunnii* and *E. archeri* whereas the glaucous population (*E. archeri* 2) from Projection Bluff (PrBl) is one extreme of a genetic and geographical continuum between *E. archeri* and *E. gunnii* on the Central Plateau of Tasmania. This cluster analysis indicated that the chemical affinities of the *E. archeri* population from Projection Bluff (PrBl) was close to *E. gunnii* to which it is clinally linked. However, the two Ben Lomond populations (StBl and CaVi) of *E. archeri* were chemically distinct from *E. gunnii* with close chemical affinities with 'west coast *ovata/brookeriana* ' populations, which are green.

The cluster analysis also indicated that the morphologically intermediate populations (*Vim/dal*) between *E. viminalis* and *E. dalrympleana* were closer to *E. dalrympleana* than to *E. viminalis*. The population StRi that was classified as *E. viminalis* was very similar to the *Vim/dal* populations and was separated from the main species cluster of *E. viminalis* populations. The chemical affinities of *Vim/dal* populations shown by this cluster analysis is similar to the previous classification using only oil data. Comparison made between the previous classification using only oil or wax data indicated that *E. viminalis* and *E. dalrympleana* had greater inter-specific difference in oil chemicals than wax chemicals. The chemical affinity indicated in this classification therefore reflects more the chemical affinity of the leaf oils.

Comparisons between classifications using juvenile and adult sample data

The dendrogram for the adult leaf samples is shown in Figure 6.2 and that for juvenile leaves in Figure 6.3. As expected, these two dendrograms differed in detail but not in overall pattern. The dendrogram using juvenile leaf samples (Fig. 6.3) classified all populations of *Symphyomyrtus* species into a continuous cluster with only four populations of *E. ovata* outside. In contrast, the dendrogram using adult leaf samples (Fig. 6.2) separated *E. dalrympleana*, the *Vim/dal* populations and one *E. gunnii* population from the main cluster of *Symphyomyrtus* populations and four *E. ovata* populations remained outside the main cluster. Thus, juvenile sample data showed a more distinct separation of subgenera than did adult samples.

However, the dendrogram derived from the adult sample data showed a stronger clustering of species compared with that from the juvenile data set. For example, all populations of *E. cordata* and *E. archeri* were grouped into specific clusters and most populations of *Piperitae* species (with the exception of *E. amygdalina)* formed identifiable species clusters in the dendrogram using adult leaf samples. In contrast, most juvenile populations of *Piperitae* species varied markedly. Hence, the marked chemical variation between populations within the *Piperitae* species appears to be mainly due to chemical variation in juvenile leaves for that of adult leaves resulted in strong species clusters.

Adult leaves of the two *Vim/dal* (V/D) populations were very similar to *E. dalrympleana.* However, the juvenile leaves of one of the *Vim/dal* (V/D) populations OlRd, fell into the pure *E. viminalis* cluster while the other LePo, fell into the mixed cluster of *E. dalrympleana, E. gunnii* and *E. viminalis*. Thus, these results indicated that the chemical affinities of adult leaves of *vim/dal* tended to be closer to *E. dalrympleana* and juvenile leaves tended to be closer to *E. viminalis*. The adult leaves of *E. dalrympleana* and the intermediate form, *Vim/dal,* were outliers from the major cluster of *Symphyomyrtus* populations but their juvenile leaves were within the general *Symphyomyrtus* cluster.

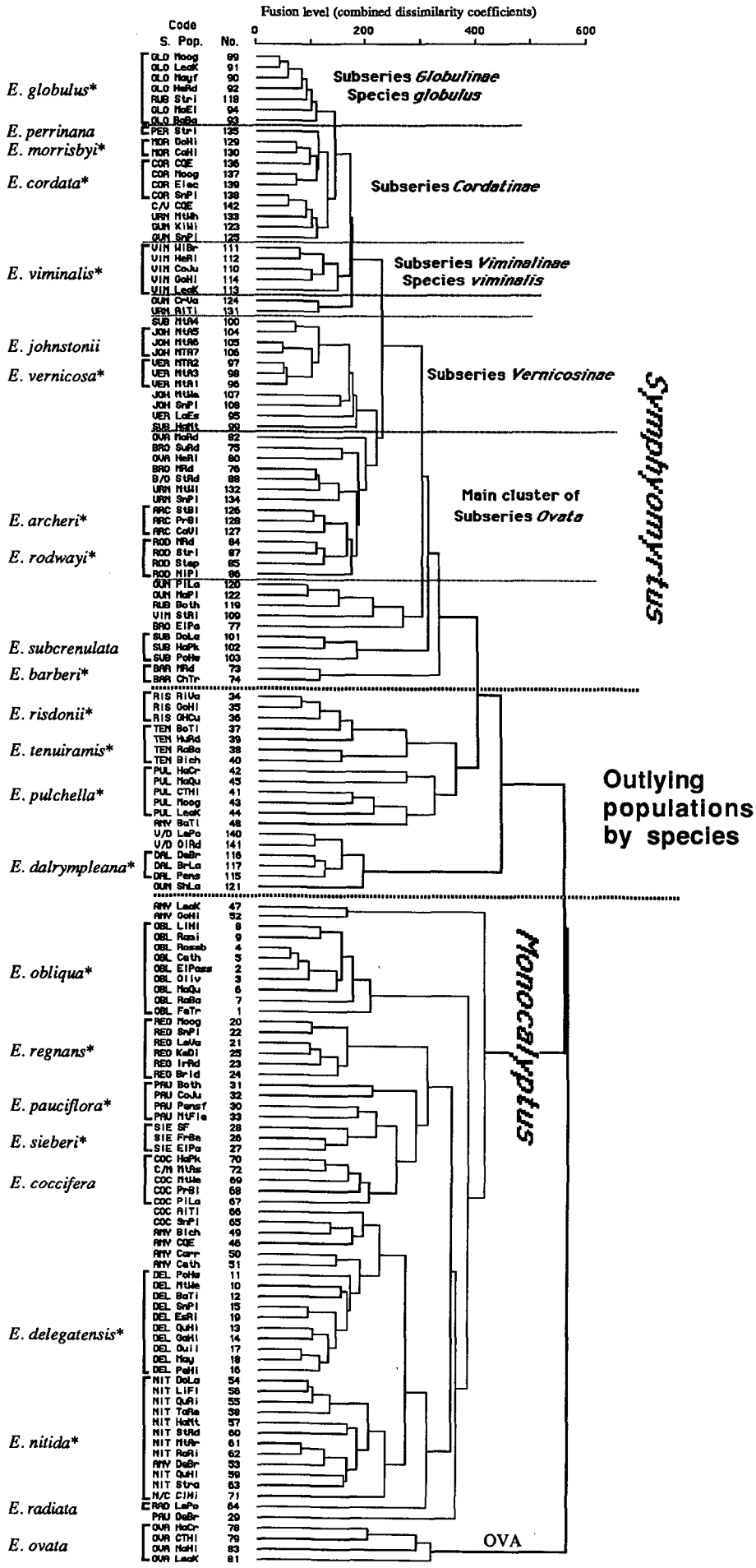


Fig. 6.2. Chemotaxonomic representation of average linkage clustering of locality populations of 29 eucalypt species, using percentage data of all components of adult leaf waxes and oils of individual populations.* All populations of a species fused in a signle specific cluster.

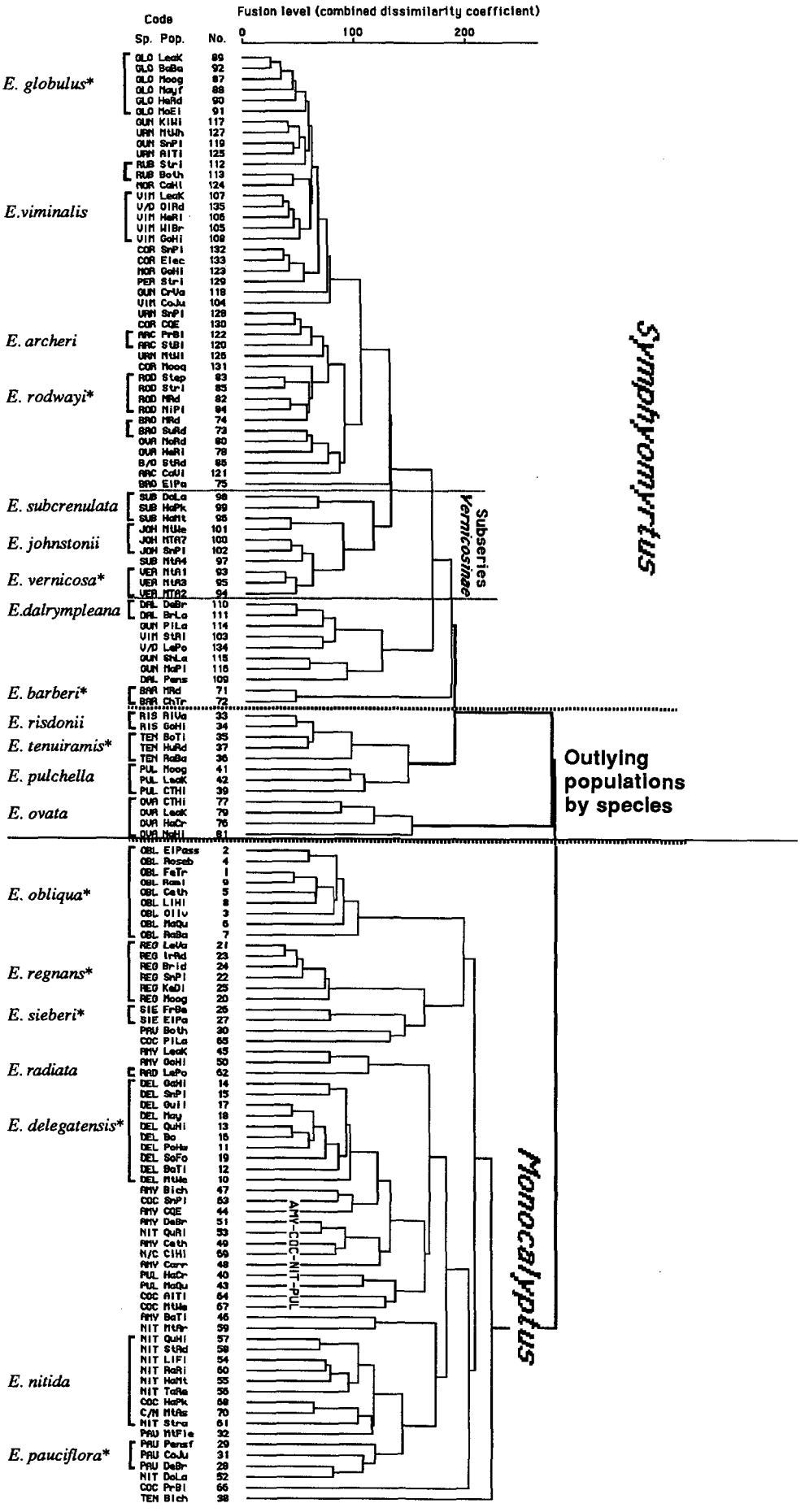


Fig. 6.3. Chemotaxonomic representation of average linkage clustering of locality populations of 29 eucalypt species, using percentage data of all components of juvenile leaf waxes and oils of individual populations.

Hence, the juvenile leaves of *E. dalrympleana* and *Vim/dal* populations had closer chemical affinities to the majority of *Symphyomyrtus* species than did adult leaves.

Moreover, adult samples assigned most *Symphyomyrtus* species into different subseries groups, although some overlap occurred. Thus, the majority of series *Viminales* was grouped into subseries *Globulinae, Vernicosinae, Viminalinae* and *Cordatinae.*

Overall, clustering of adult samples had less infraspecies variation and better reflected species and subseries taxonomy than juvenile samples.

Clustering analysis using Ward's method

Ward's clustering method indicated many more specific clusters of species populations (Figure 6.4). This technique is particularly powerful in partitioning continuous variation into equitable units. This cluster analysis grouped all atypical species populations into the outlying populations cluster, which did not exist in Average linkage analysis, and grouped all remaining species populations into typical *Monocalyptus* species group and *Symphyomyrtus* species groups respectively. The Ward's method tends to impose structure on the data resulting in groups that may not exist naturally (e.g. the outlying populations group). However, this dendrogram more clearly defined relationships between populations of species groups, and particularly the inter-specific relationships of some *Symphyomyrtus* species as defined by Pryor and Johnson (1971).

Within the typical *Symphyomyrtus* cluster, species populations were clearly separated into three species groups which could be related to Pryor and Johnson's classification. The three groups are: i) the mixed group including the majority of the subseries *Cordatinae* and all populations of *E. globulus* and *E. viminalis*, representing the subseries *Globulinae* and *Viminalinae*; ii) the subseries *Vernicosinae* and, iii) the subseries *Ovatae* cluster contained most *Ovatae* species populations, with the exception of core *E. ovata* populations.

Moreover, within the mixed group, *E. globulus* and *E. viminalis* populations were separated from the majority of *Cordatinae* species populations and further separated into individual specific clusters. Thus, the inter-specific chemical affinities among the majority of *Symphyomyrtus* species populations indicated by this dendrogram were correlated to the subseries proposed by Pryor and Johnson (1971).

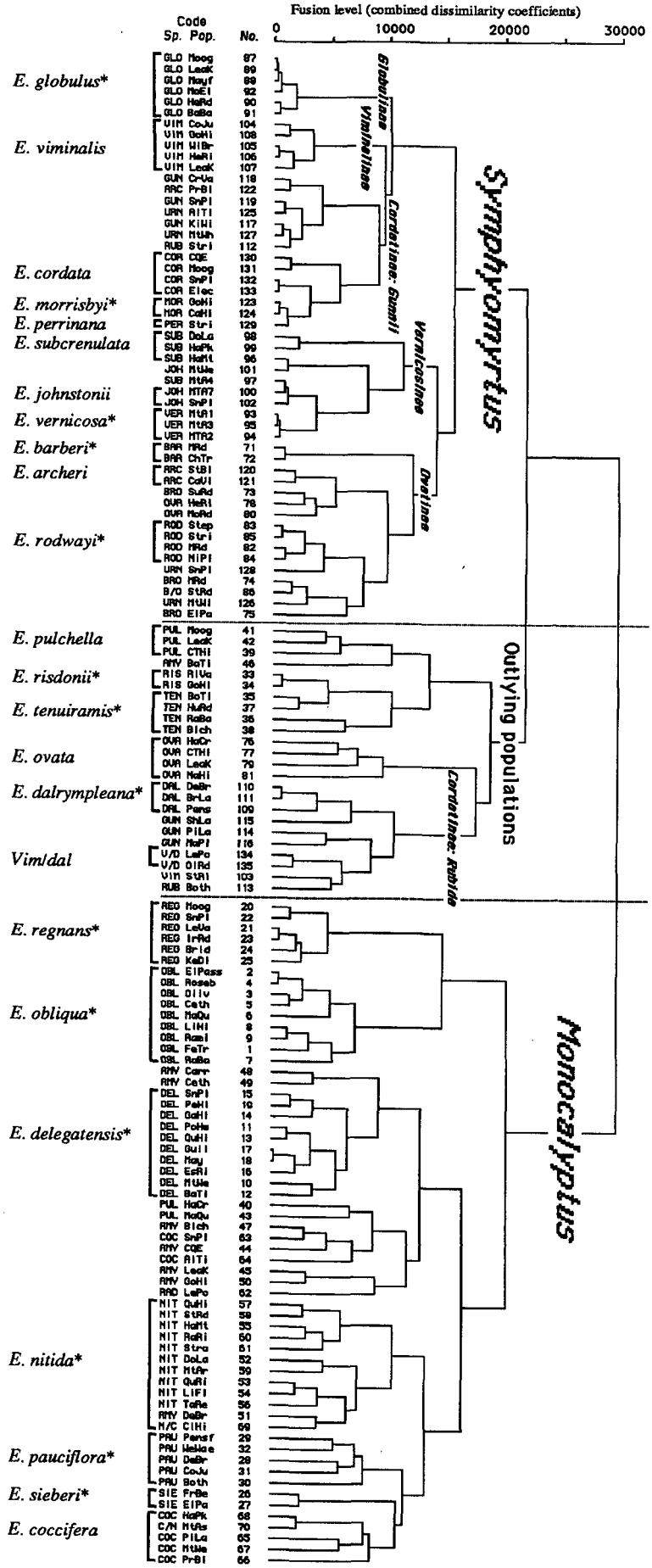


Fig. 6.4. Chemotaxonomic representation of linkage clustering of 135 populations of 29 eucalypt species, using the Ward method based on the percentage data of all components of both adult and juvenile leaf waxes and oils of individual populations.

Furthermore, this dendrogram indicated that all populations maintained in the major cluster of subseries *Cordatinae* were the species populations of superspecies *Gunnii*, with the exception of one population of *E. rubida* (StRi) from superspecies *Rubida*. Thus, this cluster could be recognised as a typical population cluster of superspecies *Gunnii*. With the exception of green populations of *E. urnigera* and *E. archeri*, all populations of *Gunnii* species were maintained in this cluster. Although the majority of *Rubida* superspecies was outlying from the main cluster of *Symphyomyrtus* species, they were all grouped into a cluster with three populations of *E. gunnii*. This indicated that the inter-specific relationships among populations of the subseries *Cordatinae* were relatively well maintained.

Populations of some individual species that were separated in the average linkage method were grouped in the dendrogram produced by Ward's method. For example, an outlier population (Both) of *E. pauciflora* in Fig. 6.1 was grouped with other populations of *E. pauciflora* in Fig. 6.4 and the four populations of *E. coccifera* formed a specific cluster, although there were two (AlTi, SnPl) still outlying .

6.1.2. Species classification

The chemical distance among the 29 species, as estimated from both the leaf oils and waxes, is shown in Figure 6.5. This dendrogram is derived from the average-linkage clustering of the mean percentage composition of all oil and wax components of both adult and juvenile leaves of individual species using unstandardised and unweighted data. As with the classification of populations, this dendrogram is also somewhat intermediate between that using only leaf oil and that using leaf wax data. With the exception of the three species, *E. risdonii*, *E. tenuiramis* and *E. pulchella*, which were grouped together with the *Symphyomyrtus* species, the close affinities that exist between certain species reflect the separation of *Monocalyptus* and *Symphyomyrtus* species. However, the interspecific structure within individual subgenera does not follow the series divisions.

This dendrogram indicated that the chemical affinities of the three species, *E. risdonii*, *E. tenuiramis* and *E. pulchella* differed markedly from other *Monocalyptus* species and varied toward *Symphyomyrtus* species. The remaining *Monocalyptus* species were not associated with the separation of species into series.

Within *Symphyomyrtus* species, two of the *Ovatae* species, *E. ovata* and *E. barberi*, differed markedly from other *Viminales* species but the other two *Ovatae* species *E. brookeriana* and *E. rodwayi*, were grouped with the *Viminales* species. One of the *Viminales* species, *E. dalrympleana*, also differed markedly from other *Viminales*

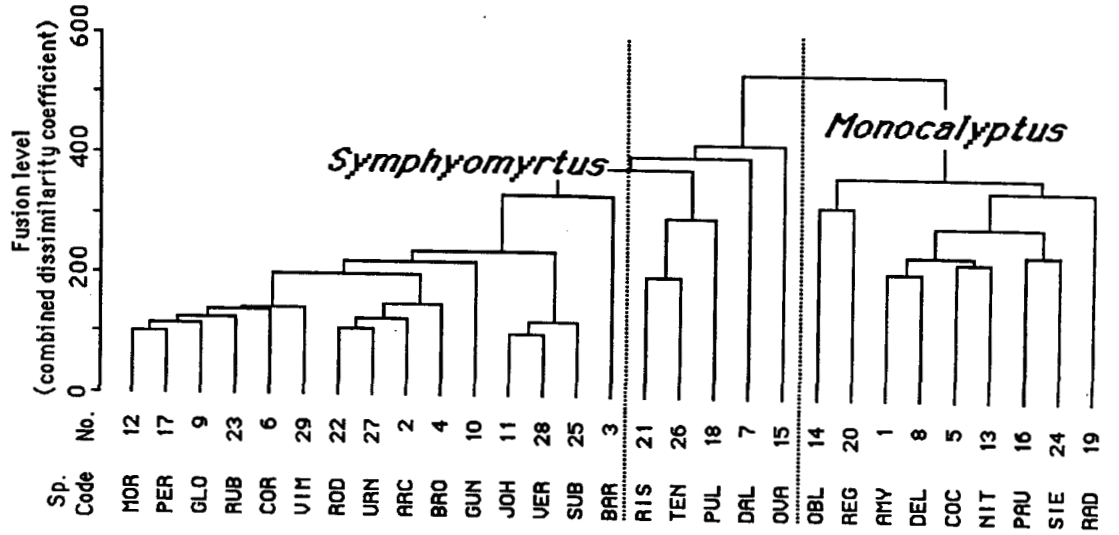


Fig. 6.5. Chemotaxonomic representation of average linkage clustering for 29 *Eucalyptus* species, using mean percentage of all wax and oil components of both adult and juvenile leaves of individual species (unstandarized and unweighted).

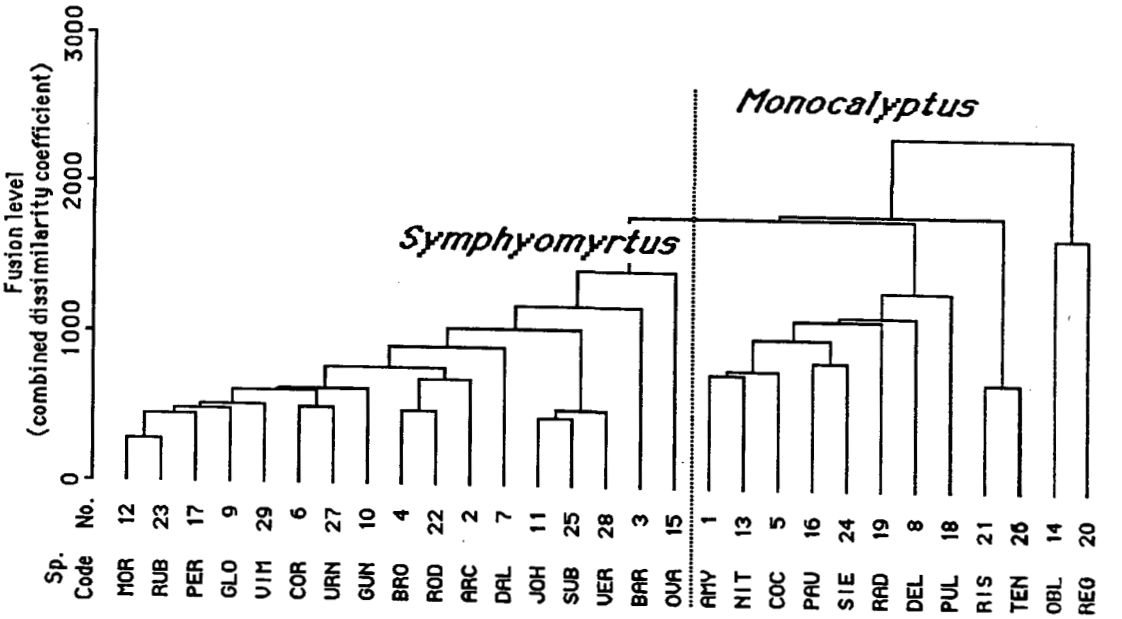


Fig. 6.6. Chemotaxonomic representation of average linkage clustering for 29 *Eucalyptus* species, based on the mean percentage of all wax and oil components of both adult and juvenile leaves of individual species (standarized and *F*-weighted).

species. The dendrogram indicated less variation amongst the yellow gum species and they were markedly different from other *Viminales* species.

The average-linkage clustering analysis using standardised and weighted data of both adult and juvenile leaf sample is shown in Fig. 6.6. This analysis weights characters in the manner which emphasises characters which show little intraspecific variation and large differences between species.

It is of interest that the three *Monocalyptus* species, *E. risdonii, E. tenuiramis* and *E. pulchella*, which were grouped with *Symphyomyrtus* species in the dendrogram using unstandardised and unweighted data, were drawn out from the *Symphyomyrtus* species cluster in this dendrogram. This indicated that the species affinities of these three species was not close to *Symphyomyrtus* species despite their similarities in some chemical characters (e.g. 1,8-cineole levels). Moreover, this dendrogram also indicated that *E. pulchella* is closer to other *Piperitae* species than to *E. tenuiramis* and *E. risdonii*. Again this dendrogram indicated that *E. dalrympleana* had close affinities with other *Viminales* species in contrast with the marked difference indicated by the dendrogram using unstandardised and unweighted data.

Comparison of species variability within subgenera indicated that the chemical differences between *Monocalyptus* species were much greater than differences within *Symphyomyrtus*. Thus, the differences between species even within series of either *Obliquae* or *Piperitae* in *Monocalyptus* were greater than that within the subgenus *Symphyomyrtus*, with the exception of *E. ovata* and *E. barberi*. This dendrogram indicated a significant species difference existing between 'green ash' and the 'blue ash' groups but also a marked difference existing between the two 'green ash' species. The close chemical affinities occurring between *E. risdonii* and *E. tenuiramis* were in agreement with the findings of other workers as described previously. However, the marked differences that occurred between these two species and the other *Piperitae* species did not agree with the proposal of Ladiges *et al.* (1983) who classified these two species with *E. nitida* and *E. coccifera* into a subseries *Cocciferinae*.

Within the *Symphyomyrtus* cluster, the differences between *Ovatae* species were greater than differences between *Viminales* species but species were not assigned into series as proposed by Pryor and Johnson (1971). In this dendrogram, the *Vernicosinae* (yellow gum) species and two of the *Ovatae* species, *E. ovata* and *E. barberi*, were clearly separated from other *Viminales* species but the other two *Ovatae* species, *E. brockeriana* and *E. rodwayi* were grouped with a *Viminales* species, *E. archeri*, to form a single cluster. With the exception of *E. dalrympleana*, the other remaining *Viminales* species were maintained in a cluster with high fusion level.

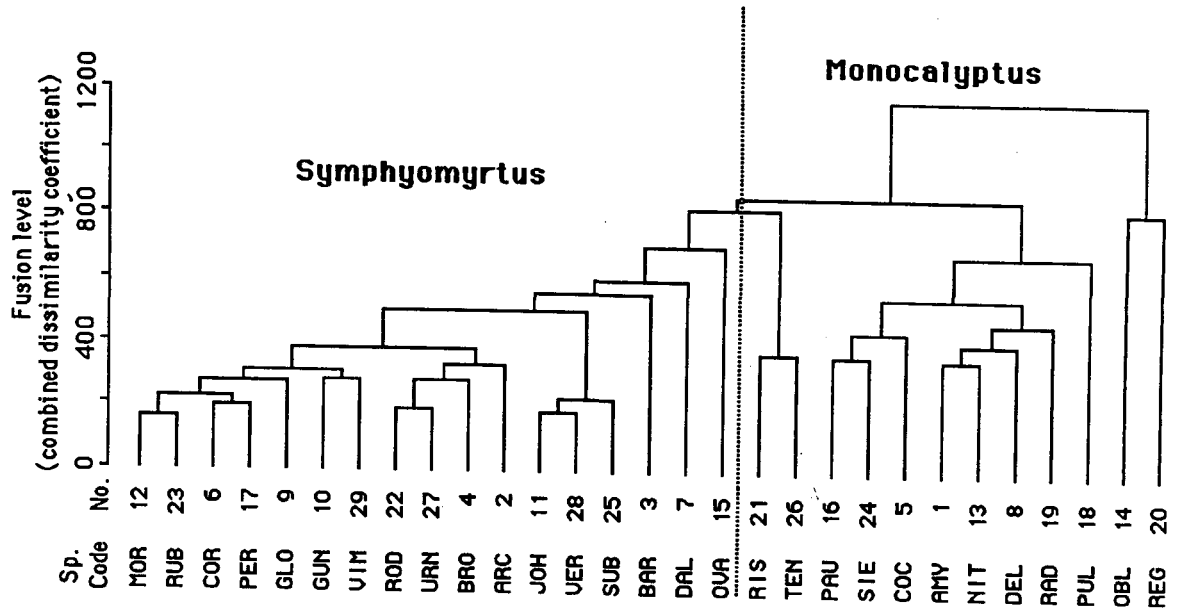


Fig. 6.7. Chemotaxonomic representation of average linkage clustering for 29 *Eucalyptus* species, using mean percentage of all wax and oil components of adult leaves of individual species (standarized and *F*-weighted).

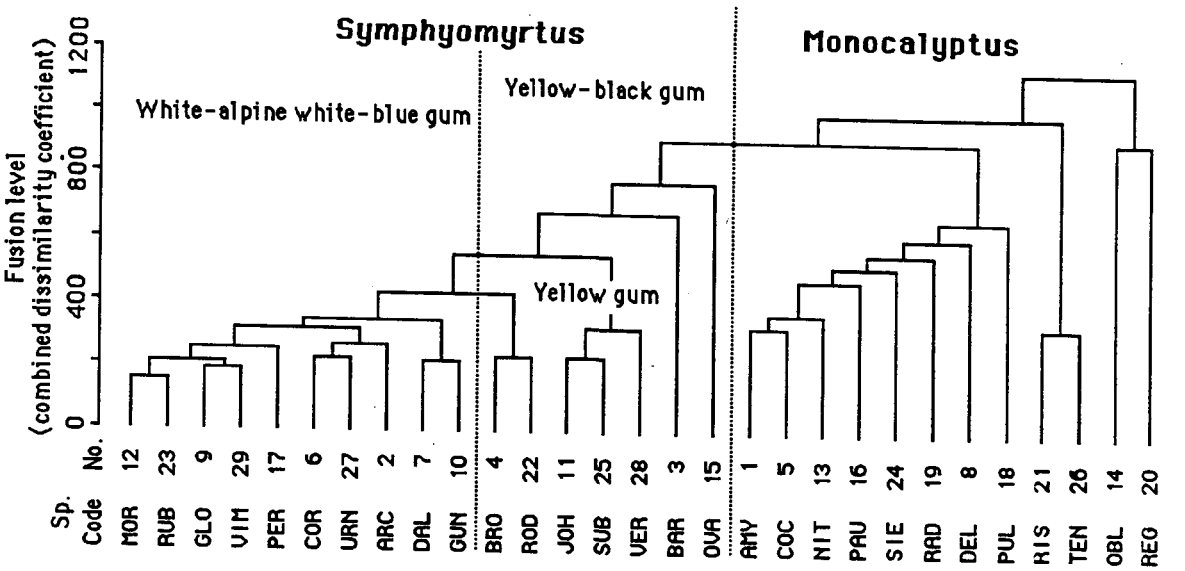


Fig. 6.8. Chemotaxonomic representation of average linkage clustering for 29 *Eucalyptus* species, based on the mean percentage of all waxand oil components of juvenile leaves of individual species (standarized and *F*-weighted).

The average-linkage clustering analysis of adult leaf samples is shown in Figure 6.7 and for juvenile leaf samples in Figure 6.8. These two dendrogram indicated that the association of oil and wax chemicals of either adult or juvenile leaf samples of the Tasmanian eucalypt species supports the morphological division into the subgenera *Monocalyptus* and *Symphyomyrtus*.

The dendrogram using adult leaf sample data indicated an interspecific relationship between *Piperitae* species which matched the classification of Pryor and Johnson (1971). It indicated that the *E. amygdalina, E. nitida* and *E. radiata* were grouped together with the differences between these three species similar to that between *E. risdonii* and *E. tenuiramis*. This agreed with the treatment of Pryor and Johnson (1971) who classified these species into the superspecies *Amygdalina* and *Risdonii* respectively. Moreover, the three *Amygdalina* species had chemical affinities intermediate between *E. coccifera* and *E. pulchella* while *E. coccifera* was most different from superspecies *Risdonii*.

6.2. A taxonomic evaluation

Similar to studies in many plant systems, results of this study indicated that the chemical characteristics of leaf oils and waxes have taxonomic value in the Tasmanian eucalypts at all levels of the taxonomic hierarchy. While chemical characteristics showed a distinct difference between subgenera and between species groups, they specifically characterised most of the Tasmanian eucalypt species. In many cases the combined chemical characteristics of leaf oils and waxes provided a "fingerprint" for individual species.

Samples used in this study were from different localities of individual species. Therefore, the variation observed in this study reflected the basic patterns within and between species and thus indicated species relationships and variability within species. Results suggested that variation in chemicals of both leaf oils and waxes were strongly correlated with the levels of species differences and infraspecies variation observed in morphological characteristics by other workers as discussed. Thus, differentiation in chemical traits in the subgenus *Monocalyptus* (Pryor and Johnson 1971) were generally greater than that in *Symphyomyrtus*. Those *Monocalyptus* species, which had greater infraspecies variation in morphology, also showed a greater variation in chemical composition (e.g. *Piperitae* species) in contrast to those species with less infraspecies variation (e.g. *Obliquae* species).

Variation in leaf chemicals could be genetic or environmental as species tend to occupy different ecological habitats. However, results of this study provide evidence to suggest

that the observed chemical variation amongst the Tasmanian eucalypt species is mainly determined by the genetic makeup of the taxa.

(i) results from previous chapters have shown that species differences are maintained in experimental trials and when the same material was planted across several sites (see chapter 3).

(ii) differences are maintained between species collected from the same locality (e.g. species from Snug Plains mixed forest).

(iii) in many cases species populations are geographically widely distributed but species similarities and differences were maintained.

Results indicated that the combinations of oil and wax data resulted in a better differentiation of species and even better differentiation may be obtained if more leaf chemicals are examined.

As observed in morphological characteristics, this study indicated that some species may share similar compounds which are the common characteristics of a species group, but individual species may differ in other compounds. For example, *E. regnans* and *E. obliqua* had close wax affinities, but differed markedly in oil chemicals. Moreover, the phenotype expression of a species must maintain the specific characteristics in different localities but individual localities of a species could differ in other chemical characteristics which may be resulted by environmental modification or genetic variation. The best example could be found in the *E. tenuiramis-E. risdonii* complex. All localities of *E. tenuiramis* had similar wax chemical composition, however, individual localities differed markedly in oil composition and this appeared to be associated with clinal variation in morphological. Again, the combination of both oil and wax data is consistent with *E. risdonii* being clearly related and clinally linked with *E. tenuiramis* as observed in morphological characteristics (Wiltshire *et al.* 1991).

Results of this study also indicated that the determination of the origin of homologues of similar compounds in different species is fundamental in the taxonomic application of leaf oil and wax chemicals as emphasised by Hegnauer (1986). For example, it was found in this study that the presence of similar compound classes or individual compounds in different species may not be biosynthetically and phylogenetically related. *E. risdonii* and *E. tenuiramis* had high contents of ß-diketones and 1,8-cineole which is similar to most of the *Symphyomyrtus* species. However, the homologous of ß-diketones in these two *Piperitae* species differed from those of *Symphyomyrtus* species. The high proportions of 1,8-cineole and α–pinene, which are biosynthetically related, is a common characteristic of leaf oils in *Symphyomyrtus* species. In contrast, α–pinene occurred only

rarely in the leaf oils of *E. risdonii* and *E. tenuiramis* suggesting that there may be a specific system which blocks its biosynthesis or accumulation. It is also probable that the 1,8-cineole could be synthesised by different pathways, since the plants of the two taxa may synthesise or accumulate the same products by different pathways (Siegler 1981).

6.3. Variation in leaf oils and waxes related to ecological formation

Results of this study indicated that variation in both leaf oils and waxes could be associated with ecological formations of eucalypts in Tasmanian which were mainly determined by water availability and temperature gradients.

First, the wet sclerophyll species in Tasmania generally exhibited less infraspecific variation. For example, *E. regnans, E. obliqua, E. delegatensis* and *E. globulus,* which occur over an extensive geographic range, exhibited less variation over the wide range of localities sampled. In contrast, species occurring in either dry sclerophyll and subalpine habitats tended to have greater infraspecific variation, in particular the *Piperitae* species.

Second, the wet sclerophyll species tended to have greater subgeneric differences and present typical subgeneric chemotypes but species from dry and subalpine, particularly dry sclerophyll conditions tended to be more variable.

Monocalyptus species occurring in wet sclerophyll forest, produced little or no 1,8-cineole and ß-diketones, e.g. 'ash' species *E. regnans, E. obliqua* and *E. delegatensis.* In contrast, those *Monocalyptus* species, which grow in dry sclerophyll and subalpine habitats tended to have higher levels of 1,8-cineole, e.g. *E. sieberi* and *E. pauciflora,* or either 1,8-cineole and ß-diketones, e.g. *Piperitae* species. However, the association between leaf chemicals and habitat is relatively poor for *Symphyomyrtus* species.

Thirdly, the change in chemical composition of leaf waxes and oils among *Monocalyptus* species appeared to associated with water availability and dryness of habitat. The change in subalpine species tended to be associated with altitude gradients. However, change in leaf chemicals by species adapted to dry sclerophyll habitats appeared to be more closely linked to aridity than to altitude. *Piperitae* species were particularly involved in such change.

Although differentiation occurs with species from the wet sclerophyll forest habitat, populations within species are relatively uniform whereas in extreme dry sclerophyll and subalpine environments species appeared to be more variable in leaf chemicals.

Monocalyptus species tended to show more distinctive changes in leaf oil and wax composition according to their habitat. However, within each ecological zone, species differences between *Monocalyptus* species were greater than between *Symphyomyrtus* species.

The mean percentage content of 1,8-cineole in leaf oils and ß-diketone contents in leaf waxes for *Piperitae* species in dry sclerophyll forests are shown in Table 6.1. This summary indicates that *Piperitae* species increased ß-diketone and 1,8-cineole contents and formed tube wax in response to drier habitats. However, *E. coccifera* populations adapted to high altitude habitats increased only ß-diketones but not 1,8-cineole. *E. nitida*, which tends to predominate in high rainfall areas of the west and south-west did not show any marked increase of ß-diketone content and did not form tube wax, except when intergrading with *E. coccifera*.

Table 6.1. The mean percentage contents of major compound classes of waxes and 1,8-cineole of oils for juvenile and adult leaves of *Piperitae* species, the altitudinal distribution of samples and the habitat in which species occurred . *J-A: juvenile - adult leaves.

	ß-Diketone J-A*	Triterpenes J-A	Aldehyde J-A	1,8-Cineole J-A	Wax Form	Altitude (m)	Habitat
E. risdonii	61-66	1-1	3-7	60-61	tube	60-100	very dry, skeletal soils on mudstone
E. tenuiramis	55-58	2-2	8-14	38-35	tube	40-400	dry soils mainly on mudstone and dolerite
E. pulchella	17-20	15-16	22-23	43-47	tube-plate	60-160	dry soils on dolerite
E. amygdalina	3-4	24-31	31-39	14-15	plate	50-750	dry sandy podsolic soils on sandstone or mudstone
E. coccifera	3-15	29-31	25-35	12-5	tube-plate	640-1150	Cold well-drained sites, form treeline on most dolerite capped mountains
E. nitida	1-1	58-61	20-17	11-1	plate	160-950	infertile skeletal soil or peaks in high rainfall areas of west and south-west

Noble (1989) stated that there is a clear difference in the distribution of the subgenera, with *Monocalyptus* species tending to grow more successfully on sites with higher water availability. Noble (1989 reference therein) also presented some workers' findings that when subjected to similar dry conditions, *Monocalyptus* species suffer either greater stress or drought damage. Some studies have suggested that *Monocalyptus* species tended to exert lesser stomatal control (Connor *et al.* 1977; Colquhoun *et al.* 1984 in

Noble 1989) or have poorer root systems (Sinclair 1980) than *Symphyomyrtus* species with which to restrict water loss. Although it remains unclear whether the variation in leaf waxes is due to habitat or population structure, it is possible that leaf waxes may be, in part, functionally important for survival of *Monocalyptus* species in dry habitat. The dense wax deposits of *E. risdonii* and *E. tenuiramis* may enable them to minimise cuticular transpiration to allow than to either successfully inhabit typical dry sclerophyll environments characterised by nutrient poor soils and low soil moisture or reflect the high irradiation from rock surfaces, which may increase the heat loading of the plant. While these typical dry sclerophyll species often have a very glaucous wax, the wet sclerophyll species (e.g. 'green ash' species of *Monocalyptus*), have large leaves with mainly an amorphous wax considered as an adaptation to wet environments.

In contrast, the *Symphyomyrtus* species appeared to have different variation in response to habitat. While the increase of ß-diketones and 1,8-cineole in species within series *Ovatae* appeared to relate to increase in moisture and frost incidence, species from series *Viminalis* showed no correlation between change of leaf chemicals and change in dryness of habitat. Moreover, increase of ß-diketones and glaucousness in some species populations appeared to be correlated with increase of altitude (e.g. *E. urnigera* and *E. gunnii*). However, species from the subseries *Vernicosinae* (yellow gum) showed no major variation in leaf chemicals with respect to altitudinal change. Glaucous waxes may be functional for some species in responding to frost or exposure, (e.g. *E. rubida* and *E. urnigera*).

Functions of glaucous wax related to altitude may be similar for both *Symphyomyrtus* and *Monocalyptus* species in subalpine environments. *E. coccifera* , *E. gunnii* and *E. urnigera* are often green at low altitudes, while at high altitude they are uniformly glaucous, such as the changes in chemicals and glaucousness in both *E. urnigera* and *E. coccifera* on Mount Wellington. It is possible that glaucousness is, from an evolutionary point of view, lethal in sheltered habitats but essential to survival when exposed at high altitudinal limit as suggested by Barber (1955, 1957, 1965). However, this did not explain the case of *E. globulus* and *E. cordata* which had very glaucous leaves and grow at low altitude with less frost.

Harborne (1988) stated that secondary plant products, no less than the morphological features of plants, are subjected to the selection pressures of the environment and ecologically may play a very important role. The above discussions suggest that the phenotypes of leaf oils and waxes among Tasmanian eucalypt species may be the result of natural selection favouring the survival and reproduction of the genotypic capacity of biochemical synthesis in certain ecological environments. The patterns of variation in the natural forests are genetically independent when the same pattern involves parallel

variation in two species from different subgenera (Barber 1955, 1957). It is possible that species from different subgenera may respond similarly to a common selective force/environment (i.e. parallel evolution of ß-diketone chemotypes and glaucous phenotypes at high altitudes for *E. coccifera* and *E. urnigera* at Mt Wellington), but the genes controlling chemical biosynthesis differed for homologous of ß-diketones of *E. coccifera* which were C_{29} dominant in contrast to C_{33} dominant in waxes of *E. urnigera* and other *Symphyomyrtus* species.

Harborne (1988) suggested that the host plant specificity of insects is the result of the coevolution of insect and plants with plant secondary compounds. In Tasmanian eucalypt forests, the major insect pests (e.g. *C. bimaculata* and *C. agricola*) are host-specific at the subgenus level. The subgeneric difference in either oil or wax chemicals suggested that these secondary compounds may be involved in the insect-host plant selection. In turn, the leaf oils and waxes may be ecologically important for survival of those eucalypt species under the insect damage stress. In particular, these subgeneric differences in leaf chemicals may help to explain why the mixed stands of eucalypts tend to be composed of species from different subgeneric groups.

6.4. Concluding remarks

Results of the chemotaxonomic study have shown that leaf oils and waxes have taxonomic value in the Tasmanian eucalypts at all levels of the taxonomic hierarchy, but particularly at the subgeneric level. The most taxonomic important results of leaf oils and waxes for Tasmanian eucalypts are:

1. In natural populations there are large differences in leaf oil and wax chemicals both at the subgenera and species levels.

2. Studies of chemical differences between species in experimental trials and between species within individual localities suggested that chemical variations were mainly genetically based.

3. Differences between species within subgenus *Monocalyptus* were generally greater than those between species within subgenus *Symphyomyrtus*. The chemical composition of leaf oils and waxes of *Monocalyptus* species tended to be more variable and much more diverse than that of *Symphyomyrtus* species.

4. Within subgenus *Monocalyptus*, *Obliquae* species are well differentiated whereas in the *Piperitae* differentiation was poor in most cases.

5. The quantity of leaf oils showed a subgeneric difference in ontogenetic change. The juvenile leaves of *Monocalyptus* species produced significantly lower oil yields than adult leaves. In contrast, the juvenile leaves of *Symphyomyrtus* species generally contained similar or higher oil yield than did adult leaves.

6. The variability of leaf oils and waxes of eucalypts appear to be related to ecological zone in Tasmanian. Wet sclerophyll species generally have less infraspecific variation and exhibit more typical subgeneric differences. In contrast, dry sclerophyll species, showed a markedly interspecific and infraspecific variations.

7. The subgeneric differences in leaf oil and wax chemicals are associated with host specificity at the subgeneric level of major eucalypt pests in Tasmania, in particular in wet sclerophyll forest. Further study to investigate the insect response relating to variation in leaf oils and waxes in foliage is important.

SECTION 2

Chapter 7

Feeding and Oviposition Response of *Chrysopharta* spp. Adults on Leaves of Different *Eucalyptus* spp.

7.1. Introduction

As reviewed previously (see Chapter 2) both *C. bimaculata* and *C. agricola* appear to be strongly host specific. The studies of this chapter were initiated to investigate the host preferences of *C. bimaculata* and *C. agricola* to different *Eucalyptus* species and to assess whether the adults could distinguish between various foliage types which differed in leaf chemistry. The approach adopted was one in which the beetles were confronted with free choice and no choice situations. A series of experiments were based on exposing adult beetles to a mix of different foliages and the beetles response measured by recording the amount of each foliage that was eaten and the egg batches deposited on each foliage in a set time. In addition, a parallel experiment was also undertaken to determine the feeding and oviposition response of adults to leaves of single species in a no choice situation. This allowed the comparison of the suitability of different foliages for beetles.

7.2. General materials and methods

7.2.1. Beetle material

C. bimaculata adults were collected from *E. obliqua, E. regnans* and *E. nitens* trees and *C. agricola* from *E. nitens* and *E. globulus* in plantations at Esperance Valley, S.E. Tasmania during the summer season (1989-1991).

Adult beetles were placed in plastic bags which were secured with rubber bands and transported to the laboratory and stored in a 4 ^{0}C cool room for a maximum of one week after which mortality occurred. Before any experiment, beetles were confined in a glass jar for a 6 hour pretest starvation period at room temperature. The sex of beetles was identified according to the method described by de Little (1979).

7.2.2. Test foliage samples

The young foliage samples were collected from different sites as listed in each experiment (see individual experiments). The leaf samples were transported in plastic bags to the laboratory, stems placed in water and foliage covered with a plastic bag in a 4 ^{0}C cool room. Proportions of the leaves of each sample were used for chemical analysis, fresh and dry weight determinations and adult testing. Prior to each test the following measurements for each leaf sample were taken:

i) Fresh (FW) and dry weight (DW) of leaves and subsequent moisture content (MC): MC(%) = (FW-DW) / FW x 100

ii) Oil yield (g/100gDW*) and oil composition (%)

iii) † Wax yield (g/100g DW) and wax composition (%)

iv) Absolute content of individual oil compounds in foliage (g/10Kg DW) = Oil yield (g/100g DW) x percentage content of compound in oil (%)

iv) Absolute content of individual wax compounds in foliage (g/10Kg DW) = wax yield (g/100g DW) x percentage content of compound in wax (%)

* DW is oven dry weight
† Oil and wax extraction and analysis as described in Chapter 4 and 5.

7.2.3. Measurements of foliage consumption and egg production

Due to the marked difference in area/weight ratio of leaves between species, foliage consumption was not measured as the area eaten but as the dry weight of leaf material consumed. Prior to the tests the areas of the original foliage were copied onto the celcast photocopier film. Then after each test the remains of leaves were again copied. Later the respective areas were measured by a Paton Electronic Planimeter (PEP). Leaf areas eaten were obtained by area-subtraction: Leaf area eaten (cm2) = Initial leaf area (cm2) - remaining leaf area (cm2). Finally, the dry weight of foliage consumed was calculated by a DW/area conversion parameter and leaf dry weight eaten: (gDW) = Leaf area eaten (cm2) x DW/Area (g/cm2).

Oviposition on foliage was recorded as egg batches.

7.2.4. Data analysis

The estimates of variance in the adult response to different foliages within an experiment and the multiple comparisons among mean responses to foliages based on equal or unequal replicate sizes were carried out by a one way ANOVA. For testing the difference in the adult feeding response to different foliage samples, Fisher's Protected Least Significant Difference (PLSD) was used.

The ANOVA and PLSD analysis described above were computed and performed using the ANOVA program of the super ANOVA (Abacus Concepts, Inc.) run on a Macintosh SE/30 computer.

7.3. Selection and oviposition behaviour of *C. bimaculata* on foliages from *Monocalyptus* and *Symphyomyrtus* species

7.3.1. Materials and method

Ten pairs of *C. bimaculata* adult beetles were used in individual cages, which were approximately 60 cm wide, 40 cm deep and 40 cm high. The framework of each cage was of angle aluminium rod which supported stainless steel netting (mesh 8-9 per cm) sewn together to form the roof and four sides. The floor of each cage was covered with a layer of paper cut to size. Six different foliages were spaced 20 cm apart in two rows on the floor of each cage. Stems of each foliage sample were placed in water in glass jars and the gap around the stems plugged with cotton-wool to prevent beetles falling into the jars. The test foliages were standardised to an approximate leaf area of 350 to 400 cm^2. A 60 hour test period at 25±2 ^0C was allowed and each test replicated 5 times. Leaves were not replaced.

The six species tested in this experiment were collected from plantation at Esperance, S.E. Tasmania during the summer season (1989-1990). Adult foliage was harvested from each.

E. nitens	Penny Saddle, Vic.	*E. regnans*	Moogara, Tas
E. globulus	Geeveston, Tas.	*E. obliqua*	Esperance Valley
E. ovata	D'entrecasteaux River, Tas.	*E. sieberi*	Tas., ex Fingal District general collection original locality unknown

7.3.2. Results

The observed data are given in Appendix 7.1. An initial examination indicated that the data were not normally distributed. The plotting of residuals against fitted values indicated that the logarithmic transformation of data [Log(x+1)] was the appropriate transformation for both foliage consumption and egg production. Therefore analyses were undertaken using transformed data.

The ANOVA of the *C. bimaculata* adult response to foliages of the six eucalypt species, as shown in Table 7.1, indicated that both feeding (foliage consumption) and oviposition (egg batch production) differed significantly between species ($p<0.01$).

Table 7.1. Analysis of variance for *C. bimaculata* adult feeding and oviposition preference to foliage of six eucalypt species using log(x+1) transformed data.

Variance of Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	5	0.782	0.156	4.625	0.0043
Residual	24	0.812	0.034		

Model summary R: 0.701 R^2: 0.491 RMS Residual: 0.184 Model F-value: 4.625 P-value: 0.0043

Variance of Egg Production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	5	0.259	0.052	4.481	0.005
Residual	24	0.278	0.012		

Model summary R: 0.685 R^2: 0.483 RMS Residual: 0.108 Model F-value: 4.481 P-value: 0.005

The multirange test for *C. bimaculata* adult response to foliage of different species is given in Fig. 7.1A and the means and standard errors for foliage consumption and egg batch production on individual species in Fig. 7.1B.

The highest foliage consumption among species occurred on *E. obliqua* foliage and the lowest on *E. globulus* foliage. Equal amounts of *E. nitens* and *E. regnans* foliage were consumed. Consumption of *E. obliqua* and *E. nitens* foliage was significantly greater than that for *E. globulus* ($p<0.01$), *E. ovata* ($p<0.01$) and *E. sieberi* ($p<0.05$) while *E. ovata* was similar to *E. globulus*. The highest numbers of egg batches occurred on *E. regnans* and *E. nitens* and *E. globulus* had the lowest. The oviposition on *E. obliqua* was not significantly different from that on *E. regnans* and *E. nitens* but significantly higher ($p<0.01$) than on foliage of *E. globulus* while egg batch production recorded on *E. sieberi* and *E. ovata* foliage did not differ significantly from that on *E. globulus*.

In summary, the multiple range test indicated that *E. obliqua*, *E. regnans* and *E. nitens* foliages were preferred and that foliage of other species was less preferred by *C. bimaculata* adults. In general, the foliages, which were preferred as food by *C.*

bimaculata, were the preferred hosts for oviposition. However, there were some unusual differences in the feeding and oviposition preferences of *C. bimaculata* adults. Beetles exhibited a much lower feeding response on *E. regnans* in comparison to that on *E. nitens*. In contrast, *E. regnans* and *E. nitens* were the most preferred hosts for oviposition. Thus, the response of feeding and oviposition could vary with these species.

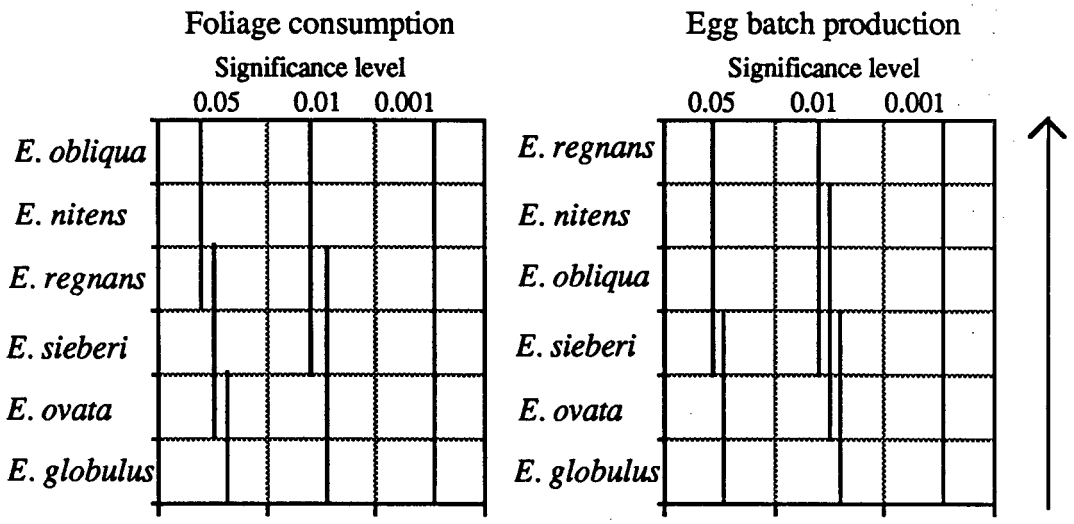


Fig. 7.1A. The multirange test (Fisher's PLSD) for *C. bimaculata* adult response to foliages of different *Eucalyptus* species based on transformed values.

The absolute contents of oil and wax compound groups in foliage samples of individual species are shown in Fig. 7.1B and indicate that levels of certain oil compounds appeared to be associated with the feeding and oviposition preference of *C. bimaculata* adults. The foliage of *E. globulus* and *E. ovata*, from *Symphyomyrtus*, were rich in group C compounds (mainly 1,8-cineole and α–pinene, see Chapter 4 for oil component groups) and not preferred by *C. bimaculata* beetles in contrast to the preferred *E. nitens* foliage which had low levels of 1,8-cineole and α–pinene.

Within the *Monocalyptus* species, the decreasing levels of Group B compounds (sesquiterpenoids) appeared to be associated with the increasing feeding of *C. bimaculata* adults, since the decrease of foliage consumption showed a rank order from *E. obliqua, E. regnans* to *E. sieberi* foliage with increase levels of group B compounds. In contrast, the increasing levels of group A compounds appeared to be positively associated with feeding. However, group B compounds did not associate with the oviposition preference of *C. bimaculata* as *E. regnans* and *E. obliqua* foliage differed significantly in amounts of group B compounds but had equal numbers of egg batches. However, the combination of high amounts of group C and group B compounds in *E. sieberi* was associated with a significantly lower feeding and oviposition response by *C. bimaculata.*

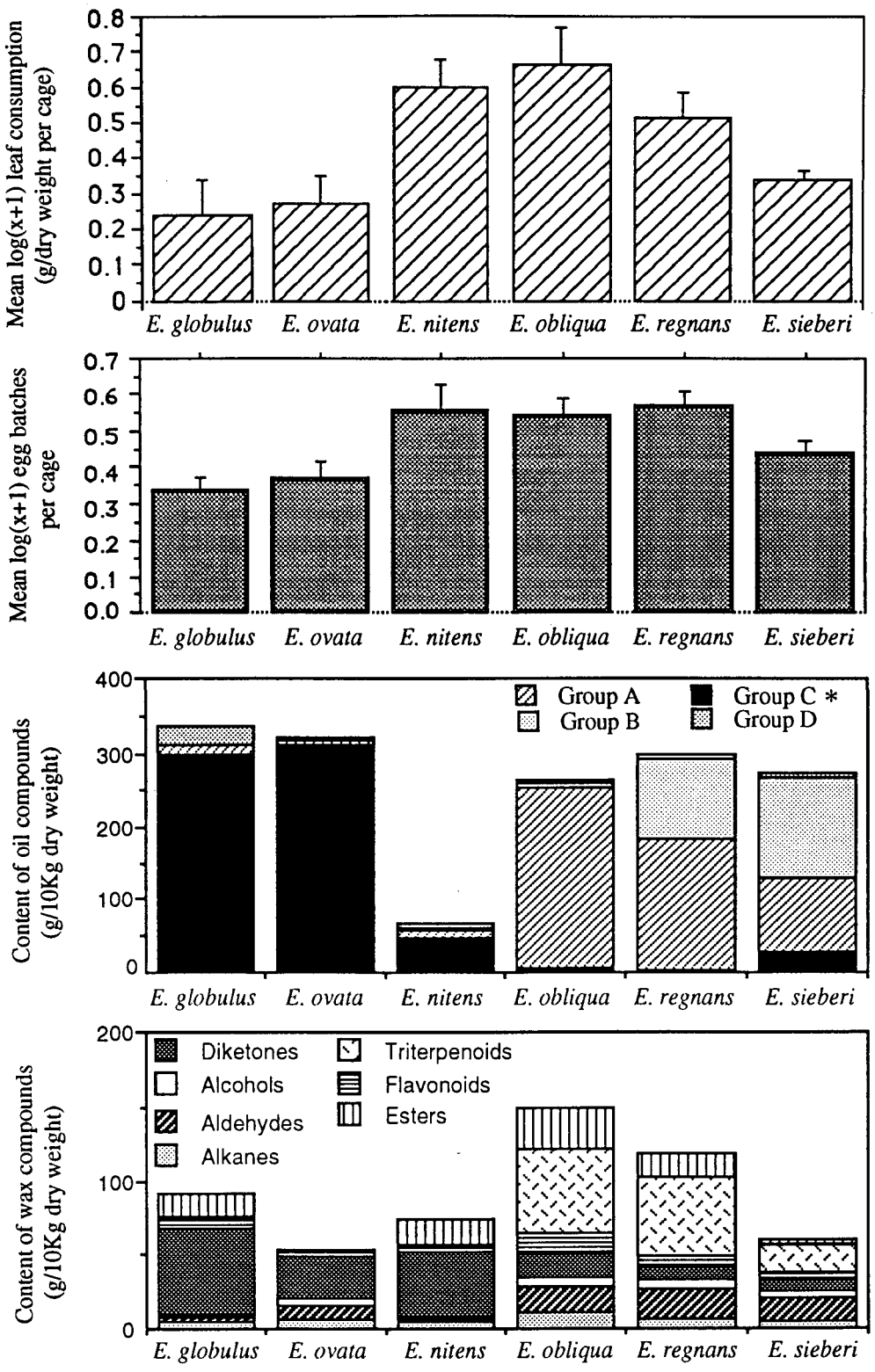


Fig. 7.1B. The means and standard errors for *C. bimaculata* adult feeding and oviposition responses to foliage of six eucalypt species [log (x+1) transformed data] and absolute contents of leaf oil and wax compounds. (see Chapter 4 for oil compound group)

* Group A: mainly α– and β–phellandrene, *cis*- and *trans*-piperitol, *cis*- and *trans*-p-menth-2-en-1-ol et al.
Group B: mainly α–, β– and γ–eudesmol, hedycaryol, elemol.
Group C: mainly 1,8-cineole, α–pinene, α–terpineol and related oil compounds.
Group D: mainly globulol and spathulenol.

In contrast, the association between wax compounds and the *C. bimaculata* adult preference was poor. The preferences of beetles differed significantly between *E. nitens* and *E. globulus* foliages which had similar amounts of ß-diketones. Nevertheless, difference in amounts of triterpenoids between the three *Monocalyptus* species were correlated to the differences in adult preference. *E. regnans* and *E. obliqua*, which had high levels of triterpenoids, were preferred in contrast to the foliage of *E. sieberi*, which had low levels of triterpenoids.

7.4. Response of *C. bimaculata* to juvenile and adult foliage of *E. nitens* and *E. globulus*

It has been reported that adult foliage of *E. nitens* in the field was intensely attacked by *C. bimaculata*, whereas the waxy juvenile foliage on the same trees was virtually untouched by this beetle species (see Chapter 2). *E. nitens* and *E. globulus*, grown in New Zealand, is subject to the same pattern of attack by *Paropsis charybdis* and it has been found that the glaucous wax layer of juvenile foliage provides protection from *P. charybdis* by preventing the attachment of adult beetles (Edwards 1982). A series of experiments were established to investigate whether the waxes of *E. nitens* and *E. globulus* juvenile foliage also had the same effect on *C. bimaculata* and *C. agricola* adults. This study also investigated the *C. bimaculata* response to dewaxed juvenile foliage of *E. nitens* and *E. globulus* and their adult foliage.

7.4.1. Materials and method

The first step in this experiment was to assess the effects of glaucous wax of juvenile foliage on the attachment of adult beetles. Adult beetles were placed onto waxy juvenile leaf surfaces which were rotated through 180^0 and disturbed suddenly by jerking the foliage. The success or otherwise of the beetle to remain on the surface was recorded and compared to that recorded on dewaxed juvenile foliage. Wax was removed by wiping the surface with moist cotton wool. In the initial experiment, it was observed that beetles could attach and cling to smooth glass. Therefore, adults were confined in a small glass jar with normal juvenile *E. nitens* foliage which touched the sides of the glass jar. This allow visual assessment of whether the adults fed on the glaucous foliage of *E. nitens* and *E. globulus* when they could reach the foliage. The response of beetles was recorded and compared with that recorded on dewaxed juvenile foliage. Results of the above experiments are described in general as the data did not lend itself to statistical analysis.

The next step compared the preference of *C. bimaculata* when presented with dewaxed juvenile foliage of *E. nitens* and *E. globulus* and their adult foliage. Four different

foliages were distributed 20 cm apart in a cage. Ten pairs of adults were used in each cage and the test replicated 4 times due to the limitation of beetle numbers. Other conditions of this experiment were similar to those of the selection experiments above (see 7.3.1).

The adult and juvenile foliage of *E. nitens* (Toorongo Vic.) was collected from Esperance, S.E. Tasmania. The adult and juvenile foliage of *E. globulus* was collected from natural stands located at Lea-Kingston, Hobart.

7.4.2. Results

A. The effects of the glaucous leaf surface on attachment and crawling behaviours of adult beetles.

C. bimaculata adults were observed to have difficulty in remaining attached to juvenile *E. nitens* and *E. globulus* foliage when held horizontally. When adults were placed on waxy juvenile foliage held at different slopes, they repeatedly slipped off. The failure of adults to attach increased with the number of times they were replaced on foliage. Adults which had fallen from glaucous leaf surface were observed under the binocular microscope and it was found that the glaucous waxes were caught and entangled in the tarsal claws and pads. The amount of waxes caught and entangled increased when adults were again placed on the glaucous foliage. However, when adults were placed on dewaxed juvenile foliage at different slopes, they were able to adhere and crawl successfully.

In contrast, *C. agricola* adults could easily attach on glaucous juvenile foliage irrespective of slope. However, with the exception that although beetles could crawl readily on roost slopes they had difficulty crawling on vertical surfaces. Examination of beetles under the binocular microscope indicated that very little wax was caught and entangled in the tarsal claws and pads of *C. agricola* adults.

C. bimaculata adults confined in a glass jar containing glaucous juvenile *E. nitens* foliage touching the sides of the jar, fed on the foliage. However, no eggs were found on the foliage but eggs did occur on the wall of jar. However, when *C. bimaculata* adults were confined to a jar containing dewaxed juvenile foliage, beetles fed and laid eggs on dewaxed juvenile *E. nitens* foliage.

B. The response of C. bimaculata adults on adult and dewaxed juvenile foliage of E. nitens and E. globulus

As it was already known that *C. bimaculata* can not attach to normal juvenile leaves of both *E. nitens* and *E. globulus*, normal juvenile leaves were not included in this experiment and only dewaxed juvenile leaves were used. The observed data is listed in Appendix 7.2. The results of the two way ANOVA (Table 7.2) for differences in the *C. bimaculata* response on adult and dewaxed juvenile foliages of *E. nitens* and *E. globulus* indicated that both foliage consumption and oviposition differed significantly ($p<0.001$) between tree species. The type of leaf did not significantly affect foliage consumption by beetles but did significantly ($p<0.01$) affect oviposition. There were no significant effects in the interaction tests.

The means and standard errors for foliage consumption and egg batch production on individual foliage samples and subsequent Fisher's PLSD tests of foliage means are shown in Fig. 7.2. The leaf consumption by beetles on both adult and dewaxed juvenile *E. nitens* foliages was significantly ($p<0.001$) higher than that recorded on adult and dewaxed juvenile *E. globulus* foliage. However, oviposition of beetles on dewaxed juvenile *E. nitens* foliage was significantly ($p<0.01$) lower than that recorded on adult foliage and significantly higher than that on dewaxed juvenile *E. globulus* foliage. Foliage consumption and oviposition on adult *E. globulus* foliage did not differ significantly from that on dewaxed juvenile *E. globulus* foliage.

Table 7.2. Analysis of variance for *C. bimaculata* adult feeding and oviposition preference to adult and dewaxed juvenile foliage of *E. nitens* and *E. globulus* using log(x+1) transformed data.

Variance of Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	1	.746	.746	50.336	.0001
Leaf type	1	.025	.025	1.669	.2147
Species * Leaf types	1	6.547E-7	6.547E-5	4.42E-5	.9948
Residual	16	.237	.015		

Model summary R: 874 R^2: 0.765 RMS Residual: 0.122 Model F-value: 17.335 P-value: 0.0001

Variance of Oviposition

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	1	.370	.370	31.246	.0001
Leaf type	1	.124	.124	10.437	.0052
Species * Leaf types	1	.019	.019	1.612	.2224
Residual	16	.189	.012		

Model summary R: 854 R^2: 0.730 RMS Residual: 0.109 Model F-value: 14.432 P-value: 0.0001

In dewaxed juvenile leaves only the outer layer of surface waxes (free waxes) was removed and solid waxes still remained on the surface. Fig. 7.2 indicated that the hexane soluble waxes of dewaxed juvenile leaves, which were extracted by washing the whole surface of leaves, had chemical composition similar to that from adult leaves. Although, the amounts of ester compounds were relative higher in adult foliage than in dewaxed juvenile foliage of either *E. nitens* or *E. globulus*, the amounts of major compounds, ß-diketones, did not differ markedly between adult and dewaxed juvenile leaves. This indicated that the wax chemicals did not correlate to the significant difference of beetle response between *E. nitens* and *E. globulus*.

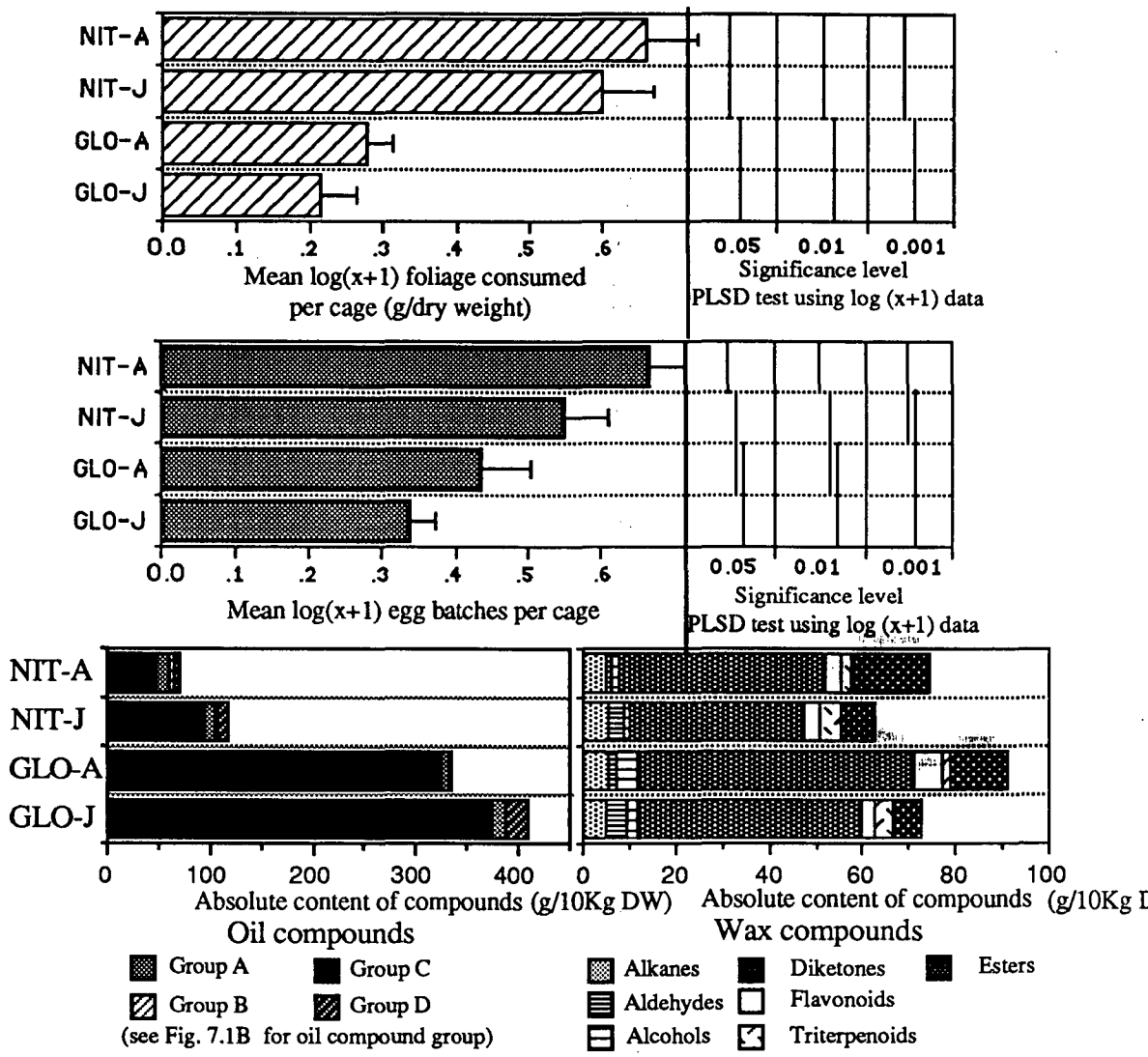


Fig. 7.2. The means, standard errors and the multiple range test for *C. bimaculata* adult feeding and oviposition preference on foliages of six eucalyptus species. The multiple range test were carried out using transformed data [log(x+1)].

Comparison between the observed differences in the *C. bimaculata* adult response and the absolute contents of oil compounds between foliages indicated that differences in amounts of group C compounds, mainly 1,8-cineole and α–pinene, between species were associated with the *C. bimaculata* adult preference. Both adult and dewaxed juvenile *E. globulus* foliage, which had high amounts of these oil compounds, were not preferred by beetles (Fig. 7.2). In contrast, adult and dewaxed *E. nitens* foliages, which contained low levels of group C compounds, were preferred.

Comparisons made between adult and dewaxed juvenile foliages within *E. nitens* indicated that the relative difference in amounts of wax esters and oil compounds did not result in a significant difference in feeding preference between adult and dewaxed foliages. However, the significantly lower oviposition response of *C. bimaculata* adults on dewaxed juvenile foliage was correlated to relatively high levels of group C compounds in dewaxed juvenile foliage. These findings indicated that oil compounds had a more significant effect on oviposition than on feeding.

Comparisons made between adult and dewaxed juvenile foliage within *E. nitens* indicated that the significantly lower oviposition response of *C. bimaculata* adults on dewaxed juvenile foliage was correlated to relatively high group C oil and low ester compound contents in dewaxed juvenile foliage.

7.4.3. Discussion

Results of this study indicated that the glaucous waxes on juvenile foliage of *E. nitens* and *E. globulus* provided protection from *C. bimaculata* adult attack by physically preventing adult attachment to these foliages. This finding is similar to the reports of Stork (1980b) for chrysomelid mustard beetles on brassicas, and Edwards (1982) for *P. charybdis* on juvenile foliage of *E. nitens* and *E. globulus*. In contrast, *C. agricola* adults were able to overcome this protection.

The tarsal adhesive setae in the beetles was studied by Stork (1980a) in which he described simple and spatulate setae as 'adhesive' setae for many plant climbing Chrysomelidae. Stork (1980b) found that the claws and the tarsal setae of *Chrysolina polita* were used to cling to rough surfaces and to adhere to smooth surfaces respectively. Edwards (1982) also suggested that the adhesion of *Paropsis charybdis* beetles was achieved by similar means. In this study, scanning electron micrographs were made of the tarsi of both *C. bimaculata* and *C. agricola* adults. Comparison indicated that these two species had different tarsal structures (Fig. 7.3).

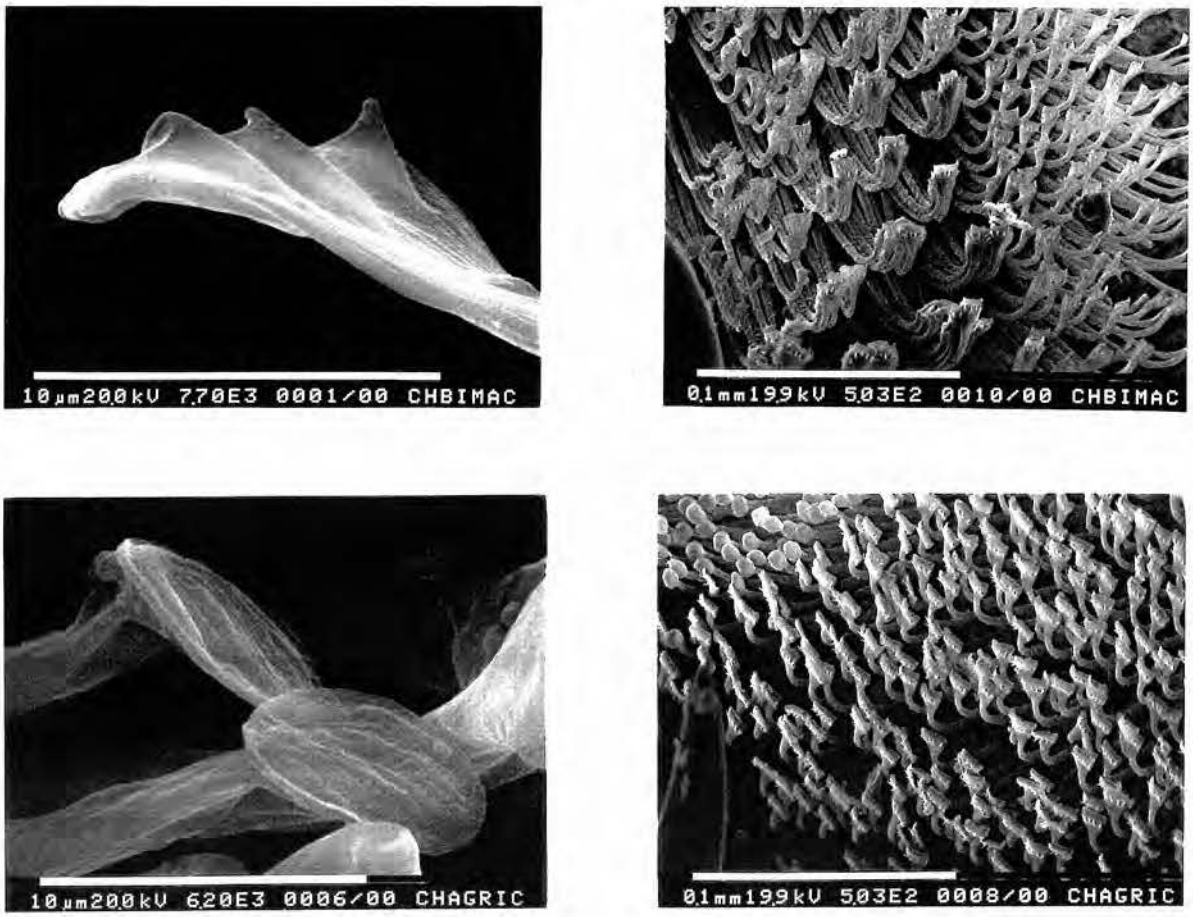


Fig. 7.3. Scanning electron micrographs of individual retineria* (left) and pulvinis** (right) on penultimate, bilobed tarsal segment of leg I of *C. bimaculata* (above) and *C. agricola* (below).

* retineriae - microscopic seta-like projections on the ventral side of the tarsus
** pulvinis - mass of retineriae covering the ventral side of the tarsus

It was found that the tarsal adhesive setae (retineriae) of *C. bimaculata* occurred in distinct clusters of variable number and were sharply bent toward the distal ends which were serrate in profile. In contrast the retineriae of *C. agricola* were more uniformly dispersed, less bent and their distal ends were plate-like (Plate A).

The inability of *C. bimaculata* adults to walk on glaucous foliage is because waxes build up in the gaps between the setal clusters and the bent ends. It is probable that the shape of the ends causes the loosening of wax.

However, the morphology and distribution of *C. agricola* tarsal setae does not favour excessive loosening and accumulation of waxes although contamination does occur.

In addition, the sites of oviposition differed between the species. *C. bimaculata* lays eggs side by side in batches on the general leaf surface. *C. agricola* females grasp the apex of the leaf and randomly deposit their egg to form a clump.

After free waxes were removed, the solid waxes, which remained on the dewaxed juvenile leaves surface, had a chemical composition similar to that of adult waxes. The results suggested that waxes did not have any significant chemical effect against *C. bimaculata.*

Beside the physical effect of glaucous waxes, the most important finding of this study was that *C. bimaculata* preferred to feed on both adult and dewaxed juvenile foliages of *E. nitens* compared with similar leaves of *E. globulus*. However, there was no significant difference in feeding preference between adult and dewaxed juvenile foliages within each species. This indicated that after the physical effect of glaucous waxes was excluded, the juvenile foliage of *E. globulus* was still not attacked by *C. bimaculata* adults.

It was considered that 1,8-cineole and related oil compounds appear to be associated, at least in part, with the chemical defence of *E. globulus*, since both juvenile and adult foliages of *E. globulus* were rich in these chemicals. In contrast, both juvenile and adult foliage of *E. nitens* contained low amounts of 1,8-cineole and related oil compounds.

The significant difference in the oviposition response between adult and dewaxed juvenile foliages of *E. nitens* indicated that the relatively higher contents of 1,8-cineole and related oil compounds in juvenile foliage may affect oviposition by *C. bimaculata* adults. However, it is possible that the relative higher amounts of ester compounds in waxes of adult *E. nitens* foliage could attract or stimulate the oviposition of beetles. Although adult foliage of *E. globulus* also contained relatively higher amounts of esters, the dominant effects of 1,8-cineole may mask any benefit of esters.

A general conclusion could not be made, because the preference of *C. bimaculata* adult may be affected by other factors beside the leaf oil and wax. However, results of this study suggest that the susceptibility of adult foliage of *E. nitens* to attack by *C. bimaculata* is associated with the lack of either physical and chemical defence. The juvenile foliage of *E. nitens* also lacked chemical defences, however, the glaucous waxes acted as a physical defence in preventing adult *C. bimaculata* attachment. This may explain why juvenile foliage of *E. nitens* was not attacked by *C. bimaculata* but adult foliage was and why both juvenile and adult foliage of *E. globulus* were not. In contrast, the *C. agricola* adult could overcome both physical and chemical defences. This may also explain why both juvenile and adult foliage of both *E. nitens* and *E. globulus* was attacked by *C. agricola.*

7.5. Comparison of responses of *C. bimaculata* and *C. agricola* on *E. nitens* and preferred and non-preferred hosts

A comparison was made of the preferences of *C. bimaculata* and *C. agricola* adults for *E. nitens, E. obliqua* and *E. globulus*. The aim of this study was to evaluate the difference in the response of both beetle species to *E. nitens* foliage in comparison with foliages from the "ash" group and the gum group of species.

7.5.1. Materials and method

The choice and no choice tests were carried out in a plastic container, which was 21 cm high and 14 cm square tapering to 15 cm at the base. A hole was cut for ventilation (3 cm diameter) in the top of each container and covered with copper mesh. Shoots were held in a glass jar containing water and the gap around the stems plugged with cotton-wool to prevent beetles falling into the glass jar. A 4 day test period at 25±2 ^{0}C was allowed and the test replicated 6 times. The measurement of the beetles response was recorded as the area of foliage consumed and egg batches deposited on foliage.

(A) No choice test with adult foliage of individual species:

Five pair of adult beetles (5male, 5 female) were confined on an approximate area of 1200 to 1500 cm^2 of foliage of a single species.

(B) Choice experiment with adult foliages of three species:

Five pairs of adult beetles were confined in 1200-1500 cm^2 foliage with 400 to 500 cm^2 foliage of each of the three species mixed together and held in water in a single glass jar. In an initial trial, it had been found that the amount of a preferred foliage with a total area 400-500cm^2 was enough to support 5 pairs of adult beetles of either *C. bimaculata* or *C. agricola* (e.g. *E. obliqua* foliage for *C. bimaculata* and *E. globulus* for *C. agricola* for 4days). Therefore, the amount of foliage of individual species was enough to avoid the possibility that the beetles could be affected by starvation and forced to eat unpalatable leaf material if most of the available palatable foliage had been consumed.

(C) Choice experiment with adult foliage of two species

Five pairs of adult beetles were provided with the adult foliage of each species in pairs that were mixed together and held in water in a glass jar. Leaf area for each species was 600-750cm^2. Due to a scarcity of beetles, each treatment was replicated 4 times for *C. bimaculata* adults only.

7.5.2. Results

A. Comparison of the feeding and oviposition response of C. bimaculata and C. agricola to foliage of individual species

The observed data was listed in Appendix 7.3 and the ANOVA (Table 7.3) for adult response of each beetle species to the foliage of different species showed that the oviposition response of both *C. bimaculata* or *C. agricola* adults differed significantly ($p<0.001$) between foliages. Foliage consumption of *C. bimaculata* adults also differed significantly ($p<0.001$) between species while that of *C. agricola* was not significant. However, the *p*-value (0.068) for *C. agricola* was close to 0.05, suggesting a species effect on the *C. agricola* adult feeding response.

The subsequent PLSD test (Fig. 7.3) of consumption means between the species foliages indicated that feeding and oviposition by *C. bimaculata* adults on *E. nitens* and *E. obliqua* foliages were similar but significantly ($p<0.001$) higher than that on *E. globulus*. In contrast, foliage consumption and egg batch production of *C. agricola* on *E. globulus* was similar to *E. nitens* and significantly lower on *E. obliqua* ($p<0.05$ and $p<0.001$ respectively).

Table 7.3. ANOVA for *C. bimaculata* and *C. agricola* adult feeding and oviposition response to mixed *E. nitens, E. obliqua* and *E. globulus* foliages based on log(x+1) transformed data.

(A) *C. bimaculata*

ANOVA for Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.960	.480	34.112	.0001
Residual	15	.211	.014		

Model summary R: 905 R^2: 0.820 RMS Residual: 0.119 Model F-value: 34.112 P-value: 0.0001

ANOVA for Oviposition

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.567	.283	18.410	.0001
Residual	15	.231	.015		

Model summary R: 843 R^2: 0.711 RMS Residual: 0.124 Model F-value: 18.410 P-value: 0.0001

(B) *C. agricola*

ANOVA for Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.059	.030	3.234	.0680
Residual	15		.00.1379		

Model summary R: .540 R^2: 0.301 RMS Residual: 0.096 Model F-value: 3.234 P-value: 0.0680

ANOVA for Oviposition

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.539	.270	16.135	.0002
Residual	15	.251	.017		

Model summary R: .826 R^2: 0.682 RMS Residual: 0.129 Model F-value: 16.136 P-value: .0002

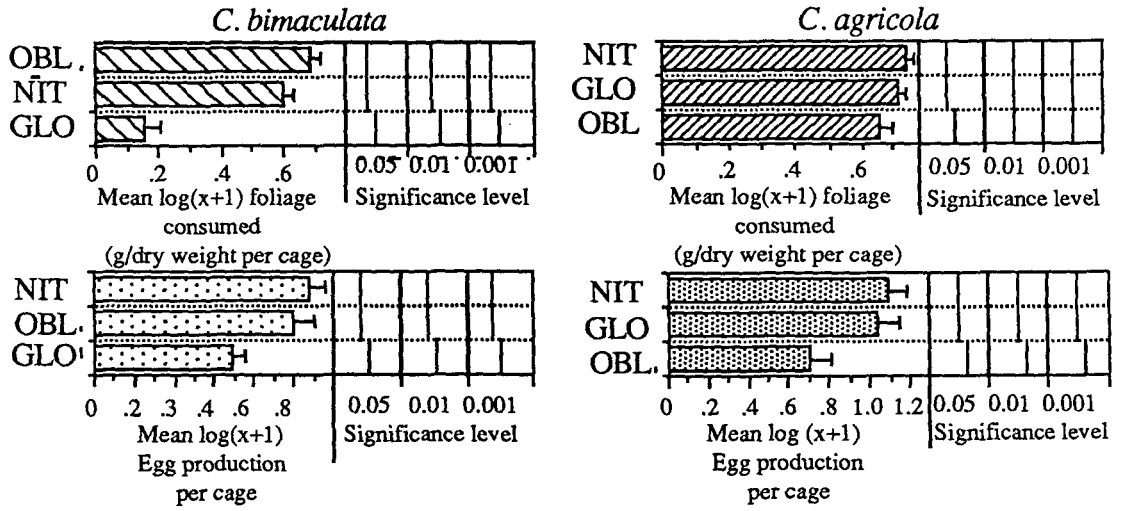


Fig. 7.3. The multiple range test (Fisher's PLSD) using transformed data for difference in feeding and oviposition response of *C. bimaculata* and *C. agricola* adults between the foliage of different species.

Comparison (Table 7.4) of the adult feeding and oviposition responses of *C. bimaculata* and *C. agricola* adults on each species indicated that the two insect species did not differ in either foliage consumption or egg batch production when feeding on *E. nitens* foliage. However, the response of *C. bimaculata* adults to *E. obliqua* foliage was significantly ($p<0.001$) higher than that recorded for *C. agricola* on this foliage (mean differences = 3.54 gms and = 5.17 batches respectively). In contrast, *C. agricola* adult foliage consumption and egg batch production were significantly ($p<0.001$) higher on *E. globulus* foliage than that presented for *C. bimaculata* (mean differences = 2.96 gms and = 6.68 batches respectively) on *E. globulus*.

The above comparisons indicated that the *E. obliqua* foliage was suitable food for *C. bimaculata* adults, *E. globulus* foliage was suitable for *C. agricola* and *E. nitens* foliage was equally accepted by both beetle species.

Table 7.4. The means and standard errors (logx+1) of *C. bimaculata* and *C. agricola* adult feeding and oviposition response on mixed foliages of *E. nitens, E. obliqua* and *E. globulus* and the ANOVA based on transformed data for differences in feeding and oviposition response between beetle species on different foliages.

(A) Foliage consumption (g dry weight)

	C. bimaculata				*C. agricola*				Mean difference	ANOVA	
	No. Cage.	Total	Mean	S.E.	No. Cage.	Total	Mean	S.E.	Δ (B-A)	F-value	P-value
Total	18	12.12	0.67	.06	18	12.64	.68	.03	-.01	0.189	0.6667ns
NIT	6	4.68	0.78	.04	6	4.57	.76	.03	.02	0.102	0.7564ns
OBL	6	5.34	0.89	.03	6	3.75	.63	.05	.26	18.370	0.0016**
GLO	6	2.10	0.35	.06	6	4.32	.72	.03	-.37	27.415	0.0004***

(B) Oviposition (batches per cage)

	C. bimaculata				*C. agricola*				Mean difference	ANOVA	
	No. Cage.	Total	Mean	S.E.	No. Cage.	Total	Mean	S.E.	Δ (B-A)	P(2-tail)	Significance
Total	18	16.07	.89	.05	18	18.07	.90	.05	-.01	0.41110	0.5257ns
NIT	6	6.23	1.04	.05	6	6.53	1.09	.05	-.05	0.53500	0.4811ns
OBL	6	5.98	1.00	.06	6	4.18	.70	.06	.30	14.4830	0.0035**
GLO	6	3.86	.64	.04	6	6.18	1.03	.06	-.39	31.0290	0.0002***

B. Feeding and oviposition response of C. bimaculata and C. agricola to mixed foliage of three species

The observed data was listed in Appendix 7.4. The ANOVA for feeding and oviposition of each beetle species on different species (Table 7.5) indicated that the feeding and oviposition choices of both *C. bimaculata* and *C. agricola* adults differed significantly ($p<0.001$) on the different foliages.

The PLSD test (Fig. 7.4) of foliage means between foliages indicated that foliage consumption and egg batch production by *C. bimaculata* adults were similar on *E. nitens* and *E. obliqua* foliages and significantly ($p<0.001$) higher than that recorded on *E. globulus*. In contrast, *C. agricola* had significantly ($p<0.01$) higher foliage consumption and egg batch production on *E. globulus* and *E. nitens* than on *E. obliqua.*

The ANOVA (Table 7.6) for feeding and oviposition response made between the *C. bimaculata* and *C. agricola* adults indicated that the mean values of foliage consumption and egg batch production of *C. bimaculata* on *E. obliqua* foliage were significantly ($p<0.01$) higher than that recorded for *C. agricola* (differences = 2.11 gms and 2.33 batch respectively). In contrast, the mean value of foliage consumption and egg batch production of *C. agricola* on *E. globulus* was significantly ($p<0.001$) higher than that recorded for *C. bimaculata* (differences = 2.23 gms and 4.33 batches respectively). However, there was no significant difference in the feeding and oviposition response of either species when exposed to *E. nitens* foliage.

In summary, the results indicated that *E. obliqua* foliage was the most preferred by *C. bimaculata* adults and *E. globulus* was the most preferred by *C. agricola* and that *E. nitens* foliage was equally accepted by both beetle species, consistent with the no choice experiment.

In order to compare the host determination by adults in choice and no choice situations, the feeding and oviposition responses of *C. bimaculata* and *C. agricola* beetles to different species in no-choice (single species) and choice (3 species) tests were converted to percentages and are listed in Table 7.7.

The response of each beetle species to foliage of different species was similar in choice and no choice experiments. These results indicated that feeding and oviposition choice of these two beetles species was related to the suitability of hosts.

Table 7.5. ANOVA for *C. bimaculata* and *C. agricola* adult feeding and oviposition choices on *E. nitens, E. obliqua* and *E. globulus* using log(x+1) transformed data.

(A) *C. bimaculata*

Variance of Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.700	.350	25.170	.0001
Residual	15	.209	.014		

Model summary R: 878 R^2: 0.770 RMS Residual: 0.118 Model F-value: 25.170 P-value: 0.0001

Variance of Oviposition

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	1.040	.625	20.033	.0001
Residual	15	.393	.026		

Model summary R: 853 R^2: 0.728 RMS Residual: 0.162 Model F-value: 20.033 P-value: 0.0001

(B) *C. agricola*

Variance of Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.349	.175	13.545	.0004
Residual	15	.193	.013		

Model summary R: 802 R^2: 0.644 RMS Residual: 0.114 Model F-value: 13.545 P-value: 0.0004

Variance of Oviposition

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Foliage species	2	.881	.440	11.92	.0008
Residual	15	.554	.037		

Model summary R: 784 R^2: 0.614 RMS Residual: 0.192 Model F-value: 11.928 P-value: 0.0008

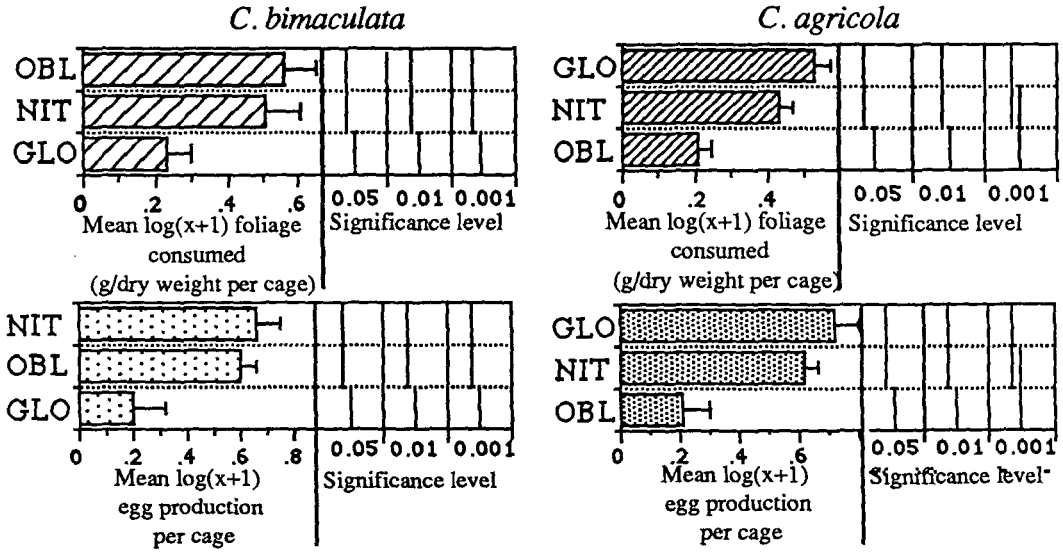


Fig. 7.4. The multirange test (Fisher's PLSD) for difference in feeding and oviposition selections of *C. bimaculata* and *C. agricola* adults on different foliage species based on transformed data.

Table 7.6. The means, standard errors of *C. bimaculata* and *C. agricola* adult feeding and oviposition selections on foliages of *E. nitens*, *E. obliqua* and *E. globulus* and the ANOVA based on transformed data for differences in feeding and oviposition response between beetle species on different foliages.

(A) Foliage consumption (g dry weight)

	C. bimaculata				*C. agricola*				Mean difference	ANOVA	
	No. Cage.	Total	Mean	S.E.	No. Cage.	Total	Mean	S.E.	Δ (B-A)	F-value	P-value
Total	6	7.11	1.18	0.03	6	7.10	1.18	0.07	0.001	4.65E-5	0.9946ns
NIT		3.07	0.51	0.05		2.63	0.44	0.05	0.07	1.013	0.3379ns
OBL		3.34	0.56	0.05		1.24	0.21	0.04	0.35	25.115	0.0005***
GLO		0.70	0.11	0.03		3.23	0.54	0.07	-0.43	54.108	0.0001***

(B) Oviposition (batches per cage)

	C. bimaculata				*C. agricola*				Mean difference	ANOVA	
	No. Cage.	Total	Mean	S.E.	No. Cage.	Total	Mean	S.E.	Δ (B-A)	F-value	P-value
Total	6	7.94	1.32	0.07	6	9.30	1.55	0.03	-0.23	0.6110	0.4397ns
NIT		3.78	0.63	0.06		3.732	0.62	0.05	0.01	0.0100	0.9210ns
OBL		3.56	0.59	0.07		1.254	0.21	0.10	0.38	10.056	0.0098**
GLO		0.60	0.10	0.06		4.314	0.72	0.08	-0.62	36.784	0.0001***

Table 7.7. Comparison of adult beetles feeding and oviposition response to single species in no choice and mixed species foliage in choice experiment. Comparisons were made using percent of adults feedings or laying eggs on the different foliages.

(A) Feeding preference

	C. bimaculata		*C. agricola*	
	Response on single foliage	Selection on mixed foliage	Response on single foliage	Selection on mixed foliage
NIT	38.51	43.32	37.65	36.40
OBL	51.30	50.60	27.17	12.89
GLO	10.19	6.08	35.18	50.71
Total	100.00	100.00	100.00	100.00

(B) Oviposition preference

	C. bimaculata		*C. agricola*	
	Response on single foliage	Selection on mixed foliage	Response on single foliage	Selection on mixed foliage
E. nitens	44.60	50.00	44.87	37.74
E. obliqua	40.29	45.24	16.03	9.43
E. globulus	15.11	4.76	39.10	52.83
Total	100.00	100.00	100.00	100.00

* Percentage distribution $(Y_1)=X_1/(X_1+X_2+X_3)$ x 100%; for example, $Y_{NIT} = X_{NIT}/(X_{NIT}+X_{GLO}+X_{OBL})$ x 100%

Moreover, a similar trend for both beetles species was that differences in either feeding and oviposition responses between foliages in choice experiments were greater than that observed in no choice experiments. In particular, the proportions of foliage consumed by *C. agricola* on *E. nitens*, *E. obliqua* and *E. globulus* in the choice experiments were 36%, 13% and 51% respectively in contrast to the proportions in the no choice experiment that were 38%, 27% and 35% respectively.

C. Feeding and oviposition response of C. bimaculata on pairs of different species.

The observed data was listed in Appendix 7.5. The means and standard errors of feeding and oviposition responses of *C. bimaculata* adults to different species pairs, the differences within each foliage species pair, the results of ANOVA for differences between species and PLSD for difference between pairs of different species are listed in Table 7.8.

Results indicated that foliage consumption of *C. bimaculata* adults was significantly higher on NIT+OBL (*E. nitens* plus *E. obliqua*) species pairs than that on OBL+GLO. The foliage consumption on NIT+GLO species pair was intermediate and did not differ significantly from either NIT+OBL or OBL+GLO. *C. bimaculata* adults also deposited more egg batches on NIT+OBL foliage pair than on NIT+GLO which, in turn, were significantly ($p<0.05$) higher than that on NIT+GLO. These results indicated that the combination of *E. nitens* plus *E. obliqua* foliage was the most suitable for *C. bimaculata* adult feeding and oviposition while the combination of *E. globulus* foliage with either *E. obliqua* or *E. nitens* resulted in lower feeding and oviposition responses respectively.

The ANOVA (Table 7.8) indicated that there was no significant difference in either the feeding or oviposition response of *C. bimaculata* between *E. nitens* and *E. obliqua* foliages when exposed to the NIT+OBL combination. However, beetles exhibited significant differences in their response when fed on NIT+GLO or OBL+GLO. The foliage consumption of *C. bimaculata* adults on *E. nitens* foliage was significantly ($p<0.01$) higher than that on *E. globulus* when fed NIT+GLO. The beetles also oviposited more on *E. nitens* foliage than on *E. globulus*, although the difference was not significant ($p=0.1002$). When *C. bimaculata* adults fed on foliage pair OBL+GLO, both foliage consumption and egg batch production were significantly ($p<0.001$ and $p<0.05$ respectively) higher on *E. obliqua* foliage than on *E. globulus*. In summary, the results indicated that *E. obliqua* and *E. nitens* foliages were more preferred by *C. bimaculata* adults and *E. globulus* the least.

To compare the responses of *C. bimaculata* adults to different foliages in choice and no choice experiments, the feeding and oviposition responses of this beetle species to different species were converted to percentages and are listed in Table 7.9. Results indicated that the response of *C. bimaculata* adults to foliage either between *E. nitens* and *E. globulus* or between *E. nitens* and *E. obliqua* in choice experiments was similar to that observed in the no choice experiments. However, the ratio of differences in the *C. bimaculata* adults feeding response to foliages between *E. obliqua* and *E. globulus* (OBL vs GLO = 20.37) in choice experiment was markedly greater than in the no choice experiment (OBL vs GLO = 5.04).

Table. 7.8. The means and standard errors [log(x+1)] of *C. bimaculata* adults feeding and oviposition response on different foliage pair combinations, the multiple range test (Fisher's Protected PLSD) for difference between foliage pairs and the ANOVA based on transformed data for difference between foliages within each foliage pair. [PLSD and *t*-test using log(x+1) transformed data].

		Foliage consumption (g/dry weight)			Egg production (batches)		
	No. Cage	Total	Mean	S.E.	Total	Mean	S.E.
NIT+OBL	4	3.87	.97	.08	5.5	1.36	.04
NIT		2.01	.50	.04	2.60	.65	.03
OBL		1.87	.47	.04	2.85	.72	.05
Mean difference (NIT-OBL)		.03			-.07		
ANOVA	F-values	0.407			1.120		
	P-values	0.5471 ns			0.3306 ns		
NIT+GLO	4	3.31	.83	.08	4.48	1.12	.11
NIT		2.21	.55	.04	2.62	.66	.08
GLO		1.10	.28	.05	1.86	.46	.06
Mean difference (NIT-OBL)		.27			.20		
ANOVA	F-values	16.86			3.714		
	P-values	0.0063**			0.1002ns		
OBL+GLO	4	2.71	.68	.07	4.55	1.14	.02
OBL		2.46	.61	.05	2.99	.75	.06
GLO		.25	.06	.02	1.56	.39	.05
Mean difference (OBL-GLO)		.55			-.36		
ANOVA	F-values	103.000			21.009		
	P-values	0.0001***			0.0499*		
Fisher's PLSD test for differences between pairs	Significance	0.05	0.01	0.001	Significance	0.05	0.01	0.001
	NIT+OBL				NIT+OBL			
	NIT+GLO				OBL+GLO			
	OBL+GLO				NIT+GLO			

Moreover, both feeding and oviposition responses of *C. bimaculata* to foliage of *E. nitens* and *E. obliqua* in choice experiments were relatively different from that observed in no choice experiments. That is the beetles consumed more *E. nitens* foliage than *E. obliqua* in choice experiment but consumed more *E. obliqua* foliage in no choice experiments. In contrast, they oviposited more egg batches on *E. obliqua* foliage in choice experiments but fewer in no choice experiments.

Table 7.9. Comparison in preference of *C. bimaculata* adults to different species foliages when foliage was mixed or separated.

A) Feeding

		NIT : GLO			OBL : GLO			NIT : OBL	
		Separated foliages	Mixed foliages		Separated foliages	Mixed foliages		Separated foliages	Mixed foliages
Percentage distribution*	NIT	79.08	73.99	OBL	83.43	95.32	NIT	42.88	53.31
	GLO	20.92	26.01	GLO	16.37	4.68	OBL	57.12	46.69
Ratio of distribution†	NIT vs GLO	3.78	2.84	OBL vs GLO	5.04	20.37	NIT vs OBL	0.75	1.14

B) Oviposition

		NIT : GLO			OBL : GLO			NIT : OBL	
		Separated foliages	Mixed foliages		Separated foliages	Mixed foliages		Separated foliages	Mixed foliages
Percentage distribution	NIT	74.70	65.22	OBL	72.73	76.00	NIT	52.54	45.16
	GLO	25.30	34.78	GLO	27.27	24.00	OBL	47.46	54.84
Ratio of distribution	NIT vs GLO	2.95	1.88	OBL vs GLO	2.67	3.17	NIT vs OBL	1.11	0.82

* Percentage distribution $(Y_1)=X_1/(X_1+X_2)$ x 100%; for example, $Y_{NIT} = X_{NIT}/(X_{NIT}+X_{GLO})$ x 100%
† Ratio of distribution $(R)=X_1/X_2$; for example, $R_{(NIT\ vs\ GLO)} = X_{NIT}/X_{GLO}$

7.5.3. Discussion

The difference between choice and no choice experiments is that the beetles can select between species in choice experiment but can not select in the latter. Therefore, results of choice experiments are more relevant to establish the preference of beetles than no choice experiments. However, the no choice experiment does reveal the ability of beetles to cope with different foliages.

Results of choice experiments indicated that the response of *C. bimaculata* and *C. agricola* was significantly different between species. *E. obliqua* foliage was preferred by *C. bimaculata* adults and *E. globulus* was least preferred. In contrast, the *E. globulus* foliage was preferred by *C. agricola* and *E. obliqua* was least preferred. However, the adult *E. nitens* foliage was preferred by both *C. bimaculata* and *C. agricola* adults. This finding is in agreement with the field observations of other workers (de Little 1989 and reference there in).

In no choice experiments, feeding response of *C. agricola* on non-preferred species, *E. obliqua*, was similar to that on preferred species. However, the acceptance of *C. bimaculata* adults to non-preferred species, *E. globulus,* was significantly lower than to preferred species. This suggested that *C. agricola* adults were more efficient in coping with the non-preferred foliage of *E. obliqua.* In contrast, *C. bimaculata* was less efficient in coping with *E. globulus*. This finding was similar to the results observed in larval feeding trials undertaken by de Little (1979) who found that *C. agricola* was a more efficient feeder than *C. bimaculata.* However, *E. obliqua* foliage was still unacceptable for *C. agricola* oviposition, since this beetles deposited significantly fewer egg batches on *E. obliqua* than on preferred *E. globulus* foliage.

The host preferences of both *C. bimaculata* and *C. agricola* were associated with chemical differences between species. Similar to the previous choice experiments, results indicated that the non-preference of *C. bimaculata* adults to *E. globulus* was associated with high amounts of 1,8-cineole and related oil compounds (oil and wax data see Fig. 7.1) which occurred in the foliage of this species. There was no correlation between wax chemicals and host preference of *C. bimaculata.* However, the preference of *C. agricola* adults appeared to be correlated with both oil and wax chemicals. Oils and waxes of *E. obliqua* foliage, were characterised by high amounts of group A oil compounds and triterpenoids respectively and was not preferred by *C. agricola* adults. In contrast, foliage of their preferred hosts, *E. globulus* and *E. nitens*, contained high amounts of ß-diketones. It was suggested that ß-Diketones may play a role in host determination of *C. agricola*, since the leaf waxes of almost all *Symphyomyrtus* species, which were preferred by this beetle, contained high amounts of ß-diketones.

The decrease in response, in particular the oviposition response, of *C. bimaculata* adults to *E. globulus* foliage in the choice experiment was correlated with the more distinct preference for *E. nitens* and *E. obliqua* foliage than in the no choice experiment. However, *C. bimaculata* did not show any difference in its response to *E. nitens* and *E. obliqua* foliage just as *C. agricola* showed no difference between *E. nitens* and *E. globulus*. Moreover, in the choice experiment with two species, the response of *C. bimaculata* adults also did not differ significantly between *E. nitens* and *E. obliqua* when the additional effect of *E. globulus* was excluded. This suggested that the *C. bimaculata* adults had similar preference to these two species.

In the choice experiment, volatile chemicals diffusing from different foliages (Appendix 7.6) may be absorbed by each other and saturate. More foliage could be eaten and more egg batches be produced if chemical stimuli diffusing from the preferred foliage was absorbed into non-preferred foliage. Conversely, deterrent chemicals diffusing into preferred foliage could result in a decrease of preference. However, the response of either *C. bimaculata* or *C. agricola* was distinctly different between species. Therefore the possible absorption of the volatiles of one species onto foliage of either a preferred or non preferred species did not influence the beetle's response significantly. That is the responses appear to be affected solely by stimuli perceived during actual feeding.

7.6. General discussion

Study of this chapter is a preliminary investigation of the responses of adult *C. bimaculata* and *C. agricola* to different *Eucalyptus* foliages. Results indicated that the response of these two beetle species observed under laboratory conditions was in agreement with the host specificities recorded in the Tasmanian forest ecosystem (deLittle 1979, 1989). It was found that leaf preferences of *C. bimaculata* and *C. agricola* adults was associated with subgeneric specific differences and with differences in leaf oil and wax chemicals between specific species. Results suggested that the host selection by *C. bimaculata* adults was more associated with the chemical difference in leaf oils whereas that of *C. agricola* appeared to be related to differences in wax chemicals.

Volatile terpenoids have been found to play an important role in host selection by insects (Harborne 1988). It has been reported that α– and β–pinene are probably major deterrents to most insects (Harborne 1988) and 1,8-cineole and α–terpineol were lethal to house flies (Shaaya *et al.* 1991). Results of this study indicated that these oil compounds may play a important role in host selection by *C. bimaculata.*

The analysis of leaf oils has indicated that the *Symphyomyrtus* species, which are not attacked by *C. bimaculata*, contain high contents of 1,8-cineole, α–pinene and α–terpineol in contrast to the preferred *Monocalyptus* species, which contained α– and β–phellandrene and related oil compounds (Fig. 7.1B). Results of this study indicated that *C. bimaculata* adults did not prefer and accept foliage which contained high amounts of 1,8-cineole and related compounds. *E. nitens* foliage containing very low contents of 1,8-cineole and related oil compounds were accepted by this beetle species but the juvenile foliage of *E. nitens* was protected physically by glaucous waxes which prevented attachment to leaves. Moreover, the *C. bimaculata* adults exhibited a similar preference to *E. nitens* and *E. obliqua* indicating that *E. nitens* did not lack appropriate stimuli or did not contain appropriate deterrents for this beetle species. *C. bimaculata* adults had significant difference in oviposition response but not feeding response between adult and dewaxed juvenile foliage of *E. nitens* indicated that ontogenetic difference had a more significant affect on oviposition response than feeding. 1,8-Cineole had been reported to inhibited the mating between the sexes in the leafhopper *Amrasca devastans* (Saxena and Kumar 1984). It is possible that 1,8-cineole may also inhibit oviposition by *C. bimaculata* beetles on *Symphyomyrtus* species.

The leaf oil chemicals may also have effects on host tree selection by *C. agricola* adults, since the leaf oil chemicals of the non-preferred host, *E. obliqua*, differed qualitatively from that of preferred hosts. Moreover, the leaf waxes may also have an effect on *C. agricola* adults. Triterpenoids have been found to be an inhibitor for many insect (Harborne 1988). Triterpenoids in waxes have been found to influence the host selection of *Plutella xylostella* on different cabbage genotypes (Eigenbrode *et al.* 1991). It is possible that triterpenoids in eucalypt leaf waxes also played a role in the host selection by *C. agricola*. Triterpenoids occurred in large amounts in leaf waxes of non-preferred host, *E. obliqua*, and other 'ash' species but the preferred hosts, *Symphyomyrtus* species, contained only minor or trace amounts of triterpenoids in leaf waxes. However, the leaf oils and triterpenoids in non-preferred hosts did not appear to active against *C. agricola* as 1,8-cineole did to *C. bimaculata*, since *C. agricola* adults could efficiently cope with *E. obliqua* foliage in the no choice experiment.

ß-Diketones may have a positively influence the host selection of *C. agricola*, since the waxes of *Symphyomyrtus* species, which are preferred by this beetle, contained high amounts of ß-diketones.

Overall results of this study indicate that individual chemicals may have different effects for different beetle species. The host specificity of individual beetles species is influenced by the combined effects of different chemicals and other factors. Only foliage of a number of species has been tested in this study, which has limited an efficient

comparison between beetle response and chemicals distribution. However, the results in conjunction with those of the chemical analyses reported in previous chapters have indicated that an important correlation may exist between the adult female response to feed and oviposit and the leaf oils and waxes of eucalypts.

Chapter 8

The Acceptance and Feeding Response of Chrysophtharta spp. Larvae to Leaves of Different *Eucalyptus* spp.

8.1 Introduction

The present research described in this chapter was initiated to determine whether the chemicals in the foliage of different *Eucalyptus* species affected those species's ability to sustain the larvae of two *Chrysophtharta* species and to compare the reaction of larvae feeding on different foliages. The approach adopted was to allow larvae to feed on leaf discs and then determine the chemicals of the corresponding foliages so as to compare chemical variables and the subsequent acceptance and feeding responses of larvae. In order to increase the efficiency of comparison, large numbers of tree species, which cover different chemotypes of the leaf oil, were used. This chapter describes the response of *C. bimaculata* and *C. agricola* larvae to leaves of different *Eucalyptus* species and the qualitative and quantitative relationships between leaf oil chemicals and larval behaviour. Results of this experiment were also discussed in conjunction with the chemotaxonomy of Tasmanian eucalypt species described in the previous chapter.

8.2. Materials and methods

8.2.1. Experimental design

In order to overcome the problems of variable supply of both insects and foliage, a standard control was run with each test. This allowed the results of different runs to be compared concurrently. Adult foliage of *E. nitens* was chosen as the control foliage as the preliminary work with foliage of several species showed that it was the most preferred by both *C. bimaculata* and *C. agricola*. Specifically, the adult foliage of the Toorongo provenance of *E. nitens*, was the most heavily defoliated by *C.* species and was therefore employed. By the use of this control in all tests, the results of different trials could be corrected for run differences and so directly compared.

8.2.2. The establishment and maintenance of test larvae

A laboratory population of *C. bimaculata* larvae was established from egg batches taken from natural forests and plantations at Snug Plains (adults of *E. regnans* and *E. delegatensis*) and Esperance Valley respectively (mainly from adult *E. regnans* and some from *E. delegatensis* and *E. nitens*) during the summer season (1990-1991). Another laboratory population of *C. agricola* larvae was established with egg batches taken from *E. nitens* and *E. globulus* trees from plantations in Esperance Valley.

Branches containing egg batches were placed in plastic bags and transported to the laboratory and stored in a 4 ^{0}C cool room (for maximum of 4 days after which mortality occurred). Large egg batches were selected and placed in a glass petri dish on damp filter paper and at least two layers of fresh leaves (adult *E. delegatensis*), before being incubated at 24±2 ^{0}C. Eggs were checked at 12 hour intervals and when longitudinal slits began to appear in the egg casing, eggs were transferred to a new glass dish. After hatching, the larvae were transferred to a third glass petri dish that was filled with two to three layers of fresh, young *E. delegatensis* leaves (no damp filter paper was added) and covered with an additional one or two layers of young leaves. These were incubated at 24±2 ^{0}C. Any larvae that did not occur on leaves but moved around the glass dish were removed within a 24 hour interval. After 3 days, larvae were checked every 8 hours to determine when the 2nd instar moult occurred. Larvae weighing between two to three mg were used in feeding tests.

During preliminary studies, it was found that the amount of free moisture in the rearing dish affected the vitality of the larvae and was a significant factor affecting larval weight within a temperature range from 22-26 ^{0}C. Furthermore, moisture directly affected larval survival rate and feeding activity. If moisture was too high the larvae became bloated in appearance and many died without feeding after they were transferred to test foliage. If moisture was limiting the leaves would dry out and larvae continuously moved around so that feeding was unbalanced resulting in large variations in weight. The above rearing schedule maintained moisture condition within acceptable limits.

8.2.3. Test foliage samples

The young foliage samples were collected from different sites. Principally, these samples were considered to cover the different chemotypes of eucalypt species in Tasmania as indicated in previous chapters and consisted of different levels of oil yield within and between species.

The leaf samples were placed in plastic bags and transported to University. When back in the laboratory, stems were placed in water and foliage covered with plastic bags in a 4 ^{0}C

cool room. Leaf discs (1.00 cm diam) were cut from young leaves with a steel punch. All discs were cut from the areas on either side of the major mid leaf vein. Proportions of the discs were used for chemical analysis, fresh and dry weight determinations and larval test food. Prior to each feeding test the following measurements were taken:

i) Fresh (FW) and dry weight (DW) of leaf discs (leaves) and subsequently moisture content (MC):

$$MC\ (\%) = (FW - DW) / FW \times 100$$

ii) Oil yield (g/100g DW*) and oil composition (%)

iv) Absolute content of individual compounds in foliage (g/10kg DW) =
Oil yield (g/100g DW) x percentage content of compound in oil (%)

*DW is oven dry weight

8.2.4. Test methods

Larvae were standardised using early-mid stage second-instar larvae of 2-3 mg weight. After a 6 hour pretest starvation period, a 48 hour feeding period at 24±2 ^{0}C was allowed. Leaf samples were offered as three leaf discs placed on moist 50mm diameter filter paper in a 60 mm diameter plastic petri dish. Fresh leaf discs were provided at the start of the assay and replaced every 24 hours during the test.

Five larvae were placed in each petri dish, and the test of each sample replicated 10 times (reduced to five when larvae were in short supply). The control reference of fresh adult *E. nitens* foliage was used in each experimental run.

To analyse the results, each replication was considered as an independent measurement of each parameter, regardless of whether the replications were from different experimental runs or within a run. A comparison of the larval feeding response between foliages used within each run was obtained while the comparison between foliages of different runs was made by comparison to the reference control (*E. nitens* adult foliage). The feeding response on the reference control was ranked as 1 and others were compared to this parameter. For example, where the leaf consumption on the control reference was 100 mg and to a foliage sample was 90 mg, the comparison ratio of this foliage sample would be 90/100 = 0.90.

8.2.5. Measurements of leaf consumption and frass/excretion production

Larval excreta was collected after 48 hours, oven dried and weighed. This estimate of larval excreta was obtained by using weight-subtraction. Thus, the petri dish plus filter paper was weighed before the test, then after the test to determine larval excreta weight. It was noted that the weight obtained in this experiment was a constant dry weight. For example, the constant weight petri dish plus filter paper was obtained by incubating them in a drying oven at 50 ^{0}C over a 24 hour period. A number of these petri dishes were selected to determine weight change at 4 hourly intervals. When the variation in weight for a petri dish was less than 1mg, then all petri dishes were weighed and recorded as quickly as possible.

Leaf consumption was also measured by drying the leaf disc remnants: Leaf consumption (mgDW) = Initial dry weight of leaf discs (mgDW) - weight of remnant discs (mgDW), where the total dry weight of leaf discs was estimated from the original fresh weight of discs and their estimated moisture contents.

8.2.6. Data analysis

The ANOVA and model used for testing the difference in the larval feeding response in different foliage samples were the same as Chapter 7. However, some tests in this experiment were of a nested design. For example, a test comparing the variability in the *C. bimaculata* larval feeding response between different levels of a taxonomic unit, for example subgenus and species. Since the species from the two subgenera of *Eucalyptus* are not the same, each species in the subgenus *Symphyomyrtus* has no relationship to species in *Monocalyptus*. Therefore, this test was a nested design with subgenera, species within subgenera and error levels.

All analyses were computed and performed using the ANOVA program of the super ANOVA (Abacus Concepts, Inc.), and were run on a Macintosh SE/30 computer.

8.2.7. Leaf materials

A. The response of C. bimaculata larvae to different eucalypt foliages

All tests for *C. bimaculata* larvae were carried out by using early to mid-stage second-instar larvae as described above. Leaf samples used for individual experiments follow:

Experiment 1: Response to *Monocalyptus* and *Symphyomyrtus* species

A total of eleven species from the two *Eucalyptus* subgenera were offered to *C. bimaculata* larvae so that a more valid comparison between species and between subgenera could be made. The leaf samples are listed in Table 8.1.

Table 8.1. Leaf samples used for *C. bimaculata* larval feeding test No.1.

Species	Locality	Leaf type	Sample Code
E. obliqua	Southern forest plantation	Adult	OBL Sout A
E. regnans	Plantation in Esperance, Tas. (Original from Moogara, Tas.)	Adult	REG Moog A
E. delegatensis	Plantation in Esperance, Tas. (Original from Guildford, Tas)	Adult	DEL Guil A
E. sieberi	Plantation in Esperance, Tas.	Adult	SIE Sout A
E. pauciflora	Mt. Field, Tas.	Adult	PAU MtFi A
E. tenuiramis	Huon Rd., Hobart, Tas.	Adult	TEN HuRd A
E. ovata	Plantation in Esperance, Tas. (Original from D'entrecasteaux river	Adult	OVA Sout A
E. globulus	Lea - Kingston, Hobart, Tas.	Adult, juvenile	GLO LeaK A
E. nitens	Plantation in Esperance, Tas. (Original form St. Gwinear, Vic.)	Adult, juvenile	NIT Refe A*
E. viminalis	Lea - Kingston, Hobart, Tas.	Adult	VIM LeaK A
E. gunnii	Snug Plains	Adult	GUN SnPl

* The leaf samples from *E. nitens* trees of this locality were used as reference control samples.

Experiment 2: Feeding on juvenile and adult foliage of three *Monocalyptus* species

This experiment compared the variability of the *C. bimaculata* larval feeding response to juvenile and adult foliage of three important *Monocalyptus* species, *E. obliqua, E. delegatensis* and *E. regnans*. The three species were sampled from two different localities, Snug Plains and Mt Wellington. Hence, this experiment also compared locality differences within each species and the variability of the larval feeding response between species within each locality. Leaf samples for this experiment are listed in Table 8.2.

Table 8.2. Leaf samples for experiment 2 of *C. bimaculata* larval test No.2.

Species	Locality	Leaf type	Code
E. obliqua	Mt Wellington	Adult	Obl M_A
		Juvenile	Obl M_J
	Snug Plains	Adult	Obl S_A
		Juvenile	Obl S_J
E. delegatensis	Mt Wellington	Adult	Del M_A
		Juvenile	Del M_J
	Snug Plains	Adult	Del S_A
		Juvenile	Del S_J
E. regnans	Mt Wellington	Adult	Reg M_A
		Juvenile	Reg M_J
	Snug Plains	Adult	Reg S_A
		Juvenile	Reg S_J

Experiment 3: Feeding on adult leaves of *E. delegatensis* from four different localities

This experiment compared the variability of the *C. bimaculata* larvae feeding response to adult foliages of *E. delegatensis* from different localities in which oil yields differed markedly but oil compositions were similar. Adult foliages of six year old trees were collected from an *E. delegatensis* provenance trial at Tarraleah, Tasmania. The original localities are listed in Table 8.3.

Table 8.3. The original location of *E. delegatensis* localities used for experiment 3 of *C. bimaculata* larval feeding test No. 3.

Species	Original locality	Leaf type	Code
E. delegatensis	Luina, Tas.	Adult	DEL Tas 1
	Ben Nevis, Tas.	Adult	DEL Tas 2
	Mt St Gwinear, Vic.	Adult	DEL Vic 1
	Mt Ellery, Vic.	Adult	DEL Vic 2

Experiment 4: Feeding on juvenile and adult leaves of *E. nitens* and *E. denticulata* from different localities

This experiment compared the variability of the *C. bimaculata* larvae feeding response to adult foliages of *E. nitens* and *E. denticulata* from different localities. At the same time, comparison was also made between normal juvenile and dewaxed juvenile leaves and adult leaves of *E. nitens*. The dewaxed juvenile foliage was treated by using wet cotton wool to physically removed the glaucous waxes from leaf surface. All leaf samples used in this experiment were collected from *E. nitens* provenance seedlots at Esperance, Tasmania. Leaf samples were listed in Table 8.4.

Table 8.4. Origins of leaf samples used for experiment 4.

Species	Original locality	Leaf type	Code
E. nitens	Connor Plain, Vic.	Adult	NIT CoPl A
		Juvenile	NIT CoPl J
E. nitens	Toorongo, Vic.	Adult	NIT Troo A
		Juvenile	NIT Troo J
E. nitens	Mt Kaye, Vic.	Adult	NIT MtKa A
E. nitens	*Noojee, Vic.	Adult	NIT Nooj A*
E. denticulata	Bendoc, Vic.	Adult	DEN Bend A

* Foliage of this locality were selected from individual trees which had higher oil yields. This foliage was used to compare other *E. nitens* foliage with normal oil levels. Therefore, it did not represent this locality.

B. Test for *C. agricola larvae*

Due to a scarcity of larval material, testing of *C. agricola* was made using third instar larvae of 4-5mg average weight and only adult foliage of ten species were tested. This feeding test of *C. agricola* included a total of ten species from the two subgenera so that valid comparison between species and between subgenera could be made. The leaf samples used are the same as those listed in Table 8.1 for *C. bimaculata* larval test.

8.3. Results

8.3.1. The response of *C. bimaculata* to foliages from *Monocalyptus* and *Symphyomyrtus* species

The results for leaf consumption, frass production, larval weight, survival rate (%) and moulting frequency (%) of *C. bimaculata* larvae feeding on foliage of the 11 eucalypt species are listed in Appendix 8.1 and the ANOVA and MANOVA of the larval feeding response between subgenera and species (within subgenera) is shown in Table 8.5. Initial examination indicated that the observed data was not distributed normally. Plotting residuals against fitted values indicated that the logarithmic transformation of data [Log(x+1)] was the appropriate transformation for leaf consumption, frass production and larval weight and the arcsin($\sqrt{x}$) transformation for survival (%) and moulting frequency (%) data.

The variation between species within subgenera was the most significantly ($p<0.001$) affected for all components of the *C. bimaculata* larval feeding response (Table 8.5A). Subgenera also had a significant effect on frass production ($p<0.05$). The F-ratios for the effect of subgenera on leaf consumption (3.790) and moulting frequency (4.407) were relatively higher but not quite significant at the $p<0.05$ level. The mean response values for both subgenera are shown in Table 8.6 and indicate that leaf consumption, frass production and moulting frequency of *C. bimaculata* on *Monocalyptus* was higher than on *Symphyomyrtus*.

The MANOVA (Table 8.5B) indicated that species (within subgenera) significantly affected ($p<0.001$) the larval feeding response. However, the effect of subgenera was not significant.

The means and standard errors for leaf consumption, frass production, larval weight, survival and moulting frequency on individual species and the multirange test, using transformed data, are shown in Fig. 8.1.

The highest leaf consumption, frass production, larval weight, moulting frequency and survival rate among species generally occurred on *E. nitens* adult foliage while *E. globulus* juvenile foliage had the lowest response in all categories. *E. delegatensis* had the same moulting frequency and survival rate as adult foliage of *E. nitens*.

Table 8.5. ANOVA for *C. bimaculata* larval response on foliage of 11 eucalypt species

A) ANOVA

ANOVA for Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	3.724	3.724	3.790	0.0775	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	11	10.808	0.983	29.901	0.0001	Residual
Residual	117	3.844	0.033			

Model summary R: 0.889 R^2: 0.791 RMS Residual: 0.181 Model F-value: 36.853 P-value: 0.0001

ANOVA for Frass Production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	4.33	4.33	5.551	0.0381	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	11	8.581	0.78	29.433	0.0001	Residual
Residual	117	3.101	0.027			

Model summary R: 0.898 R^2: 0.806 RMS Residual: 0.163 Model F-value: 40.595 P-value: 0.0001

ANOVA for Larval Weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	2.306	2.306	3.044	0.1088	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	11	8.333	0.758	22.454	0.0001	Residual
Residual	117	3.947	0.034			

Model summary R: 0.854 R^2: 0.729 RMS Residual: 0.184 Model F-value: 26.28 P-value: 0.0001

ANOVA for moulting frequency (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	1.136	1.136	4.407	0.0597	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	11	2.836	.258	8.573	0.0001	Residual
Residual	117	3.518	.030			

Model summary R: 0.728 R^2: 0.530 RMS Residual: 0.173 Model F-value: 11.007 P-value: 0.0001

ANOVA for Survival Rate (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	1.605	1.605	2.061	0.1789	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	11	8.567	0.779	9.305	0.0001	Residual
Residual	117	9.792	0.084			

Model summary R: 0.714 R^2: 0.51 RMS Residual: 0.289 Model F-value: 10.128 P-value: 0.0001

Table 8.5. Continued.

B) MANOVA#

Effect: Subgenera			Value	F-Value	Num DF	Den DF	P-Value
S	1	Wilks' Lambda	.427	1.882	5.000	7.000	2.159
M	1.5	Hotelling-Lawley Trace	1.344	1.882	5.000	7.000	2.159
N	2.5	Pillai Trace	.573	1.882	5.000	7.000	2.159

Effect: Species(subgenera)			Value	F-Value	Num DF	Den DF	P-Value
S	5	Wilks' Lambda	.122	5.505	55.000	526.638	0.0001
M	2.5	Hotelling-Lawley Trace	3.991	8.084	55.000	557.000	0.0001
N	55.5	Pillai Trace	1.35	3.935	55.000	585.000	0.0001

For MANOVA refer to Harris (1975). S = min. (df_{effect}, p); M = ($|df_{effect}$ - p| -1) / 2; N= (df_{error}-p-1) / 2; Num DF = Numerator degrees of freedom; Den DF = Denominator degrees of freedom.

Table 8.6. The untransformed mean values of the *C. bimaculata* larval feeding response on the two different subgenera of *Eucalyptus*.

		Leaf consumption (mg)		Frass production (mg)		Larval weight (mg)		Survival rate (%)		Moulting frequency (%)	
Subgenus	No. of Rep.	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)
Symphyomyrtus	70	10.48	1.32	6.13	0.77	13.87	1.30	62.57	3.48	24.00	2.95
Monocalyptus	60	17.48	1.10	11.98	0.69	20.93	1.02	79.33	2.51	43.33	3.20

The multiple range test (Fig. 8.1B) indicated that leaf consumption and frass production on adult foliage of the four 'ash' species, *E. delegatensis*, *E. pauciflora*, *E. obliqua* and *E. regnans* and the juvenile foliages of *E. nitens* were not significantly different from adult *E. nitens*, the most preferred foliage. However, they were significantly higher ($p<0.05$) than on foliage of two other *Monocalyptus* species, *E. tenuiramis* and *E. sieberi*, and on the other *Symphyomyrtus* species.

Leaf consumption on foliage of *E. tenuiramis* did not differ significantly to that on *E. viminalis* and foliage consumption on *E. sieberi* did not differ significantly to that on *E. viminalis* and *E. gunnii*. Frass production on *E. tenuiramis* foliage was significantly lower ($p<0.05$) than that on other *Monocalyptus* species, with the exception of *E. sieberi*. However, frass production following feeding on *E. sieberi* foliage did not differ significantly from that on *E. gunnii*. Therefore, rates of leaf consumption and frass production on the foliage of *E. tenuiramis* and *E. sieberi* tended to be more similar to that observed on the foliage of *Symphyomyrtus* species.

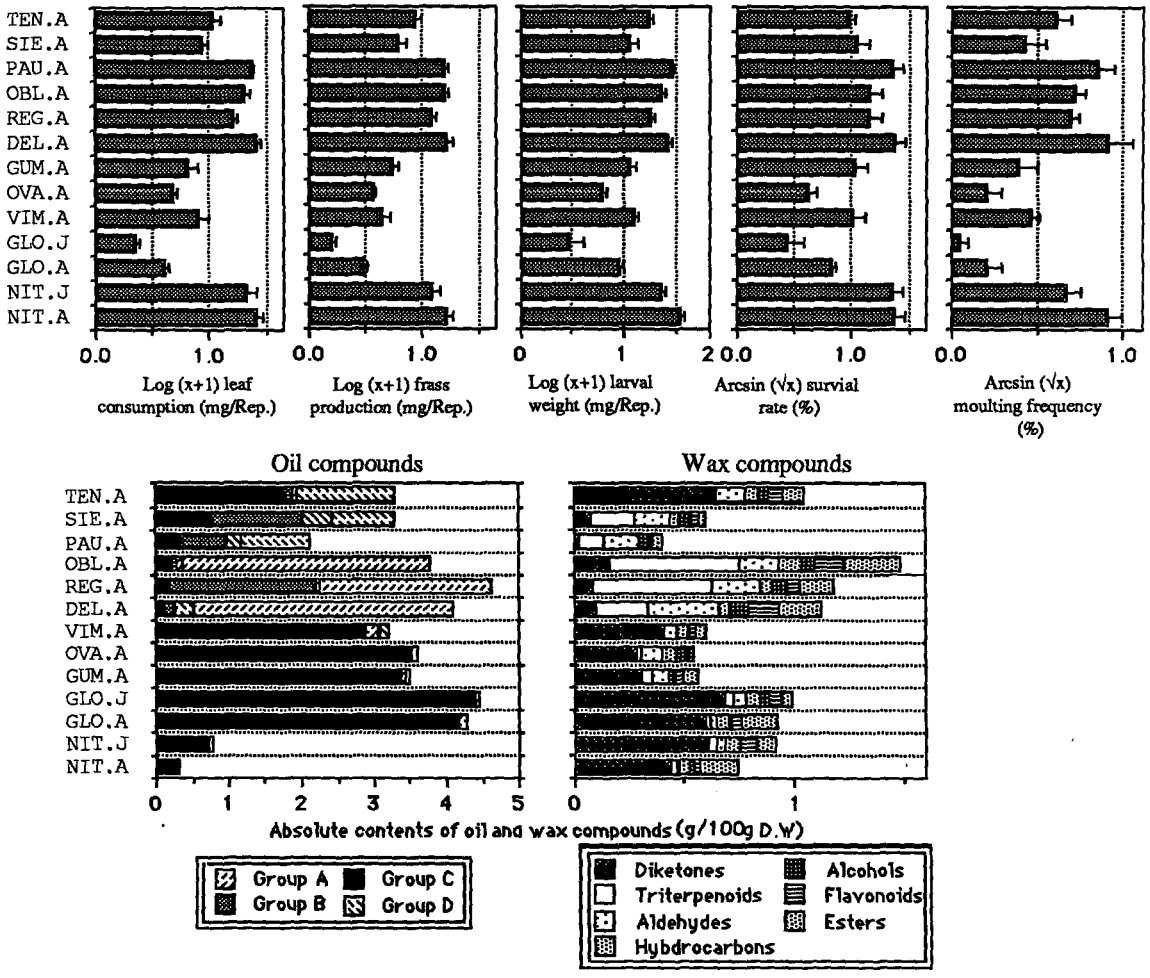


Fig. 8.1.A. The means and standard errors of *C. bimaculata* larval feeding response to foliage of 11 eucalypt species (transformed data) and the absolute contents of oil and wax compounds of these foliage.

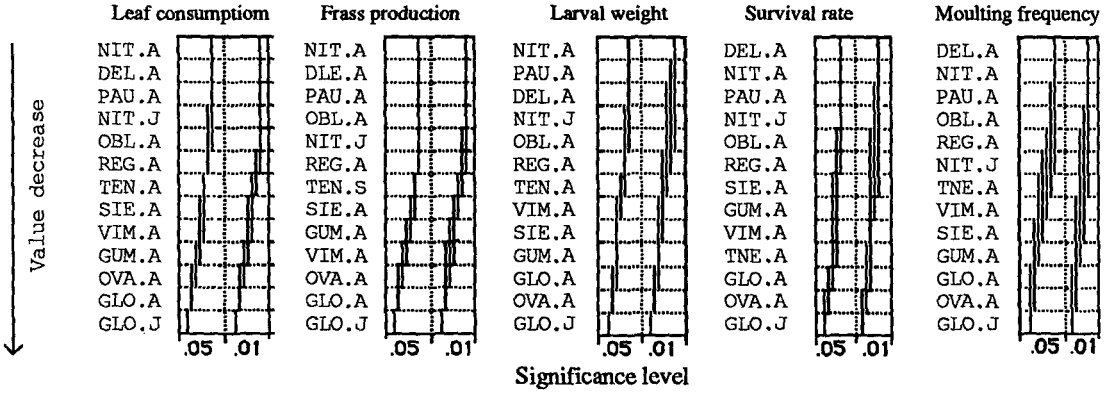


Fig. 8.1B. The multiple range test (Fisher's PLSD) for *C. bimaculata* larval feeding response on foliage of 11 eucalypt species using transformed data [log(x+1)] for leaf consumption, frass production, and larval weight and transformed data [arcsin(√x)] for survival rate (%) and moulting frequency (%).

C. bimaculata larvae that fed on the foliage of *E. nitens, E. delegatensis, E. pauciflora, E. obliqua* and *E. regnans* were significantly ($p<0.05$) heavier than those on *E. sieberi* and all other *Symphyomyrtus* species. However, there was no significant difference in weights between larvae fed on either *E. tenuiramis, E. regnans* and *E. obliqua* or between *E. tenuiramis* and *E. viminalis* foliages.

Survival of *C. bimaculata* larvae fed on the foliage of *E. nitens, E. delegatensis* and *E. pauciflora* was significantly higher ($p<0.05$) than on *E. sieberi* and *E. tenuiramis* and other *Symphyomyrtus* species. Survival on foliage of *E. obliqua* and *E. regnans* did not differ significantly from that on *E. nitens, E. delegatensis* and *E. pauciflora.* In addition, survival on foliage of *E. obliqua* and *E. regnans* did not differ significantly from that on *E. sieberi, E. tenuiramis, E. gunnii* and *E. viminalis.*

Moulting frequency following consumption of foliage of *E. nitens, E. delegatensis* and *E. pauciflora* was significantly higher ($p<0.05$) than that on *E. sieberi* (*Monocalyptus*) and other *Symphyomyrtus* species but did not differ from moults on *E. obliqua, E. regnans* and the juvenile foliage of *E. nitens.* Moulting frequency on foliage of *E. tenuiramis* did not differ significantly from that on the preferred foliages of *E. pauciflora, E. obliqua* and *E. regnans* and the juvenile foliage of *E. nitens* and the non-preferred foliage of *E. sieberi, E. viminalis* and *E. gunnii.*

In summary, the multiple range test indicated that the *Symphyomyrtus* species *E. nitens,* which is not indigenous to Tasmania, and the four commercial 'ash' species, *E. delegatensis, E. pauciflora, E. obliqua* and *E. regnans* were suitable hosts for *C. bimaculata* larvae. In contrast, other *Symphyomyrtus* species, which are indigenous to Tasmania were less suitable. The non-preference of *C. bimaculata* larvae for the foliage of the 'ash' species, *E. sieberi,* and peppermint species, *E. tenuiramis* of *Monocalyptus,* tended to be similar to that on Tasmanian *Symphyomyrtus* species.

To further describe the effects of individual foliage samples on feeding responses, the detailed behaviour of *C. bimaculata* larvae during the test period is given in Table 8.7. In comparing the biting intensity of *C. bimaculata* larvae among individual samples, it was found that the total biting response on preferred foliage was generally lower than those on the non-preferred foliage samples (Table 8.7), particularly for foliage of adult and juvenile *E. nitens* and adult *E. delegatensis* and *E. pauciflora.* Moreover, the biting-feeding index indicated that the bite/feeding ratio for preferred foliage was notably lower than those on non-preferred foliages. This further indicates that *C. bimaculata* larvae made fewer test bites on preferred foliage but soon accepted them as a favourable food

resource and fed on. Thus, foliage of *E. nitens*, *E. delegatensis* and *E. pauciflora* had fewer test bites and higher leaf consumption than any other sample (Fig. 8.1 and Table 8.7). In the comparison of the biting and feeding intensities at different periods of a test, foliage samples of these three species received most test bites during the first 12 hr. When new leaf discs were added at 24 hr, the larvae made fewer test bites but fed more than in the first 12 hr. *C. bimaculata* larvae also favoured the foliages of adult *E. regnans* and *E. obliqua* and continuously fed throughout the test but also made many test bites throughout the test. Observations on the behaviour of larvae during the test also indicated that *C. bimaculata* larvae moved less when they fed on foliages of *E. nitens*, *E. delegatensis* and *E. pauciflora*, but moved more when feeding on *E. regnans* and *E. obliqua*. Figure 8.2 indicates that faeces of *C. bimaculata* larvae were concentrated around the leaf discs when they fed on the leaf discs of adult *E. nitens* but that the faeces were distributed over the entire petri dish when they fed on *E. obliqua*.

Table 8.7. Larval biting response of second-instar *C. bimaculata* larvae to foliages of 11 species of eucalypts during a 48 hr. test period. The number of test bites and leaf discs consumed were recorded every 12 hours. Numbers listed in here were the total numbers of 10 replicates of each samples.

* The numbers of biting lesions

** Leaf discs consumed recorded as consumption index: No. discs consumed x 10

Discs 1 were those discs provided at start of test and discs 2 were new discs replaced at 24 hr..

$ the column of 0-12 is the first 12 hr. and 12-24 is the number of additional bites or consumption index during second 12 hr.

	*No. of test bites					** Consumption index					Biting-feeding index (ratio: bites/consumption)				
Discs	Discs 1#		Discs 2			Discs 1		Discs 2			Discs 1		Discs 2		
Time (hr.)	0-12$	12-24	24-36	36-48	Total	0-12	12-24	24-36	36-48	Total	0-12	12-24	24-36	36-48	Total
Nit.A	48	5	17	5	75	103	80	132	102	417	0.5	0.1	0.1	0.0	0.2
Nit.j	44	4	19	6	73	98	72	155	108	433	0.4	0.1	0.1	0.1	0.2
Glo.A	104	27	43	8	182	12	17	23	6	58	8.7	1.6	1.9	1.3	3.1
Glo.j	52	8	30	9	99	13	8	4	1	26	4.0	1.0	7.5	9.0	3.8
Vim.A	58	18	41	30	147	53	31	51	41	176	1.1	0.6	0.8	0.7	0.8
Ova.A	57	13	61	42	173	24	26	6	7	63	2.4	0.5	10.2	6.0	2.7
Gun.A	50	7	42	38	137	31	26	26	36	119	1.6	0.3	1.6	1.1	1.2
Del.A	37	2	20	8	67	115	83	143	150	491	0.3	0.0	0.1	0.1	0.1
Reg.A	34	11	45	25	115	68	100	63	73	304	0.5	0.1	0.7	0.3	0.4
Obl.A	48	12	36	17	113	99	47	139	118	403	0.5	0.3	0.3	0.1	0.3
Pau.A	39	1	19	7	66	93	127	124	117	461	0.4	0.0	0.2	0.1	0.1
Ten.A	77	17	22	16	132	55	45	105	117	322	1.4	0.4	0.2	0.1	0.4
Sie.A	84	24	48	35	191	46	49	40	28	163	1.8	0.5	1.2	1.3	1.2

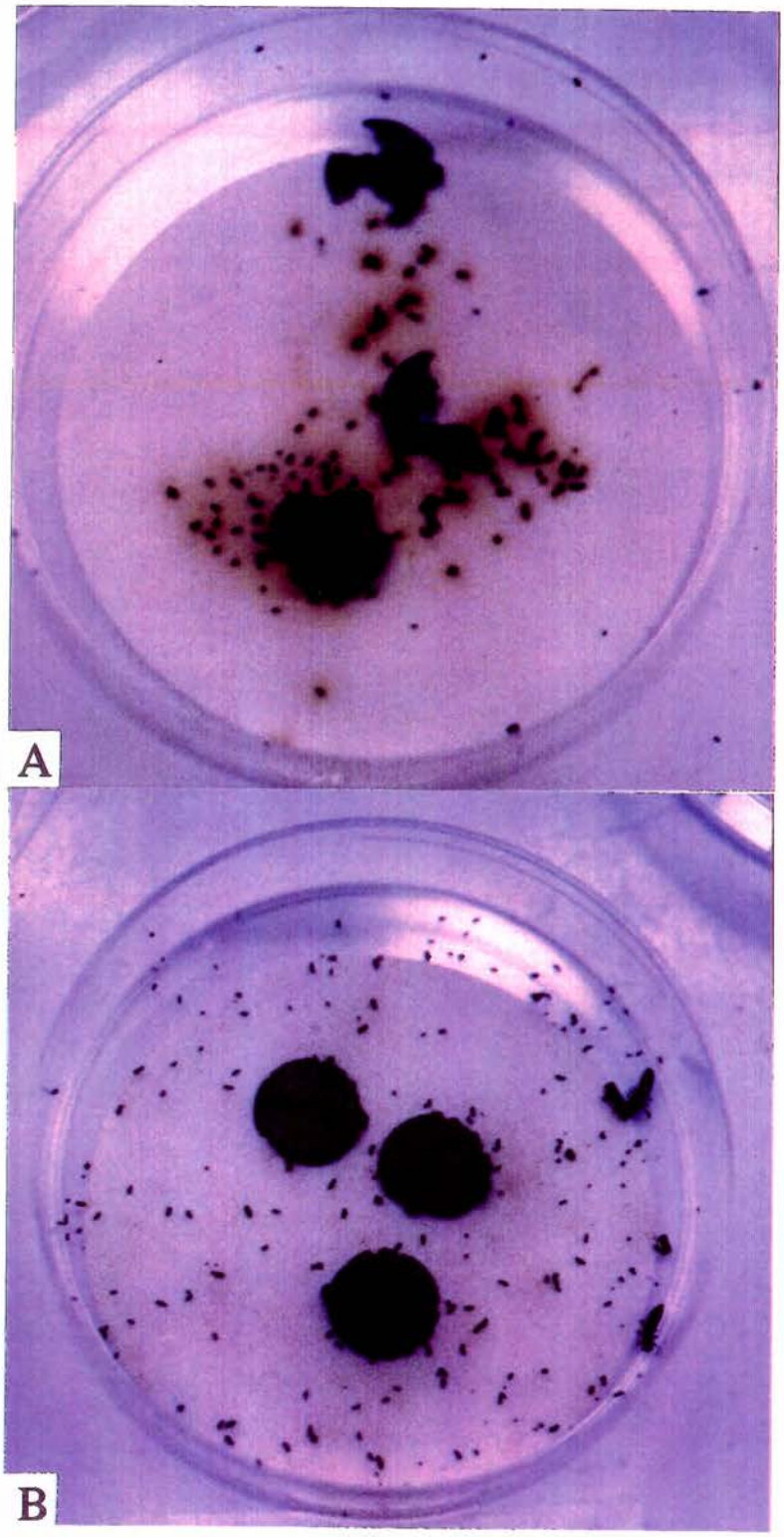


Fig. 8.2. *C. bimaculata* larval feeding response on *E. nitens* (A) and *E. obliqua* (B) adult leaf discs.

Note greater feeding and localised dispersion of frass and larvae on *E. nitens* in contrast to increased dispersion of frass and larvae and less feeding on *E. obliqua*.

On non-preferred foliage, *C. bimaculata* larvae exhibited intense biting activity but less feeding. This indicated that the larvae could not accept these foliages as favourable food.

The absolute content of different oil and wax compound groups in individual species foliage samples are listed in Fig. 8.1. Comparison made between *C. bimaculata* larval feeding response and distributions of these chemical compounds indicated that the differences of larval response appeared to be more associated with the differences of oil compounds between species than that of wax compounds.

The results indicated that the levels of compound group C (mainly 1,8-cineole and α–pinene, see Fig. 4.3 in chapter 4) in foliage were negatively associated with the feeding preference of *C. bimaculata* larvae. All high oil yielding foliage samples from *Symphyomyrtus,* which were rich in group C compounds, were not accepted by *C. bimaculata* larvae with the exception of adult and juvenile foliage of the *E. nitens* cultivar that had the lowest oil yield and levels of group C compounds. These findings suggested that absolute amounts of oil compounds but not the percentage composition of oils in the foliage of *Symphyomyrtus* species were associated with the feeding response of *C. bimaculata*, since the leaf oils of *E. nitens* contained both 1,8-cineole and α–pinene as major components of comparable percentage to the non-preferred *Symphyomyrtus* species.

For *Monocalyptus* species, the feeding response of *C. bimaculata* larvae does not appear to be associated with oil yields. The oil yields of the two most preferred *Monocalyptus* species, *E. delegatensis* and *E. pauciflora*, differed markedly, with *E. pauciflora* having the lowest oil yield among the *Monocalyptus* species, while *E. delegatensis* had the highest oil yield. The interaction between oil compounds and the feeding response of *C. bimaculata* larvae on *Monocalyptus* appears to involve a more complex situation than that operating in *Symphyomyrtus* species, since the leaf oils of *Monocalyptus* species are composed of quite different compounds.

In comparing *Monocalyptus* species, the leaf oils of *E. delegatensis* and *E. obliqua* were dominated by group A compounds (mainly α– and β–phellandrene, *cis*- and *trans*-piperitol and *cis*- and *trans*-menth-2-en-1-ol see chapter 4). The other preferred species, *E. regnans* also had high levels of group A compounds and in addition, had high levels of group B compounds, eudesmol and related sesquiterpenoids. These differences were most related to the lower feeding response to *E. regnans* than to *E. delegatensis*. Again, a mixture of relatively high levels of groups C and B compounds in *E. sieberi* and the relatively high level of group C compounds in *E. tenuiramis* correlated to the relatively low preference for these species by *C. bimaculata* larvae than that recorded on *E. delegatensis, E. regnans* and *E. obliqua.* Therefore, the distribution of oil chemicals and

C. bimaculata preference may involve a complex interaction where some compounds might act to deter, others to stimulate, and together to result in the observed grade in the feeding response to different species.

Comparisons made between all species also indicated that the acceptance of eucalypt foliages by *C. bimaculata* larvae was not necessarily stimulated by oil compounds, since the most preferred foliage of adult *E. nitens* had the lowest oil amount among all species and the preferred foliage of *E. pauciflora* had the lowest amount of oil among *Monocalyptus* species.

In contrast, the correlation between larval feeding response and distribution of wax compounds was relatively poor. For *Symphyomyrtus* species, the most preferred foliage of adult *E. nitens* had similar wax compounds to the least preferred foliages of *Symphyomyrtus* species. In comparing *Obliquae* species, results indicated that the most preferred species, *E. delegatensis* and *E. pauciflora,* had low triterpenoids. However, *E. pauciflora* and *E. sieberi* have similar wax content and compositions yet *E. pauciflora* is significantly more preferred than *E. sieberi*. Therefore, it was considered that the lesser preference of *E. sieberi* by larvae was not determined by differences in wax chemicals.

8.3.2. The feeding response of *C. bimaculata* larvae to juvenile and adult foliage of *E. delegatensis, E. obliqua* and *E. regnans*

The results for larval feeding response were listed in Appendix 8.2A and the data analysis for results of this experiment was separated into two steps. The first step (ANOVA and MANOVA) was to test the effects of species, leaf types (juvenile and adult) and localities in which reference foliage of adult *E. nitens* was excluded. The second step was to compare the larval feeding response on individual foliage samples in which reference foliage of adult *E. nitens* was included.

The results of the three way ANOVA (Table 8.8) indicated that species significantly affected all five components of the larval response ($p<0.001$ for leaf consumption, frass production and larval weight; $p<0.05$ survival rate and $p<0.01$ for moulting frequency) while leaf type (adult and juvenile) also significantly ($p<0.01$) affected leaf consumption, frass production and larval weight.

Leaf type did not significantly affect larval survival and moulting frequency ($p<0.072$ and 0.0715 respectively), although these values were close to the 0.05 level of significance.

Tree locality did not significantly affect any component of the larval feeding response and none of the interaction effects were significant. These results indicated that species and

leaf type were the major factors affecting larval feeding and their effects were independent.

The results of the ANOVA also indicated that tree species mainly affected the food consumption rates of *C. bimaculata* larvae, since the F-values for the species effect on leaf consumption, frass production and larval weight (F=33.969, 23.658 and 14.013 respectively) were much greater than that for survival and moulting rate (F=3.319 and 4.878 respectively). The comparison of F-values for the leaf type effect on these components of the larval feeding response also showed a similar trend with that of the species effect.

Again, the MANOVA (Appendix 8.2B), which considered the correlation among all five variables simultaneously, indicated that species and leaf type effects were significant ($p<0.001$ and $p<0.05$ respectively) and the effect of locality was not.

Table 8.8. ANOVA for *C. bimaculata* feeding response on foliages of *E. obliqua, E. delegatensis* and *E. regnans.*

ANOVA for Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	2	1.027	0.514	33.969	0.0001
Leaf type	1	0.139	0.139	9.208	0.003
Localities	1	0.033	0.033	2.169	0.1437
Species * Leaf types	2	0.0003757	0.0001879	0.012	0.9877
Species * Localities	2	0.05	0.025	1.64	0.1988
Leaf type * Localities	1	0.00005946	0.00005946	0.004	0.9501
Species * Leaf types * Localities	2	0.042	0.021	1.402	0.2505
Residual	108	1.633	0.015		

Model summary R: 0.661 R^2: 0.437 RMS Residual: 0.123 Model F-value: 7.628 P-value: 0.0001

ANOVA for Frass Production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	2	0.988	0.494	25.658	0.0001
Leaf type	1	0.198	0.198	10.284	0.0018
Localities	1	0.029	0.029	1.481	0.2263
Species * Leaf types	2	0.007	0.003	0.17	0.844
Species * Localities	2	0.043	0.022	1.129	0.3271
Leaf type * Localities	1	0.003	0.003	0.173	0.6786
Species * Leaf types * Localities	2	0.036	0.018	0.935	0.3958
Residual	108	2.079	0.019		

Model summary R: 0.604 R^2: 0.365 RMS Residual: 0.140 Model F-value: 5.64 P-value: 0.0001

Table 8.8. Continued.

ANOVA for Larval Weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	2	0.401	0.2	14.013	0.0001
Leaf type	1	0.139	0.139	9.715	0.0023
Localities	1	0.044	0.044	3.093	0.0815
Species * Leaf types	2	0.004	0.002	0.135	0.8736
Species * Localities	2	0.002	0.001	0.074	0.9292
Leaf type * Localities	1	0.004	0.004	0.289	0.5917
Species * Leaf types * Localities	2	0.012	0.006	0.404	0.6687
Residual	108	1.544	0.014		

Model summary R: 0.531 R^2: 0.282 RMS Residual: 0.120 Model F-value: 3.85 P-value: 0.0001

ANOVA for Survival Rate

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	2	0.615	0.308	3.319	0.0399
Leaf type	1	0.306	0.306	3.301	0.072
Localities	1	0.118	0.118	1.273	0.2617
Species * Leaf types	2	0.066	0.033	0.356	0.7015
Species * Localities	2	0.119	0.06	0.643	0.5279
Leaf type * Localities	1	0.132	0.132	1.42	0.236
Species * Leaf types * Localities	2	0.092	0.046	0.494	0.6114
Residual	108	10.013	0.093		

Model summary R: 0.355 R^2: 0.126 RMS Residual: 0.304 Model F-value: 1.42 P-value: 0.1743

ANOVA for moulting frequency

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Species	2	0.476	0.238	4.878	0.0094
Leaf type	1	0.162	0.162	3.313	0.0715
Localities	1	0.004	0.004	0.085	0.7717
Species * Leaf types	2	0.014	0.007	0.142	0.8678
Species * Localities	2	0.101	0.05	1.03	0.3606
Leaf type * Localities	1	0.026	0.026	0.524	0.4706
Species * Leaf types * Localities	2	0.014	0.007	0.14	0.8693
Residual	108	5.272	0.049		

Model summary R: 0.362 R^2: 0.131 RMS Residual: 0.221 Model F-value: 1.482 P-value: 0.1486

The multiple range test (Fisher's PLSD) (Fig. 8.3A) using transformed data indicated that the foliages of *E. delegatensis* had significantly ($p<0.01$) higher larval feeding response in all aspects to *E. regnans*, with the exception of larval weight. The leaf consumption, frass production and larval weight on *E. delegatensis* was also significantly ($p<0.05$) higher than that on *E. obliqua*. Hence, this experiment further confirmed *E. delegatensis* as the most preferred species for *C. bimaculata* larval feeding among the three 'ash' species.

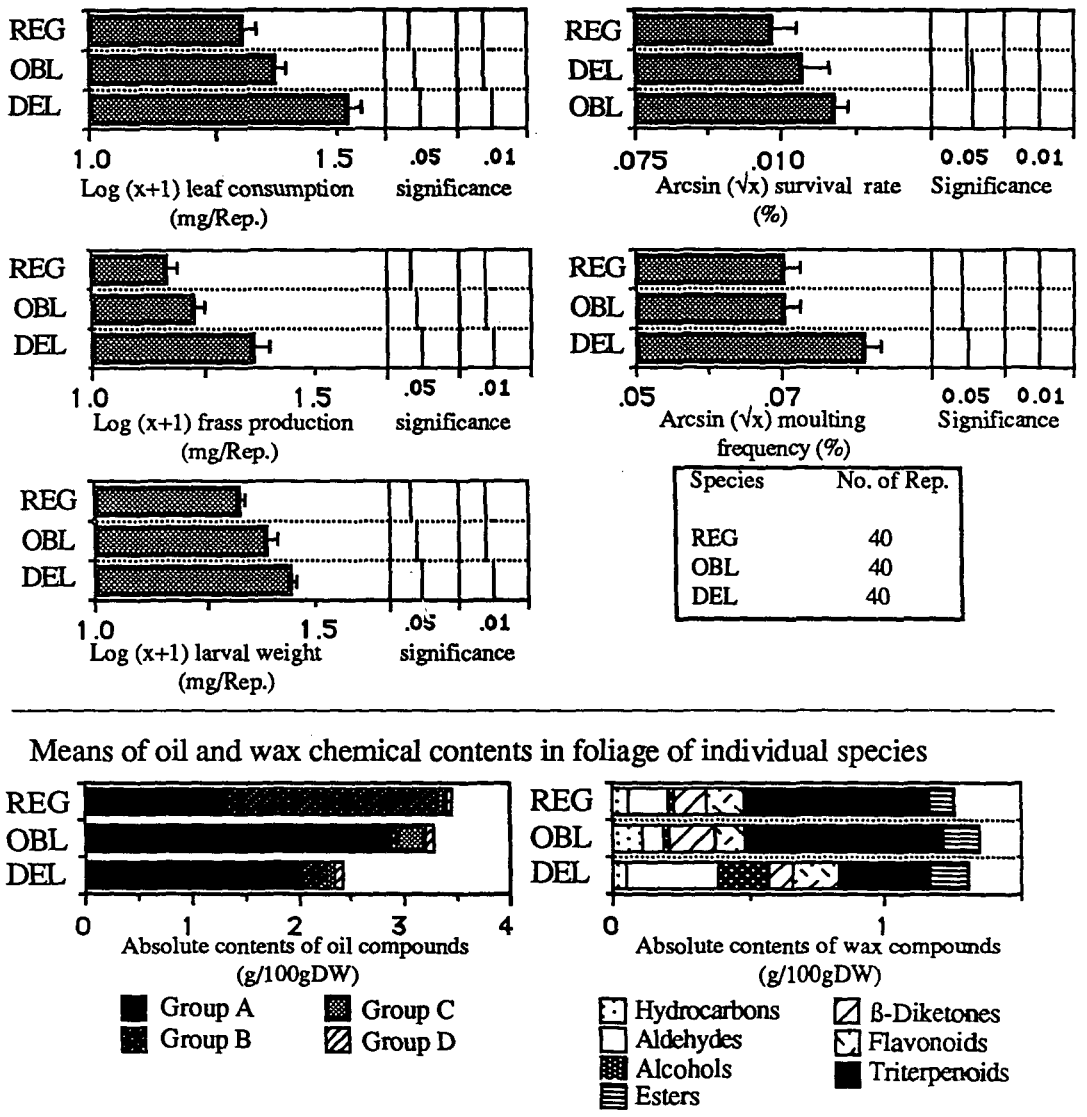


Fig. 8.3A. Species mean and standard errors and results of multiple range test (Fisher's PLSD) for differences in *C. bimaculata* larval feeding responses between foliages of *E. delegatensis*, *E. obliqua* and *E. regnans* based on transformed data.

* For oil compounds group see Fig. 7.1.

Comparison between leaf chemicals and larval feeding response indicated that the highest feeding response on *E. delegatensis* was associated with the lowest oil yield. The lowest feeding response on *E. regnans* was associated with a high amounts of group B oil compounds (sesquiterpenoids). However, wax yield did not differ markedly between species. The most preferred foliage, *E. delegatensis*, had markedly lower content of triterpenoids and high contents of alkanals than *E. obliqua* and *E. regnans*. However, there was no marked difference in wax composition between *E. obliqua* and *E. regnans* foliage which differed significantly ($p<0.05$) in leaf consumption, frass production and larval weight.

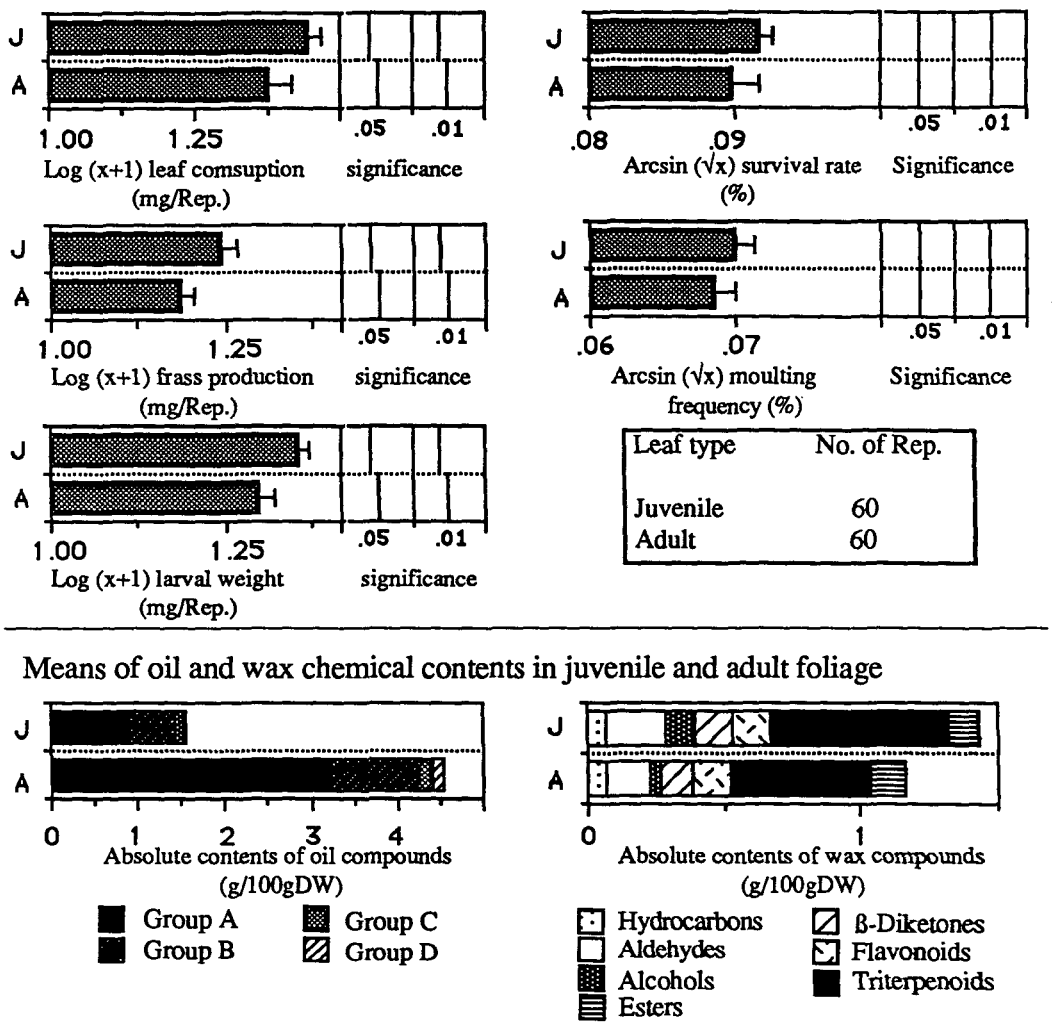


Fig. 8.3B. Species means and standard errors and results of multiple range tests (Fisher's PLSD) for differences of *C. bimaculata* larval feeding responses between adult and juvenile foliage of *E. delegatensis*, *E. obliqua* and *E. regnans* based on transformed data.

Comparison (Fig. 8.3B) of the larval feeding response between the juvenile and adult foliages indicated that the leaf consumption, frass production and larval weight of *C. bimaculata* larvae were significantly ($p<0.01$) higher on juvenile foliage than on adult foliage. The significantly higher feeding on juvenile foliage was associated with the markedly lower oil yield in juvenile foliage while the wax yield was relatively higher in juvenile foliage.

Further comparison of the larval feeding response between juvenile and adult foliages within individual species indicated that the juvenile foliage of each species had higher average values for all aspects of the *C. bimaculata* larval feeding response to adult foliage (Appendix 8.2B). However, the ANOVA indicated that the differences in larval feeding

response did not differ significantly between leaf types within individual species, although they had relatively close *p*-values to 0.05.

Overall, results indicated that the *C. bimaculata* larvae had significantly higher feeding on juvenile foliage among the "ash" species localities. *C. bimaculata* larvae also had higher feeding response on juvenile foliage within each species, although these differences were not significant.

8.3.3. Larval feeding test on *E. delegatensis* foliage from four localities

The observed means of individual replicates of *C. bimaculata* larval feeding response to foliages from the four *E. delegatensis* localities and the reference *E. nitens* foliage are listed in Appendix 8.3A and the results of the ANOVA and MANOVA (using transformed data) are shown in Appendix 8.3B. The means and standard errors of individual localities (transformed data) and the results of the Multiple range test (transformed data) are given in Fig. 8.4.

Significant variation ($P<0.001$) in all aspects of the larval feeding response was indicated between the foliages from the four localities (Appendix 8.3B). The MANOVA (Appendix 8.3B) further indicated that the localities effect was significant ($P<0.001$).

The Multiple range test indicated that *C. bimaculata* larvae have significantly higher levels of leaf consumption, frass production, larval weight, survival and moulting frequency on foliage from the two Tasmanian localities (Tas 1 and Tas 2) than on the foliage from the two Victorian localities (Vic 1 and Vic 2) which had very high oil yields (Fig. 8.4B). However, there was no significant difference in any aspects of the larval feeding response between either the two Tasmanian or the two Victorian localities.

The oil yield and larval preference for foliage indicated that the significantly higher oil yield of foliage (over 10% of dried leaf weight) of the two Victorian localities was correlated to a significantly lower feeding response by *C. bimaculata* larvae compared to the two Tasmanian localities. These results suggested that high oil yields (particularly group A compounds) in *E. delegatensis* could deter *C. bimaculata* larval feeding. There were no obvious association between the distribution of wax compounds and larval feeding response.

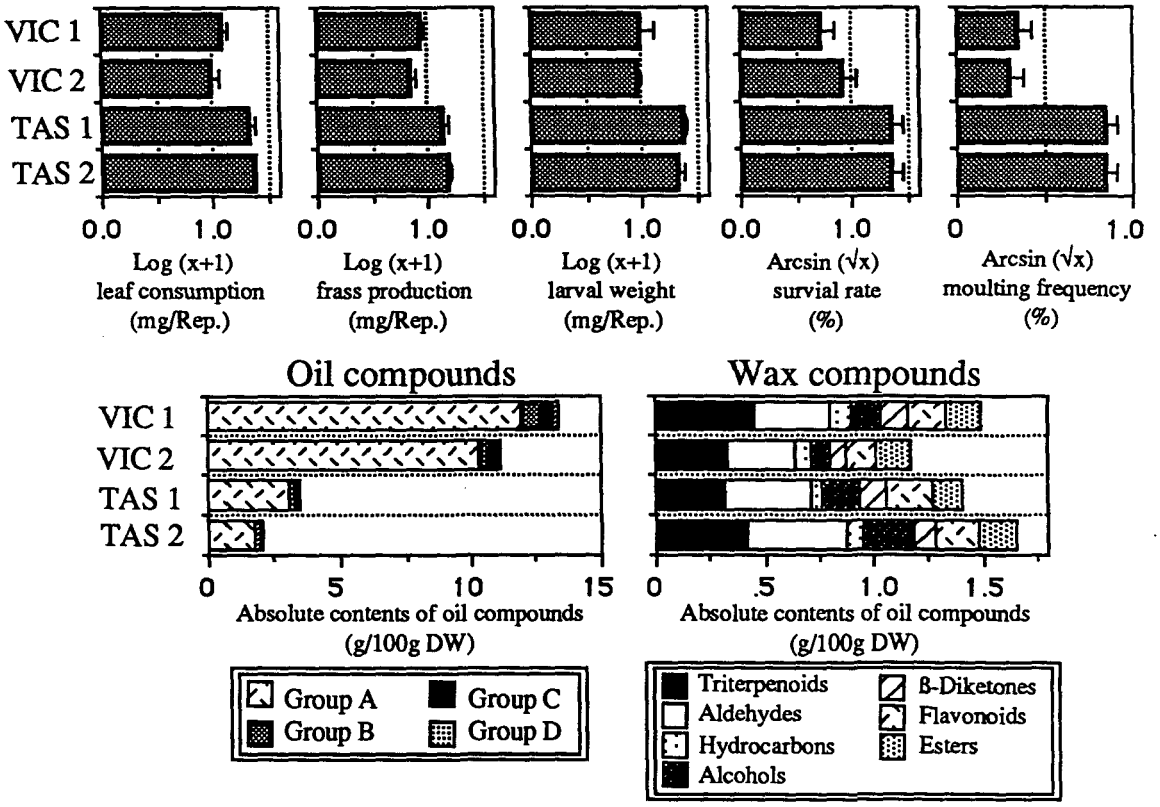


Fig. 8.4A. The means, standard errors and the multiple range test for *C. bimaculata* larvae feeding response on adult foliage of different *E. delegatensis* localities based on transformed data and the absolute contents of oil and wax compounds of these foliage.

* For oil compound groups see Fig. 7.1.

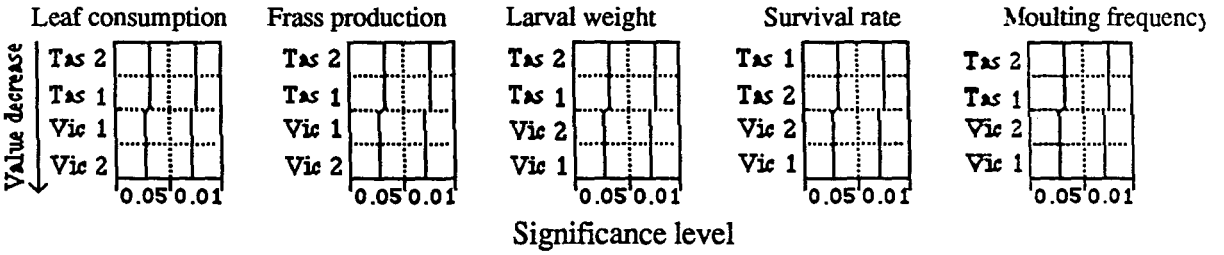


Fig. 8.4B. The multiple range test (Fisher's PLSD) for *C. bimaculata* larvae feeding response on foliage of different localities of *E. delegatensis* using transformed data [log(x+1)] for leaf consumption, frass production and larval weight and transformed data [arcsin(√x)] for survival rate (%) and moulting frequency (%).

8.3.4. Larval feeding on foliage of *E. nitens* and *E. denticulata*

The oil yield and the means and standard errors of the *C. bimaculata* larval feeding response on foliages of *E. nitens* from four localities and foliage of *E. denticulata* are shown in Table 8.10 and observed data listed in Appendix 8.4A.

From this data, two comparisons were made: (1) between leaf types (adult, juvenile and dewaxed juvenile foliages) of the two *E. nitens* localities, Connor Plains (N1) and Toorongo (N2) and , (2) between adult foliages of all four localities of *E. nitens* and the foliage of *E. denticulata.* (Bendoc locality).

Table 8.10. *C. bimaculata* larval feeding response on adult, juvenile and dewaxed juvenile foliage of Connor Plain (N1) and Toorongo (N2) localities and adult foliage of Mt Kaye (N3) and Noojee (N4) of *E. nitens* and adult foliage of *E. denticulata* (Den).

		Leaf consumption		Frass production		Larval Weight		Survival		Exuviae		Oil yield (% DW)	Wax yield (% DW)
	No. of Rep.	Mean (mg)	S.E.	Mean (mg)	S.E.	Mean (mg)	S.E.	Mean (%)	S.E.	Mean (%)	S.E.		
(a) N1-Adult	10	1.458	.024	1.26	.03	1.481	.036	1.318	.087	1.025	.076	0.47	0.72
(b) N2-Adult	10	1.484	.023	1.305	.029	1.532	.03	1.432	.070	1.071	.093	0.85	0.63
Adult leaves (a+b)	20	1.471	.017	1.282	.021	1.502	.024	1.375	.056	1.048	.059	0.66	0.68
(c) N1-Juvenile	10	1.324	.031	1.138	.040	1.383	.037	1.182	.090	0.853	.085	1.11	0.78
(d) N2-Juvenile	10	1.373	.038	1.193	.051	1.447	.028	1.342	.096	.087	.038	1.38	0.97
Juvenile leaves (c+d)	20	1.349	.025	1.166	.032	1.415	.024	1.262	.067	.862	.046	1.25	0.88
(e) N1-Juvenile (Dewaxed)	10	1.38	.022	1.189	.035	1.435	.031	1.274	.102	.829	.056	1.11	0.67
(f) N2-Juvenile (Dewaxed)	10	1.287	.067	1.128	.066	1.406	.037	1.274	.102	.896	.087	1.38	0.72
Juvenile leaves (dewaxed) (e+f)	20	1.333	036	1.159	.037	1.420	.024	1.274	.070	.863	.051	1.25	0.70
(G) N3-Adult	10	1.332	.036	1.118	.049	1.383	.029	1.16	.094	.913	.060	1.39	0.68
(h) N4-Adult	10	1.071	.044	1.879	.051	1.121	.045	.913	.060	.892	.045	5.60	0.59
(i) Den-Adult	10	1.348	.031	1.133	.061	1.305	.048	1.207	.107	.678	.045	0.85	0.59

Variation between leaf types from the Connor Plains and Toorongo localities of E. nitens

The two way ANOVA (Appendix 8.4B) indicated that there was significant variation in leaf consumption ($p<0.001$), frass production ($p<0.05$) and moulting frequency ($p<0.05$) between leaf types (adult, normal juvenile and dewaxed juvenile leaves) for samples from the N1 and N2 localities. However, there was no significant variation in

aspects of the larval feeding response between these two localities. The leaf type x locality interactions were also not significant.

Further tests indicated that the leaf consumption, frass production and moulting frequency on adult foliage were all significantly higher ($p<0.05$ or $p<0.01$) than on juvenile and dewaxed juvenile foliage (Fig. 8.5), where the oil yield was lower than in adult leaves (Table. 8.10). However, there was no significant difference in any component of the larval feeding response between juvenile and dewaxed juvenile leaves.

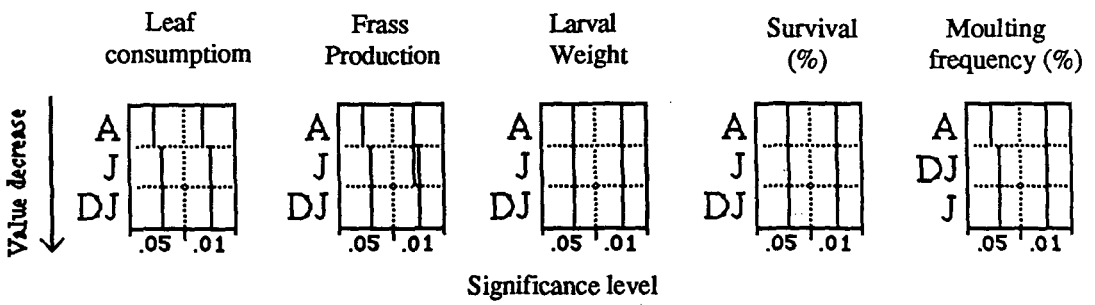


Fig. 8.5. The multiple range test (Fisher's PLSD) for *C. bimaculata* larvae feeding response to different leaf types of *E. nitens* using transformed data of means.

A= Adult leaves (N1+N2); J= Juvenile leaves (N1+N2); DJ= Dewaxed of juvenile leaves (N1+N2)

Variation between adult foliages from E. nitens localities and E. denticulata

Comparisons made between *C. bimaculata* larvae fed on adult foliages of *E. nitens* from four different localities and *E. denticulata* from one locality were significantly different ($p<0.001$) for all components of *C. bimaculata* larval feeding response (Appendix 8.4C). Leaf consumption, frass production, larval weight, survival rate and moulting frequency on the foliage of Noojee locality (N4) were all significantly lower compared to the other *E. nitens* localities, with the exception that there was no difference in the survival rate between Noojee and Mt Kaye localities (Fig. 8.6). Leaf consumption on foliage from the Mt Kaye (N3) locality was significantly lower than on foliages from the Connor Plain (N1) and Toorongo (N2) localities, while frass production and survival rate on Mt Kaye (N3) foliage was significantly lower than on that from Toorongo (N2). However, there were no significant difference in larval weight and moulting frequency between Mt Kaye, Connor Plain and Toorongo localities.

Comparisons made between foliages of *E. denticulata* and *E. nitens* indicated that the leaf consumption, frass production and survival rates on foliage of *E. denticulata* were intermediate between the most highly preferred localities of *E. nitens*, Connor Plain and Toorongo and the least preferred localities of Mt Kaye and Noojee. Larval weights and moulting frequencies on *E. denticulata* were lower than those recorded

on foliage from Mt Kaye. Comparisons made between foliages from different localities within the species *E. nitens* indicated that the larval feeding response was the lowest on foliage of Noojee locality which had a high oil yield and levels of group C compounds. In addition, the larval feeding response on foliage of the Mt Kaye locality was also lower than that on Connor Plain and Toorongo, since the oil yield of Mt Kaye foliage was relatively high. Thus, the differences in the larval feeding response of *C. bimaculata* appears to be associated to difference in oil yield between localities within *E. nitens*. It was also considered that the differences between *E. denticulata* and other *E. nitens* foliage may be also be affected by the differences of oil composition (see Fig. 8.7 and Chapters 3 and 11) and also other chemicals (for example, difference in polyphenols as found by Pederick and Lennox 1979).

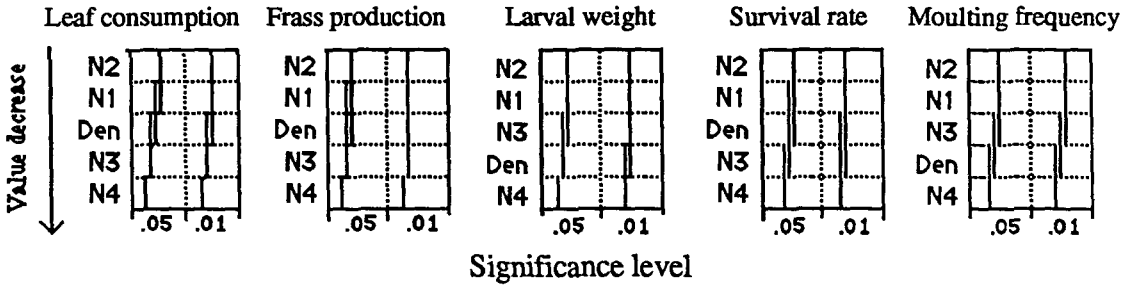


Fig. 8.6. The multiple range test (Fisher's PLSD) for *C. bimaculata* larvae feeding response on different adult foliage of *E. nitens* using transformed data.

8.3.5. Comparison of *C. bimaculata* larval feeding response on all foliages under investigation

The *C. bimaculata* larval feeding response to the foliage of 12 different eucalypt species including different foliages of *E. nitens* and three important 'ash' species was compared using ratios related to the adult *E. nitens* reference foliage [NIT.A(ref)]. The results are given in Fig. 8.7A. The results from different feeding tests showed that the response of larvae on all foliage of *E. nitens*, with the exception of Noojee individual tree (NIT Noojee A), was similar to that on 'ash' species, *E. delegatensis, E. obliqua* and *E. regnans*. Comparing the results from each foliage showed that the larvae ate markedly less of the foliages of *Symphyomyrtus* species, which had high levels of group C compounds (mainly 1,8-cineole and α–pinene). In addition, the larval feeding response to foliages of two *Monocalyptus* species, *E. sieberi* and *E. tenuiramis*, was significantly lower than on other *Monocalyptus* species.

The results of the analysis for the correlation between the *C. bimaculata* larvae feeding response and leaf oil amongst all foliage samples indicated that variation in amounts of group C compounds (mainly 1,8-cineole and α–pinene) was significantly ($p<0.001$) negatively correlated to all components of the larval feeding response recorded (Table 8.11). Changes in the different components of the larval feeding response were significantly negatively correlated ($p<0.01$ for leaf consumption, frass production and larval weight; $p<0.001$ for survival rate and moulting frequency) with variation in total oil yield among foliages. However, there was no significant correlation between variation in amounts of other groups of oil compounds and the larval feeding response.

Changes in the different components of the larval feeding response were significantly correlated ($p<0.05$ for leaf consumption, larval weight, survival rate and moulting frequency; $p<0.01$ for frass production) with variation in wax yield among foliages. Changes in the leaf consumption and frass production were mainly significantly ($p<0.01$) positively correlated with variation in amounts of alkanols, flavonoids and esters. Variation in amounts of esters was significantly ($p<0.05$) correlated with changes in larval weight, survival rate and moulting frequency and flavonoids significantly ($p<0.05$) correlated with larval weight and survival and alkanals with larval weight ($p<0.05$). However, none of these correlations were as high as that observed for the group C oil components and many may in fact simply reflect the covariance of these components with the group C oil component rather than reflects a causal relationship.

Moreover, the results of analysis for correlation between larval feeding response and leaf chemicals among foliage within individual subgenera indicated that variation in oil yield was significantly negatively correlated with all components of larval feeding response among foliage within *Monocalyptus* ($p<0.05$ or $p<0.01$) and *Symphyomyrtus* species ($p<0.001$) samples. In contrast, there was no significant correlation between total wax yield and larval feeding response among foliage within each subgenus.

Within *Monocalyptus* species, the feeding behaviour responses of leaf consumption and frass production of larvae were most highly negatively correlated with variation in group C oil compounds whereas changes in larval weight, survival and moulting frequency were most highly correlated with variation in amounts of group A compounds (monoterpenoids). All components of larval feeding response significantly correlated with group C and D compounds, with the exception of the correlation between moulting rate and group C compounds. Variation in group B compounds was also significantly correlated with leaf consumption, frass weight and larval weight. In addition, variation in amounts of alkanals, alkanols and flavonoids were significantly ($p<0.05$, $p<0.01$ or $p<0.001$) correlated with feeding behaviour responses of leaf consumption and frass

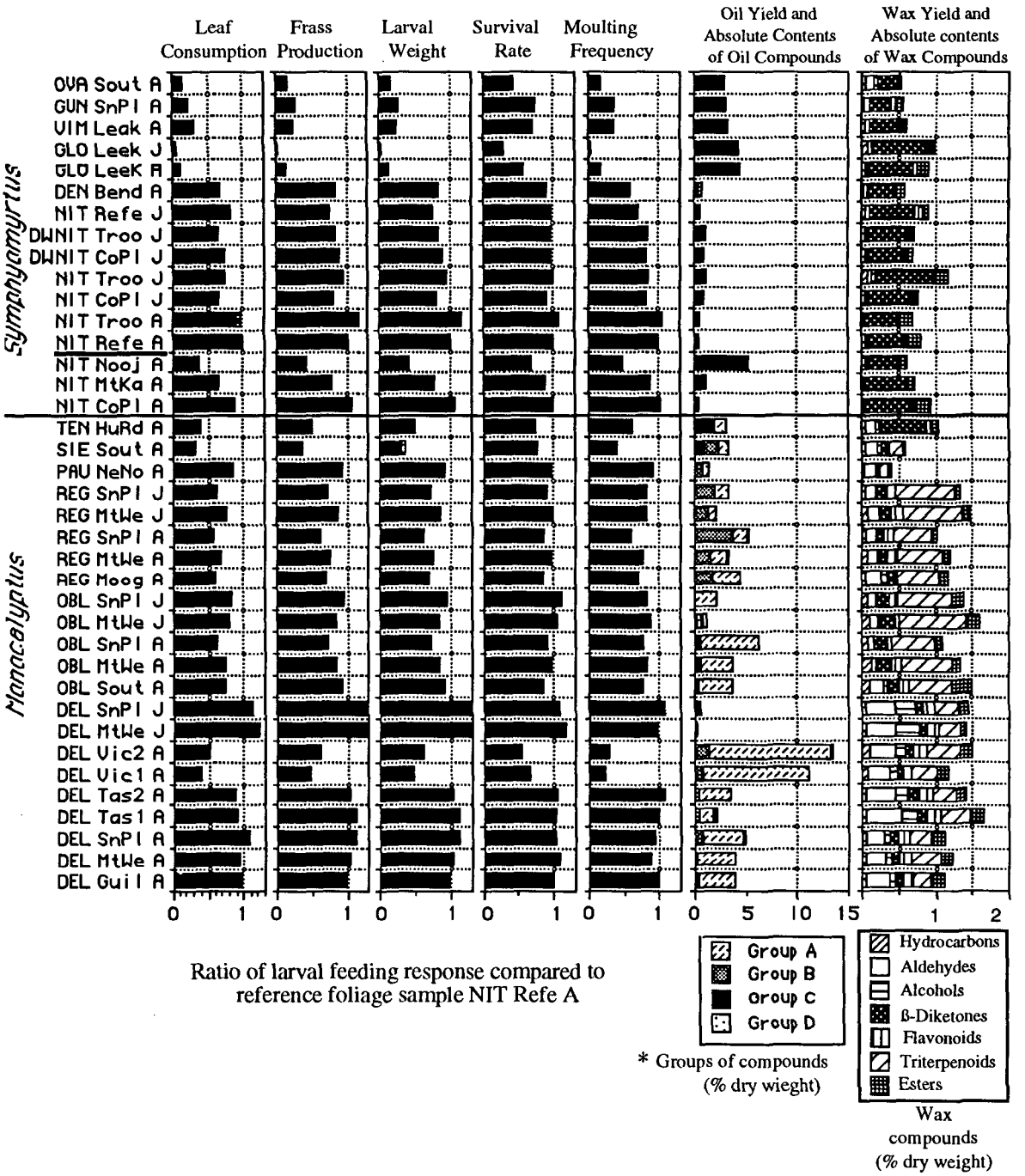


Fig. 8.7A. Comparison of *C. bimaculata* larvae feeding response between foliage of *E. nitens* and different eucalypt species.

The larval feeding response on reference foliage, adult foliage of *E. nitens* (NIT Refe A), was recorded at 1 and others were compared to this parameter. For example, where the leaf consumption on the reference was 100 mg and on a foliage sample was 90 mg, the comparison ratio of this foliage sample would be 90/100 = 0.90.

* Groups of oil compounds:

Group A: α– and β–phellandrene, *cis*- and *trans*-menth-2-en-1-ol, *cis*- and *trans*-piperitol, *p*-cymene, α– and γ–terpinene, terpinolene and terpinen-4-ol *et al.*.

Group B: α– , β– and γ– eudesmol, hedycaryol, elemol.

Group C: 1,8-cineole, α– pinene, limonene, α– terpineol and related oil compounds.

Group D: globulol, viridiflorol, aromadendrene, *allo*-aromadendrene and spathulenol.

Fig. 8.7B. Average linkage clustering of *C. bimaculata* larval feeding response on all foliage samples tested, using ratio data (compared to NIT Refe.A) of all five components of feeding response.

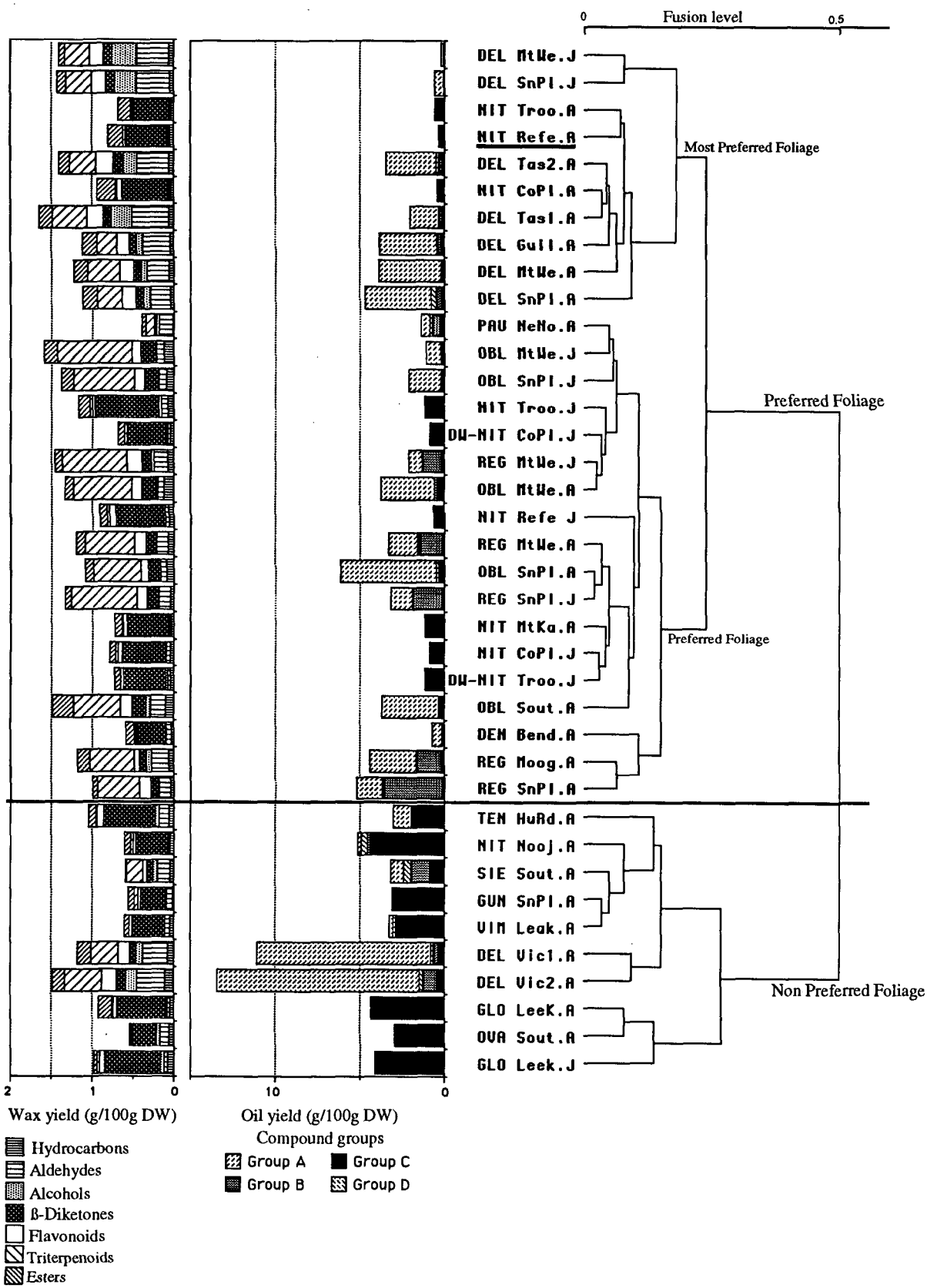


Table 8.11. Correlation between *C. bimaculata* larval feeding response and leaf oil content. ns: p>0.05; *: 0.01<p<0.05; **: 0.01<p<0.001; ***: p<0.001.

Compound group	Leaf consumption		Frass Production		Larval Weight		Survival Rate		Moulting Frequency	
	R-values	Sign.	R-values	Sign.	R-values	Sign.	R-values	Sign.	R-values	Sign.
Correlation among all foliage samples *d.f.*=37										
Oil compounds										
group A	-0.018	ns	-0.011	ns	-0.097	ns	-0.179	ns	-0.194	ns
group B	-0.104	ns	-0.107	ns	-0.092	ns	-0.033	ns	-0.085	ns
group C	-0.77	***	-0.774	***	-0.753	***	-0.73	***	-0.699	***
group D	-0.246	ns	-0.268	ns	-0.284	ns	-0.26	ns	-0.275	ns
Oil yield	-0.412	**	-0.408	**	-0.479	**	-0.532	***	-0.546	***
Wax compounds										
Hydrocarbons	0.014	ns	0.011	ns	0.034	ns	-0.028	ns	-0.053	ns
Aldehydes	0.38	*	0.392	*	0.286	ns	0.2	ns	0.213	ns
Alcohols	0.44	**	0.472	**	0.343	*	0.229	ns	0.265	ns
ß-Diketones	-0.295	ns	-0.277	ns	-0.242	ns	-0.26	ns	-0.181	ns
Flavonoids	0.432	**	0.419	**	0.367	*	0.312	*	0.291	ns
Triterpenoids	0.247	ns	0.238	ns	0.272	ns	0.303	ns	0.22	ns
Esters	0.449	**	0.46	**	0.417	*	0.338	*	0.386	*
Wax yield	0.407	*	0.421	**	0.389	*	0.319	*	0.316	*
Correlation among *Monocalyptus* species samples *d.f.*=21										
Oil compounds										
group A	-0.35	ns	-0.344	ns	-0.504	*	-0.654	**	-0.612	**
group B	-0.427	*	-0.447	*	-0.416	*	-0.299	ns	-0.346	ns
group C	-0.556	**	-0.543	**	-0.474	*	-0.487	*	-0.397	ns
group D	-0.447	*	-0.487	*	-0.522	*	-0.499	*	-0.518	*
Oil yield	-0.611	**	-0.608	**	-0.552	**	-0.559	**	-0.481	*
Wax compounds										
Hydrocarbons	-0.139	ns	-0.11	ns	-0.091	ns	-0.126	ns	-0.165	ns
Aldehydes	0.449	*	0.514	*	0.285	ns	0.099	ns	0.22	ns
Alcohols	0.565	**	0.649	***	0.406	ns	0.258	ns	0.322	ns
ß-Diketones	-0.302	ns	-0.272	ns	-0.184	ns	-0.215	ns	-0.113	ns
Flavonoids	0.488	*	0.52	*	0.377	ns	0.268	ns	0.305	ns
Triterpenoids	-0.007	ns	-0.01	ns	0.081	ns	0.202	ns	0.076	ns
Esters	0.24	ns	0.259	ns	0.124	ns	0.031	ns	0.119	ns
Wax yield	0.341	ns	0.412	ns	0.314	ns	0.234	ns	0.261	ns
Correlation among *Symphyomyrtus* species samples *d.f.*=15										
Oil compounds										
group A	0.049	ns	0.079	ns	-0.063	ns	0.085	ns	-0.048	ns
group B	-0.11	ns	-0.085	ns	-0.148	ns	-0.045	ns	-0.058	ns
group C	-0.915	***	-0.904	***	-0.884	***	-0.848	***	-0.858	***
group D	-0.185	ns	-0.204	ns	-0.204	ns	-0.165	ns	-0.142	ns
Oil yield	-0.903	***	-0.893	***	-0.873	***	-0.836	***	-0.845	***
Wax compounds										
Hydrocarbons	-0.215	ns	-0.261	ns	-0.212	ns	-0.328	ns	-0.275	ns
Aldehydes	-0.544	*	-0.53	*	-0.577	**	-0.505	*	-0.605	*
Alcohols	-0.299	ns	-0.226	ns	-0.302	ns	-0.462	ns	-0.283	ns
ß-Diketones	0.187	ns	0.177	ns	0.212	ns	0.119	ns	0.179	ns
Flavonoids	-0.103	ns	-0.184	ns	-0.135	ns	-0.125	ns	-0.16	ns
Triterpenoids	-0.12	ns	-0.165	ns	-0.111	ns	-0.114	ns	-0.167	ns
Esters	0.587	**	0.584	**	0.62	**	0.542	*	0.579	*
Wax yield	0.191	ns	0.176	ns	0.214	ns	0.107	ns	0.162	ns

production. However, no wax chemicals were correlated with the concomitant physiological reaction responses of larvae (i.e. larval weight, survival rate and moulting frequency). The most important association with wax compounds was the positive association between alcohol levels and leaf consumption and frass production.

Among *Symphyomyrtus* foliage samples, all components of larval feeding response were significantly negatively correlated with the variation in group C oil compounds ($p<0.001$) and wax alkanals ($p<0.005$ or $p<0.001$) and positively correlated with wax esters ($p<0.05$ or $p<0.01$). There were no significant correlations between the larval feeding and amounts of other oil and wax compounds.

Comparing the significant levels of correlation between the changes in larval feeding response and variation in oil and wax compounds indicated that the group C oil compounds and oil yield were the most significantly correlated with the larval feeding response among all foliage and among foliage within individual subgenera, particularly among foliage of *Symphyomyrtus* species.

The overall variation in the *C. bimaculata* larval response between the foliages tested, as estimated from the ratio data in comparison to NIT Refe.A, is summarised in the dendrogram in Fig. 8.7B. This dendrogram, derived by average linkage clustering from the Manhattan matrix distance analysis, indicates that the division of non-preferred and preferred foliages corresponded to differences in the quality and quantity of leaf oil. Thus, with the exception of Noojee locality, both the adult and juvenile foliages of *E. nitens*, the foliage of *E. denticulata* and most *Monocalyptus* species were grouped into the major cluster of preferred foliages. Within this cluster, three of the *E. nitens* foliages, which had very low oil yields, and all foliages of *E. delegatensis*, were separated from the other *E. nitens* and *Monocalyptus* species to form a most preferred cluster. However, most species of *Symphyomyrtus* and the two *Monocalyptus* species, *E. tenuiramis* and *E. sieberi*, which had high amounts of group C compounds, formed a single cluster with the two Victorian localities of *E. delegatensis*, which had uncommonly high oil yields (mainly group A compounds). Comparison of oil chemicals indicated that the amounts of group C compounds significantly affected the larval feeding response when the absolute content was over 2.5% in contrast to the group A compounds, which only significantly affect larval feeding at high amounts (over 10%). The content of group C compounds in *E. sieberi* did not appear to reach a level sufficient to affect larval feeding when compared to other non-preferred foliage. However, it is possible that the low response of larvae on *E. sieberi* may be due to other effects in conjunction with leaf oils, although it is notable that *E. sieberi* has the highest level of group C compounds of all the ash samples examined (Fig. 8.7B).

8.3.6. The response of *C. agricola* larvae to foliage of 10 eucalypt species from *Monocalyptus* and *Symphyomyrtus* subgenera

The means for leaf consumption, frass production, larval weight and survival rate (percent) of *C. agricola* larvae on foliages of the 10 eucalypt species and results of the analysis of variance are shown in Table 8.12 and observed data listed in Appendix 8.5. Due to a scarcity of larval material, this test was made using third instar larvae of 4-5mg average weight.

The ANOVA (Table 8.12A) indicated that the species (within subgenera) most significantly ($p<0.001$) affected all components of the *C. agricola* larval feeding response. Subgenera also had a significant ($p<0.05$) effect on leaf consumption, frass production and larval weight. The F-value (4.969) for the subgenera effect on survival rate was close to significance suggesting that the subgenera also had a slight effect on survival rate with the larval survival rate on *Symphyomyrtus* species higher than on *Monocalyptus*. The mean response values for both subgenera shown in Table 8.12B indicated that leaf consumption, frass production, larval weights and survival rate of *C. agricola* on *Symphyomyrtus* species were much higher than on *Monocalyptus*.

The MANOVA (Table 8.12C) further indicated that species (within subgenera) significantly affected ($p<0.001$) the larval feeding response. However, the effect of subgenera was not significant as indicated by this test.

The means and standard errors for leaf consumption, frass production, larval weight and survival on individual species and the multiple range test, using transformed data, are shown in Fig. 8.8.

Foliage of *E. nitens* promoted the highest leaf consumption, frass production and larval weight increase and foliage of *E. regnans* the lowest. In addition, survival of *C. agricola* larvae was highest (100%) on foliage of *E. gunnii* and lowest on *E. sieberi* and *E. regnans*.

The multiple range test for the response of *C. agricola* larval feeding response on the different foliage showed no clear separation of response between subgenera (Fig. 8.8). There was no significant difference in any of the components of the larval feeding response between foliage of the two *Symphyomyrtus* species, *E. globulus* and *E. ovata* and the two *Monocalyptus* species, *E. pauciflora* and *E. delegatensis*. Larval weight and survival on foliage of *E. sieberi* (SG *Monocalyptus*) differed significantly from that recorded on *E. globulus* and *E. delegatensis* foliage, but levels of leaf consumption and frass production were not.

The leaf consumption, frass production and larval weight increase on foliage of *E. nitens* was significantly higher than on all other species, with the exceptions of leaf consumption and larval weight on *E. gunnii* and in frass production on *E. viminalis*. These results indicated that larval feeding on foliages of the two *Symphyomyrtus* species, *E. viminalis* and *E. gunnii*, were similar to that on the most preferred foliage of *E. nitens*.

Table 8.12. Analysis of variance for *C. agricola* larval feeding response on adult foliages of 10 eucalypt species.

A). ANOVA

ANOVA for Leaf Consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	5.268	5.268	5.45	0.0478	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	8	7.732	0.967	29.656	0.0001	Residual
Residual	90	2.933	0.033			

Model summary R: 0.903 R^2: 0.816 RMS Residual: 0.181 Model F-value: 44.319 P-value: 0.0001

ANOVA for Frass Production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	7.619	7.619	6.332	0.036	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	8	9.625	1.203	19.288	0.0001	Residual
Residual	90	5.614	0.062			

Model summary R: 0.869 R^2: 0.754 RMS Residual: 0.250 Model F-value: 30.715 P-value: 0.0001

ANOVA for Larval Weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	3.214	3.214	10.026	0.0133	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	8	2.565	0.321	4.974	0.0001	Residual
Residual	90	5.8	0.064			

Model summary R: 0.706 R^2: 0.499 RMS Residual: 0.254 Model F-value: 9.962 P-value: 0.0001

ANOVA for Survival Rate (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
SUBGENERA	1	1.948	1.948	4.969	0.0564	SPECIES (SUBGENERA)
SPECIES (SUBGENERA)	8	3.136	0.392	3.652	0.001	Residual
Residual	90	9.661	0.107			

Model summary R: 0.587 R^2: 0.345 RMS Residual: 0.328 Model F-value: 5.262 P-value: 0.0001

B). The mean values of the *C. agricola* larval feeding response on foliage of the two different subgenera of *Eucalyptus*.

		Leaf consumption (mg)		Frass production (mg)		Larval weight (mg)		Survival (%)	
Subgenus	No. of Rep.	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)	Mean	SE(±)
Symphyomyrtus	50	58.83	3.16	33.34	2.12	55.20	2.78	90.00	2.30
Monocalyptus	50	26.70	2.85	14.02	2.00	27.88	2.189	72.00	3.96

Table 8.12. Continued.

C). MANOVA

Effect: Subgenera

			Value	F-Value	Num DF	Den DF	P-Value
S	1	Wilks' Lambda	.435	1.624	4.00	5.00	.3010
M	1.0	Hotelling-Lawley Trace	1.299	1.624	4.00	5.00	.3010
N	1.5	Pillai Trace	.565	1.624	4.00	5.00	.3010

Effect: Species(subgenera)

			Value	F-Value	Num DF	Den DF	P-Value
S	5	Wilks' Lambda	.092	9.173	32.00	322.435	0.0001
M	2.5	Hotelling-Lawley Trace	5.246	14.015	32.000	342.000	0.0001
N	55.5	Pillai Trace	1.422	6.203	32.000	360.000	0.0001

Leaf consumption and frass production by larvae fed on foliage of the two *Monocalyptus* species, *E. regnans* and *E. obliqua,* differed significantly from all other species. The leaf consumption and frass production of larvae on *E. sieberi* did not differ from *E. delegatensis* and *E. pauciflora* and some other *Symphyomyrtus* species. However, larval weight and survival rate on *E. sieberi* were similar to that on *E. regnans* and *E. obliqua.*

The highest feeding response by *C. agricola* occurred on the *E. nitens* which had the lowest oil yield. The foliage of *E. pauciflora,* which was the most consumed among *Monocalyptus* species, also had the lowest oil yields. However, comparisons made between the amounts of oil and individual oil compounds and the larval feeding response, indicated that differences in the larval feeding response between eucalypt species were not generally associated with differences in oil yield and amounts of individual oil compounds.

While *C. agricola* slightly favoured foliage of *Symphyomyrtus* species, *C. agricola* larvae also fed on and survived on foliages of *Monocalyptus* species *E. delegatensis* and *E. pauciflora,* in which the leaf oils belong to different chemotypes. *C. agricola* larvae did not like and suffered a physiological disability on foliage of *E. regnans* and *E. obliqua.* However, the leaf oil of *E. obliqua* was similar to that of *E. delegatensis.* In contrast, the feeding response of *C. agricola* larvae appeared to be associated with the differences in wax chemicals in which the two 'green ash' species, *E. regnans* and *E. obliqua,* had significantly higher amounts of triterpenoids than *E. delegatensis* and *E. pauciflora.*

However, at least within the *Monocalyptus* species, it was of interest that the larvae fed on *E. sieberi* foliage but suffered the physiological disability of decreased weight and survival. This foliage had similar amounts of sesquiterpenoids to *E. regnans* but the wax of *E. sieberi* was similar to *E. pauciflora.* It is possible that the sesquiterpenoids also affected the *C. agricola* larvae.

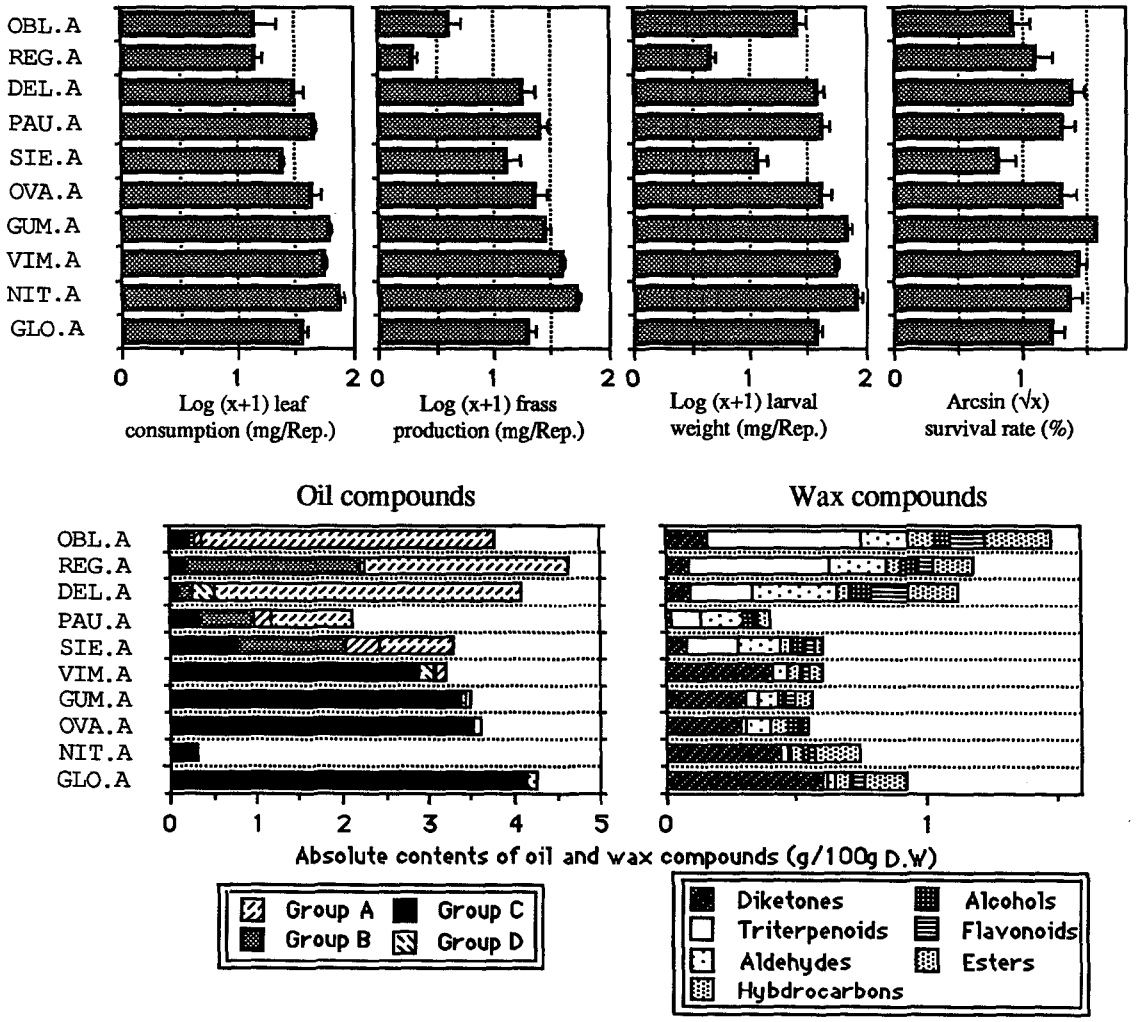


Fig. 8.8A. The means and standard errors (transformed data) for *C. agricola* larvae feeding response to foliage of 10 eucalypt species and the absolute contents of oil and wax compounds of these foliage.

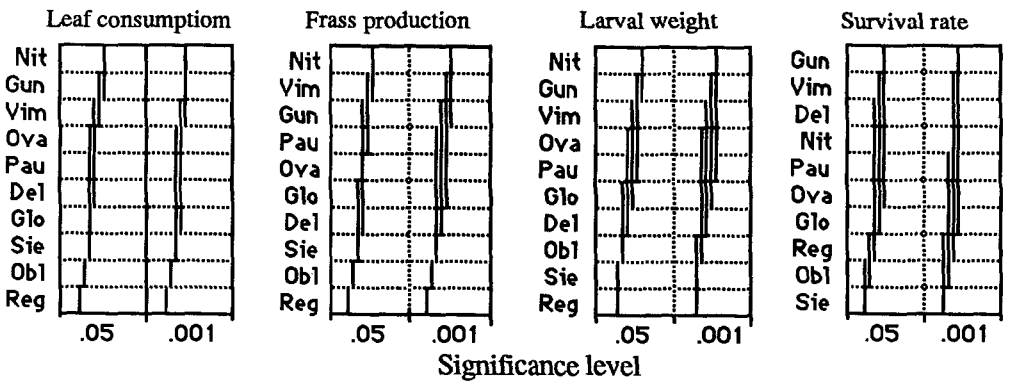


Fig. 8.8B. The multiple range test (Fisher's PLSD) for *C. agricola* larvae feeding response on foliage of 10 eucalyptus species using transformed data [log(x+1)] for leaf consumption, frass production, and larval weight and transformed data [arcsin(√x)] for survival rate (%).

8.4. Discussion

The objective of this study was to describe the feeding response of *C. bimaculata* and *C. agricola* larvae to the foliage of different eucalypt species especially with regards to the effect of foliage oils and waxes. Foliages from both *Symphyomyrtus* and *Monocalyptus* species were tested. Results highlighted a number of interesting phenomena.

1. A fundamental finding is that the feeding response of both *C. bimaculata* and *C. agricola* larvae showed no clear difference between the foliage of *Symphyomyrtus* and *Monocalyptus* species. However, leaf chemicals appeared to be the important factors influencing the larval feeding response by both beetle species.

2. The feeding response of larvae to foliage of different species was basically correlated with qualitative differences in leaf oils and wax chemicals and subsequently correlated with quantitative variation in these leaf chemicals.

3. *C. bimaculata* larval feeding responses were more correlated with variation in leaf oils. In contrast, *C. agricola* larval feeding response appeared to be more associated to differences in leaf waxes only.

4. The feeding response of larvae to species within the subgenus *Monocalyptus* appeared to be more correlated with the qualitative differences of leaf chemicals while differences in the feeding response to *Symphyomyrtus* species were more correlated with quantitative differences in leaf oils.

5. The different types of leaf oils may affect the feeding responses of larvae at different quantitative thresholds. Group C compounds could affect larvae at relative low contents in contrast to group A compounds did not affect larvae significantly until much higher contents.

6. The differences in feeding response of larvae between juvenile and adult leaves corresponded to quantitative differences in leaf oils.

7. The larval feeding response to different foliages was generally similar to the preference of adult beetles. However, some exceptions occurred in that some foliage accepted by adults was not suitable for larvae feeding. For example, *C. agricola* adults could feed on *E. obliqua* in no choice experiment but its larvae could not.

Comparisons made between results of this study and chemotaxonomic studies (Chapter 4, 5 and 6) indicated that a distinct correlation exists between larval feeding responses and chemical relationships among Tasmanian eucalypt species. With the exception of *E. sieberi*, the feeding response of *C. bimaculata* larvae corresponded to the major division

between the 'ash' species and the 'gum' species populations in classifications using leaf oil (Fig. 4.6 in Chapter 4), wax (Fig. 5.4 in Chapter 5) and oil plus wax chemical data (Fig. 6.1 in Chapter 6). *C. bimaculata* larvae did not like foliage of the *Piperitae* species, *E. tenuiramis*, the oils of which tended to be close to 'gum' species. In contrast, the *C. agricola* larvae did not follow the major division of species populations in all those classifications but they did correlate with the classification using wax or wax plus oil data at lower taxonomic levels.

Of the group C compounds, 1,8-cineole, α– and β–pinene, limonene and α–terpineol, occur in substantial amounts in non preferred foliages tested in this study. Both α– and β–pinene have been reported to be major deterrents in pine trees as they are highly obnoxious to most arthropods (Harborne 1988), α–terpineol may exert an antifeedant effect (Gombos and Gasko *et al.* 1977) and 1,8-cineole, α–pinene and limonene have been found to possess a lethal action against house flies (Saxena and Koul 1982) and bark beetles (Shaaya *et al.* 1991). It is considered from their studies that 1,8-cineole and α–pinene, which occur in high amounts in non-preferred species are the major chemical compounds determining host tree selection by *C. bimaculata* in eucalypts. Thus, *C. bimaculata* does not attack foliage of most *Symphyomyrtus* species and some peppermint species of *Monocalyptus*, for they contain substantial amounts of 1,8-cineole and α–pinene. However, for those *Symphyomyrtus* species, the larval feeding response appeared to be correlated with the oil yields of foliage. Although, the leaf oils of *E. nitens* had a chemical composition similar to other *Symphyomyrtus* species, the foliage of *E. nitens* contained only very low amount of leaf oil and therefore was attacked by *C. bimaculata.*

The presence of mainly group A compounds, α– and β–phellandrene, *cis*- and *trans*-meth-2-en-1-ol and *cis*- and *trans*-piperitol, did not have positive effects for *C. bimaculata* as they are major compounds in the leaf oils of *Monocalyptus* species. However, *C. bimaculata* larvae did not like and suffered a physiological disability on foliage of the two Victorian localities of *E. delegatensis*, which had very high amounts of group A compounds. This suggested that the group A compounds may also possess deterrent and toxic effects at high amounts.

These results suggested that the different types of eucalypt leaf oil all could have negative effects on *C. bimaculata* larvae. However, the leaf oil characterised by 1,8-cineole and related oil compounds could act at a relatively lower quantitative threshold in contrast to leaf oils of *E. delegatensis* and other no or low 1,8-cineole leaf oils.

Results of this study indicated that the differences in the *C. bimaculata* larval feeding response between juvenile and adult foliage were associated with differences in oil yield.

The *C. bimaculata* larval feeding response to juvenile foliage of the three 'ash' species, *E. delegatensis, E. obliqua* and *E. regnans*, was significantly greater than to adult foliages. It has been found (Chapter 4) that the oil yield of juvenile foliage of all *Monocalyptus* species was significantly lower than that of adult foliage. This quantitative difference could be one of the factors explaining why *C. bimaculata* attacks young regeneration of the 'ash' species more heavily than adult trees (de Little 1979), although they may also be affected by other factors.

On the other hand, the *C. bimaculata* larval feeding response to adult foliage of *E. nitens* and *E. globulus* appears to be higher than to juvenile foliage as oil yields of juvenile foliage in these two species were higher than in adult foliage.

In contrast to the correlation of *C. bimaculata* larval feeding response to leaf oils, the association between *C. agricola* larval feeding response and leaf oil was relatively poor. However, the variation in the larval feeding response of *C. agricola* was more associated with variation in leaf wax chemicals among 'ash' species. The feeding behaviour response of leaf consumption and frass weight [Fig. 8.9A-(a)] of *C. agricola* larvae on 'ash' species clearly corresponded to the separation of 'green ash' and 'blue ash' species populations in the classification of wax chemical data [Fig. 9.8-(b)]. The concomitant physiological reaction responses of change in survival rate of larvae [Fig. 9.8A-(a)] tended to associated with the chemical relationship in the classification of leaf oil plus wax data [Fig. 8.9B-(c)]. However, there was no association between feeding responses of *C. agricola* larvae and the classification using only oil chemicals. Therefore, the feeding behaviour of *C. agricola* larvae on the 'ash' species group followed the classification using leaf wax chemicals while the physiological reactions followed the classification based on the combined effects of leaf waxes and oils. However, it should be noted that these associations are only based on a single foliage sample test for larvae and more leaf samples of "ash" species require testing to calculate correlations.

Moreover, the larval feeding response of *C. bimaculata* larvae to 'ash' species also showed a difference between the two 'green ash' species and two of the 'blue ash' species, *E. delegatensis* and *E. pauciflora*, in which differences associated with differences in alkanals, alkanols and triterpenoids were shown. However, the marked low feeding response to *E. sieberi* may be due to foliage of this species containing relatively higher 1,8-cineole or possibly the additional effect of chemicals other than leaf oils and waxes.

It should be noted that the two 'green ashes' were the preferred hosts for *C. bimaculata* larvae but not for *C. agricola*. Therefore, the effect of leaf chemicals of 'green ash' species for *C. agricola* were much greater that *C. bimaculata*. Triterpenoids in waxes have

been found to influence the host selection of *Plutella xylostella* on different cabbage genotypes (Eigenbrode *et al.* 1991). If the wax chemicals of the *Monocalyptus* species have a role in affecting *C. agricola*, then it is possible that those triterpenoids, which are present in high amounts in foliage of *E. regnans* and *E. obliqua*, but low in 'blue ash' species, may deter the feeding of *C. agricola* larvae while the combined effect of both wax and oil chemicals may lead to negative physiological effects.

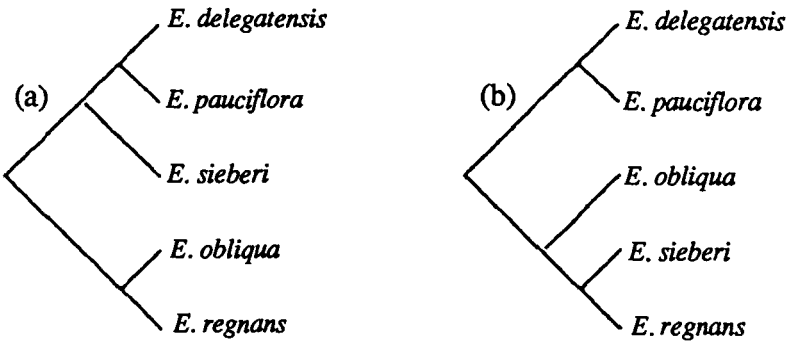


Fig. 8.9A. The two hypothetical trees according to the *C. agricola* larval feeding response to foliage of 'ash' species referred to in Fig. 8.8. (a) tree of feeding behaviour referring to leaf consumption and frass production and (b) tree of physiological effects referring to changes in larval survival rate.

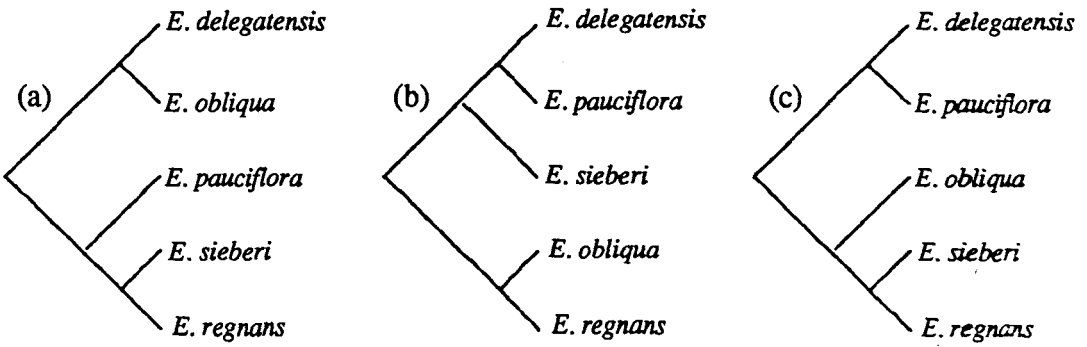


Fig. 8.9B. The three hypothetical trees from the cluster analysis based on percentage composition data of leaf oils and waxes for Tasmanian 'ash' species. (a) the tree based on clustering using leaf oil data referred to in Fig. 4.12, (b) tree based on clustering using leaf wax data referred to in Fig. 5.6 and, (c) tree based on clustering using leaf oil plus waxes data referred to in Fig. 6.1.

As fewer types of foliage were tested in adult acceptance and preference experiments, it is difficult to make complete comparisons between adult and larval feeding responses by *C. agricola*. However, results indicated that foliage, which was not preferred by adults, was subsequently not accepted by larvae. However, the foliage accepted by adults due to

starvation were rejected by larvae. For example, *C. agricola* adults could feed on non-preferred foliage, *E. obliqua*, but this foliage was rejected by their larvae. This indicated that *C. agricola* adults were more efficient in coping with non-preferred foliage than larvae. Whereas, adult *C. agricola* laid fewer eggs on *E. obliqua* foliage, the non-preferred host, indicating that adults could identify the suitability of foliage for larvae.

de Little (1979) suggested that inter-specific relationships among the *Eucalyptus*-defoliating paropsids could be seen to consist of a mosaic of strategies which permit the survival and "co-existences" of many species in a multiplicity of niches in a relatively harsh environment. In this study, the case of true "co-existence" was observed on *E. nitens*, since the adult foliage of *E. nitens* is most preferred by both *C. bimaculata* and *C. agricola* in either larval or adult experiments and has been reported to be attacked by many other paropsine species (de Little 1989). It is considered that a lack of chemical defence in *E. nitens* foliages through a low oil content, makes it a suitable host for a wide range of paropsids and other insects.

Not only *E. nitens*, but also *E. pauciflora* and *E. delegatensis* foliages were preferred by both *C. bimaculata* and *C. agricola* larvae. Although, the adult response to these two species was not assessed, this result reconfirms the findings of de Little (1979), who found that *C. agricola* larvae fed on *E. delegatensis* foliage and developed as well as on the preferred *Symphyomyrtus* host. In addition, he recorded that adults, eggs and larvae of *C. agricola* occurred on *E. delegatensis* in the field. In this study, regeneration of *E. pauciflora*, when mixed with *E. viminalis*, was found to be heavily attacked by *C. bimaculata* and *C. agricola* in conjunction with other pests. Adults, eggs and larvae of *C. agricola* occurred together with *C. bimaculata* on *E. pauciflora* foliage. Thus, in addition to *E. delegatensis*, *E. pauciflora* also is a potential alternative host for both *C. bimaculata* and *C. agricola*. It is considered that the lack of chemical defence in waxes, which occurred in 'green ashes', allowed their utilization by *C. agricola*.

A major problem of this study was the failure to use, through non-availability, larvae of similar instar in order to make a true comparison between *C. bimaculata* and *C. agricola* feeding. However, the observed differences in feeding responses between species using third instar larvae of *C. agricola* were similar to the results of de Little (1979) using first instar larvae and the preliminary findings of this thesis in which different instar larvae were tested on foliage of *E. globulus*, *E. nitens* and *E. delegatensis*. It was found that the sensitivity of the feeding response by third instar larvae was less than second and first instar larvae respectively but the overall response to different foliages was similar between different instar larvae. Therefore, the comparison between *C. bimaculata* and *C. agricola* based in this study was considered valid.

Although host-plant selection by insects may be affected by many other factors, results of this study suggested that the host-plant selection by both *C. bimaculata* and *C. agricola* were associated with variations in leaf oil and wax chemicals between Tasmanian eucalypt species in which they may play a role as chemical defences. The non-preference of *C. bimaculata* was correlated to the presence of leaf oils with high 1,8-cineole and related oil compounds while the non-preference of *C. agricola* was correlated to the presence of leaf wax with high triterpenoid contents in 'green ash' species. The leaf oils and wax chemicals also may have a combined effect on host-plant selection. Species that lack both leaf oils and wax chemical defences would allow both beetle species "co-existence".

Chapter 9

Induced Effects of Leaf Oils and Some of Their Major Constituents on *C. bimaculata* and *C. agricola* Larvae

9.1. Introduction

Essential oils have been considered to play an important role in plant-insect interactions (Harborne 1988). In some studies, the principal chemical compounds of essential oils, have been tested individually against insect pests (Sharma 1974; Shaaya at al. 1991). However, little work has been undertaken on the effects of eucalypt oils. Studies in the previous chapter indicated that the qualitative and quantitative variations in leaf oils of some Tasmanian eucalypt foliages were correlated to differences in the feeding response of *C. bimaculata* adult and larvae. This chapter reports on tests conducted on the effect of eucalypt leaf oil and its principal compounds on *C. bimaculata* and *C. agricola* larvae.

9.2. Materials and methods

A laboratory population of *C. bimaculata* and *C. agricola* larvae was established similar to that used for the larval feeding tests in Chapter 8.

The use of the agar-medium assay to determine the influence of chemicals on larval feeding responses has been discussed by Hsiao and Fraenkel (1968a,b and c), who found that the addition of host foliage homogenate to the agar diet gave a satisfactory growth response in larvae tested. In this study, an adult *E. nitens* foliage-agar diet was used, since the adult foliage of *E. nitens* was the most preferred foliage for *C. bimaculata*. A preliminary study compared the growth response of *C. bimaculata* larvae to adult *E. nitens* foliage-agar diets to that on *E. delegatensis* and *E. obliqua* foliage-agar diets and found a similar response. Consequently the addition of different leaf oils and chemicals to a standard *E. nitens* foliage-agar diet would discriminate the effect of those added materials, because adult foliage of *E. nitens* contained only low oil yields.

The leaf oils were obtained from fresh foliage by steam distillation as in chapter 4. The pure chemicals were purchased commercially (Sigma Chemical Company; Ajax Chemical Ltd.). Monoterpenoid and sesquiterpenoid fractions were separated from *E. regnans* leaf oil.

The basic agar diet consisted of 4% bacto-agar (g/100ml), 5% cellulose (Whatman no. 1. Chromatographic pure) and 6% D-sucrose and the adult *E. nitens* foliage-agar diet consisted of the basic diet made up to 20% with fresh foliage. Cellulose was used in order to obtain a frass weight at the end of each test.

In the preparation of the foliage-agar diet, fresh foliage was cut into small pieces. Foliage pieces were placed in a mortar and ground into a fine powder while liquid nitrogen was added continuously during the procedure. Two hundred grams of fresh foliage powder were mixed with 150 ml distilled water and stirred in a homogenizer. The agar, sucrose and cellulose were mixed and distilled water was added to make up half volume of the foliage agar diet. This mixture was kept in a boiling-water bath until the agar formed a consistent solution. Leaf oils or pure oil compounds for testing were dissolved in a little alcohol and homogenised into 6 ml distilled water using a sonicator and again added to 14 ml of foliage homogenate in a flask which had been warmed to 50 ^{0}C in a water bath. Next, 20 ml aliquots of the hot agar solution (about 80 ^{0}C) were mixed with the foliage homogenate and then transferred into glass petri dishes as soon as possible to a depth of about two cm. After solidification, the foliage-agar diet was stored in a refrigerator.

In the larval test, the medium was cut into discs of 1 cm diameter with a cork borer and placed on pieces of wax paper. One such agar disc was placed in the centre of a 6-cm petri dish lined with moist filter paper. The larvae were first starved in a moist dish for 4-6 hr so as to eliminate food retained in the gut in order to standardise the physiological condition. Ten larvae were placed into each test dish and allowed to feed on the test medium. The experiment was carried out at 24.5 ^{0}C in total darkness for 48 hr. The agar disc was replaced at 12 hr intervals. Afterward, the frass weight was measured as in the larval feeding test for foliage in chapter 8. Larvae mortality was also recorded.

9.3. Results

9.3.1. The response of *C. bimaculata* larvae

Leaf oils

To compare the feeding response to different leaf oils, *C. bimaculata* larvae were reared for 48 hr on the diets: fresh *E. nitens* adult foliage, the basic agar diet (see materials and methods section), foliage diet (basic agar diet plus adult *E. nitens* foliage homogenate) and three different concentrations (0.25%, 1.00% and 2%) of each of four different leaf oils plus foliage diet. The means and standard errors (untransformed data) for frass production and mortality of *C. bimaculata* on different oils in foliage agar diet are shown in Table 9.1 and Fig. 9.1.

Table 9.1. The feeding response of second instar *C. bimaculata* larvae to different leaf oils in *E. nitens* foliage-agar diet at 24.5 ^{0}C, 48 hours test period (10 larvae/per Reps., 5 Reps./per treatment)

Diet type	Concen. %(mg/ml)	No. Reps.	Frass production (mg)		Mortality (no. deaths)		
			Mean	S.E (±)	Mean	S.E (±)	(%)
Fresh foliage		5	30.20	3.37	1.40	0.42	14
Basic agar		5	7.8	1.15	5.00	0.59	50
Foliage + agar		5	12.2	1.03	2.60	0.36	26
Subgenus *Monocalyptus*							
Foliage + agar + *E. delegatensis* Oil	0.25	5	11.8	1.86	2.80	0.26	28
	1.00	5	13.4	1.42	2.40	0.36	24
	2.00	5	12.0	1.50	4.00	0.92	40
Foliage + agar + *E. regnans* Oil	0.25	5	10.8	1.10	2.60	0.48	26
	1.00	5	11.4	1.32	3.00	0.45	30
	2.00	5	8.8	0.72	3.60	0.76	36
Subgenus mean		30	11.37	0.76	3.07	0.33	30
Subgenus *Symphyomyrtus*							
Foliage + agar + *E. globulus* Oil	0.25	5	13.6	1.68	2.20	0.41	22
	1.00	5	9.6	1.32	3.00	0.74	30
	2.00	5	4	0.63	6.80	0.65	68
Foliage + agar + *E. nitens* Oil	0.25	5	11	1.07	3.00	0.32	30
	1.00	5	10.6	1.62	3.60	0.85	36
	2.00	5	5.2	1.56	6.20	0.82	62
Subgenus mean		30	9.00	0.95	4.13	0.47	41

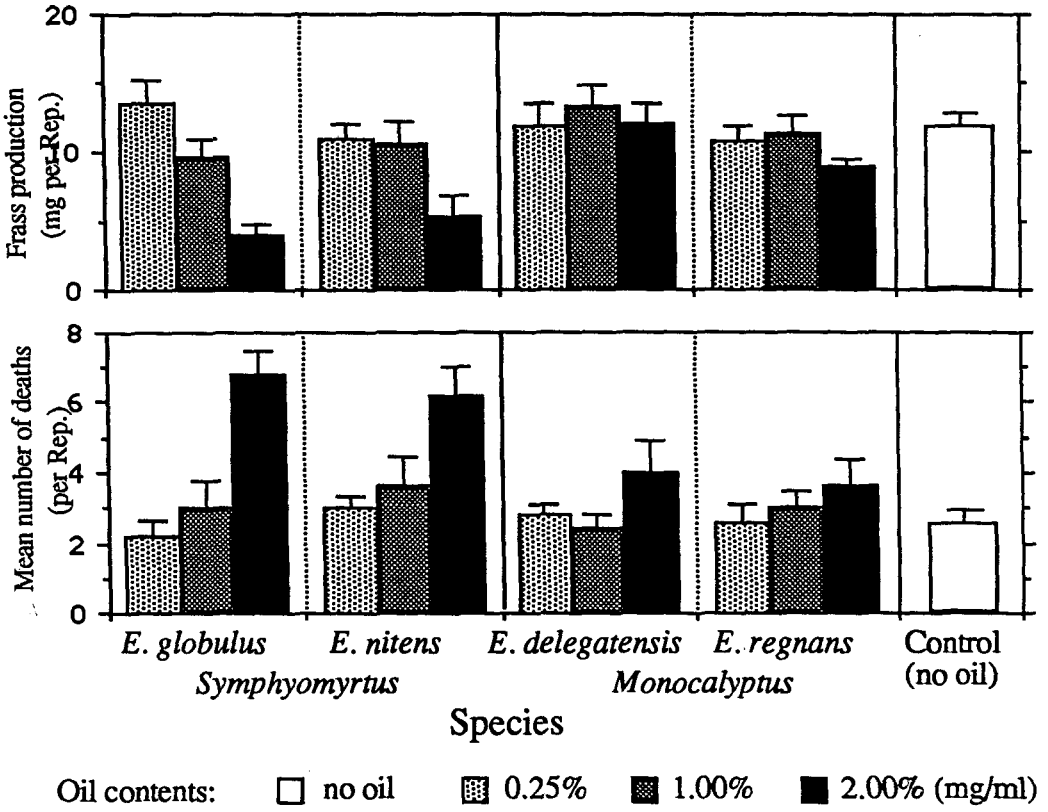


Fig. 9.1. Plot of feeding response of second instar *C. bimaculata* larvae (2-3mg) to leaf oils of four eucalypt species at 24.5 ^{0}C, 48 hours test period (10 larvae/per Rep., 5 Reps./per treatment).

As indicated in Table 9.1, fresh *E. nitens* adult foliage was the best diet for assessing the *C. bimaculata* larvae feeding response (highest frass weight and lowest mortality). The addition of *E. nitens* adult leaf homogenate to the basic agar diet was not as good a diet as fresh foliage but much better than the basic agar diet alone. Larvae feeding on the basic agar diet alone produced low frass weights and experienced high mortality. The addition of any leaf oil at any concentration did not improve the larval feeding response markedly. At high concentrations (2%), the leaf oils of *E. globulus* and *E. nitens* had marked negative effects (Table 9.1 and Fig. 9.1).

The ANOVA of the larval feeding response between subgenera, between species (within subgenera) and between oil concentrations (within species, within subgenera) using transformed data are shown in Table 9.2A. Results indicated that subgenera significantly affected mortality ($p<0.01$) and frass production ($p<0.05$) of the *C. bimaculata* larval feeding response. Changes of oil concentration also significantly affected ($p<0.001$) these two components of larval response. However, the species (within subgenera) effect were not significant.

The two way ANOVA for larval feeding response to different leaf oils within each subgenus (Table 9.2B and C) further confirmed that the *C. bimaculata* larval feeding response was not significantly affected by leaf oils from different species within each subgenus. The concentration of leaf oils of *Symphyomyrtus* species differed significantly ($p<0.001$) in affecting frass production and mortality of *C. bimaculata* larvae. However, the larval feeding response to *Monocalyptus* species leaf oils was not significantly affected by oil concentrations.

Table 9.2. ANOVA for *C. bimaculata* larval feeding response to different leaf oils in *E. nitens* foliage-agar diet using transformed data [log(x+1) for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%).

A) Variance between subgenera, between species within subgenera, between concentrations within species within subgenera.

ANOVA of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Subgenera	1	0.214	0.214	126	0.0079	Species (Subgenera)
Species (Subgenera)	2	0.003	0.002	0.01	0.9899	Concentration (Spp., Subg.)
Concentration (Spp., Subg.)	8	1.342	0.168	3.194	0.0055	Residual
Residual	48	2.52	0.053			

Model summary R: 0.618 R^2: 0.382 RMS Residual: 0.229 Model F-value: 2.7 P-value: 0.0087

ANOVA of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Subgenera	1	0.276	0.276	19.2	0.0483	Species (Subgenera)
Species (Subgenera)	2	0.029	0.014	0.106	0.9006	Concentration (Spp., Subg.)
Concentration (Spp., Subg.)	8	1.085	0.136	3.733	0.0018	Residual
Residual	48	1.743	0.036			

Model summary R: 0.666 R^2: 0.444 RMS Residual: 0.191 Model F-value: 3.478 P-value: 0.0013

B) ANOVA of feeding response of *C. bimaculata* to different concentration between species within subgenus *Monocalyptus*.

ANOVA of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
between Species	1	0.0004107	0.0004107	0.01	0.9228
between Concentration	2	0.096	0.048	1.124	0.3416
Species*concentration	2	0.023	0.012	0.27	0.7657
Residual	24	1.028	0.043		

Model summary R: 0.323 R^2: 0.104 RMS Residual: 0.207 Model F-value: 0.559 P-value: 0.73

ANOVA of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
between Species	1	0.028	0.028	1.028	0.3207
between Concentration	2	0.027	0.013	0.485	0.6213
Species*concentration	2	0.012	0.006	0.219	0.8049
Residual	24	0.663	0.028		

Model summary R: 0.304 R^2: 0.092 RMS Residual: 0.166 Model F-value: 0.487 P-value: 0.7822

Table 9.2. Continued.

C) ANOVA of feeding response of *C. bimaculata* to different concentration between species within subgenus *Symphyomyrtus*.

ANOVA of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
between Species	1	0.003	0.003	0.048	0.828
between Concentration	2	1.164	0.582	9.363	0.001
Species*concentration	2	0.058	0.029	0.466	0.633
Residual	24	1.492	0.062		

Model summary R: 0.671 R^2: 0.451 RMS Residual: 0.249 Model F-value: 3.941 P-value: 0.0095

ANOVA of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
between Species	1	0.0003745	0.0003745	0.008	0.9281
between Concentration	2	1.028	0.514	11.412	0.0003
Species*concentration	2	0.018	0.009	0.199	0.8206
Residual	24	1.081	0.045		

Model summary R: 0.701 R^2: 0.492 RMS Residual: 0.212 Model F-value: 4.646 P-value: 0.0042

Comparisons of the larval feeding response made between the control foliage agar diet and various concentrations of each oil indicated that frass production of *C. bimaculata* larvae was significantly lower ($p<0.05$) feeding on 2.00% concentration of both *E. globulus* and *E. nitens* leaf oils than that recorded on the control foliage-agar diet and 1.00% and 0.25% of concentrations (Table 9.3 and Fig. 9.1). In contrast, mortality rates were significantly higher ($P<0.05$) at 2.00% of concentration. Thus, the leaf oils of *E. globulus* and *E. nitens* significantly affected *C. bimaculata* larval survival at 2.00% concentration.

The *C. bimaculata* larval feeding response to all concentrations of *E. delegatensis* and *E. regnans* leaf oils did not differ significantly from that recorded on the control foliage-agar diet (Table 9.3). However, comparisons of mean values of the larval feeding response (Fig. 9.1) indicated that mortality of *C. bimaculata* larvae at 2.00% concentration of both *E. delegatensis* and *E. regnans* leaf oils was relatively higher than that recorded on lower concentrations and the control foliage-agar diet.

Comparisons made between species at different oil concentrations indicated that the four leaf oils, *E. globulus, E. nitens, E. delegatensis and E. regnans,* did not significantly affect frass production and mortality of larvae (Table 9.3) at 0.25 and 1.00% concentrations. However, frass production on the two foliage-agar diets, which contained 2.00% of *E. globulus and E. nitens* leaf oils was significantly ($p<0.05$) lower than that of 2.00% *E. regnans, E. delegatensis* leaf oils and no-oil foliage agar diet. At 2.00% concentration, the mortality rate of *C. bimaculata* larvae on *E. globulus* leaf oil

agar diet was significantly higher than that on *E. regnans* leaf oil diet and control foliage-agar diet while mortality rate on *E. nitens* leaf oil diet was significantly higher than that on control foliage-agar diet.

In summary, results indicated that the *E. delegatensis* and *E. regnans* leaf oils did not significantly affect the *C. bimaculata* larvae feeding response. However, *E. globulus* and *E. nitens* oils were found to be active at a concentration of 2.00%.

Table 9.3. The multiple range test (Fisher's PLSD) for difference between oil concentrations of four eucalypt species in larval feeding response using transformed data [log(x+1) for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%).

(significant level = 5%)
Oil concentration: Control (no oil foliage-agar diet alone) = N; 0.25% = A; 1.00% = B; 2.00% = C

E. globulus	C B N A	A N B C
E. nitens	C B A N	N A B C
E. delegatensis	A C N B	B N A C
E. regnans	C A B N	N A B C
	Frass production increase →	Mortality increase →

Table 9.4. The multiple range test (Fisher's PLSD) for difference between leaf oil of different species in larval feeding response when oil was added at 0.25%, 1.00% and 2.00% of concentration (using transformed data [log(x+1) for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%).

(Significant level = 5%)
Control (no oil) = Non; *E. globulus* = Glo; *E. nitens* = Nit; *E. regnans* = Reg; *E. delegatensis* = Del.

	Frass production	Mortality rate
0.25%	Reg Nit Del Non Glo	Glo Reg Non Del Nit
1.00%	Glo Nit Reg Non Del	Dle Non Glo Reg Nit
2.00%	Glo Nit Reg Del Non	Non Reg Del Nit Glo
	Frass production increase →	Mortality increase →

Oil compounds

The effects of various oil compounds on feeding and mortality of *C. bimaculata* larvae are showed in Table 9.5 and Fig. 9.2. The two way ANOVA of larval feeding response between oil compounds and between compound concentrations in foliage agar diet using transformed data are showed in Table 9.6. Results indicated that both oil compound and concentration of oil compound significantly ($p<0.001$) affected either mortality and frass production of the *C. bimaculata* larval feeding response. The interaction of oil compound and concentration effects were also significant ($p<0.001$).

Further tests (Fisher's PLSD in Table 9.7A) made between control foliage-agar diet (control) and various concentrations of oil compounds indicated that frass production was significantly ($p<0.05$) lower when larvae fed on foliage-agar diets which contained concentrations of 1.00, 1.50, 2.00 and 2.5% α–pinene, α–terpineol and piperitone when compared to the control foliage-agar diet. The frass production of larvae on the foliage-agar diets, which contained 1,8-cineole at 2.00 and 2.50% and the sesquiterpenoid fraction of *E. regnans* oil at 1.00, 2.00 and 2.50% were also significantly lower ($p<0.05$) than on the control diet. In contrast, frass production of larvae on the foliage-agar diet, which contained α–phellandrene at 2.00% was significantly higher ($p<0.05$) than on the control diet.

α–Terpineol was found to be the most active compound affecting *C. bimaculata* larvae, since the addition of this compound to foliage-agar diet at any concentration level (with the exception of 0.25%) resulted in significantly higher ($p<0.05$) mortality rates in comparison to that observed on the control diet (Table 9.7B). The compounds, α–pinene and piperitone, were also active on *C. bimaculata* larvae at concentrations of 1.00 to 2.50%, 1,8-cineole was active at 2.00 and 2.50% and the sesquiterpenoid fraction of *E. regnans* oil at 2.50%.

Moreover, comparison of the larval feeding response made between different oil compounds at each concentration (Table 9.8A) indicated that, at 2.00 and 2.50% concentration, the oil compounds separated into two activity groups according to the mortality rates of *C. bimaculata* larvae. The low activity group, was associated with the foliage diet alone (control) and the compounds limonene, α–phellandrene, monoterpenoid and the sesquiterpenoid fraction of *E. regnans* leaf oils. The high activity group, which significantly ($p<0.05$) increased mortality over the control diet was associated with the addition of α–terpineol, α–pinene, piperitone and 1,8-cineole. Similarly frass production (Table 9.8B) of larvae fed on foliage agar diet containing high

concentrations of high activity compounds was significantly lower than on the control diet and the diet containing low activity compounds.

Table 9.5. The feeding response of *C. bimaculata* second instar larvae (2-3mg) to different oil compounds in *E. nitens* foliage-agar diet at 24.5 ^{0}C, 48 hours photophase (10 larvae/per Rep., 5 Reps/per treatment)

	Concn (%)	Frass production mg		Mortality (%)		
		Mean	S.E (±)	Mean	S.E (±)	(%)
No leaf ash		3.60	1.13	4.00	0.32	40
No chemical diet		9.20	1.29	2.20	0.35	22
1,8-Cineole	0.25	6.60	0.94	2.00	0.32	20
	0.5	7.60	0.82	1.60	0.48	16
	1	10.40	1.26	2.80	0.69	28
	1.5	6.40	0.91	4.00	0.50	40
	2	3.80	0.41	7.60	0.73	76
	2.5	1.60	0.48	9.60	0.28	96
α–Pinene	0.25	5.40	0.66	2.40	0.66	24
	0.5	5.80	0.69	1.80	0.41	18
	1	3.20	0.41	4.80	0.41	48
	1.5	2.60	0.53	5.80	0.69	58
	2	2.00	0.63	8.60	0.53	86
	2.5	2.40	0.57	9.60	0.28	96
Limonene	0.25	7.00	0.81	1.40	0.42	14
	0.5	7.40	1.13	1.80	0.57	18
	1	8.80	1.03	2.00	0.32	20
	1.5	9.00	1.07	2.40	0.36	24
	2	10.00	1.12	2.40	0.53	24
	2.5	9.40	0.53	3.60	0.36	36
α–Terpineol	0.25	5.20	0.69	3.00	0.59	30
	0.5	5.80	0.88	5.60	0.48	56
	1	4.40	0.76	7.00	0.67	70
	1.5	2.60	0.36	8.80	0.57	88
	2	1.40	0.53	9.80	0.14	98
	2.5	0.80	0.41	10.00	0.00	100
α–Phellandrene	0.25	9.80	0.91	1.20	0.14	12
	0.5	9.20	1.13	1.80	0.47	18
	1	12.00	1.22	1.80	0.35	18
	1.5	13.40	1.01	2.20	0.52	22
	2	14.80	1.10	2.20	0.35	22
	2.5	12.20	1.31	2.60	0.36	26

Table 9.5. Continued.

	Concn (%)	Frass production mg		Mortality		
		Mean	S.E (±)	Mean	S.E (±)	(%)
Piperitone	0.25	6	0.97	1.80	0.35	18
	0.5	5.4	0.96	3.00	0.39	30
	1	3.4	0.76	6.60	0.48	66
	1.5	3.2	0.75	8.60	0.53	86
	2	1.6	0.48	9.80	0.14	98
	2.5	1.4	0.53	9.80	0.14	98
Monoterpenoid fraction *(E. regnans)*	0.25	10.00	0.67	1.60	0.53	16
	0.5	10.80	0.61	2.00	0.59	20
	1	12.00	0.77	2.60	0.69	26
	1.5	9.60	1.57	1.80	0.35	18
	2	12.40	0.85	2.80	0.52	28
	2.5	10.40	1.01	2.80	0.47	28
Sesquiterpenoid fraction *(E. regnans)*	0.25	7.80	0.75	3.20	0.35	32
	0.5	7.00	0.74	2.60	0.48	26
	1	5.80	0.82	2.40	0.36	24
	1.5	6.20	0.57	3.80	0.65	38
	2	4.40	0.53	3.20	0.41	32
	2.5	5.00	0.50	4.40	0.36	44

Table 9.6. ANOVA for *C. bimaculata* larval feeding response to different oil compounds at different concentrations in *E. nitens* foliage-agar diets using transformed data [log(x+1) for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%).

Variance of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Compound	7	15.947	2.278	48.667	0.0001
Concentration	5	12.448	2.49	53.186	0.0001
Compound * Concentration	35	7.478	0.214	4.564	0.0001
Residual	192		8.988	0.047	

Model summary R: 0.894 R^2: 0.800 RMS Residual: 0.216 Model F-value: 16.305 P-value: 0.0001

Variance of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Compound	7	10.402	1.486	40.356	0.0001
Concentration	5	1.91	0.382	10.374	0.0001
Compound * Concentration	35	3.505	0.1	2.72	0.0001
Residual	192		7.07	0.037	

Model summary R: 0.831 R^2: 0.691 RMS Residual: 0.192 Model F-value: 9.139 P-value: 0.0001

Table 9.7. The multiple range test (Fisher's PLSD) for difference in larval feeding response between different concentrations of individual oil compounds.

{using transformed data [log(x+1)] for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%)}

Cont = Control (no oil compound added) Significance ($p<0.05$)

A. Frass production

Compounds	Concentration (%)						
1,8-Cineole	2.50	2.00	1.50	0.25	0.50	Cont	1.00
α– Pinene	2.00	2.50	1.50	1.00	0.25	0.50	Cont
d-Limonene	0.25	0.50	1.00	1.50	Cont	2.50	2.00
α–Terpineol	2.50	2.00	1.50	1.00	0.25	0.50	Cont
α–Phellandrene	0.50	Cont	0.25	1.00	2.50	1.50	2.00
Piperitone	2.50	2.00	1.00	1.50	0.25	0.50	Cont
Monoterpenoids	1.50	Cont	0.25	2.50	0.50	1.00	2.00
Sesquiterpenoids	2.00	2.50	1.00	1.50	0.50	0.25	Cont

Frass production weight increasing →

B. Mortality rate

Compounds	Concentration (%)						
1,8-Cineole	0.50	0.25	Cont	1.00	1.50	2.00	2.50
α– Pinene	0.50	Cont	0.25	1.00	1.50	2.00	2.50
d-Limonene	0.25	0.50	1.00	Cont	2.00	1.50	2.50
α–Terpineol	Cont	0.25	0.50	1.00	1.50	2.00	2.50
α– Phellandrene	0.25	0.50	1.50	1.00	Cont	2.00	2.50
Piperitone	0.25	Cont	0.50	1.00	1.50	2.50	2.00
Monoterpenoids	1.50	0.25	0.50	Cont	1.00	2.50	2.00
Sesquiterpenoids	Cont	1.00	0.50	2.00	0.25	1.50	2.50

Mortality rate increasing →

Table 9.8. The multiple range test (Fisher's PLSD) for difference in larval feeding response between different concentrations of individual oil compounds.

Cont = control. Significance ($p<0.05$)

A. Mortality rate

Concentration (%)	Compounds
0.25	Lim Phe Mon Pip Cin Cont Pin Ter Ses
0.50	Cin Lim Phe Pin Mon Cont Ses Pip Ter
1.00	Lim Phe Cont Cin Ses Mon Pin Pip Ter
1.50	Mon Phe Cont Lim Ses Cin Pin Pip Ter
2.00	Cont Lim Phe Mon Ses Cin Pin Pip Ter
2.50	Cont Mon Phe Lim Ses Pin Cin Pip Ter

Mortality rate increasing →

B. Frass production

Concentration (%)	Compounds
0.25	Ter Pin Pip Cin Lim Ses Cont Phe Mon
0.50	Pip Ter Pin Ses Lim Cin Phe Con Mon
1.00	Pip Pin Ter Ses Lim Cont Cin Phe Mon
1.50	Pin Ter Pip Cin Ses Lim Mon Cont Phe
2.00	Ter Pip Pin Cin Ses Cont Lim Mon Phe
2.50	Ter Pip Cin Pin Ses Cont Lim Mon Phe

Frass production weight increasing →

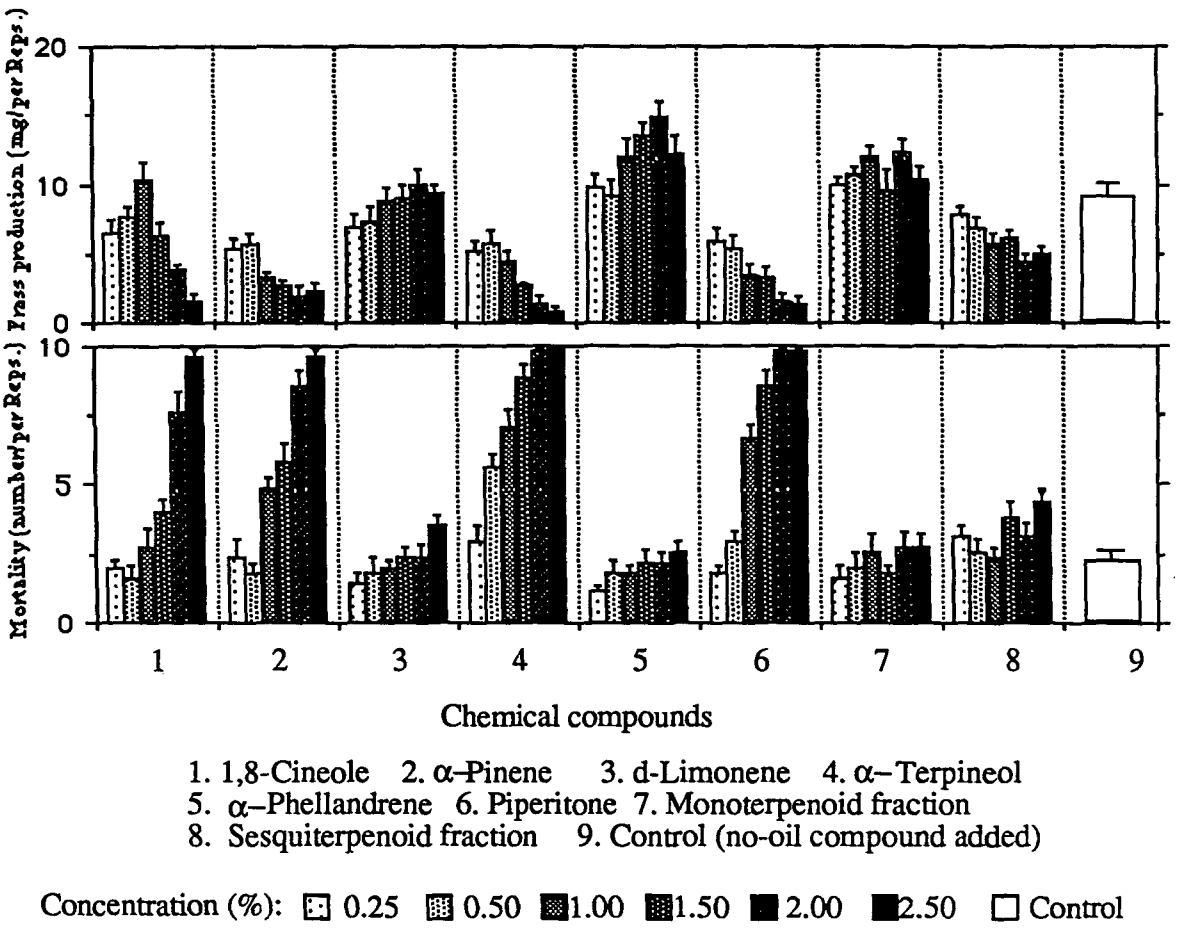


Fig. 9.2. Plot of feeding response of second instar *C. bimaculata* larvae to different oil compounds in *E. nitens* foliage-agar diet (data from Table 10.2).

9.3.2. The response of *C. agricola* larvae

Leaf oils

To compare the feeding response to different leaf oils, second instar larvae of *C. agricola* (4-5mg average weight) were reared for 48 hr on each of 14 diets: fresh *E. nitens* adult foliage, the basic agar diet (see materials and methods section), foliage diet (basic agar diet plus adult *E. nitens* foliage homogenate) and the addition of three different concentrations of each of four different leaf oils to the foliage diet. The effects of various leaf oils on frass production and larval mortality are shown in Table 9.9 and Fig. 9.3.

As for *C. bimaculata*, fresh *E. nitens* adult foliage provided the best diet to assess the *C. agricola* larval feeding response (i.e. high frass production and low mortality) (Table 9.9). The addition of *E. nitens* adult leaf homogenate to the basic agar diet was not as

acceptable as fresh foliage but much better than basic agar diet. The larval feeding response on the basic agar diet alone resulted in low in frass weight and high mortality.

However, in contrast to the response of *C. bimaculata* larvae, ANOVA indicated that the leaf oil from different subgenera did not significantly affect either frass production or mortality of *C. agricola* larvae (Table 9.10). The effects of species (within subgenera) and oil concentration (within species, within subgenera) were also not significant.

Comparisons in the larval feeding response made between the control foliage agar diet and various concentrations of each oil further indicated that the larval feeding response of *C. agricola* larvae at any concentration of each oil did not differ significantly to that recorded on control foliage-agar diet (Table 9.11A). Comparisons made between the control foliage agar diet and different leaf oil at each concentration also showed no significant difference (Table 9.1B). However, at 2.00%, the frass production of *C. agricola* larvae when fed on foliage-agar diet containing *E. delegatensis* leaf oil was significantly higher than that containing *E. regnans* leaf oils.

Table 9.9. The feeding response of *C. agricola* second instar larvae (4-5mg) to different leaf oils in *E. nitens* foliage-agar diet at 24.5 ^{0}C, 48 hours test period (5 larvae/per Rep., 5 Reps./per treatment)

	Con.(%)	Frass production mg		Mortality		
		Mean	S.E (±)	Mean	S.E (±)	(%)
Fresh leaves		43.48	3.59	0.40	0.24	8.00
No foliage diet		11.34	1.23	1.40	0.68	28.00
No oil foliage diet		22.76	1.96	0.80	0.37	16.00
E. globulus	0.25	23.84	2.15	0.40	0.24	8.00
	1.00	24.99	1.81	0.40	0.24	8.00
	2.00	19.75	2.87	1.20	0.58	24.00
E. nitens	0.25	22.00	2.92	0.80	0.58	16.00
	1.00	24.19	2.69	0.40	0.40	8.00
	2.00	21.05	1.78	0.80	0.37	16.00
E. delegatensis	0.25	23.33	1.93	0.20	0.20	4.00
	1.00	20.61	1.58	0.80	0.58	16.00
	2.00	25.44	1.83	0.40	0.24	8.00
E. regnans	0.25	20.94	1.78	0.80	0.37	16.00
	1.00	7.51	1.48	0.40	0.24	8.00
	2.00	8.04	2.20	1.00	0.45	20.00

Table 9.10. ANOVA of *C. agricola* larval feeding response to different leaf oils in *E. nitens* foliage-agar diet using transformed data [log(x+1) for frass production and [arcsin(√x)] for mortality rate (%).

Variance between subgenera, between species within subgenera, between concentrations within species within subgenera.

Variance of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Subgenera	1	0.003	0.003	0.066	0.8218	Species (Subgenera)
Species (Subgenera)	2	0.091	0.045	0.859	0.4594	Concentration (Spp., Subg.)
Concentration (Spp., Subg.)	8	0.423	0.053	0.536	0.8234	Residual
Residual	48	4.73	0.099			

Model summary R: 0.314 R^2: 0.098 RMS Residual: 0.314 Model F-value: 0.476 P-value: 0.9089

Variance of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Subgenera	1	0.001	0.001	0.126	0.7564	Species (Subgenera)
Species (Subgenera)	2	0.016	0.008	0.643	0.5508	Concentration (Spp., Subg.)
Concentration (Spp., Subg.)	8	0.098	0.012	1.303	0.2648	Residual
Residual	48	0.449	0.009			

Model summary R: 0.45 R^2: 0.203 RMS Residual: 0.097 Model F-value: 1.11 P-value: 0.3747

Table 9.11A. The multiple range test (Fisher's PLSD) for differences in the larval feeding response between oil concentration of four eucalypt species (significance level = 5%) using transformed data [log(x+1) for frass production and [arcsin(√x)] for mortality rate (%)

Oil concentration: Control (no oil) = N; 0.25% = A; 1.00% = B; 2.00% = C

E. globulus	C N A B	A B N C
E. nitens	N C A B	B A C N
E. delegatensis	B N C A	A B C N
E. regnans	B N A C	B C A N
	Frass production increase →	Mortality increase →

Table 9.11B. The multiple range test (Fisher's PLSD) for differences in larval feeding response between leaf oils of four species when oil added at 0.25, 1.00 and 2.00% (analysis to control foliage diet). (Significant level = 5%)

Control (no oil) = Non; *E. globulus* = Glo; *E. nitens* = Nit; *E. regnans* = Reg; *E. delegatensis* = Del.

	Frass production	Mortality rate
0.25%	Non Reg Nit Del Glo	Del Non Glo Nit Reg
1.00%	Del Non Reg Nit Glo	Nit Glo Reg Del Non
2.00%	Reg Glo Non Nit Del	Del Nit Non Reg Glo
	Frass production increase →	Mortality increase →

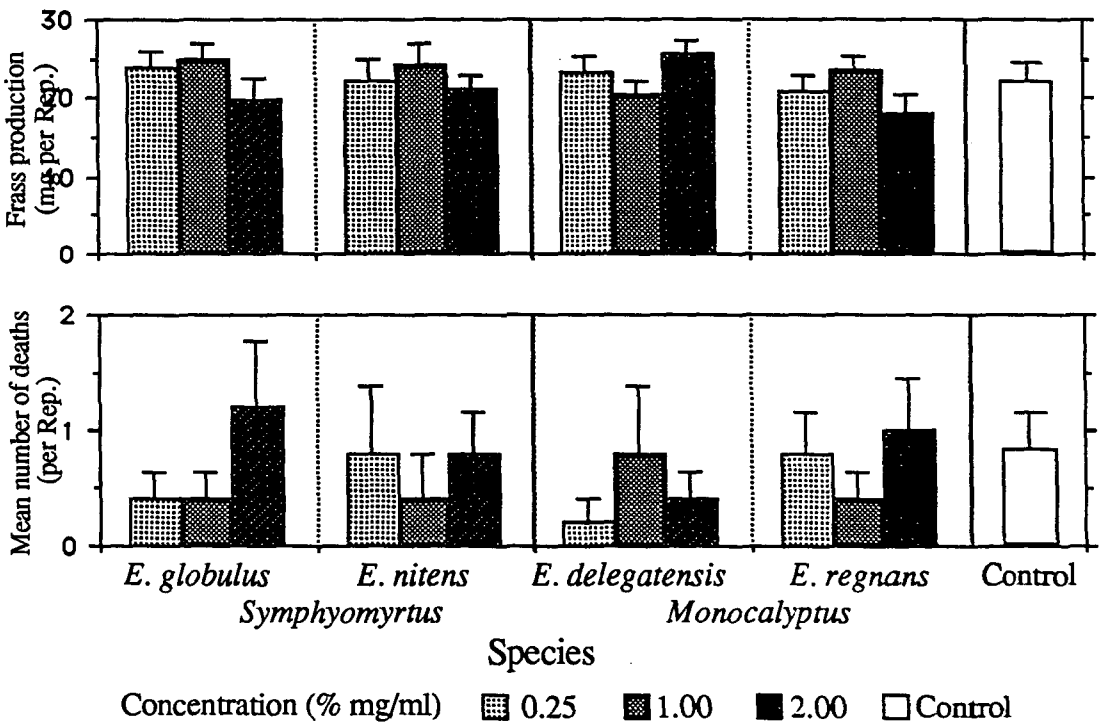


Fig. 9.3. Plot of feeding response of *C. agricola* third instar larvae (4-5mg) to leaf oils of four eucalypt species at 24.5 0C, 28 hours photophase (5 larvae/per rep., 5 reps./per treatment).

Oil compounds

The effects of various oil compounds on the larval feeding response of *C. agricola* are shown in Table 9.12 and Fig. 9.4. The two way ANOVA (Table 9.13) indicated that both mortality rate and frass production differed significantly ($p<0.001$) when larvae fed on foliage-agar diets containing different oil compounds. The F-ratios for the effect of concentration (3.079) on frass production had an associated *p*-value of 0.0521, which is close to 0.05, indicating a concentration effect. However, concentration did not significantly affect mortality rate of larvae. The interactions of oil compounds and concentrations were not significant for both mortality and frass production.

Table 9.12. The feeding response of third instar *C. agricola* larvae (4-5mg) foliage agar plus different oil compounds at 24.5 ^{0}C, 48 hours test period (5 larvae/per Rep., 5 Reps./per treatment).

	Concn. (%)	Frass production (mg)		Mortality		
		Mean	S.E (±)	Mean	S.E (±)	(%)
Fresh leaves		40.65	3.33	0.60	0.40	12
No chemical foliage-agar diet		20.90	1.82	1.00	0.45	20
1,8-Cineole	0.25	24.58	1.39	0.40	0.24	8
	1.00	24.46	0.87	0.40	0.24	8
	2.00	24.96	0.78	0.60	0.24	12
α–Pinene	0.25	21.51	1.64	0.60	0.40	12
	1.00	20.48	1.95	1.00	0.32	20
	2.00	20.37	1.75	0.80	0.37	16
α–Phellandrene	0.25	24.43	1.51	0.20	0.20	4
	1.00	26.56	1.00	0.00	0.00	0
	2.00	22.79	1.77	0.40	0.24	8
Piperitone	0.25	14.16	2.51	1.80	0.73	36
	1.00	10.48	1.43	3.00	0.45	60
	2.00	7.79	1.16	3.20	0.20	64
Monoterpenoids	0.25	21.93	1.84	0.60	0.40	12
	1.00	22.80	2.76	0.80	0.37	16
	2.00	20.58	2.35	0.80	0.37	16
Sesquiterpenoids	0.25	19.96	1.72	0.80	0.37	16
	1.00	20.48	1.95	1.00	0.32	20
	2.00	18.05	2.13	0.80	0.37	16

Table 9.13. ANOVA for *C. agricola* larval feeding response to different oil compounds at different concentrations in *E. nitens* foliage-agar diet using transformed data [log(x+1) for frass production and [arcsin($\sqrt{x}$)] for mortality rate (%).

Variance of mortality (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Compound	5	4.398	0.88	11.736	0.0001
Concentration	2	0.244	0.122	1.625	0.2041
Compound * Concentration	10	0.376	0.038	0.502	0.883
Residual	72	5.396	0.075		

Model summary R: 0.694 R^2: 0.482 RMS Residual: 0.096 Model F-value: 3.938 P-value: 0.0001

Variance of frass production (%)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Compound	5	1.385	0.277	30.326	0.0001
Concentration	2	0.056	0.028	3.079	0.0521
Compound * Concentration	10	0.097	0.01	1.067	0.399
Residual	72	0.658	0.009		

Model summary R: 0.837 R^2: 0.701 RMS Residual: 0.274 Model F-value: 9.909 P-value: 0.0001

Comparisons in the larval feeding response made between the control foliage agar diet and various concentrations of each oil compound indicated that, piperitone was active against *C. agricola* larvae, since the addition of this compound to foliage-agar diet at 1.00 and 2.00% resulted in significantly higher ($p<0.05$) mortality rate and lower ($p<0.05\%$) frass production in comparison to that observed on the control diet (Table 9.14). In contrast, when larvae fed on foliage-agar diet containing 1.00% α–phellandrene, the frass production was significantly higher (0.05%) and mortality rate significantly lower ($p<0.05$) than that on control foliage-agar diet. The frass production of larvae fed on foliage agar diet containing 2.00% of 1,8-cineole was also significantly higher (0.05%) than that on control. However, other oil compounds did not significantly affect either frass production or mortality of *C. agricola* larvae at any concentration.

Moreover, comparison of the *C. agricola* larval feeding response made between different oil compound at each concentration (Table 9.15) indicated that when larvae fed on foliage-agar diet containing piperitone at 1.00 and 2.00% frass production was significantly lower ($p<0.05$) and mortality significantly higher ($p<0.05$) than that on control diet and foliage-agar diets containing any other compound at any concentration.

Table 9.14. The multiple range test (Fisher's PLSD) for differences in *C. agricola* larval feeding response to different concentrations of each oil compound.

Cont = Control (foliage diet alone) Significance ($p<0.05$)

	Frass production	Mortality rate
1,8-Cineole	Cont 1.00 0.25 2.00	1.00 0.25 2.00 cont
α–Pinene	2.00 1.00 Cont 0.25	0.25 2.00 Cont 1.00
α–Phellandrene	Cont 2.00 0.25 1.00	1.00 0.25 2.00 Cont
Piperitone	2.00 1.00 0.25 Cont	Cont 0.25 1.00 2.00
Monoterpenoids	2.00 Cont 0.25 1.00	0.25 2.00 1.00 Cont
Sesquiterpenoids	2.00 0.25 1.00 Cont	2.00 0.25 Cont 1.00
	Frass production increasing →	Mortality rate increasing →

Table 9.15. The multiple range test (Fisher's PLSD) for differences in *C. agricola* larval feeding response between oil compounds at different concentration levels.

Cont = control (foliage diet alone) Significance ($p<0.05$)

Frass production

Concentration (%)	Compounds
0.25	Pip Ses Cont Pin Mon Phe Cin
1.00	Pip Ses Pin Cont Mon Cin Phe
2.00	Pip Ses Mon Pin Cont Phe Cin

Frass production increasing →

Mortality rate

Concentration (%)	Compounds
0.25	Phe Cin Pin Mon Ses Cont Pip
1.00	Phe Cin Mon Cont Pin Ses Pip
2.00	Phe Cin Pin Ses Mon Cont Pip

Mortality rate increasing →

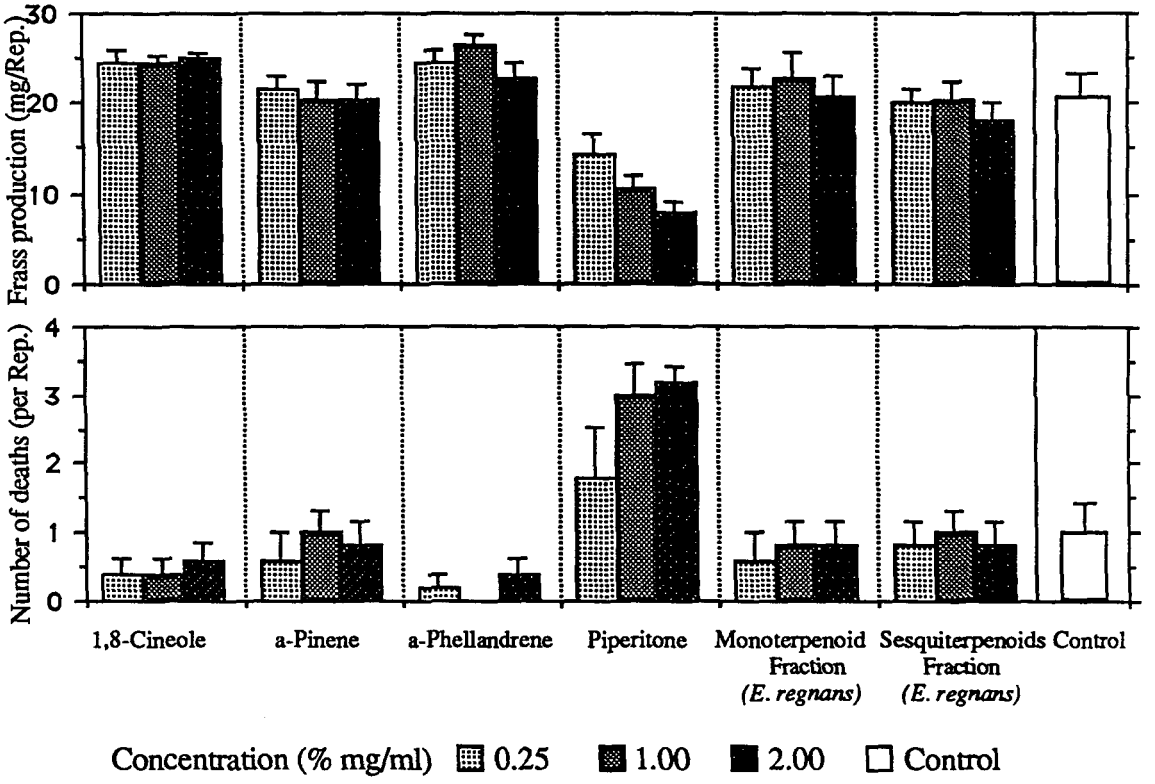


Fig. 9.4. Plot of *C. agricola* larval feeding response to different oil compounds in *E. nitens* foliage-agar diet supplemented with different oil compounds (data from Table 9.12).

9.4. Discussion

The objective of this study was to determine the effects of different types of eucalypt leaf oils and their major constituents on *C. bimaculata* and *C. agricola* larvae. Leaf oils from both *Symphyomyrtus* and *Monocalyptus* species were tested. The chemotypes of the two leaf oils, *E. globulus* and *E. nitens*, are the typical chemotype of *Symphyomyrtus* leaf oil, which is characterized by high percentages of 1,8-cineole and α–pinene. The leaf oils of *E. delegatensis* and *E. regnans* represented two different chemotypes of *Monocalyptus* leaf oils, the high monoterpenoid chemotype and the high sesquiterpenoid type respectively. Results indicated that the effects of leaf oils on the *C. bimaculata* larval feeding response differed significantly between subgenera while effects on *C. agricola* did not. Certain leaf oils from the two *Symphyomyrtus* species, *E. globulus* and *E. nitens*, significantly affected the *C. bimaculata* larval feeding response when at high concentration but did not affect *C. agricola* larvae. However, the leaf oils from the two

Monocalyptus species did not significantly affect the larval feeding responses of either *C. bimaculata* or *C. agricola.*

Three groups of oil compounds could be distinguished in the compounds tested. Group 1, compounds α–terpineol, α–pinene and 1,8-cineole, were active against *C. bimaculata* larvae as antifeedants and toxicants; the group 2 compound piperitone, was active against both *C. bimaculata* and *C. agricola* larvae as both an antifeedant and toxicant; group 3, sesquiterpene fractions of *E. regnans* oil, acted as an antifeedant for *C. bimaculata* larvae only and group 4, compounds α–phellandrene, limonene and the monoterpene fraction of *E. regnans* oil, had low activity.

The *E. globulus* and *E. nitens* oils and their major constituents, 1,8-cineole, α–pinene and α–terpineol showed greater activity against *C. bimaculata* larvae with increasing concentration. At low concentrations, *E. globulus* and *E. nitens* oil and compounds of this group did not affect *C. bimaculata* larvae. This result confirmed that substantial amounts of *E. globulus* oils could act against *C. bimaculata* larvae and that 1,8-cineole and α–pinene were the principal compounds affecting larval feeding and survival. The results also suggested that the leaf oils of *Symphyomyrtus* species played an important role in protecting foliage from *C. bimaculata* attack, since those leaf oils contained substantial amounts of 1,8-cineole and α–pinene. One important finding obtained in this study was that the leaf oils of *E. nitens* also had the same properties as *E. globulus* leaf oils. Thus, these two oils were active against *C. bimaculata* larvae when in high amounts but not when in low amounts.

It is of interest that the monoterpene limonene, which is one of major compounds occurring with 1,8-cineole, α–pinene and α–terpineol in *E. globulus and E. nitens* leaf oils showed no negative effect on *C. bimaculata* larvae. Therefore, this further indicated that the active effect of *E. globulus* and *E. nitens* oils on *C. bimaculata* larvae was due to levels of 1,8-cineole, α–pinene and α–terpineol.

In contrast to these effects of *Symphyomyrtus* leaf oils, the leaf oils of *E. delegatensis* and *E. regnans* showed little to no activity against *C. bimaculata* while α–phellandrene, which is one of the principal compounds of *E. delegatensis* oil, stimulated the response of both *C. bimaculata* and *C. agricola* larvae on the foliage agar-diet. The monoterpene fraction of *E. regnans*, which contained compounds similar to *E. delegatensis* oil, also promoted the feeding response of *C. bimaculata* larvae. This suggested that the presence of α–phellandrene and related monoterpene compounds in the foliage of 'ash' species may possibly induce *C. bimaculata* feeding. The *C. bimaculata* larval feeding response to the sesquiterpene fraction of *E. regnans* leaf oils was significantly lower than to the monoterpene fraction at high concentration. However, these sesquiterpene compounds

did not result in high larval mortality. This suggested that the sesquiterpene from *E. regnans* oil was possibly a feeding inhibitor for *C. bimaculata* larvae but not a toxicant.

Only one compound, piperitone, was found to be active against both *C. bimaculata* and *C. agricola* larvae. This compound not only acted as an inhibitor to feeding but also as a toxicant for both species. However, the effect of these compounds on the *C. agricola* larval response was much more significant than on *C. bimaculata* at low concentration. *C. agricola* larvae died at a concentration of 0.25% in foliage agar-diet, while for *C. bimaculata* larvae the effect was at a concentration of 1.00%. This could explain why *E. obliqua* foliage, which contained a high level of piperitone, was rejected by *C. agricola*. However, this possibility did not agree in the case of *C. bimaculata* for *E. obliqua* foliage is one of its most preferred, since this species contained about 0.2-0.4 % of absolute contents in fresh adult and juvenile foliage (16% and 30% of the adult and juvenile leaf oil composition respectively; see Chapter 4). It is possible that the combination effects of different chemicals in leaf oils may reduce the toxicant effect of piperitone for *C. bimaculata*.

In contrast with the significant effects of leaf oils on *C. bimaculata larvae*, the effect of leaf oils on *C. agricola* was relatively poor. With the exception of piperitone, no other oil compound tested was found to affect *C. agricola* larvae. Furthermore, the addition of leaf oils to the foliage-agar diet did not significantly affect the *C. agricola* larval response. However, 1,8-cineole was found to increase *C. agricola* larval feeding at high concentration. This suggested that the 1,8-cineole in leaves of *Symphyomyrtus* species may possibly induce *C. agricola* feeding. The positive effects (high frass production and survival) of α–phellandrene with *C. agricola* larvae also suggested that the occurrence of this compound in *E. delegatensis* and *E. pauciflora* leaves benefited the feeding of *C. agricola* larvae. However, this does not agree in the case of other *Monocalyptus* species which are not preferred by *C. agricola*.

Although, a conclusion of the overall effect of oil compounds on the *C. bimaculata* larval response could not be made due to the limited number of different monoterpene compounds used in this study, it can be seen from the relationship of monoterpenoid structure and their biological activity, that alcohol, ether, ketone and bicyclic monoterpene hydrocarbons, such as α–terpineol, 1,8-cineole, piperitone and α–pinene show high activity against *C. bimaculata* larvae. However, the effect of monoterpene hydrocarbons, such as α–phellandrene, limonene and monoterpene fraction of *E. regnans* leaf oil (mainly monoterpene hydrocarbons, such as α– and β–phellandrene, α–terpinene, *p*-cymene and terpinolene) was low. Thus, oxygenated monoterpenoids were active and monoterpene hydrocarbons were not. This result is similar to the finding by Shaaya *et al.* (1991), who reported that the fumigant toxicity of oil compounds against stored-product

insects was related to the structure of the monoterpenoids. They found that the monoterpene alcohols, such as 1,8-cineole, linalool, α–terpineol and terpinen-4-ol were active but the monoterpene hydrocarbons, such as *p*-cymene, α–terpinene, sabinene and myrcene were not.

Chapter 10

Inheritance of Feeding Resistance in Interspecific F1 Hybrids of *Eucalyptus*

10.1. Introduction

Improvement of insect resistance to *C. bimaculata* in *Eucalyptus* is important to the Tasmanian forest industry since this species is the major pest of many important eucalypt species. As described in Chapter 8, the feeding of *C. bimaculata* adult and larvae on different *Eucalyptus* spp. is very variable and that these difference could be related to distribution of terpenoid compounds of foliage. A further series of experiments were performed to determine the inheritance of such chemicals in interspecific F1 hybrids and whether variability in larval feeding could be related to chemical variable among parental species and their F1 hybrid. The present chapter covers these experiments using *C. bimaculata* larvae as test individuals feeding on the species *E. ovata*, *E. globulus* and their F1 hybrids. The inheritance of oil chemicals in F1 hybrids was determined and the feeding response of *C. bimaculata* described. The potential for the development of resistance in trees to *C. bimaculata* is discussed. Due to the available of samples from successful hybrid families, only 3 tree/family was analysis. Therefore, study of this chapter described only the trend oil inheritability and larval response among samples rather than a true family inheritability.

10.2. Materials and methods

Two hybrid combinations using *E. ovata* and *E. globulus* were used in this experiment. One F1 hybrid was between *E. ovata* female, Ov5 and *E. globulus* male, Glo4, whereas the other involved Ov4xGlo5. In each case open pollinated seed has been collected from each parent and these families grown as controls with the F1 hybrid. At the same time, two foliage samples were added to this experiment as reference. A sample was a polymix family of *E. ovata* female (Ov4pl) grown in the same trial whereas the other was a *E. ovata* sample, OVA-Sout-A (Ovs), from the southern forest trial plots. This sample, Ovs, had been tested for its effects on *C. bimaculata* larval and adult feeding in study of the previous chapter. Results had indicated that the Ovs sample was one of the samples that was least preferred by *C. bimaculata*. Field observations also indicated that trees of this *E.*

ovata provenance in southern forest (originally from D'entrecasteaux River) have not been defoliated by *C. bimaculata,* even when the provenance trial of *E. ovata* was next to the *E. nitens* provenance trial. Therefore, the sample of Ovs was used as a resistant type of *E. ovata* to compare with other *E. ovata* families.

The families of *E. ovata*, Ov4op, Ov5op and Ov4pl, were originally from Kingston, Hobart. Family Glo5op of the *E. globulus* was from Proctor Rd., Hobart and family Glo4op from an ornamental near the Hobart airport. Progenies from interspecific pollinations of these families were planted on a single quality site of APPM Forest Products at West Ridgley in October 1988 by Dr. B. M. Potts. From the successful hybrids and out crosses, adult leaf samples of three trees of each family were sampled in December 1990. These families were: Ov4op, Ov5op, Glo4op, Glo5op and the F1 hybrid progenies of Ov5xGlo4 and Ov4xGlo5 and the polymix *E. ovata* family Ov4pl. Leaf samples of three trees from Ovs were sampled at the same time from the Tasmanian Forest Commission *E. ovata* provenance trial located in the Esperance Valley, Tasmania. In this experiment, all foliage consisted of young adult leaves and the same materials were used for both larval feeding bioassay and oil analysis.

The larval feeding test for *C. bimaculata* on the foliage of F1 hybrids, parent families and the reference samples were carried out in the same way using second-instar larvae of 2-3 mg average weight as the main series of larval feeding experiments described in Chapter 8. However, this experiment involved presenting each group of larvae to a number of foliage discs from a certain tree and measuring the average response and reaction of the larvae to foliage samples of individual trees of different families of the two species and their F1 hybrids where each test for each tree was replicated five times.

Thus in this chapter, parental families and their F1 hybrid were categorised as families, eg. Ov4op, Glo4op etc. Hybrids were categorised as hybrid combinations, eg. Ov5xGlo4, etc. The basic unit here is the tree and there were three trees, A, B and C, within individual families. The larval feeding tests were replicated five times for each tree of each family.

The ANOVA of the larval feeding response to foliage samples was carried out in the same manner as that used previously. However, the foliage samples of this experiment involved a complete mixture of different taxonomic units where hybrid combinations, families and trees were all different from each other. Thus, this experiment involves a nested design where different categories occur. At the same time, a correlation analysis for interaction between oil chemicals of different foliages and the respective larval feeding responses wa made.

The inheritance of oil chemicals and the variability of feeding preference of *C. bimaculata* larvae in hybrids were estimated using the same test method as used previously for heritability frost resistance (Tibbits *et al.* 1991). The deviation of each F1 hybrid family from the corresponding midparent value was tested using a two-tailed t-test and, where parental progenies differed significantly, the degree of dominance (DD) of oil chemicals and feeding response in the F1 hybrid was calculated as DD = Deviance of F1 from mp/(P2-mp), where F1 is the F1 hybrid value, mp is the midparent value [(parent 1+parent 2)/2] and P2 is the value of the most resistant parent or high oil chemical parent such that -1 or +1 corresponds to complete dominance toward the least or most inhibiting or lowest or highest oil parent, respectively, and 0 represents no dominance.

10.3. Data analysis and results

10.3.1. The inheritance of oil chemicals in F1 hybrids of *E. globulus* and *E. ovata*

Chemical composition of leaf oils

Similar leaf oils were identified from the original plants and the seedling progenies planted in trial plots for the two families of *E. ovata* (Appendix 10.1). The leaf oils of these two *E. ovata* families were characterised by the acyclic sesquiterpenoid, nerolidol, as principal compound and the presence of the acyclic monoterpenoid, linalool, as a minor compound while the cyclic monoterpenoids and sesquiterpenoids occurred in very low or trace amounts. It was found that the percentage content of nerolidol was higher in family Ov5op (mean percentage = 81.1%) than that in family Ov4op (67.4%) while linalool was higher in family Ov4op (23.2%) than Ov5op (6.1%). Comparison of the leaf oils between the progenies and the corresponding original plants of *E. ovata* indicated that the leaf oils of individual families were strongly inherited. The leaf oils of the two families of *E. globulus* (Glo4op and Glo5op) were characterised by the cyclic monoterpenoids 1,8-cineole and α–pinene as major compounds, nerolidol and linalool occurred in only trace amounts. Similarly, the leaf oils of the individual progenies of the two families of *E. globulus* also showed strong inheritance from the original plants.

Inheritance of leaf oils in F1 hybrids

Significant differences in the percentage contents of ten compounds were observed between the parents in hybrid combination Ov5xGlo4 and between parents in Ov4xGlo5 (Table 10.1.A) while α– and γ–terpinene, *p*-cymene, α–terpineol and aromadendrene did not differ significantly. For the ten compounds that differed significantly between the

Table 10.1. Mean oil yield (%), oil composition (%) and absolute contents of oil compounds (g/10g D.W) for parental control *E. globulus* (Glo)and *E. ovata* (Ov) and their F1 hybrid.

The significance of the difference between parental species and the difference of the F1 hybrid mean from the midparent value (mp) is indicated . Where the parental species differ significantly in the absolute contents of compounds, the degree of dominance (D.D.) of the inheritance of the F1 hybrid has been calculated (0 = no dominace, +1 = complete dominace toward the *E. globulus*, -1 = complete dominance toward the *E. ovata*. # = F1 hybrid mean over range of parental means. (ns P>0.05, * p<0.05 ** p<0.01 *** p<0.001).

(A) Oil yield and oil compositions

Compounds	Ov5 X Glo4						OV4 X Glo5					
	Glo4	F1	Ov5	Difference between parents	Difference between F1 vs mp	D.D.	Glo5	F1	Ov4	Difference between parents	Difference between F1 vs mp	D.D.
OIL YIELD	5.54	4.60	2.60	**	*	0.34	4.17	2.83	2.72	*	ns	0.73
α–Pinene	19.36	11.23	0.20	***	ns	0.15	23.60	12.07	0.00	***	ns	0.03
β–Pinene	0.52	0.19	0.00	***	ns	-0.20	0.82	0.21	0.00	***	ns	0.40
α–Phellandrene	0.83	0.25	0.03	***	**	-0.25	1.02	0.44	0.21	***	ns	-0.50
α–terpinene	0.10	0.00	0.33	ns	***	#	0.25	0.00	0.22	ns	***	#
Limonene	7.23	3.57	0.39	***	ns	-0.06	6.89	3.71	0.32	***	ns	0.03
1,8-Cineole	58.09	28.38	1.23	***	ns	-0.04	52.87	28.36	0.09	***	ns	-0.02
γ–Terpinene	0.45	0.21	1.50	ns	**	#	0.57	0.55	0.33	ns	ns	1.00
p-Cymene	0.13	0.11	0.61	ns	*	1.00	0.15	0.12	0.16	ns	ns	#
Linalool	0.07	0.03	6.15	*	***	#	0.06	7.05	23.19	***	ns	0.40
Un	2.72	1.86	0.31	ns	ns	0.33	0.95	2.57	0.09	ns	***	#
Terpinyl acetate	1.41	0.85	0.36	**	ns	0.00	2.56	2.78	0.32	**	***	#
α–Terpineol	0.00	0.28	0.49	ns	ns	-0.20	0.04	0.14	1.17	ns	*	0.83
Nerolidol	0.37	46.72	81.09	***	ns	-0.15	0.22	36.14	67.41	***	ns	-0.07
Globulol	5.60	2.64	1.57	**	ns	-0.50	8.07	2.36	1.11	**	*	-0.63
Viridiflorol	0.88	0.53	0.23	**	ns	-0.14	1.04	0.50	0.26	**	ns	-0.43

(B) Absolute contens (g/10kg dried leaf weight).

Compounds	Ov5 X Glo4						Ov4 X Glo5					
	Glo4	F1	Ov5	Difference between parents	Difference between F1 vs mp	D.D.	Glo5	F1	Ov4	Difference between parents	Difference between F1 vs mp	D.D.
α–Pinene	107.42	50.06	0.48	***	ns	-0.07	98.59	32.10	0.00	***	ns	-0.35
β–Pinene	2.90	0.86	0.00	***	**	-0.41	3.41	0.56	0.00	***	**	-0.67
α–Phellandrene	4.61	1.16	0.06	***	*	-0.52	4.26	1.10	0.58	***	**	-0.72
α–Terpinene	0.57	0.00	0.82	ns	ns	#	1.03	0.00	0.63	ns	***	#
Limonene	40.06	16.22	1.02	***	ns	-0.22	28.77	9.70	0.91	***	*	-0.53
1,8-Cineole	321.79	129.40	3.09	***	ns	-0.22	221.01	75.64	0.24	***	ns	-0.29
γ–Terpinene	2.50	0.93	3.83	ns	**	#	2.33	1.79	0.92	*	ns	0.23
p-Cymene	0.72	0.51	1.56	ns	**	#	0.65	0.29	0.44	ns	*	#
Linalool	0.41	0.13	15.76	**	***	#	0.25	22.15	62.38	**	ns	0.30
Un	15.27	8.70	0.87	***	ns	0.09	3.64	4.52	0.25	ns	ns	#
Terpinyl acetate	7.79	3.81	0.92	**	ns	-0.16	10.57	8.67	0.92	***	ns	0.61
α–Terpineol	0.00	1.39	1.23	*	ns	#	0.14	0.32	2.83	*	***	0.87
Nerolidol	2.06	217.37	213.11	**	ns	(-1.04)#	0.91	109.54	184.18	***	ns	-0.19
Globulol	31.04	10.63	3.90	***	ns	-0.50	33.38	6.21	2.84	***	**	-0.78
Viridiflorol	4.89	2.15	0.58	***	ns	-0.27	4.30	1.34	0.65	***	*	-0.62

parental families, only two compounds, linalool and nerolidol, were significantly higher ($p<0.001$) in the female parents of *E. ovata* but all other eight compounds were significantly higher ($p<0.01$ to $p<0.001$ respectively) in male parents of *E. globulus*.

Within the Ov5xGlo4 hybrid combination, for those compounds where the F1 hybrid mean was intermediate between the parental means, only the minor compound, α–phellandrene, differed significantly ($p<0.01$) from the midparent value and tended toward the female parent Ov5op [degree of dominance (DD) = - 0.25]. However, the mean of linalool of the F1 hybrid of Ov5xGlo4 differed significantly ($p<0.001$) from the midparent value and was outside the range of the parental means. In the hybrid combination Ov4xGlo5, for those compounds where the F1 hybrid means were intermediate between the parent means, only the compound globulol differed significantly ($p<0.05$) from the midparent value and tended toward the female parent Ov4op (DD =- 0.63). At the same time, the mean value of terpinyl acetate in the F1 hybrid Ov4 x Glo5 was significantly different ($p<0.001$) from the midparent value and outside the range of parental means. The analysis for inheritance of individual compounds thus indicated that the leaf oils of the two F1 hybrids were strongly inherited from their parents with the mean percentage contents of most compounds in the F1 hybrids intermediate between their parental values. Nevertheless, one compound, linalool, from *E. ovata* showed poor inheritance in the F1 hybrids since this compound occurred in only trace amounts in the F1 hybrid of Ov5 x Glo4 and outside the range of parental means. Linalool also showed 0.4 degrees of dominance toward the male parent Glo5op in the F1 hybrid of Ov4 x Glo5.

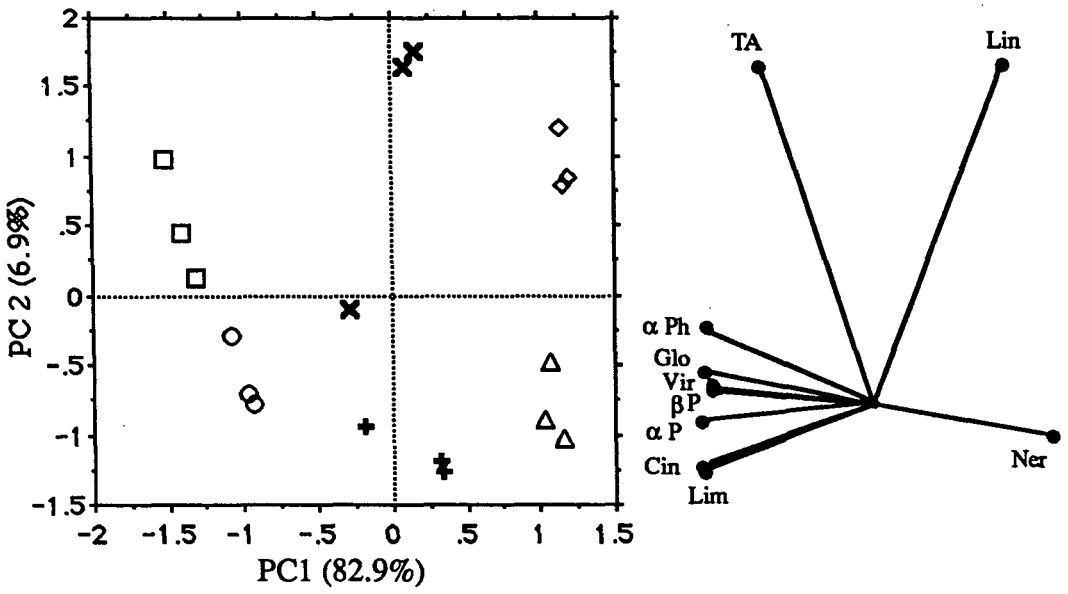
In the principal component analysis using percentage content data of all compounds that showed a significant difference between both parents (Fig.10.1.A), the first axis represented 82.9% of the total variation and the second axis 6.9%, so that the ordination provides a convenient summary of the data. While the leaf samples of *E. ovata* families had high levels of linalool and nerolidol and the *E. globulus* samples had high levels of 1,8-cineole, α–pinene and other compounds, the samples of the F1 hybrids were intermediate between their parents on the first (and only major) axis and showed no significant difference in distance from each parent. There is again much variation between families within the female parent *E. ovata* where samples of family Ov4op had higher levels of linalool in contrast to the Ov5op which had higher levels of nerolidol. Two of the F1 hybrid Ov4xGlo5 samples had higher levels of both linalool and terpinyl acetate than the samples of F1 hybrid Ov5xGlo4 while one leaf sample of the Ov4xGlo5 was distinctly separated from the other two samples of its family. Overall, the PCA confirmed the genetic inheritance in leaf oil composition for F1 hybrids where the F1 hybrids were intermediate between their parents.

While the percentage contents of most oil compounds of the F1 hybrids showed values close to the midparent values, marked differences between the two F1 hybrids in the inheritance of oil yields were observed. The mean value of the oil yield in the F1 hybrid family Ov5xGlo4 differed significantly ($p<0.05$) from the midparent value and tended toward the pollen parent *E. globulus*, whereas, the hybrid mean of Ov4xGlo5 was significantly different ($p<0.05$) from the midparent value and outside the parental range.

Further tests (Table 10.1B) indicated that the absolute contents of most compounds were significantly different between parents for both hybrid combinations. Within the Ov5xGlo4 combination, in those cases where the F1 hybrid mean was intermediate between the parental means, most compounds did not differ significantly from the midparent values but tended toward Ov5op, with the exception of β–pinene, α–phellandrene and linalool. It is of interest that the F1 hybrid mean of nerolidol (217.4) in Ov5xGlo4 was very close to the female parent value (213.1) although it was slightly over the range of parental means. The F1 hybrid mean of linalool was also outside the parental means but close to Glo4op. Within hybrid combination Ov4xGlo5, in those cases where the F1 hybrid mean was intermediate between the parental means and significantly different to the midparent values, α–pinene, α–phellandrene, limonene, globulol and viridiflorol tended toward Ov4op and only one compound, α–terpineol, toward to Glo5op. In contrast to Ov5xGlo4, the F1 hybrid means of linalool and nerolidol in Ov4xGlo5 were intermediate between parental values and did not differ significantly from the midparent values.

The PCA, using absolute content data (Fig.10.1.B), indicated that the first axis represents 83.1% of the total variation and the second axis 6.8%. All samples of the F1 hybrids were intermediate between their parents on the first axis and tended to be more close to the female parents. The second axis again separated the two female families of *E. ovata* and indicated that the samples of the F1 hybrid of Ov5xGlo4 had a close affinity to the female parent Ov5op and two of the F1 hybrid of Ov4xGlo5 samples were close to the female parent Ov4op. However, the third sample of F1 hybrid of Ov4xGlo5 was separated from the other two samples of F1 hybrid and close to the Ov5op family and F1 hybrid of Ov5xGlo4. The PCA using absolute content data of oil compounds thus indicated that the F1 hybrids of both hybrid combinations were closer to female parent *E. ovata* than to male parent *E. globulus* in contrast to the result of the PCA using percentage composition data.

A. Oil composition:



B. Absolute contents:

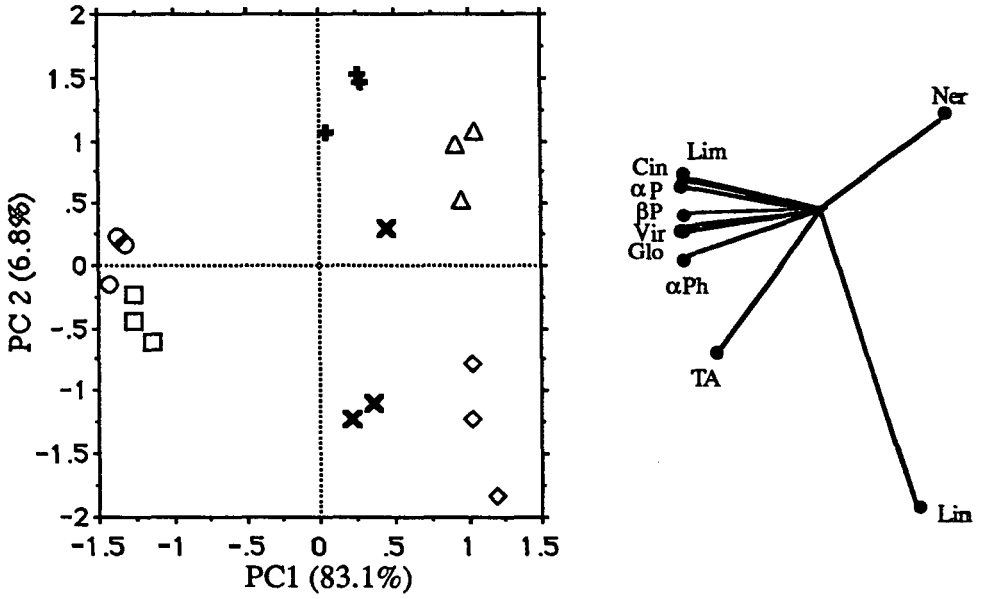


Fig. 10.1. Variation in the percentage composition of leaf oils among different families of *E. globulus* (Glo), *E. ovata* (Ov) and their F1 hybrids at the first and second principal components.

Again, the plot of absolute mean values of the oil chemicals of individual parental families and F1 hybrid for each hybrid combination (Fig.10.2) does indicate that the foliage of the F1 hybrid of Ov5xGlo4 contained similar amounts of nerolidol to that in the female parent Ov5op but does not contain substantial amount of linalool. Thus, the F1(G4O5) has inherited nearly 100% of the nerolidol from female parent Ov5op, since the degree of

dominance in absolute content of nerolidol was -1.04 (Table 10.1B) for F1(G4O5). At the same time, the F1 hybrid (G4O5) has inherited approximately 40% of the total amount of the major chemicals from the male parent Glo4op. Notice in Table 10.1.A that over 80% of the total oil yield of Ov5op was contributed by the absolute amount of nerolidol. However, the F1 hybrid Ov4xGlo5 contained only about half the amounts of chemicals from the female parent Ov4op and small amount from the pollinated parent Glo5 where the total oil yield of F1(G5O4) was similar to female parent Ov4op.

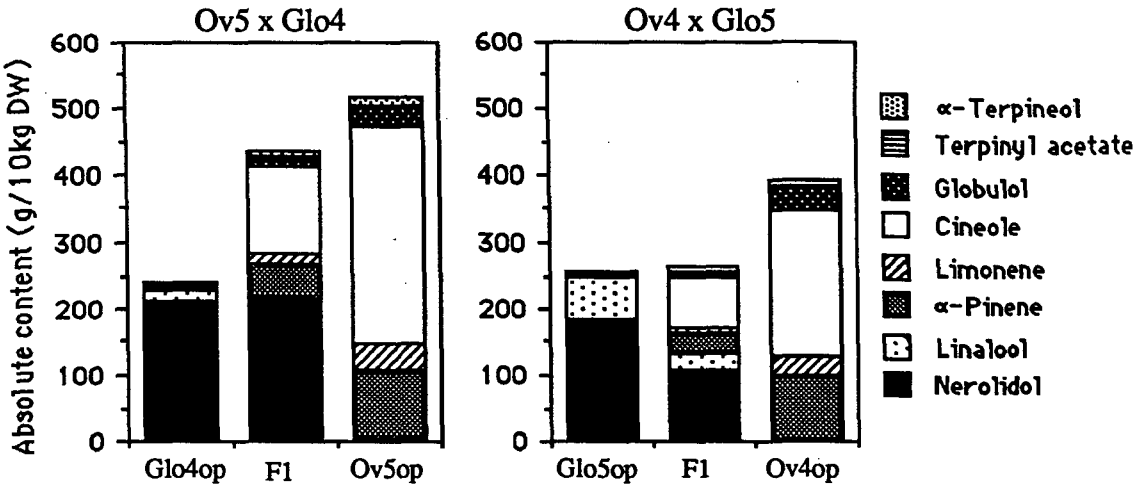


Fig. 10.2. The plot of absolute contents of major oil chemicals in foliage of parental families and F1 hybrids of the two hybrid combinations.

10.3.2. The inheritance of beetle feeding deterrence in F1 hybrids of *E. globulus* and *E. ovata*

Variation in larval feeding response

Experiments were designed to examine the inheritance of larval feeding deterrence in F1 hybrids between *E. globulus* and *E. ovata* and the relationship between chemical inheritance and deterrence. In this larval feeding test, three factors may affect the larval feeding response: hybrid combination, families within hybrid combinations and trees within individual families. Analysis of variance here is most interested in the variation in the feeding response between families within hybrid combinations since the oil chemicals have been found to be markedly different between parent families for each hybrid combination. Because the trees represent a sub-sampling unit within each parental family and F1 hybrid and trees from each family or F1 hybrid had no relationship to others since the trees were randomly selected, hence the appropriate error term for testing the effect of families is tree (family, hybrid combination), not the residual error. The residual error serves as an appropriate error term for the tree (families, hybrid combination).

Hybrid combination	Glo4 x Ov5			Glo5 x Ov4		
Family	Glo4	F1(G4O5)	Ov5	Glo5	F1(G5O4)	Ov4
Tree	3	3	3	3	3	3
	X	X	X	X	X	X
No. of Rep. for each tree	5	5	5	5	5	5

Initial examination indicated that the observed data was not distributed normally, so the dependent variables (larval feeding responses) were transformed. Further examination of the results of the model by plotting residual against fitted values indicated that the logarithmic transformation of data [Log (x+1)] was the appropriate transformation for leaf consumption, frass production and larval weight and the arcsin[$\sqrt{(x)}$] was the appropriate transformation for mortality rate. Therefore, all data for the larval feeding response was transformed for data analysis.

The results of the analysis of variance of the *C. bimaculata* larval feeding response for the two hybrid combinations of *E. globulus* and *E. ovata* are presented in Table 10.2 (transformed data) and the observed means, standards error and standard deviation of individual trees listed in Appendix 10.2 (untransformed data).

Table. 10.2. Analysis of variance for *C. bimaculata* larvae feeding response

ANOVA of leaf consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Hybrid combination	1	0.039	0.039	0.192	0.6836	Residual
Family (hybrid combination)	4	0.819	0.205	5.73	0.0081	Tree (family, H.C.)
Trees (family, hybrid combination)	12	0.429	0.036	3.013	0.0018	Residual
Residual	72	0.854	0.012			

Model summary R: 0.775 R^2: 0.601 RMS Residual: 0.109 Model F-value: 6.384 P-value: 0.0001

ANOVA of frass production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Hybrid combination	1	0.038	0.038	0.216	0.6665	Residual
Family (hybrid combination)	4	0.697	0.174	8.551	0.0017	Tree (family, H.C.)
Trees (family, hybrid combination)	12	0.244	0.02	1.893	0.0494	Residual
Residual	72	0.775	0.011			

Model summary R: 0.747 R^2: 0.558 RMS Residual: 0.104 Model F-value: 5.351 P-value: 0.0001

ANOVA of larval weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Hybrid combination	1	0.008	0.008	0.154	0.7149	Residual
Family (hybrid combination)	4	0.203	0.051	3.921	0.0292	Tree (family, H.C.)
Trees (family, hybrid combination)	12	0.155	0.013	3.735	0.0002	Residual
Residual	72	0.249	0.003			

Model summary R: 0.771 R^2: 0.595 RMS Residual: 0.059 Model F-value: 6.215 P-value: 0.0001

Table 10.2. Continued.

Variance of mortality rate

Source	df	Sum of Squares	Mean Square	F-Value	P-Value	Error Term
Hybrid combination	1	0.038	0.038	0.011	0.9206	Residual
Family (hybrid combination)	4	13.614	3.404	12.712	0.0003	Tree (family, H.C.)
Trees (family, hybrid combination)	12	3.213	0.268	0.8	0.6491	Residual
Residual	72	24.096	0.335			

Model summary R: 0.642 R^2: 0.412 RMS Residual: 0.579 Model F-value: 2.964 P-value: 0.0007

The P-value was larger than 0.05 for variance in leaf consumption, frass production, larval weight and mortality rate between hybrid combinations (Table 10.2) and indicated that the hybrid combination did not significantly affect the larval feeding response. In contrast, families(hybrid combination) significantly affected leaf consumption ($p<0.01$), frass production ($p<0.01$), larval weight ($p<0.05$) and mortality rate ($p<0.001$) of larvae. At the same time, tree(family, hybrid combination) also significantly affected leaf consumption ($p<0.01$), frass production ($p<0.05$) and larval weight ($P<0.001$) but did not significantly affect larval mortality.

As many aspects of larval feeding response are incorporated in this experiment, then correlations among these dependent variables are correctly taken into account by data analysis. Here, the models for analysis of multiple variance (Wilks 1932, Rao 1973; Bock 1975) were employed. Results of the MANOVA (Table 10.3) confirmed family and tree effects on the larval feeding responses when all four aspects were correctly taken into account. However, results also indicated that the family(hybrid combination) exhibited a more significant effect than tree(family, hybrid combination).

Table 10.3. The MANOVA for *C. bimaculata* larvae feeding response

Num DF = Number for degrees of freedom; Den DF = Denomination degrees of freedom.

Effect: Families (hybrid combination)			Value	F-Value	Num DF	Den DF	P-Value
S	4	Wilks' Lambda	0.034	3.581	16	28.133	0.0014
M	-0.5	Hotelling-Lawley Trace	8.693	4.075	16	30	0.0004
N	3.5	Pillai Trace	1.791	2.432	16	48	0.009

Effect: Trees (family, hybrid combination)			Value	F-Value	Num DF	Den DF	P-Value
S	4	Wilks' Lambda	0.36	1.696	48	267.334	0.005
M	3.5	Hotelling-Lawley Trace	1.275	1.794	48	270	0.002 1
N	33.5	Pillai Trace	0.841	1.597	48	288	0.011

Variation in the larval feeding response on F1 hybrids

The plot of the larval feeding responses of leaf consumption, frass production, larval weight and mortality rate on different foliage samples, oil yields and amounts of 1,8-cineole and α–pinene are shown in Fig. 10.4.

The highest leaf consumption, frass production, larval weight and survival rate among foliage samples occurred on family Ov4pl of *E. ovata,* which had the lowest oil yield and contained only traces of 1,8-cineole. In contrast, the foliage of Ovs, which had the lowest response in all categories, had high contents of 1,8-cineole and the highest oil yield. Thus, the Ovs is the most resistant and the Ov4pl the most susceptible foliage to larval feeding response.

The comparison made between species *E. ovata* and *E. globulus* indicated that the larval feeding response on the two *E. globulus* families was similar to Ovs, the most resistant *E. ovata* sample. Larval feeding responses on the two *E. ovata* families Ov4op and Ov5op, which were used for hybridisation, were much higher than that on the Ovs and the two *E. globulus* families but were closer to the response to the family Ov4pl of *E. ovata.* Thus, the larval feeding response on the *E. ovata* families Ov5op, Ov4op and Ov4pl, which were originally from Proctor Rd., Hobart, were markedly higher than Ovs samples, which were originally from Old Bridge on the D'entrecasteaux River. Moreover, within the Proctor Rd. families the larval feeding response on Ov5op and Ov4op was relatively lower than on Ov4pl and this was correlated to the relatively higher oil yield in foliage of Ov5op and Ov4op.

Tests for the larval feeding response of *C. bimaculata* on the F1 hybrid combinations, using transformed data based on tree means, are presented in Table 10.4. The results indicated that leaf consumption, frass production and mortality rate on the parents of each hybrid combination differed significantly but did not differ significantly in larval weight. For the Ov5xGlo4 hybrid combination, mean values of leaf consumption, frass production and mortality rates on the F1 hybrid were not significantly different to the midparent value but tended slightly toward the male parent Glo4op (0.305, 0.439 and 0.536 degrees of dominance for leaf consumption, frass production and mortality rates respectively). However, the F1 hybrid mean of Ov4xGlo5 deviated toward *E. ovata* but was outside the range of parental means for leaf consumption and frass weight. Only mean mortality rate on the F1 hybrid of Ov4xGlo5 was intermediate between that on the parental means (0.587 degree of dominance toward Ov4op).

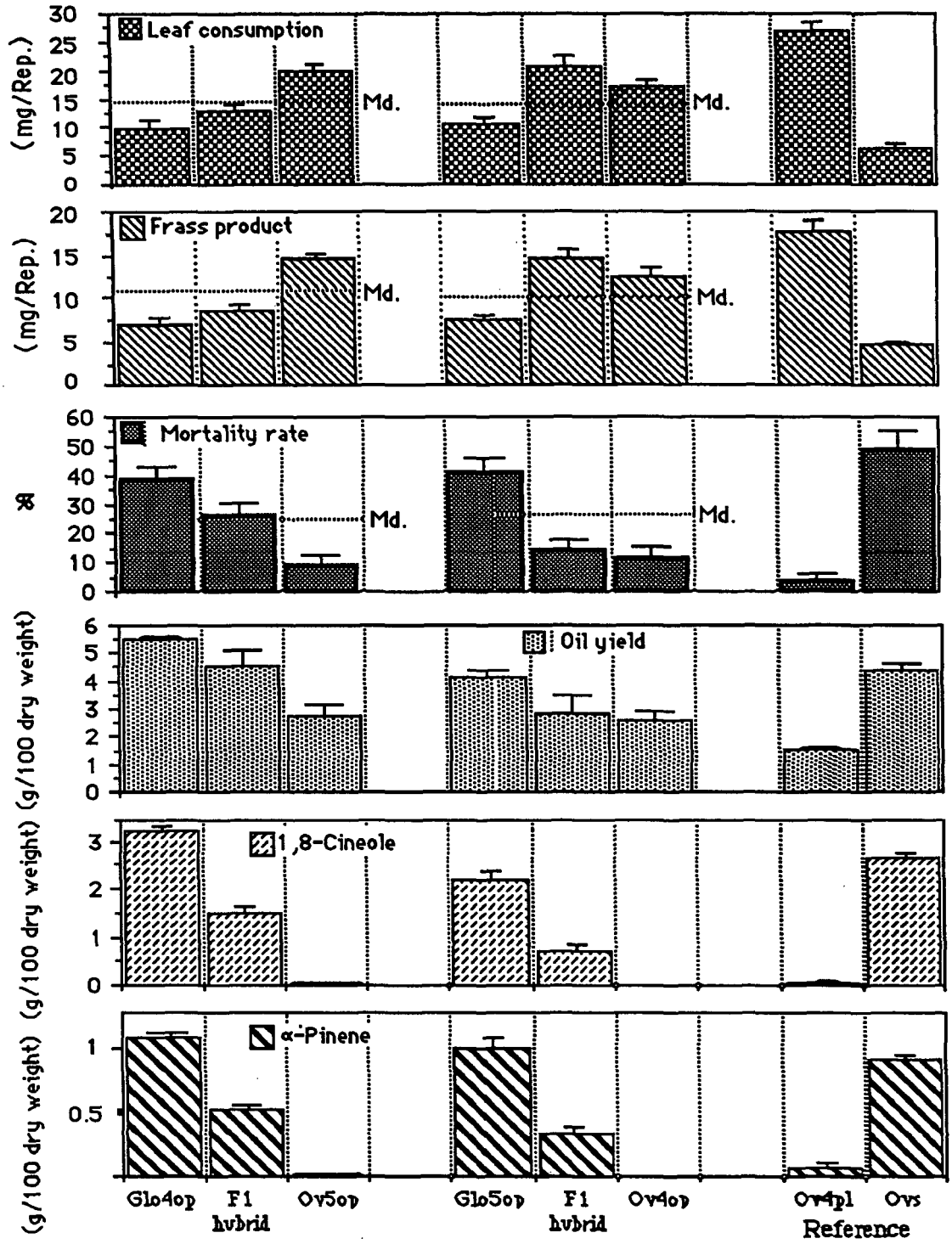


Fig. 10.4. Plot of mean values of larval feeding response of *C. bimaculata* larvae on foliage of the two cross of *E. globulus* and *E. nitens* and the reference types Ov4pl and Ovs and mean values of oil yields and chemicals in these foliage.

Table 10.4. Mean leaf consumption, frass production, larval weight [log(x+1)] and mortality rate [arcsin(√x)] of *C. bimaculata* larvae feeding response for *E. globulus* (Glo)and *E. ovata* (Ov) and their F1 hybrid.

The significance of the difference between parental species and the difference of the F1 hybrid mean from the midparent value (mp) is indicated. Where the parental species differ significantly in the absolute contents of compounds, the degree of dominance (d) of the inheritance of the F1 hybrid has been calculated (0 = no dominance, +1 = complete dominance toward the *E. globulus*, -1 = complete dominance toward the *E. ovata*, # = F1 hybrid mean over range of parental means. (ns P>0.05, * p<0.05 ** p<0.01 *** p<0.001).

Larval response	Ov5xGlo4						Ov4xGlo5					
	Glo4 op	F1	Ov5 op	Difference between		DD	Glo5 op	F1	Ov4 op	Difference between		DD
				parents	F1 vs mp					parents	F1 vs mp	
Leaf consumed (mg DW/larva)	.449	.532	.688	*	ns	0.305	.474	.691	.631	*	ns	#
Frass production (mg/larva)	.359	.421	.580	*	ns	0.439	.381	.576	.526	*	ns	#
Larval weight (mg/larva)	.729	.691	.825	ns	-	-	.717	.800	.784	ns	-	-
Mortality rate (%)	1.501	1.281	.548	*	ns	0.538	1.582	.901	.724	**	ns	-.587

The comparison of the chemical inheritance of the two F1 hybrids indicated that the mean values for oil yield, α–pinene and 1,8-cineole in the F1(O5G4) were close to the midparent value but for F1(O4G5) were more toward to the female parent Ov4op (Fig. 10.4). Therefore, the amounts of these chemicals in the latter F1 hybrid are relevant since the regression analysis had indicated that the variables of α–pinene and 1,8-cineole in foliage were attributed to the two-order poly-regression line where the amount of these chemicals under a threshold point may increase the leaf consumption and frass production (see Appendix 10.3). Thus, a slight increase in oil yield, α–pinene and 1,8-cineole in the Ov4xGlo5 F1 hybrid increased the feeding of *C. bimaculata* larvae. In contrast, the higher levels of inheritance in oil yields, α–pinene and 1,8-cineole contents in Ov5xGlo4 F1 hybrid decreased feeding by *C. bimaculata*.

Finally, variation in the C. *bimaculata* larval response between all foliage samples under this experiment (including the two reference samples) were summarised by the ANOVA as showed in Appendix 10.4 and by the multiple range test using Fisher's Protected LSD in Fig.10.5. Results of ANOVA showed in Appendix indicated that the variation in the *C. bimaculata* larval response was significantly different (p<0.001) between families in all respects while variation between trees (families) also significantly affected leaf consumption (p<0.001), frass production (p<0.05) and larval weight (p<0.01).

Nevertheless, the values of the variation between families were much greater than the variation between trees (families) in all cases.

As shown by the multiple range test (Fig. 10.5), the two families of *E. globulus*, Glo4op and Glo5op, and the F1 hybrid of Ov5xGlo4 have been ranked into a group with Ovs foliage which was most resistant to leaf consumption by *C. bimaculata*. The two families of *E. ovata*, Ov4op and Ov5op, and the F1 hybrid of Ov4xGlo5 have been ranked into a group with the most preferred family Ov4pl ($P<0.001$). At the same time, similar ranked groups were formed with respects to frass production ($P<0.01$). While detailed information concerning leaf consumption and frass production on different leaf types were indicated by linkage groups at other significance levels, these results have indicated that the F1 hybrid of Ov5xGlo4 was linked into the family group resistant to *C. bimaculata* larval feeding the F1 hybrid of Ov4xGlo5 was not. The multiple range linkages of larval weight at different significance levels also showed similar trends to that of leaf consumption and frass production.

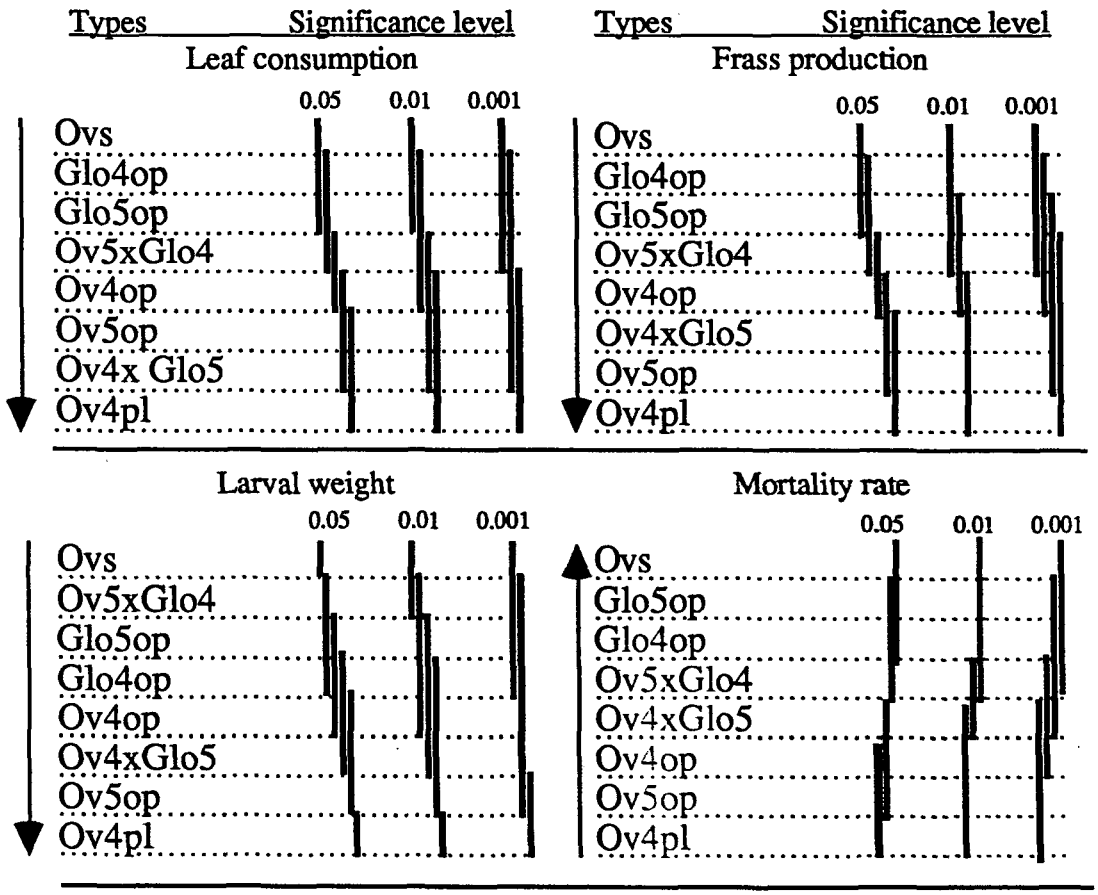


Fig. 10.5. The activity of different foliage types on the response of C. bimaculata larvae tested by Fisher's Protected LSD.

(Arrow indicates increased response)

It is of interest that the mortality rate of *C. bimaculata* larvae clearly divided the foliage samples into different groups. Thus, at the 0.05 significance level, the two female parent families of hybrids, Ov4op and Ov5op, which were linked with acceptable family Ov4pl while the two male parents, Glo4op and Glo5op, were linked with the most resistant type Ovs. Moreover, F1 hybrid of Ov4xGlo5 was linked with Ov5op and Ov4op and the F1 hybrid of Ov5xGlo4 was linked with the Glo4op. These linkages indicated that larval mortality rate on the F1 hybrid of Ov5xGlo4 was linked to a resistant group and the F1 hybrid of Ov4xGlo5 to a preferred group.

10.4. Discussion

Results of this experiment have indicated that the F1 hybrid of Ov5 x Glo4 had increased resistance to *C. bimaculata* larval feeding over the preferred parent Ov5op. In other words, samples from the F1 hybrid of Ov5xGlo4 inherited feeding deterrents from the more resistant parent Glo4op. The increase in resistance to *C. bimaculata* larvae of the Ov5xGlo4 F1 hybrid associated with a significant increase in amounts of α–pinene and 1,8-cineole in the F1 hybrid which were inherited from the high oil parent Glo4op. However, F1 hybrid Ov4 x Glo5 did not significantly affect feeding from that resulted on the susceptible parent Ov4op for the increase of chemicals in the F1 hybrid was not sufficient to act as a deterrent factor but actually tended to increase larval feeding. These results suggested that interspecific F1 hybrids between resistant and susceptible parents could have different results on the feeding responses by *C. bimaculata* and such differences are associated with the inheritance of leaf oils from hybrid parents.

Results of this experiment also indicated the significant difference in *C. bimaculata* larval feeding between *E. ovata* populations. The *E. ovata* population, Ovs, from a plantation in the southern forest, which had a high levels of 1,8-cineole and related oil compounds, was resistant to *C. bimaculata* larvae (also see Chapter 8) in contrast to the *E. ovata* populations from Hobart (Proctors Rd) which was susceptible to damage by larvae of *C. bimaculata* which had low oil yield and 1,8-cineole contents. These further suggested that these leaf chemicals also affected the larval feeding of *C. bimaculata* within species.

It should be noted that the sampling of only 3 trees/family is very limited for a genetic study. Therefore, further study requires the use of more progeny to determine true family inheritability.

Chapter 11

A Survey for Leaf Oils and Waxes of *E. nitens*

11.1 Introduction

Shining gum (*Eucalyptus nitens* Deane & Maiden) is a species belonging to the subseries *Globulinae*, series *Viminales*, subgenus *Symphyomyrtus* (Pryor and Johnson, 1971) and the series *Viminales* of the most recent classification of Chippendale (1988). Its foliage characteristics are similar to southern blue gums (*E. globulus*), but the general properties of the timber are similar to those of the ash group (series *Obliquae*, subgenus *Monocalyptus*, Pryor and Johnson, 1971). Shining gum is a fast-growing species of the mountain ranges of the south-eastern Australian mainland and frequently occurs mixed with mountain ash (*E. regnans* F. Muell.), or alpine ash (*E. delegatensis* R. T. Baker) in Victoria (Pederick 1979; Cook and Ladiges 1991).

The distribution and variation patterns within *E. nitens* on the mainland have already been investigated by Pederick (1979). He reported that two different forms could be identified among the populations although there was continuous variation in growth rates and foliage characteristics. The 'juvenile-persistent' form is characterized by broad, glaucous juvenile leaves produced for 2-4 years after planting, followed by adult leaves with a smooth margin, and high growth rates in both height and diameter up to at least 5 years of age. In contrast, the Errinundra 'mature' form produces relatively narrow, green juvenile leaves for 1-2 years after planting and in relatively slow growing. Moreover, the variation in a polyphenol constituent, which was identified as stilbene, also complements differences in foliage morphology and rate of growth as a basis of separation for the two different forms (Pederick and Lennox 1979). Studies of the two forms of *E. nitens* in field trials showed consistent differences in floral morphology, flowering time and maturity (Tibbits 1989). More recently, Cook and Ladiges (1991) determined the pattern of variation in adult and seedling morphology for the complete geographic range of *E. nitens* and reported the presence of two distinct taxa: one is found in disjunct stands in New South Wales and Victoria, which is characterised by entire leaf margins, and was recognised as *E. nitens* s. str.; the other occurs on the Errinundra Plateau, east Gippsland, with limited occurrences in the Baw Baw Ranges and Central Highlands of Victoria. This form is characterised by denticulate adult leaf margins, and was described as *E. denticulata* sp. nov..

Provenances of shining gum have been successfully transplanted to Tasmania some of which are regarded as potentially important in the Tasmanian forest industry. The leaf oils of some commercially important "ash" species and pulp species in Tasmanian forest industry have been compared (Li *et al.* 1990). The yields of juvenile leaf oils from *E. nitens* and *E. globulus* were higher than the corresponding adult leaves while the oil yields of both juvenile and adult leaves of *E. nitens* were significantly lower than *E. globulus*. A similar composition of *E. nitens* leaf oil has been reported from the Australian mainland and overseas (Yatagai and Takahashi 1983; Franich 1986; Brophy *et al.* 1991). Furthermore, the adult leaf oils of two *E. nitens* provenances, Bendoc (Errinundra) and Penny Saddle (Toorongo), have been compared with *E. globulus* and two *Monocalyptus* species in a provenance trial where genetic differences in oil yield and composition between species and between provenances of *E. nitens* were found (see Chapter 3). However, information on essential oils of *E. nitens* provenances is still lacking and the chemical composition of leaf waxes of *E. nitens* have not been investigated.

This chapter investigates the yields and composition of the leaf oils from both adult and juvenile leaves from twenty populations of *E. nitens* in a provenance trial established to assess provenance variation in growth. At the same time, ten families of *E. nitens* trees grown in two family trials and F1 hybrid between *E. nitens* and *E. globulus* were examined to determine the inheritance of the leaf oils. These leaf oils were then compared to the leaf oils of the 29 Tasmanian *Eucalyptus* spp.. The chemical composition and morphology of the leaf waxes of *E. nitens* were also investigated and compared with those from *E. globulus*.

11.2. Materials and methods

11.2.1. Collection of Leaf samples

1) Experiment 1 - Leaf sample collection from twenty populations of E. nitens

The *E. nitens* provenance trial is located in the Esperance Valley, SE Tasmanian (c. lat. 43^{0}18' S; long. 146^{0}54'; alt. 150m). Planting stock was raised from seed collected from mainland Australia (regions and provenances detailed in Table 11.1 and Fig. 11.1) and seedlings were planted in August 1982 by the Tasmanian Forest Commission. This trial contained materials from 20 provenances, each provenance represented by 5 randomised blocks. In each randomised block each provenance was represented by 9 trees in 3 x 3 randomized plots with 3 x 3m spacing between trees.

Table 11.1. Details of leaf samples from TFC *E. nitens* provenance seedlots at Esperance, Tasmania. (seed origin data - Forestry and Timber Bureau, Canberra, ACT, Australia).

Code	Sample*	Provenance	Region of Provenance	Lat. (S)	Long (E)	Alt. (m)
1	A	Tweed Spur	Rubicon (Vic)	37°21'	145°47'	1000
2	A,J	Blue Range	Rubicon (Vic)	37°23'	145°48'	1000
3	A,J	Mt Torbrek	Rubicon (Vic)	37°22'	145°56'	1220
4	A	Toolangi	Rubicon (Vic)	37°32'	145°34'	610
5	A	Federation Range	Rubicon (Vic)	37°27'	145°57'	1100
6	A	Mt Skene	Macalister (Vic)	37°28'	146°23'	1160
7	A,J	Mt Wellington	Macalister (Vic)	37°27'	146°52'	1200
8	A,J	Connors Plain	Macalister (Vic)	37°32'	146°28'	1310
9	A,J	Mt Kaye	Errinundra (Vic)	37°25	149°07'	1000
10†	A	Bendoc	Errinundra (Vic)	37°12'	148°52'	1070
11†	A	Gunmark Range	Errinundra (Vic)	37°18'	148°51'	1000
12	A,J	Powelltown	Toorongo (Vic)	37°47'	145°49'	900
13	A,J	Toorongo	Toorongo (Vic)	37°49'	146°07'	860
14	A,J	Mt Erica	Toorongo (Vic)	37°54'	146°21'	1080
15	A,J	Noojee	Toorongo (Vic)	37°48'	146°04'	960
16	A,J	Mt St Gwinear	Toorongo (Vic)	37°50'	146°21'	1175
17	A,J	Tallaganda	Southern NSW	35°37'	149°31'	1200
18	A	Badja Mt	Southern NSW	36°01'	149°34'	1250
19	A,J	Anembo Trig	Southern NSW	35°52'	149°30'	1400
20	A	Ebor	Northern NSW	30°40'	150°00'	1460
E. nitens provenance seedlots in Esperance, Tasmania				43°18'	146°54'	150

* A=Adult leaves; J=Juvenile leaves; Lat. = latitude; Long. = longtitude; Alt. = altitude
† These provenances were classified as *E. denticulata* by Cook and Ladiges (1991)

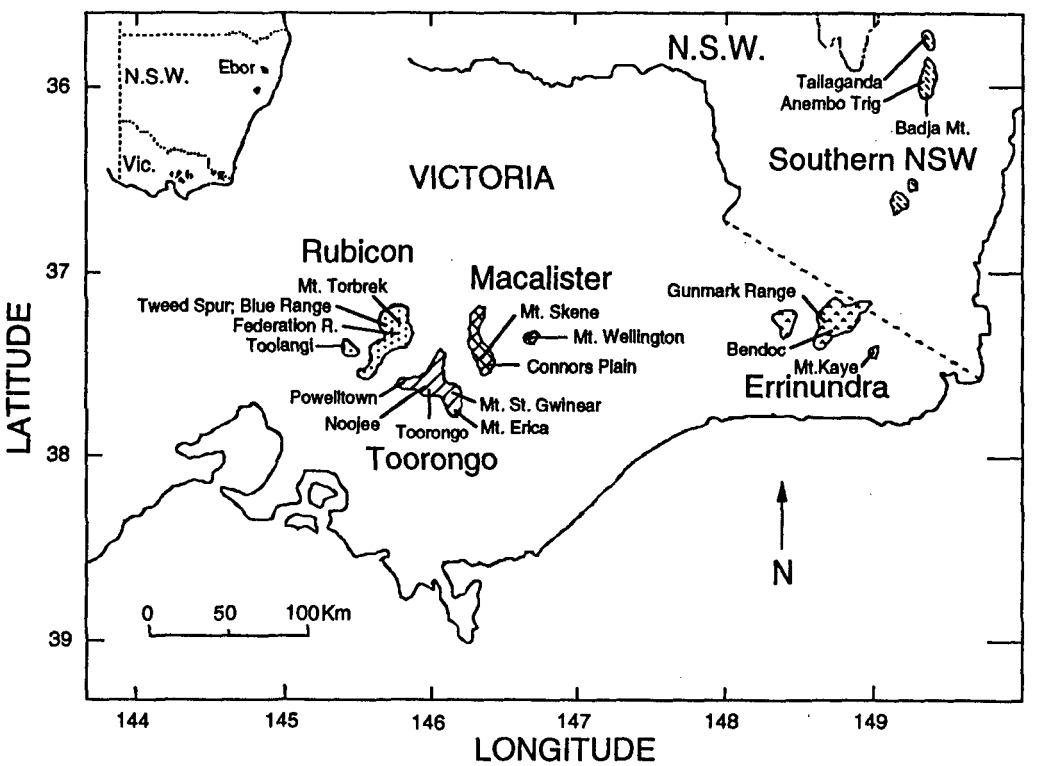


Fig. 11.1. The original locations of the populations sampled from provenance trial.

Branches bearing mature adult leaves of each provenance were collected from two trees in each of five replicate plots and branches bearing juvenile leaves from one tree from each of the five replicate plots in November 1988 (due to a six years growing, trees of some provenances have no juvenile leaves). Fresh leaf samples of each juvenile and adult leaf type of each provenance were mixed and sealed in plastic bags. On return to the laboratory individual leaves were plucked from the upper 30-40 cm of the sample branches, cut into 1-2 cm^2 pieces and 150 g lots taken for oil extraction. Another 150g lot of chopped leaves was prepared for each sample and dried to constant weight at 50 ^0C for determination of moisture content.

2). Experiment 2- Sampling leaves for study of inheritance of leaf oils in ten families of E. nitens at two sites.

E. nitens family trials were established at Huntsman (Site 1) and Burnie (Site 2), Northern Tasmania by APPM in 1986. Trees of different families were planted randomly within each site and the location, soil type and climate of both sites and the original seed collection of these families are listed in Table 11.2A and B. Adult leaves were randomly collected from three trees of each family within each site in November 1990. Fresh leaf samples of each sampled tree were examined individual. The *t*-tests for differences between diferent population regression slopes or elevations were calculated following Zar (1974).

Table 11.2. Site characteristics and original collection of *E. nitens* families.

A. The localities, soil types and climate of two sites.

	Lat. (S)	Long (E)	Alt. (m)	Soil Types
Site 1 (Huntsman)	41^0 39'	146^0 37'	290	Stoney, brown clay loam developed on colluvium/alluvium from predominantly Jurassic dolerite
Site 2 (Round Hill)	42^0 04'	145^0 57'	10	Krasnozem (Oxisol) on Tertiary basalt

	Mean Temperature (^0C)		Mean Rainfall (ml)
	Maximum	Minimum	
Site 1 (Huntsman)	15.8	5.5	90
Site 2 (Round Hill)	16.9	9.2	82

* Climatic data was recorded in 1990 and provided by Associate Paper and Pulp Mill (APPM) Forest Research Unit, Ridgley, Tasmania.

B. The origin of seed of 10 families of E. nitens at used the APPM selection trials at Huntsman (Site 1) and Round Hill (Site 2), Northern Tasmania.

Code of families	Type of collection	Provenances	Location
135	Single tree	Toorongo	Toorongo Pl. Loch V. Rd. Site 2
145	Single tree	Toorongo	Toorongo Pl. Loch V. Rd. Site 3
177	Single tree	Toorongo	Toorongo Plateau
71	Single tree	Rubicon	Con Gap Rd./Snobs Creek
264	Single tree	Toorongo	Mt. Toorongo
136	Single tree	Toorongo	Toorongo Pl. Loch V. Rd. Site 2
283	Single tree	Toorongo	Mt. St. Gwynear
316	Single tree	Toorongo	Mt. Toorongo MMBW Rd.
312	Single tree	Toorongo	Mt. Toorongo MMBW Rd.
340	Single tree	Toorongo	Loch Valley Rd.

3). Experiment 3 - sampling for study of inheritance of leaf oils in interspecific F1 hybrids of E. nitens and E. globulus

Leaf samples used in this study resulted from interspecific controlled pollinations on *E. nitens* and *E. globulus* reported by Tibbits (1988, 1989). The F1 hybrid combination was the cross between open pollinated *E. nitens* family 123 (female) and the *E. globulus* family 281 (female). The parent seed origin are:

	E. nitens family 123	*E. globulus* family 281
Seed collector	L. Pederick No. 123	Tas. F. C.
Latitude	$37^{0}47'$	$43^{0}05'$
Longitude	$146^{0}03'$	$147^{0}46'$
Altitude	850m	200m

Samples of both young and matured leaves from five or four trees of parent families and F1 hybrid were collected at the end of April 1989 from APPM St Georges Rd Hybrid Trial. Fresh leaf samples of young and mature leaves (c.a. 100gm) of each sampled tree were examined individually.

4). Experiment 4 - Collection of leaf samples for the comparison of leaf waxes between E. nitens and E. globulus.

The adult and juvenile leaves of *E. nitens* (origin - Toorongo, Vic.) and *E. globulus* (Tasmania) were collected from 10 individual trees of each species from APPM *E. nitens* and *E. globulus* provenance trials respectively at Ridgley, northern Tasmanian in June 1987. Comparison of leaf oils between *E. nitens* and *E. globulus* using these samples had been published in Li *et al.* (1991).

11.2.2. Extraction and analysis of oil and wax

Methods of oil extraction and analysis were as described in chapter 3. Analysis of wax chemistry and examination of wax morphology were as described in chapter 5. The yields of leaf oils and waxes were calculated on a dry weight basis (g/100g).

11.3. Results

11.3.1. Experiment 1 - Variation in the leaf oils of *E. nitens* from 20 provenances

Yield and composition of leaf oil

The yields and composition of the leaf oils are listed in Table 11.3. The percentage yields of leaf oils of juvenile leaf samples ranged from 0.7-1.5% and adult leaf samples from 0.3-1.1%. The composition of adult leaf oils from different provenances of *E. nitens* varied widely. Leaf oils from 17 provenances from six regions contained the monoterpenoids, 1,8-cineole (30-48%) and α–pinene (14-33%), as major components with variable amounts of the monoterpene hydrocarbons, limonene (1-4%), *cis*–β–ocimene and *p*-cymene (2-13%), the oxygenated monoterpenes, pinocarvone (1-5%), terpinyl acetate (2-5%) and α–terpineol (1-4%), and the sesquiterpene alcohol, spathulenol (0.3-9%) as their minor components (Table 11.3).

In contrast, the adult leaf oils from the two provenances Bendoc and Gunmark Range, which form the Errinundra region and had denticulate adult leaf margins, were characterized by higher percentages of *p*-cymene (29-30%) and γ–terpinene (18-22%) as well as by the presence of low amounts of 1,8-cineole (11-15%) and a very small amounts of α–pinene (0.4-0.5%). These two oils were distinct from the other 18 provenances. At the same time, the adult leaf oil from one provenances, Connors Plain, of the Macalister region, was different from the above provenances. This leaf oil was characterized by 1,8-cineole (27%) as well as by the presence of higher percentages of spathulenol (16%) and *p*-cymene (14%) as minor components and a low percentage of α–pinene (4%). In contrast, the compositions of the juvenile leaves of 13 provenances of were very similar. All juvenile leaf oils contained high percentages of 1,8-cineole (40-56%) and α–pinene (18-38%) as major components.

Table 11.3. The chemical composition of individual locality populations of *E. nitens*.

Locality code	Leaf type *	Oil yield (g/100g DW)	α–Pinene	Isobutyl isobutanoate	α–Phellandrene	Limonene#	1,8-Cineole	cis–β–Ocimene	Terpinene	*p*-Cymene	Isobutyl isovalerate	Pinocarvone	Terpinen-4-ol	Aromadendrene	Alloaromadendrene	α–Terpinyl acetate	α–Terpineol	Bicyclogermacrene	Globulol	Viridiflorol	Spathulenol	Σ Eudesmol **
Rubicon Populations																						
1	A	0.3	14.3	0.1	3.0	2.0	32.0	3.7	0.2	12.4	1.4	1.6	0.4	0.2	0.9	2.5	1.8	3.5	1.9	1.5	6.8	5.0
	J	1.5	23.1	0.7	0.2	4.9	54.6	-	1.5	4.5	2.3	0.5	0.3	0.1	0.1	1.1	2.7	0.2	0.2	0.2	0.6	0.9
2	A	0.4	18.0	0.2	0.3	3.6	36.6	1.0	0.7	8.3	3.7	2.1	0.3	0.1	0.7	2.2	4.0	0.8	0.9	0.5	9.3	3.4
	J	0.7	27.1	0.2	-	5.0	48.1	-	1.4	4.2	2.4	0.8	0.3	0.1	0.3	0.9	2.4	0.2	0.4	0.3	1.2	0.3
3	A	0.4	28.4	0.6	0.5	2.6	33.7	2.2	0.2	5.4	5.3	1.9	0.3	0.2	0.6	3.0	1.9	1.8	0.6	0.4	5.1	1.9
	J	1.0	28.8	0.4	0.1	4.5	48.8	-	0.7	2.8	2.5	0.8	0.1	0.4	0.2	1.6	4.2	0.2	0.3	0.2	0.8	1.1
4	A	0.8	20.6	0.1	0.2	2.8	49.3	2.0	0.1	2.8	1.6	3.3	1.2	1.7	0.3	3.2	2.1	0.6	0.7	0.5	4.4	1.7
5	A	0.6	18.1	-	0.8	2.7	35.4	0.3	2.2	13.6	3.3	2.8	0.9	0.9	0.3	3.3	2.5	0.7	0.9	0.5	2.8	3.1
Macalister Populations																						
7	A	0.4	22.8	0.1	1.3	2.3	35.6	1.3	0.1	7.8	2.8	2.3	0.3	0.2	0.6	3.1	2.0	1.4	1.1	0.7	6.2	4.4
	J	1.3	22.6	0.2	0.1	5.5	46.1	0.1	4.5	5.6	2.5	0.3	0.8	0.2	0.2	0.5	4.7	0.2	0.3	0.2	0.5	1.1
8	A	0.3	4.2	-	0.3	2.2	26.5	1.6	-	13.9	4.5	3.3	0.7	0.3	2.1	5.3	4.7	2.7	3.4	2.2	16.4	2.1
	J	1.1	23.1	0.4	-	4.6	51.3	0.3	2.0	5.9	2.2	0.6	0.7	-	0.1	1.1	4.0	0.1	0.2	0.1	0.4	0.4
6	A	0.5	14.0	0.4	1.3	1.4	41.8	2.6	0.3	6.9	5.0	3.8	0.4	0.4	0.5	4.6	1.4	2.2	1.3	0.9	4.7	2.8
Errinundra Populations																						
9	A	1.1	27.7	0.2	0.3	4.2	47.4	1.2	0.1	1.9	1.6	1.0	0.5	0.2	0.4	1.8	1.7	1.1	0.7	0.5	2.9	1.5
	J	1.1	23.1	0.2	-	4.4	48.9	-	2.3	4.7	2.4	0.8	0.5	0.1	0.2	1.8	3.2	0.1	0.3	0.1	0.4	0.9
10§	A	0.7	0.4	4.6	1.1	0.9	11.3	7.4	22.3	29.6	0.7	0.2	4.3	0.2	0.6	1.2	2.0	0.7	0.4	0.2	2.7	2.0
11§	A	0.3	0.5	8.7	1.4	1.6	14.6	5.6	17.8	30.3	0.5	0.7	3.8	0.3	0.3	0.8	4.2	0.7	0.4	0.1	1.8	2.4
Toorongo Populations																						
12	A	0.7	20.6	0.1	0.2	3.1	39.7	0.1	0.1	4.2	2.7	2.4	0.3	0.2	0.1	3.8	4.8	0.7	1.3	0.8	5.9	4.7
	J	1.2	37.6	0.2	0.1	4.1	41.9	0.1	0.1	0.5	2.3	1.1	0.3	0.1	0.1	1.9	4.8	0.1	0.2	0.1	0.2	0.5
13	A	0.6	21.6	0.2	1.7	1.8	33.6	1.3	0.1	5.4	1.9	4.9	0.2	0.2	0.5	5.0	1.4	2.2	1.7	1.0	5.4	5.8
	J	1.4	24.6	0.5	-	4.9	51.2	-	1.7	3.9	3.1	0.8	0.3	0.1	0.1	1.2	3.6	0.1	0.3	0.2	0.7	1.1
14	A	0.5	13.3	0.2	0.6	1.0	47.2	1.0	0.1	8.1	1.4	3.9	0.2	0.1	0.4	5.3	1.1	1.1	1.1	0.7	5.1	3.3
	J	1.0	21.9	0.3	-	4.5	55.2	-	-	1.1	3.6	1.2	0.5	0.2	0.2	1.6	2.9	0.1	0.3	0.2	0.6	1.2
15	A	0.6	26.1	0.3	0.2	2.5	46.2	1.6	0.1	1.8	2.9	3.5	0.2	0.2	0.3	4.8	2.3	0.5	0.4	0.3	1.8	0.6
	J	1.2	31.2	0.6	-	4.5	49.7	0.1	0.2	0.4	2.0	1.1	0.2	0.2	0.1	1.8	4.4	0.1	0.1	0.1	0.2	0.4
16	A	0.5	26.6	0.2	0.7	3.1	30.7	1.7	0.5	9.7	1.7	1.2	0.4	0.2	0.8	1.5	1.0	2.3	1.0	0.6	8.2	3.3
	J	0.9	26.4	1.2	-	5.2	48.2	-	0.2	0.7	3.0	0.5	0.1	0.3	-	0.6	8.0	0.3	0.1	-	0.5	0.4
Southern NSW Populations																						
17	A	0.6	15.5	-	0.5	1.1	40.4	2.2	-	4.6	2.4	5.1	0.4	0.6	0.8	8.6	1.3	1.4	1.6	1.1	7.1	2.0
	J	1.1	18.4	0.3	0.4	3.7	39.7	0.1	9.5	11.4	2.0	0.5	0.1	1.9	0.2	0.4	3.7	0.5	0.4	0.3	0.8	1.6
19	A	1.1	33.4	0.2	0.2	2.7	39.6	1.1	0.1	2.6	1.1	2.6	2.0	1.2	0.3	3.5	1.4	0.7	0.7	0.5	0.3	4.7
	J	0.9	17.9	0.7	0.2	4.1	56.1	-	1.2	4.3	1.6	1.3	0.4	0.3	0.3	1.9	3.6	0.2	0.4	0.2	1.2	0.9
18	A	0.8	15.4	-	0.4	2.7	48.2	1.1	-	5.0	0.4	2.3	0.3	0.4	0.8	2.3	3.3	1.5	1.5	1.1	8.2	1.5
Northern NSW Populations																						
20	A	0.6	18.1	-	0.4	2.3	45.2	1.4	0.2	5.0	3.8	3.4	1.4	1.3	0.6	3.9	2.5	0.9	1.3	1.0	4.9	0.8

* Leaf types: A = adult; J = juvenile. No juvenile leaves were sampled from locality populations 4, 5, 6, 10, 11, 18 and 20.

\# Mixed with isobutyl isopentanoate but peak was dominated by limonene.

** ΣEudesmol = sum of α–, β– and γ–eudemols.

§ These localities were classified as *E. denticulata* by Cook and Ladiges (1991)

β–Pinene, terpinolene, α–gurjunene, linalool and rosifoliol were present in trace amounts.

Results of the *t*-tests for differences in yields and percentage composition of oils between adult and juvenile foliage from 13 provenances of *E. nitens* are shown in Table 11.4. The results indicated that the oil yields of juvenile leaves were significantly higher than the corresponding adult leaves ($p<0.001$). With the exception of α–pinene, *p*-cymene, isoamyl isovalerate, terpinen-4-ol and aromadendrene, the percentage contents of most components were significantly different between adult and juvenile leaf oils. The juvenile leaf oils contained significantly higher 1,8-cineole (Δ_{J-A} =11.6; $p<0.001$), limonene (Δ_{J-A} = 2.1; $p<0.001$), γ–terpinene (Δ_{J-A} = 1.8; $p<0.05$), and α–terpineol (Δ_{J-A} = 1.7; $p<0.01$) than adult leaf oils.

Table 11.4. Comparisons of the oil yield and percentage content of oil components between juvenile and adult leaves from 13 *E. nitens* provenances#.
(This comparison is confounded by genotype and is not a direct comparison between trees.

Components	Mean Percentage Contents		Differences	*t*-Test Paired	Significance
	Juvenile	Adult	Δ (J-A)	*t*-Value	
Oil yields (%DW)	1.1	0.6	0.5	-4.7	***
α–Pinene	25.1	20.9	4.1	-1.7	ns
Isobutyl isobutanoate	0.5	0.2	0.3	-3.1	**
α–Phellandrene	0.1	0.7	-0.7	3.0	*
Limonene†	4.6	2.5	2.1	-8.0	***
1,8-Cineole	49.2	37.6	11.6	-5.0	***
cis–β–Ocimene	0.1	1.6	-1.6	6.6	***
γ–Terpinene	1.9	0.2	1.8	-2.4	*
p-Cymene	3.9	6.6	-2.8	2.2	ns
Isoamyl isovalerate	2.5	2.6	-0.1	0.8	ns
Pinocarvone	0.8	2.7	-2	5.4	***
Terpinen-4-ol	0.4	0.5	-0.1	1.0	ns
Aromadendrene	0.3	0.3	0	-0.1	ns
Allo-aromadendrene	0.2	0.6	-0.5	3.4	**
Terpinyl acetate	1.2	3.9	-2.7	4.7	***
α–Terpineol	4.0	2.3	1.7	-3.3	**
Bicyclogermacrene	0.2	1.6	-1.4	5.5	***
Globulol	0.3	1.3	-1.0	4.5	***
Viridiflorol	0.2	0.8	-0.7	4.5	***
Spathulenol	0.6	6.2	-5.6	5.0	***
ΣEudesmols	0.8	3.2	-2.4	4.6	***

Comparison made using 13 provenance means for *E. nitens* populations which were sampled for both juvenile and adult leaves (see Table 11.2).
Significance of difference: *** $p<0.001$; ** $0.001<p<0.01$; * $0.01<p<0.05$; ns $p>0.05$.
† Mixed with isobutyl isopentanoate but peak was dominated by limonene.

In contrast, percentage contents of all sesquiterpenoids and several monoterpenoids, α–phellandrene, *cis*–β–ocimene, terpinyl acetate and pinocarvone, were significantly higher in the juvenile leaf oils (see Table 11.4).

Although, this study did not include juvenile leaves from all 20 provenances due to the tree growth stage, the comparison between adult and juvenile leaf samples of the 13 provenances indicated the significant difference between adult and juvenile leaf oils.

Classification and ordination of populations

Variations in the leaf oil components among populations were summarised by Principal Component Analysis (PCA) and the population scores on the first two principal component axes are shown in Fig. 11.2.

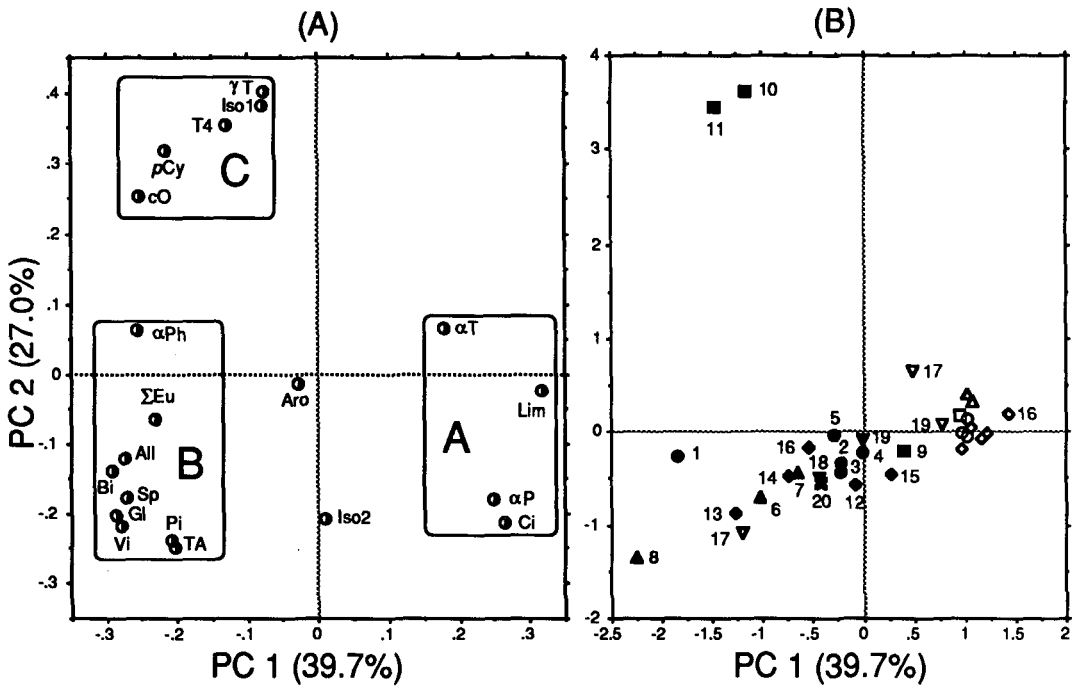


Fig. 11.2. Scatter plot of the oil parameter (A) and locality populations (B) on the axes of the first two principal components (PC1 and PC2).

Lenges: ○ Juvenile and ● adult leaves of Rubicon; △ Juvenile and ▲ adult leaves of Macalister;
□ Juvenile and ■ adult leaves of Errinundra; ◇ Juvenile and ◆ adult leaves of Toorogo;
▽ Juvenile and ▼ adult leaves of Southern NSW; ✖ Adult leaves of Northern NSW.

Abbreviations: αP = α–pinene; Isol = isobutanoate; αPh = α–phellandrene; Lim = limonene; Ci = 1,8-cineole; γT = γ–terpinene; Cy = *p*–cymene; Iso2 = isoamyl isovalerate; Pi = pinocarvone; T4 = terpinen–4-ol; Aro = aromadendrene; All = alloaromadendrene; TA = α–terpinyl; Bi = bicyclogermacrene; Gl = globulol; Vi = viridiflorol; Sp = spathulenol; ΣE = sum of α–, β– and γ–eudesmols.

The ordination of components (Fig. 11.2a) indicates that variation along the major axis of variation PC1 (38%) is mainly due to variation in group A compounds in contrast to variation of group B and C compounds. Variation along PC2 (22%) is mainly due to increasing levels of group C compounds. The group A compounds included 1,8-cineole, α– and β–pinene, limonene and α–terpineol and group B consisted of mainly sesquiterpenoids while the monoterpenoids *p*-cymene, γ–terpinene, terpinen-4-ol, *cis*–β–ocimene and terpinolene and ester compounds, isobutyl isobutanoate, formed group

C. As indicated in Fig. 11.2B, the major axis of variation (PC1) clearly separated the juvenile and adult leaves of *E. nitens*. The juvenile leaves have higher levels of monoterpenoids of group A and lower contents of components of the other two groups than did adult leaves. There was also continuous variation in oil components among adult leaves from the 17 provenances of the five regions, Rubicon, Macalister, Toorongo, Southern and Northern NSW and the Mt. Kaye provenances on the Errinundra Plateau along the first axis (PC1). The Mt. Kaye (9a) and Noojee (15a) provenances have the highest level of group A compounds and Tweed Spur (1a) and Connors Plain (8a) the lowest. However, there was no clear separation between regions. There was marked variation in the oil components of adult leaves between provenances of the Errinundra region. The second axis of variation (PC2) separated the Bendoc (10a) and Gunmark Range (11a) from the Mt. Kaye (8a) provenance of Errinundra and the 17 other provenances. The Bendoc and Gunmark Range provenances had high levels of group C components and low levels of group A and B compounds.

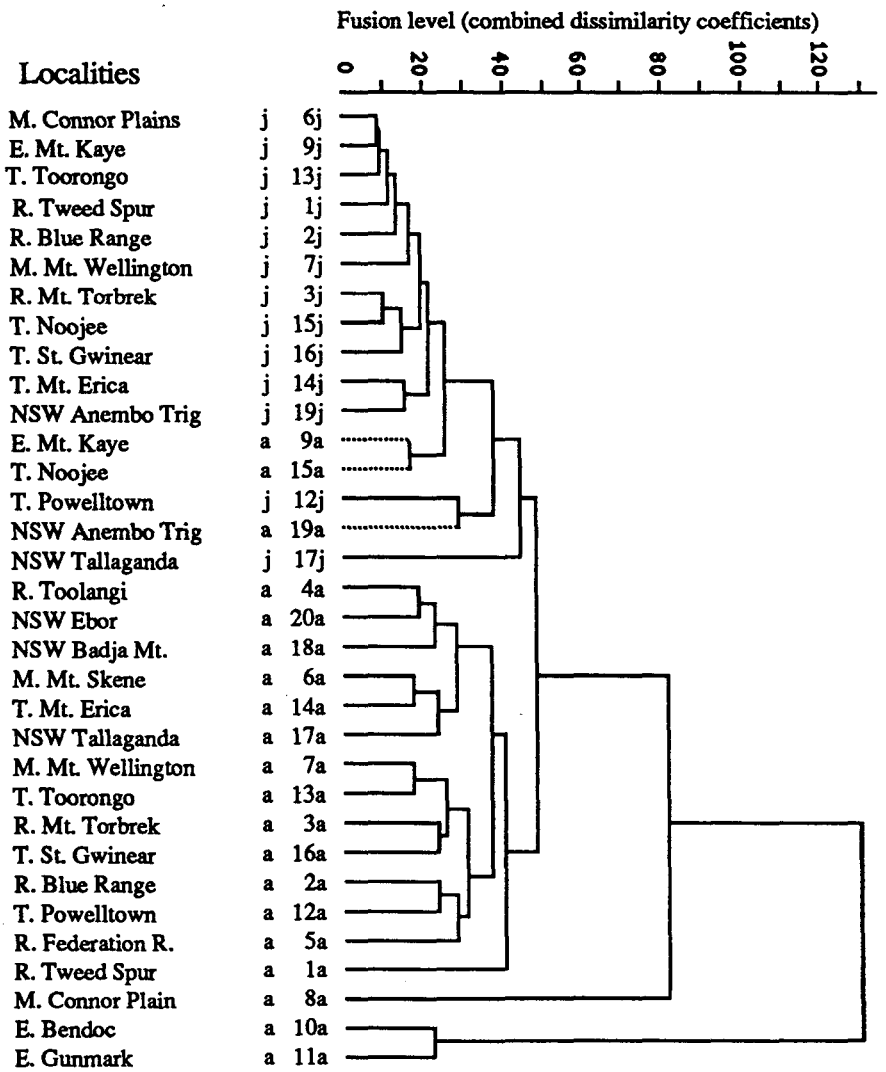


Fig. 11.3. Average linkage clustering of *E. nitens* populations based on leaf oil components.

The average linkage clustering resulting from the Manhattan distance matrix (Fig. 11.3) confirmed the separation of populations. The major dichotomy in the dendrogram is between the adult leaves of Bendoc and Gunmark Range of Errinundra and all other provenances. The adult leaves of the Connors Plain provenance from Macalister is also clearly an outlier to the core populations of both juvenile and adult leaves. Within the core populations, adult and juvenile leaf samples are relatively well separated (Fig. 11.3), although the variation is continuous (Fig. 11.2b) and the adult leaves of Mt. Kaye, Noojee and Anembo Trig provenances have an oil composition most similar to the juvenile leaves. The pattern of chemical variations among core populations can be summarised as having high levels of 1,8-cineole and α–pinene and continuous variation with reducing amounts of these compounds and increasing in the content of group B and C compounds as indicated in Fig. 11.2a.

Comparison of the leaf oil of the 20 E. nitens provenances and Tasmanian eucalypts

The overall variation in oil composition of both adult and juvenile leaves of the *E. nitens* populations and all populations of the 29 Tasmanian eucalypt species were summarised by the Principal Component Analysis (PCA) as shown in Fig. 11.4 (a). The two first PCs accounted for 29.8% and 12.1% of total variance of the oil components among populations indicating that all *E. nitens* populations were closely grouped with the main populations groups of the *Symphyomyrtus* species and clearly distinguished from the *Monocalyptus* species while few of the adult leaf samples tended to be outlying. Furthermore, the overall variation in oil composition of both adult and juvenile leaves of the *E. nitens* populations and all Tasmanian *Symphyomyrtus* populations were summarised by the PCA as shown in Fig. 11.4 (b). The two first PCs accounted for 34.6% and 10.9% of total variance of the oil components among these populations indicating that there was a continuous variation among the *E. nitens* populations while all adult leaf samples which were characterised by the entire adult leaf form were separated from the main population group of the *Symphyomyrtus*. The corresponding juvenile leaf samples were grouped together with the main population group. However, the adult leaf samples of the Bendoc and Gunmark Range provenances of the Errinundra Plateau were clearly distinguished from the other populations of *E. nitens* and the main population group of the *Symphyomyrtus*. This indicated that the adult leaf oil of the *E. nitens* of both entire and denticulate adult leaf forms were not like the majority of populations of *Symphyomyrtus* species in Tasmania due to the decrease in levels of 1,8-cineole and related compounds.

As described previously, the oil yields of *E. nitens* populations were in general low, 0.7-1.5% for juvenile leaves and 0.3-1.1% for adult leaves. In order to compare the absolute

contents of monoterpenoid compounds in *E. nitens* leaves with the Tasmanian eucalypts, the absolute content data of the major monoterpenoids (calculated from the percentage oil yield and ratio of individual compounds in oil) of *E. nitens* populations together with the absolute content data of all the Tasmanian eucalypts were subjected to PCA analysis. As indicated in Fig. 11.5, the two first PCs accounted for 54.4% and 19.3% of the total variance of the major monoterpenoids among the populations. Variation along the major axis of variation PC1 is mainly due to variation in absolute amounts of 1,8-cineole, α–pinene, limonene, terpinyl acetate and α–terpineol and along the second axis variation PC2 is mainly due to variation in α– and β–phellandrene, *cis*- and *trans*-*p*-menth-2-en-1-ol, *cis*- and *trans*-piperitol, piperitone, *p*-cymene, terpinolene and terpinen-4-ol.

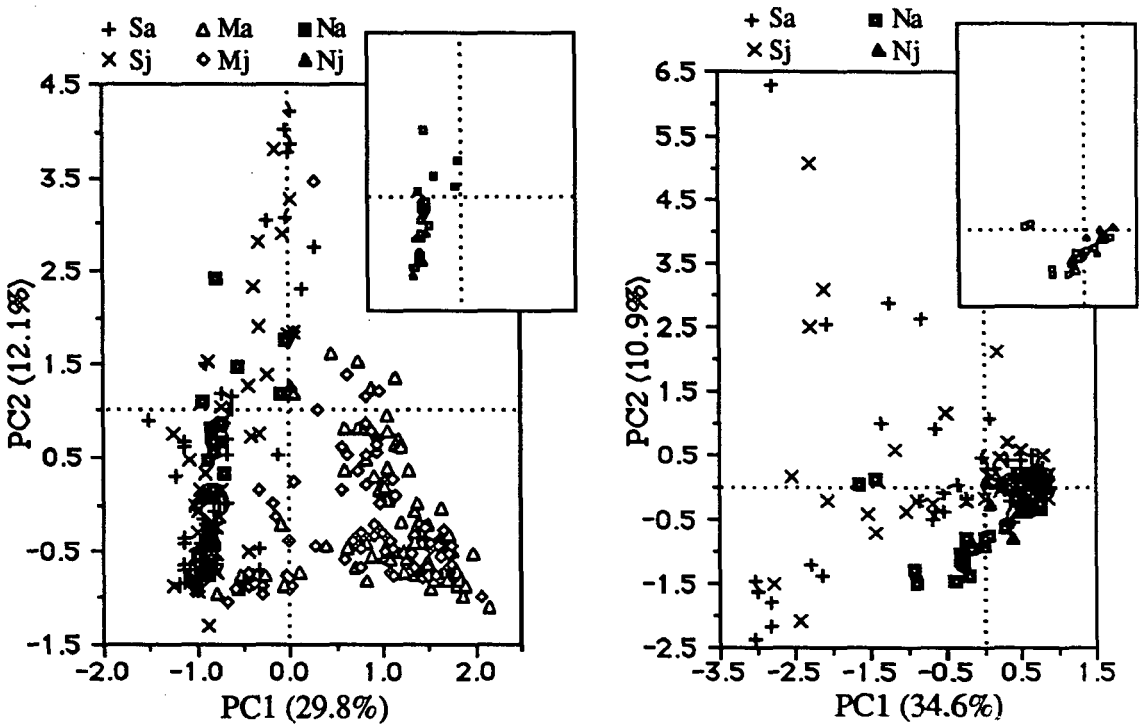


Fig. 11.4. Comparison of the chemical composition in leaf oils of *E. nitens* populations and populations of Tasmanian eucalypts by the PCA.

(a) Left figure is the scatter plot of the population scores on the first two PCs of PCA for all populations of 29 Tasmanian eucalypt species and *E. nitens* populations.
(b) Right figure is the scatter plot of the population scores on the first two PCs of PCA for all populations of *Symphyomyrtus* species and *E. nitens* populations.

Plots of both adult and juvenile leaf samples of all Tasmanian populations and *E. nitens* populations indicated that there were two continuous variations in absolute amounts amongst leaf samples. That is one of the variations in populations of Tasmanian *Monocalyptus* species along the major axis increased with α– and β–phellandrene and

related compounds and the other variation in populations of mainly Tasmanian *Symphyomyrtus* species and *E. nitens* occurred along the second axis with increases in 1,8-cineole and related compounds. This plot indicates that the adult and juvenile leaf samples of all *E. nitens* provenances were within the variation of *Symphyomyrtus* species populations but not *Monocalyptus*. Thus, variation in absolute amounts of the major monoterpenoids of both adult and juvenile leaves of the *E. nitens* showed no affinities with *Monocalyptus* species. However, as indicated in the extracting plot of *E. nitens* scores (upper right corner), the sample scores of the *E. nitens* populations were located at the point of lowest variation of increased 1,8-cineole and related compounds. That means that the absolute amounts of 1,8-cineole and related compounds in all *E. nitens* leaves were very low when compared with the Tasmanian *Symphyomyrtus* populations.

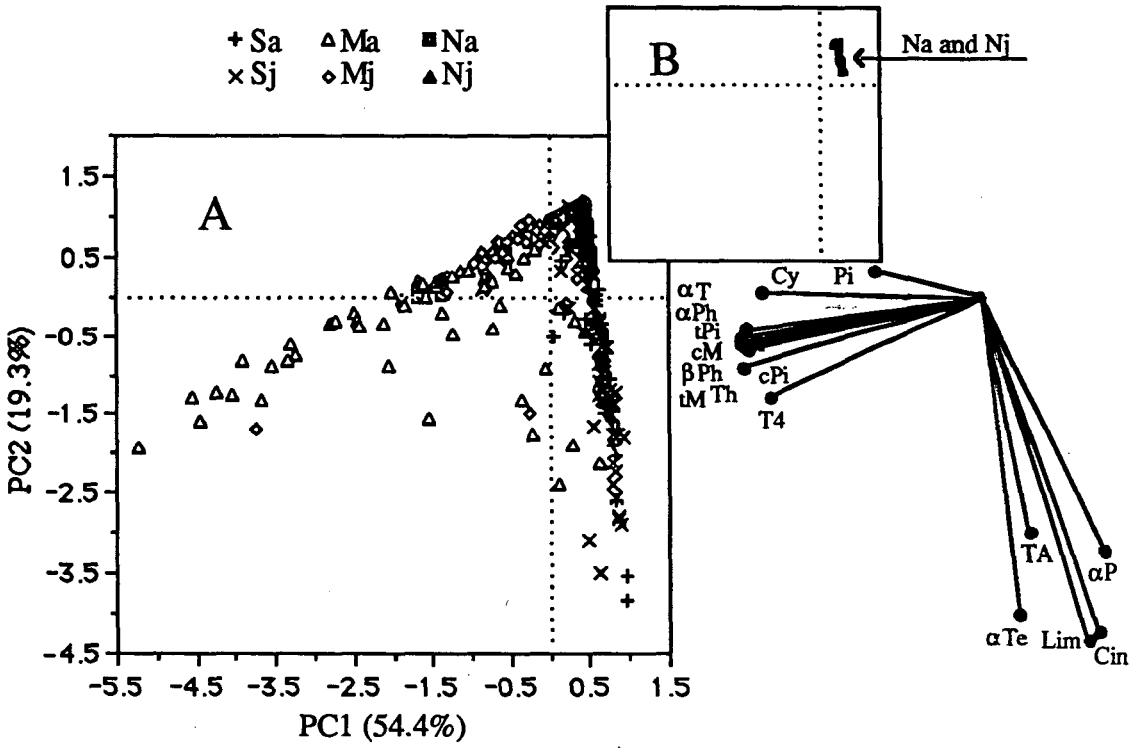


Fig. 11.5. (A) Comparison of the absolute contents of the major monoterpenoids of *E. nitens* and all populations of the 29 Tasmanian eucalypt species by the PCA; (B) The actual scores location of *E. nitens* populations within the cluster.

Sa = Adult leaves of *Symphyomyrtus* species; Sj = Juvenile leaves of *Symphyomyrtus* species; Ma = Adult leaves of *Monocalyptus* species; Mj = Juvenile leaves of *Monocalyptus* species; Na = Adult leaves of *E. nitens*; Nj = Juvenile leaves of *E. nitens*.

11.3.2. Comparison of the leaf oils of 10 *E. nitens* families between sites

The oil yield and chemical composition of 10 families of *E. nitens* at two different sites are given in Appendix 11.1. The mean value of oil yields for the 10 families of *E. nitens* at site 1 ranged from 0.6 to 0.9 percent and for those at site 2 from 0.6 to 1.4 percent. The two way ANOVA (Table 11.5) indicated that the oil yields were highly variable between sites ($p<0.001$). However, there was no significant variation in the oil yield between families and in the site by family interaction. This analysis thus indicated that the effect of site on oil yield was significant, since both of the family main effect and the site by family interaction were not significant. Moreover, the t-test (Table 11.5), based on family means, indicated that the mean values of oil yield at site 2 were significantly higher than those at site 1 ($\Delta_{S1\text{-}S2} = -0.24$; $p<0.001$).

In contrast, the percentage composition of leaf oils varied significantly between families of *E. nitens*. As shown in Table 11.5, all leaf oil chemicals varied significantly between families (see Table 11.5 for significance level of individual compounds). There were only five monoterpenoids, β–pinene, 1,8-cineole, *p*-cymene, α–terpineol and terpinyl acetate that varied significantly between sites, whereas, all sesquiterpenoids varied significantly between sites, with the exception of *allo*-aromadendrene. In the comparison between sites and families, the variation between families was much greater than variation between sites. However, all compounds, with the exception of *p*-cymene, which varied significantly between sites showed no significant variation for the site by family interaction. This confirmed the independent effect of site on these compounds.

In addition, the analysis indicated that both the site and family effect acted independently on oil composition rather than in combination, since all compounds showed no significance in the site by family interaction effect, with the exception of *p*-cymene, *cis*–β–ocimene and terpinolene. The significant family effect indicated that the difference in oil composition among these ten families of *E. nitens* was genetically inherited, and these differences were maintained at each site.

The t-test based on family means of individual compounds indicated that the two compounds, 1,8-cineole and α–terpinyl acetate were significantly higher at site 2 than at site 1 ($\Delta_{S1\text{-}S2} = -4.7$ and $\Delta_{S1\text{-}S2} = -0.65$, $p<0.05$ respectively) and that sesquiterpenoids were significantly higher at site 1 than at site 2 (see Table 11.5 for details). Thus, the difference in the principal compound of *E. nitens* leaf oil, 1,8-cineole, between sites followed positively the site differences in oil yield, since the oil yields were also significantly higher at site 2.

Table 11.5. Analysis of variance of oil yield (g/100g dried leaves) and percentage composition (% in oil) of leaf oil between sites and families and the t-test for difference between sites.

	Analysis of variance						Test for difference between site				
Source of Variance	Between sites		Between families		Interaction		(based on provenance means)				
Degree of freedom	1		9		9		d.f.=9				
Model error	40		40		40		Site 1	Site 2	ΔS1-S2	Pair t-Value	Sign.
	F-Value	Sign.	F-Value	Sign.	F-Value	Sign.					
Oil yields	11.240	**	1.257	ns	0.478	ns	0.71	0.95	-0.24	-4.3	**
α–Pinene	3.220	ns	4.022	**	1.186	ns	17.93	20.73	-2.8	-1.7	ns
Isobutyl isobutanoate	0.221	ns	4.126	***	0.132	ns	0.55	0.46	0.09	0.8	ns
β–Pinene	5.982	*	3.434	**	1.684	ns	0.72	0.52	0.21	1.9	ns
α–Phellandrene	3.793	ns	3.584	**	0.980	ns	3.14	3.6	-0.46	-1.9	ns
Isobutyl isopentanoate	0.672	ns	4.547	***	0.281	ns	0.59	0.8	-0.21	-1.5	ns
Limonene	1.903	ns	2.723	*	0.544	ns	0.16	0.11	0.05	1.9	ns
1,8-Cineole	7.94	**	2.719	*	1.098	ns	30.45	35.16	-4.71	-2.7	*
cis–β–Ocimene	3.707	ns	7.054	***	2.138	*	0.73	1.34	-0.62	-1.3	ns
γ–Terpinene	0.856	ns	5.140	***	0.999	ns	2.36	2.91	-0.55	-1	ns
trans–β–Ocimene	0.013	ns	2.485	*	1.670	ns	0.46	0.45	0	0.1	ns
p-Cymene	4.768	*	6.213	***	2.182	*	8.42	6.28	2.14	1.5	ns
Terpinolene	1.921	ns	5.892	***	2.873	*	0.2	0.18	0.03	0.9	ns
Isobutyl isovalerate	0.041	ns	6.144	***	0.443	ns	2.06	2.11	-0.05	-0.4	ns
Linalool	1.304	ns	2.288	*	0.809	ns	0.29	0.21	0.09	-0.7	ns
Pinocarvone	0.095	ns	2.661	*	1.001	ns	1.11	1.25	-0.15	-0.4	ns
Terpinen-4-ol	0.001	ns	8.206	***	1.296	ns	0.55	0.56	0	0	ns
Aromadendrene	14.41	***	5.083	***	0.775	ns	0.55	0.39	0.17	4	**
Allo-aromadendrene	0.324	ns	6.497	***	0.440	ns	2.03	1.86	0.17	0.9	ns
Terpinyl acetate	7.016	*	10.610	***	0.892	ns	3.14	3.8	-0.65	-2.8	*
α–Terpineol	6.813	*	7.244	***	1.146	ns	0.86	0.58	0.28	2.5	*
Globulol	12.6	**	2.614	*	1.62	ns	1.54	0.8	0.74	2.7	*
Spathulenol	5.256	*	7.137	***	0.702	ns	5.14	3.71	1.43	3.5	**
α–Eudesmol	6.469	*	2.691	*	0.702	ns	1.22	0.87	0.35	2.7	*
γ–Eudesmol	5.407	*	2.796	*	0.815	ns	0.73	0.53	0.2	2	*
β–Eudesmol	5.038	*	2.915	**	1.286	ns	0.84	0.58	0.25	-0.6	ns

The linear regression of the family mean values of oil yield and tree diameter at breast height over bark (DBHob) between sites (Fig. 11.6A and B) were significant ($p<0.01$), indicating that both oil yield and volume growth of the *E. nitens* families are highly inherited in the different sites. Moreover, the linear regression between oil yield and DBHob among families (Fig. 11.6C) is significant for site 1 ($p<0.01$) and site 2 ($p<0.05$). Again, the *t*-test for comparing the two regression equations indicated that the two populations slopes were not significantly different while their elevations differed significantly ($p<0.01$).

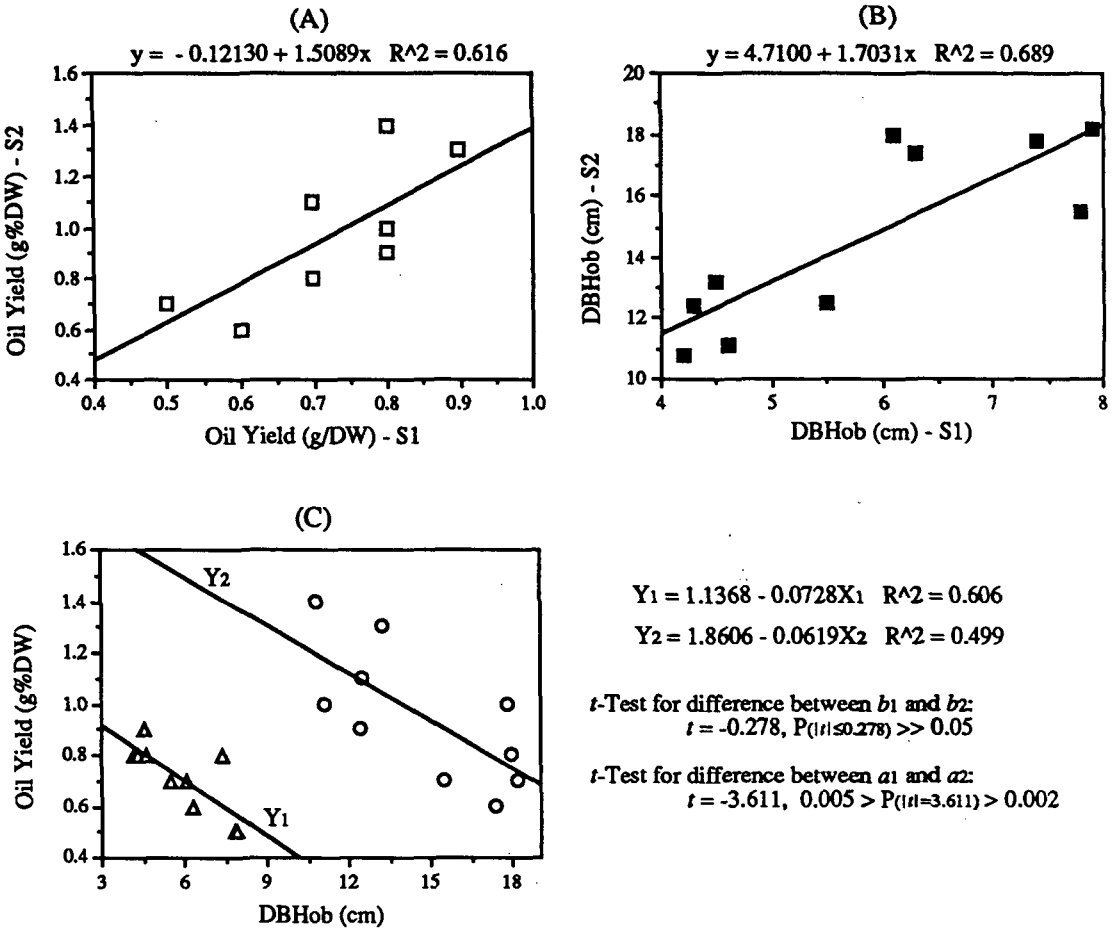


Fig.11.6. Linear regression of mean oil yield and tree diameter at breast heigh over bark (DBHod) of *E. nitens* families between sites.

(A) Mean oil yield of individaul families in site 1 plotted against site 2; (B) Mean DBHob of individual families in site 1 plotted against site 2; (C) Mean oil yield plotted against DBHob in site 1 (Y_1) and site 2 (Y_2) and *t*-test for differences between two population slopees (b_1 and b_2) and between two elevations (a_1 and a_2).

Overall, the data analysis has indicated that the chemical composition of the leaf oil of *E. nitens* varied significantly between families and showed strong inheritance at different sites, whereas, the site effect on the oil composition was poor when compared to the family effect. Site was a significant factor affecting the oil yield where it influenced the quantity of the major compound, 1,8-cineole. Therefore, variation in leaf oils of the ten families of *E. nitens* at two sites can be summarised as follow - 1) oil composition and oil yield of *E. nitens* were strongly inherited, 2) the site had a quantitative affect on oil yield and amounts of major compounds and 3) the oil yield of families was negatively correlated with the diameter growth.

11.3.3. Inheritance of leaf oils in interspecific F1 hybrids of *E. nitens* and *E. globulus*

The leaf oil yields and chemical compositions of individual trees of parent families and their F1 hybrid are listed in Appendix 11.2 and the principal components using percentage content data and absolute content data are given in Fig. 11.7A and B.

Both young and mature leaf samples of all hybrid trees were intermediate concerning their parental controls, as determined by separate discriminate function of PCA analysis (Fig. 11.7A and B). While the PCA using percentage composition data indicated that the young and mature leaf samples of the *E. nitens* parents was clearly separated and differed from the *E. globulus* parents, the F1 hybrids had closer chemical composition to the *E. globulus* parents with some samples overlapping. However, the PCA using absolute content data, which comprised of both qualitative and quantitative factors, clearly separated F1 hybrid samples from their parent controls and indicated that F1 hybrid samples were closer to the *E. nitens* parents. Thus, although the leaf oils of F1 hybrids were similar to *E. globulus* in chemical composition, the quantities of the principal oil components in the F1 hybrid were closer to *E. nitens*.

Detailed tests (Table 11.6) for assessing inheritances of F1 hybrid oil traits indicated that the oil yields and the absolute contents of most oil compounds were significantly difference between parent controls while these characteristics in their F1 hybrid were generally intermediate, with the exception of few compounds. However, the inheritance of leaf oils of F1 hybrids differed between young and mature leaf samples. The mean oil yield of F1 hybrid young leaves differed significantly from the midparent value of parent controls and was closer to *E. nitens* while that of mature leaves did not. The amounts of the major monoterpenoid, 1,8-cineole, and related oil components α–pinene, limonene, α–terpineol of both young and mature leaves of F1 hybrid did not differ from the midparent values with the exception of α–pinene. The amount of α–pinene in young leaves of F1 hybrid differed significantly from midparent value and tended toward *E. nitens*. The amounts of the major sesquiterpenoid, globulol, in young leaves of F1 hybrid were out of the range of parent values and that in mature leaves was similar to the *E. nitens* parent while aromadendrene was similar to *E. nitens* for both young and mature leaves.

Table 11.6. Mean oil yield (%), oil composition (%) and absolute contents of oil compounds (g/10g D.W) for parental control *E. nitens* (Nit) and *E. globulus* (Glo) and their F1 hybrid.

The significance of the difference between parental species and the difference of the F1 hybrid mean from the midparent value (mp) is indicated . Where the parental species differ significantly in the absolute contents of compounds, the degree of dominance (D.D.) of the inheritance of the F1 hybrid has been calculated (0 = no dominace, +1 = complete dominance toward the *E. nitens*, -1 = complete dominance toward the *E. globulus*. (ns P>0.05, * p<0.05 ** p<0.01 *** p<0.001)

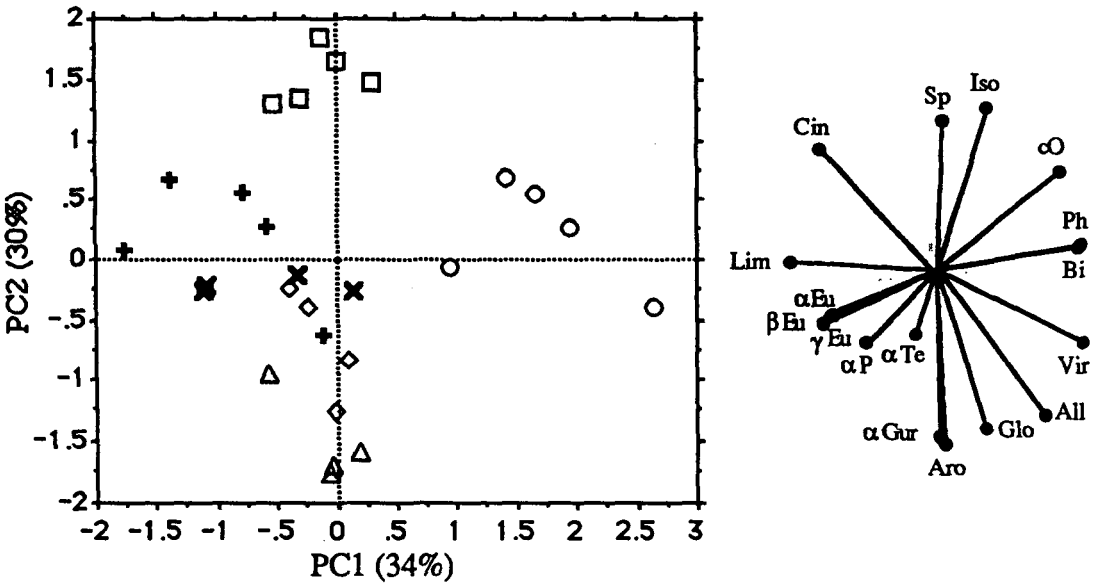
(A) Oil yield and oil compositions

Compounds	Young leaves						Mature leaves					
	Nit	F1	Glo	Difference between parents	Difference between F1 vs mp	D.D.	Nit.	F1	Glo.	Difference between parents	Difference between F1 vs mp	D.D.
Oil Yield	0.62	1.12	3.03	***	***	0.59	0.76	1.64	3.20	***	ns	0.28
α–Pinene	16.76	23.94	28.62	**	ns	-0.21	22.01	22.97	20.68	ns	ns	-
α–Phellandrene	7.27	0.43	0.10	***	***	-0.91	0.62	1.74	0.00	*	ns	#
Limonene	3.36	5.97	4.72	*	***	#	4.86	5.67	4.97	ns	**	#
1,8-Cineole	29.20	46.37	28.09	ns	**	#	46.41	41.13	39.72	**	ns	-0.58
cis-β–Ocimene	5.24	0.33	0.41	**	***	#	3.38	0.34	0.24	*	***	-0.94
Isopentyl isopentanoate	1.26	0.39	0.09	**	*	-0.49	2.24	0.34	0.09	***	**	-0.77
α–Gurjunene	0.49	0.76	2.10	***	ns	0.67	0.06	0.91	0.78	ns	ns	-
Aromadendrene	1.15	1.02	5.73	***	**	#	0.17	1.35	4.15	**	**	#
Allo-aromadendrene	1.13	0.43	1.15	ns	**	#	0.28	0.65	1.05	**	ns	#
α–Terpineol	5.71	6.08	6.73	ns	ns	-	5.37	6.58	4.68	ns	*	#
Bicyclogermacrene	7.94	0.72	0.15	***	***	-0.86	1.14	2.72	0.11	*	*	#
Globulol	3.95	1.60	6.86	***	**	#	0.60	2.05	8.04	***	**	0.61
Viridiflorol	2.17	0.51	1.03	**	**	#	0.39	1.06	1.31	*	**	#
Spathulenol	0.53	0.47	0.28	**	ns	0.52	1.79	0.21	0.61	**	***	#
γ–Eudesmol	0.30	0.96	0.79	*	ns	#	0.28	1.30	0.68	*	ns	#
α–Eudesmol	0.26	1.21	1.04	ns	ns	-	0.41	1.45	1.16	*	ns	#
β–Eudesmol	0.38	1.35	0.97	ns	ns	-	0.22	1.61	1.06	*	ns	#

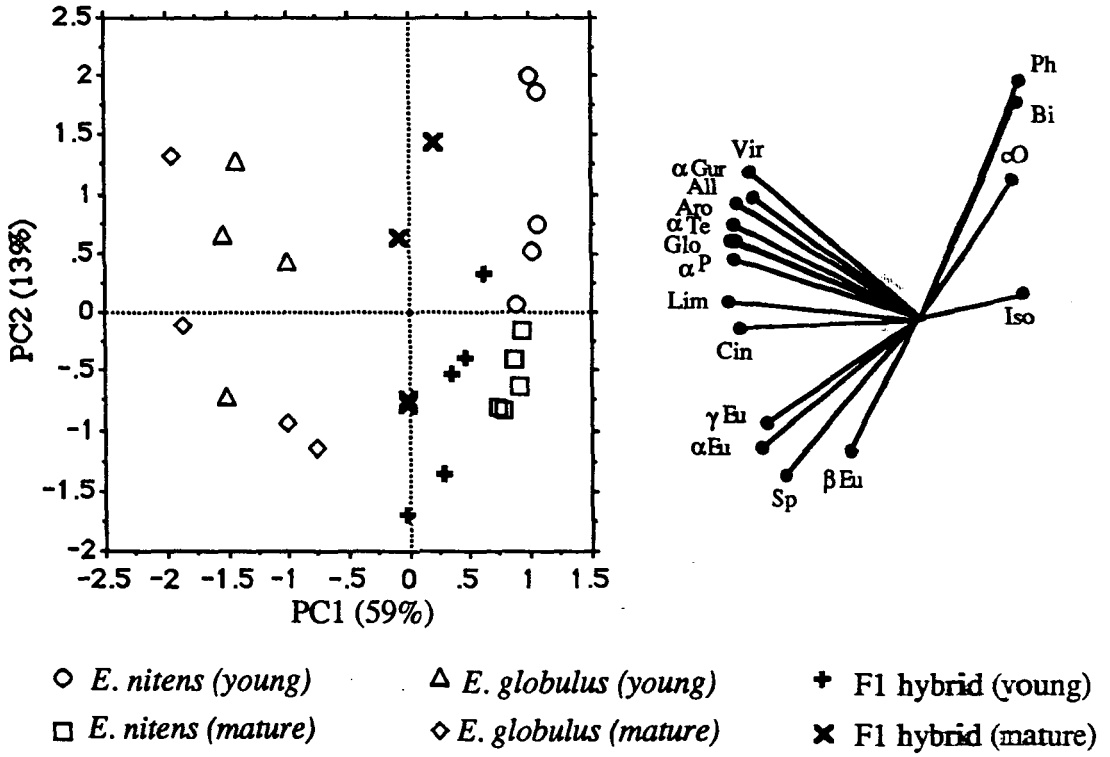
(b) Absolute contents (g/10kg dried leaf weight).

Compounds	Young leaves						Mature leaves					
	Nit	F1	Glo	Difference between parents	Difference between F1 vs mp	D.D.	Nit	F1	Glo	Difference between parents	Difference between F1 vs mp	D.D.
α–Pinene	10.43	25.42	87.61	***	**	0.61	16.86	38.57	66.21	**	ns	0.12
α–Phellandrene	4.79	0.96	0.30	**	*	-0.71	0.44	2.87	0.00	*	*	#
Limonene	2.07	6.71	14.23	***	ns	0.24	3.75	9.36	16.03	**	ns	0.09
1,8-Cineole	18.07	53.23	85.00	***	ns	-0.04	35.36	67.42	126.55	***	ns	0.3
cis-β–Ocimene	3.23	0.40	1.15	ns	***	#	2.62	0.59	0.78	ns	*	#
Isopentyl isopentanoate	0.84	0.45	0.26	ns	ns	-	1.79	0.62	0.27	*	ns	-0.54
α–Gurjunene	0.29	0.65	6.28	***	***	0.88	0.05	1.62	2.81	ns	ns	-
Aromadendrene	0.68	0.91	17.25	***	***	0.97	0.12	2.30	13.72	**	**	0.75
Allo-aromadendrene	0.69	0.44	3.44	***	***	#	0.20	1.09	3.41	**	*	0.45
α–Terpineol	3.51	7.07	20.33	***	***	0.58	4.02	10.72	15.21	**	ns	-0.2
Bicyclogermacrene	4.96	1.16	0.46	***	ns	-0.69	0.81	4.33	0.34	ns	*	#
Globulol	2.44	1.63	20.78	***	***	#	0.43	3.44	26.03	***	***	0.77
Viridiflorol	1.35	0.59	3.10	**	***	#	0.27	1.74	4.29	**	ns	0.27
Spathulenol	0.32	0.52	0.85	*	ns	0.25	1.32	0.35	1.76	ns	***	#
γ–Eudesmol	0.21	1.17	2.35	**	ns	0.1	0.22	1.88	2.12	**	ns	-0.75
α–Eudesmol	0.16	1.44	3.11	**	ns	0.13	0.31	2.09	3.48	***	ns	-0.12
β–Eudesmol	0.22	1.64	2.88	**	ns	-0.68	0.16	2.32	3.04	*	ns	-0.5

A. Oil composition:



B. Absolute contents of compounds:



○ *E. nitens (young)* △ *E. globulus (young)* + F1 hybrid (young)

□ *E. nitens (mature)* ◇ *E. globulus (mature)* × F1 hybrid (mature)

Fig. 11.7. Directions of the terpenoid parameters on the first two principal components (PC1 and PC2) and scatter plot of the young and mature leaf samples of the parental control and F1 hybrid.

11.3.4. The leaf waxes of *E. nitens*

The wax chemistry and morphology of adult and juvenile leaves of seven provenances and the adult leaves of another three provenances of *E. nitens* were examined. The wax yield, the percentage ratio of the major wax compound classes of these waxes are shown in Table 11.7 and the percentage compositions of the these waxes in Appendix 11.3. As indicated in Table 11.7, the hexane-soluble waxes of the *E. nitens* provenances were qualitatively similar in the percentage distribution of different classes of chemical compound. Thus, the leaf waxes were characterised by a high percentage of ß-diketones as the major compounds (57% to 76%), the presence of alkanes (3% to 10%), alkanals (3%t to 11%) and flavonoids (2% to 9%). Free n-alkanols, triterpenoids and long chain esters occurred in very low amounts (<2.5%). Aromatic esters were identified from these waxes, however, they were included in the unidentified category as some peaks of these aromatic esters were obscured by other compound peaks (detailed information of these aromatic esters see Appendix 11.3).

The distributions of the homologues of individual classes of compounds for these leaf waxes were also similar (Appendix 11.3). The major diketones were the homologues, C_{29}, C_{31} and C_{33} and the C_{27} and C_{29} homologues were the major constituent of alkanes while the alkanals were dominated by the C_{26}, C_{28} and C_{30} homologues. The flavonoids identified from these waxes were the common compounds eucalyptin and demethyl eucalyptin of eucalypt leaf waxes.

Although the adult leaves of the Bendoc and Gunmark Range provenances of the Errinundra region, had leaf oils distinctly different from the other provenances, the chemical composition of leaf waxes was qualitatively similar.

The wax yield of adult leaves of the seven *E. nitens* provenances were significantly ($\Delta_{A-J} = -0.3$; $p<0.001$) lower than their corresponding juvenile leaves (Table 11.7). The t-test indicated that the percentage ratio of the ß-diketones was significantly higher ($\Delta_{A-J} = -5.26$; $p<0.001$) in juvenile waxes than that in adult waxes while the percentage ratios of the alkanes were significantly higher ($\Delta_{A-J} = 2.48$; $p<0.05$) in adult waxes.

To make a detailed comparison of the hexane soluble leaf waxes between *E. nitens* and *E. globulus*, both adult and juvenile leaf waxes of the *E. nitens* and *E. globulus* were subjected to column chromatography and the individual fractions of each wax were analysed.

Table 11.7. The yield and percentage ratio of the major compound classes of leaf waxes from *E. nitens* populations. A=adult, J=juvenile; ns $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$. (t-test for difference between adult and juvenile leaves do not include the three localities which had only adult leaves; that are Bendoc, Ebor and Gunmark Range).

Localities	Leaf type	Yield	Alkanes	Aldehydes	Alcohols	Diketones	Flavonoids	Triterpenoids	Long chain esters	Aromatic esters+ un
Tweed Spur	A	0.68	5.66	4.14	0.49	68.43	6.94	0.96	1.13	12.25
	J	0.76	3.38	5.14	0	75.34	1.78	0.25	1.17	12.94
Mt. Torbrek	A	0.76	8.86	9.04	0.43	65.87	5.03	1.23	1.1	8.44
	J	0.68	6.96	3.36	0.19	76.34	4.24	0.85	0.71	7.35
Mt. Wellington	A	0.59	9.37	7.8	0.73	58.36	8.37	2.35	1.11	11.91
	J	0.86	10.46	7.84	0.25	66.77	6.5	0.34	1.26	6.58
Connor Plain	A	0.68	7.18	5.84	1.09	67.64	5.82	0.34	0.06	12.03
	J	1.32	5.05	2.98	0.49	75.63	3.4	0.65	0.76	11.04
Tootogo	A	0.93	8.04	4.65	0.12	64.55	9.3	0.63	0.75	11.96
	J	0.86	3.73	6.4	0	75.8	3.93	1.3	1.92	6.92
St. Gwinear	A	1.08	6.4	4.86	0.71	71.56	4.23	1.35	0	10.89
	J	0.96	4.16	4.12	0	74.27	6.39	0.5	1.02	9.54
Tallaganda	A	0.82	3.64	7.43	1.45	69.56	5.6	0.43	0.46	11.43
	J	1.12	3.47	4.74	0.13	84.17	2.06	1.23	1.39	2.81
Ebor	A	0.68	6.15	10.49	0.92	60.08	5.38	0.53	0.89	15.56
Bendok	A	0.76	9.22	8.1	0.5	56.57	9.48	2.36	0.47	13.3
Gunmark Range	A	0.64	10.31	6.94	0.46	59.62	9.25	0	0.19	13.23
t-Test for Adult vs Juvenile	No.									
Mean of adult	7	0.72	7.41	6.17	0.45	68.39	5.53	0.90	0.93	
Mean of juvenile	7	1.01	4.93	5.03	0.41	73.65	4.99	0.87	0.90	
Difference (A-J)		-0.30	2.48	1.14	0.04	-5.26	0.54	0.03	0.03	
Significance of difference		***	*	ns	ns	***	ns	ns	ns	

The yields and chemical composition of the hexane-soluble waxes of both adult and juvenile leaves of *E. nitens* and *E. globulus* are shown in Table 11.8. The chemical composition of leaf waxes of these two species had a similar composition. Thus, these leaf waxes were characterised by high percentage of long chain ß-diketones as major components and long chain n-alkanes, alkanals, long chain esters and flavonoids as minor components. There was a consistent difference in wax composition between juvenile and adult leaf samples for both species. That is the percentage contents of ß-diketones of juvenile leaf waxes in both species were higher than those for adult leaf waxes (67.4% and 67.8% for juvenile leaves of *E. nitens* and *E. globulus* and 54.7 and 58.9% for adult leaves respectively) while the n-alkanes were higher in adult leaves. The wax yields of juvenile leaves of both species were also higher than those from adult leaves.

Table 11.8. The yields and chemical compositions (%) of leaf waxes from *E. nitens* and *E. globulus*.

Compound classes	*E. nitens*		*E. globulus*	
	Juvenile	Adult	Juvenile	Adult
Wax yields	1.00	0.56	0.93	0.74
Hydrocarbons	6.6	8.5	2	5.2
Aldehydes	6.5	7.2	2.3	4.3
Free alcohols	t	2.7	2.3	2.5
Free acids	t	2.1	1	t
Esters	2.2	7	7.2	9.3
β–Diketones	67.4	54.7	67.8	58.9
Flavones	4.5	7.9	8.3	7.9
Triterpenoids	t	0.5	0.1	0.1
unidentified	12	9	8	11

Results of the examination of the major classes of compounds of the juvenile and adult waxes of both species, indicated that the homologues of the different compounds had a similar distribution in both species. (see Appendix 11.3 for detailed comparison).

Overall, results of experiments indicated that the leaf waxes of both *E. nitens* and *E. globulus* were very similar in both wax chemistry and morphology.

11.4. Discussion

11.4.1. The description of the chemotypes in leaf oils of *E. nitens*

The work reported in this chapter is the first investigation of the adult and juvenile leaf oils of many provenances of this species. The leaf samples of this study included all major provenances of *E. nitens* (Pederick 1979; Pederick and Lennox 1979; Cook and Ladiges 1991) and therefore provide a comprehensive survey of the leaf oils of this species.

While the different leaf oils of the Penny Saddle and Bendoc provenances of *E. nitens* were identified in Chapter 3, this chapter further confirms that there are two different chemotypes of leaf oils occurring in *E. nitens*. One is the typical chemotype of *E. nitens* leaf oil characterised by high percentages of 1,8-cineole (35-56%) and α–pinene (14-29%) and the other an atypical chemotype which was characterised by high percentages of *p*-cymene (30%) and γ–terpinene (18-22%) as major components and by the presence of 1,8-cineole (11-15%) and *cis*–β–ocimene (5-7%). Both juvenile and adult leaf oils of all 'juvenile persistent' forms of *E. nitens* were characterised by the typical chemotype and the adult leaf oils of an 'early adult form' belong to the atypical chemotype.

As discussed previously, the chemotype of the leaf oil characterised by 1,8-cineole and α–pinene has been regarded as a chemogenetic marker for most *Symphyomyrtus* species of the Tasmanian eucalypts since these two compounds are the end products in the biosynthetic pathway of monoterpenoids. Thus, the typical chemotype of leaf oils of the *E. nitens* is similar to that of most *Symphyomyrtus* species of Tasmanian eucalypts and 1,8-cineole and α–pinene can also be regarded as genetic markers for the typical leaf oil of *E. nitens*.

However, the atypical leaf oils of *E. nitens* are uncommon in *Eucalyptus* species. It should be noted that the atypical chemotype of *E. nitens* leaf oils differed from the typical chemotype not only in the presence of *p*-cymene and γ–terpinene but also the occurrence of α–pinene in only trace amounts. α–pinene was recorded previously in this study to occur in trace amounts in the leaf oil of the Bendoc provenance in leaves of different ages and growth seasons (see chapter 3). The virtual absence of α–pinene in the atypical chemotype of *E. nitens* leaf oil is clearly not the result of leaf age or seasonal variation effects but associated with biosynthetic divergence of monoterpenoids (see discussion of Chapter 3).

Doran (1990) summarised genetic variation within some species of eucalypts and suggested that the chemotypes (or chemical forms) are generally well defined and readily distinguishable from each other and that they do not appear to be the result of site

differences, seasonal variation, leaf ageing effects or hybridisation. Analysis of the leaf oils of *E. nitens* as described above has indicated that the two chemotypes of *E. nitens* leaf oils are sufficiently different in chemical composition to be regarded as distinct chemical forms. Moreover, the results of correlation analysis of oil components among all provenances of *E. nitens* and the proposed biochemical relationships between these oil components (see chapters 3 and 4) suggested that the monoterpenoids of the two chemotypes of *E. nitens* differed biosynthetically. Nevertheless, the presence of 1,8-cineole in both the typical and atypical forms of *E. nitens* leaf oil does indicate some affinity between the leaf oils of the typical and atypical chemotypes.

As the trees were grown in a provenance trial by which the differences in environmental effects have been removed, the divergence in leaf oils of *E. nitens* has been confirmed to have arisen through genetic variation within the species *E. nitens*. Moreover, this divergence is in agreement with the finding of oil differences between the two provenances of this species which was based on analysis of individual tree samples (chapter 3). However, this is inconsistent with the findings on *E. punctata* spp. *punctata* where the wide variation in the leaf oil composition between individual trees showed no grounds for the establishment of chemical varieties within the subspecies (Southwell 1974).

11.4.2. The variation between adult and juvenile leaves

Comparison of the leaf oils of juvenile and adult samples of *E. nitens* s. lat. (entire adult leaf form) indicated that juvenile oil was qualitatively similar to adult oil since all juvenile leaf oils examined showed the same chemotype as the corresponding adult leaves. However, oil yields of juvenile *E. nitens* were significantly higher than from adult leaf oils. Thus, the leaf oils of *E. nitens* differed quantitatively but not qualitatively between juvenile and adult leaves among provenances. This was consistent with the previous findings in other Tasmanian eucalypt species, such as *E. globulus* and *E. viminalis*, (chapter 5) and in other studies of eucalypt leaf oils (Boland *et al.* 1991). However, it should be noted that the comparison made in this study is confounded by provenance genotype. Further study is needed to make comparisons based on individual tree samples within and between provenances.

The juvenile leaf samples of *E. nitens* populations examined in this chapter were of the entire adult form only. As samples of juvenile leaves from the samples of the *E. nitens* populations with denticulate adult form were not available for examination. Therefore, the conclusion in the comparison of juvenile and adult leaf oils only applies to *E. nitens* s. lat. populations. Further study of the comparison between juvenile and adult leaf samples of the *E. nitens* populations with the denticulate adult form may provide

interesting information since the adult leaf of the denticulate adult leaf form produces the leaf oil with the atypical chemotype.

11.4.3. Pattern of variation in leaf oils compared to morphological patterns

Analysis of the leaf oils indicated that *E. nitens* is a highly variable species since the two different chemotypes were identified from different provenances. Thus, all provenances from the regions of Rubicon, Macalister and Toorongo in Victoria, Southern and Northern NSW and one provenance (Mt Kaye) from Errinundra region in Victoria, in which the adult leaf is characterised by an entire leaf margin, produced leaf oil of the typical chemotype. The leaf oils of two provenances, Bendoc and Gunmark Range from Errinundra, which had adult leaves with denticulate leaf margins, were of the atypical chemotype. These results are in agreement with the division in morphological characteristics described by Pederick (1979) and Cook and Ladiges (1991) and differences in leaf polyphenols (Pederick and Lennox 1979).

The results of *E. nitens* growth in provenance trials undertaken by both Pederick (1979) and Cook and Ladiges (1991) indicated that the adult denticulate form of *E. nitens* occured on Errinundra Plateau. The leaf samples of Bendoc and Gunmark Range provenances from Errinundra examined in this thesis were the only provenances from the Errinundra Plateau which had the typically denticulate leaf form. The leaf samples of the Mt Kaye provenance from Errinundra had the entire form when grown in a provenance trial in Tasmania and these differences corresponded to the observation by Cook and Ladiges (1991) who identified the adult leaves of Mt Kaye provenance to be of the entire margin form in contrast to the other provenances of Errinundra. The results of the oil analyses of this study further confirmed a genetic difference between the provenances from the Errinundra plateau where the adult leaf oils of the Bendoc and Gunmark Range provenances were of the atypical chemotype in contrast to the typical chemotype of the Mt Kaye leaf oil.

Overall variation in leaf oils between populations of *E. nitens* in this study have indicated that the adult leaf oils of *E. nitens* parallel the genetic variation in morphology that has been discussed by Cook and Ladiges (1991). According to the difference in many morphological characteristics between the entire and denticulate adult leaf forms of *E. nitens*, Cook and Ladiges (1991) proposed that the denticulate adult leaf form of *E. nitens* be made a new species, *E. denticulata*. The differences in the leaf oils between the leaf forms found in this study have provided further chemical evidence to support this proposal. Therefore, the two chemotypes of leaf oils *E. nitens* could be recognised as leaf oils of two different species. Thus, the typical chemotype of adult leaf oil could be

used as a genetic characteristic of *E. nitens* and named as the *E. nitens* leaf oil, whereas, the atypical chemotype of adult leaf oil would be the *E. denticulata* leaf oil.

It is of interest that the division in adult leaf oil also responded to the division in polyphenols between leaf forms (Pederick and Lennox 1979). This suggests that variation in leaf chemicals is not simple, for other classes of chemicals, when present, might also be related to the variation in leaf oil components or polyphenols. Further studies of other chemicals may provide more information for the study of *E. nitens* and *E. denticulata,* since this study has indicated that the leaf oil appears to provide a potentially useful marker for genetic experiments.

However, the association between oil composition and leaf morphological characteristics found in this study is inconsistent to the findings in leaf oil of *E. dives* (Hellyer *et al.* 1969) and *E. punctata* (Southwell 1974) where the marked differences in oil composition of different varieties cannot be separated on morphological evidence.

11.4.4. Relationships between oil characteristics and phylogenetic history

As shown in the above section, *E. nitens* leaf oil is similar to most *Symphyomyrtus* species in which leaf oil is characterised by high percentages of 1,8-cineole and α–pinene as major components. However, the chemotype of the adult leaf oil identified from the *E. denticulata* species in this study is uncommon in eucalypts. Only the leaf oil from *E. quadrangulata* has been reported to have a oil composition similar to that of *E. denticulata.* In addition, two other chemotypes of leaf oil were identified from *E. quadrangulata.* Consequently the variation in the leaf oil within *E. quadrangulata* is somewhat similar to the variation between *E. nitens* and *E. denticulata.* This indicates that certain chemical affinities may exist between these two sets of species. Some insight into the possible correlation of leaf oils among these species is indicated in Table 11.10.

The percentage contents of the major components shown in the above table clearly indicate that adult leaf oils of *E. nitens* had high percentages of 1,8-cineole and α–pinene but low percentages of γ–terpinene and *p*-cymene which is similar to the juvenile leaf oils of *E. nitens* and *E. quadrangulata.* However, the composition of the adult leaf oils of the *E. denticulata* is similar to adult leaf oils of *E. quadrangulata.* This comparison in adult leaf oils indicates that *E. denticulata* has a close affinity to *E. quadrangulata.*

The phylogenetic relationships between *E. nitens, E. denticulata* and *E. quadrangulata* have been discussed by Chappil (1988) who proposed that the species *E. denticulata* and *E. nitens* appear to be paraphyletic, with *E. denticulata* a sister species to *E. quadrangulata.* Cook and Ladiges (1991) summarised the cladogram and present-day

distribution of these species and have suggested that they are an ancestral species which may have been split by climatic change into two species, with *E. nitens* in areas with colder climates, and the common ancestor of *E. quadrangulata* and *E. denticulata* in regions of milder climates. The close affinity in adult leaf oils of the *E. denticulata* and *E. quadrangulata* supports the proposals of Chappil and Cook and Ladiges.

Table 11.10. Comparison of leaf oil composition of *E. nitens*, *E. denticulata* and *E. quadrangulata*.

	E. nitens#		*E. denticulata**	*E. quadrangulata*†		
	Juvenile	Adult	Adult	Adult		Juvenile
Compounds (%)				Chem.1	Chem.2	Chem.3
α–Pinene	25.1	20.9	0.4	0.2	10.5	25.4
Limonene	4.6	2.5	1.3	0.5	2.7	3.4
1,8-Cineole	49.2	37.6	13.0	3.6	25.3	55.0
cis–β–Ocimene	0.1	1.6	6.5	-	-	-
γ–Terpinene	1.9	0.2	20.1	4.9	19.1	0.1
p-Cymene	3.9	6.6	30.0	33.7	17.0	0.3
α–terpineol	4.0	2.3	3.1	-	2.2	4.0
Globulol	0.3	1.3	0.4	0.0	6.3	1.7
Spathulenol	0.6	6.2	2.3	0	0.7	0.1
γ–Eudesmol	0.3	1.1	0.4	6.0	0.1	0.3
α–Eudesmol	0.2	1.2	1.1	13.7	0.5	0.4
β–Eudesmol	0.3	0.9	0.8	21.6	0.1	1.2

The juvenile leaf and adult leaf oils are extracted from the mean percentage composition of the 13 provenances of *E. nitens* leaf oils as shown in Table 11.4. * the adult leaf oil of *E. denticulata* is the mean percentage for provenances Bendoc and Gunmark Range; † the leaf oil of *E. quadrangulata* is obtained from Boland *et al.* (1991).

In addition, the comparison of leaf oils between *E. denticulata* and *E. quadrangulata* suggest that there may be some continuous variation in populations between these two species since the leaf oil of *E. denticulata*, identified in this study, was intermediate between the two chemotypes of the *E. quadrangulata* leaf oils identified in N.S.W. In addition, continuous variation between *E. nitens* and *E. denticulata* may also occur since the leaf oil of the Penny Saddle provenance of *E. nitens* (see chapter 3) appears to be intermediate between other *E. nitens* and *E. denticulata* populations. As discussed by Cook and Ladiges (1991), the current distributions of *E. nitens* - *E. denticulata* and *E.*

quadrangulata are mutually exclusive and there is no significant introgression between *E. nitens* and *E. denticulata*, however, the first two species do overlap in the area of the Baw Baw Ranges and further west and a few individual trees of intermediate morphology have been observed. Further investigation into leaf oil characteristics may be useful in the identification of variation between these three species.

11.4.5. Comparison in the leaf oils and waxes of *E. nitens, E. denticulata* and *Symphyomyrtus* species in Tasmania

Due to an absence of juvenile leaves of *E. denticulata* and some of the *E. nitens* populations, it was not possible to make a complete analysis and classification of the adult and juvenile leaf oils of these two species and the 29 Tasmanian eucalypt species. However, comparison of major trends in variation in both oil composition and absolute contents of oil compounds by Principal Component Analysis (PCA) indicated that leaf oils of *E. nitens* showed no tendency toward *Monocalyptus* but were closely related to *Symphyomyrtus* species. Hence, the leaf oils of *E. nitens* are qualitatively similar to most of the Tasmanian *Symphyomyrtus* species while the leaf oils of *E. denticulata* are different. However, oil yields of both *E. nitens* and *E. denticulata* are relatively low in contrast to the oil yields of most Tasmanian *Symphyomyrtus*.

Results also indicated that the surface waxes of both juvenile and adult leaves of both species contained high percentage contents of ß-diketones and had the tube type of wax. These findings are in agreement with Hallam and Chambers (1970) who described the leaf waxes of *E. nitens* to be tube type with high levels of ß-diketones. Since many populations of *E. nitens* were examined, results indicated that there was no significant variation in either leaf wax chemistry or morphology between regions or provenances within this species. Therefore, the chemical and morphological characteristics described by Hallam and Chambers (1970) and in this chapter could be regarded as the general characteristics of *E. nitens* leaf waxes. In addition, the adult leaf waxes of *E. denticulata* were similar to those in *E. nitens*.

Comparison of leaf waxes between *E. nitens* and *E. globulus* indicated that the waxes of these two species not only had similar compounds but also had a similar composition within each compound class and had similar morphological characteristics. Thus, *E. nitens* and *E. denticulata* had a close affinity to the leaf waxes to *E. globulus* and most Tasmanian *Symphyomyrtus* species. However, the oil yield of *E. nitens* leaves was significantly lower than the majority of Tasmanian *Symphyomyrtus* species.

11.4.6. Inheritance of leaf oils of *E. nitens* with respect to site.

Although, the family heritabilities in this study were based on a comparison between two family trials it is not possible to predict whether the leaf oil characteristics of different families were inherited from the original natural stands or not. However, results indicated that the leaf oils of *E. nitens* progeny had strong family heritabilities in oil yield irrespective of site as family differences in oil composition were maintained at both sites. The family heritabilities of progeny provide encouraging evidence that there is strong genetic base to the control of the production of leaf oils of *E. nitens*.

The correlation of family means of leaf oils and diameter growth presented in this study represent the first report of this trend in eucalypt species or any other plant species. The strong and inverse correlation of leaf oils and diameter growth strongly suggests that leaf oil and diameter growth of *E. nitens* are genetically related. Therefore leaf oil characteristics could serve as an indicator of diameter and general growth of trees between families. However, the negative correlation between oil yield and volume growth in *E. nitens* differed from *E. delegatensis* trees in which the leaf oil yields from faster grown trees had correspondingly high oil yields and slow grown trees produced low oil yields (Li, unpublished data). It is possible that these relationships are charactersistics of the subgenera. Further study is needed to confirm.

It should also be noted that the correlation between leaf oil and wood growth found in this study is based on a very small sample size. Further study is needed to determine such a trend.

It was found that site differences significantly affected quantities of leaf oil and tree volume growth of the different families. Thus, tree volume growth and oil yield of different families were significantly higher at Round Hill (Site 2) where the soil and temperature conditions were much better for *E. nitens* tree growth. This suggests that diameter growth and leaf oil production of *E. nitens* may have similar niche requirements. Results indicated that quantitative differences in both oil yield and diameter growth between families were maintained in the two different sites. Therefore, site did not significantly affect their potential value to taxonomic studies.

11.4.7. Inheritance of leaf oils in interspecific F1 hybrids of *E. nitens* and *E. globulus*.

The results suggested that the variation in leaf oil composition and yield between *E. nitens* and *E. globulus* parent families and their F1 hybrids is under genetic control, which is in agreement with the findings for variation in morphological and freezing resistance among these trees (Tibbits 1989; Tibbits *et al.* 1991) and the heritabilities of oil components in open pollinated families of *E. kochii* (Barton *et al.* 1991). With the exception of some minor components, the present study indicated that the yield of major oil compounds in F1 hybrids was intermediate between the parent controls and not one compound of this interspecific hybrid was ever significantly higher than the parent with the highest content.

Results also indicated that F1 hybrids tended to inherit the major oil components of the dominant oil parent, i.e., 1,8-cineole and α–terpineol (Table 11.6). The heritabilities of hybrid progeny may exhibit incomplete dominance of terpenoids producing a chemotype of leaf oils more toward the male *E. globulus* parent as found in other species (Lincoln and Langenheim 1980; Snajberk *et al.* 1982). However, the heritabilities of quantities of compounds tended to be intermediate between the parents. Comparisons made using oil composition and absolute content data indicated that the absolute oil content was a better indicator to identify the F1 hybrid. It appears that the inheritance of F1 hybrids may be reliably evaluated by using mature leaf samples for they display a more complete profile of oil composition and yield.

Chapter 12
General Discussion and Conclusions

The thesis has attempted to describe the chemotaxonomic relationships amongst the Tasmanian eucalypts and evaluate the relationship between chemotaxonomy and insect preferences. Results indicate that large subgeneric differences in leaf oils and wax chemicals are of ecological importance. The subgeneric differences in these leaf chemicals were correlated to the insect-plant relationships that exist in the Tasmanian eucalypt forest and suggest that leaf oil and wax chemicals are important mediators of the insect-eucalypt interactions. The major aspects of chemotaxonomic studies have been discussed in Chapter 6. This discussion emphasises aspects of leaf chemicals and insect-plant relationships.

12.1. Chemical basis of beetle host-selection strategies

The picture that emerged of the different interactions of *C. bimaculata* and *C. agricola* beetles and variations in leaf oils and wax chemicals indicated that the host-range of the major eucalypt pest, *C. bimaculata,* appears to be mainly imposed by components of the leaf oil. In contrast, the host-range of *C. agricola* was not explained by variation in leaf oil components but rather it appears to be limited by leaf surface wax components. In general, these secondary plant compounds appear to act as *allomones* (Lincoln *et al.* 1982) which function as deterrents, repellents or inhibitors of feeding and oviposition.

C. bimaculata adults showed a distinctive oviposition selection behaviour in relation to the suitability of foliage for their larvae. Thus, the chemoreceptors of *C. bimaculata* females permit a high degree of discrimination in oviposition made on potential host plants. This suggests that adult females might also display positive or negative anemotaxis if plant odours diffuse in air-currents.

However, the comparison of the composition of volatiles diffusing from foliage of different species (see Appendix 7.6) suggested that host-finding by *C. bimaculata* adults appeared to be not due to specific odours but rather the presence of repellents. The chemical composition of odours that diffuse from foliage of preferred species differed markedly and volatiles of the most preferred foliage of *E. nitens* diffused at very low concentrations which differed markedly in composition to the preferred *Monocalyptus*

species. However, the foliage of non-preferred species diffused consistently high concentrations of 1,8-cineole and α–pinene.

Some oil compounds, e.g. α–phellandrene, may promote feeding, but *C. bimaculata* larvae do not appear to require specific oil and wax compounds to act as feeding excitants. However, the foliage rejected by larvae was consistently characterized by the presence of high amounts of 1,8-cineole, α–pinene and related compounds which have been found to act as both deterrents and toxicants.

Therefore, the host-range of *C. bimaculata* appears to be imposed by the nature of feeding deterrents and toxicants, rather than the presences of excitants.

C. bimaculata does not appear to have evolved mechanisms (e.g. to sequester, by-pass, or detoxify) to protect it from the potential toxicity of 1,8-cineole, α–pinene and related oil compounds. Most Tasmanian *Symphyomyrtus* species have high concentrations of these compounds and therefore, the host range of *C. bimaculata* is subgenerically restricted to the *Monocalyptus* species which do not have high concentrations of any of these components. The few *Monocalyptus* species (e.g. *E. tenuiramis*) which appear to have independently evolved 1,8-cineole are not hosts of *C. bimaculata*. Moreover, even populations from the most preferred *Monocalyptus* species could be excluded if their foliage contained extremely high oil contents, as did Victorian populations of *E. delegatensis*.

In contrast, *C. agricola* displayed a higher degree of adaptation to the presence of different oil compounds. It's potential host-range appears to be imposed by the nature of wax chemicals or a combination of oil and waxes rather than variation in leaf oils. Moreover, the host preference of *C. agricola* appears to be restricted to the presence of 1,8-cineole and ß-diketones. *C. agricola* adults are highly discriminatory and their observed host range is restricted to *Symphyomyrtus* species.

Although results of the larval feeding experiments and occasionally field observations indicated that *E. delegatensis* and *E. pauciflora* could be attacked by this beetle species, there is no evidence of large populations of *C. agricola* occurring on these species. In mixed species trials (see Chapter 3) *C. agricola* only attacked and oviposited on *E. nitens* and *E. globulus* but not on *E. delegatensis* and *E. regnans*. Moreover, in no choice experiments *C. agricola* adults could feed on foliage of the non-preferred *E. obliqua* but laid fewer eggs compared to oviposition on *E. globulus* and *E. nitens*. It is possible that the occasionally 'overflow' of *C. agricola* onto less preferred species may be due to food availability as suggested by Morrow (1977b). Therefore, the preferred hosts of *C. agricola* appear to be restricted to the presence of ß-diketones and/or 1,8-cineole and

related oil compounds while it's potential host-range is limited by the presence of high triterpenoids, although further study is needed.

While host-plant relationships may not be completely mediated by leaf chemicals, the leaf oils and wax chemicals seem to, at least, play an important role in host selection. These compounds acting as *allomones* provide an adaptive advantage to the plant in limiting the host range of different beetle species in Tasmanian eucalypts. Moreover, the leaf chemicals may be fundamental in defining the realisation of insect feeding niches (Thorsteinson 1960; Fraenkel 1969; Schoonhoven 1972; Beck 1974) which may be further determined in natural populations by factors such as competition and predation as defined by Schowalter *et al.* (1986).

The host preference list given by de Little (1979) is the only extensive survey for paropsid fauna collected in natural eucalypt forests to date in Tasmania. This list indicates that not only *C. bimaculata* and *C. agricola*, but also other *Chrysophtharta* species (e.g. *C. aurea*) and species from the genus, *Paropsis* (e. g. *P. charybdis*, *P. sp (Ps7)*), were found to occur within a distinct subgeneric taxon. It is of interest that some species (e.g. *Paropsis rubidipes*) were found to occur on a wide range of *Monocalyptus* species, but were also found on *E. dalrympleana* and *E. ovata*, which are outliers from the majority of *Symphyomyrtus* species in term of leaf oils and/or waxes chemicals. Thus, it appears that large differences exist between the host-selection of specialist paropsid insects and variation in leaf oils and wax chemicals among Tasmanian eucalypts.

Some workers have found that the degree of insect attack on eucalypt species was related to the nitrogen content of leaves rather than to secondary compounds, e.g. oil chemicals (Fox and Macauley 1977; Morrow and Fox 1980; Ohmart *et al.* 1985). This does not explain subgeneric differences in the response to different eucalypts. Noble (1989) noted that 'I have not found evidence of systematic differences between the subgenera in leaf N contents in the above studies' and Lambert and Turner (1983) found no significant difference between the two subgenera in foliar N. Nutrient value may influence the degree of insect feeding in some cases but it certainly could not explain the systematic host-plant selection by the major pests occurring in eucalypt forests in Tasmania.

While further work is clearly required, particularly for *C. agricola*, this study is the first systematic study to help explain host-selection by the major pests in Tasmanian eucalypt forest and it appears, as suggested by Fraenkel (1969) and Harborne (1988), that secondary plant compounds play a key role.

12.2. The ecological impact of insects on eucalypt species

A comparison of forest structures provided by Duncan and Brown (1985) indicated that eucalypt species richness tends to be greater in dry sclerophyll compared to wet sclerophyll forests in Tasmania and this also appears to be the case on mainland Australia (e.g. Margules *et al.* 1986). In contrast, the wet sclerophyll forests tend to be less mixed and, in particular, the dominant species, *E. obliqua* and *E. regnans,* tend to occur in mono-specific stands in Tasmania.

Dry forest *Symphyomyrtus* species (e.g. *E. viminalis* and *E. ovata*) carry more paropsid species than do the *Monocalyptus* species (de Little 1979). In contrast in wet forests, it is the *Monocalyptus* species from the 'ash' group (*E. obliqua, E. regnans* and *E. delegatensis*) which carry most insect species, although a high diversity was also recorded for *E. dalrympleana,* which has a low oil yield. Thus, the diversity level of paropsids between subgenera could differ completely in different forest types. It suggests that the comparison of the ecological impact of insects between subgenera should take account of individual forest types and it would appear to be difficult to make a general comparison, as predicted by Noble (1989), that *Monocalyptus* species carry a lower diversity of leaf herbivores and suffer less leaf loss and damage by them than do *Symphyomyrtus* species.

Based on chemical data provided by this study, it is difficult to predict such issues in an overall comparison between subgenera, because the essential situations of each species are differentiated in the different forest types. However, results of this study have provided a chemical basis for comparison between subgenera in individual cases.

It has been suggested that the *Symphyomyrtus* species should have a greater investment in anti-herbivore defences, in particular quantitative defences, because they are slower growing species after an initial period (Coley *et al.* 1985). This appear to be similar to the comparison in oil yield during the juvenile growth stage. However, results of this study indicated that many *Monocalyptus* species appear to have oil yields similar to those of *Symphyomyrtus* species in mature forests. This suggests that any comparison between subgenera should consider the ontogenetic stage of tree growth.

It is possible that *Monocalyptus* species have become more successful competitors than *Symphyomyrtus* species after early growth in mixed forests because the leaf chemicals of *Monocalyptus* species are more complex than these in *Symphyomyrtus* species. From the viewpoint of coevolution (Harborne 1988), the more complex chemical composition of *Monocalyptus* should make it more difficult for insect adaptation.

It has been speculated that herbivores and parasitic insects may play a role in the dominance changeover in subgeneric mixed forests because *Symphyomyrtus* species suffer more insect damage (Chilvers and Brittain 1972; Morrow 1977; Duff *et al.* 1980). Results of this study suggest that the change over in quantitative levels of leaf chemicals is more likely the mechanism, at least in part, by which the *Monocalyptus* species eventually gain dominance (Davidson and Reid 1980; Duff *et al.* 1983). Thus, the oil yield of some *Symphyomyrtus* species decline with ontogenetic change from juvenile to adult leaves in contrast to *Monocalyptus* species increase with ontogenetic change. Further observations of an association of leaf chemicals and the age and stage of different species within individual mixed forests need to be carried out.

An overall comparison between subgenera and the ecological impact of insects remains unclear due to the lack of published data. However, *Monocalyptus* species in Tasmania clearly suffer greater insect damage than do *Symphyomyrtus* species. Results of this study suggest that subgeneric differences, ontogenetic changes in leaf chemicals and the presence of mono-specific stands are an array of factors which affect major insects in wet sclerophyll forests. Thus, the lack of chemical defences and a suitable environmental niche may explain the ecological impact of major insect pests on some commercially important eucalypt species in Tasmania.

Young regeneration of *Monocalyptus* species in wet forests is more susceptible to outbreaks of specialist insects, particular the major pest *C. bimaculata*. However, this is not the case in mature forests. This may be explained by the lower oil yield in juvenile foliage of 'ash' species which are more preferred by *C. bimaculata*. The ontogenetic change and concomitant increase in oil yields could render foliage of mature trees less susceptible. Moreover, the intense wild fire frequency in Tasmania could make up large new generations of 'ash' stands (Jackson 1965), which are more attractive and susceptible. Therefore, insect populations in mature forest of 'ash' species could decline as oil yield increases.

The life history and population biology of *C. bimaculata* described by de Little (1979) indicated that populations of *C. bimaculata* were present in outbreak proportions on *E. delegatensis* in young plantations but rapidly declined after a few years as the tree matured and beetles moved to other new 'ash' regeneration. It is clear that this shift was associated with ontogenetic changes in oil yield with tree growth. Therefore, it is suggested that while *C. bimaculata* beetles have a specific host-range, which is determined by systematic differences in oil quality, they also have an preferred-stage which is associated with low oil yields in the ontogeny of young 'ash' species.

In contrast, juvenile plantations of *Symphyomyrtus* species (e.g. *E. globulus*) are less susceptible to outbreaks of specialist herbivores, because their juvenile foliage has high quantities of oils and waxes.

It is difficult to predict whether leaf oil is important for eucalypt species in responding to insect stress on the mainland or other places. It is notable that *E. nitens* from the mainland, which lacks leaf oils, carries a higher diversity of insect species in plantations and has suffered greater insect damage (de Little 1989). Thus, leaf oil content is important in the survival of *Symphyomyrtus* species in Tasmania.

Because of the important status of *E. nitens* to the Tasmanian forest industry, a detailed study of the leaf oils and waxes of this species was undertaken. Results indicated that a significant low oil yield was a common characteristic of this species. However, the major pest, *C. bimaculata*, cannot walk on the juvenile glaucous waxes of this species. Therefore, *E. nitens* trees which retain their juvenile foliage sustain relatively less damage and this should be an important consideration in tree selection.

The use of hybrids appears to be a feasible way to increase oil yields, since results indicated that interspecific hybrids between *E. globulus* and *E. nitens* had intermediate oil yields. Analysis of leaf oils of infraspecific hybrids of *E. nitens* need to be done, since other studies have indicated that interspecific hybrids of *E. kochii* could produce higher oil yields and 1,8-cineole contents than either of the parents (Barton *et al.* 1989). However, it should be noted that hybrid trees need to increase oil yields up to a resistance threshold otherwise, they may suffer more insect damage, since results of this study indicated that 1,8-cineole may promote the feeding of *C. bimaculata* on F1 hybrids which contain low amounts of 1,8-cineole.

Furthermore, it is notable that the host-tree and leaf chemical relationships among Tasmanian eucalypts appear to have coevolved and exist in equilibrium. Large industrial actions disturb this equilibrium. In particular, clear-felling and regeneration of many stands of 'ash', which mimic natural fires in Tasmania, appear to be one of the factors that favour the increase of *C. bimaculata* populations.

Again, the low oil yield of adult *E. nitens* foliage, which is susceptible to *C. bimaculata*, could provide a relatively stable host refuge for *C. bimaculata* beetle in comparison to 'ash' regeneration. This may affect the life and population biology of this species. The *E. nitens* forest could also provide a food source for all those less damaging insect species that can cope with low levels of 1,8-cineole and related oil compounds. It may lead to *Monocalyptus* specialist insect species overcoming *Symphyomyrtus* species. from the viewpoint of coevolution.

The situation with *E. nitens* in Tasmania suggests that any new tree species should be examined for leaf chemicals to evaluate their potential resistance to major pests.

Data provided by de Little (1979) also indicated that the three *Symphyomyrtus* species, *E. viminalis*, *E. ovata* and *E. dalrympleana*, carry the greatest diversity of paropsid fauna among *Symphyomyrtus* species in Tasmania. Many insect species found on a wide range of *Monocalyptus* species only overlap into the three *Symphyomyrtus* species. *Paropsis charybdis* has a very broad host range (both *Symphyomyrtus* and *Monocalyptus* species of *Eucalyptus*) in New Zealand (Steven 1973) where it was introduced. However, in Tasmania, its host range is confined to the above three given species (de Little 1979). It is possible that the host-plant relationship among Tasmanian eucalypts and paropsid fauna has had sufficient time to undergo evolutionary convergence toward these three species. It is of interest that the classification using leaf oil plus wax data indicated that *E. ovata* and *E. dalrympleana* were outliers from the majority of *Symphyomyrtus* species. Thus, leaf chemicals appear to have played a considerable role in this evolution. In conclusion, this study suggests that insect-host tree selection is influenced by different leaf chemicals, either singly or in combination, in different amounts.

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(a) *E. regnans* and *E. delegatensis*

	Species	*E. regnans*								*E. delegatensis*							
	Provenances	A1				A2				C1				C2			
	Sites	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
	Oil Yield (g/100g dried leaves)	6.5	4.4	4.5	3	4.8	4.2	3.9	2.5	4.4	4.3	2.1	2.4	3.1	3	2	2.2
1	α–Pinene	-	-	-	-	0.3	-	0.1	-	-	-	-	-	-	-	-	-
2	α–Thujene	1.1	1.1	1	1.4	0.5	0.7	0.6	0.9	2.5	2.5	4.3	2.2	2	1.8	1.6	1.9
3	Camphene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	β–Pinene	-	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t
5	Isobutyl isobutanoate	0.7	0.2	0.3	0.4	0.4	0.3	0.4	0.4	-	-	-	-	-	-	-	-
6	Sabinene	-	-	-	-	-	-	-	-	0.1	t	t	t	t	t	t	t
7	α–Phellandrene	7.1	10.7	9.3	7.5	4.7	5.1	5.4	4.4	17.2	13.7	13.7	14.6	19.7	13.5	20.2	21.6
8	α–Terpinene	1.4	1.8	1.8	1.4	1.1	0.8	1.1	0.9	3.6	3.1	3.5	3.3	3.3	3.4	4	4.1
9	Limonene	0.3	0.2	0.3	0.3	0.5	0.5	0.1	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
10	β–Phellandrene	3.8	5	4.6	4	3.8	2.1	3.8	2.6	10.7	9.7	10.9	10.1	9.9	10.1	10.3	10.9
11	1,8-Cineole	-	-	-	-	-	-	-	-	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
12	*cis*-β–Ocimene	0.2	0.3	0.2	0.3	0.5	0.2	0.6	0.4	0.4	0.4	0.7	0.5	0.5	0.6	0.7	0.6
13	γ–Terpinene	0.2	0.3	0.2	0.2	0.3	0.1	0.3	0.1	0.7	0.6	0.6	0.7	0.7	0.6	0.8	0.9
14	*trans*-β–ocimene	0.1	0.1	t	0.3	0.1	0.1	0.1	0.1	0.8	0.3	0.3	0.5	0.7	0.3	0.8	0.9
15	*p*-Cymene	3.3	4	4.4	1.8	4	1.6	4.1	6	9.1	12	13.9	11.1	7.5	12.2	7.5	7.5
16	Terpinolene	0.5	0.7	0.7	0.4	0.6	0.4	0.3	0.5	1.2	0.9	0.8	1	1.4	0.9	1.4	1.6
17	Isoamyl isovalerate	-	-	-	-	t	-	-	-	-	-	-	-	-	-	-	-
18	Linalool	0.3	0.1	0.2	0.2	0.6	0.2	0.4	0.5	0.7	0.7	0.4	0.7	0.5	1.1	0.7	0.5
19	*trans-p*-Menth-2-en-1-ol	4.2	5.2	6.2	2.8	4.1	2.4	3.2	3.7	9.1	11.7	9.4	11.8	9.2	9.2	5.9	8.4
20	Pinocarvone	0.1	0.1	0.1	0.3	-	-	-	-	-	-	-	-	-	-	-	-
21	b-Caryophyllene	0.2	0.1	t	t	0.1	0.3	0.2	0.2	-	-	-	-	-	-	-	-
22	Aromadendrene	0.1	t	0.1	0.3	0.1	0.2	0.2	0.1	-	-	-	-	-	-	-	-
23	Terpinen-4-ol	1.2	1.6	1.3	0.9	1.1	0.8	1	1.5	3.5	2.8	3	1.9	2.6	2.8	2.7	3.2
24	*cis-p*-Menth-2-en-1-ol	3.3	3.9	4.3	2.1	3.1	2.1	2.7	2.8	7	8.9	7.3	9.3	7.1	6	4.8	6.8
25	*Allo*-aromadendrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	*trans*-Pinocarveol	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	*cis*-Piperitol	1.1	1.2	1.4	0.8	1	0.7	0.6	0.7	2.2	3	2.5	2.9	2.3	2.1	1.7	2.4
28	Terpinyl acetate	0.1	-	-	-	0.1	0.1	0.1	0.1	0.1	-	-	-	-	-	-	-
29	α–Terpineol	0.5	0.5	0.3	0.5	0.6	0.3	0.5	0.4	1	1.1	1	1.1	1.1	1.1	2	1
30	Piperitone	1.7	0.3	0.6	5.6	1.7	0.3	1.6	8.5	0.8	0.8	1	0.9	1	2.3	0.9	2.4
31	*trans*-Piperitol	1.9	2	1.9	1.4	2	1.2	1.9	1.5	3.4	4.6	4	5.1	3.9	3.5	2.8	3.8
32	Citronellal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	*cis*-Sabinol	0.2	0.1	0.2	0.2	0.3	0.1	0.2	0.4	0.5	0.4	0.5	0.5	0.4	0.7	0.5	0.4
34	*p*-Cymene-8-ol	0.1	0.1	0.2	0.1	0.8	0.1	0.1	0.3	0.3	0.4	0.5	0.4	0.3	0.5	0.3	0.3
35	Elemol	7	5	3.8	5.2	6.4	8.7	6.8	5	-	-	-	-	-	-	-	-
36	Globulol	-	-	-	-	-	-	-	-	2.1	1.6	1.5	1.6	2.6	2.2	3.4	2.1
37	Viridiflorol	0.7	0.6	0.5	0.7	0.5	0.9	0.6	0.5	0.6	0.5	0.5	0.4	0.8	0.8	1.1	0.7
38	Methyl cinnamate	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	2.4	1.5	1.5	2.3	0.8	0.2	0.2	1.3
39	Spathulenol	1.6	0.9	1.5	1.2	1.3	1.1	1.4	1.2	3.4	3.9	3.8	3.2	2.1	2.3	2.1	2.5
40	α–Eudesmol	17	14.5	17.1	16.2	19.4	26.7	24	19.5	3.6	3	3.5	2.6	5.2	4.5	7.1	3.4
41	γ–Eudesmol	12.1	9.3	10.5	12.2	14.4	17.4	14.3	13	3.3	2.8	3.1	2.4	3.9	3.5	4.3	2.5
42	β–Eudesmol	13.7	10.5	13.3	12.5	15.6	19.3	16.5	13.9	2.4	2	2.5	1.6	3.3	2.8	3.6	1.7
43	Unidentified	5.5	12.9	8.2	11.2	2.1	2	2.5	1.8	-	-	-	-	-	-	-	-

Appendix 3.1. The oil yields and percentage chemical composition (%) of leaf oils from leaves of different provenances of four eucalypt species at Sites 1, 2, 3 and 4. t = trace (<0.1); - = 0

(b) *E. nitens* and *E. globulus*

	Species	*E. nitens*								*E. globulus*							
	Provenances	D1				D2				B1				B2			
	Sites	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
	Oil Yield (g/100g dried leaves)	0.6	0.5	0.8	1.3	1	1.1	0.9	0.8	3.1	3.4	3	2.6	4.1	4.9	4.4	4
1	α–Pinene	11.5	8.8	17.3	11.2	1.6	3.7	1.6	2	13.7	10.4	15.2	19.3	10.2	9.8	10.9	13.5
2	α–Thujene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Camphene	t	0.1	0.3	0.2	-	-	-	-	0.1	0.1	t	t	t	t	t	t
4	β–Pinene	0.1	0.1	0.1	t	-	-	-	-	0.3	0.2	0.3	0.6	0.3	0.3	0.3	0.4
5	Isobutyl isobutanoate	1.2	1.7	2.9	2.3	8.1	7.3	8.4	5.9	-	-	-	-	-	-	-	-
6	Sabinene	0.1	t	0.1	t	-	-	-	-	-	-	-	-	-	-	-	-
7	α–Phellandrene	0.5	0.4	0.5	1.4	0.7	0.2	t	0.7	-	t	0.2	0.6	-	0.2	1	1
8	α–Terpinene	0.1	0.1	t	0.1	0.2	0.2	0.2	0.1	-	-	-	-	-	t	0.4	0.3
9	Limonene	3.5	4.9	6.6	5.4	6.5	7	5.7	5.5	2.8	1.3	3.2	4.6	1.9	2.3	5.1	3.9
10	β–Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1,8-Cineole	28.9	26.7	26.2	25.4	16.6	18.8	16.8	17.3	52.1	47.1	48.5	47.1	60.5	62.3	50.6	43
12	*cis*-β–Ocimene	2.5	4	3.2	10.9	10	6.2	7.4	9.8	0.5	0.6	0.5	0.7	0.5	0.4	0.4	0.4
13	γ–Terpinene	6.2	9.3	3.7	12	14.7	15.3	19.6	18.7	0.1	0.1	0.2	0.3	0.1	0.2	6.7	4.2
14	*trans*-β–ocimene	0.3	0.4	0.3	1	0.7	0.5	-	-	0.1	0.2	0.1	0.1	0.3	0.2	0.1	0.1
15	*p*-Cymene	16.4	17.9	13	6	20.5	21.2	21.1	17.2	0.4	0.9	0.9	0.6	0.9	1.2	4	8.5
16	Terpinolene	0.2	0.3	0.3	0.8	0.4	0.3	0.4	0.6	0.1	0.1	0.2	0.2	0.1	0.1	0.7	0.5
17	Isoamyl isovalerate	2.2	1.3	1.1	2.1	0.1	0.2	0.1	0.3	-	0.1	0.1	t	0.1	t	0.1	t
18	Linalool	0.2	0.3	0.2	0.2	0.4	0.3	0.3	0.2	t	t	t	-	t	t	t	0.1
19	*trans-p*-Menth-2-en-1-ol	t	t	-	t	0.1	-	t	t	0.1	0.1	t	-	0.1	t	t	t
20	Pinocarvone	1.1	1.2	0.9	0.3	0.2	0.2	0.2	0.3	1.7	3.5	2.2	0.9	2.3	3	0.2	0.6
21	b-Caryophyllene	-	-	0.3	t	t	0.1	t	0.1	3.4	4	1.4	0.4	1.6	0.6	0.1	0.1
22	Aromadendrene	1.3	0.5	0.2	0.3	-	-	t	0.1	1.6	2.7	2.2	2.2	1.6	1	1.9	2.5
23	Terpinen-4-ol	1.4	1.2	0.7	1.6	2.3	2.2	2.6	2.1	0.2	0.1	0.2	0.3	0.1	0.2	1.1	1.4
24	*cis-p*-Menth-2-en-1-ol	0.1	0.1	0.1	t	t	t	t	0.1	-	-	-	-	-	-	-	-
25	*Allo*-aromadendrene	0.5	0.5	0.6	0.5	0.2	0.2	0.2	0.2	0.5	0.8	0.7	0.5	0.5	0.3	0.5	0.6
26	*trans*-Pinocarveol	2.8	1.6	1.5	0.5	0.1	0.2	0.2	0.5	4.9	6.7	3.4	1.2	3.8	3.3	0.3	0.7
27	*cis*-Piperitol	-	-	-	-	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.1	0.3	0.2	0.1	-
28	Terpinyl acetate	0.4	0.5	0.5	-	0.5	0.5	0.5	0.4	1.1	1.2	4.2	2.4	0.1	-	-	-
29	α–Terpineol	4.2	3	4.1	3.6	4.1	5.3	4	3.7	1.6	0.9	1.5	1.5	0.9	0.9	1.6	1.1
30	Piperitone	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.4	-	0.1	t	0.2	-	t	t
31	*trans*-Piperitol	0.1	0.1	0.1	t	t	t	t	t	-	0.3	0.4	0.1	0.3	0.3	0.1	0.2
32	Citronellol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	*cis*-Sabinol	0.2	0.2	0.3	0.1	0.2	0.2	0.2	0.1	-	-	t	-	t	t	t	0.1
34	*p*-Cymene-8-ol	0.3	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
35	Elemol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	Globulol	0.7	0.9	1.3	1.2	0.5	0.3	0.5	0.6	6.5	8.6	7.4	8.1	5.1	3.1	4.5	6.2
37	Viridiflorol	0.5	0.7	1.1	0.7	0.4	0.2	0.4	0.4	0.9	1.1	0.9	1.1	0.7	0.5	0.8	0.9
38	Methyl cinnamate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	Spathulenol	3.6	4.7	4.1	1.9	3	1.5	2.2	2.5	0.3	0.8	1	1	0.5	0.4	0.6	1
40	α–Eudesmol	0.7	0.7	0.8	0.3	0.7	0.4	1	0.9	0.1	0.1	0.1	0.2	0.5	1.2	1.1	0.3
41	γ–Eudesmol	1.1	1.2	1.3	0.6	1.1	0.5	1.1	0.9	0.1	0.2	0.3	0.3	0.3	0.7	1.2	0.6
42	β–Eudesmol	0.8	0.8	1	0.3	0.6	0.4	0.8	0.7	0.1	0.3	0.2	0.4	1.5	3.7	1.6	0.6
43	Unidentified	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 3.2A. Variation in percentage oil yield (dry leaf weight) and percentage contents of major oil components in leaves of *E. nitens* (D2) over a year (1988-1989) at two months intervals.

H-values and probabilities listed in here are the Kruskal-Wallis H-statistic and probability. If there are tied groups the H-values listed here are Kruskal-Wallis H corrected for ties and probability.
Significance: ns : p>0.05; * : 0.05>p>0.01; ** : 0.01>p>0.001; *** : p<0.001
t = trace (<0.3); - = error; -- = no statistic was made

	Components		Sample time 1 Nov.	2 Jan.	3 Mar.	4 May	5 Jul.	6 Sep.	7 Nov.	Analysis of variance (between times/trees) No. (tree)	*H*-value	Sign.
	Oil Yield	Mean	0.75	0.80	0.97	0.89	0.84	0.80	0.70	5	11.5	ns
		S.D.	0.07	0.21	0.16	0.17	0.09	0.09	0.04			
1	α–Pinene	Mean	0.54	0.59	0.62	0.58	0.62	0.58	0.73	5	1.7	ns
		S.D.	0.29	0.23	0.47	0.19	0.31	0.21	0.31			
2	Isobutyl isobutanoate	Mean	2.0	2.7	5.1	6.8	7.3	6.5	6.8	5	22.5	**
		S.D.	0.7	0.8	1.0	2.4	2.0	1.5	1.7			
3	α–Phellandrene	Mean	3.4	1.7	0.7	0.6	0.4	0.4	0.3	5	24.9	***
		S.D.	0.8	0.8	0.2	0.4	0.4	0.2	0.2			
4	α–Terpinene	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
5	Isobutyl isopentanoate	Mean	3.20	4.03	4.96	5.20	4.46	3.89	3.49	5	5.1	ns
		S.D.	0.83	0.78	1.21	2.18	2.20	2.45	1.83			
6	Limonene	Mean	1.57	1.28	1.48	1.13	1.49	1.28	1.37	5	6.0	ns
		S.D.	0.40	0.54	0.14	0.32	0.42	0.34	0.22			
7	β–Phellandrene	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
8	1,8-Cineole	Mean	13.5	13.9	14.1	14.0	14.7	14.9	14.0	5	2.8	ns
		S.D.	2.5	2.5	1.8	1.3	0.8	1.6	1.1			
9	*cis*-β–Ocimene	Mean	27.5	21.5	12.8	8.8	7.3	7.9	6.8	5	30.5	***
		S.D.	3.5	3.0	1.8	0.5	0.7	0.8	1.1			
10	γ–Terpinene	Mean	27.8	26.7	23.2	20.3	18.9	18.7	17.6	5	20.9	**
		S.D.	4.0	2.6	3.2	3.5	2.7	3.0	3.6			
11	*p*-Cymene	Mean	5.6	11.9	19.2	24.3	25.8	26.1	28.5	5	28.1	***
		S.D.	1.6	3.7	2.4	2.3	2.5	4.8	3.4			
12	*trans*-*p*-Menth-2-en-1-ol	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
13	Pinocarvone	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	-	-	-	-	-	-	-			
14	Aromadendrene	Mean	t	-	t	-	t	t	t	5	–	
15	Terpinen-4-ol	Mean	1.53	1.63	1.74	1.87	2.02	2.24	2.52	5	6.6	ns
		S.D.	0.47	0.61	0.86	0.61	0.58	0.75	0.80			
16	*cis*-*p*-Menth-2-en-1-ol	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
17	trans-Pinocarveol	Mean	t	t	t	t	t	-	-	5	–	
		S.D.	-	-	-	-	-	-	-		–	
18	*trans*-Piperitol	Mean	t	-	t	t	-	t	t	5	–	
		S.D.	t	-	t	t	-	t	t			
19	α–Terpineol	Mean	2.85	3.29	3.47	4.07	4.24	4.45	4.38	5	10.9	ns
		S.D.	0.60	0.92	0.53	1.21	0.99	0.95	0.94			
20	Piperitone	Mean	t	t	-	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
21	*trans*-Piperitol	Mean	-	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
22	Globulol	Mean	0.96	0.96	1.03	1.04	1.04	0.94	1.21	5	2.4	ns
		S.D.	0.31	0.26	0.30	0.28	0.23	0.48	0.36			
23	Methyl cinnamate	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
24	Spathulenol	Mean	2.62	3.11	3.55	4.07	4.29	4.83	4.86	5	17.9	**
		S.D.	0.72	1.01	1.03	0.96	0.88	0.73	0.51			
25	α–Eudesmol	Mean	t	t	t	t	t	0.40	0.44	5	10.6	ns
		S.D.	t	t	t	t	t	0.15	0.28			
26	γ–Eudesmol	Mean	0.79	0.46	0.24	0.37	0.27	0.49	0.36	5	6.6	ns
		S.D.	0.70	0.16	0.11	0.15	0.13	0.37	0.12			
27	β–Eudesmol	Mean	t	t	t	t	t	0.30	0.38	5	3.8	ns
		S.D.	t	t	t	t	t	0.15	0.19			
28	Other components	Mean	5.35	6.37	7.45	6.19	6.73	6.01	6.27	5	–	

Appendix 3.2B. Variation in percentage oil yield (dry leaf weight) and percentage contents of major oil components in leaves of *E. delegatensis* (C2) over a year (1988-1989) at two months intervals.

H-values and probabilities listed in here are the Kruskal-Wallis H-statistic and probability. If there are tied groups the H-values listed here are Kruskal-Wallis H corrected for ties and probability.
Significance: ns : p>0.05; * : 0.05>p>0.01; ** : 0.01>p>0.001; *** : p<0.001
t = trace (<0.3); - = error; -- = no statistic was made

	Components		Sample time 1 Nov.	2 Jan.	3 Mar.	4 May	5 Jul.	6 Sep.	7 Nov.	Analysis of variance (between times/trees) No. (tree)	*H*-value	Sign.
	Oil Yield	Mean	2.62	2.72	3.14	3.07	2.88	2.82	2.73	5	3.6	ns
		S.D.	0.59	0.57	0.66	0.56	0.19	0.42	0.27			
1	α–Pinene	Mean	-	-	-	-	-	-	-	5	--	
		S.D.	-	-	-	-	-	-	-			
2	Isobutyl isobutanoate	Mean	-	-	-	-	-	-	-	5	--	
		S.D.	-	-	-	-	-	-	-			
3	α–Phellandrene	Mean	27.3	25.0	20.9	18.4	19.6	18.2	17.1	5	21.7	**
		S.D.	3.7	2.6	2.8	1.6	2.2	1.9	3.2			
4	α–Terpinene	Mean	3.9	3.7	3.0	3.1	2.6	2.1	2.1	5	8.7	ns
		S.D.	0.9	1.1	1.4	1.6	1.3	1.4	1.1			
5	Isobutyl isopentanoate	Mean	-	-	-	-	-	-	-	5	--	
		S.D.	-	-	-	-	-	-	-			
6	Limonene	Mean	0.49	0.46	0.32	0.51	0.40	0.43	0.48	5	1.6	ns
		S.D.	0.24	0.22	0.18	0.14	0.12	0.11	0.12			
7	β–phellandrene	Mean	13.2	12.8	10.6	11.1	9.7	9.1	9.0	5	8.6	ns
		S.D.	2.8	2.7	2.6	2.6	2.7	2.9	2.5			
8	1,8-Cineole	Mean	t	t	t	t	t	t	t	5	--	
		S.D.	t	t	t	t	t	t	t			
9	*cis*-β–Ocimene	Mean	0.33	0.31	0.39	0.41	0.38	0.36	0.43	5	2.9	ns
		S.D.	0.13	0.14	0.07	0.14	0.16	0.05	0.14			
10	γ–Terpinene	Mean	1.30	1.10	1.23	1.03	0.76	0.61	0.54	5	26.9	***
		S.D.	0.32	0.32	0.13	0.32	0.13	0.16	0.20			
11	*p*-Cymene	Mean	1.5	2.7	5.0	6.6	7.8	7.4	8.5	5	27.0	***
		S.D.	0.8	1.1	0.8	1.5	1.4	0.5	1.4			
12	*trans*-*p*-Menth-2-en-1-ol	Mean	8.6	8.8	9.1	10.5	10.8	11.3	11.4	5	8.1	ns
		S.D.	2.1	2.1	2.5	2.7	1.9	2.8	2.3			
13	Pinocarvone	Mean	-	-	-	-	-	-	-	5	-	
		S.D.	-	-	-	-	-	-	-			
14	Aromadendrene	Mean	-	-	-	-	-	-	-	5	-	
		S.D.	-	-	-	-	-	-	-			
15	Terpinen-4-ol	Mean	1.5	1.6	2.7	3.1	3.5	3.3	3.8	5	19.2	**
		S.D.	0.6	0.4	0.7	1.1	1.0	0.9	1.0			
16	*cis*-*p*-Menth-2-en-1-ol	Mean	6.2	6.3	6.9	7.1	7.4	7.1	7.9	5	10.6	ns
		S.D.	0.9	1.7	1.1	0.8	1.3	1.1	1.4			
17	trans-Pinocarveol	Mean	-	-	-	-	-	-	-	5	-	
		S.D.	-	-	-	-	-	-	-			
18	*cis*-Piperitol	Mean	2.2	2.3	2.6	3.2	3.2	3.4	3.8	5	12.4	ns
		S.D.	0.8	0.8	0.8	1.0	1.0	0.6	1.1			
19	α–Terpineol	Mean	1.22	1.17	1.05	1.35	0.98	1.27	1.11	5	3.5	ns
		S.D.	0.47	0.26	0.51	0.50	0.27	0.28	0.19			
20	Piperitone	Mean	1.7	1.8	2.0	2.2	2.4	2.3	2.2	5	4.8	ns
		S.D.	0.5	0.8	0.4	0.4	0.8	0.4	0.5			
21	*trans*-Piperitol	Mean	6.5	6.6	7.7	6.4	6.0	6.6	6.3	5	4.3	ns
		S.D.	1.7	1.1	1.5	1.1	1.3	1.7	0.5			
22	Globulol	Mean	1.59	1.49	1.53	1.32	1.12	1.62	1.18	5	11.7	ns
		S.D.	0.50	0.61	0.62	0.76	0.43	0.99	0.77			
23	Methyl cinnamate	Mean	1.77	1.69	1.59	1.28	1.10	1.12	1.06	5	11.5	ns
		S.D.	0.57	0.26	0.35	0.31	0.39	0.45	0.39			
24	Spathulenol	Mean	2.76	2.45	2.36	1.87	1.54	1.72	1.81	5	13.7	*
		S.D.	0.65	0.26	0.43	0.32	0.45	0.25	0.95			
25	α–Eudesmol	Mean	4.05	3.67	4.39	4.40	4.30	4.63	5.42	5	5.3	ns
		S.D.	1.46	0.94	0.92	1.16	1.42	1.69	1.53			
26	γ–Eudesmol	Mean	2.67	2.99	2.75	2.94	3.15	3.59	3.99	5	7.6	ns
		S.D.	0.88	0.67	0.60	0.92	0.98	0.99	0.72			
27	β–Eudesmol	Mean	2.65	2.83	2.44	2.86	2.55	3.05	2.99	5	6.1	ns
		S.D.	0.25	0.55	0.31	0.64	0.39	0.63	0.22			
28	Other components	Mean	8.12	10.22	10.78	9.87	12.11	9.95	8.54	5	--	

Appendix 3.2C. Variation in percentage oil yield (dry leaf weight) and percentage contents of major oil components in leaves of *E. globulus* (B2) over a year (1988-1989) at two months intervals.

H-values and probabilities listed in here are the Kruskal-Wallis H-statistic and probability. If there are tied groups the H-values listed here are Kruskal-Wallis H corrected for ties and probability.
Significance: ns : p>0.05; * : 0.05>p>0.01; ** : 0.01>p>0.001; *** : p<0.001
t = trace (<0.3); - = error; -- = no statistic was made

	Components		Sample time 1 Nov.	2 Jan.	3 Mar.	4 May	5 Jul.	6 Sep.	7 Nov.	Analysis of variance (between times/trees) No. (tree)	*H*-value	Sign.
	Oil Yield	Mean	3.56	3.72	4.37	4.27	4.21	4.03	3.82	5	5.0	ns
		S.D.	0.80	0.46	0.70	0.81	0.72	0.82	0.72			
1	α–Pinene	Mean	22.1	19.2	15.6	13.9	14.6	13.3	12.8	5	22.0	**
		S.D.	3.8	2.2	2.5	2.3	2.0	2.0	2.4			
2	Isobutyl isobutanoate	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
3	α–Phellandrene	Mean	t	t	t	-	-	t	t	5	–	
		S.D.	t	t	t	-	-	t	t			
4	α–Terpinene	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
5	Isobutyl isopentanoate	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
6	Limonene	Mean	5.04	4.78	5.34	5.65	5.56	5.92	5.82	5	9.6	ns
		S.D.	0.59	0.67	0.68	0.87	0.71	0.92	0.60			
7	β–phellandrene	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
8	1,8-Cineole	Mean	34.9	38.3	45.6	48.8	48.0	51.0	50.1	5	19.9	**
		S.D.	6.1	4.9	4.1	5.2	5.0	6.8	5.3			
9	*cis*-β–Ocimene	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
10	γ–Terpinene	Mean	0.98	0.70	0.82	0.67	0.68	0.59	0.49	5	6.9	ns
		S.D.	0.36	0.32	0.31	0.31	0.26	0.36	0.21			
11	*p*-Cymene	Mean	0.7	0.5	0.7	1.2	1.4	1.6	1.6	5	16.8	*
		S.D.	0.4	0.4	0.2	0.6	0.5	0.7	0.6			
12	*trans-p*-Menth-2-en-1-ol	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
13	Pinocarvone	Mean	1.2	0.9	1.8	2.2	2.0	2.4	2.6	5	–	
		S.D.	0.3	0.3	0.4	1.1	1.5	1.5	0.8			
14	Aromadendrene	Mean	3.1	3	3.2	4	3.06	3.3	3.9	5	–	
			0.8	0.8	1.3	1.6	0.934	1.5	1.9			
15	Terpinen-4-ol	Mean	0.93	0.77	0.75	0.77	0.66	0.69	0.66	5	3.4	ns
		S.D.	0.19	0.50	0.30	0.16	0.24	0.34	0.40			
16	*cis-p*-Menth-2-en-1-ol	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
17	trans-Pinocarveol	Mean	1.5	2.0	2.2	1.7	2.8	2.6	3.1	5	–	
		S.D.	0.7	0.7	0.2	1.0	1.0	0.6	0.8		–	
18	*cis*-Piperitol	Mean	t	t	t	t	t	-	-	5	–	
		S.D.	t	t	t	t	t	-	-			
19	α–Terpineol	Mean	7.0	5.5	2.6	3.0	2.5	2.1	1.8	5	20.6	**
		S.D.	2.4	2.1	1.1	1.2	1.0	1.6	1.1			
20	Piperitone	Mean	t	t	t	t	t	t	t	5	–	
		S.D.	t	t	t	t	t	t	t			
21	*trans*-Piperitol	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
22	Globulol	Mean	6.33	6.55	7.39	7.32	8.05	7.86	8.32	5	1.9	ns
		S.D.	1.55	1.74	2.66	2.33	2.64	2.83	3.26			
23	Methyl cinnamate	Mean	-	-	-	-	-	-	-	5	–	
		S.D.	-	-	-	-	-	-	-			
24	Spathulenol	Mean	0.58	0.59	0.56	0.70	0.71	0.55	0.64	5	1.7	ns
		S.D.	0.30	0.19	0.36	0.26	0.26	0.25	0.36			
25	α–Eudesmol	Mean	0.15	0.09	0.10	0.20	0.16	0.20	0.20	5	5.5	ns
		S.D.	0.08	0.12	0.07	0.11	0.15	0.14	0.15			
26	γ–Eudesmol	Mean	0.32	0.26	0.19	0.34	0.34	0.19	0.28	5	4.5	ns
		S.D.	0.20	0.18	0.14	0.23	0.05	0.15	0.16			
27	β–Eudesmol	Mean	0.09	0.17	0.16	0.14	0.13	0.15	0.08	5	1.5	ns
		S.D.	0.07	0.14	0.11	0.16	0.11	0.12	0.03			
28	Other components	Mean	20.47	22.32	19.57	17.81	17.06	15.96	16.72	5	–	

Appendix 3.3A. Variation in major chemical components of *E. nitens* oils with time and leaf age during growth season (1987 to 1988).

(1) Sample time 1 (8/11/1987) to sample time 4 (3/2/1988)

	Sample Time		1 (8/11)	2 (15/12)		3 (12/1)			4 (3/2)			
	Age Code		A	A	B	A	B	C	A	B	C	D
	Leaf age (days)		leaves	28	56	leaves	28	56	leaves	28	56	84
	Oil yield	Mean	0.44	0.52	0.62	0.59	0.49	0.68	0.54	0.61	0.46	0.55
		S.D	0.09	0.19	0.24	0.22	0.26	0.16	0.28	0.23	0.16	0.25
1	α–Pinene	Mean	0.83	0.60	0.85	0.89	0.74	1.02	0.90	1.17	1.18	1.18
		S.D	0.09	0.29	0.17	0.17	0.08	0.55	0.68	0.84	0.69	0.87
2	Isobutyl	Mean	1.59	1.45	1.94	2.03	2.83	2.99	3.88	5.10	3.87	6.09
	isobutanoate	S.D	1.33	1.37	1.72	1.23	1.45	1.79	3.11	4.05	3.35	3.80
3	α–Phellandrene	Mean	3.81	3.87	2.93	3.36	3.08	2.43	2.20	1.70	2.12	0.91
		S.D	1.29	1.53	1.83	1.35	1.86	1.75	2.40	1.65	1.96	1.06
4	Limonene	Mean	3.93	4.76	4.98	3.76	4.19	4.44	3.93	4.94	5.75	4.76
		S.D	0.85	0.92	1.11	1.08	0.51	0.81	0.71	0.76	0.57	0.58
5	Isobutyl	Mean	1.39	1.61	1.76	1.63	1.46	1.55	1.47	1.47	1.32	1.67
	isopentanoate	S.D	0.33	0.33	0.34	0.36	0.18	0.28	0.32	0.39	0.60	0.28
6	1,8-Cineole	Mean	10.79	9.52	12.89	10.69	9.47	10.79	12.85	11.54	12.55	12.31
		S.D	3.41	4.99	2.61	1.91	2.67	1.98	2.39	2.62	2.32	2.81
7	*cis*-β–Ocimene	Mean	25.52	28.86	24.58	26.38	21.93	21.37	21.40	18.77	16.61	15.74
		S.D	5.59	3.81	2.19	6.14	3.83	4.29	3.66	4.99	7.59	4.76
8	γ–Terpinene	Mean	26.81	23.87	24.75	26.31	26.47	26.03	25.15	25.28	24.94	24.46
		S.D	3.80	4.65	3.08	6.29	3.37	4.43	4.42	2.90	3.41	2.95
9	*p*-Cymene	Mean	6.17	5.88	6.95	7.54	9.08	9.64	8.37	11.47	11.42	14.39
		S.D	1.76	1.03	1.61	2.84	5.56	4.37	3.43	3.65	5.10	2.30
10	Terpinen-4-ol	Mean	1.08	1.65	1.37	1.13	1.25	1.47	1.56	1.60	1.73	1.31
		S.D	0.57	1.38	0.80	0.61	0.67	0.83	0.66	0.97	0.96	0.62
11	α–Terpineol	Mean	2.42	3.07	2.94	2.35	2.66	2.65	3.84	3.53	3.02	3.24
		S.D	0.42	0.69	0.69	0.40	0.48	0.34	0.99	1.07	0.67	0.63
12	Globulol	Mean	0.83	1.05	0.92	0.75	0.69	0.52	0.81	0.72	0.61	0.68
		S.D	0.63	0.73	0.47	0.38	0.45	0.43	0.50	0.38	0.20	0.19
13	Spathulenol	Mean	0.66	0.85	0.83	1.10	0.92	0.76	1.50	1.57	1.16	1.73
		S.D	0.55	0.72	0.87	1.08	0.79	0.70	0.86	0.99	1.06	0.93

Appendix 3.3A. Variation in major chemical components of *E. nitens* oils with time and leaf age during growth season (1987 to 1988).

(2) Sample time 5 (2/3/1987) to sample time 6 (30/3/1988)

	Sample Time		5 (2/3/88)					6 (30/3/88)					
	Age Code		A	B	C	D	E	A	B	C	D	E	F
	Leaf age (days)		leaves	28	56	84	114	leaves	28	56	84	114	140
	Oil yield	Mean	0.64	0.58	0.55	0.46	0.52	0.50	0.52	0.70	0.60	0.57	0.66
		S.D	0.32	0.23	0.18	0.18	0.27	0.18	0.13	0.32	0.26	0.31	0.23
1	α–Pinene	Mean	0.62	0.83	0.71	0.88	0.79	0.58	0.81	0.75	0.69	0.51	0.55
		S.D	0.47	0.47	0.21	0.68	0.20	0.18	0.56	0.22	0.48	0.36	0.46
2	Isobutyl	Mean	3.02	4.74	4.62	3.57	5.32	2.91	2.75	3.49	3.55	3.78	3.82
	isobutanoate	S.D	1.59	1.94	2.03	2.94	2.41	1.83	1.17	2.56	2.89	2.11	2.47
3	α–Phellandrene	Mean	1.49	0.51	0.43	0.28	0.40	0.56	0.59	0.32	0.21	0.29	0.14
		S.D	2.44	0.29	0.49	0.29	0.48	0.15	0.56	0.19	0.15	0.23	0.14
4	Limonene	Mean	3.66	5.31	5.98	6.38	7.07	4.56	5.22	5.47	6.22	6.64	6.88
		S.D	1.18	0.77	1.13	0.65	0.80	0.80	1.10	0.83	0.70	0.51	0.51
5	Isobutyl	Mean	1.40	1.30	1.47	1.52	1.53	1.32	1.48	1.54	1.27	1.68	1.48
	isopentanoate	S.D	0.39	0.26	0.14	0.33	0.16	0.29	0.52	0.33	0.48	0.32	0.41
6	1,8-Cineole	Mean	12.09	12.19	11.74	12.00	14.19	13.26	10.23	12.09	10.33	11.99	10.64
		S.D	4.28	2.95	3.64	3.38	2.72	2.86	4.49	2.94	2.86	1.71	5.36
7	*cis*-β–Ocimene	Mean	19.39	12.61	10.61	9.74	8.06	17.66	11.43	7.90	8.32	7.35	5.48
		S.D	5.63	3.27	3.67	2.18	6.28	2.33	2.28	2.89	4.92	3.29	4.47
8	γ–Terpinene	Mean	22.29	23.66	23.26	23.49	19.82	20.86	19.39	20.65	18.93	23.94	19.90
		S.D	3.59	6.44	4.52	8.73	2.83	5.96	5.07	7.63	5.73	7.33	7.97
9	*p*-Cymene	Mean	8.75	13.98	17.56	20.84	22.58	11.54	20.26	21.07	22.42	25.29	26.28
		S.D	2.62	4.62	5.02	3.85	7.23	2.26	2.14	4.10	3.47	5.94	5.81
10	Terpinen-4-ol	Mean	1.73	1.67	1.97	1.93	1.55	2.13	1.74	1.93	1.83	1.55	1.45
		S.D	0.66	0.59	1.20	1.02	0.84	0.84	0.94	0.44	0.24	0.57	0.61
11	α–Terpineol	Mean	5.52	3.45	4.17	3.70	3.54	5.71	5.66	5.55	4.74	4.01	3.65
		S.D	2.59	1.34	2.02	1.41	0.32	1.83	1.21	1.68	2.18	1.19	1.11
12	Globulol	Mean	1.11	0.95	0.89	0.89	0.64	1.46	1.29	1.64	1.05	0.94	1.90
		S.D	0.54	0.58	0.59	0.40	0.50	0.46	0.69	1.30	1.11	0.35	2.17
13	Spathulenol	Mean	3.70	3.29	2.80	3.44	2.14	4.94	4.95	5.50	4.72	4.20	2.94
		S.D	2.62	1.20	2.27	0.47	0.74	2.00	1.59	1.89	4.09	1.22	0.86

Appendix 3.3B. Variation in major chemical components of *E. delegatensis* oils with time and leaf age during growth season (1987 to 1988).

(1) Sample time 1 (8/11/1987) to sample time 4 (3/2/1988)

Sample Time			1 (8/11)	2 (15/12)		3 (12/1)			4 (3/2)			
Age Code			A	A	B	A	B	C	A	B	C	D
Leaf age (days)			leaves	28	56	leaves	28	56	leaves	28	56	84
	Oil Yield	Mean	1.75	1.93	2.10	1.85	2.09	2.37	2.07	2.29	2.60	2.76
		S.D	0.37	0.54	0.17	0.19	0.23	0.37	0.34	0.65	0.67	0.24
1	α–Phellandrene	Mean	28.43	27.46	28.79	26.65	25.75	25.87	26.51	23.85	20.32	17.59
		S.D	2.20	2.02	0.89	1.38	1.20	1.99	2.60	2.43	2.85	3.55
2	α–Terpinene	Mean	2.08	1.94	2.37	1.77	1.56	1.62	1.66	1.29	1.17	1.13
		S.D	0.44	0.31	0.14	0.34	0.28	0.36	0.40	0.51	0.66	0.31
3	β–Phellandrene	Mean	9.22	7.52	8.95	7.00	5.26	6.56	6.86	6.33	5.34	6.45
		S.D	1.46	1.53	3.27	1.39	0.86	0.94	0.80	1.64	1.30	1.24
4	γ–Terpinene	Mean	1.59	1.31	1.16	1.03	0.94	0.88	0.84	0.71	0.73	0.58
		S.D	0.39	0.45	0.25	0.68	0.65	0.48	0.42	0.38	0.33	0.33
5	*p*-Cymene	Mean	1.03	1.08	0.59	0.74	1.18	1.73	1.45	1.81	3.28	3.56
		S.D	1.00	0.61	0.19	0.30	0.65	1.32	0.60	0.37	0.69	0.47
6	Terpinolene	Mean	2.49	2.14	2.33	2.10	1.85	1.79	2.13	1.70	1.50	1.44
		S.D	0.42	0.79	0.12	0.23	0.59	0.63	0.27	0.72	0.51	0.77
7	*trans-p*-Menth-	Mean	10.27	9.90	11.49	11.23	11.78	12.39	10.06	10.82	12.49	13.37
	2-en-1-ol	S.D	1.02	2.83	0.63	1.91	1.46	1.95	2.12	1.11	1.35	2.19
8	Terpinen-4-ol	Mean	1.65	1.60	1.75	1.90	1.95	2.10	1.89	2.07	2.44	2.42
		S.D	0.56	0.78	0.70	0.54	0.93	0.69	0.89	0.94	0.99	0.80
9	*cis-p*-Menth-	Mean	7.54	7.15	7.59	7.58	8.13	8.85	7.37	8.37	9.79	9.87
	2-en-1-ol	S.D	1.31	1.38	0.94	0.66	0.77	0.57	1.69	0.68	0.82	0.91
10	*cis*-Piperitol	Mean	2.28	2.63	2.77	2.78	2.86	3.11	3.07	3.18	3.47	3.58
		S.D	0.52	0.76	0.42	0.54	0.37	0.40	0.33	0.46	0.52	0.64
11	Piperitone	Mean	2.68	2.72	2.97	3.53	3.94	3.66	3.57	3.75	4.03	4.05
		S.D	2.78	1.93	2.45	1.57	2.55	2.96	1.75	1.93	2.20	1.80
12	*trans*-Piperitol	Mean	7.71	9.85	7.17	8.83	9.21	8.52	8.74	8.36	8.71	8.48
		S.D	2.19	2.40	2.41	3.68	2.94	2.49	2.27	1.46	1.05	0.21
13	Methyl	Mean	2.11	2.37	1.58	2.95	2.89	3.04	2.94	3.19	3.48	3.30
	cinnamate	S.D	1.04	1.57	0.58	1.99	1.36	1.40	1.54	1.67	1.40	1.63
14	Spathulenol	Mean	2.12	2.71	2.20	2.98	2.25	2.28	2.44	2.48	2.18	2.11
		S.D	0.24	0.71	1.09	0.19	0.46	0.60	0.54	0.77	0.60	0.68
15	α–Eudesmol	Mean	3.94	4.67	3.39	4.22	3.04	3.28	3.22	3.83	3.52	4.03
		S.D	0.09	1.31	0.30	1.44	0.57	0.34	0.46	0.68	0.91	0.93
16	γ–Eudesmol	Mean	1.87	1.99	1.73	1.96	1.97	1.90	2.09	2.75	2.52	2.56
		S.D	0.40	0.62	0.11	0.77	0.36	0.25	0.12	0.12	0.54	0.46
17	β–Eudesmol	Mean	1.75	1.71	1.68	2.14	2.00	1.95	1.93	2.73	2.22	2.55
		S.D	0.47	0.60	0.01	0.46	0.34	0.39	0.34	0.78	0.60	0.29

Appendix 3.3B. Variation in major chemical components of *E. delegatensis* oils with time and leaf age during growth season (1987 to 1988).

(2) Sample time 5 (2/3/1987) to sample time 6 (30/3/1988)

Sample Time			5 (2/3/88)					6 (30/3/88)					
Age Code			A	B	C	D	E	A	B	C	D	E	F
Leaf age (days)			leaves	28	56	84	114	leaves	28	56	84	114	140
	Oil Yield	Mean	1.81	2.53	2.61	2.67	2.65	2.15	2.32	2.86	2.65	2.73	2.43
		S.D	0.41	0.42	0.34	0.54	0.42	0.19	0.18	0.68	0.68	0.27	0.34
1	α–Phellandrene	Mean	23.86	21.89	18.99	17.36	15.05	22.23	20.26	16.25	17.42	14.71	15.06
		S.D	1.49	2.03	2.70	3.59	6.01	1.81	2.92	4.24	5.41	5.34	4.61
2	α–Terpinene	Mean	1.67	1.32	1.08	1.13	0.81	1.27	1.20	0.85	0.91	0.74	0.88
		S.D	0.21	0.23	0.45	0.74	0.37	0.26	0.51	0.40	0.80	0.34	0.56
3	β–Phellandrene	Mean	6.78	6.64	5.22	5.76	5.10	6.79	6.14	5.93	5.83	5.74	5.48
		S.D	1.19	1.37	1.12	1.26	1.33	0.91	0.69	1.96	0.89	1.01	1.49
4	γ–Terpinene	Mean	0.77	0.60	0.61	0.46	0.42	0.73	0.60	0.71	0.50	0.45	0.38
		S.D	0.38	0.28	0.36	0.33	0.25	0.27	0.21	0.39	0.31	0.28	0.11
5	*p*-Cymene	Mean	1.68	2.48	3.78	4.01	5.21	2.13	3.34	4.68	4.97	6.46	6.08
		S.D	0.45	0.76	0.58	0.72	1.25	0.44	1.70	2.48	0.75	1.88	1.08
6	Terpinolene	Mean	1.85	1.70	1.60	1.22	1.26	1.90	1.67	1.56	1.54	1.22	1.39
		S.D	0.26	0.32	0.09	0.84	0.44	0.28	0.22	0.49	0.85	0.31	0.75
7	*trans-p*-Menth-	Mean	9.75	10.05	12.18	13.58	12.80	8.95	10.43	10.72	12.28	12.11	12.82
	2-en-1-ol	S.D	1.70	2.32	3.02	1.23	2.75	2.32	2.40	1.70	2.92	2.29	2.36
8	Terpinen-4-ol	Mean	1.95	2.00	2.36	2.54	2.46	1.83	2.01	2.32	2.67	2.69	3.20
		S.D	0.61	0.74	0.26	0.90	0.78	0.23	0.30	0.89	0.31	0.45	0.80
9	*cis-p*-Menth-	Mean	7.72	9.04	10.41	11.05	10.59	7.43	7.58	8.41	9.80	9.48	10.33
	2-en-1-ol	S.D	1.03	2.07	1.49	1.01	1.17	1.30	1.76	1.17	2.58	1.67	1.00
10	*cis*-Piperitol	Mean	2.89	3.30	3.64	3.90	4.10	2.68	3.04	3.29	4.00	3.80	4.40
		S.D	0.68	0.52	0.86	1.34	0.56	0.71	0.70	0.79	0.63	0.41	0.83
11	Piperitone	Mean	4.61	3.24	3.26	3.98	3.95	3.93	4.92	4.11	3.45	3.41	3.50
		S.D	2.19	0.67	0.67	0.91	1.33	0.70	0.69	0.99	1.31	1.25	1.03
12	*trans*-Piperitol	Mean	8.39	7.96	8.92	8.53	6.62	8.51	8.26	8.83	7.86	7.25	6.79
		S.D	2.22	0.60	1.41	1.90	1.53	1.90	2.75	4.86	2.01	1.65	1.49
13	Methyl	Mean	3.28	3.01	3.34	3.81	3.46	3.65	3.57	3.85	2.90	2.84	3.47
	cinnamate	S.D	0.65	0.89	0.79	1.72	1.23	0.54	0.56	1.30	1.43	1.49	1.65
14	Spathulenol	Mean	1.84	2.18	1.33	1.30	1.78	2.12	2.07	2.12	1.65	1.55	1.32
		S.D	1.08	1.11	0.64	0.46	0.45	0.95	0.85	0.51	0.85	0.15	0.38
15	α–Eudesmol	Mean	4.71	5.10	3.79	5.00	5.08	4.82	6.07	5.49	4.21	5.41	4.71
		S.D	0.92	1.94	1.70	1.38	1.96	1.05	0.62	0.54	1.80	0.88	0.77
16	γ–Eudesmol	Mean	2.31	2.26	3.06	3.29	3.59	3.33	3.53	3.79	2.80	3.66	2.90
		S.D	0.58	0.68	0.95	0.66	0.73	0.59	0.38	0.64	0.68	0.60	0.46
17	β–Eudesmol	Mean	2.47	1.99	2.10	2.47	2.65	2.91	3.86	2.94	2.59	3.06	2.78
		S.D	0.61	0.61	0.90	0.67	0.48	0.99	1.10	0.33	0.52	0.71	0.57

Appendix 3.3C. Variation in major chemical components of *E. globulus* (B1) oils with time and leaf age during growth season (1987 to 1988).

(1) Sample time 1 (4/11/87) to time 4 (26/1/88)

Sample Time			1 (4/11)	2 (2/12)		3 (29/12)			4 (26/1)			
Age Code			A	A	B	A	B	C	A	B	C	D
Leaf age (days)			leaves	28	56	leaves	28	56	leaves	28	56	84
	Oil Yield	Mean	2.93	2.90	3.56	2.92	3.85	4.17	3.48	3.91	4.09	4.23
		S.D	1.12	0.95	0.65	0.51	0.70	0.52	0.44	0.32	0.67	0.83
1	α–Pinene	Mean	24.42	22.23	21.76	21.97	19.29	18.23	21.73	20.73	20.16	18.99
		S.D	4.73	6.31	5.92	7.80	6.03	4.98	6.93	7.58	6.17	7.01
2	Limonene	Mean	6.26	4.92	6.32	6.42	6.30	6.41	5.52	6.30	6.35	6.71
		S.D	1.61	1.64	0.87	0.69	0.64	1.19	1.41	1.13	1.05	0.60
3	1,8-Cineole	Mean	29.23	26.41	32.11	34.00	39.51	41.01	33.77	37.37	41.10	42.30
		S.D	6.51	8.59	7.63	9.56	8.37	8.87	9.92	10.14	9.17	6.90
4	γ–Terpinene	Mean	0.47	0.40	0.89	0.97	0.84	0.96	0.95	0.91	0.63	0.76
		S.D	0.15	0.19	1.11	1.43	0.98	1.32	1.18	1.00	0.71	0.91
5	p-Cymene	Mean	0.58	0.40	0.65	0.49	0.46	0.35	0.72	0.69	0.77	0.89
		S.D	0.47	0.21	0.63	0.42	0.44	0.31	0.40	0.39	0.45	0.90
6	Aromadendrene	Mean	5.51	5.93	5.9	4.55	4.78	4.22	5.32	5.83	5.14	4.96
		S.D	1.54	0.93	1.32	1.26	1.39	1.52	2.50	2.21	2.03	1.71
7	Terpinyl acetate	Mean	3.77	4.60	3.27	3.31	2.35	2.84	3.64	2.82	2.74	2.35
		S.D	3.44	3.72	3.20	2.48	2.05	2.53	2.04	1.69	1.45	0.86
8	α–Terpineol	Mean	4.62	5.81	5.53	4.62	5.10	5.25	4.38	3.24	2.77	2.79
		S.D	2.86	3.77	2.88	4.14	2.92	1.85	3.30	2.27	2.36	2.36
9	Globulol	Mean	10.33	11.55	9.84	9.20	7.91	8.21	9.13	8.70	8.28	7.70
		S.D	2.04	3.00	1.53	2.74	1.71	3.00	2.44	2.69	2.97	2.44
10	Spathulenol	Mean	1.42	1.49	1.26	1.09	0.94	0.88	1.02	0.96	1.01	1.00
		S.D	1.27	1.15	0.99	0.84	0.69	0.68	0.85	0.60	0.76	0.70

Appendix 3.3C. Continued

(2) Sample time 5 (24/2/88) to time 6 (23/3/88)

Sample Time			5 (24/2)					6 (23/3/88)					
Age Code			A	B	C	D	E	A	B	C	D	E	F
Leaf age (dasy)			leaves	28	56	84	112	leaves	28	56	84	114	140
	Oil Yield	Mean	3.67	4.04	4.43	4.32	4.21	4.20	4.59	4.87	4.67	4.74	4.81
		S.D	0.70	0.52	0.48	0.91	0.86	0.79	0.95	0.94	0.78	1.08	1.37
1	α–Pinene	Mean	23.45	18.60	17.48	15.63	18.24	21.58	18.78	18.52	19.48	16.83	18.38
		S.D	11.73	7.00	5.91	2.11	5.07	7.44	6.59	5.73	5.84	5.85	4.32
2	Limonene	Mean	6.95	6.11	6.27	6.66	6.37	6.24	6.41	6.75	6.14	5.32	6.42
		S.D	2.11	1.68	1.41	0.80	1.14	2.00	1.61	1.87	1.60	3.04	1.31
3	1,8-Cineole	Mean	37.41	39.15	43.89	42.33	41.00	37.14	41.57	44.12	45.10	46.28	46.16
		S.D	12.29	10.07	10.01	7.66	7.36	10.86	10.59	8.58	9.56	9.26	7.38
4	γ–Terpinene	Mean	0.79	0.69	0.77	0.63	0.59	0.56	1.27	0.70	0.58	0.50	0.44
		S.D	1.05	0.83	0.87	0.71	0.55	0.67	1.33	0.56	0.42	0.23	0.38
5	p-Cymene	Mean	0.98	1.19	0.84	1.05	0.83	1.23	1.22	1.04	1.36	1.17	1.50
		S.D	0.49	0.48	0.41	0.35	0.19	0.74	0.69	0.56	0.89	0.62	0.75
6	Aromadendrene	Mean	5.35	5.85	5.18	6.25	6.11	5.35	5.63	4.83	5.75	5.47	5.65
		S.D	2.79	2.10	1.89	1.89	1.63	3.54	2.93	2.82	2.37	2.54	1.92
7	Terpinyl acetate	Mean	2.86	2.71	2.47	2.55	2.60	2.75	2.78	2.72	2.80	2.75	2.95
		S.D	1.66	1.20	0.94	0.92	0.98	1.49	1.49	1.06	1.11	0.79	0.88
8	α–Terpineol	Mean	3.61	3.20	2.25	2.23	1.99	3.38	2.41	1.58	1.93	1.43	0.80
		S.D	3.10	1.84	1.11	1.22	0.99	2.99	1.52	0.61	1.21	1.15	0.74
9	Globulol	Mean	8.73	9.15	7.77	9.11	8.02	8.30	7.86	7.99	7.21	7.92	7.26
		S.D	3.60	3.49	2.79	2.72	2.55	2.73	2.33	3.70	4.09	3.58	2.55
10	Spathulenol	Mean	1.18	1.21	0.86	1.19	1.01	0.97	1.00	1.07	1.01	1.02	1.13
		S.D	0.89	0.72	0.52	0.74	0.51	0.65	0.70	0.71	0.75	0.59	0.67

Appendix 3.3C. Continued.

(3) Sample time 7 (20/4/88)

Sample Time			7 (20/4/88)						
Age Code			A	B	C	D	E	F	G
Leaf age (days)			leaves	28	56	84	114	140	168
	Oil Yield	Mean	4.34	4.79	4.65	4.91	4.71	4.66	4.27
		S.D	0.98	0.78	1.35	1.02	1.09	1.51	1.17
1	α–Pinene	Mean	19.92	17.26	15.93	17.81	15.80	17.45	17.17
		S.D	6.68	5.62	5.08	4.68	5.15	5.19	3.79
2	Limonene	Mean	6.88	7.02	6.85	6.48	6.99	6.61	6.60
		S.D	2.27	1.99	1.65	1.48	1.43	1.55	1.28
3	1,8-Cineole	Mean	40.49	43.70	46.46	43.43	48.40	44.36	45.37
		S.D	11.61	10.88	11.41	10.88	9.03	9.21	8.32
4	γ–Terpinene	Mean	0.38	0.35	0.27	0.52	0.47	0.39	0.43
		S.D	0.35	0.18	0.13	0.17	0.25	0.23	0.33
5	p-Cymene	Mean	1.12	1.21	1.29	0.94	1.10	1.27	1.46
		S.D	0.76	0.84	0.61	0.74	0.34	0.68	0.71
6	Aromadendrene	Mean	5.61	5.21	7.78	7.18	5.81	6.55	6.06
		S.D	3.37	3.55	5.35	4.77	1.92	2.80	2.05
7	Terpinyl acetate	Mean	3.05	3.36	3.17	3.20	2.55	2.75	2.44
		S.D	1.96	1.72	0.81	1.19	0.97	0.89	0.33
8	α–Terpineol	Mean	3.03	1.98	1.66	1.71	1.50	1.17	1.26
		S.D	1.95	1.15	0.93	0.72	0.52	0.55	0.45
9	Globulol	Mean	7.06	7.64	7.13	7.68	6.95	7.28	7.32
		S.D	3.00	3.24	3.06	3.36	2.69	2.73	2.56
10	Spathulenol	Mean	0.89	0.93	1.13	1.05	1.12	0.97	0.80
		S.D	0.66	0.73	0.78	0.76	0.60	0.52	0.37

Table 4.1. The leaf sample locations of 29 *Eucalyptus* species in Tasmania Altitude and geographic position of populations were ascertained from maps (Tas map 1:100,000 topographic); * P&J code = Pryor and Johnson's species code (1971).
† A = adult leaves; J = juvenile leaves.

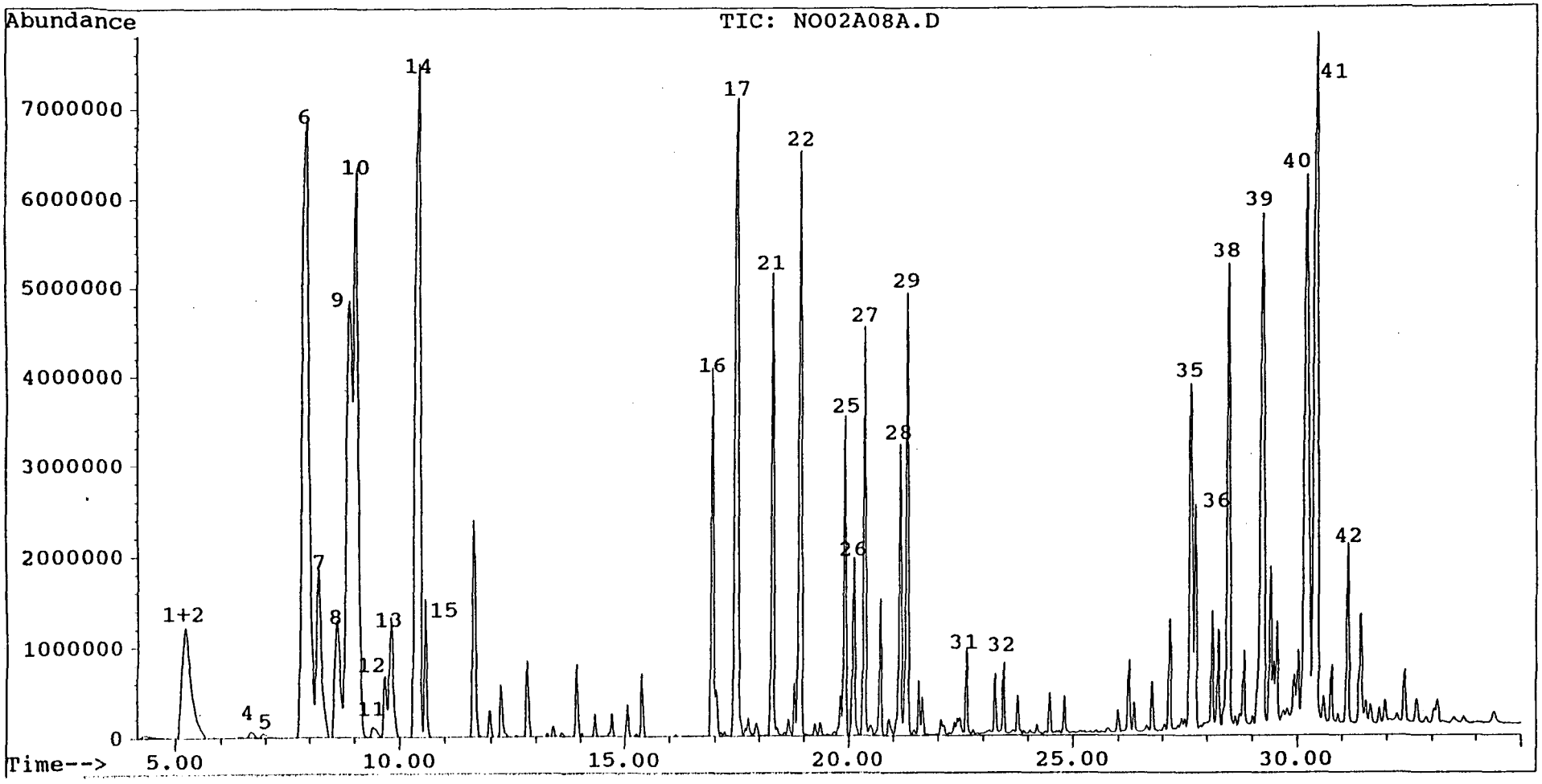
(1) Subgenus *Monocalyptus*

Species	Locality	Population code on map	Altitude (m)	Grid Reference E/W	Grid Reference N/S	Species Code	Population Code	P&J Code*	Foliage† Type
Series *Obliquae*									
E. obliqua	Summerleas Rd.	O1	440	5213	52475	OBL	OBL FeTr	MAKAA	A, J
E. obliqua	Elephants Pass	O2	470	6018	53909	OBL	OBL ElPass	MAKAA	A, J
E. obliqua	Olivers Road	O3	500	4328	54982	OBL	OBL Oliv	MAKAA	A, J
E. obliqua	Roseberry	O4	160	3767	53724	OBL	OBL Roseb	MAKAA	A, J
E. obliqua	Cethana	O5	320	4290	54086	OBL	OBL Ceth	MAKAA	A, J
E. obliqua	Margate Quarry	O6	160	5189	52333	OBL	OBL MaQu	MAKAA	A, J
E. obliqua	Randalls Bay	O7	80	5104	52132	OBL	OBL RaBa	MAKAA	A, J
E. obliqua	Little Hill	O8	100	5342	54582	OBL	OBL LiHi	MAKAA	A, J
E. obliqua	Raminea	O9	60	4945	52030	OBL	OBL Rami	MAKAA	A, J
E. delegatensis	Mt. Wellington	D1	550	5203	51483	DEL	DEL MtWe	MAKBE	A, J
E. delegatensis	Poatina High way	D2	1140	4870	53658	DEL	DEL PoHw	MAKBE	A, J
E. delegatensis	Bakers Tier	D3	760	4910	53345	DEL	DEL BaTi	MAKBE	A, J
E. delegatensis	Quailes Hill	D4	850	4113	53931	DEL	DEL QuHi	MAKBE	A, J
E. delegatensis	Gads Hills	D5	740	4303	54965	DEL	DEL GaHi	MAKBE	A, J
E. delegatensis	Snug Plains	D6	600	5153	52298	DEL	DEL SnPl	MAKBE	A, J
E. delegatensis	Guildford	D7	580	3759	54255	DEL	DEL Guil	MAKBE	A, J
E. delegatensis	Maydena	D8	660	4767	52896	DEL	DEL Mayd	MAKBE	A, J
E. delegatensis	Esperance River	D9	550	4865	52108	DEL	DEL EsRi	MAKBE	A, J
E. delegatensis	Pecks Hill	D10	470	5267	54253	DEL	DEL PeHi	MAKBE	A, J
E. regnans	Moogara	R1	500	4923	52630	REG	REG Moog	MAKCA	A, J
E. regnans	Leslie Vale	R2	340	5191	52444	REG	REG LeVa	MAKCA	A, J
E. regnans	Snug Plains	R3	480	5157	52319	REG	REG SnPl	MAKCA	A, J
E. regnans	Irocliffe Rd.	R4	210	4185	54447	REG	REG IrRd	MAKCA	A, J
E. regnans	Briddale	R5	590	5307	54583	REG	REG Brid	MAKCA	A, J
E. regnans	Ker Mandie Divide	R6	250	4904	52125	REG	REG KeDi	MAKCA	A, J
E. sieberi	Friendly Beach	S1	40	6053	53491	SIE	SIE FrBe	MAKED	A, J
E. sieberi	Elephants Pass	S2	160	6050	53863	SIE	SIE ElPa	MAKED	A, J
E. pauciflora	Derwent Bridge	P1	750	4363	53347	PAU	PAU DeBr	MAKHA	A, J
E. pauciflora	Pensford	P2	960	4838	53479	PAU	PAU Pensf	MAKHA	A, J
E. pauciflora	Bothwell	P3	380	4989	53107	PAU	PAU Both	MAKHA	A, J
E. pauciflora	Conara Junction	P4	200	5413	53690	PAU	PAU CoJu	MAKHA	A, J
E. pauciflora	WesterWay	P5	250	4815	52740	PAU	PAU WeWa	MAKHA	A, J
Series *Piperitae*									
E. risdonii	Warrane/Risdon Vale	r1	100	5293	52609	RIS	RIS RiVa	MATEB	A, J
E. risdonii	Government Hills	r2	100	5268	52585	RIS	RIS GoHi	MATEB	A, J
E. risdonii	Cultivated	r3	60	5230	52397	RIS	RIS GHCu	MATEB	A, J
E. tenuiramis	Bothwell tip	T1	400	5030	53071	TEN	TEN BoTi	MATEC	A, J
E. tenuiramis	Randalls Bay	T2	80	5104	52132	TEN	TEN RaBa	MATEC	A, J
E. tenuiramis	Huon Rd.	T3	280	5233	52493	TEN	TEN HuRd	MATEC	A, J
E. tenuiramis	Bicheno	T4	40	6057	53650	TEN	TEN Bich	MATEC	A, J
E. pulchella	Cherry Tree Hill	p1	60	5925	53511	PUL	PUL CTHi	MATEG	A, J
E. pulchella	Harris Creek	p2	20	5116	52110	PUL	PUL HaCr	MATEG	A, J
E. pulchella	Moogara	p3	320	4946	52624	PUL	PUL Moog	MATEG	A, J
E. pulchella	Lea-Kingston	p4	50	5254	52438	PUL	PUL LeaK	MATEG	A, J
E. pulchella	Margate quarry	p5	160	5189	52333	PUL	PUL MaQu	MATEG	A, J
E. amygdalina	Cape Queen Elizabeth	A1	20	5324	52122	AMY	AMY CQE	MATEH	A, J
E. amygdalina	Lea-Kingston	A2	70	5235	52407	AMY	AMY LeaK	MATEH	A, J
E. amygdalina	Baker Tier	A3	640	4920	53300	AMY	AMY BaTi	MATEH	A, J
E. amygdalina	Bicheno	A4	40	6041	53658	AMY	AMY Bich	MATEH	A, J
E. amygdalina	Carrick-217Km	A5	140	4976	54024	AMY	AMY Carr	MATEH	A, J
E. amygdalina	Cethana	A6	50	4355	54300	AMY	AMY Ceth	MATEH	A, J
E. amygdalina	Government	A7	80	4265	52584	AMY	AMY GoHi	MATEH	A, J
E. amygdalina	Derwent Bridge	A8	750	4398	53368	AMY	AMY DeBr	MATEH	A, J
E. nitida	Dove Lake Road	N1	950	4132	53887	NIT	NIT DoLa	MATEJ	A, J
E. nitida	Que River	N2	640	3900	53961	NIT	NIT QuRi	MATEJ	A, J
E. nitida	Little Florentine	N3	440	4528	52385	NIT	NIT LiFl	MATEJ	A, J
E. nitida	Hartz Mt. Rd.	N4	780	4812	52163	NIT	NIT HaMt	MATEJ	A, J
E. nitida	Tahune roserve Rd.	N5	160	4841	52218	NIT	NIT TaRe	MATEJ	A, J
E. nitida	Quailes Hill	N6	850	4113	53931	NIT	NIT QuHi	MATEJ	A, J
E. nitida/amyg	Claude Hill	N7	600	4310	54090	NIT	NIT ClHi	MATEJ	A, J
E. nitida	Strahan road	N8	160	4644	53618	NIT	NIT StRd	MATEJ	A, J
E. nitida	Mt. Arrowsmith Rd	N9	580	4235	53256	NIT	NIT MtAr	MATEJ	A, J
E. nitida	Raglan River	N10	480	4007	53361	NIT	NIT RaRi	MATEJ	A, J
E. nitida	Strahan Road	N11	200	3664	53327	NIT	NIT Stra	MATEJ	A, J
E. radiata	Lemonthyme	RA	330	4282	53937	RAD	RAD LeMo	MATEL	A, J
E. coccifera	Snug Tiers	C1	640	5138	52308	COC	COC SnPl	MATES	A, J
E. coccifera	Alma Tier	C2	1040	5044	53377	COC	COC AlTi	MATES	A, J
E. coccifera	Pine Lake	C3	1150	4760	53777	COC	COC PiLa	MATES	A, J
E. coccifera	Projection Bluff	C4	1100	4755	53783	COC	COC PrBl	MATES	A, J
E. coccifera	Mt. Wellington	C5	1100	5200	52513	COC	COC MtWe	MATES	A, J
E. cocci/niti	Mt. Arrowsmith	C6	860	4242	53263	COC	COC MtAr	MATES	A, J
E. coccifera	Hansens' Peak	C7	1080	4140	53868	COC	COC HaPk	MATES	A, J
E. cocci/niti	Mt. Arrowsmith	C8	970	4237	53263	COC	COC MtAs	MATES	A, J

Table 4.1. Continued.

(2) Subgenus *Symphyomyrtus*

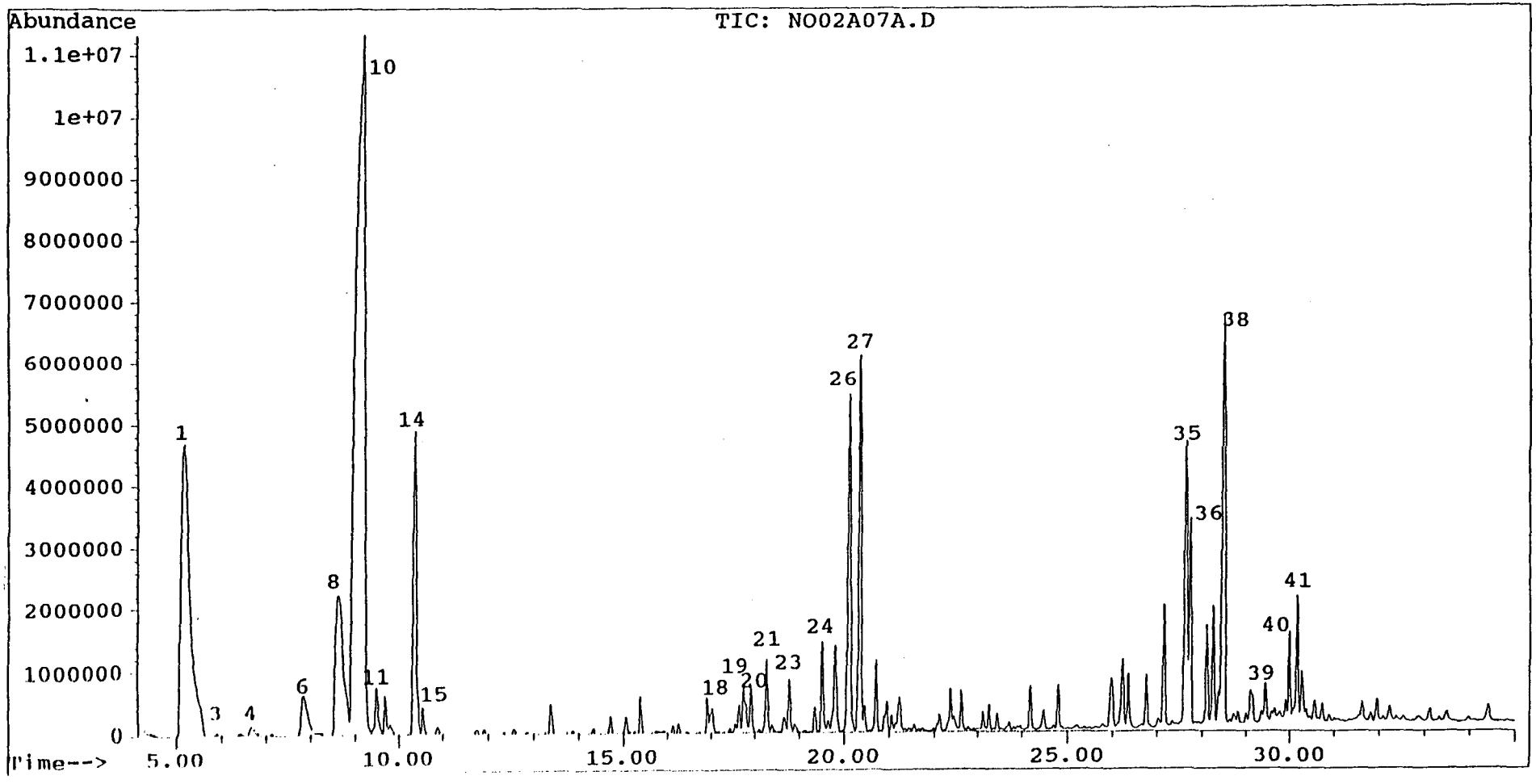
Species	Locality	Map Code	Altitude (m)	Grid Reference E/W	Grid Reference N/S	Species Code	Population Code	P&J Code*	Foliage Type
Series *Ovatae*									
E. barberi	M. Road	B1	330	5715	53162	BAR	BAR MRd	SPEA1	A, J
E. barberi	Cherry tree Hill	B2	170	5947	53517	BAR	BAR ChTr	SPEA1	A, J
E. brookeriana	Sumach Road (NW)	b1	160	3342	54421	BRO	BRO SuRd	SPEA2	A, J
E. brookeriana	Rocka Rurlet	b2	440	5700	53206	BRO	BRO MRd	SPEA2	A, J
E. brookeriana	Elephant Pass	b3	460	6018	53909	BRO	BRO ElPa	SPEA2	A, J
E. brookeriana/ovata	Strahan road	b4	160	4644	53618	BRO	BRO StRd	SPEA2	A, J
E. ovata	Harris Creek	o1	20	5116	52110	OVA	OVA HaCr	SPEAB	A, J
E. ovata	Cherry tree Hill	o2	120	5925	53504	OVA	OVA CTHi	SPEAB	A, J
E. ovata	Henty River	o3	40	3575	53418	OVA	OVA HeRi	SPEAB	A, J
E. ovata	Lea-Kingston	o4	50	5253	52447	OVA	OVA LeaK	SPEAB	A, J
E. ovata	Nations Hill	o5	150	4783	52742	OVA	OVA NaHi	SPEAB	A, J
E. ovata	Montagu Rd.	o6	30	3122	54835	OVA	OVA MoRd	SPEAB	A, J
E. rodwayi	M. Road	Ro1	540	5710	53253	ROD	ROD MRd	SPEAH	A, J
E. rodwayi	Steppes	Ro2	820	4909	53377	ROD	ROD Step	SPEAH	A, J
E. rodwayi	Nive Plains	Ro3	680	4506	53330	ROD	ROD NiPl	SPEAH	A, J
E. rodwayi	Strictland	Ro4	550	4717	52115	ROD	ROD Stri	SPEAH	A, J
Series *Ovatae*									
E. globulus	Moogara	G1	500	4910	52610	GLO	GLO Moog	SPIFL	A, J
E. globulus	Mayfield	G2	10	5833	53225	GLO	GLO Mayf	SPIFL	A, J
E. globulus	Lea-Kingston	G3	50	5259	52424	GLO	GLO LeaK	SPIFL	A, J
E. globulus	Hermons Rd.	G4	40	4940	52164	GLO	GLO HeRd	SPIFL	A, J
E. globulus	Mount Elephant	G5	280	6043	53905	GLO	GLO MoEl	SPIFL	A, J
E. globulus	Barnes Bay	G6	30	5295	52258	GLO	GLO BaBa	SPIFL	A, J
E. vernicosa	Lake Esperance	V1	970	4812	52137	VER	VER LaEs	SPIJAA	A, J
E. vernicosa (Big expose W. side)	Mt. Arrowsmith (b)	V2	960	4235	53263	VER	VER MtA1	SPIJAA	A, J
E. vernicosa (Small expose W. side)	Mt. Arrowsmith(s)	V3	960	4236	53263	VER	VER MTA2	SPIJAA	A, J
E. vernicosa (parvula)	Mt. Arrowsmith	V4	910	4235	53262	VER	VER MtA3	SPIJAA	A, J
E. subcrenulata	Hartz Mt. Rd.	s1	780	4812	52163	SUB	SUB HaMt	SPIJAB	A, J
E. subcrenulata	Mt. Arrowsmith	s2	860	4234	53263	SUB	SUB MtA4	SPIJAB	A, J
E. subcrenulata	Dove Lake	s3	950	4128	53888	SUB	SUB DoLa	SPIJAB	A, J
E. subcrenulata	Hansan's peak	s4	1040	4139	53874	SUB	SUB HaPk	SPIJAB	A, J
E. subcrenulata	Poatina High way	s5	1140	4874	53669	SUB	SUB PoHw	SPIJAB	A, J
E. johnstonii (columnaris)	Mt. Arrowsmith	J1	720	4234	53258	JOH	JOH MtA5	SPIJAC	A, J
E. johnstonii (columnaris)	Mt. Arrowsmith	J2	620	4235	53258	JOH	JOH MtA6	SPIJAC	A, J
E. johnstonii (columnaris)	Mt. Arrowsmith	J3	580	4234	53257	JOH	JOH MTA7	SPIJAC	A, J
E. johnstonii	Mt. Wellington	J4	600	5203	51483	JOH	JOH MtWe	SPIJAC	A, J
E. johnstonii	Snug Tiers	J5	660	5142	52314	JOH	JOH SnPl	SPIJAC	A, J
E. viminalis	Stony river	v1	20	5886	53326	VIM	VIM StRi	SPIKK	A, J
E. viminalis	Conara Junction	v2	200	5413	53690	VIM	VIM CoJu	SPIKK	A, J
E. viminalis	Wilmot Bridge	v3	150	4299	54217	VIM	VIM WiBr	SPIKK	A, J
E. viminalis	Henty river	v4	50	3567	53460	VIM	VIM HeRi	SPIKK	A, J
E. viminalis	Lea-Kingston	v5	40	5253	52441	VIM	VIM LeaK	SPIKK	A, J
E. viminalis	Government Hills	v6	100	5260	52581	VIM	VIM GoHi	SPIKK	A, J
Vim/Dal	Lemonthyme	Vd1	330	4282	53937	V/D	V/D LePo	SPIKK	A, J
Vim/Dal	Olivers Road	Vd2	500	4328	54982	V/D	V/D OlRd	SPIKK	A, J
E. dalrympleana	Pensford	d1	960	4838	53479	DAL	DAL Pens	SPINCA	A, J
E. dalrympleana	Derwent Bridge	d2	750	4363	53347	DAL	DAL DeBr	SPINCA	A, J
E. dalrympleana	Brady's Lake	d3	680	4592	53275	DAL	DAL BrLa	SPINCA	A, J
E. rubida	Strickland	Ru1	550	4717	52115	RUB	RUB Stri	SPINF	A, J
E. rubida	Bothwell	Ru2	380	5052	53071	RUB	RUB Both	SPINF	A, J
E. gunnii	Pine Lake	g1	1150	4760	53777	GUN	GUN PiLa	SPINI	A, J
E. gunnii	Shannon Lagoon	g2	1050	4805	53513	GUN	GUN ShLa	SPINI	A, J
E. gunnii	Mayday Plain	g3	720	4028	54002	GUN	GUN MaPl	SPINI	A, J
E. gunnii	King William Saddle	g4	800	4268	53263	GUN	GUN KiWi	SPINI	A, J
E. gunnii	Cradle Valley Road	g5	880	4122	53903	GUN	GUN CrVa	SPINI	A, J
E. gunnii	Snug Plains	g6	600	5132	52304	GUN	GUN SnPl	SPINI	A, J
E. archeri	Stacks Bluff	a1	1120	5588	53917	ARC	ARC StBl	SPNIB	A, J
E. archeri	Carr Villa	a2	1230	5526	54041	ARC	ARC CaVi	SPNIB	A, J
E. archeri	Projection Bluff	a3	1100	4770	53809	ARC	ARC PrBl	SPNIB	A, J
E. morrisbyi	Government Hills	M1	80	5268	52581	MOR	MOR GoHi	SPNK	A, J
E. morrisbyi	Calverts Hill	M2	70	5432	52454	MOR	MOR CaHi	SPNK	A, J
E. urnigera	Alma Tiers	U1	1010	5037	53375	URN	URN AlTi	SPNL	A, J
E. urnigera	Mt.Wellington	U2	600	5203	51483	URN	URN MtWl	SPNL	A, J
E. urnigera	Mt.Wellington	U3	1100	5203	52513	URN	URN MtWh	SPNL	A, J
E. urnigera	Snug Tiers	U4	640	5138	52311	URN	URN SnPl	SPNL	A, J
E. perriniana	Strickland	Pe	550	4717	52115	PER	PER Stri	SPNN	A, J
E. cordata	Cape Queen Elizabeth	c1	100	5345	52109	COR	COR CQE	SPNO	A, J
E. cordata	Moogara	c2	460	4930	52613	COR	COR Moog	SPNO	A, J
E. cordata	Chimney Pot Hill	c3	560	5120	52317	COR	COR CPH	SPNO	A, J
E. cordata	Snug Plains	c4	580	5125	52309	COR	COR SnPl	SPNO	A, J
E. cordata	Electrona	c5	140	5200	52335	COR	COR Elec	SPNO	A, J



Appendix 4.2A. GLC separation of the components from the oil of *E. coccifera* (AlTi) adult leaves.

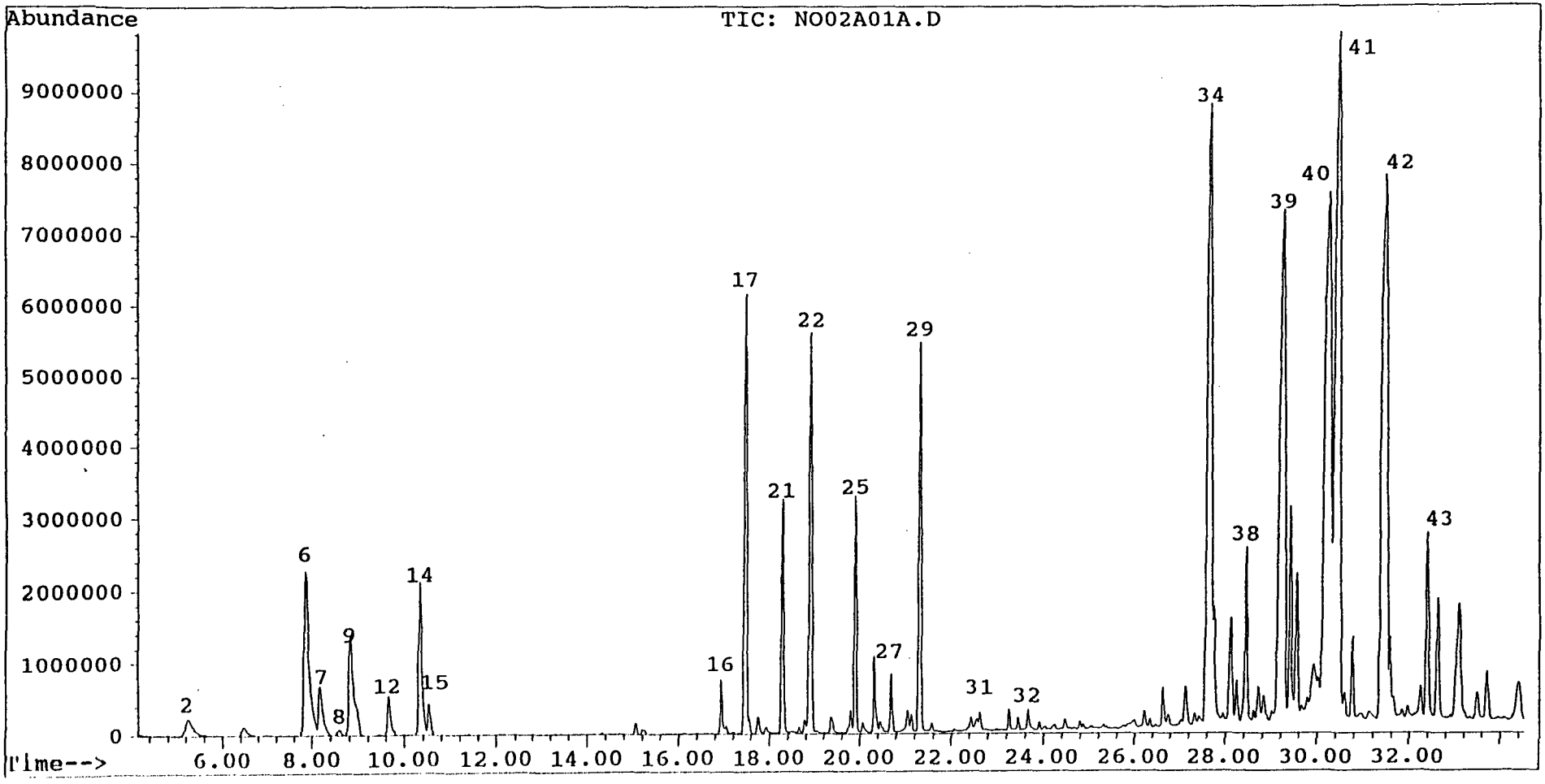
Compound peaks:

1	α-Pinene	8	Limonene	14	p-Cymene	22	cis-p-Menth-2-en-1-ol	29	trans-Piperitol	38	Spathulenol
2	α-Thujene	9	β-Phellandrene	15	Terpinolene	25	cis-Piperitol	31	cis-Sabinol	39	α-Eudesmol
4	β-Pinene	10	1,8-Cineole	16	Linalool	26	Terpinyl acetate	32	p-Cymene-8-ol	40	γ-Eudesmol
5	Sabinene	11	cis-β-Ocimene	17	trans-p-Menth-2-en-1-ol	27	α-Terpineol	35	Globulol	41	β-Eudesmol
6	α-Phellandrene	12	γ-Terpinene	21	Terpinen-4-ol	28	Piperitone	36	Viridiflorol	42	Tasmanone Type 1
7	α-Terpinene	13	trans-β-Ocimene								



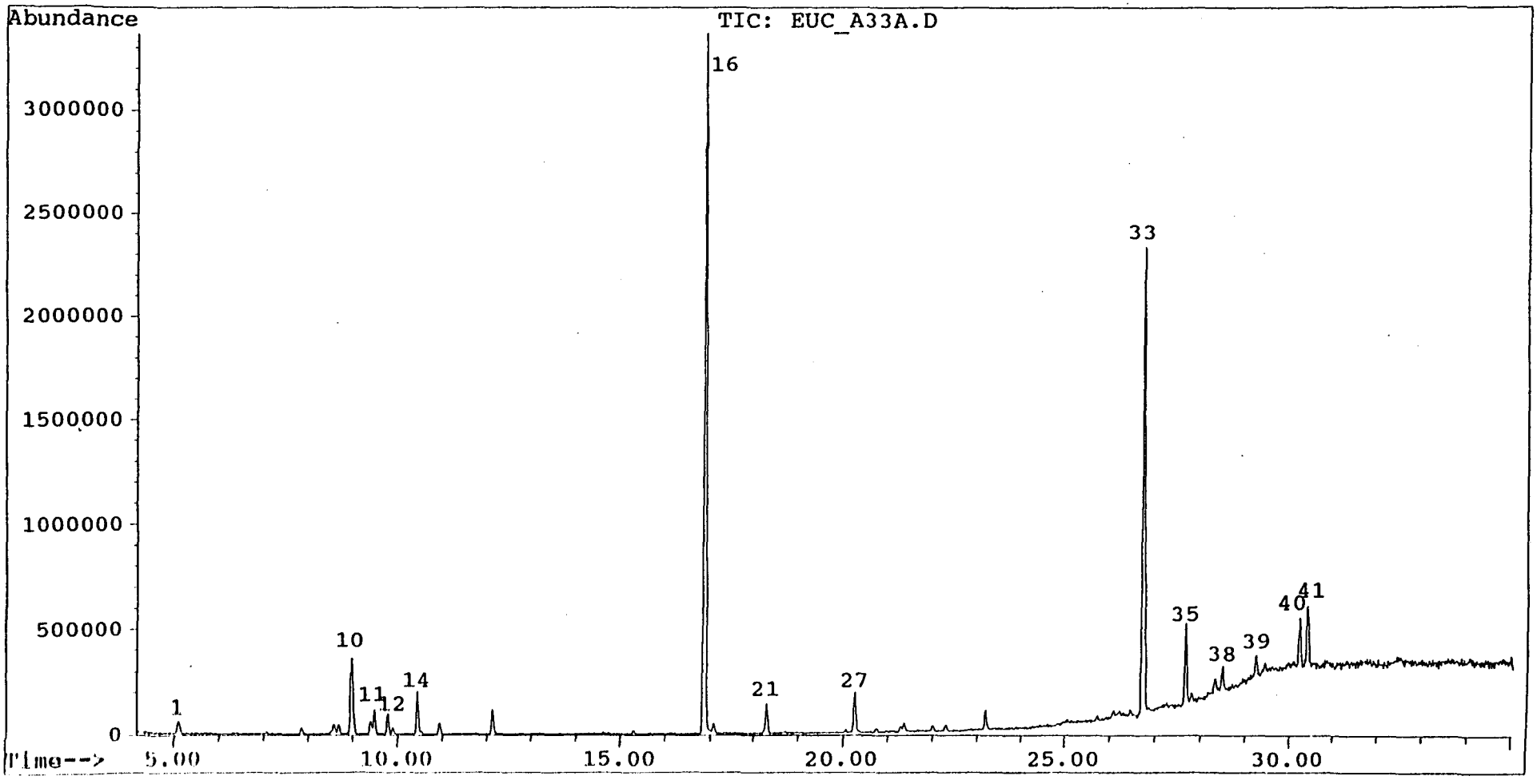
Appendix 4.2B. GLC separation of the components from the oil of *E. viminalis* adult leaves. (see Table 4.4A in chapter 4 for compound name)

Peak 1: α–pinene; 8: limonene; 10: 1,8-cineole; 14: p-cymene; 26: terpinyl acetate; 27: α–terpineol; 35: globulol; 38: spathulenol.



Appendix 4.2C. GLC separation of the components from the oil of *E. regnans* adult leaves. (see Table 4.4A in Chapter 4 for compound name)

Peak 6: α–phellandrene; 9: β–phellandrene; 14: p-cymene; 17: trans-p-menth-2-en-1-ol; 21: terpinen-4-ol; 22: cis-p-Menth-2-en-1-ol; 25: cis-piperitol; 27: α–terpineol; 29: trans-piperitol; 34: elemol; 39: α–eudesmol; 40: γ–eudesmol; 41: β–eudesmol; 42: Tasmanone type compounds (1).



Appendix 4.2D. GLC separation of the components from the oil of *E. ovata* adult leaves. (see Table 4.4A in Chapter 4 for compound name)

Peak 16: linalool; 33: nerolidol

Appendix 4.3A. Part 1. The correlation matrix of chemical components based on percentage composition data of the adult leaf oils of Tasmanian eucalypt species populations.
n = 146 *V* = 145, (F> 0.1614 P<0.05; F > 0.2111, P < 0.01; F > 0.2678, P < 0.001) +++ : p<0.001, ++ : 0.01>p>0.001, + : 0.05>p>0.01, ns : p>0.05. + = positively correlated - = negatively correlated

	Compounds	1 αP	2 αTh	3 Cam	4 βP	5 S	6 αPh	7 αT	8 Lim	9 βPh	10 Ci	11 cO	12 γT	13 tO	14 Cy	15 Te	16 Lin	17 tM	18 Pi	19 Ca	20 Aro	21 T4	22 CM	23 All
1	α–Pinene	1.00	—	+++	+++	—	—	—	+++	—	+++	ns	ns	—	—	—	ns	—	+++	+	ns	—	—	+
2	α–Thujene	-.62	1.00	—	—	+++	+++	+++	—	+++	—	ns	ns	+++	+++	+++	ns	+++	—	-	-	+++	+++	ns
3	Camphene	.63	-.43	1.00	+++	—	—	—	+++	—	+++	ns	ns	—	—	—	ns	—	+++	++	ns	—	—	ns
4	β–Pinene	.56	-.55	.37	1.00	-.14	—	—	+++	—	+++	ns	ns	ns	—	—	ns	—	+++	+	ns	—	—	++
5	Sabinene	-.35	.28	-.23	-.14	1.00	ns	+	–	+	–	ns	ns	ns	++	ns	ns	+++	–	ns	ns	+++	+++	ns
6	α–Phellandrene	-.59	.80	-.39	-.48	.16	1.00	+++	—	+++	—	ns	ns	+++	+++	+++	ns	+++	—	ns	ns	+++	+++	ns
7	α–Terpinene	-.60	.79	-.43	-.53	.19	.87	1.00	—	+++	—	ns	ns	+++	+++	+++	ns	+++	—	-	-	+++	+++	–
8	Limonene	.68	-.65	.48	.52	-.26	-.61	-.64	1.00	—	+++	ns	ns	–	—	—	ns	—	+++	ns	ns	—	—	ns
9	β–Phellandrene	-.59	.75	-.43	-.52	.18	.77	.91	-.65	1.00	—	ns	ns	+++	+++	+++	ns	+++	—	-	–	+++	+++	-
10	1,8-Cineole	.71	-.67	.50	.54	-.25	-.65	-.66	.95	-.67	1.00	-	ns	–	—	—	-	—	+++	+	ns	—	—	ns
11	*cis*-β–Ocimene	-.16	-.02	-.14	-.05	-.09	-.09	-.10	-.16	-.03	-.17	1.00	+	ns	++	ns	+++	ns	ns	ns	ns	ns	ns	+
12	γ–Terpinene	.09	-.15	.11	.11	-.11	-.07	-.07	.11	-.07	.05	.21	1.00	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns
13	*trans*-β–Ocimene	-.33	.29	-.23	-.13	.16	.37	.30	-.25	.29	-.25	-.01	-.07	1.00	ns	+++	ns	+	-	ns	ns	++	+	ns
14	*p*-Cymene	-.55	.51	-.36	-.47	.26	.42	.52	-.70	.56	-.73	.27	.04	.01	1.00	++	ns	+++	—	ns	ns	+++	+++	ns
15	Terpinolene	-.50	.73	-.36	-.38	.11	.90	.76	-.53	.65	-.55	-.10	.02	.41	.23	1.00	ns	+++	—	-	-	+++	+++	ns
16	Linalool	-.15	-.04	-.08	-.04	-.07	-.04	-.02	-.14	.04	-.20	.37	.45	.07	.10	-.02	1.00	ns	ns	ns	++	ns	ns	ns
17	*trans-p*-Menth-2-en-1-ol	-.63	.68	-.42	-.54	.31	.58	.69	-.68	.67	-.68	-.08	-.12	.21	.56	.54	-.03	1.00	—	-	–	+++	+++	—
18	Pinocarvone	.54	-.40	.39	.31	-.24	-.39	-.38	.32	-.37	.48	-.09	-.05	-.20	-.37	-.35	-.11	-.39	1.00	+++	+	—	—	+++
19	b-Caryophellene	.17	-.18	.22	.19	-.07	-.16	-.19	.07	-.19	.18	.05	-.04	-.10	-.13	-.18	-.05	-.20	.52	1.00	+++	-	-	+++
20	Aromadendrene	.10	-.18	.05	.14	-.13	-.16	-.21	.07	-.22	.07	.17	.02	-.12	-.02	-.19	.22	-.24	.20	.31	1.00	-	–	+++
21	Terpinen-4-ol	-.67	.65	-.44	-.49	.33	.70	.78	-.64	.73	-.67	.08	.09	.22	.66	.63	.16	.70	-.45	-.20	-.18	1.00	+++	–
22	*cis-p*-Menth-2-en-1-ol	-.62	.66	-.42	-.53	.32	.56	.66	-.68	.63	-.69	-.08	-.12	.20	.55	.52	-.02	.99	-.39	-.20	-.24	.67	1.00	—
23	*Allo*-aromadendrene	.17	-.10	.16	.24	-.15	-.10	-.24	.01	-.21	.02	.20	.05	-.15	.12	-.16	.00	-.29	.29	.32	.55	-.22	-.29	1.00
24	*trans*-Pinocarveol	.59	-.43	.42	.33	-.21	-.43	-.42	.38	-.41	.53	-.11	-.08	-.23	-.40	-.39	-.13	-.42	.87	.64	.21	-.48	-.42	.20
25	*cis*-Piperitol	-.57	.58	-.34	-.44	.24	.52	.54	-.65	.53	-.66	.01	-.13	.18	.56	.46	-.05	.83	-.34	-.19	-.18	.57	.83	-.14
26	Terpinyl acetate	.05	-.32	-.04	.09	.08	-.30	-.26	.40	-.22	.39	.09	-.05	.00	-.18	-.22	-.04	-.23	.03	-.02	-.11	-.16	-.23	-.10
27	α–Terpineol	.25	-.42	.18	.38	-.19	-.32	-.36	.54	-.33	.52	.01	.09	-.07	-.37	-.28	.14	-.36	.03	-.06	-.04	-.26	-.37	-.12
28	Piperitone	-.29	.20	-.22	-.27	.23	.25	.20	-.33	.17	-.33	.02	.00	.04	.38	.33	-.02	.23	-.19	-.09	-.13	.47	.24	-.15
29	*trans*-Piperitol	-.56	.51	-.37	-.48	.24	.42	.50	-.58	.49	-.59	-.02	-.07	.21	.47	.39	.04	.81	-.34	-.17	-.21	.58	.81	-.25
30	Citronellal	-.24	.19	-.16	-.02	.20	.04	.06	-.07	.11	-.09	.04	.09	.15	.05	.03	.34	.11	-.17	-.09	-.05	.12	.12	-.07
31	*cis*-Sabinol	-.35	.23	-.23	-.18	.68	.21	.24	-.26	.16	-.28	-.10	-.05	.17	.31	.13	-.05	.36	-.26	-.06	-.07	.45	.39	-.13
32	*p*-Cymene-8-ol	-.42	.24	-.29	-.38	.42	.16	.39	-.49	.42	-.49	.08	-.01	.02	.73	.07	.03	.52	-.26	-.13	-.14	.57	.54	-.21
33	Nerolidol	-.02	-.11	.00	.00	-.09	-.10	-.10	-.02	-.06	-.08	.25	.27	.02	-.03	-.06	.79	-.11	-.05	-.02	.12	.01	-.10	.07
34	Elemol	-.22	-.01	-.14	-.10	.18	-.03	.02	-.27	.01	-.27	-.06	-.13	.08	-.06	-.05	-.02	.09	-.13	-.07	-.10	-.02	.10	-.13
35	Globulol	-.09	-.05	.01	.10	-.17	-.03	-.20	-.18	-.16	-.21	.46	.14	.03	.21	-.12	.34	-.21	.02	.27	.56	-.11	-.20	.59
36	Viridiflorol	-.12	.07	-.07	.04	-.13	.03	-.16	-.25	-.10	-.29	.45	.01	.09	.33	-.06	.06	-.18	-.09	.07	.27	-.10	-.17	.60
37	Methyl cinnamate	-.22	.31	-.13	-.21	.09	.33	.33	-.26	.31	-.26	-.05	-.01	-.01	.10	.37	-.02	.43	-.13	-.06	-.09	.25	.42	-.14
38	Spathulenol	-.12	.10	-.07	.00	-.07	.00	-.12	-.30	-.10	-.32	.42	-.02	-.09	.46	-.08	-.01	-.10	-.11	.02	.23	-.06	-.09	.57
39	α–Eudesmol	-.42	.20	-.30	-.28	.11	.17	.21	-.51	.23	-.54	-.07	-.21	.26	.02	.13	-.04	.25	-.27	-.14	-.19	.07	.27	-.21
40	γ–Eudesmol	-.38	.18	-.28	-.23	.04	.11	.15	-.54	.15	-.56	-.04	-.23	.15	.08	.07	-.03	.22	-.28	-.13	-.13	.04	.24	-.13
41	β–Eudesmol	-.34	.14	-.25	-.21	.04	.07	.12	-.48	.12	-.49	-.04	-.21	.12	.01	.03	-.02	.19	-.22	-.12	-.16	.01	.21	-.17
42	Tasmanone Type 1	-.16	.01	-.09	.01	.21	-.02	.03	-.20	-.01	-.20	-.11	-.09	-.08	-.07	-.02	-.02	.07	-.10	-.05	-.07	-.01	.08	-.10
43	Tasmanone Type 2	-.15	.22	-.09	-.08	.30	.01	.00	.06	.01	.06	-.05	-.07	.01	-.04	-.03	-.03	.00	-.11	-.05	-.07	.06	.01	-.09
44	Tasmanone Type 3	-.10	-.01	-.07	.02	.15	-.06	-.05	.04	-.04	.04	-.04	-.07	.09	-.03	-.09	-.01	.01	-.07	-.04	-.05	.06	.02	-.08
45	Tasmanone Type 4	-.20	.23	-.11	-.05	.33	.11	.06	.00	.06	-.01	-.09	-.07	.17	-.01	.11	-.03	.07	-.14	-.07	-.07	.12	.09	.01
46	Tasmanone Type 5	-.13	.26	-.07	-.07	.33	-.05	-.05	.09	-.05	.08	-.04	-.06	.00	-.06	-.08	-.02	-.01	-.09	-.05	-.06	.03	.00	-.08

Appendix 4.3A. Part 2. The correlation matrix of chemical components based on percentage composition data of the adult leaf oils of Tasmanian eucalypt species populations.
n = 146 *V* = 145, (F> 0.1614 P<0.05; F > 0.2111, P < 0.01; F > 0.2678, P < 0.001) +++ : p<0.001, ++ : 0.01>p>0.001, + : 0.05>p>0.01, ns : p>0.05. + = positively correlated - = negatively correlated

	Compounds	24 tPi	25 Cpip	26 TA	27 αTe	28 Pip	29 tPip	30 Cit	31 cS	32 Cy8	33 Ner	34 El	35 Gl	36 Vir	37 MC	38 Sp	39 αE	40 γE	41 βE	42 Ta1	43 Ta2	44 Ta3	45 Ta4	46 Ta5
1	α–Pinene	+++	—	ns	++	—	—	–	—	—	ns	–	ns	ns	–	ns	—	—	—	ns	ns	ns	-	ns
2	α–Thujene	—	+++	—	—	+	+++	+	++	++	ns	ns	ns	ns	+++	ns	+	+	ns	ns	++	ns	+	+
3	Camphene	+++	—	ns	+	–	—	ns	–	—	ns	ns	ns	ns	ns	ns	—	—	–	ns	ns	ns	ns	ns
4	β–Pinene	+++	—	ns	+++	–	—	ns	-	—	ns	ns	ns	ns	—	ns	—	–	-	ns	ns	ns	ns	ns
5	Sabinene	-	++	ns	-	++	++	+	+++	+++	ns	+	-	ns	ns	ns	ns	ns	ns	+	+++	ns	+++	+++
6	α–Phellandrene	—	+++	—	—	++	+++	ns	+	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns
7	α–Terpinene	—	+++	–	—	+	+++	ns	++	+++	ns	ns	-	ns	+++	ns	+	ns	ns	ns	ns	ns	ns	ns
8	Limonene	+++	–	+++	+++	–	—	ns	–	—	ns	–	-	–	—	—	—	—	-	ns	ns	ns	ns	ns
9	β–Phellandrene	—	+++	–	—	+	+++	ns	ns	+++	ns	ns	ns	ns	+++	ns	++	ns	ns	ns	ns	ns	ns	ns
10	1,8-Cineole	+++	—	+++	+++	—	—	ns	—	—	ns	–	-	—	–	—	—	—	—	-	ns	ns	ns	ns
11	*cis*-β–Ocimene	ns	ns	ns	ns	ns	ns	ns	ns	ns	++	ns	+++	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
12	γ–Terpinene	ns	ns	ns	ns	ns	ns	ns	ns	ns	++	ns	ns	ns	ns	ns	-	–	-	ns	ns	ns	ns	ns
13	*trans*-β–Ocimene	–	+	ns	ns	ns	+	ns	+	ns	ns	ns	ns	ns	ns	ns	++	ns	ns	ns	ns	ns	+	ns
14	*p*-Cymene	—	+++	-	—	+++	+++	ns	+++	+++	ns	ns	+	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
15	Terpinolene	—	+++	–	—	+++	+++	ns	ns	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns
16	Linalool	ns	ns	ns	ns	ns	ns	+++	ns	ns	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
17	*trans-p*-Menth-2-en-1-ol	—	+++	–	—	++	+++	ns	+++	+++	ns	ns	-	-	+++	ns	++	++	+	ns	ns	ns	ns	ns
18	Pinocarvone	+++	—	ns	ns	-	—	-	–	–	ns	ns	ns	ns	ns	ns	—	—	–	ns	ns	ns	ns	ns
19	b-Caryophellene	+++	-	ns	ns	ns	-	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
20	Aromadendrene	+	-	ns	ns	ns	-	ns	ns	ns	ns	ns	+++	++	ns	++	-	ns	ns	ns	ns	ns	ns	ns
21	Terpinen-4-ol	—	+++	ns	–	+++	+++	ns	+++	+++	ns	ns	ns	ns	++	ns	ns	ns	ns	ns	ns	ns	ns	ns
22	*cis-p*-Menth-2-en-1-ol	—	+++	–	—	++	+++	ns	+++	+++	ns	ns	-	-	++	ns	+++	++	+	ns	ns	ns	ns	ns
23	*Allo*-aromadendrene	+	ns	ns	ns	ns	–	ns	ns	-	ns	ns	+++	+++	ns	+++	–	ns	-	ns	ns	ns	ns	ns
24	*trans*-Pinocarveol	1.00	—	ns	ns	-	—	-	–	—	ns	ns	ns	ns	ns	ns	—	—	—	ns	ns	ns	ns	ns
25	*cis*-Piperitol	-.37	1.00	–	—	+	+++	ns	++	+++	ns	ns	ns	ns	+++	ns	+++	++	+	ns	ns	ns	ns	ns
26	Terpinyl acetate	.04	-.25	1.00	ns	ns	-	ns	ns	ns	ns	ns	-	ns	ns	ns	—	—	–	ns	ns	ns	ns	ns
27	α–Terpineol	.07	-.36	.12	1.00	-	—	+	-	–	ns	–	ns	ns	-	-	—	—	—	-	ns	ns	ns	ns
28	Piperitone	-.20	.18	.02	-.17	1.00	+	ns	++	+++	ns	ns	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
29	*trans*-Piperitol	-.37	.69	-.18	-.30	.18	1.00	ns	+++	+++	ns	ns	ns	ns	+++	ns	+++	++	++	ns	ns	ns	ns	ns
30	Citronellal	-.19	.04	-.02	.18	-.03	.06	1.00	++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	+++	+	+++	+++
31	*cis*-Sabinol	-.25	.27	.05	-.18	.24	.34	.24	1.00	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	++	+	+++	++
32	*p*-Cymene-8-ol	-.28	.43	.01	-.26	.53	.48	.07	.43	1.00	ns	ns	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
33	Nerolidol	-.08	-.11	.06	.14	-.04	-.03	.31	-.08	-.04	1.00	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
34	Elemol	-.14	.10	-.15	-.24	-.06	.12	-.03	.11	.00	-.04	1.00	ns	ns	ns	ns	+++	+++	+++	+++	ns	ns	ns	ns
35	Globulol	.03	-.01	-.17	-.07	-.17	-.15	.00	-.11	-.18	.16	-.10	1.00	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
36	Viridiflorol	-.10	.05	-.16	-.14	-.12	-.13	-.04	-.15	-.16	.02	-.03	.78	1.00	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
37	Methyl cinnamate	-.14	.36	-.15	-.18	-.06	.35	-.08	.03	.00	-.03	.02	-.10	-.08	1.00	ns	ns	ns	ns	ns	ns	ns	ns	ns
38	Spathulenol	-.13	.07	-.14	-.20	-.06	-.10	-.07	-.09	-.04	-.02	-.04	.62	.89	-.03	1.00	ns	ns	ns	ns	ns	ns	ns	ns
39	α–Eudesmol	-.30	.28	-.28	-.36	-.09	.32	.01	.06	.05	-.07	.66	.02	.07	.09	-.01	1.00	+++	+++	+++	ns	ns	ns	ns
40	γ–Eudesmol	-.32	.23	-.29	-.38	-.08	.25	-.02	.03	.05	-.05	.58	.09	.16	.07	.16	.91	1.00	+++	+++	ns	ns	ns	ns
41	β–Eudesmol	-.27	.19	-.26	-.35	-.09	.22	-.01	.03	.04	-.04	.57	.03	.07	.05	.05	.90	.98	1.00	+++	ns	ns	ns	ns
42	Tas 1	-.10	.06	-.11	-.18	-.04	.07	-.06	.09	-.04	-.03	.80	-.14	-.10	.03	-.04	.42	.41	.40	1.00	ns	ns	ns	ns
43	Tas 2	-.11	-.03	-.01	-.01	.01	-.01	.39	.24	-.03	-.03	-.05	-.11	-.08	-.05	-.08	-.08	-.09	-.08	-.04	1.00	+++	+++	+++
44	Tas 3	-.08	-.01	.06	.00	.03	.01	.20	.19	-.05	-.02	-.04	-.07	-.04	-.03	-.06	-.04	-.05	-.04	-.03	.64	1.00	+++	+++
45	Tas 4	-.15	.01	.07	.01	.11	.04	.45	.42	.05	-.04	-.07	-.11	-.07	-.07	-.09	-.09	-.10	-.09	-.05	.60	.45	1.00	+++
46	Tas 5	-.10	-.04	-.01	.04	-.01	-.02	.44	.23	-.04	-.02	-.05	-.10	-.07	-.04	-.07	-.07	-.08	-.06	-.03	.90	.52	.61	1.00

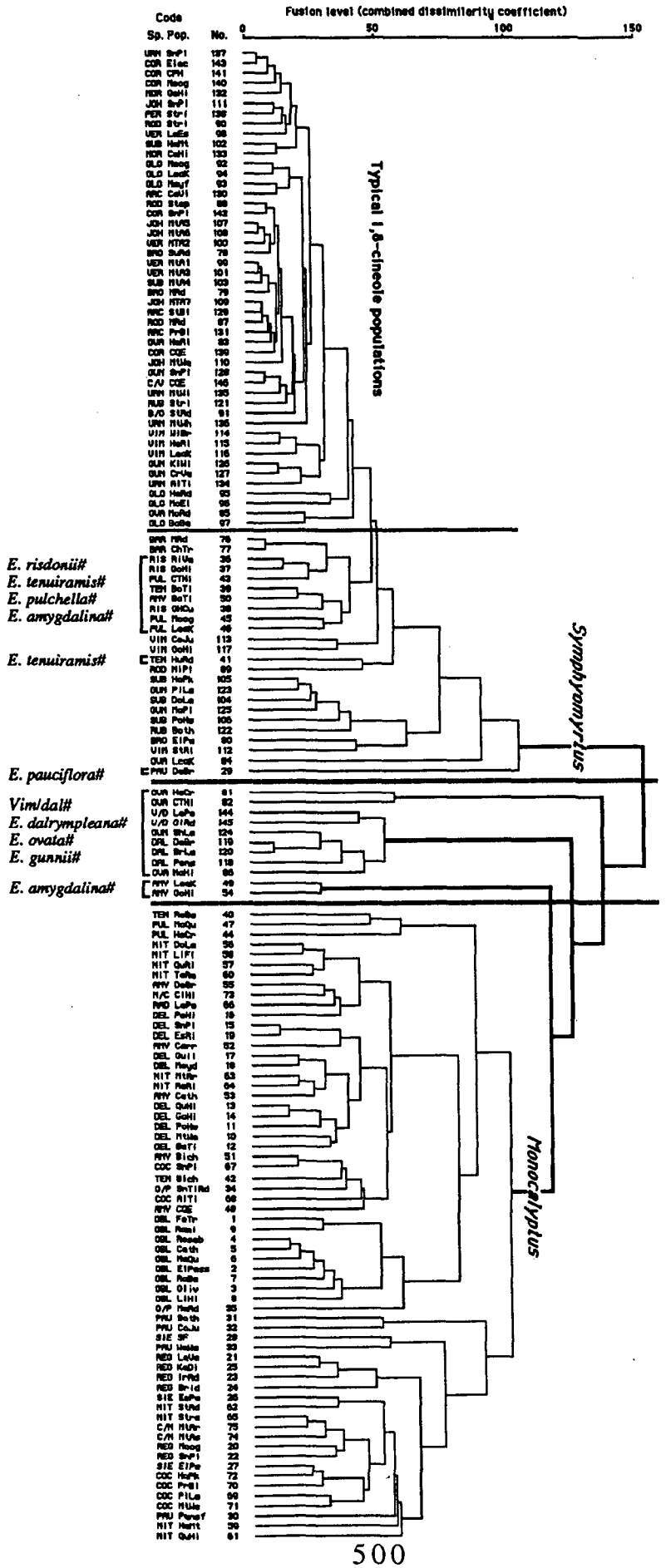
Appendix 4.3B. Part 1. The correlation matrix of chemical components based on percentage composition data of the juvenile leaf oils of Tasmanian eucalypt species populations.
n = 137 *V* = 136, (F> 0.1672 P<0.05; F > 0.2186, P < 0.01; F > 0.2771, P < 0.001) +++ : p<0.001, ++ : 0.01>p>0.001, + : 0.05>p>0.01, ns : p>0.05. + = positively correlated - = negatively correlated

	Compounds	1 αP	2 αTh	3 Cam	4 βP	5 S	6 αPh	7 αT	8 Lim	9 βPh	10 Ci	11 αO	12 γT	13 tO	14 Cy	15 Te	16 Lin	17 tM	18 Pi	19 Ca	20 Aro	21 T4	22 CM	23 All
1	α–Pinene	1.00	—	+++	++	ns	—	—	+++	—	+++	—	ns	—	—	—	ns	—	+++	+++	+	—	—	ns
2	α–Thujene	-.65	1.00	—	–	ns	+++	+++	—	+++	—	+	-	++	+++	+++	ns	+++	—	—	ns	+++	+++	–
3	Camphene	.46	-.35	1.00	+++	*ns*	–	—	+++	—	+++	–	ns	-	–	—	ns	—	+++	ns	ns	—	—	ns
4	β–Pinene	.22	-.25	.38	1.00	ns	ns	-	-	ns	ns	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	-	-	ns
5	Sabinene	-.14	.07	-.02	-.05	1.00	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	α–Phellandrene	-.53	.76	-.26	-.06	.01	1.00	+++	—	+++	—	+	ns	+++	++	+++	ns	+++	—	–	ns	+++	+++	ns
7	α–Terpinene	-.59	.76	-.31	-.17	.03	.77	1.00	—	+++	—	ns	ns	++	+++	+++	ns	+++	—	–	*ns*	+++	+++	-
8	Limonene	.64	-.68	.46	.20	-.10	-.55	-.63	1.00	—	+++	+++	ns	ns	—	—	-	—	+++	++	ns	—	—	ns
9	β–Phellandrene	-.60	.71	-.31	-.16	.01	.69	.81	-.67	1.00	—	+	ns	+++	+++	+++	ns	+++	—	–	ns	+++	+++	-
10	1,8-Cineole	.70	-.67	.42	.12	-.12	-.59	-.63	.95	-.70	1.00	—	ns	–	—	—	-	—	+++	+++	ns	—	—	ns
11	*cis*-β–Ocimene	-.31	.17	-.23	-.14	-.03	.20	.15	-.31	.18	-.32	1.00	ns	+++	+++	++	+	ns	—	-	ns	+	ns	+
12	γ–Terpinene	.04	-.17	.16	-.04	-.07	-.04	-.03	.11	-.10	.06	.05	1.00	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns
13	*trans*-β–Ocimene	-.32	.24	-.15	-.05	.04	.36	.25	-.11	.32	-.23	.39	-.05	1.00	ns	+++	ns	ns	—	-	ns	ns	ns	ns
14	*p*-Cymene	-.49	.38	-.29	-.17	-.02	.24	.43	-.71	.47	-.67	.33	-.02	-.08	1.00	ns	ns	+++	—	-	ns	+++	+++	ns
15	Terpinolene	-.51	.73	-.27	-.05	.04	.90	.74	-.49	.67	-.55	.24	-.06	.52	.14	1.00	ns	+++	—	–	ns	+++	+++	ns
16	Linalool	-.15	-.07	-.09	-.07	-.04	-.06	-.04	-.17	-.03	-.19	.17	.51	.03	.03	-.06	1.00	ns	ns	ns	ns	ns	ns	+++
17	*trans-p*-Menth-2-en-1-ol	-.58	.71	-.31	-.19	.03	.54	.72	-.67	.71	-.64	.13	-.10	.13	.50	.49	-.05	1.00	—	—	ns	+++	+++	–
18	Pinocarvone	.72	-.49	.42	.12	-.12	-.39	-.43	.53	-.44	.57	-.34	.14	-.27	-.41	-.39	-.12	-.42	1.00	+++	ns	—	—	ns
19	b-Caryophellene	.46	-.30	.09	.10	-.09	-.25	-.28	.25	-.27	.30	-.18	-.09	-.17	-.18	-.24	-.08	-.27	.38	1.00	+++	—	—	+++
20	Aromadendrene	.18	-.14	.11	.07	-.06	-.14	-.15	.11	-.16	.11	-.06	-.05	-.09	-.09	-.12	.02	-.15	.03	.52	1.00	ns	ns	+++
21	Terpinen-4-ol	-.63	.57	-.32	-.19	.05	.46	.65	-.65	.61	-.63	.19	.15	.13	.67	.43	.12	.66	-.45	-.31	-.16	1.00	+++	-
22	*cis-p*-Menth-2-en-1-ol	-.61	.74	-.33	-.20	.02	.53	.73	-.70	.74	-.68	.14	-.11	.15	.54	.50	-.04	.95	-.44	-.29	-.15	.67	1.00	–
23	*Allo*-aromadendrene	.13	-.22	-.08	.04	-.12	-.06	-.21	-.04	-.20	-.05	.17	.09	-.04	.05	-.11	.32	-.23	-.06	.41	.42	-.20	-.23	1.00
24	*trans*-Pinocarveol	.72	-.50	.47	.08	-.11	-.44	-.45	.57	-.45	.63	-.33	.11	-.27	-.44	-.42	-.13	-.44	.89	.36	.05	-.48	-.46	-.05
25	*cis*-Piperitol	-.58	.67	-.33	-.20	.03	.49	.71	-.66	.71	-.64	.18	-.10	.16	.56	.46	-.07	.90	-.43	-.29	-.17	.71	.93	-.24
26	Terpinyl acetate	-.02	-.29	.08	.07	.00	-.26	-.25	.42	-.30	.41	-.02	-.03	-.01	-.18	-.24	-.06	-.25	.06	-.09	-.04	-.13	-.27	-.07
27	α–Terpineol	.30	-.38	.06	.08	-.13	-.28	-.34	.54	-.29	.48	-.03	.26	.14	-.40	-.18	.05	-.34	.27	.07	.00	-.24	-.34	-.03
28	Piperitone	-.44	.36	-.23	-.14	.16	.29	.28	-.52	.23	-.48	.21	-.07	.00	.49	.30	-.05	.25	-.32	-.20	-.12	.49	.27	-.20
29	*trans*-Piperitol	-.64	.58	-.32	-.18	.03	.40	.61	-.64	.68	-.66	.22	-.12	.23	.53	.42	.02	.81	-.45	-.31	-.16	.62	.85	-.25
30	Citronellal	-.19	.06	-.11	-.01	.06	.05	.01	.08	-.03	-.01	.05	.09	.24	-.09	.09	.16	-.03	-.17	-.11	-.03	.03	-.04	.02
31	*cis*-Sabinol	-.35	.25	-.15	-.12	.47	.14	.20	-.22	.22	-.26	.12	-.14	.10	.24	.12	-.06	.22	-.26	-.16	-.08	.26	.19	-.19
32	*p*-Cymene-8-ol	-.35	.17	-.20	-.13	.05	.06	.28	-.49	.25	-.43	.21	.01	-.07	.76	.04	-.02	.39	-.26	-.16	-.09	.56	.40	-.17
33	Nerolidol	-.11	-.10	-.08	-.05	-.04	-.11	-.08	-.13	-.10	-.15	.16	.41	-.01	.00	-.09	.89	-.11	-.10	-.02	.12	-.02	-.10	.46
34	Elemol	-.20	.09	-.12	-.05	.24	-.02	.02	-.20	.02	-.28	-.06	-.15	.07	-.09	.02	-.03	.05	-.16	-.10	-.07	-.03	.07	-.10
35	Globulol	-.04	-.13	-.06	.06	-.13	.01	-.20	-.13	-.07	-.18	.24	.17	.08	.13	-.07	.31	-.20	-.12	.11	.34	-.14	-.19	.73
36	Viridiflorol	-.11	.03	-.12	.07	-.10	.11	-.13	-.26	.05	-.32	.28	-.06	.15	.25	.07	.05	-.13	-.20	.03	.13	-.11	-.11	.55
37	Methyl cinnamate	-.25	.46	-.15	-.11	.03	.30	.55	-.33	.46	-.31	.02	.00	-.01	.20	.31	-.02	.56	-.17	-.11	-.08	.27	.53	-.14
38	Spathulenol	-.10	.06	-.13	.06	-.06	.05	-.08	-.32	.01	-.34	.23	-.12	-.04	.42	-.01	-.02	-.07	-.18	.04	.07	-.06	-.07	.45
39	α–Eudesmol	-.39	.30	-.22	-.03	.16	.12	.21	-.45	.31	-.54	-.03	-.24	.17	.03	.16	-.03	.29	-.31	-.22	-.13	.11	.34	-.16
40	γ–Eudesmol	-.33	.24	-.21	.04	.14	.09	.14	-.49	.24	-.56	-.04	-.25	.11	.08	.12	-.01	.23	-.29	-.21	-.13	.09	.27	-.08
41	β–Eudesmol	-.32	.23	-.20	-.01	.14	.08	.14	-.46	.24	-.53	-.04	-.24	.11	.02	.12	.01	.24	-.27	-.21	-.12	.07	.29	-.08
42	Tas 1	-.15	.12	-.09	-.07	.56	-.02	.05	-.17	-.01	-.23	-.12	-.12	-.07	-.07	-.01	-.03	.03	-.12	-.08	-.06	.01	.03	-.10
43	Tas 2	-.15	.05	-.10	-.06	.04	.05	.01	.04	-.02	.03	.02	-.06	.07	-.09	.02	-.04	.01	-.12	-.08	-.05	.02	.01	-.10
44	Tas 3	-.10	.08	-.05	-.03	.04	.03	-.01	.00	.00	-.01	.05	-.05	.16	-.04	.02	-.02	.01	-.08	-.05	-.03	.01	.01	-.06
45	Tas 4	-.18	.14	-.12	-.07	.07	.12	.04	.05	.00	.02	.02	-.06	.16	-.09	.11	-.04	-.04	-.15	-.09	-.07	.05	.00	-.12
46	Tas 5	-.14	.04	-.09	-.06	.06	.03	.00	.05	-.02	.04	.02	-.06	.08	-.10	.00	-.03	.01	-.11	-.07	-.05	.01	.00	-.09

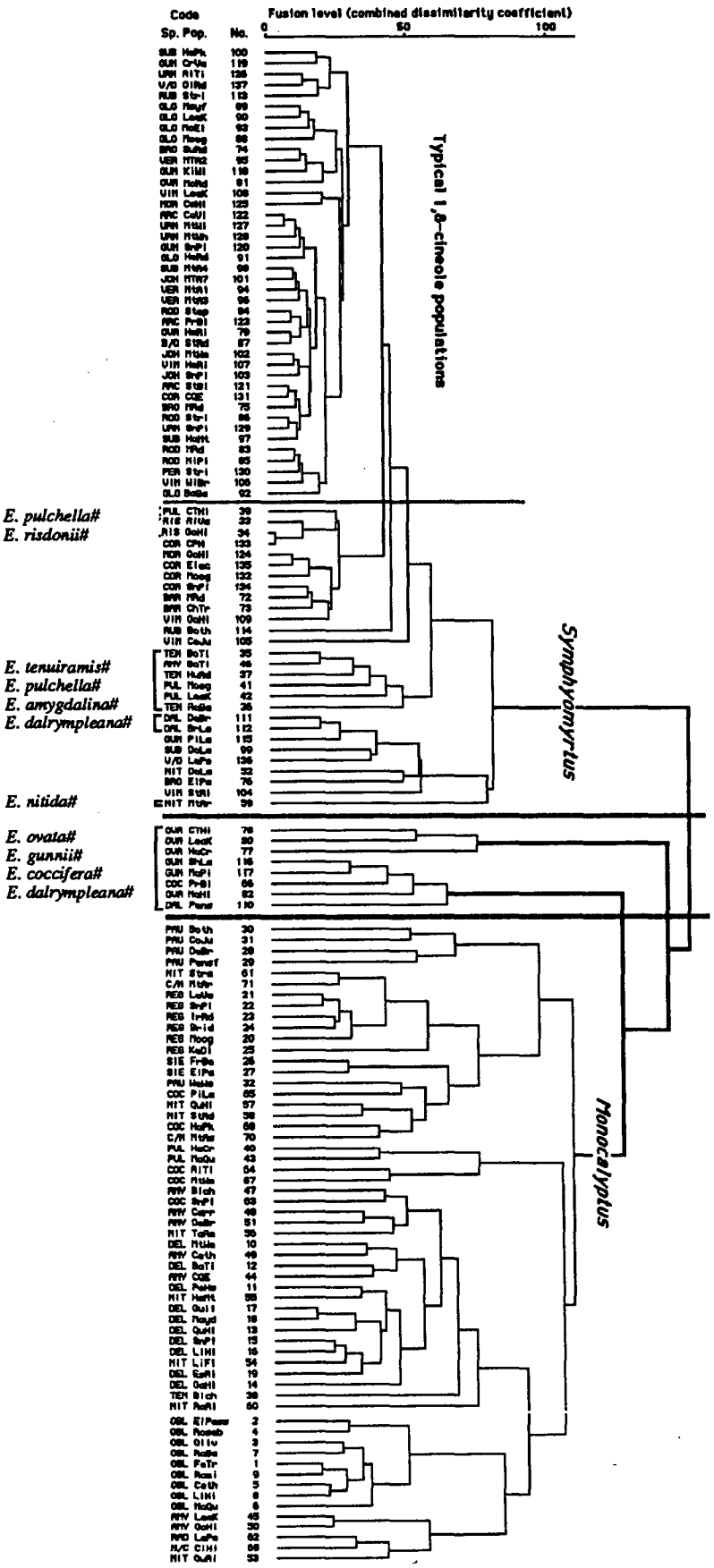
Appendix 4.3B. Part 2. The correlation matrix of chemical components based on percentage composition data of the juvenile leaf oils of Tasmanian eucalypt species populations.
n = 137 *V* = 136, (F> 0.1672 P<0.05; F > 0.2186, P < 0.01; F > 0.2771, P < 0.001) +++ : p<0.001, ++ : 0.01>p>0.001, + : 0.05>p>0.01, ns : p>0.05. + = positively correlated - = negatively correlated

	Compounds	24 tPi	25 Cpip	26 TA	27 αTe	28 Pip	29 tPip	30 Cit	31 cS	32 Cy8	33 Ner	34 El	35 Glo	36 Vir	37 MC	38 Spa	39 αE	40 γE	41 βE	42 Ta1	43 Ta2	44 Ta3	45 Ta4	46 Ta5
1	α–Pinene	+++	---	ns	+++	---	---	-	---	---	ns	-	ns	*ns*	–	*ns*	---	---	---	ns	ns	ns	-	ns
2	α–Thujene	---	+++	---	---	+++	+++	ns	++	+	ns	ns	ns	ns	+++	ns	+++	++	++	ns	ns	ns	ns	ns
3	Camphene	+++	---	ns	ns	--	---	ns	ns	-	ns	ns	ns	ns	ns	ns	–	-	-	ns	ns	ns	ns	ns
4	β–Pinene	ns	-	ns	ns	ns	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
5	Sabinene	ns	ns	ns	ns	ns	ns	ns	+++	ns	ns	++	ns	ns	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns
6	α–Phellandrene	---	+++	–	---	+++	+++	ns	ns	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns
7	α–Terpinene	---	+++	–	---	+++	+++	ns	++	+++	ns	ns	-	ns	+++	ns	+	ns	ns	ns	ns	ns	ns	ns
8	Limonene	+++	---	+++	+++	---	---	ns	–	---	ns	-	ns	--	---	---	---	---	---	-	*ns*	*ns*	*ns*	ns
9	β–Phellandrene	---	+++	---	---	+++	+++	ns	++	++	ns	ns	ns	ns	+++	ns	+++	++	++	ns	ns	ns	ns	ns
10	1,8-Cineole	+++	---	+++	+++	---	---	ns	–	---	ns	---	-	---	---	---	---	---	---	++	ns	ns	ns	ns
11	*cis*–β–Ocimene	---	+	*ns*	*ns*	+	+	ns	ns	+	ns	ns	++	+++	ns	++	ns	ns	ns	ns	ns	ns	ns	ns
12	γ–Terpinene	ns	ns	ns	++	ns	ns	ns	ns	ns	+++	ns	+	ns	ns	ns	–	–	–	ns	ns	ns	ns	ns
13	*trans*–β–Ocimene	---	ns	ns	ns	ns	++	++	ns	ns	ns	ns	ns	ns	ns	ns	+	ns	ns	ns	ns	ns	ns	ns
14	*p*-Cymene	---	+++	-	---	+++	+++	ns	++	+++	ns	ns	ns	++	+	+++	ns	ns	ns	ns	ns	ns	ns	ns
15	Terpinolene	---	+++	–	-	+++	+++	ns	ns	ns	ns	ns	ns	ns	++	ns	ns	ns	ns	ns	ns	ns	ns	ns
16	Linalool	ns	ns	ns	ns	ns	ns	ns	ns	ns	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
17	*trans-p*-Menth-2-en-1-ol	---	+++	–	---	++	+++	ns	++	+++	ns	ns	-	ns	+++	ns	+++	++	++	ns	ns	ns	ns	ns
18	Pinocarvone	+++	---	*ns*	+++	---	---	-	–	–	ns	ns	ns	-	-	-	---	---	---	ns	ns	ns	ns	ns
19	b-Caryophellene	+++	---	ns	ns	-	---	ns	ns	ns	ns	ns	ns	ns	ns	ns	–	-	–	ns	ns	ns	ns	ns
20	Aromadendrene	ns	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
21	Terpinen-4-ol	---	+++	ns	–	+++	+++	ns	++	+++	ns	ns	ns	ns	++	ns	ns	ns	ns	ns	ns	ns	ns	ns
22	*cis-p*-Menth-2-en-1-ol	---	+++	---	---	++	+++	ns	+	+++	ns	ns	-	ns	+++	ns	+++	++	+++	ns	ns	ns	ns	ns
23	*Allo*-aromadendrene	-.05	–	ns	ns	-	–	ns	-	-	+++	ns	+++	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
24	*trans*-Pinocarveol	1.00	---	*ns*	+++	---	---	-	–	---	ns	ns	ns	-	-	-	---	---	---	ns	ns	ns	ns	ns
25	*cis*-Piperitol	-.44	1.00	–	---	+++	+++	*ns*	++	+++	*ns*	*ns*	–	ns	+++	ns	+++	+	++	ns	ns	ns	ns	ns
26	Terpinyl acetate	.09	-.24	1.00	ns	ns	–	ns	ns	ns	ns	ns	ns	ns	ns	ns	---	---	---	ns	ns	ns	ns	ns
27	α–Terpineol	.27	-.33	.06	1.00	---	---	++	ns	---	ns	–	ns	ns	–	--	---	---	---	–	ns	ns	ns	ns
28	Piperitone	-.33	.31	-.11	-.31	1.00	+++	ns	+++	+++	ns	ns	–	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
29	*trans*-Piperitol	-.47	.80	-.17	-.27	.38	1.00	ns	+++	+++	ns	ns	-	ns	+++	ns	+++	++	+++	ns	ns	ns	ns	ns
30	Citronellal	-.17	-.06	-.06	.26	-.05	.04	1.00	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	+++	ns	+	+++
31	*cis*-Sabinol	-.25	.22	-.03	-.07	.31	.33	.03	1.00	+++	ns	ns	-	ns	ns	ns	ns	ns	ns	++	ns	+++	+	ns
32	*p*-Cymene-8-ol	-.28	.47	-.07	-.28	.67	.50	-.06	.38	1.00	*ns*	*ns*	-	*ns*	*ns*	*ns*	*ns*	ns	ns	ns	ns	ns	ns	ns
33	Nerolidol	-.10	-.12	-.05	.01	-.08	-.07	.12	-.04	-.03	1.00	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
34	Elemol	-.16	.05	-.14	-.23	.07	.11	-.01	.09	-.07	-.04	1.00	ns	ns	ns	ns	+++	+++	+++	+++	ns	ns	ns	ns
35	Globulol	-.15	-.22	-.10	-.02	-.22	-.18	.08	-.19	-.18	.33	-.11	1.00	+++	ns	+++	ns	ns	ns	ns	ns	ns	ns	ns
36	Viridiflorol	-.21	-.13	-.13	-.08	-.14	-.10	.10	-.11	-.13	.09	-.05	.82	1.00	ns	+++	ns	+	ns	ns	ns	ns	ns	ns
37	Methyl cinnamate	-.18	.48	-.15	-.22	-.06	.40	-.07	.03	.01	-.04	.00	-.11	-.11	1.00	ns	+	ns	ns	ns	ns	ns	ns	ns
38	Spathulenol	-.18	-.08	-.10	-.22	-.08	-.10	.02	-.02	.01	.06	-.02	.63	.88	-.03	1.00	ns	+	ns	ns	ns	ns	ns	ns
39	α–Eudesmol	-.34	.28	-.27	-.27	.05	.34	-.01	.05	-.07	-.06	.74	-.05	.06	.17	.02	1.00	+++	+++	+++	*ns*	*ns*	*ns*	*ns*
40	γ–Eudesmol	-.34	.21	-.28	-.29	.04	.26	-.03	.03	-.04	-.02	.62	.06	.21	.12	.17	.93	1.00	+++	+++	ns	ns	ns	ns
41	β–Eudesmol	-.31	.21	-.27	-.27	.01	.27	-.02	.01	-.08	-.01	.60	.03	.15	.13	.08	.94	.98	1.00	+++	ns	ns	ns	ns
42	Tas 1	-.13	.02	-.11	-.24	.09	-.01	-.05	.27	-.05	-.03	.62	-.16	-.13	.03	-.03	.55	.49	.49	1.00	ns	ns	ns	ns
43	Tas 2	-.12	.00	-.05	-.07	-.01	-.02	.29	.15	-.06	-.03	-.05	-.11	-.06	-.05	-.07	-.08	-.09	-.09	-.04	1.00	+++	+++	+++
44	Tas 3	-.08	.01	.02	-.07	.04	-.01	.02	.28	-.03	-.02	-.03	-.07	-.05	-.03	-.05	-.05	-.06	-.06	-.02	.60	1.00	+++	+++
45	Tas 4	-.15	-.01	-.01	-.04	.06	-.02	.34	.17	-.06	-.04	-.06	-.10	-.03	-.06	-.08	-.09	-.11	-.10	-.05	.82	.56	1.00	+++
46	Tas 5	-.11	-.01	.00	-.04	-.02	-.01	.26	.15	-.06	-.03	-.04	-.10	-.06	-.05	-.07	-.08	-.09	-.09	-.03	.93	.51	.77	1.00

Appendix 4.4A. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percent composition data of all components of adult leaf oils of individual populations. #: Species populations lying outside the subgenus cluster. Sp.= species code; Pop.= population code (see Appendix 4.1).



Appendix 4.4B. Chemotaxonomic representation of average linkage clustering of 135 populations of 29 eucalypt species, using percent composition data of all components of juvenile leaf oils of individual populations. #: Species populations lying outside the subgenus cluster. Sp.= species code; Pop.= population code (see Appendix 4.1).



Appendix 4.5. The ordinations from PCA of variation in terpenoid components between populations within the series *Obliquae*.

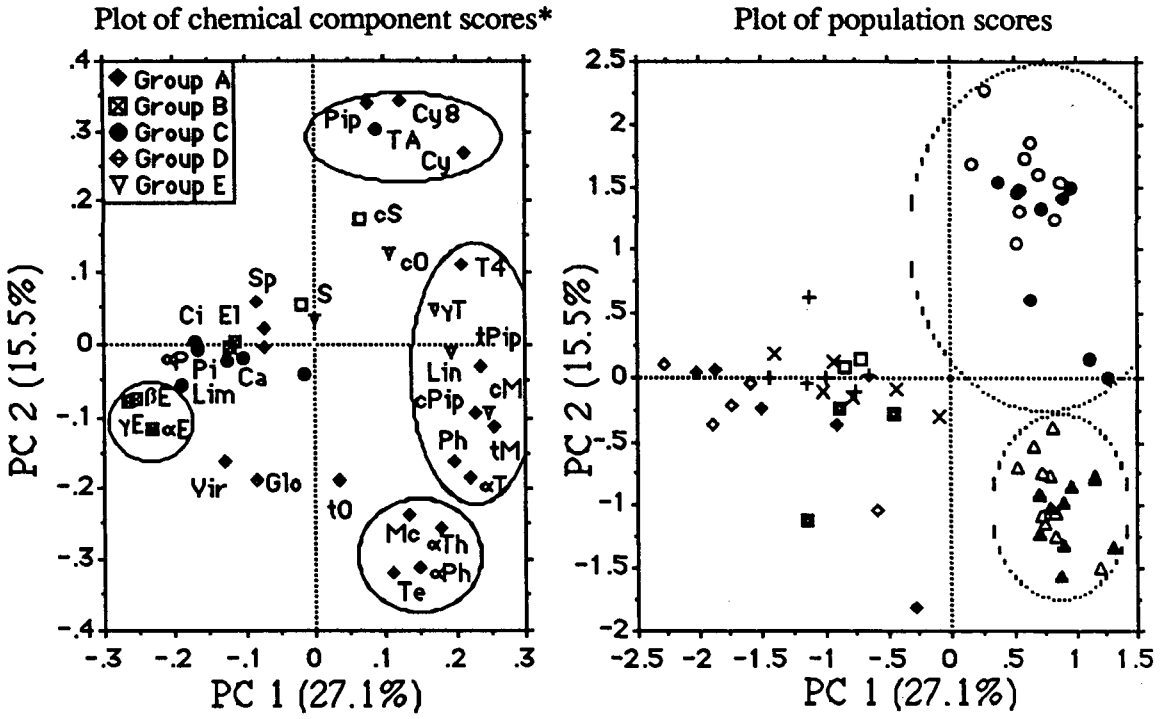
The ordinations of compounds (left plot of Appendix Fig. 4.5A) indicate that the first two PCs accounted for 27.0% and 15.5%, respectively, of the total variance in terpenoid compounds among *Obliquae* populations. The variation along PC 1 was mainly due to variations between the sesquiterpenoids of compound groups B and D and monoterpenoids of group C, located on the left hand side of PC 1 and the monoterpenoids of group A, located on the right hand side of horizontal axis. The sesquiterpenoids of group B had the greatest negative values and some monoterpene alcohols, cis- and trans-piperitol and cis- and trans-p-menth-2-en-1-ol, of group A had high positive values along PC 1 and contribute most to variation along PC 1. The positive variation along PC 2 was mainly due to increasing levels of the four monoterpenoids, piperitone, p-cymene, p-cymene-8-ol and terpinyl acetate and decreasing levels of the other four monoterpenoids, α–phellandrene, terpinolene and α–thujene.

Plots of the two factors separated the *Obliquae* populations into three groups (right plot of Appendix Fig. 4.5A). PC 1 separated all populations of *E. regnans*, *E. sieberi* and *E. pauciflora* (left hand side) from all populations of *E. delegatensis* and *E. obliqua* (right hand side). The populations of *E. regnans, E. sieberi* and *E. pauciflora* had high levels of sesquiterpenoids of group B and D and monoterpenoids of group C. *E. delegatensis* and *E. obliqua* had high levels of monoterpenoids of group A. Furthermore, PC 2 separated all populations of *E. delegatensis* from *E. obliqua*. The *E. obliqua* populations had higher levels of piperitone, p-cymene, p-cymene-8-ol and terpinyl acetate (upper right quadrant) and the *E. delegatensis* populations had higher levels of α–phellandrene, α–thujene and terpinolene (lower right quadrant).

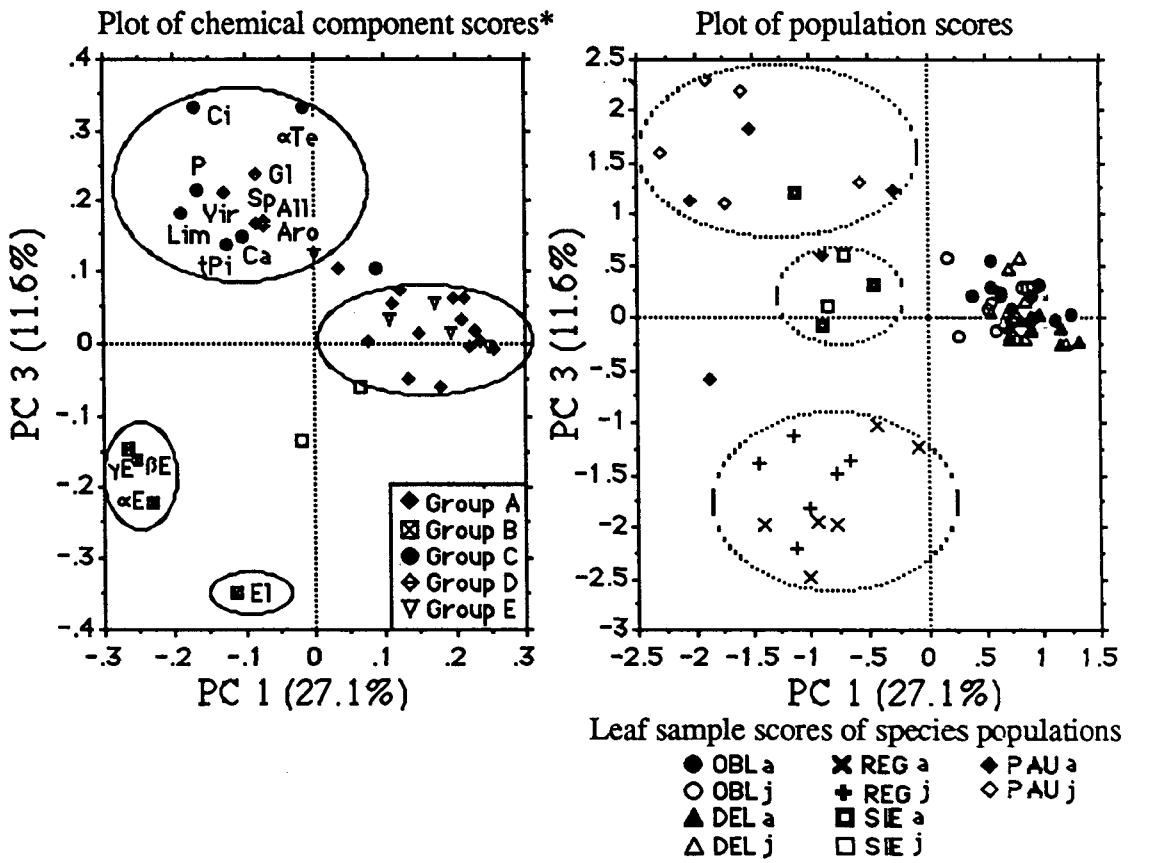
PC 1 plus PC 3 (11.6%) separated all populations of *E. regnans* from *E. sieberi* and *E. pauciflora* (Appendix Fig. 4.5B) mainly due to the variation between the sesquiterpenoids of group B that is located in the lower left quadrant and the sesquiterpenoids of group D and monoterpenoids of group C and located in the upper left quadrant of the right plot in this (Appendix Fig. 4.5B). Thus *E. regnans* has higher levels of sesquiterpenoids of group B compared to the *E. pauciflora* and *E. sieberi* populations which have high levels of sesquiterpenoids of group D and monoterpenoids of group C.

Appendix Fig. 4.5. Scatter plot of the terpenoid parameters (leaf) and samples of the 12 *Obliquae* species (right) on the first three principal components (PC1, PC2 and PC3) derived from analysis of juvenile and adult leaf oils.

A: PC 1 and PC2



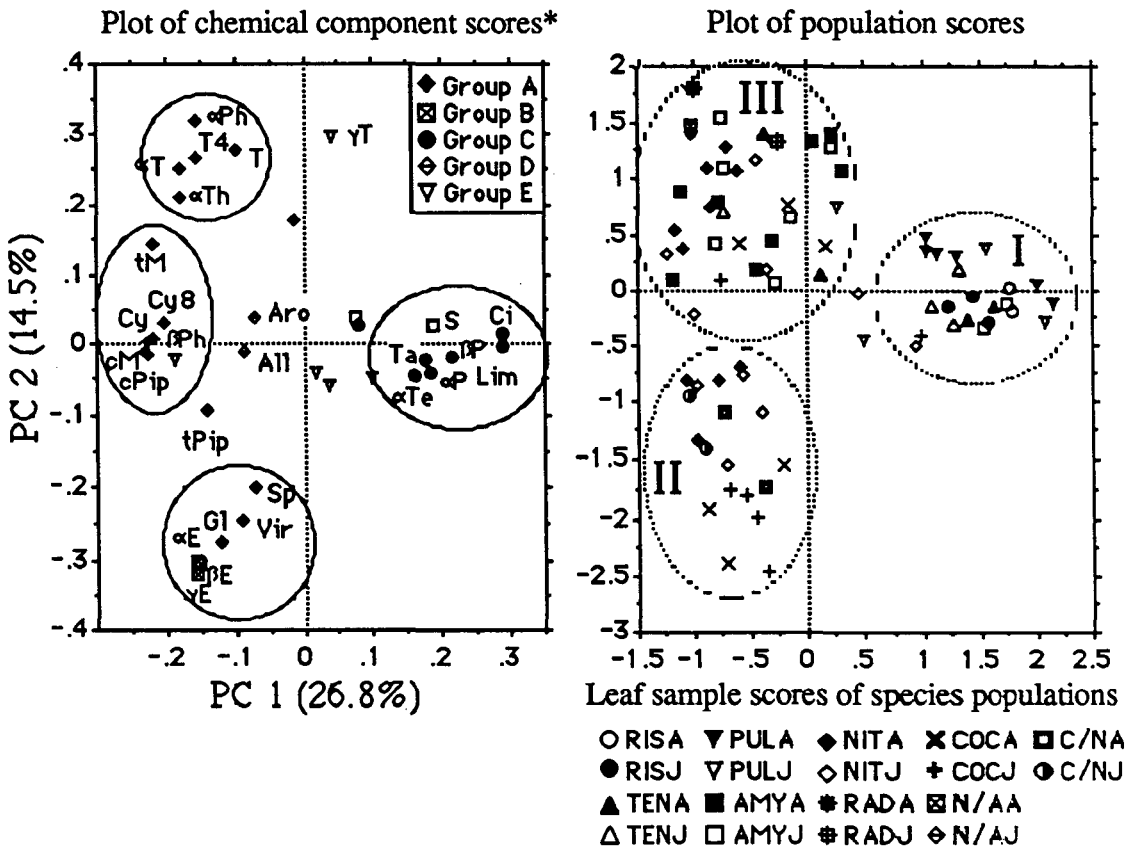
B: PC 1 and PC3



Appendix 4.6. The ordinations from PCA of variation in terpenoid compounds between populations of the series *Piperitae*.

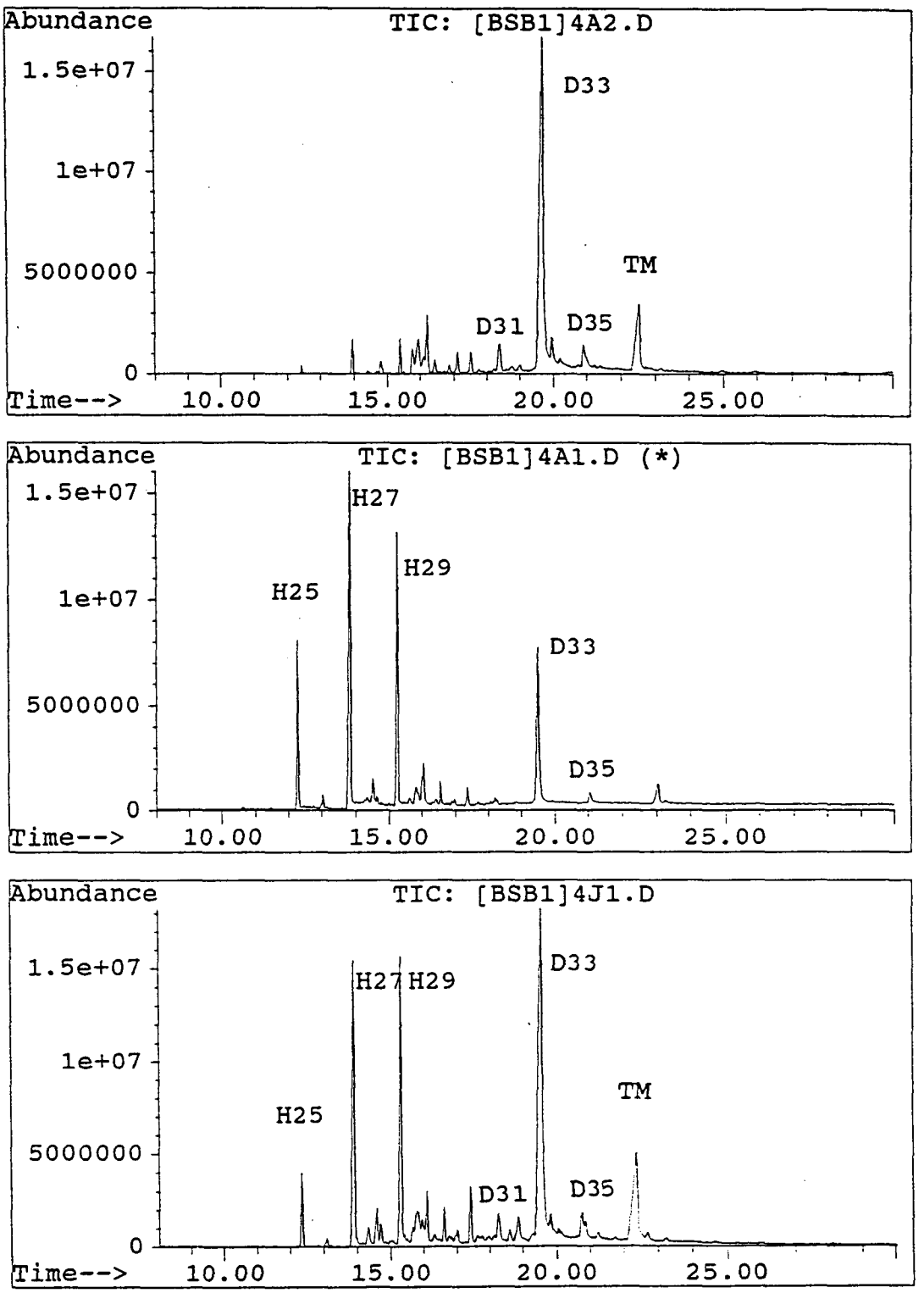
As indicated in Appendix Fig. 4.6, the eigenvalues of the PCA indicated that the first two PCs accounted for 26.8 and 14.5 percent of the total variance of terpenoids of leaf oils among *Piperitae* species populations. The variation along the horizontal axis (PC 1) is mainly due to the variation between the monoterpenoids of group A and sesquiterpenoids of group B and D which are located on the left hand side of the horizontal axis and the monoterpenoids of group C which are located on the right hand side of the horizontal axis. The variation along the vertical axis is mainly due to variation between the sesquiterpenoids of groups B and D, located in the lower left quadrant, and the five monoterpenoids of group A located in the upper right quadrant.

Appendix Fig. 4.6. Scatter plots of the terpenoid parameters (leaf) and samples of the 12 *Piperitae* species (right) on the first two principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf oils.



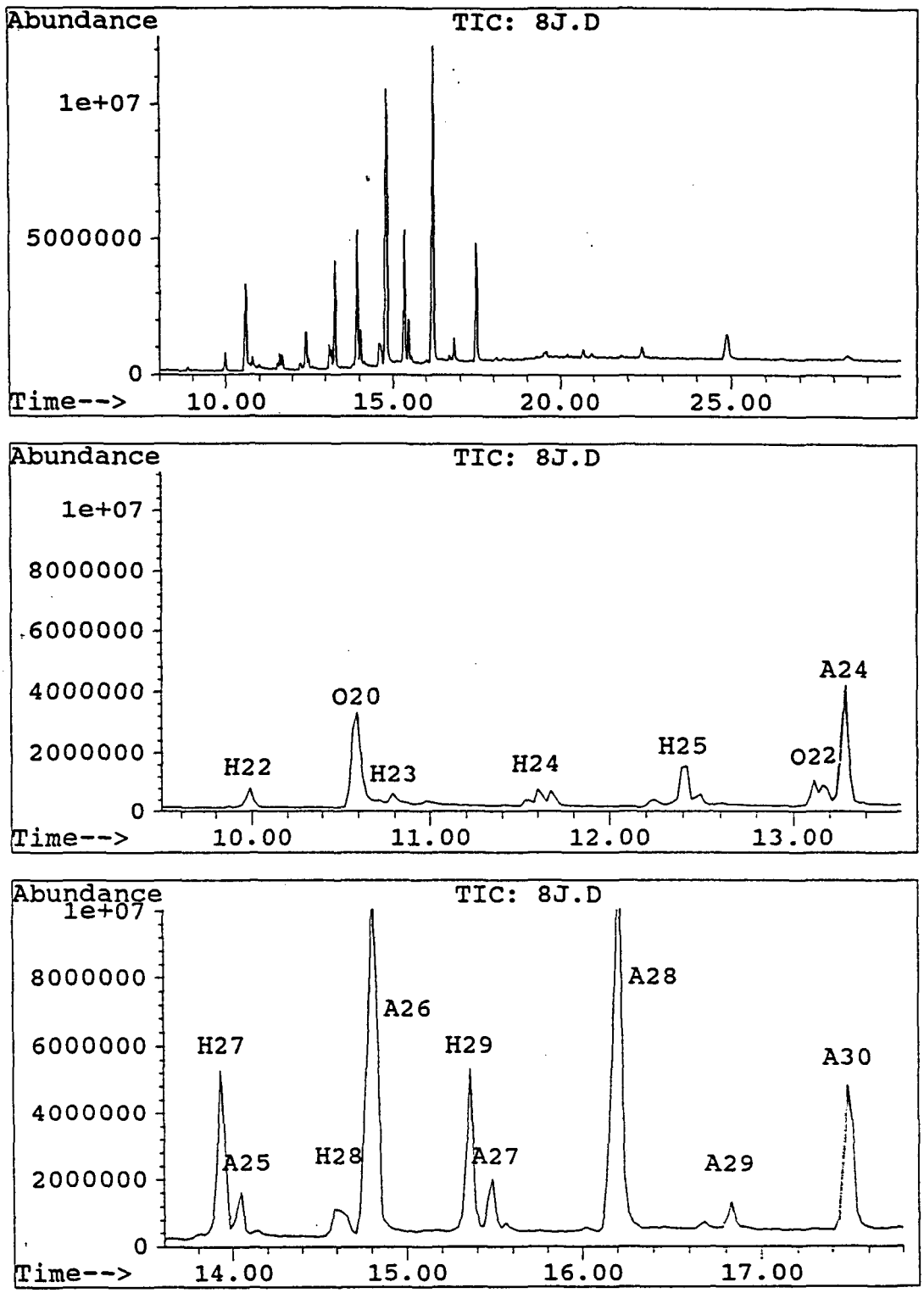
The plot of the population scores on these two factor axes (right plot of Appendix Fig. 4.6) indicated that the populations of *Piperitae* species were separated into three major groups. All populations of *E. risdonii* and some of the *E. tenuiramis*, *E. pulchella*

and few *E. amygdalina* populations had high levels of the compounds of group C and were grouped into the population group I on the right hand side of the horizontal axis. Some populations of *E. nitida*, *E. coccifera* and an intermediate form of *E. nitida*/*E. amygdalina* had high levels of sesquiterpenoids of groups B and D and were grouped into the population group II in the lower left quadrat. Most populations of *E. amygdalina* and *E. nitida*, some populations of *E. coccifera* and the single population of *E. radiata* sampled which had high levels of the compounds of group A, were grouped into the population group III.

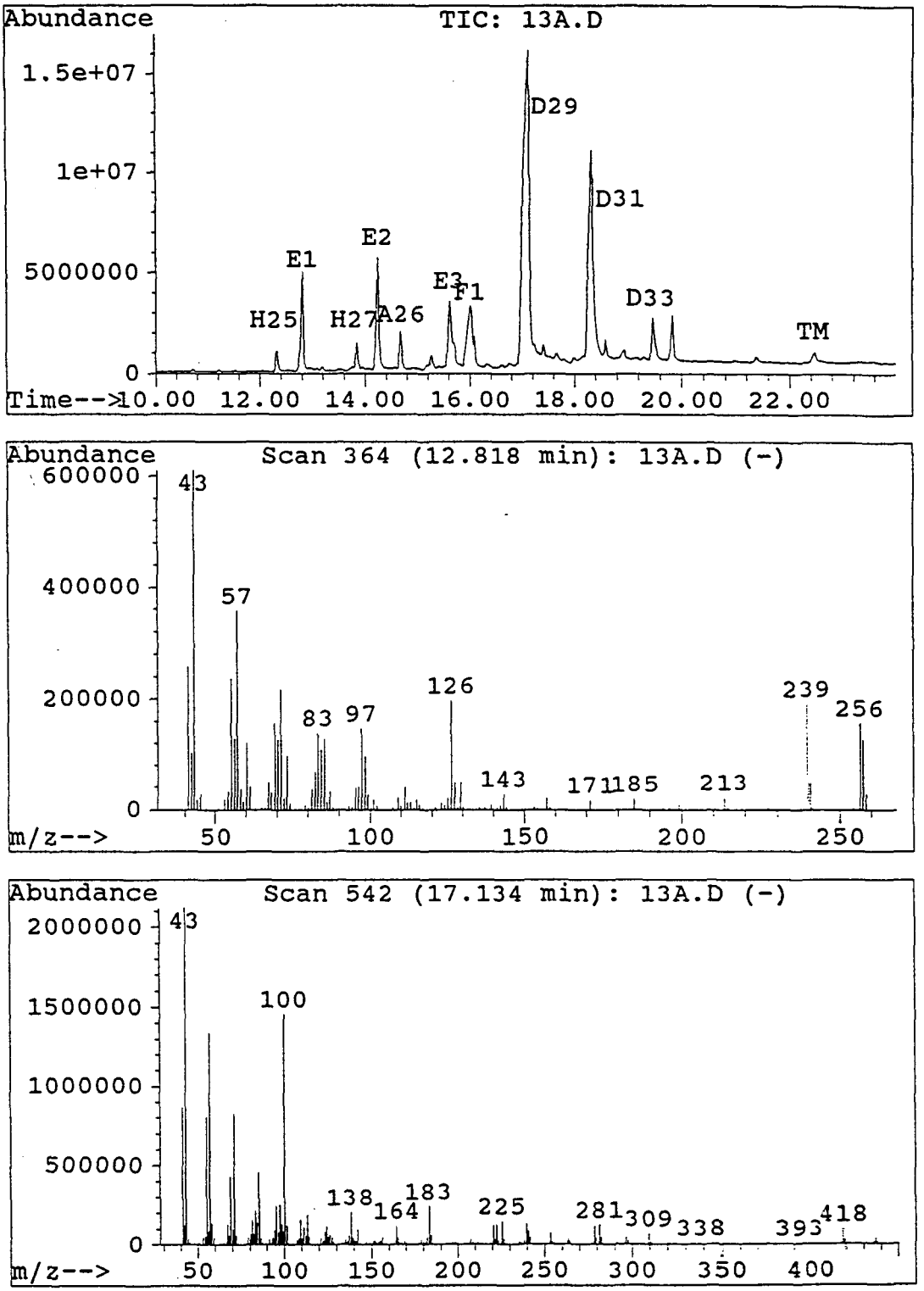


Appendix 5.1A. GLC separation of the components from the waxes of *E. urnigera* adult and juvenile leaves. Picture 4A2.D is the adult leaves of glaucous populations of *E. urnigera* at Mt Wellington (altitude 1100 m); Picture 4A1.D and 4J1.D are the adult and juvenile leaves of green populations of *E. urnigera* at Mt Wellington (altitude 600 m). (see Table 5.2 in chapter 5 for compound name)

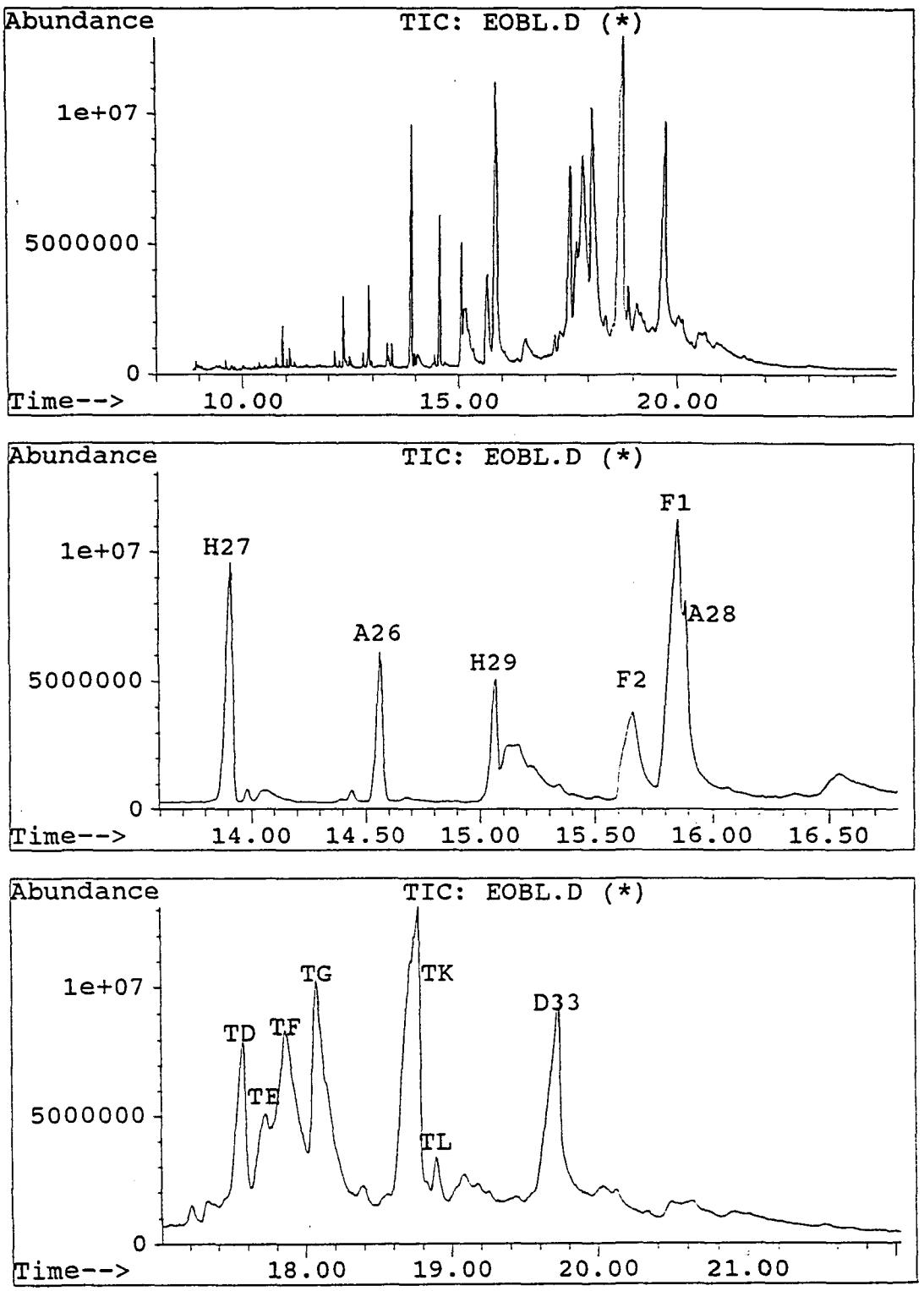
Peaks H25, H27 and H29 are n-alkanes; Peaks D31, D33 and D35 are ß-diketones; Peak TM is 11,12-dehydroursolic lactone acetate (triterpenoid).



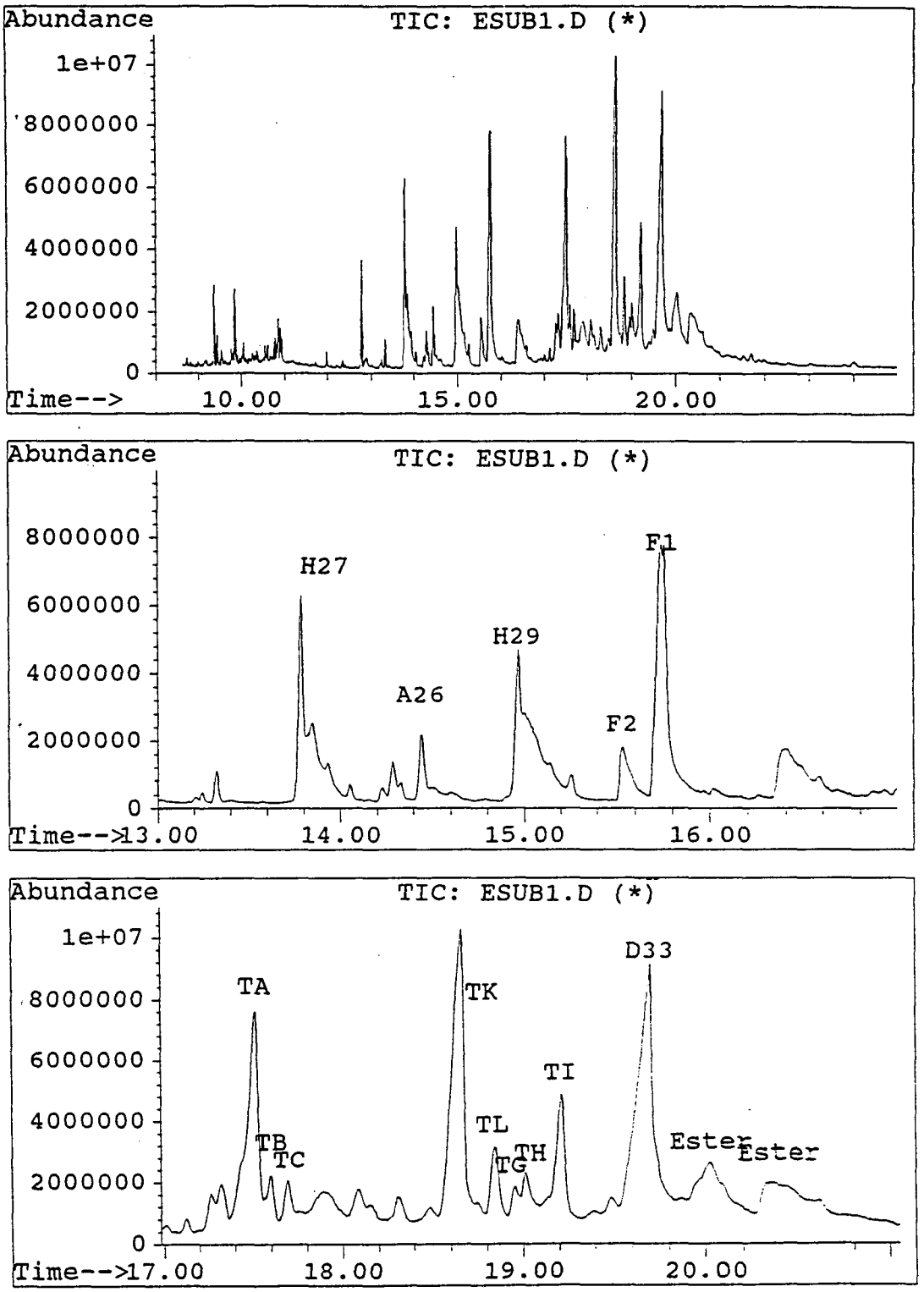
Appendix 5.1B. GLC separation of the components from the waxes of *E. ovata* (Leak) juvenile leaves (see Table 5.2 for name of individual compounds). Peaks H22, H23, H24, H 25, H27, H28 and H29 are n-alkanes; peaks O20 and O22 are n-alcohols; peak O20 is n-C_{20} alcohol; peaks A 25, A26, A27, A28, A29 and A30 are alkanals; peak A28 is C_{28} alkanal.



Appendix 5.1C. GLC separation of the components from the waxes of *E. risdonii* juvenile leaves (top). Peaks H25 and H27 are n-alkanes; peaks E1, E2 and E3 are alkan-2-ol long chain esters: peak E2 (14.245 min.) is ses. Undecanoyl hexadecanoate; peaks D29, D31 and D35 are ß-dikesones: peak D29 (17.134 min.) is n-nonacosane-12,14-dione (C_{29}); peak F1 is eucalyptin.

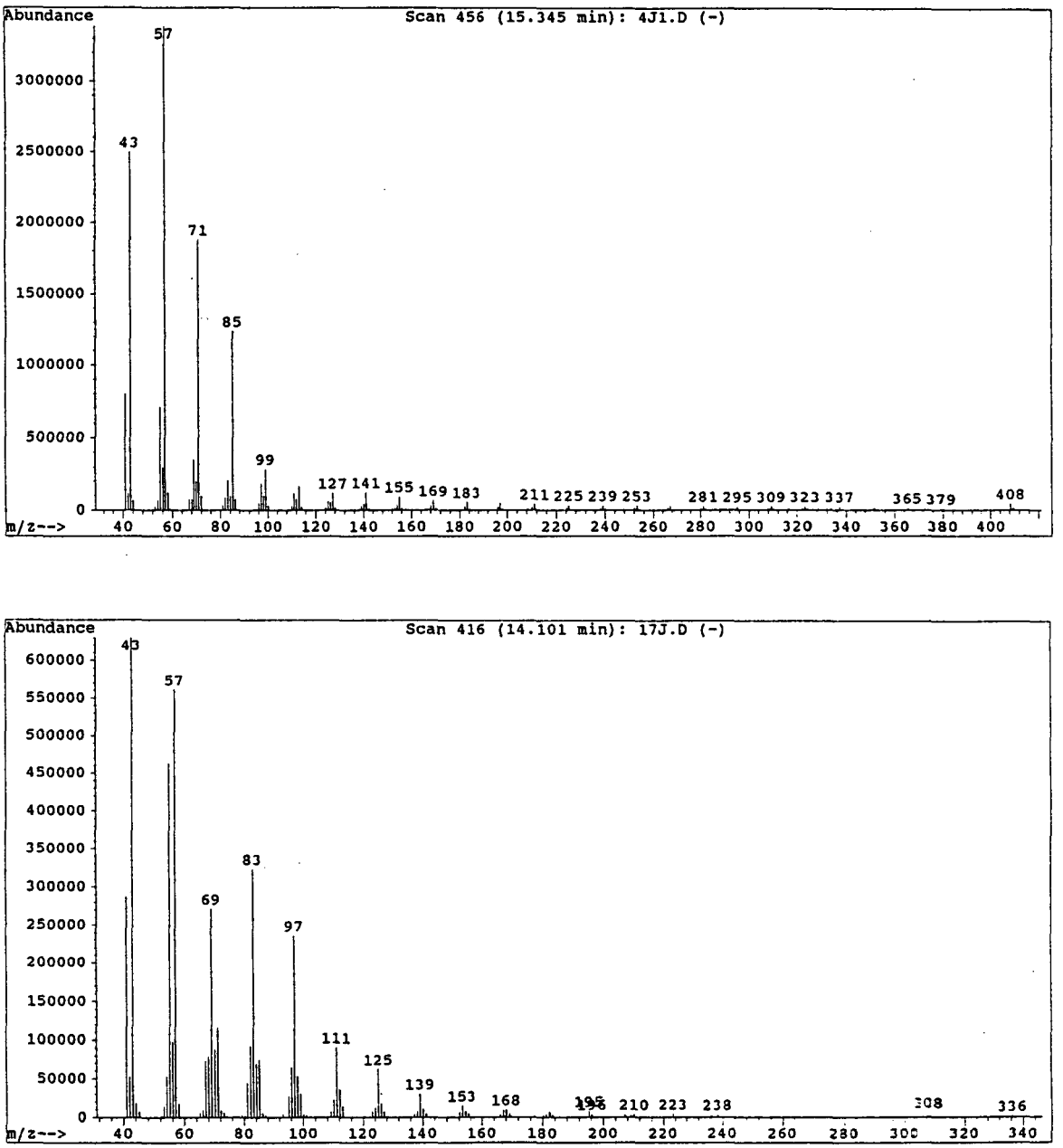


Appendix 5.1D. GLC separation of the components from the wax of *E. obliqua* adult leaves. Peaks H27 and H29 are n-alkanes; peaks A26 and A28 are alkanals; peaks F1 and F2 are eucalyptin (5-hydroxy-4',7-dimethoxy-6,8-dimethylflavone) and demethyl eucalyptin (5-hydroxy-4',7-dimethoxy-6-methylflavone) respectively; peaks TD, TE, TF, TG, TK and TL are triterpenoids; peak TD is 28-nor-Δ17-oleanene; peak TG is ß-amyrin; peak TK is methyl moronate. D33 is ß-diketone (C_{33}).

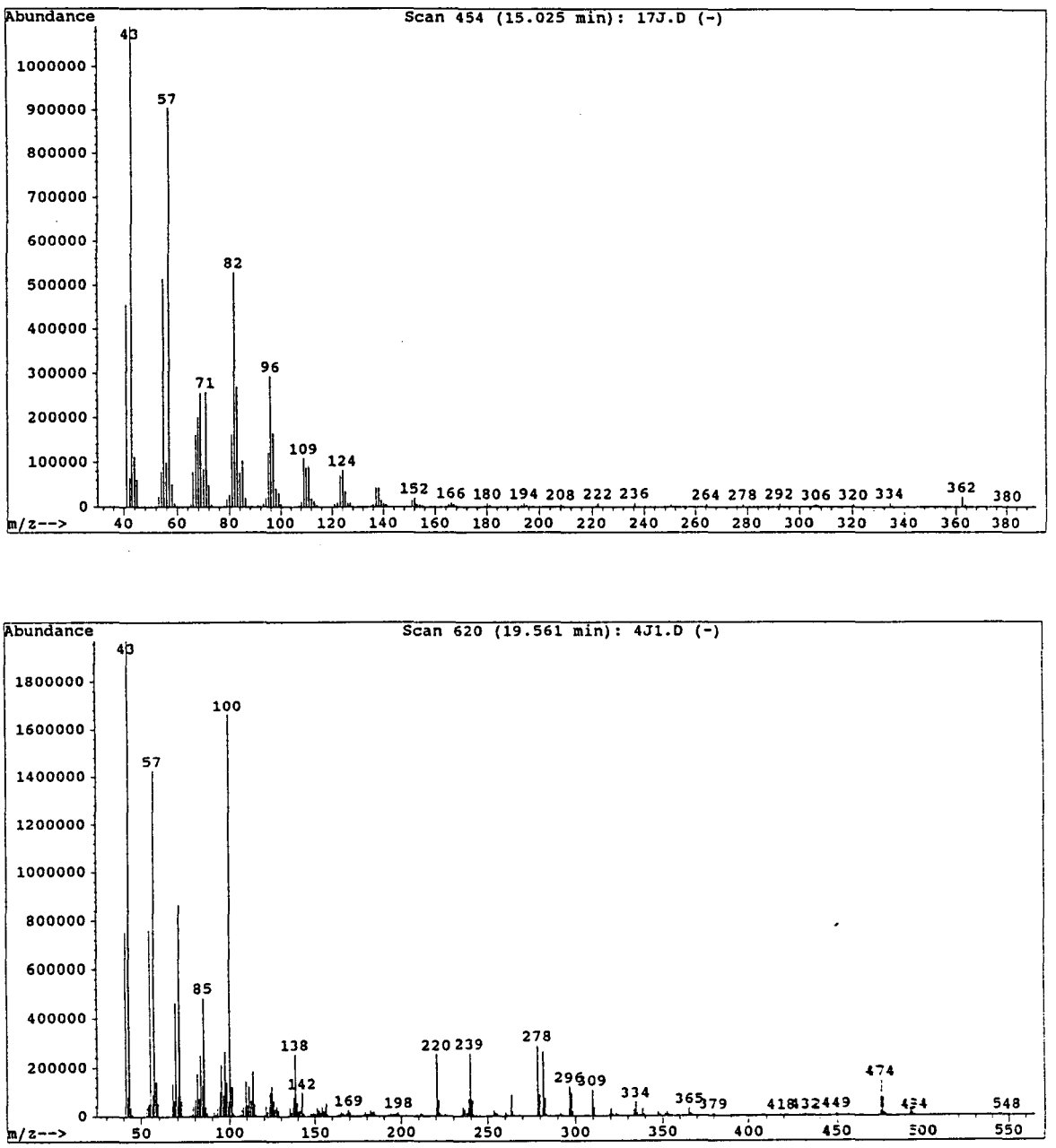


Appendix 5.1E. GLC separation of the components from the wax of *E. subcrenulata* juvenile leaves. Peaks H27 and H29 are n-alkanes; peaks A26 are alkanal (C_{26}); peaks D33 is ß-diketone (C_{33}); Peaks TA, TB, TC, TK, TL, TG, TH and TI are triterpenoids.

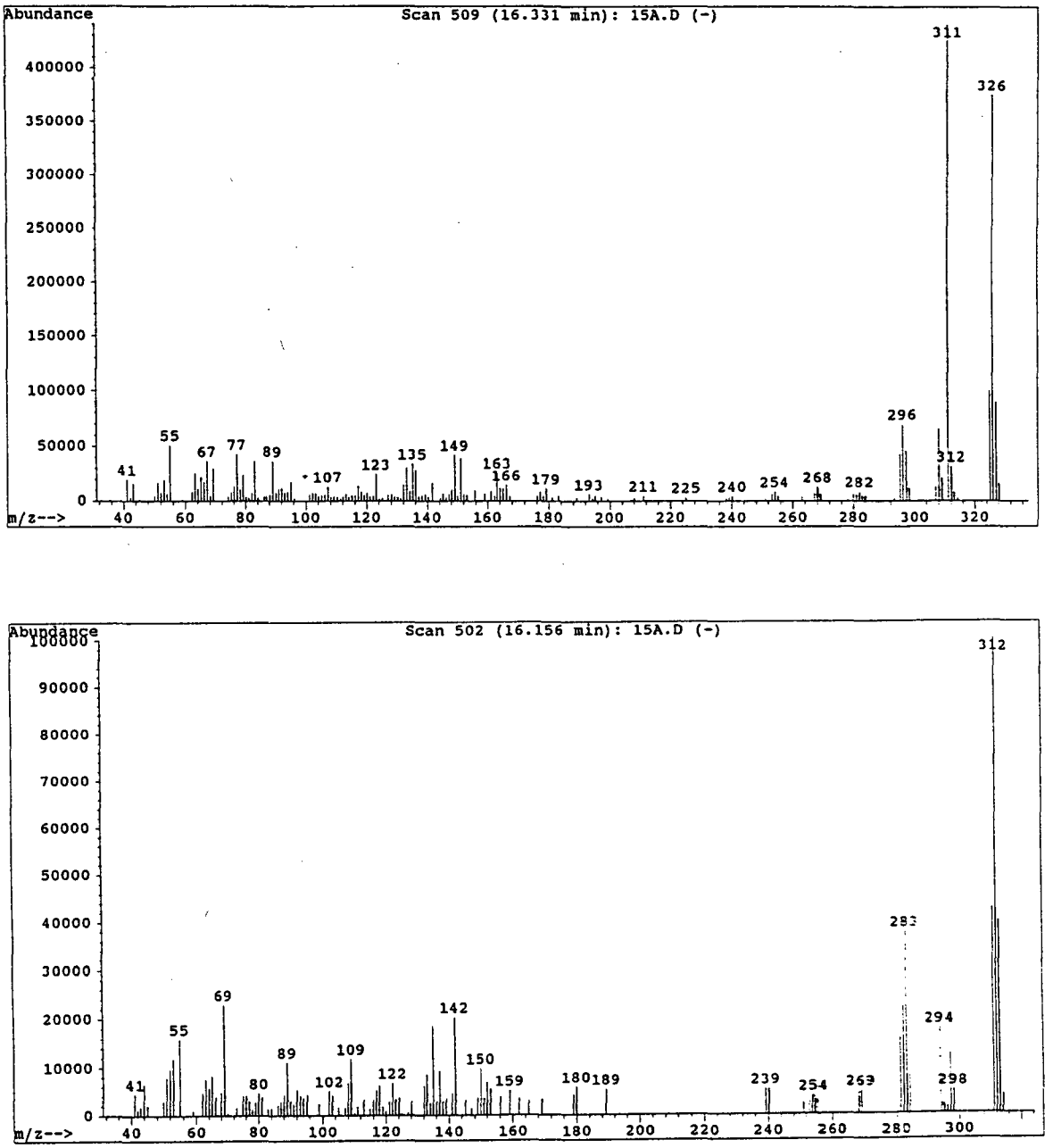
Appendix 5.1F. Representative mass spectra of the major compound classes



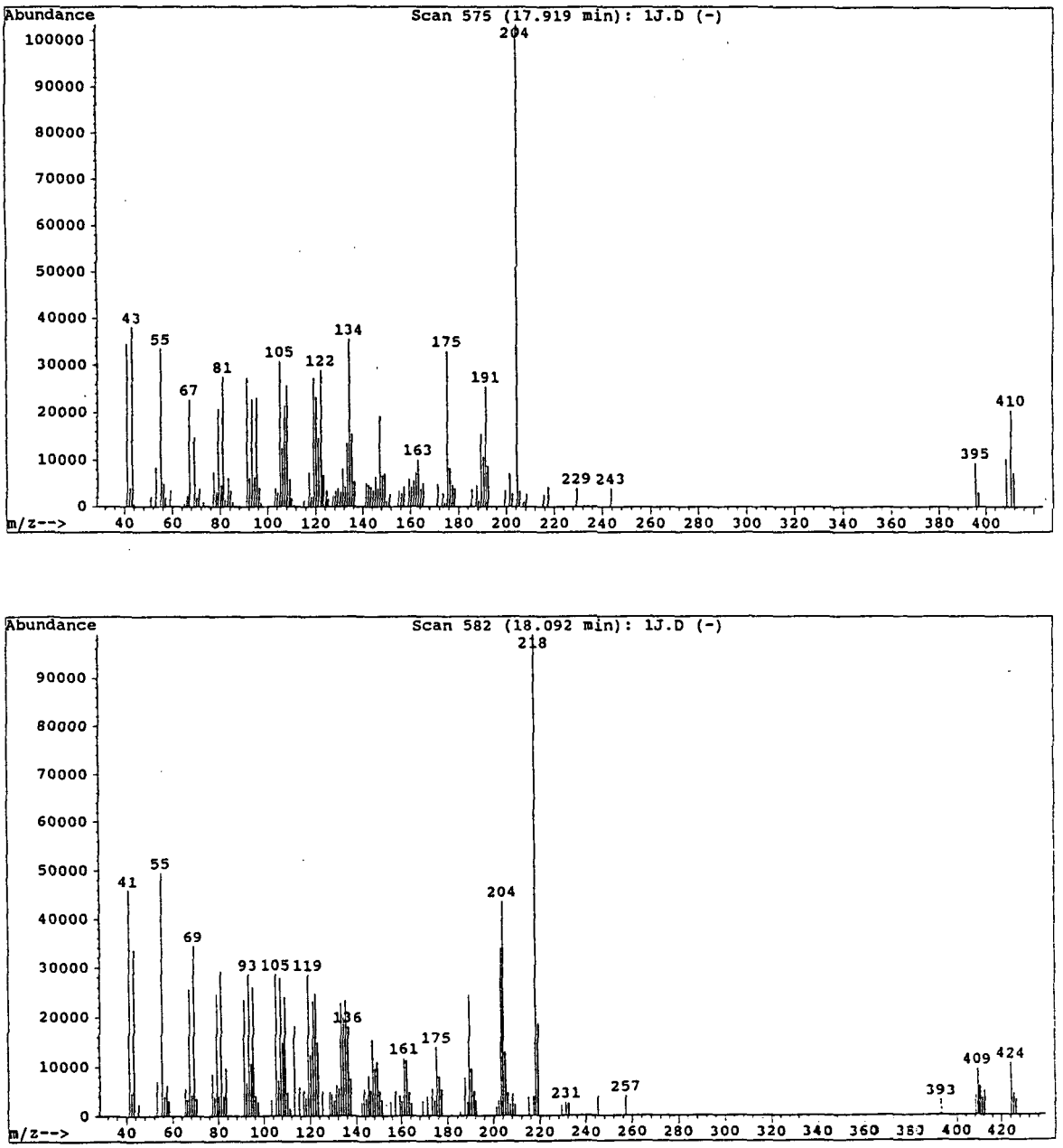
Appendix 5.1F-1. Mass spectra of the n-C_{29} alkane from the wax of *E. urnigera* leaves (above) and the n-C_{24} alcohol from the wax of *E. amygdalina* leaves (below).



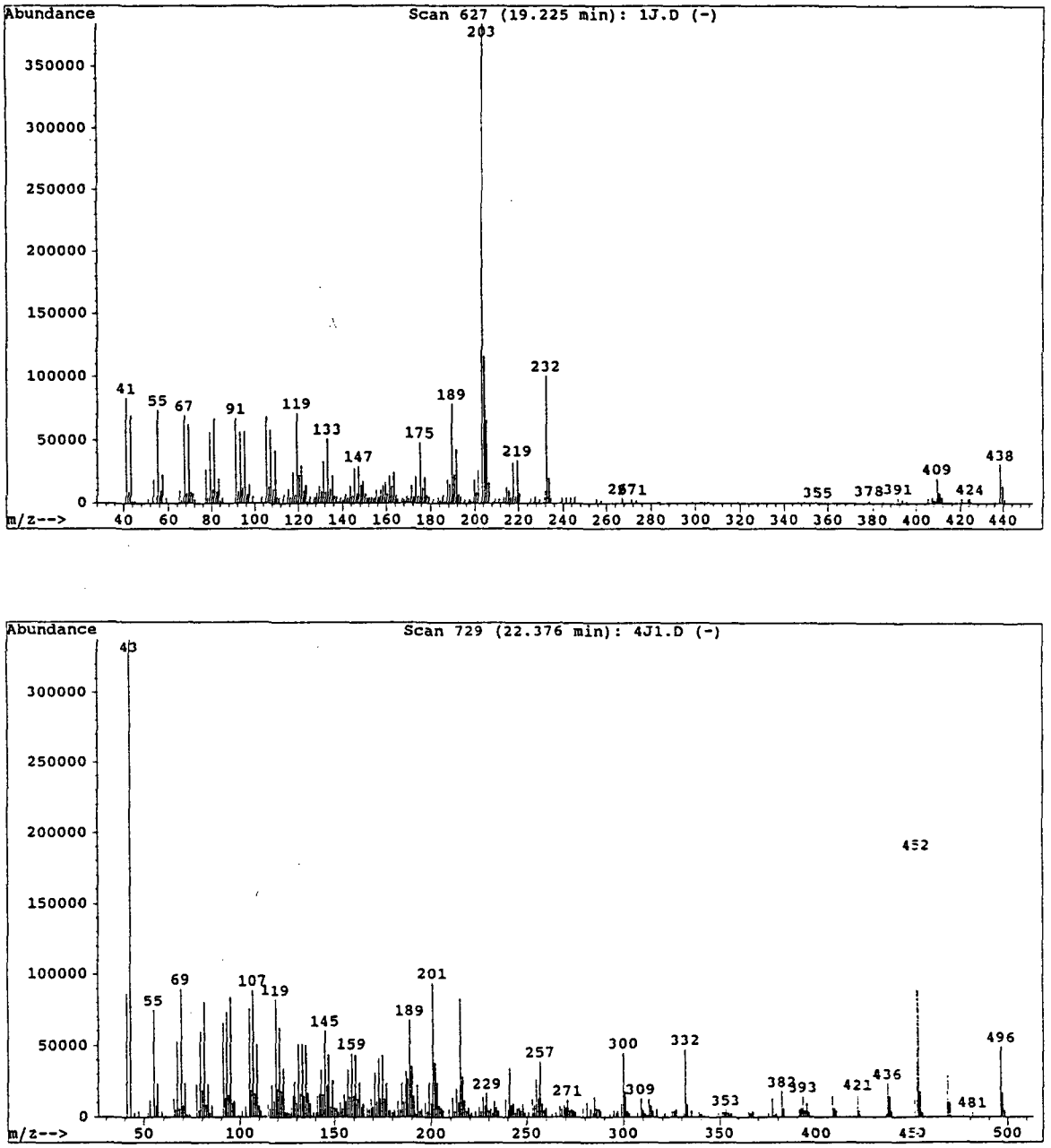
Appendix 5.1F-2. Mass spectra of the C_{26} alkanal from the wax of *E. amygdalina* leaves (above) and the C_{33} ß-diketone from the wax of *E. urnigera* (below).



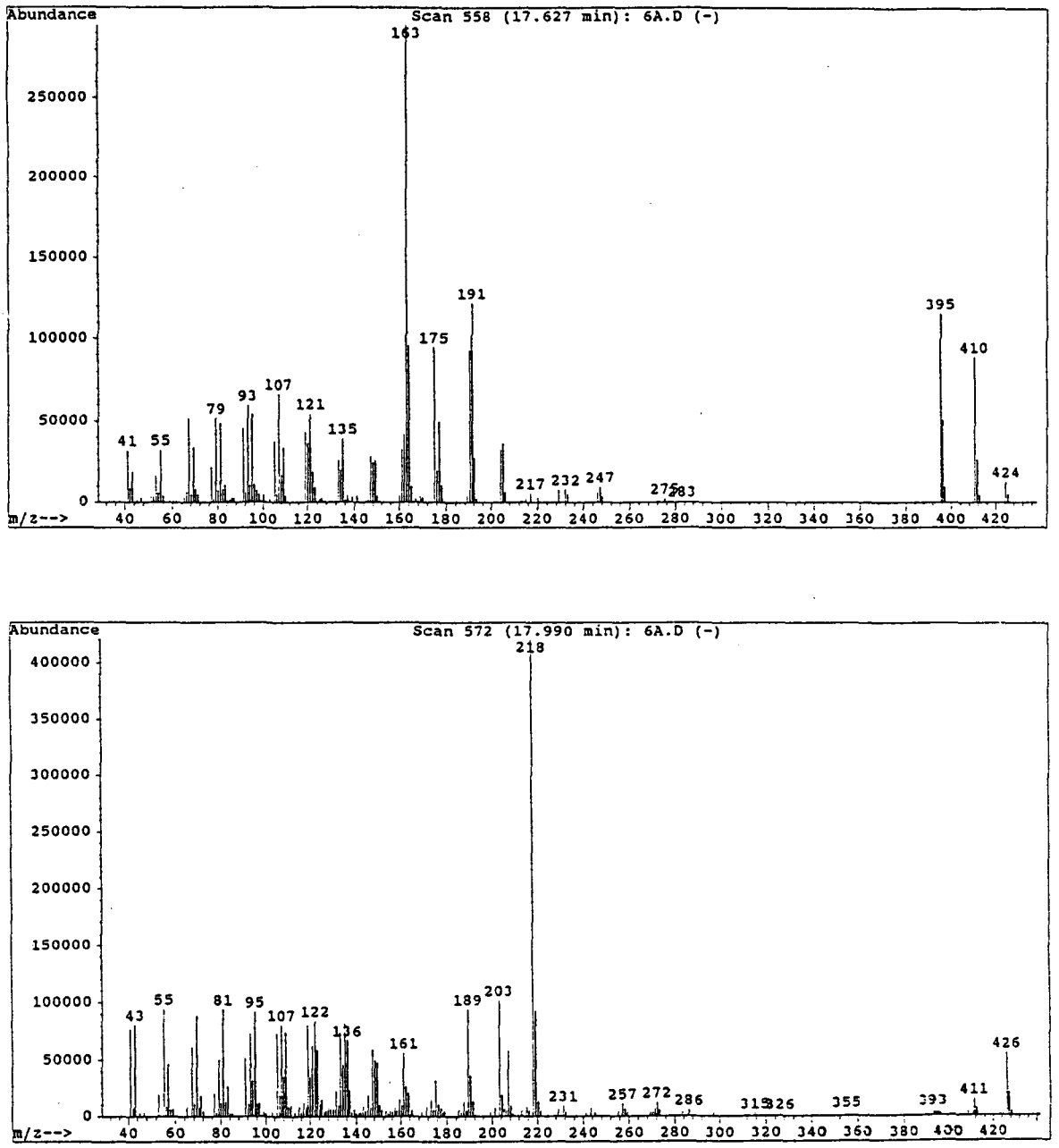
Appendix 5.1F-3. Mass spectra of the eucalyptin (5-hydroxy-4',7--dimethoxy-6,8-dimethylflavone) (above) and demthyl eucalyptin (5-hydroxy-4',7--dimethoxy-6-methylflavone) from the wax of *E. nitida* leaves (below).



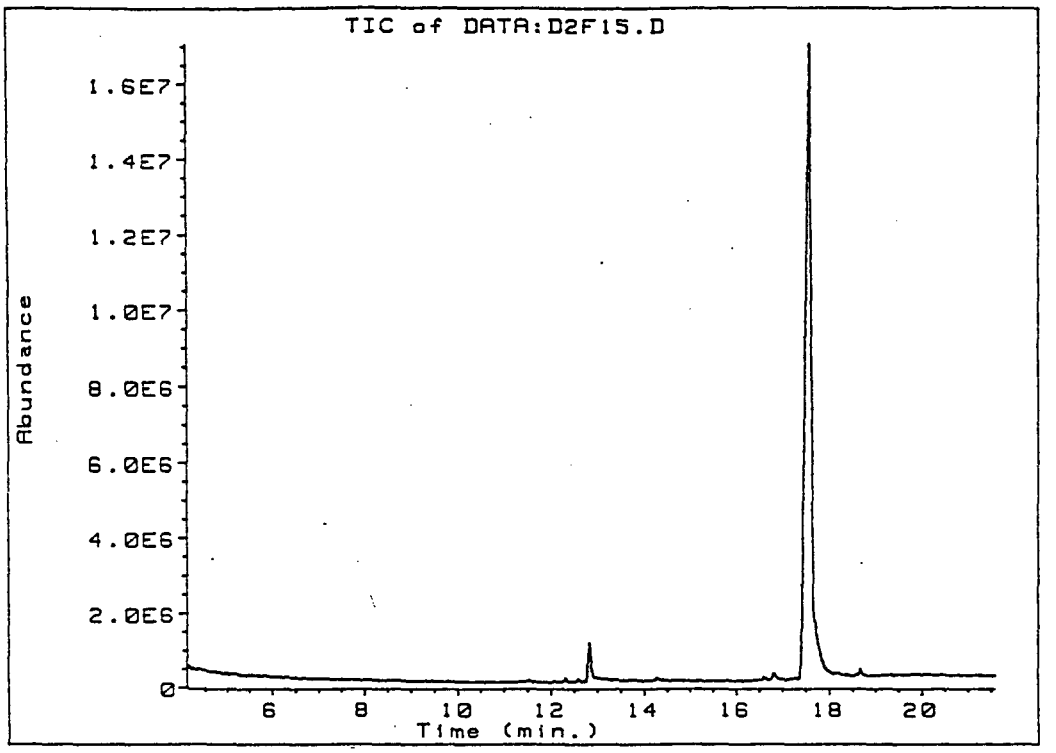
Appendix 5.1F-4. Mass spectra of the triterpenoid compound A (TA) (above) and C (TC) (below) from the wax of *E. johnstonii* leaves.

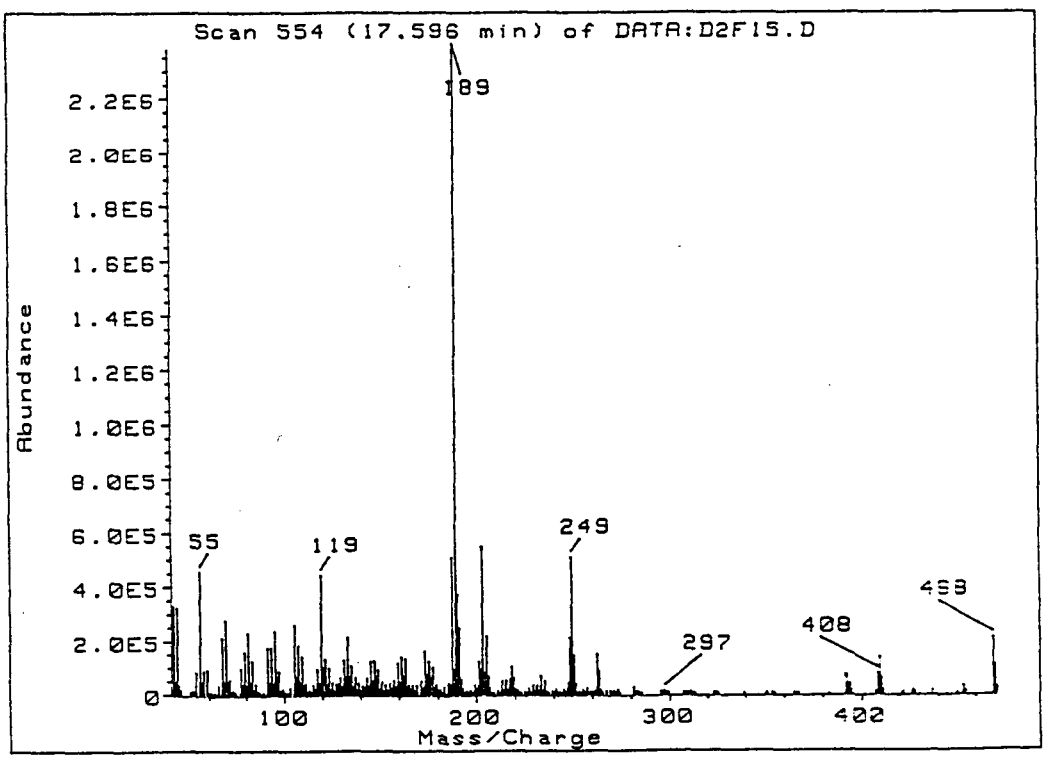


Appendix 5.1F-5. Mass spectra of the triterpenoid compound H (TH) from the wax of *E. johnstonii* leaves (above) and 11,12-dehydroursolic lactone acetate (TM) from the wax of *E. urnigera* leaves (below).



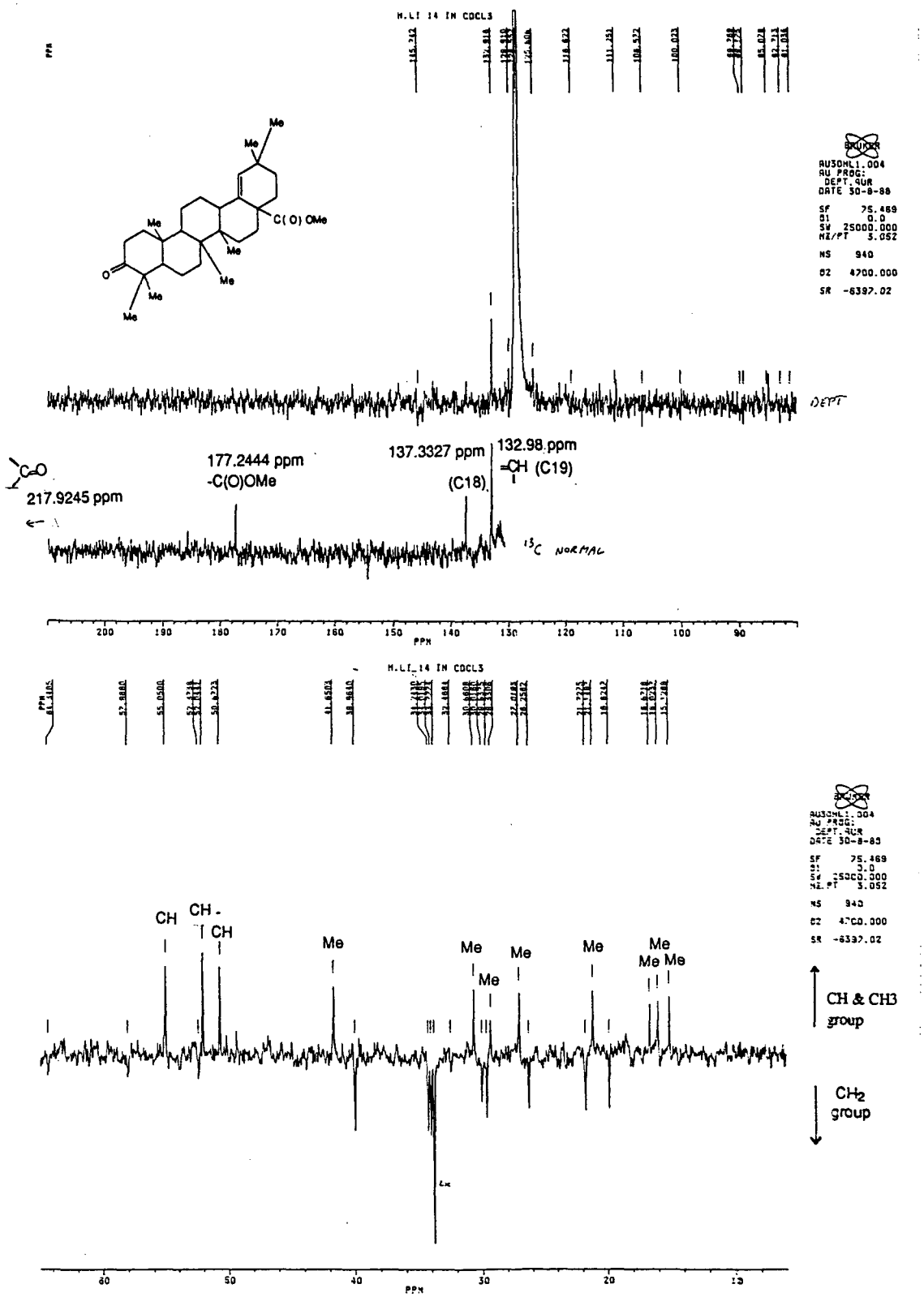
Appendix 5.1F-6. Mass spectra of the triterpenoids D (above) (28-norolean-17en type) and ß-amyrin (below) from the wax of *E. obliqua* leaves.





Appendix 5.1F-7. Methyl moronate* isolated from the wax of *E. delegœnsis* (TK) (above) and its mass spectrum (below).

* This compound was obtained from the benzene fraction by chromatography on alumina following Horn *et al.* (1964).

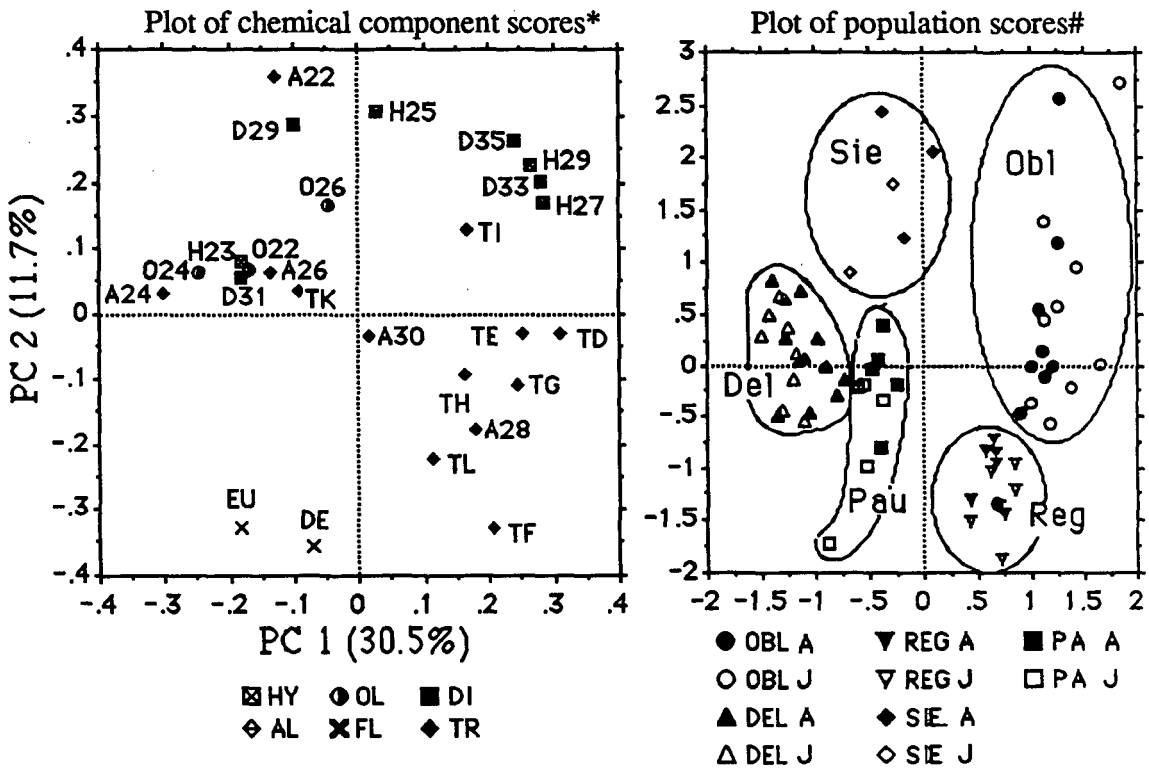


Appendix 5.1F-8. The ^{13}C-NMR spectrum of methyl moronate.

Appendix 5.2. The PCA of *Obliquae* species based on wax chemical data.

The PCA of *Obliquae* species alone indicated that both juvenile and adult leaf samples of each species were grouped together and discriminated from other species in the space defined by variation of wax compounds in the first two PCs, with the exception of few samples (right figure). The *E. obliqua* samples were separated from *E. regnans* samples due to higher levels of ß-diketones D33 and D35 and hydrocarbons H29 and H27 while other species were also separated by variation in compounds as indicated in the plot of chemical component scores (left figure).

Appendix Fig 5.2. Scatter plot of the wax chemicals (left) and samples of the 5 *Obliquae* species (right) on the first two principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf waxes.



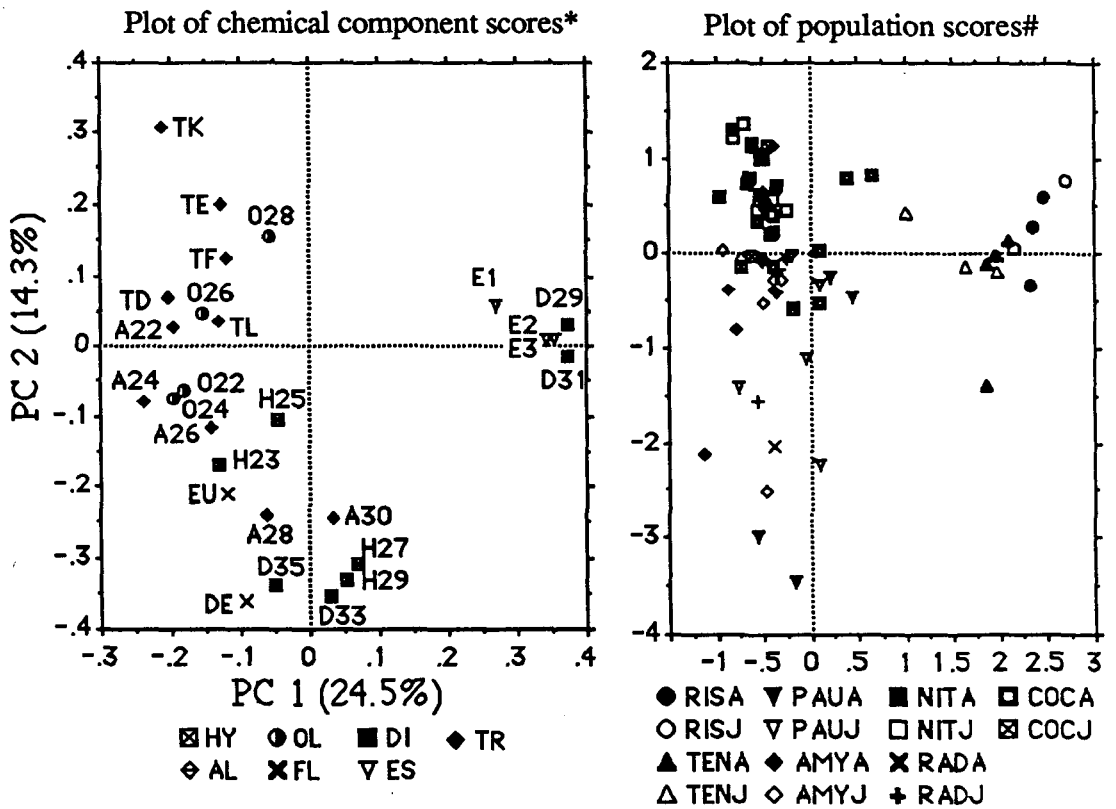
* Compound scores are indicated as in Fig. 5.3.

Juvenile and adult leaf sample scores of individual species were indicated by the species code listed in Table 4.1.

Appendix 5.3. The PCA of *Piperitae* species based on wax chemicals.

The main trends of variation in wax chemicals among *Piperitae* species on the first two PCs (24.5% and 14.3%) of PCA is shown in Appendix Fig. 5.3. All samples of *E. tenuiramis* and *E. risdonii* had high levels of esters and ß-diketones, D33 and D35, and were clearly separated from samples of other species by PC 1. All samples of *E. nitida* had higher levels of triterpenoids and were grouped together in the upper left quadrant. There was a continuous variation, which was mainly associated with samples of *E. pulchella, E. amygdalina* and *E. radiata,* along PC 2 with increasing levels of alkanes H27 and H29, aldehydes A28 and A30, ß-diketones D33 and D35 and flavonoids associated with decreasing values along PC2.

Appendix Fig. 5.3. Scatter plot of the wax chemical parameters (leaf) and samples of *Piperitae* the 7 species (right) on the two first principal components (PC1 and PC2) derived from analysis of juvenile and adult leaf oils.

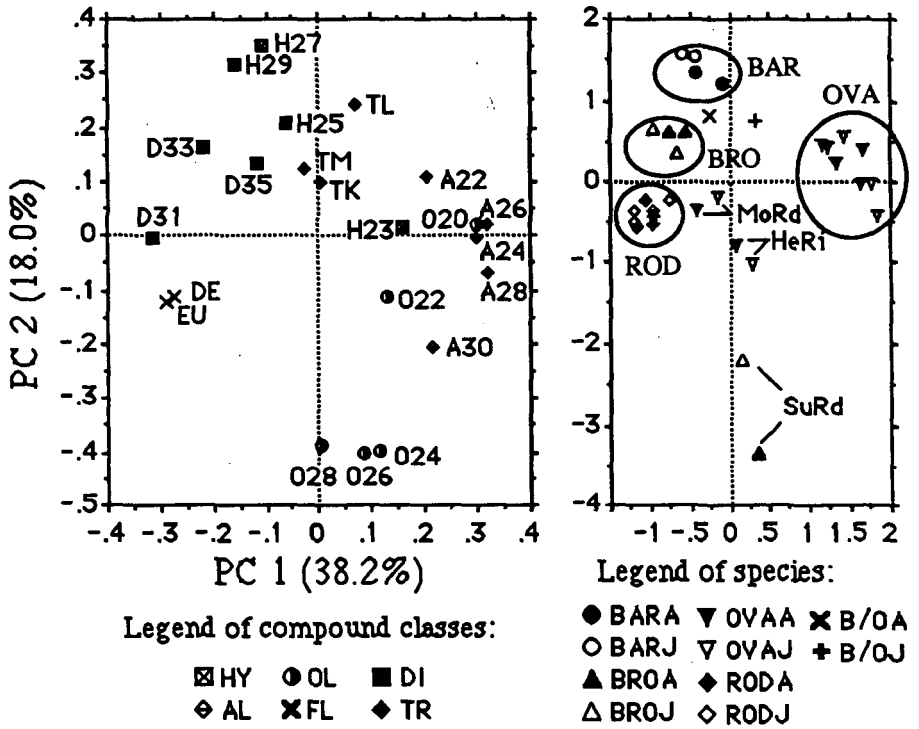


* Compound scores are indicated as in Fig. 5.3.
# Juvenile and adult leaf sample scores of individual species were indicated by species codes listed in Table 4.1.

Appendix 5.4. The PCA of *Ovatae* species based on wax chemical data.

A PCA analysis for variation within black gum (series *Ovatae*) species, shown in Appendix Fig. 5.4, indicated that the majority of samples of individual species were separated into specific groups on the first two PCs. Both juvenile and adult samples of all four *E. rodwayi* localities had high levels of ß-diketones in contrast with samples of the

Appendix Fig. 5.4. Scatter plot of the wax chemical parameters (left) and samples of the *Ovatae* species (right) on the first two principal components (PC1 and PC2) derived from analysis of juvenile adult leaf waxes.



* Compound scores are indicated as in Fig. 5.13.
# Juvenile and adult leaf sample scores of individual species are indicated by the species code listed in Table 4.1.

two *E. barberi* localities, which had high levels of hydrocarbons. Both juvenile and adult samples of the eastern localities of *E. brookeriana* (MRd and ChTr) were intermediate between *E. rodwayi* and *E. barberi* samples while samples of the SuRd locality, classified as *E. brookeriana*, were separated from the above two localities due to high levels of alcohols. The majority of *E. ovata* had high levels of aldehydes in contrast to samples of the other three species while samples of *E. ovata* from the western Tasmania (MoRd, HeRi) were separated from majority of eastern populations of *E. ovata* and tended toward *E. rodwayi or E. brookeriana.*

Appendix 7.1. Data for *C. bimaculata* adult feeding and oviposition responses to foliage of six eucalypt species (60 hour test period).

Species Code	Cage	Leaf consumption (g dry weight)	No. Egg batches
GLO	A	1.4	1
OVA	A	1.6	1
NIT	A	3.8	3
OBL	A	4.6	2
REG	A	1.8	3
SIE	A	1.4	1
GLO	B	1.6	1
OVA	B	0.0	2
NIT	B	4.8	4
OBL	B	2.4	2
REG	B	2.6	2
SIE	B	1.4	2
GLO	C	0.0	1
OVA	C	1.0	1
NIT	C	2.0	2
OBL	C	1.0	3
REG	C	4.6	4
SIE	C	1.2	2
GLO	D	0.0	1
OVA	D	1.8	2
NIT	D	1.2	4
OBL	D	6.4	2
REG	D	2.2	3
SIE	D	.8	2
GLO	E	1.6	2
OVA	E	.6	1
NIT	E	4.4	1
OBL	E	6.2	4
REG	E	1.0	2
SIE	E	1.2	2
Total	GLO	4.6	6
	OVA	5.0	7
	NIT	16.2	14
	OBL	20.6	13
	REG	12.2	14
	SIE	6.0	9
Means	GLO	0.9	1.2
	OVA	1.0	1.4
	NIT	3.2	2.8
	OBL	4.1	2.6
	REG	2.4	2.8
	SIE	1.2	1.8

Appendix 7.2. Data for *C. bimaculata* adult feeding and oviposition responses to adult and dewaxed juvenile foliage of *E. nitens* and *E. globulus* (60 hour test period).

Species	Cage	Leaf consumption (g dry weight)	No. Egg batches
NIT-A	A	2.13	5
NIT-J	A	3.25	2
GLO-A	A	0.68	2
GLO-J	A	0.12	1
NIT-A	B	4.53	3
NIT-J	B	1.23	3
GLO-A	B	0.86	1
GLO-J	B	0.77	2
NIT-A	C	4.26	6
NIT-J	C	4.06	2
GLO-A	C	1.12	2
GLO-J	C	0.56	1
NIT-A	A	6.33	7
NIT-J	A	3.58	4
GLO-A	A	1.25	1
GLO-J	A	0.78	1
NIT-A	C	2.13	4
NIT-J	C	3.25	2
GLO-A	C	0.65	3
GLO-J	C	0.98	1
Total	NIT-A	19.38	25
	NIT-J	15.37	13
	GLO-A	4.56	9
	GLO-J	3.21	6
Means	NIT-A	3.88	5.5
	NIT-J	3.07	2.6
	GLO-A	0.91	1.8
	GLO-J	0.64	1.2

Appendix 7.3. The feeding and oviposition responses of *C. bimaculata* and *C. agricola* adults to foliage of individual species with no choice (96 hour test period).

C. bimaculata			*C. agricola*		
Foliage species	Leaf consumption (g/dry weight)	No. egg batches	Foliage species	Leaf consumption (g/dry weight)	No. egg batches
E. nitens	5.60	7	*E. nitens*	4.50	10
	3.40	16		3.30	17
	4.30	8		6.30	11
	7.90	10		5.80	8
	5.30	8		4.30	15
	4.50	13		5.01	9.12
Rep.	6	6	Rep.	6	6
Mean	5.17	10.33	Mean	4.87	11.69
E. obliqua	6.90	9	*E. obliqua*	4.20	2
	4.80	13		2.40	5
	10.00	10		3.90	5
	5.80	12		1.90	3
	7.50	7		4.80	6
	6.30	5		2.86	4
Rep.	6	6	Rep.	6	6
Mean	6.88	9.33	Mean	3.34	4.17
E. globulus	0.80	4	*E. globulus*	4.50	7
	0.40	3		2.80	14
	1.90	3		3.56	10
	0.90	5		5.20	15
	2.50	2		4.90	7
	1.70	4		5.02	8
Rep.	6	6	Rep.	6	6
Mean	1.37	3.50	Mean	4.33	10.17

Appendix 7.4. The feeding and oviposition response of *C. bimaculata* and *C. agricola* adults to mixed foliage of three species (96 hour test period).

	C. bimaculata				*C. agricola*		
Cage	Species	Leaf consumptio (g/dry weight)	No. egg batches	Cage	Species	Leaf consumption (g/dry weight)	No. egg batches
1	GLO	0.5	1	1	GLO	3.98	2
1	NIT	2.1	6	1	NIT	1.52	3
1	OBL	1.5	2	1	OBL	0.36	2
2	GLO	0.1	0	2	GLO	3.0	5
2	NIT	3.8	3	2	NIT	3.1	5
2	OBL	2.1	4	2	OBL	1.3	1
3	GLO	0.7	0	3	GLO	2.4	7
3	NIT	1.4	2	3	NIT	0.9	2
3	OBL	2.9	5	3	OBL	1.0	0
4	GLO	0.3	0	4	GLO	1.4	2
4	NIT	3.2	3	4	NIT	2.4	4
4	OBL	1.9	4	4	OBL	0.4	0
5	GLO	0	1	5	GLO	2.7	5
5	NIT	1.3	5	5	NIT	1.3	4
5	OBL	4.9	1	5	OBL	0.7	2
6	GLO	0.4	0	6	GLO	2.0	7
6	NIT	2.4	2	6	NIT	1.9	2
6	OBL	3.3	3	6	OBL	0.2	0
Total	GLO	2.0	2	Total	GLO	15.3	28
	NIT	14.2	21		NIT	11.0	20
	OBL	16.6	19		OBL	3.9	5
Means	GLO	0.3	0.33	Means	GLO	2.6	4.67
	NIT	2.4	3.50		NIT	1.8	3.33
	OBL	2.8	3.17		OBL	0.7	0.83

Appendix 7.5. The feeding and oviposition response of *C. bimaculata* adults on pairs of different species (96 hour test period)

Species pair	Species	Cage	Leaf consumption (g/dry weight)	No. egg batches
N-O	NIT	1	3.02	4
N-O		2	2.50	3
N-O		3	1.86	3
N-O		4	1.56	4
N-O	OBL	1	2.53	3
N-O		2	1.86	6
N-O		3	2.12	4
N-O		4	1.32	4
Total	NIT		8.94	14
	OBL		7.83	17
Means	NIT		2.24	3.50
	OBL		1.96	4.25
N-G	NIT	1	2.50	3
N-G		2	3.10	6
N-G		3	1.70	4
N-G		4	3.20	2
N-G	GLO	1	0.50	2
N-G		2	1.60	3
N-G		3	0.90	1
N-G		4	0.70	2
Total	NIT		10.50	15
	GLO		3.70	8
Means	NIT		2.63	3.75
	GLO		0.93	2.00
O-G	OBL	1	3.50	3
O-G		2	4.50	6
O-G		3	2.50	6
O-G		4	2.30	4
O-G	GLO	1	0.10	2
O-G		2	0.30	1
O-G		3	0.15	1
O-G		4	0.08	2
Total	OBL		12.80	19
	GLO		0.63	6
Means	OBL		3.20	4.75
	GLO		0.16	1.50

Appendix 7.6. The volatile chemicals in air samples from enclosed fresh eucalypt leaves and solvent extractions of fresh leaves.

A. The volatile chemicals in arie samples from enclosed fresh leaves.

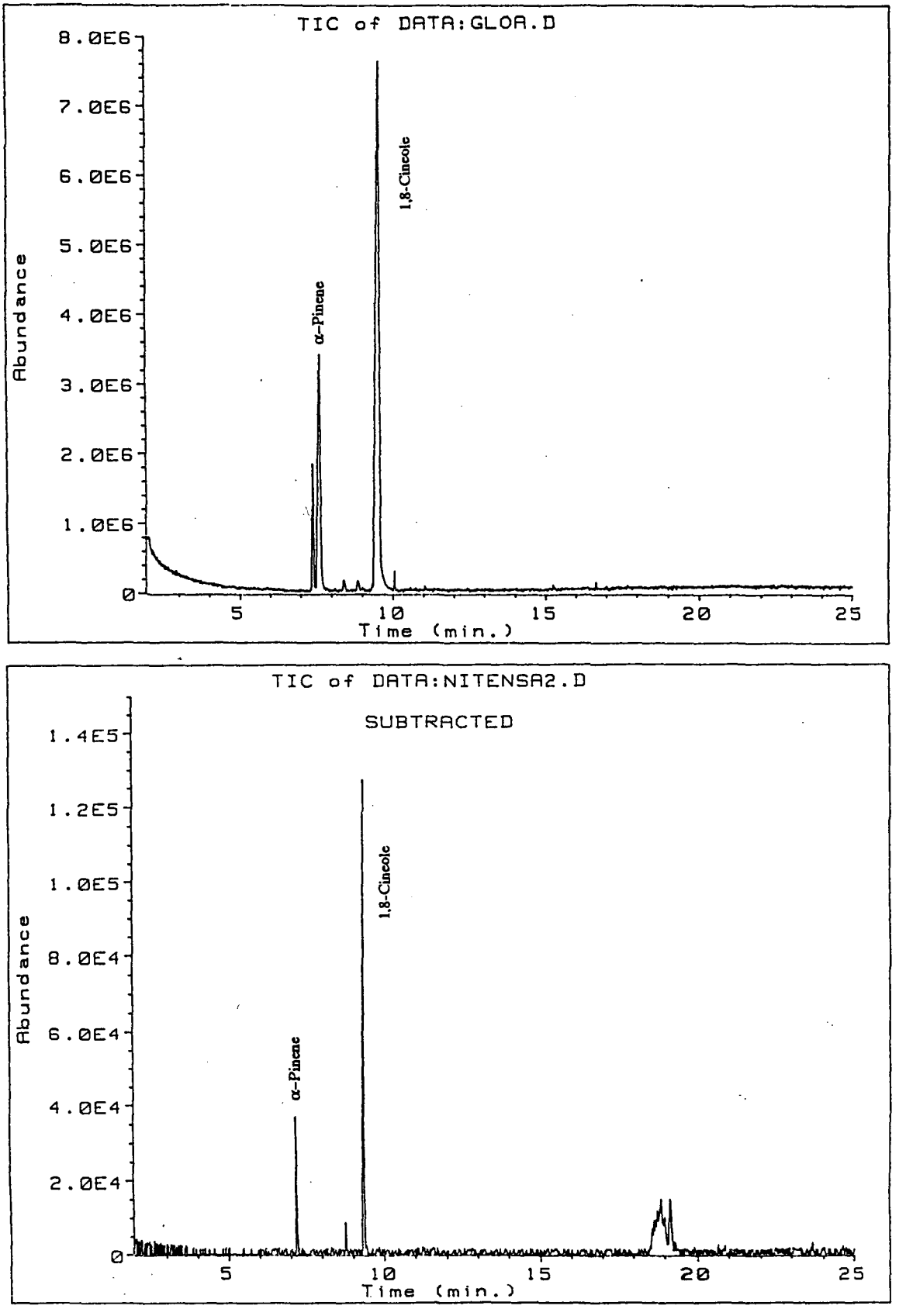
Method and analysis

Fresh adult leaves of each sample (approximately 30 cm^2 of leaf area) were sealed in a glass vial (35 ml volume) with a plastic cap and equilibrated at 35^0C for 5 minutes. Head space in the vial was sampled directly piercing the cap by a 5 ml plastic syringe. The air sample was then injected slowly into a pre-column (50 cm) which passed through a liquid nitrogen trap. The pre-column was attached to the front of the analytical GC column and immersed in liquid nitrogen. After injection the liquid nitrogen trap was removed and the temperature program commenced immediately. The accumulated volatiles were released into the standard column. Care was taken to void blocking the pre-column with ice from condensed water vapour of air samples.

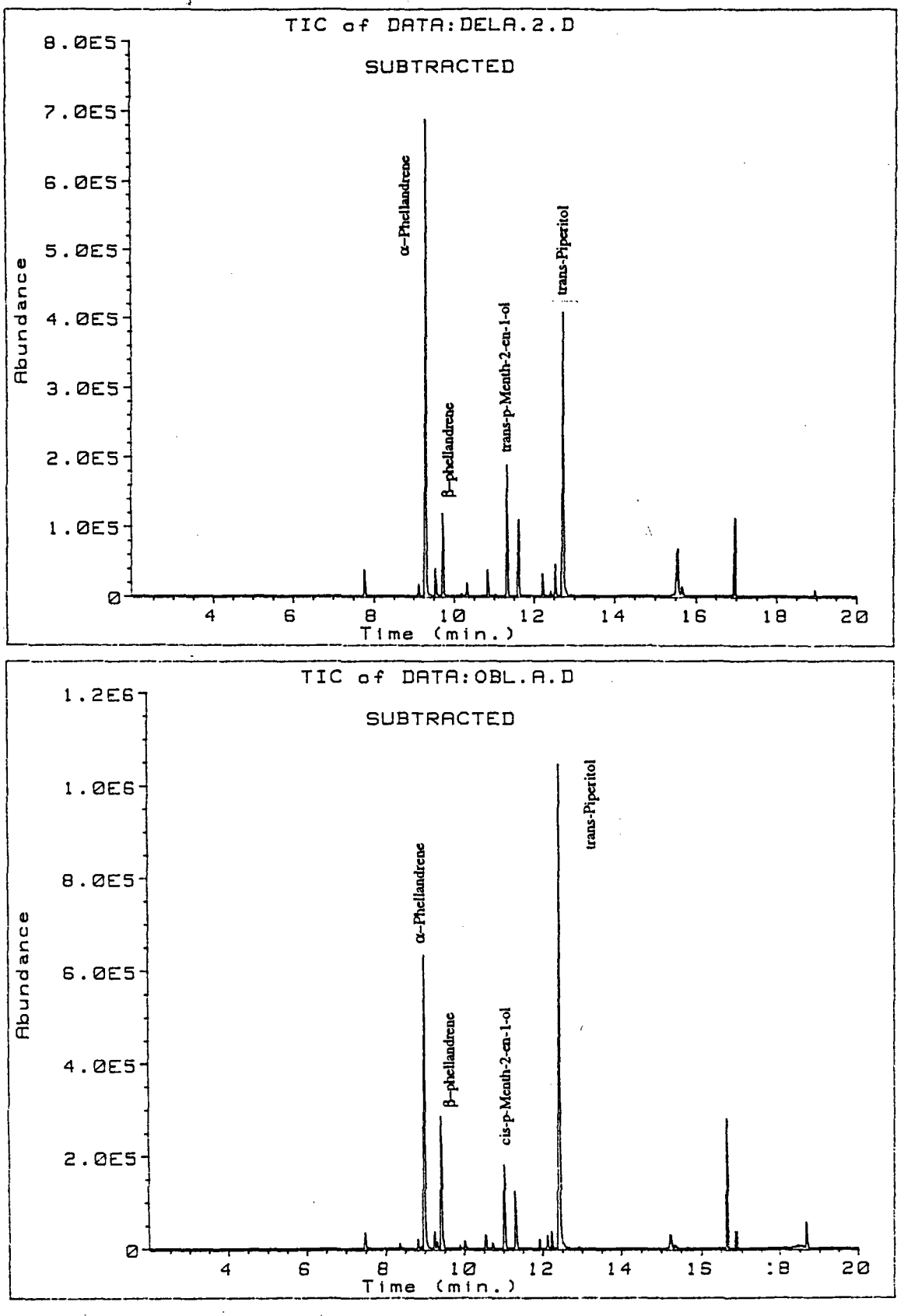
The samples were analysed by combined GC-MS using a HP-5890 GC directly coupled to a VG 707F mass spectrometer with 2035 data system as used for oil and wax analysis (see methods in chapter 3 and chapter 5). The GC column was a 24m x 0.32mm x 0.17 um film thickness Hewlett Packard (HP) 1 cross linked methyl-silica capillary column (SGE Pty Ltd). The oven temperature was programmed from 35 to 200 ^0C at 6^0C/minute and 200 to 290 ^0C at 30^0C/minute for 3 minutes using helium as carrier gas (70KPa and a flow rate of approximately 2ml/minute).

Results

The GLC separation of volatile compounds indicated that 1,8-cineole and α–pinene were the major volatile compounds in air samples of both adult and juvenile foliages of *E. globulus* and *E. nitens* (Appendix 7.6A-1). However, the concentration of these compounds was high for *E. globulus* but very low for *E. nitens*. (total air volume injected for each sample was 3 ml. In contrast, ß-phellandrene and trans-piperitol were the major volatile chemicals of *E. delegatensis* and *E. obliqua* respectively (Appendix 7.6A-2). Results for the other 26 species also indicated that the major compounds of volatiles from air samples are similar to those of essential oil samples and will be reported separately.



Appendix 7.6A-1. GLC separation of the components from liquid nitrogen trapped materials of air samples for *E. globulus* (above) and *E. nitens* (below) leaves. *Same volumes of air samples (3 ml) were injected and analysed with a limited interval between each run. Therefore, the concentrations of different samples could be compared approximately using scale value.



Appendix 7.6A-2. GLC separation of the components from liquid nitrogen trapped materials of air samples for *E. delegatensis* (above) and *E. obliqua* (below) fresh leaves.

Appendix 7.6B. Comparison of chemical composition (%) of leaf oils between solvent extraction and steam distillate for eucalypt species.

In this experiment, solvent extraction and steam distillation for ten species were compared. In order to avoid experiment error, oil yield was determined by internal standard for both steam distillation and solvent extraction.

Method and analysis

Adult leaf sample of each species were collected from a single tree. Fresh immature leaves of each sample were cut into pieces of 2 x 2 cm^2 or less and thoroughly mixed. This sample was than divided and utilised as described below. Fifteen 2 g lots were taken for solvent extraction. 10 g lots of chopped leaves was prepared for each sample for steam distillation and another 10 g lots was dried to for moisture determination. Ten species, *E. delegatensis, E. regnans, E. obliqua, E. coccifera, E. tenuiramis, E. globulus, E. viminalis, E. ovata, E. nitens* and *E. gunnii* were examined in this experiment.

Solvent extraction

Hexane, dichloromethane (CH_2Cl_2) and methanol were used for solvent extraction and each solvent extraction was replicated for five times.

Hexane and dichloromethane extraction:

2 g fresh leaf pieces were ground to a pulp using a mortar and pestle. 2 g of granular anhydrous sodium sulphate was used during grinding to absorb water and to act as an abrasive agent. The leaf pieces were added to the sodium sulphate and finely ground before the addition of a volume of hexane, followed by a known amount of tetradecane, the internal standard, in 5 ml of solvent. The mortar and pestle was then washed with two further 3 ml of solvent and washed solvent was added to the glass vial. The glass vial contained leaf pulp was allowed to stand at ambient temp. for one days. The clear liquid was drawn off using a pipette for oil analysis.

Methanol extraction:

2 g fresh leaf pieces and 2 g of granular anhydrous sodium sulphate were placed into a glass vial and 10 ml of methanol was added, followed by a same amount of tetradecane with above two solvent extractions. The glass vial was than treated in same way as above.

Steam distillation

10 g fresh leaf pieces were steam distilled for six hours, using a small glass steam-distillation unit in which 1ml hexane had been added. The oils were collected in the hexane and added into a standardised hexane with a known amount of tetradecane.

Oil analysis

The oils were analysed by combined GC-Ms using A HP-5890 GC directly coupled to a VG 707F mass spectrometer with 2035 dada system. The GC column was a 24m x 0.32mm x 0.17 um film thickness Hewlett Packard (HP) 1 cross linked methyl-silica capillary column (SGE Pty Ltd). The oven temperature was programmed from 35 to 200 ^{0}C at 6 ^{0}C/minute and 200 to 290 ^{0}C at 30 ^{0}C/minute for 3 minutes using helium as carrier gas. The injection temperature was 190 ^{0}C and the detector temperature was 270 ^{0}C.

The oils were sun under same conditions using a FID on a HP5890 Gas chromatography to give relative peak areas for individual compounds. The yield determination of these leaf oils was made possible by the use of n-tetradecane as an internal standard. The internal standard chosen had a retention time which did not interfere with peaks being measured.

Results

Result indicated that steam distillation gave the highest oil yield than solvent extraction for most species, in particular those *Monocalyptus* species while dichloromethane gave the highest recovery of oil than other two solvents for all species. However, the capabilities of hexane and methanol differed between species. For example, hexane extraction gave lower oil yield for *E. regnans* leaves than methanol extraction, whereas the reverse were found for *E. delegatensis* and *E. obliqua* (see table).

Result showed that the α– and β–phellandrene and other monoterpene hydrocarbons were found to occur in all solvent extraction of *E. delegatensis, E. obliqua* and *E. regnans* leaves (see table). This clearly indicated that these monoterpene hydrocarbons are original compounds in the leaves. Results of comparison in leaf oil between solvent extraction and steam distillation for all ten species will be reported in another publication.

Appendix 7.6B Table. The monoterpenoids and major sesquiterpenoids from solvent extractions of *E. delegatensis, E. regnans* and *E. obliqua* adult leaves.

Species	*E. delegatensis*			*E. regnans*			*E. obliqua*		
Solvent	H	D	M	H	DD	M	H	D	M
α–Thujene	1.8	2.2	3.5	1.6	1.7	1.6	2.6	2.0	3.4
Sabinene	0.3	0.3	0.4	0.2	0.2	0.3	1.0	1.1	1.2
Myrcene	0.6	0.8	1.2	0.3	0.4	0.6	0.9	1.1	1.4
α–Phellandrene	19.9	18.7	22.2	17.1	17.2	10.1	25.8	23.9	21.9
α–Terpinene	1.1	0.9	1.6	0.9	0.4	0.3	1.1	0.9	0.6
p-Cymeme	0.0	0.0	1.2	0.5	0.4	0.8	1.1	0.5	1.8
β–Phellandrene	4.3	5.6	4.7	2.0	1.6	1.7	12.0	11.7	13.9
Limonene	0.4	0.6	0.8	0.2	0.3	0.3	0.5	0.6	0.5
Terpinolene	1.6	1.4	1.5	1.1	0.7	0.7	1.4	1.4	1.3
trans-p-Menth-2-en-1-ol	7.2	3.0	1.9	1.1	0.6	0.4	1.1	0.8	0.7
cis-p-Menth-2-en-1-ol	6.8	4.4	1.9	2.3	1.7	1.4	3.0	2.9	2.7
Terpinen-4-ol	2.4	2.0	3.0	0.9	0.7	0.8	0.6	0.5	0.8
α–Terpineol	0.5	0.5	0.9	0.4	0.4	0.6	1.3	1.4	1.8
cis-Piperitol	1.8	0.9	0.0	0.3	0.2	0.0	0.2	0.0	0.0
trans-Piperitol	35.1	38.3	30.5	26.2	22.8	18.9	33.2	33.7	31.2
Germacrene	2.1	3.8	3.3	9.3	10.0	10.3	8.4	9.3	9.1
Elemol	0.3	0.6	-	2.3	1.6	2.7	-	-	-
Σ α–, β–, γ–Eudesmols	2.6	3.2	1.5	11.9	14.9	3.1	0.0	0.3	1.2
Hedycaryol	6.3	8.5	12.5	21.0	18.4	34.1	1.7	2.0	2.2

Appendix 8.1. Data of *C. bimaculata* larval feeding response to foliage of 11 eucalypt species. **E. nitens* control.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
NITa1*	41.2	25.2	35.6	5	2
	29.0	21.0	40.0	4	2
	29.7	18.9	31.6	5	4
	32.8	22.6	37.4	4	3
	39.1	21.7	38.1	5	4
	20.7	11.0	26.9	5	3
	10.7	5.6	16.5	4	2
	14.9	7.8	19.3	4	2
	26.3	17.3	37.6	5	5
	28.6	14.2	39.9	5	3
No. of Rep.	10	10	10	10	10
Mean	27.3	16.5	32.3	4.6	3
				0	
Glo.a1	2.0	2.0	9.6	3	1
	2.0	1.3	7.2	3	0
	3.0	2.1	7.3	2	2
	6.5	3.1	11.7	3	1
	1.2	1.6	6.1	2	0
	2.4	2.0	7.7	3	0
	5.1	3.9	14.2	3	1
	3.3	1.3	5.0	2	0
	2.3	1.2	7.6	3	0
	4.7	2.4	6.4	3	0
No. of Rep.	10	10	10	10	10
Mean	3.3	2.1	8.3	2.7	0.5
Vim.a1	17.3	7.8	22.1	5	2
	4.9	2.6	12.0	4	1
	11.9	5.1	17.9	5	1
	2.5	0.8	7.0	2	0
	5.5	2.9	11.6	4	1
	15.7	6.8	13.4	3	2
	7.1	2.8	9.5	3	1
	3.6	2.3	8.0	2	1
	8.0	5.5	11.8	2	1
	6.0	2.6	9.0	3	1
No. of Rep.	10	10	10	10	10
Mean	8.3	3.9	12.2	3.3	1.1
Gum.a1	1.1	2.0	4.9	2	0
	3.6	2.3	5.2	2	0
	5.8	5.5	13.5	3	3
	8.2	6.4	14.4	4	1
	14.2	7.8	18.5	4	2
	5.9	5.2	15.7	5	2
	3.2	3.5	7.8	3	0
	7.5	4.0	7.3	2	1
	5.7	5.0	13.6	4	0
	8.5	5.7	16.9	5	2
No. of Rep.	10	10	10	10	10
Mean	6.4	4.7	11.8	3.4	1.1

Appendix 8.1. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
NITj1	29.1	12.0	24.9	5	3
	41.2	21.8	34.2	5	3
	3.1	1.5	9.3	4	0
	22.5	13.2	23.3	5	2
	23.4	13.7	24.4	5	2
	19.9	11.5	22.4	4	3
	22.2	8.3	22.6	4	2
	26.1	15.8	27.7	5	3
	23.6	14.4	25.0	5	1
	17.9	11.0	21.4	3	2
No. of Rep.	10	10	10	10	10
Mean	22.9	12.3	23.5	4.5	2.1
Glo.j1	0.7	0.9	5.7	3	0
	1.7	0.7	0.0	0	0
	1.9	1.1	7.0	3	0
	1.8	0.4	5.4	2	0
	0.6	0.3	0.0	0	0
	1.5	0.6	3.2	1	0
	0.6	0.2	0.0	0	0
	2.9	1.4	10.8	4	1
	1.1	0.5	2.0	1	0
	0.7	0.4	0.0	0	0
No. of Rep.	10	10	10	10	10
Mean	1.4	0.7	3.4	1.4	0.1
Ov.a1	5.2	2.0	2.4	0	0
	2.6	3.3	5.6	2	0
	3.7	2.1	4.3	2	0
	4.0	2.6	4.5	2	0
	2.2	2.6	7.5	2	1
	5.6	3.1	5.6	2	1
	3.7	2.1	5.3	2	0
	3.2	3.1	5.0	2	1
	3.8	2.7	5.2	3	2
	5.5	3.3	10.3	3	0
No. of Rep.	10	10	10	10	10
Mean	4.0	2.7	5.6	2	0.5
Del.a1	30.5	13.0	23.0	4	2
	19.6	12.6	23.5	4	2
	21.6	19.8	27.4	5	3
	26.1	16.7	26.8	4	2
	38.1	23.6	37.1	5	4
	32.6	19.0	33.1	5	4
	11.1	5.4	17.6	4	0
	30.5	16.5	25.4	5	3
	30.7	19.3	28.1	5	5
	19.9	15.7	26.9	5	5
No. of Rep.	10	10	10	10	10
Mean	26.1	16.2	26.9	4.6	3

Appendix 8.1. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Reg.a1	12.9	7.8	17.5	4	2
	17.6	11.4	19.6	4	2
	20.2	14.5	9.9	2	2
	11.5	7.6	15.7	4	2
	9.0	7.5	12.4	3	1
	13.2	8.9	14.3	4	1
	14.1	14.9	28.8	5	3
	19.9	12.9	23.9	5	3
	20.2	14.8	25.4	5	3
	19.4	15.8	14.4	3	2
No. of Rep.	10	10	10	10	10
Mean	15.8	11.6	18.2	3.9	2.1
Pau.a1	16.1	10.8	21.1	3	3
	21.6	13.3	27.9	5	4
	28.8	18.5	36.3	5	3
	16.1	10.8	20.8	4	2
	32.4	20.1	31.4	5	2
	20.7	16.2	29.9	5	5
	26.8	17.9	36.5	5	2
	22.7	15.2	28.2	5	3
	23.7	15.6	28.6	4	1
	22.7	12.6	24.9	4	2
No. of Rep.	10	10	10	10	10
Mean	23.2	15.1	28.6	4.5	2.7
Sie.a1	11.5	5.2	8.4	3	0
	4.3	4.2	12.9	4	1
	5.7	4.7	12.2	4	1
	9.2	9.3	17.2	5	2
	7.0	6.2	12.7	4	1
	3.1	1.6	2.1	1	0
	8.2	4.1	8.8	3	0
	4.0	3.5	6.7	2	2
	17.8	11.6	18.5	4	1
	14.4	8.2	20.8	5	4
No. of Rep.	10	10	10	10	10
Mean	8.5	5.9	12.0	3.5	1.2

Appendix 8.1. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Obl.a1	35.0	24.8	31.9	5	4
	22.7	15.2	17.2	3	2
	10.8	18.4	18.3	4	2
	26.0	12.1	26.8	4	1
	11.0	9.7	15.6	4	3
	24.7	18.4	26.1	5	2
	16.3	8.7	12.9	3	1
	20.2	12.9	24.8	5	3
	18.7	13.5	32.1	2	2
	19.3	16.2	24.2	4	2
No. of Rep.	10	10	10	10	10
Mean	20.5	15.0	23.0	3.9	2.2
Ten.a1	10.1	8.8	21.0	4	3
	2.5	1.5	9.2	2	0
	11.9	11.0	22.3	4	1
	6.4	6.6	14.7	3	3
	11.7	8.0	14.7	3	3
	16.8	11.7	21.0	4	2
	8.8	8.7	15.7	3	2
	13.1	6.2	14.0	3	2
	9.9	8.0	18.3	4	1
	17.6	11.1	18.4	4	1
No. of Rep.	10	10	10	10	10
Mean	10.9	8.2	16.9	3.4	1.8

Appendix 8.2A. Data of *C. bimaculata* larval feeding response to adult and juvenile foliage of three 'ashes' species. Locality 1 = Mt Wellington; Locality 2 = Snug Plains.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Obl-Adults	38.2	29.8	32.0	5	3
Locality 1	17.5	11.2	16.3	3	3
	21.5	18.4	28.0	5	2
	24.4	15.1	21.9	3	2
	20.3	14.7	23.0	4	2
	31.4	20.3	28.1	5	3
	14.9	10.7	16.4	3	2
	18.8	10.9	24.8	4	1
	30.6	17.5	26.7	3	3
	20.5	16.2	28.5	4	3
No. of Rep.	10	10	10	10	10
Mean	23.7963	16.48	24.57	3.9	2.4
				0	
Obl-Adults	30.3	20.1	21.2	4	4
Locality 2	10.3	6.9	7.6	1	1
	22.3	18.3	26.0	4	2
	17.2	11.3	15.8	3	1
	28.6	20.5	25.8	5	1
	23.2	17.4	27.0	5	3
	13.2	10.2	21.0	3	3
	14.9	8.9	18.2	3	2
	16.8	11.2	21.1	4	2
	24.0	16.2	25.6	4	3
No. of Rep.	10	10	10	10	10
Mean	20.075	14.095	20.929	3.6	2.2
Del-Adult	34.3	19.0	32.7	5	2
Localitiy 1	28.7	21.6	30.2	5	2
	20.3	15.8	20.2	4	1
	23.5	15.7	20.2	3	3
	42.6	30.6	38.7	5	4
	32.8	22.0	26.5	5	4
	23.6	16.4	22.8	4	1
	34.6	24.5	30.8	5	3
	35.3	22.3	25.3	4	3
	21.3	14.4	23.4	3	3
No. of Rep.	10	10	10	10	10
Mean	29.704	20.23	27.08	4.3	2.6
Del-Adult	32.9	21.0	25.7	4	2
Locality 2	26.9	14.6	16.3	3	2
	26.9	18.8	23.9	4	2
	26.4	17.7	20.2	3	2
	51.0	38.6	39.9	5	4
	25.9	16.0	23.4	4	3
	35.0	20.4	21.7	4	2
	35.9	22.5	30.9	5	4
	47.1	30.3	36.5	5	3
	31.9	18.6	25.1	4	3
No. of Rep.	10	10	10	10	10
Mean	33.99	21.85	26.36	4.1	2.7

Appendix 8.2A. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Obl-Juvenile	36.0	25.8	34.7	5	4
Locality 1	23.4	16.2	24.2	4	3
	26.4	16.3	27.3	4	2
	19.4	13.1	23.7	4	2
	14.4	9.2	20.4	4	3
	34.1	25.4	31.2	5	4
	22.1	16.3	23.3	4	1
	19.3	10.3	25.7	4	3
	24.8	17.1	26.7	4	2
	29.0	16.8	25.8	4	2
No. of Rep.	10	10	10	10	10
Mean	24.885	16.648	26.3	4.2	2.6
Obl-Juvenile	26.7	21.8	34.4	5	3
Locality 2	20.0	14.2	19.7	3	2
	35.4	28.4	30.5	5	4
	25.0	18.1	22.8	4	1
	26.7	19.9	23.7	5	1
	32.2	22.4	26.5	5	4
	24.7	14.7	23.5	4	3
	13.9	8.9	15.4	3	1
	23.1	16.5	24.4	5	2
	33.7	21.2	31.7	5	3
No. of Rep.	10	10	10	10	10
Mean	26.14	18.61	25.259	4.4	2.4
Del-Juvenile	34.1	21.0	23.5	4	2
Locality 1	45.1	30.6	35.8	5	2
	29.1	22.8	31.2	5	2
	27.5	17.7	25.5	4	2
	46.1	37.6	40.1	5	4
	47.4	34.0	37.9	5	2
	23.3	16.4	21.6	3	3
	39.7	29.5	34.6	5	4
	48.6	31.3	39.4	5	3
	42.3	32.6	34.2	5	4
No. of Rep.	10	10	10	10	10
Mean	38.32	27.35	32.38	4.6	2.8
Del-Juvenile	41.1	31.0	34.8	5	2
Locality 2	32.9	22.6	27.2	4	2
	19.1	14.8	17.6	3	2
	22.1	15.7	20.4	3	2
	44.1	35.6	38.7	5	4
	32.0	23.0	22.7	4	2
	36.1	24.4	26.8	4	3
	44.5	33.5	35.3	5	4
	47.2	33.3	38.9	5	5
	40.6	26.6	29.6	5	5
No. of Rep.	10	10	10	10	10
Mean	35.97	26.05	29.2	4.3	3.1

Appendix 8.2A. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Reg-Adult	28.8	18.8	25.1	5	1
Locality 1	19.4	12.4	17.0	3	1
	13.6	7.9	15.1	3	2
	22.2	16.5	23.1	5	3
	35.2	25.5	29.0	5	4
	17.8	10.5	18.7	4	3
	27.2	21.9	26.7	5	3
	17.4	10.6	16.6	3	2
	23.4	17.5	27.9	5	2
	8.7	5.8	6.5	1	1
No. of Rep.	10	10	10	10	10
Mean	21.385	14.74	20.573	3.9	2.2
Reg-Adult	17.0	13.8	19.1	4	1
Locality 2	17.6	11.4	17.0	3	2
	18.6	12.9	18.3	4	1
	18.3	13.5	21.4	4	2
	22.0	16.5	22.5	5	4
	9.3	5.5	13.6	3	1
	21.4	16.9	24.6	4	2
	11.4	5.6	6.0	1	1
	24.5	15.6	26.1	4	2
	10.7	6.8	11.1	2	1
No. of Rep.	10	10	10	10	10
Mean	17.044	11.85	17.966	3.4	1.7
Control	35.9	20.0	38.4	5	4
Nit-Adult	21.9	12.6	18.9	3	2
	19.9	14.8	20.6	3	1
	34.4	22.7	33.6	4	3
	41.0	26.6	39.6	5	4
	32.7	19.0	25.2	4	2
	26.0	15.4	28.5	4	3
	41.9	27.5	35.1	5	3
	35.1	23.3	40.3	4	4
	24.9	16.6	27.6	3	3
No. of Rep.	10	10	10	10	10
Mean	31.37	19.85	30.78	4	2.9

Appendix 8.2A. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Del-Juvenile	23.3	16.8	22.8	4	2
Locality 1	17.5	11.4	15.8	3	2
	19.9	15.9	21.4	4	3
	18.3	12.5	16.1	3	1
	34.3	23.5	29.3	5	3
	25.3	16.1	26.8	4	3
	34.3	26.9	27.5	5	3
	25.5	16.6	22.7	4	3
	23.5	15.8	20.0	4	2
	22.6	16.8	24.3	4	2
No. of Rep.	10	10	10	10	10
Mean	24.45	17.23	22.67	4	2.4
Reg-Juvenile	21.5	14.8	25.8	5	1
Locality 2	23.1	15.4	26.2	5	2
	19.6	17.5	23.5	3	2
	21.8	16.5	21.2	3	2
	16.5	10.5	18.6	3	4
	30.8	20.5	31.2	5	1
	11.9	8.9	16.5	3	3
	22.9	18.6	27.5	5	2
	21.1	14.3	17.5	3	4
	12.2	5.8	15.1	2	2
No. of Rep.	10	10	10	10	10
Mean	20.108	14.28	22.31	3.7	2.3

Appendix 8.2B. MANOVA for *C. bimaculata* feeding response to foliage of *E. obliqua, E. delegatensis* and *E. regnans.*

Effect: Species			Value	F-Value	Num DF	Den DF	P-Value
S	2	Wilks' Lambda	0.47	9.553	10	208	0.0001
M	1	Hotelling-Lawley Trace	1.057	10.888	10	206	0.0001
N	51	Pillai Trace	0.564	8.257	10	210	0.0001

Effect: Leaf types			Value	F-Value	Num DF	Den DF	P-Value
S	1	Wilks' Lambda	0.888	2.613	5	104	0.0288
M	1.5	Hotelling-Lawley Trace	0.126	2.613	5	104	0.0288
N	51	Pillai Trace	0.112	2.613	5	104	0.0288

Effect: Localities			Value	F-Value	Num DF	Den DF	P-Value
S	1	Wilks' Lambda	0.964	0.767	5	104	0.5757
M	1.5	Hotelling-Lawley Trace	0.037	0.767	5	104	0.5757
N	51	Pillai Trace	0.036	0.767	5	104	0.5757

Effect: Species * Leaf types			Value	F-Value	Num DF	Den DF	P-Value
S	2	Wilks' Lambda	0.92	0.891	10	208	0.5427
M	1	Hotelling-Lawley Trace	0.086	0.887	10	206	0.5461
N	51	Pillai Trace	0.082	0.894	10	210	0.5394

Effect: Species * Localities			Value	F-Value	Num DF	Den DF	P-Value
S	2	Wilks' Lambda	0.817	2.218	10	208	0.018
M	1	Hotelling-Lawley Trace	0.213	2.197	10	206	0.0193
N	51	Pillai Trace	0.193	2.239	10	210	0.0169

Effect: Leaf types * Localities			Value	F-Value	Num DF	Den DF	P-Value
S	1	Wilks' Lambda	0.962	0.813	5	104	0.5431
M	1.5	Hotelling-Lawley Trace	0.039	0.813	5	104	0.5431
N	51	Pillai Trace	0.038	0.813	5	104	0.5431

Effect: Species * Leaf types * Localities			Value	F-Value	Num DF	Den DF	P-Value
S	2	Wilks' Lambda	0.93	0.768	10	208	0.6596
M	1	Hotelling-Lawley Trace	0.074	0.767	10	206	0.6607
N	51	Pillai Trace	0.071	0.769	10	210	0.6586

Appendix 8.2C. The means and standard errors and the ANOVA for differences of *C. bimacluata* larval feeding response between juvenile and adult foliages of *E. obliqua, E. regnans* and *E. delegatensis* from two localities based on transformed data.

Species	No. Rep.	Adult		Juvenile		Difference	ANOVA		
		Mean	S.E.	Mean	S.E.	Δ (A-J)	F-values	P-values	Sign.
Log(x+1) leaf consumptiom									
E. obliqua	20	1.341	0.03	1.41	0.021	-0.069	3.11	0.0859	ns
E. regnans	20	1.281	0.034	1.353	0.026	-0.072	2.795	0.1028	ns
E. delegatensis	20	1.504	0.023	1.568	0.026	-0.064	3.234	0.0801	ns
Log(x+1) frass production									
E. obliqua	20	1.19	0.032	1.252	0.029	-0.062	2.042	0.1611	ns
E. regnans	20	1.122	0.04	1.206	0.03	-0.084	2.762	0.1048	ns
E. delegatensis	20	1.329	0.024	1.427	0.027	-0.098	7.179	0.0108	*
Log(x+1) larval weight									
E. obliqua	20	1.36	0.029	1.421	0.018	-0.061	3.312	0.0848	ns
E. regnans	20	1.278	0.04	1.362	0.021	-0.084	3.552	0.0671	ns
E. delegatensis	20	1.432	0.022	1.491	0.023	-0.059	3.327	0.076	ns
Arcsin√x mortality rate									
E. obliqua	20	1.114	0.069	1.271	0.058	-0.157	3.007	0.091	ns
E. regnans	20	1.106	0.082	1.148	0.068	-0.042	0.157	0.6941	ns
E. delegatensis	20	1.248	0.063	1.352	0.063	-0.104	1.346	0.2533	ns
Arcsin√x mouting frequency									
E. obliqua	20	0.742	0.041	0.786	0.05	-0.044	0.449	0.507	ns
E. regnans	20	0.669	0.048	0.754	0.041	-0.085	1.833	0.1837	ns
E. delegatensis	20	0.818	0.044	0.909	0.064	-0.091	1.374	0.2485	ns

Appendix 8.3A. Data of *C. bimaculata* larval feeding response to adult foliage *E. delegatensis* from Tasmanian and Victorian localities.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Del	25.3	16.2	25.9	5	6
Tas 1	18.8	7.8	11.2	3	4
	21.4	16.3	21.7	5	4
	21.1	13.3	17.8	4	6
	17.7	12.6	18.6	4	6
	21.8	12.3	24.8	5	8
	23.7	14.7	21.1	4	4
	24.1	19.3	23.5	5	6
	26.3	21.6	29	5	8
	26.4	14.5	23.2	5	4
No. of Rep.	10	10	10	10	10
Mean	22.66	14.86	21.68	4.5	5.6
				0	
Del	6.28	6.3	5.9	2	0
Vic 1	16.68	10.7	11.1	3	2
	8.28	6	5.5	2	0
	8.48	3.5	6.8	2	0
	4.58	2.3	6.7	2	2
	14.82	8	9.8	3	2
	4.58	3.7	6.7	2	2
	12.58	9.6	14.4	5	2
	12.28	8.6	9.3	3	0
	9.28	5.5	8	5	2
No. of Rep.	10	10	10	10	10
Mean	9.784	6.42	8.42	2.9	1.2
Control	35.26	19.63	36.96	5	3
Nit.a	13.25	3.56	18.25	3	2
	33.25	24.58	41.25	5	4
	16.35	7.58	22.35	4	2
	30.25	18.96	36.36	5	5
	24.56	13.25	26.39	4	3
	27.58	15.25	32.58	5	2
	22.15	11.25	29.36	4	3
	27.56	14.25	33.25	5	2
	15.38	6.2	20.23	4	2
No. of Rep.	10	10	10	10	10
Mean	24.559	13.451	29.698	4.4	2.8

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
Del	19.22	9.6	21.9	5	4
Tas 2	25.62	16	22.9	4	4
	24.22	22	28.9	5	8
	21.42	13	25.3	4	6
	29.3	16	23.1	5	8
	24.76	13	22.3	5	6
	12.52	6.3	18.2	4	4
	25.52	14	23.3	4	4
	12.3	9	17.9	5	10
	23.22	18.3	26.6	5	6
No. of Rep.	10	10	10	10	10
Mean	21.81	13.72	23.04	4.6	6
Del	16.26	13.1	13.3	2	2
Vic 2	15.26	8.4	9	3	0
	16.26	9.1	13	3	2
	5.26	5	0	0	0
	8.26	6.3	15	4	2
	11.36	7	13.4	2	2
	8.26	4.7	5.9	1	2
	19.26	11.6	18.9	4	4
	8.26	5.6	9.4	2	2
	13.36	9.5	11.9	3	0
No. of Rep.	10	10	10	10	10
Mean	12.18	8.03	10.98	2.4	1.6

Appendix 8.3B. Analysis of variance for *C. bimaculata* larval feeding response to foliages of four localities of *E. delegatensis*.

A. ANOVA

Variance of survival rate

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
localities	3	3.004	1.001	10.416	0.0001
Residual	36	3.461	0.096		

Model summary R: 0.465 R2: 0.42 RMS Residual: 0.31 Model F-value: 10.416 P-value: 0.0001

Variance of moulting frequency

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
localities	3	2.803	0.934	20.548	0.0001
Residual	36	1.637	0.045		

Model summary R: 0.631 R2: 0.601 RMS Residual: 0.213 Model F-value: 20.548 P-value: 0.0001

Variance of leaf consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
localities	3	0.992	0.331	17.434	0.0001
Residual	36	0.683	0.019		

Model summary R: 0.592 R2: 0.558 RMS Residual: 0.138 Model F-value: 17.434 P-value: 0.0001

Variance of frass production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
localities	3	0.822	0.274	13.081	0.0001
Residual	36	0.754	0.021		

Model summary R: 0.522 R2: 0.482 RMS Residual: 0.145 Model F-value: 13.081 P-value: 0.0001

Variance of larval weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
localities	3	1.492	0.497	11.87	0.0001
Residual	36	1.509	0.042		

Model summary R: 0.497 R2: 0.455 RMS Residual: 0.205 Model F-value: 11.87 P-value: 0.0001

B. MANOVA

Effect: localities * Leaf types			Value	F-Value	Num DF	Den DF	P-Value
S	3	Wilks' Lambda	0.207	4.554	15	88.739	0.0001
M	0.5	Hotelling-Lawley Trace	3.147	6.434	15	92	0.0001
N	15	Pillai Trace	0.937	3.09	15	102	0.0004

Appendix 8.4A. Data of *C. bimaculata* larval feeding response to adult, juvenile and dewaxed juvenile foliage of *E. nitens* and adult *E. denticulata.*

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
N1-Adult	31.1	23.3	35	5	4
	32.6	24.1	44.1	5	5
	25.6	15.2	25.4	4	3
	20.9	14.7	17.6	3	2
	29.2	19.6	27.4	5	3
	34.2	15	32.9	4	4
	33.2	14	34.2	5	4
	22.2	18	23.7	4	3
	23.2	12	24.3	4	3
	28.2	20	37.5	5	4
No. of Rep.	10	10	10	10	10
Mean	28.04	17.59	30.21	4.4	3.5
				0	
N3-Adult	25.9	17.3	27.1	5	3
	21.3	15.1	27	4	3
	11.3	6.2	15.7	3	2
	23.6	16.7	28.4	4	3
	17.9	10	21.6	3	3
	18.9	8	21.1	3	3
	15.9	8	16.2	3	2
	24.9	16	27.9	5	4
	22.9	13	25.2	4	3
	27.9	18	26	5	4
No. of Rep.	10	10	10	10	10
Mean	21.05	12.83	23.62	3.9	3
Den-Adult	24.4	16.3	24.1	5	2
	25.9	20.1	30.2	5	3
	23.9	15.2	19.2	4	2
	30.6	19.7	24.9	5	2
	21.5	14	24.1	5	2
	14.5	5	9.2	2	1
	20.5	10	20.4	4	2
	23.5	21	23.8	4	3
	15.5	8	12.1	3	1
	17.5	8	14.5	3	2
No. of Rep.	10	10	10	10	10
Mean	21.78	13.73	20.25	4	2
N1-Jucenile	28.7	18.3	31.3	5	4
Dewaxed	31.2	20.1	35.9	5	4
	24.2	16.2	28.5	5	2
	22.5	15.7	32.8	5	3
	21.8	8	17.8	3	2
	20.8	18	31	5	3
	18.8	13	20.5	3	2
	19.8	14	21.7	4	2
	19.8	14	24.3	4	3
	24.8	11	24.3	3	2
No. of Rep.	10	10	10	10	10
Mean	23.24	14.83	26.81	4.2	2.7
N2-Juvenile	29.1	23.3	31.6	5	4
Dewaxed	26.6	21.1	36.3	5	5
	28.6	12.2	26.2	5	3
	21.9	16.7	26.1	4	3
	25.2	20	28.9	5	3
	13.2	8	19.4	3	2
	29.2	17	30.4	5	3
	10.2	7	15.4	3	2
	12.2	7	17.9	3	2
	6.88	6	20.8	4	2
No. of Rep.	10	10	10	10	10
Mean	20.308	13.83	25.3	4.2	2.9

Appendix 8.4A. Continued.

	Leaf consumption	Frass production	Larval weight	No. survival	No. moulting
N2-Adult	35.8	20.3	40.3	5	5
	27.3	13.1	27.7	5	3
	32.3	21.2	38.6	5	4
	33.6	27.7	40.9	5	5
	21.9	17	24.2	4	3
	27.9	23	33.7	5	4
	33.9	17	37.2	5	3
	25.9	15	22.7	4	2
	23.9	18	26.8	4	3
	35.9	23	38.5	5	4
No. of Rep.	10	10	10	10	10
Mean	29.84	19.53	33.06	4.7	3.6
N4-Adult	13.2	9.3	14.6	3	2
	17.7	9.1	19.6	4	3
	9.7	4.2	9.6	3	1
	12	9.7	14.5	4	2
	12.3	6	13.1	3	2
	9.3	6	11.7	3	1
	5.3	3	5.3	1	1
	7.4	4	11.2	3	0
	15.1	11	17.4	4	3
	11.3	8	11.2	3	1
No. of Rep.	10	10	10	10	10
Mean	11.33	7.03	12.82	3.1	1.6
N1-Juvenile	17	11.3	24	4	3
	31.5	28.1	43.5	5	5
	20.5	11.2	24.3	5	2
	18.8	12.7	20.2	4	2
	21.1	13	20.3	4	2
	17.1	11	19.6	3	3
	25.1	14	29.7	5	3
	19.1	13	16.4	3	2
	13.1	9	19.3	3	3
	23.1	10	22.6	4	2
No. of Rep.	10	10	10	10	10
Mean	20.64	13.33	23.99	4	2.7
N2-Juvenle	26	18.3	32.1	5	3
	27.5	15.1	34.6	5	4
	28.5	13.2	28.1	5	3
	27.8	25.7	33.4	5	3
	12.1	8	20.5	4	2
	24.1	15	23.9	4	3
	32.1	24	32.7	5	3
	18.1	8	20	3	2
	20.1	15	21.7	3	3
	18.1	13	27.7	5	3
No. of Rep.	10	10	10	10	10
Mean	23.44	15.53	27.47	4.4	2.9
Control	23.5	11.3	29.6	5	2
Nit.a	41.3	22.7	49.0	5	4
	18.6	9.6	17.6	3	1
	35.4	14.6	42.4	5	4
	25.7	11.0	32.6	4	3
	37.6	19.6	43.9	5	5
	35.7	22.4	50.2	5	5
	24.3	13.3	29.6	4	4
	31.7	17.4	39.4	5	4
	38.4	25.4	20.2	3	2
No. of Rep.	10	10	10	10	10
Mean	31.198	16.701	35.433	4.4	3.4

Appendix 8.4B. Analysis of variance in *C. bimaculata* larval feeding response between adult, juvenile and dewaxed juvenile leaves of Connor Plain (N1) and Toorongo (N2) localities of *E. nitens*.

Variance of leaf consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Leaf types	2	0.351	0.175	8.997	0.0004
Localities	1	0.002	0.002	0.079	0.7799
Type * Locality	2	0.085	0.043	2.192	0.1215
Residual	54	1.052	0.019		

Variance of frass production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Type	2	0.200	0.100	3.850	0.0274
Locality	1	0.001	0.001	0.045	0.8321
Type * Locality	2	0.062	0.031	1.198	0.3097
Residual	54	1.405	0.026		

Variance of Larval weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Type	2	0.068	0.034	1.68	0.196
Locality	1	0.006	0.006	0.321	0.5735
Type * Locality	2	0.045	0.022	1.11	0.337
Residual	54	1.085	0.02		

Variance of survival

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Type	2	0.177	0.089	0.901	0.4123
Locality	1	0.196	0.196	1.985	0.1646
Type * Locality	2	0.092	0.046	0.469	0.6284
Residual	54	5.319	0.099		

Variance of moulting frequency

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Type	2	0.406	0.203	3.176	0.0496
Locality	1	0.074	0.074	1.165	0.2853
Type * Locality	2	0.003	0.001	0.021	0.9789
Residual	54	3.453	0.064		

Appendix 8.4 C. Analysis of variance in *C. bimaculata* larval feeding response between adult foliages of Connor Plain (N1), Toorongo (N2), Mt Kaye (N3) and Noojee (N4) localities of *E. nitens* and adult foliage of *E. denticulata.*

Variance of leaf consumption

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Localities	4	.992	.248	18.745	.0001
Residual	45	.596	.013		

Variance of frass production

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Localities	4	.910	.227	8.743	.0001
Residual	45	1.170	.026		

Variance of larval weight

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Localities	4	.831	.208	10.506	.0001
Residual	45	.890	.020		

Variance of survival

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Localities	4	1.450	.363	4.476	.0040
Residual	45	3.645	.081		

Variance of moulting frequency

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Localities	4	1.741	.435	7.679	.0001
Residual	45	2.550	.057		

Appendix 8.5. Data of *C. agricola* larval feeding response to adult foliage of 10 eucalypt species.

	Leaf consumption	Frass production	Larval weight	No. survival		Leaf consumption	Frass production	Larval weight	No. survival
Glo.a	59.256	30.117	39.6	4	Nit.a	85.099	52.333	73.8	5
	42.451	25.485	38.3	4		98.77	62.241	103.2	5
	37.815	23.322	41.4	5		58.02	39.926	78.2	4
	35.224	18.32	38.7	5		78.068	43.463	58.1	4
	22.878	8.705	16.4	2		100.391	65.834	84.2	5
	38.264	18.498	35.07	4		93.624	57.479	73.705	5
	46.753	28.512	42.81	4		113.815	65.374	104.97	5
	49.35	24.468	42.926	5		59.814	41.954	50.17	4
	26.29	15.293	32.655	5		73.131	39.179	54.89	3
	25.182	12.305	25.792	3		85.298	56.148	84.117	5
No. of Rep.	10	10	10	10	No. of Rep.	10	10	10	10
Mean	38.3463	20.5025	35.3653	4.1	Mean	84.603	52.3931	76.5352	4.5
				0					
Vim.a	37.107	25.002	40	4	Gum.a	54.081	19.797	51.1	5
	55.754	39.087	57.5	5		68.343	26.606	66.5	5
	59.814	42.954	61.5	5		82.176	33.277	68.4	5
	58.566	40.02	62.4	5		90.201	33.674	71	5
	63.863	49.816	58.4	5		56.412	19.701	54.7	5
	38.994	26.227	31.275	4		52.703	18.409	52.04	5
	53.075	37.107	57.55	5		73.131	28.444	68.32	5
	53.2	38.264	51.714	4		73.131	29.62	66.141	5
	64.917	44.394	64.922	5		86.297	37.459	75.297	5
	62.68	48.888	61.512	5		57.479	29.409	46.37	5
No. of Rep.	10	10	10	10	No. of Rep.	10	10	10	10
Mean	54.797	39.1759	54.6773	4.7	Mean	69.3954	27.6396	61.9868	5
Ova.a	52.456	27.119	51.9	5	Del.a	45.666	26.102	35.9	5
	34.237	17.113	40.4	4		61.806	40.879	47.9	5
	62.096	33.914	59.9	5		24.235	9.495	13.4	3
	75.384	50.88	69.4	5		53.325	30.189	53.3	5
	13.223	6.295	13.7	2		19.701	5.295	25.6	5
	59.256	36.239	51.89	5		49.699	28.444	33.885	5
	37.371	18.634	35.44	3		56.28	37.194	48.54	5
	53.954	29.479	60.658	5		27.84	5.934	5	3
	68.663	46.315	72.08	5		45.345	25.669	57.355	5
	13.488	3.943	19.324	3		19.989	5.353	26.996	4
No. of Rep.	10	10	10	10	No. of Rep.	10	10	10	10
Mean	47.0128	26.9931	47.4692	4.2	Mean	40.3886	21.4554	34.7876	4.5
Obl.a	8.727	0.3	21.1	2	Reg.a	1.871	0.199	9.2	3
	20.528	6.396	25.9	1		2.273	0.698	4.5	5
	7.831	2.499	17.9	3		5.776	1.5	18.2	1
	17.836	6.396	26.5	2		5.067	0.799	11.1	5
	10.117	4.2	22.7	5		4.07	2.304	25.3	3
	9.765	0.337	22.13	3		2.119	0.225	13.24	3
	18.679	5.637	27.38	1		1.979	0.69	5.4	5
	7.67	2.451	17.855	4		5.138	1.333	22.276	2
	18.187	6.534	28.095	3		5.637	0.888	14.025	5
	1.636	4.408	21.118	5		3.989	2.251	24.581	3
No. of Rep.	10	10	10	10	No. of Rep.	10	10	10	10
Mean	12.0976	3.9158	23.0678	2.9	Mean	3.7919	1.0887	14.7822	3.5
Sie.a	19.701	8.908	19.2	3	Pau.a	51.481	29.62	46.3	4
	13.997	3.699	0	0		67.707	45.559	51.5	5
	49.466	26.99	35.7	4		30.046	15.293	31.9	3
	47.417	28.717	27.9	4		59.117	35.224	53.3	5
	37.282	18.409	33.2	4		25.546	12.305	55.5	5
	22.442	1.148	18.195	3		42.551	29.62	50	4
	15.558	4.164	0	0		56.28	43.157	48	5
	44.499	24.293	25.371	4		22.281	8.354	22.1	3
	47.641	24.704	25.045	3		61.23	45.345	55.3	5
	38.446	18.77	31.654	3		19.606	12.305	37.5	4
No. of Rep.	10	10	10	10	No. of Rep.	10	10	10	10
Mean	33.6449	15.9802	21.6265	2.8	Mean	43.5845	27.6782	45.14	4.3

Appendix 10.1. The yields and chemical compositions of the leaf oils and the absolute contents of individual oil compounds in leaves for *E. globulus*, *E. ovata* and their F1 hybrid progenies.

(A) Oil yield and chemical composition

Species		*E. globulus*				*E. ovata*				*F1 hybrid*				*E. ovata*	
Families		Glo4op		Glo5op		Ov5op		Ov4op		F1(G5O4)		F1(G4O5)		OV4pl	
No. of trees		3		3		3		3		3		3		3	
Statistics		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
	OIL YIELD	5.5	0.2	4.2	0.3	2.6	0.3	2.7	0.5	4.6	0.5	2.8	0.7	1.5	0.2
1	α–Pinene	19.4	0.8	23.6	2.3	0.2	0.1	-	-	12.1	2.9	11.2	2.2	1.6	1.3
2	β–Pinene	0.5	t	0.8	t	-	-	-	-	0.2	0.1	0.2	t	-	-
3	α–Phllanderne	0.8	t	1.0	t	t	t	0.2	t	0.4	0.2	0.3	t	0.3	0.1
4	α–terpinene	0.1	t	0.2	0.2	0.3	0.3	0.2	t	-	-	-	-	20.6	14.0
5	Limonene	7.2	0.1	6.9	0.2	0.4	t	0.3	0.1	3.7	1.1	3.6	0.2	0.9	0.5
6	1,8-Cineole	58.1	2.3	52.9	2.9	1.2	1.0	0.1	t	28.4	6.6	28.4	1.0	3.6	3.3
7	γ–Terpinene	0.5	0.1	0.6	0.2	1.5	0.8	0.3	t	0.6	0.4	0.2	0.1	3.7	5.4
8	p-Cymene	0.1	t	0.2	t	0.6	0.7	0.2	t	0.1	t	0.1	t	7.1	2.6
9	Linalool	0.1	0.1	0.1	0.1	6.1	2.5	23.2	2.3	7.0	3.3	t	t	8.7	2.2
10	Un	2.7	2.0	0.9	1.5	0.3	0.1	0.1	t	2.6	3.8	1.9	0.6	0.2	t
11	Terpinyl acetate	1.4	0.3	2.6	0.5	0.4	0.1	0.3	0.1	2.8	1.1	0.9	0.1	0.9	0.6
12	α–Terpineol	-	-	t	0.1	0.5	0.3	1.2	0.8	0.1	0.1	0.3	0.5	12.8	6.9
13	Nerolidol	0.4	0.3	0.2	0.1	81.1	2.2	67.4	2.6	36.1	10.5	46.7	5.4	26.7	16.8
14	Globulol	5.6	0.3	8.1	1.4	1.6	0.9	1.1	0.4	2.4	0.7	2.6	2.4	9.9	5.7
15	Viridiflorol	0.9	t	1.0	0.1	0.2	0.1	0.3	0.1	0.5	0.1	0.5	0.4	1.2	0.7

(B) Absolute contents

Species		*E. globulus*				*E. ovata*				*F1 hybrid*				*E. ovata*	
Families		Glo4op		Glo5op		Ov5op		Ov4op		F1(G5O4)		F1(G4O5)		OV4pl	
No. of trees		3		3		3		3		3		3		3	
Statistics		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	α–Pinene	107.4	7.9	98.6	15.1	0.5	0.2	0.0	0.0	32.1	7.9	50.1	1.6	2.7	2.2
2	β–Pinene	2.9	0.0	3.4	0.3	0.0	0.0	0.0	0.0	0.9	0.1	0.56	0.1	0.0	0.0
3	α–Phllandrene	4.6	0.1	4.3	0.4	0.1	0.1	0.6	0.2	1.1	0.1	1.2	0.3	0.5	0.2
4	α–terpinene	0.6	0.1	1.0	0.8	0.8	0.8	0.6	0.3	0.0	0.0	0.0	0.0	33.4	23.6
5	Limonene	40.1	0.8	28.8	2.9	1.0	0.1	0.9	0.5	9.7	2.1	16.2	2.4	1.3	0.5
6	1,8-Cineole	321.8	6.4	221.0	28.1	3.1	2.1	0.2	0.1	75.6	20.1	129.4	22.7	5.3	4.3
7	γ–Terpinene	2.5	0.3	2.3	0.8	3.8	1.7	0.9	0.3	1.8	1.3	0.9	0.3	5.1	7.2
8	p-Cymene	0.7	0.1	0.6	0.2	1.6	1.5	0.4	0.2	0.3	0.0	0.5	0.1	11.2	5.1
9	Linalool	0.4	0.7	0.3	0.2	15.8	5.5	62.4	14.9	22.2	14.7	0.1	0.2	13.1	1.7
10	Un	15.3	11.3	3.6	5.6	0.9	0.5	0.3	0.1	4.5	5.4	8.7	3.7	0.3	0.1
11	Terpinyl acetate	7.8	1.7	10.6	1.1	0.9	0.1	0.9	0.5	8.7	5.5	3.8	0.4	1.3	0.7
12	α–Terpineol	0.0	0.0	0.1	0.2	1.2	0.6	2.8	1.6	0.3	0.0	1.4	2.4	20.5	12.4
13	Nerolidol	2.1	1.9	0.9	0.2	213.1	44.9	184.2	58.9	109.5	62.5	217.4	65.8	39.3	20.4
14	Globulol	31.0	0.9	33.4	3.8	3.9	1.7	2.8	0.5	6.2	1.5	10.6	7.1	15.9	10.3
15	Viridiflorol	4.9	0.1	4.3	0.3	0.6	0.2	0.7	0.2	1.3	0.3	2.2	1.2	1.9	1.2

Appendix 10.2. The leaf consumption, frass production, larval weight and mortality rate of *C. bimaculata* larvae feeding on *E. globulus* and *E. ovata* and their F1 hybrids.

Type	Tree	No. of Rep.	Leaf consumption (mg)		Frass product (mg)		Larval weight (mg)		Mortality (%)	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Glo4op	A	5	5.45	0.50	4.20	0.65	17.35	1.10	44.00	4.00
	B	5	16.00	2.20	10.20	1.55	25.70	1.35	24.00	7.48
	C	5	8.10	0.70	6.10	0.75	23.35	1.60	48.00	4.90
	Total	15	9.85	1.40	6.85	0.85	22.15	1.20	38.67	4.13
Ov5xGlo4	A	5	13.90	2.75	9.05	1.55	19.95	1.75	24.00	7.48
	B	5	13.95	2.30	8.85	1.55	22.25	1.65	28.00	8.00
	C	5	10.15	1.05	7.75	1.40	17.20	0.70	28.00	4.90
	Total	15	12.70	1.25	8.55	0.80	19.80	0.95	26.67	3.74
Ov5op	A	5	20.10	1.80	14.95	1.25	27.80	2.30	4.00	4.00
	B	5	20.60	2.35	14.40	1.65	29.30	2.20	12.00	4.90
	C	5	18.75	2.50	13.55	1.85	29.00	1.25	12.00	8.00
	Total	15	19.80	1.20	14.30	0.85	28.70	1.10	9.33	3.31
Glo5op	A	5	9.05	1.80	6.60	1.00	22.35	2.80	44.00	7.48
	B	5	13.35	2.10	9.50	1.25	24.45	1.15	28.00	4.90
	C	5	8.90	1.60	5.95	1.30	17.60	0.80	52.00	10.20
	Total	15	10.45	1.15	7.35	0.75	21.50	1.25	41.33	4.96
Ov4xGlo5	A	5	19.50	1.35	14.00	0.90	25.50	0.85	8.00	4.90
	B	5	15.20	3.10	11.30	2.35	23.25	2.25	20.00	6.33
	C	5	27.00	2.80	18.10	2.00	32.10	2.00	16.00	4.00
	Total	15	20.55	1.90	14.45	1.25	26.95	1.40	14.67	3.07
Ov4op	A	5	16.15	3.15	11.80	2.80	25.35	2.30	16.00	7.48
	B	5	16.30	1.40	12.15	1.20	23.65	1.50	8.00	4.90
	C	5	18.85	2.80	13.35	2.10	28.05	1.60	12.00	4.90
	Total	15	17.10	1.40	12.45	1.15	25.70	1.10	12.00	3.27
Ov4pl	A	5	29.40	3.35	17.50	2.50	40.50	3.20	8.00	4.90
	B	5	22.45	1.85	14.75	1.95	40.25	1.55	8.00	4.90
	C	5	31.40	1.60	22.65	2.15	39.10	2.50	4.00	4.00
	Total	15	27.75	1.65	0.00	0.00	39.95	1.35	6.67	2.52
Ovs	A	5	7.80	1.45	4.95	0.75	14.60	1.00	60.00	14.14
	B	5	6.20	0.90	5.05	1.05	15.75	1.75	40.00	6.33
	C	5	4.80	1.10	3.90	0.70	16.25	2.70	52.00	12.00
	Total	15	6.25	0.70	4.65	0.55	15.60	1.10	50.67	6.43

Appendix 10.3. Correlation of larval feeding response of C. bimaculata and oil composition in foliage of *E. globulus* and *E. ovata* hybrid combinations

The transformed data of individual tree means of each of the components of the larval feeding response were subjected to correlation analysis with the absolute content of major oil components and results shown in Table 1. The correlation coefficients indicated that all major oil constituents were significantly correlated with the different components of the feeding response, with the exception of nerolidol which was not significantly correlated with larval weight. The comparison of the correlation coefficients indicated that the chemical components were most significantly correlated to mortality rate of larvae followed by frass production and leaf consumption.

Table 1. Correlationships between variables of major oil chemical compounds and larval feeding response. ns $p>0.05$ * $0.05>p>0.01$ ** $0.01>p>0.001$ *** $p<0.001$

	Leaf consumption		Frass production		Larval weight		Mortality rate	
α–Pinene	-0.586	**	-0.636	**	-0.55	*	0.817	***
1,8-Cineole	-0.564	*	-0.61	**	-0.529	*	0.789	***
Linalool	0.637	**	0.694	***	0.589	**	-0.717	***
Nerolidol	0.603	**	0.624	**	0.309	ns	-0.68	**
Globulol	-0.674	**	-0.72	***	-0.471	*	0.811	***

Further regression analysis (Table 2.A) indicated that both of the correlation coefficients (R) and the coefficients of determination (R^2) between chemical variables and the dependent variables of the larval feeding response were generally higher for mortality rate regression. In contrast, R^2 were low for larval weight although the R values showed a significance at 0.05 level for five of the six compounds. This indicated that the proportion of variance of the mortality rate of *C. bimaculata* larvae was highly predictable due to variation in chemical compounds but the variability in larval weights was poorly predictable.

Moreover, the R and R^2 values of the two order polynomial regression models increased for all chemical variables (Table 2.B). Thus, the variances of the feeding response were more predictable from the chemical variables in the two order polynomial regression models than from the one order simple regressions. In the two order polynomial regression model, assuming a critical probability level of 0.05, only the regression coefficients for X and X^2 of the two chemical variables, α–pinene and 1,8-cineole, were significant for leaf consumption and frass production regressors and the regression coefficients for X^2 of these two chemicals were significantly for mortality rate regressor. One other chemical variable, linalool, was significantly regressed with frass production. The two order polynomial regression does indicated that α–pinene and 1,8-cineole were the most effective chemical compounds that were highly correlated

to the larval feeding response with the exception of larval weight. The model summary for the two order regression indicated that the variability of mortality rate of larvae was significantly correlated to variation of these two chemical compounds (R = 0.90 and 0.89 for α–pinene and 1,8-cineole respectively) while the proportion of variance of the mortality rate was highly predictable (R^2 = 0.81 and 0.78) from these two chemical variables. These R^2 values of the two order polynomial regression model for larval weight again indicated that the proportion of variance of the larval weight was not significantly associated with the chemical variables.

The fitted regression line for predicting larval feeding response for the chemical variable 1,8-cineole and α–pinene is shown in Fig. 1. Notice that in these plots the regression line for leaf consumption and frass production appears to increase when the log(x+1) values of amounts of 1,8-cineole and α–pinene between 0 and 0.5 [inverse log(x+1) = 2.16 g/kg dried leaves] and to decrease when the log(x+1) values of these two chemical variables become greater than 1 (9.00g). Most of the points associated with α–pinene over 1.25 (16.78g) and 1,8-cineole over 1.5 (30.62g) decreased significantly in the fitted regression line for leaf consumption and frass weight. In contrast, some of the points associated with low values of α–pinene under 0.25 (0.78g) and 1,8-cineole under 1 (9.00g) increased in the regression line for leaf consumption and frass weight. However, most of the points associated with high values of α–pinene and 1,8-cineole increased significantly in the fitted regression for mortality rate increased significantly and some points associated with low values of the two chemical variables decreased for mortality. Therefore, the fitted regression lines did indicate that low levels of α–pinene and 1,8-cineole could benefit leaf consumption and frass production and that increase in these two chemical compounds to a high level could decrease leaf consumption and frass production. In contrast, changes in these two chemical variables had the opposite effect on mortality rate. In the comparison of the points in the above figure and the original chemical data of individual trees, those points associated with low values of α–pinene and 1,8-cineole were trees from the two *E. ovata* families Ov4op and Ov5op respectively and those points associated with high values of α–pinene and 1,8-cineole were from trees of F1 hybrids and the two *E. globulus* families Glo4op and Glo5op. Hence, this analysis has indicated that variation in chemicals among trees of the F1 hybrids and their parents resulted in large differences in the larval feeding response. Furthermore, the threshold value to elicit the negative effect on leaf consumption and frass production and increase in mortality rate was indicated by the fitted regression line to be approximately 16.78g/kg of the absolute content of α–pinene and 30.62g/kg for 1,8-cineole.

Table. 2.A. The polynomial regression between *C. bimaculata* larval feeding response (Y) and absolute contents of major oil compounds (X) in leaves.

$$Y = a + bX$$

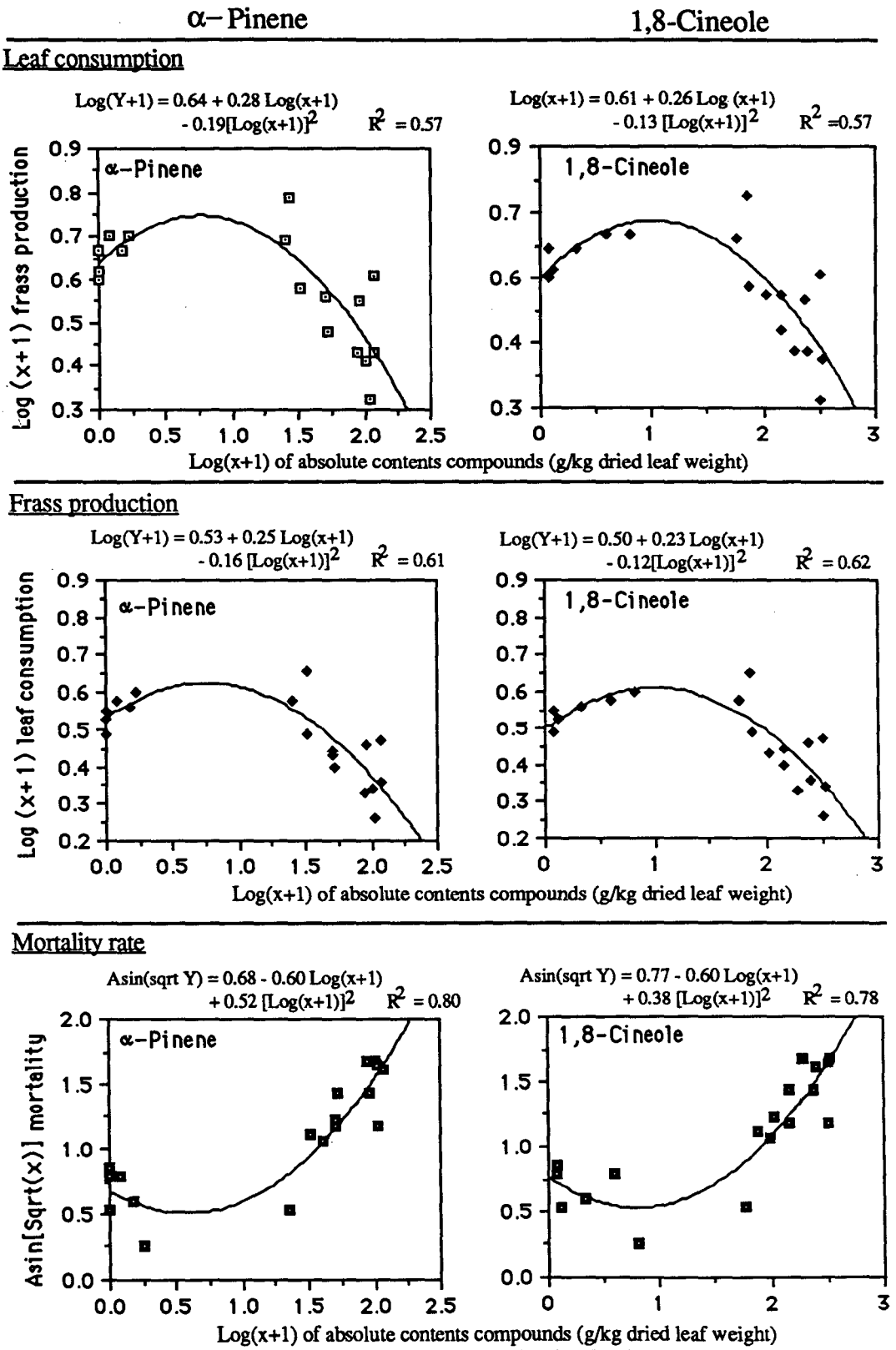
	Leaf consumption							Frass production						
	X				Model summary			X				Model summary		
	F-value	T-test	P-value		R	R2	RMS	F-value	T-test	P-value		R	R2	RMS
Oil yield (X)	1.78	-3.28	.0047	**	.63	.40	.10	13.21	-3.63	.0022	**	.67	.45	.08
α–Pinene (X1)	8.37	-2.89	.0105	*	.59	.34	.10	10.85	-3.29	.0046	**	.64	.4	.09
Limonene (X2)	11.73	-3.43	.0350	*	.65	.42	.10	15.10	-3.42	.0013	**	.7	.42	.08
1,8-Cineole (X3)	7.48	-2.73	.0147	*	.56	.32	.11	9.47	-2.73	.0072	**	.61	.37	.09
Linalool (X4)	10.92	3.30	.0045	**	.64	.48	.10	14.90	3.30	.0045	**	.69	.48	.08
Nerolidol (X5)	9.13	3.02	.0019	**	.60	.36	.10	10.18	3.19	.0057	**	.62	.49	.86
Globulol (X6)	13.30	-3.65	.0022	**	.67	.45	.10	17.19	-4.15	.0008	***	.72	.52	.08
	Larval weight							Mortality rate						
	X				Model summary			X				Model summary		
	F-value	T-test	P-value		R	R2	RMS	F-value	T-test	P-value		R	R2	RMS
Oil yield (X)	7.13	-2.67	.0168	*	.56	.31	.06	24.05	4.90	.0002	***	.78	.60	.29
α–Pinene (X1)	6.94	-2.64	.0181	*	.55	.30	.06	32.11	5.67	.0001	***	.82	.67	.27
Limonene (X2)	7.91	-2.81	.0125	*	.58	.33	.06	41.75	6.46	.0001	***	.85	.73	.24
1,8-Cineole (X3)	6.22	-2.50	.0239	*	.53	.28	.06	26.34	5.13	.0001	***	.79	.62	.28
Linalool (X4)	8.50	2.92	.0101	*	.59	.35	.06	16.87	-4.11	.0008	***	.72	.51	.32
Nerolidol (X5)	1.68	1.30	.2128		.31	.10	.07	13.78	-3.71	.0019	**	.68	.46	.34
Globulol (X6)	4.56	-2.13	.0486	*	.47	.22	.06	30.78	5.55	.0001	***	.81	.66	.27

Table 2.B. The two order regression between *C. bimaculata* larval feeding response (Y) and absolute contents of major oil components (X) in leaves

$$Y = a + bX + CX^2$$

	Coefficient						Model summary				
	X d.f.= 1 residual: 15			X^2 d.f.= 1 residual: 15			Count = 18		d.f.	model=2 error=15 total=17	
	F-value	T-test	P-value	F-value	t-Test	P-value	R	R2	RMS	F-value	P-value
(Y) Leaf consumption											
Oil yield (X)	0.29	0.54	0.5967	0.70	-0.84	0.4149	0.66	0.43	0.10	5.64	0.149
α–Pinene (X_1)	4.57	2.03	0.0493 *	7.21	-2.68	0.017 *	0.75	0.56	0.09	9.41	0.0022
Limonene (X_2)	1.53	1.24	0.2347	3.81	-1.95	0.0698	0.74	0.54	0.09	8.80	0.003
1,8-Cineole (X_3)	4.82	2.20	0.0443 *	8.26	-2.87	0.0116 *	0.75	0.56	0.09	9.56	0.0021
Linalool (X_4)	3.42	1.85	0.0841	1.22	-1.10	0.2872	0.67	0.45	0.1	6.14	0.0133
Nerolidol (X_5)	0.41	0.64	0.5307	0.07	-0.27	0.7913	0.61	0.37	0.1	4.34	0.0327
Globulol (X_6)	0.08	0.28	0.7808	0.45	-0.67	0.5132	0.69	0.47	0.1	6.64	0.0096
(Y) Frass product											
Oil yield (X)	0.23	0.54	0.6365	0.66	-0.84	0.4300	0.69	0.48	0.08	6.79	0.0079
α–Pinene (X_1)	4.67	2.16	0.0472 *	8.56	-2.93	0.0104 *	0.78	0.82	0.07	12.27	0.0007
Limonene (X_2)	1.38	1.18	0.2578	3.94	-1.99	0.0657	0.77	0.54	0.09	10.91	0.0012
1,8-Cineole (X_3)	5.47	2.34	0.0336 *	9.74	-3.12	0.007 **	0.79	0.62	0.07	12.19	0.0007
Linalool (X_4)	5.40	2.32	0.0345 *	2.05	-1.43	0.1723	0.74	0.55	0.08	8.97	0.0027
Nerolidol (X_5)	0.59	0.77	0.4547	0.14	-0.37	0.7137	0.63	0.4	0.09	4.89	0.0232
Globulol (X_6)	0.03	0.18	0.8613	0.38	-0.62	0.5475	0.73	0.53	0.08	8.45	0.0035
(Y) Larval Weight											
Oil yield (X)	0.14	0.48	0.7174	0.37	-0.81	0.5521	0.57	0.33	0.06	3.61	0.0526
α–Pinene (X_1)	0.04	0.19	0.8495	0.45	-0.67	0.5143	0.57	0.32	0.06	3.57	0.0539
Limonene (X_2)	0.39	-0.62	0.544	0.02	0.12	0.904	0.58	0.33	0.06	3.72	0.0488
1,8-Cineole (X_3)	0.26	0.51	0.6163	1.01	-1.00	0.3316	0.57	0.33	0.06	3.62	0.0523
Linalool (X_4)	2.38	1.54	0.144	0.79	-0.89	0.3877	0.62	0.38	0.06	4.59	0.0278
Nerolidol (X_5)	0.18	0.42	0.6749	0.07	-0.26	0.7964	0.32	0.1	0.07	0.83	0.4526
Globulol (X_6)	0.62	-0.78	0.445	0.32	0.57	0.5807	0.49	0.24	0.06	2.34	0.1305
(Y) mortality rate											
Oil yield (X)	0.28	0.53	0.6065	0.01	-0.10	0.9244	0.78	0.60	0.30	11.29	0.0010
α–Pinene (X_1)	3.40	-1.84	0.0851	10.02	3.17	0.0064 **	0.90	0.8	0.21	30.11	0.0001
Limonene (X_2)	0.02	-0.14	0.8917	1.90	1.38	0.1878	0.87	0.75	0.24	23.01	0.0001
1,8-Cineole (X_3)	4.11	-2.03	0.0609	11.09	3.33	0.0046 **	0.89	0.78	0.22	27.02	0.0001
Linalool (X_4)	4.23	-2.36	0.0695	1.99	1.41	0.1784	0.76	0.57	0.31	9.96	0.0018
Nerolidol (X_5)	1.69	-1.30	0.2131	0.70	0.84	0.4155	0.7	0.49	0.34	7.11	0.0067
Globulol (X_6)	0.32	0.56	0.5809	0.00	0.01	0.9926	0.81	0.66	0.28	14.43	0.0003

Fig. 1. Scattergram of the effect of the chemicals 1,8-cineole and α–pinene on leaf consumption, frass production and mortality rate of *C. bimaculata* larvae on foliage of *E. globulus* and *E. ovata* and their F1 hybrids.



Appendix 11.1. The mean oil yield and chemical compositions of leaf oils from different families of *E. nitens* at two experimental sites. DBHob values are mean value of all trees of individual families (Data provided by APPM Forest Research Units).

a) Huntsman (Site 1)

Code of Family		135	145	177	71	264	136	283	316	312	340
No. of trees		3	3	3	3	3	3	3	3	3	3
DBHob* (cm)		5.5	4.2	6.3	7.8	4.3	4.5	7.4	6.1	7.9	4.6
Oil yields	Mean	0.7	0.8	0.6	0.5	0.8	0.9	0.7	0.7	0.5	0.7
	S.D.	0.3	0.3	0.1	0.1	0.3	0.3	0.3	0.4	0.2	0.1
α–Pinene	Mean	20.0	19.4	13.0	14.7	24.6	5.6	19.1	26.6	13.5	22.7
	S.D.	3.3	4.2	5.6	3.8	10.9	5.3	1.9	8.9	6.1	1.1
Isobutyl isobutaboate	Mean	0.1	0.9	0.0	0.0	2.4	1.5	0.2	0.3	0.1	0.0
	S.D.	0.1	0.8	0.0	0.0	2.1	1.4	0.1	0.3	0.0	0.0
β–Pinene	Mean	0.5	0.4	0.5	0.9	0.7	0.4	1.4	0.6	1.3	0.5
	S.D.	0.1	0.3	0.3	0.5	0.4	0.1	0.5	0.2	0.1	0.2
α–Phellandrene	Mean	3.8	3.5	1.6	3.7	3.6	2.7	2.3	3.5	2.5	4.3
	S.D.	0.4	1.4	0.6	1.8	1.1	0.7	0.3	1.1	1.5	0.9
Isobutyl isopentanoate	Mean	0.4	1.5	0.0	0.0	1.8	2.1	0.0	0.0	0.1	0.0
	S.D.	0.8	1.4	0.0	0.0	1.7	1.8	0.0	0.0	0.3	0.0
Limonene	Mean	0.2	0.0	0.3	0.2	0.1	0.1	0.3	0.2	0.2	0.0
	S.D.	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1,8-Cineole	Mean	32.3	40.6	25.7	28.2	26.2	24.0	29.3	32.8	29.2	36.1
	S.D.	4.0	5.6	8.0	9.1	3.3	5.9	7.0	5.8	7.6	0.8
cis–β–Ocimene	Mean	0.5	0.4	0.1	0.1	0.6	2.2	0.4	0.1	1.0	2.0
	S.D.	0.1	0.3	0.1	0.0	0.5	1.7	0.5	0.0	0.7	2.2
γ–Terpinene	Mean	1.5	1.4	0.6	2.1	2.5	8.0	1.8	0.9	1.9	2.8
	S.D.	1.1	1.0	0.4	1.4	1.4	5.8	0.5	0.1	0.8	2.0
trans–β–Ocimene	Mean	0.3	0.2	0.3	1.3	0.8	0.3	0.3	0.3	0.4	0.5
	S.D.	0.0	0.1	0.1	0.9	0.6	0.1	0.1	0.2	0.1	0.4
p-Cymene	Mean	6.9	2.3	11.8	7.3	6.3	22.1	7.4	4.9	10.8	4.3
	S.D.	2.1	1.3	5.9	3.2	5.8	6.5	1.7	3.9	5.4	0.0
Terpenolene	Mean	0.3	0.2	0.1	0.2	0.5	0.2	0.2	0.1	0.2	0.2
	S.D.	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Isoamyl isovalerate	Mean	1.9	2.5	1.8	2.6	0.8	1.1	2.1	2.8	2.1	2.9
	S.D.	0.5	0.8	0.5	2.0	0.2	0.9	0.2	0.9	0.6	0.1
Linalool	Mean	0.4	0.2	0.0	0.1	0.8	0.4	0.4	0.1	0.2	0.2
	S.D.	0.2	0.2	0.1	0.2	0.2	0.1	0.8	0.1	0.3	0.2
Pinocarvone	Mean	0.7	2.2	2.1	0.7	0.7	0.1	2.0	1.3	0.5	0.7
	S.D.	0.2	2.0	1.1	0.2	0.7	0.1	0.7	0.6	0.2	0.8
Terpinen-4-ol	Mean	0.5	0.2	0.3	0.4	0.8	1.9	0.4	0.3	0.4	0.5
	S.D.	0.3	0.1	0.1	0.1	0.5	1.0	0.2	0.1	0.1	0.2
Aromadendrene	Mean	0.5	0.3	0.9	0.8	0.5	0.4	0.5	0.4	0.8	0.4
	S.D.	0.2	0.1	0.2	0.3	0.2	0.2	0.1	0.2	0.2	0.1
Allo-aromadendrene	Mean	1.1	3.6	3.7	1.4	1.9	0.4	3.2	2.5	1.5	1.0
	S.D.	0.2	2.7	0.9	0.3	1.1	0.2	1.4	0.9	0.7	0.8
Terpinyl acetae	Mean	4.7	3.7	1.2	2.5	5.0	4.6	2.1	2.7	1.5	3.5
	S.D.	0.5	1.8	0.8	0.7	0.4	2.1	0.5	0.5	0.4	0.5
α–Terpineol	Mean	0.6	0.3	1.1	1.6	0.3	0.2	1.6	0.9	1.4	0.7
	S.D.	0.4	0.1	0.5	0.6	0.1	0.2	0.3	0.5	0.4	0.3
Globulol	Mean	1.4	0.9	1.0	2.8	0.8	0.8	2.2	1.1	3.2	1.1
	S.D.	0.9	0.3	0.4	1.5	0.3	0.3	1.7	0.6	0.7	0.5
Spathulenol	Mean	3.9	1.9	13.1	6.5	3.0	3.5	5.4	4.2	7.2	2.7
	S.D.	2.0	0.8	5.8	3.4	1.1	2.7	2.2	3.4	1.5	1.1
α–Eudesmol	Mean	0.9	0.6	1.7	1.7	0.8	1.4	1.6	1.0	1.7	0.8
	S.D.	0.2	0.3	0.7	1.0	0.5	0.5	0.7	0.6	0.8	0.1
γ–Eudesmol	Mean	0.7	0.4	1.2	0.8	0.5	0.7	0.9	0.4	1.3	0.5
	S.D.	0.3	0.2	0.6	0.4	0.3	0.4	0.3	0.3	0.5	0.2
β–Eudesmol	Mean	0.7	0.6	0.4	1.3	0.5	0.7	1.5	0.7	1.3	0.6
	S.D.	0.2	0.4	0.2	0.9	0.2	0.2	0.8	0.3	0.4	0.1

Appendix 11.1. The mean oil yield and chemical compositions of leaf oils from different families of *E. nitens* at two experimental sites.

b) Bround Hill (Site 2).

Code of Family		135	145	177	71	264	136	283	316	312	340
No. of trees		3	3	3	3	3	3	3	3	3	3
DBHob (cm)		12.5	10.83	17.35	15.47	12.38	13.17	17.28	17.95	18.24	11.11
Oil yields	Mean	1.1	1.4	0.6	0.7	0.9	1.3	0.9	0.8	0.7	0.9
	S.D.	0.3	0.5	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.5
α–Pinene	Mean	25.1	21.3	14.0	20.0	23.4	16.6	25.3	28.2	19.7	13.8
	S.D.	6.0	7.1	2.5	4.9	11.8	3.3	3.7	2.5	0.5	10.2
Isobutyl isobutanoate	Mean	0.2	1.2	0.1	0.0	2.1	0.8	0.1	0.2	0.1	0.0
	S.D.	0.1	1.6	0.1	0.0	2.3	0.6	0.1	0.1	0.1	0.0
β–Pinene	Mean	0.2	0.3	0.6	0.6	0.6	0.2	0.4	0.6	0.9	0.7
	S.D.	0.1	0.3	0.3	0.4	0.5	0.1	0.3	0.1	0.5	0.4
α–Phellandrene	Mean	4.8	3.2	1.9	4.3	3.7	3.3	4.0	3.7	3.7	3.4
	S.D.	0.7	1.3	0.7	0.8	0.7	0.9	0.2	0.1	0.5	0.8
Isobutyl isopentanoate	Mean	0.5	1.9	0.0	0.0	2.4	1.8	0.0	0.0	1.4	0.0
	S.D.	0.9	1.1	0.0	0.0	1.1	1.7	0.0	0.0	2.4	0.0
Limonene	Mean	0.0	0.0	0.3	0.1	0.0	0.1	0.1	0.1	0.2	0.1
	S.D.	0.1	0.0	0.2	0.1	0.0	0.2	0.1	0.0	0.1	0.1
1,8-Cineole	Mean	36.2	40.9	34.9	41.0	30.2	25.3	39.6	37.1	36.6	29.9
	S.D.	4.5	7.6	13.1	6.2	5.3	1.0	1.7	1.1	5.1	10.4
cis–β–Ocimene	Mean	2.3	0.5	0.0	0.1	1.4	6.5	0.1	0.0	0.3	2.3
	S.D.	1.7	0.3	0.1	0.1	0.1	3.8	0.1	0.1	0.3	2.2
γ–Terpinene	Mean	2.5	1.2	1.4	1.1	2.8	6.6	2.2	1.5	1.6	8.2
	S.D.	0.7	0.4	0.8	0.5	2.1	2.0	0.4	0.6	0.4	6.8
trans–β–Ocimene	Mean	0.6	0.2	0.2	0.5	0.5	0.8	0.3	0.2	0.8	0.5
	S.D.	0.3	0.0	0.0	0.3	0.2	0.1	0.3	0.1	0.5	0.4
p-Cymene	Mean	3.2	3.5	8.3	3.9	6.2	11.3	5.2	4.1	5.9	11.2
	S.D.	0.8	2.5	3.8	1.3	6.0	1.0	2.0	0.7	1.2	6.9
Terpinolene	Mean	0.2	0.2	0.1	0.1	0.3	0.5	0.1	0.1	0.1	0.1
	S.D.	0.0	0.1	0.0	0.0	0.1	0.4	0.0	0.1	0.1	0.0
Isoamyl isovalerate	Mean	2.8	2.2	2.2	2.6	0.7	0.7	2.3	2.6	2.2	2.8
	S.D.	0.4	0.8	0.8	0.2	0.4	0.2	0.3	0.2	0.8	0.2
Linalool	Mean	0.5	0.1	0.0	0.0	0.4	0.2	0.0	0.0	0.7	0.3
	S.D.	0.2	0.1	0.0	0.0	0.3	0.1	0.1	0.0	0.9	0.1
Pinocarvone	Mean	0.5	1.6	6.3	0.5	0.6	0.2	0.8	1.3	0.6	0.3
	S.D.	0.3	0.9	7.5	0.3	0.2	0.1	0.2	0.2	0.6	0.3
Terpinen-4-ol	Mean	0.4	0.2	0.3	0.3	0.4	1.6	0.4	0.3	0.3	1.5
	S.D.	0.1	0.0	0.1	0.1	0.3	0.3	0.1	0.1	0.0	1.2
Aromadendrene	Mean	0.2	0.2	0.7	0.5	0.4	0.3	0.3	0.3	0.5	0.5
	S.D.	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.3
Allo-aromadendrene	Mean	1.2	4.1	4.2	1.5	1.0	0.4	1.7	2.5	1.4	0.6
	S.D.	0.5	2.5	2.5	0.6	0.4	0.1	0.4	0.7	0.3	0.6
Terpinyl acetae	Mean	4.6	3.7	1.5	3.3	5.3	5.9	4.2	3.0	2.8	3.6
	S.D.	1.0	1.9	0.3	1.0	0.9	0.5	0.5	0.8	0.4	0.9
α–Terpineol	Mean	0.3	0.1	0.7	1.1	0.3	0.0	0.5	0.6	1.0	1.1
	S.D.	0.2	0.1	0.3	0.9	0.3	0.1	0.3	0.1	0.3	0.7
Globulol	Mean	0.3	0.4	1.0	1.1	0.7	0.5	0.6	0.7	1.1	1.6
	S.D.	0.1	0.2	0.5	0.9	0.4	0.2	0.4	0.1	0.7	1.7
Spathulenol	Mean	1.8	1.8	9.1	3.4	3.8	2.4	2.9	3.1	5.0	3.8
	S.D.	0.9	0.8	4.1	1.1	2.4	1.0	1.9	0.3	1.2	2.5
α–Eudesmol	Mean	0.4	0.4	1.3	1.0	0.8	0.9	0.7	0.8	1.3	1.0
	S.D.	0.1	0.2	0.5	0.4	0.5	0.1	0.2	0.1	0.8	0.5
γ–Eudesmol	Mean	0.3	0.3	0.9	0.5	0.6	0.4	0.3	0.4	0.8	0.7
	S.D.	0.1	0.1	0.5	0.3	0.4	0.2	0.3	0.0	0.3	0.6
β–Eudesmol	Mean	0.3	0.3	0.3	0.9	0.5	0.7	0.4	0.6	1.1	0.9
	S.D.	0.0	0.1	0.0	0.5	0.2	0.1	0.1	0.2	1.0	0.4

Appendix 11.2. The chemical composition of leaf oils from *E. nitens*, *E. globulus* and their F1 hybrid trees.

Species		*E. nitens*				*E. globulus*				F1 Hybrid			
Leaf age		Young		Mature		Young		Mature		Young		Mature	
No. of trees		5		5		4		4		5		4	
No.	Components	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	α–Pinene	16.8	2.0	22.0	3.4	28.6	4.3	20.7	1.3	23.9	4.9	23.0	5.7
2	Isobutyl isobutanoate	t	t	0.4	0.1	0.1	t	0.1	t	t	0.1	-	-
3	β–Pinene	0.2	t	0.1	t	0.8	0.1	0.6	0.1	0.3	0.1	0.4	0.1
4	Sabinene	-	-	0.1	t	-	-	-	-	-	-	-	-
5	Myrcene	0.2	0.2	0.1	0.1	0.8	0.1	0.6	0.4	0.2	0.1	0.4	0.1
6	α–Phellandrene	7.3	1.9	0.6	0.5	0.1	t	-	-	0.4	0.3	1.7	1.3
7	α–Terpinene	t	t	-	-	0.1	t	0.1	0.1	-	-	-	-
8	Limonene	3.4	0.7	4.9	0.6	4.7	0.6	5.0	0.4	6.0	0.3	5.7	0.2
9	1,8-Cineole	29.2	5.8	46.4	2.6	28.1	3.7	39.7	2.4	46.4	7.9	41.1	4.4
10	*trans*-hex-2-enal	0.2	t	0.8	0.2	0.1	t	0.2	0.1	0.3	0.1	0.2	0.2
11	*cis*–β–Ocimene	5.2	3.0	3.4	2.1	0.4	0.3	0.2	0.1	0.3	0.2	0.3	0.2
12	γ–Terpinene	0.7	1.2	0.7	1.3	0.2	0.1	0.2	0.1	0.8	0.3	0.6	0.2
13	trans–β–Ocimene	0.2	0.1	0.5	0.3	0.1	t	0.1	t	-	-	-	-
14	p-Cymene	0.8	0.3	1.6	0.6	0.1	t	0.2	0.1	0.7	0.3	0.5	0.2
15	Terpinolene	0.5	0.1	0.1	t	0.2	t	0.1	0.1	0.1	0.1	0.2	0.1
16	Isopentyl isopentanoate	1.3	0.6	2.2	0.8	0.1	t	0.1	t	0.4	0.2	0.3	0.2
17	α–Cubene	0.2	0.1	-	-	0.2	t	0.2	0.1	t	t	-	-
18	α–Gurjunene	0.5	0.1	0.1	0.1	2.1	0.5	0.8	0.7	0.8	0.8	0.9	0.5
19	Linalool	0.5	0.4	0.6	0.7	0.2	0.1	0.1	t	0.2	0.2	0.3	0.1
20	*p*-Menth-2-en-1-ol	0.1	t	-	-	t	t	t	t	t	t	t	t
21	β–elemene	0.2	0.1	-	-	-	-	t	t	-	-	-	-
22	Terpinen-4-ol	0.8	0.2	0.4	0.2	0.5	0.1	0.4	t	0.3	0.1	0.5	0.1
23	Aromadendrene	1.1	0.3	0.2	0.1	5.7	1.3	4.1	1.7	1.0	1.0	1.3	0.4
24	*Allo*-aromadendrene	1.1	0.3	0.3	0.1	1.1	0.2	1.1	0.3	0.4	0.3	0.6	0.2
25	Terpinyl acetate	0.2	0.1	0.2	0.1	0.2	t	0.2	0.1	0.1	0.1	t	0.1
26	α–Terpineol	5.7	1.2	5.4	0.9	6.7	1.3	4.7	1.1	6.1	0.3	6.6	0.9
27	Boreneol	0.1	t	t	t	0.1	t	0.1	t	t	t	t	t
28	Cineol acetate	0.2	0.1	0.2	0.1	0.2	t	0.2	t	0.1	0.1	-	-
29	Bicyclogermacrene	7.9	3.1	1.1	0.9	0.1	0.1	0.1	t	0.7	0.5	2.7	1.2
30	Geranyl acetate	0.1	0.1	0.1	0.1	0.2	t	0.3	0.2	0.2	0.1	0.1	0.2
31	omega-Cadinene	0.2	t	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
32	Nerol	0.1	0.1	0.1	0.1	0.1	t	0.1	t	0.1	0.1	t	t
33	Anethole	0.1	0.1	t	t	t	t	0.1	0.1	t	t	t	t
34	Geraniol	0.6	0.2	0.4	0.1	0.7	0.2	0.5	0.4	0.8	0.2	1.0	0.3
35	Benzyl isopentanoate	0.1	t	0.1	0.1	t	t	t	t	t	t	t	t
36	Geranyl isopentanoate	0.8	0.2	0.7	0.2	t	t	t	t	0.3	0.1	0.4	0.2
37	Palustrol	0.4	0.1	0.1	t	0.1	t	0.1	t	t	t	0.1	0.1
38	Phenzyl ethyl isopentanoate	0.1	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.1
39	Globulol	4.0	1.0	0.6	0.3	6.9	0.4	8.0	0.4	1.6	1.1	2.0	0.6
40	Viridiflorol	2.2	0.6	0.4	0.2	1.0	0.1	1.3	0.2	0.5	0.4	1.1	0.2
41	Spathulenol	0.5	0.1	1.8	0.4	0.3	0.1	0.6	0.3	0.5	0.3	0.2	0.1
42	γ–Eudesmol	0.3	0.2	0.3	0.1	0.8	0.3	0.7	0.2	1.0	0.9	1.3	1.0
43	α–Eudesmol	0.3	0.1	0.4	0.1	1.0	0.5	1.2	0.5	1.2	1.1	1.5	1.1
44	β–Eudesmol	0.4	0.1	0.2	t	1.0	0.5	1.1	0.7	1.4	1.4	1.6	1.3

Appendix 11.3. The chemical composition of different compound classes of *E. nitens* and *E. globulus* waxes

Appendix table 11.3-1 indicates that the homologues of the principal compound class, the ß-diketones, were very similar for both adult and juvenile waxes of both *E. nitens* and *E. globulus*. The ß-diketones of all waxes were dominated by n-tritriacontan-16,18-dione (84 to 95% of the ß-diketone homologues) and by the presence of n-hentriacontan-14,-16-dione (1.6 to 10%) and β–pentatriacontane-16,20-dine (3 to 9%) as minor homologues. The relative percentage of individual homologues in the n-alkane and aldehyde fractions of both adult and juvenile leaf waxes of *E. nitens* and *E. globulus* are shown in Appendix table 11.3-2.. In all waxes of the two species the chain length of n-alkane homologues ranged from 12 to 31 carbon numbers with C_{29} accounting for 62.4 to 76% of the alkane fraction. There was a similar difference between juvenile and adult waxes of both species as the relative percentage of C_{27} was markedly higher in adult waxes (15.3 and 19.4%) than that in juvenile waxes (4.4 and 7.6% for *E. nitens* and *E. globulus* respectively).

The distribution of aldehyde homologues was also similar in both species with an identical difference between juvenile and adult waxes. Thus, the chain length of aldehydes from juvenile waxes of both species ranged from 22 to 30 carbon numbers with the homologues, C_{30}, accounting for 65.8% for *E. nitens* and 52.4% for *E. globulus*. However, the chain length of aldehydes from adult waxes of both species ranged fro 20 to 30 carbon numbers with similar percentages of C_{28} and C_{30} (35.3 to 40%) as dominate homologues. The percentage of C_{24} and C_{26} homologues in adult waxes of both species was also markedly higher than that in the juvenile waxes for both species.

As shown in Appendix table 11.3-3, there were four classes of esters to be detected in the leaf waxes of *E. nitens* and *E. globulus*. Results indicated that the percentage distribution of individual esters was similar between species and an identical difference between adult and juvenile leaf waxes was found. Thus, the wax of juvenile leaves contained mainly a series of long chain esters (alkane-2-ol esters) which accounted for 98.5% of the total esters of juvenile *E. nitens* and 92.5% for *E. globulus*. However, the other three series of aromatic esters, the benzyl, phenyl and benzoate esters occurred only in low to trace amounts in the juvenile waxes. In contrast, the adult leaf waxes of both species contained higher percentages of aromatic esters and substantial amounts of alkan-2-ol esters.

The distribution of homologues of individual ester homologues was also similar in both *E. nitens* and *E. globulus*. The composition of alkane-2-ol esters from both juvenile and adult waxes of the two species are shown in Appendix table 11.3-4. These alkane-2-ol esters are compounds in which carbon numbers ranged from 25 to 35 with individual esters consisting of mixture of long chain acids and secondary alcohols of different chain lengths. The carbon numbers of individual long chain acids in esters had even numbers ranged from 14 to 24 with C_{16}, C_{20} and C_{22} the most common and the carbon numbers of their secondary alcohols had odd numbers ranging from 9 to 15 with C_9 and C_{11} predominate. There was a similar difference in the composition of alkane-2-ol long chain esters between juvenile and adult waxes in both species. The composition of alkan-2-ol long chain ester homologues in juvenile leaves mainly consisted of the five compounds, *ses.* nonanoyl hexadecanoate (C_{16} acid; C_9 alcohol), *ses.* undecanoyl octadecanoate (C_{16}; C_9), *ses.* nonaoyl eicosanoate (C_{20}; C_9), *ses.* undecanoyl docosanoate (C_{20}; C_{11}), and undecanoyl docosanoate (C_{22}; C_{11}), as major components and *ses.* tridecanoyl docosanoate and *ses.* pentadecanoyl eicosanoate as minor components. The composition of alkan-2-ol ester homologues in adult waxes was similar with that in juvenile waxes, whereas, the adult also contained high parentage of *ses.* nonaoyl docosanoate, *ses.* nonaoyl tetracosanoate and *ses* undecanoyl tetracosanoate which were absent in juvenile waxes. At the same time, *ses.* tridecanoyl decosanoate and *ses.* pentadecanoyl eicosanoate occurred in trace amounts in adult leaf waxes.

The composition of benzyl ester and phenyl ethyl ester homologues in adult leaf waxes of *E. nitens* and *E. globulus* are shown in Appendix table 11.3-5 indicated that the carbon number of long chain acids in these two series of esters ranged from 20 to 30 with even numbers predominating. It is considered that the composition of these two series of ester homologues was very similar between species. Again, Appendix table 11.3-6 indicates that the composition of benzoate ester homologues from adult waxes were also similar in both species.

Appendix table 11.3-1. Composition (%) of leaf waxes from *E. nitens* and *E. globulus*.

Compounds	*E. nitens*		*E. globulus*	
	Juvenile	Adult	Juvenile	Adult
n-Hentriacontan-14,16,dione	7.5	10.3	8.3	1.6
n-Triancotan-16,18-dione	83.7	83.8	84.9	95.2
n-Pentatriacontan-16,20-dione	8.8	5.8	6.7	3.1

Appendix table 11.3-2. Homologue composition (%) of n-alkanes and aldehydes in leaf waxes of *E. nitens* and *E. globulus*.

n-Alkanes					Aldehydes				
Carbon No.	*E. nitens*		*E. globulus*		Carbon No.	*E. nitens*		*E. globulus*	
	Juvenile	Adult	Juvenile	Adult		Juvenile	Adult	Juvenile	Adult
12	0.19	0.34	0.12	0.15	12	-	-	-	-
13	0.17	0.38	t	0.08	13	-	-	-	-
14	1.12	0.57	0.35	0.32	14	-	-	-	-
15	1.24	0.96	0.98	0.15	15	-	-	-	-
16	5.15	3.47	3.09	1.35	16	-	-	-	-
17	1.21	0.54	1.32	0.86	17	-	-	-	-
18	1.07	0.62	0.83	0.56	18	-	-	-	-
19	0.29	0.28	0.16	0.02	19	-	-	-	-
20	0.16	0.09	0.15	0.12	20	-	0.91	-	0.86
21	0.35	0.23	0.16	0.21	21	-	0.28	-	0.12
22	0.23	0.18	0.45	0.09	22	-	0.15	0.35	0.78
23	0.64	0.69	0.96	0.53	23	0.13	0.54	0.25	0.25
24	0.69	0.52	0.38	0.65	24	0.71	4.79	1.96	3.25
25	0.77	1.36	3.58	2.85	25	0.71	0.92	0.63	0.96
26	1.34	1.57	1.98	1.68	26	1.61	7.66	4.56	12.35
27	4.37	15.27	7.58	19.36	27	2.57	2.29	1.86	2.16
28	2.57	3.94	3.85	4.25	28	22.37	39.69	31.25	35.28
29	75.79	66.42	69.35	62.35	29	5.39	3.92	6.25	4.28
30	0.93	0.69	1.02	0.86	30	65.82	37.05	52.36	39.36
31	1.16	1.84	3.15	1.98	31	-	-	-	-

Appendix table 11.3-3. Composition (%) of esters in the leaf waxes from *E. nitens* and *E. globulus*.

Types of ester compounds	*E. nitens*		*E. globulus*	
	Juvenile	Adult	Juvenile	Adult
Alkan-2-ol esters	98.5	16.09	92.5	12.12
Benzyl esters	t	41.29	2.3	31.21
Phenyl ethyl esters	t	33.93	t	35.34
Benzoate esters	1.5	8.69	4.5	20.45

Appendix table 11.3-4. Homologue composition (%) of alkan-2-ol long chain esters in leaf waxes of *E. nitens* and *E. globulus*.

Compounds	Carbon No. of esters	Carbon No. of acids	Carbon No. of alcohols	*E. nitens*		*E. globulus*	
				Juvenile	Adult	Juvenile	Adult
ses. Nonanoyl hexadecanoate	25	16	9	10.86	12.01	4.86	7.25
ses. Undecanoyl tetradecanoate	25	14	11	-	-	t	t
ses. Undecanoyl hexadecanoate	27	16	11	16.24	13.25	21.21	18.58
ses. Nonamoyl octadecanoate	27	18	9	t	t	t	t
ses. Undecanoyl octadecanoate	29	18	11	t	0.4	t	4.25
ses. Nonamoyl eicosanoate	29	20	9	12.16	6.62	18.25	10.25
ses. Undecanoyl eicosanoate	31	20	11	27.66	9.35	28.58	13.56
ses. Nonamoyl docosanoate	31	22	9	t	8.21	t	7.25
ses. Undecanoyl docosanoate	33	22	11	24.69	12.62	18.69	8.59
ses. Nonanoyl tetracosanoate	33	24	9	t	14.56	t	11.25
ses. Undecanoyl tetracosanoate	35	24	11	-	22.36	-	18.58
ses. Tridecanoyl decosanoate	35	22	13	3.38	-	4.29	t
ses. Pentadecanoyl eicosanoate	35	20	15	3.42	-	3.85	t

Appendix table 11.3-5.. Homologue composition (%) of benzyl esters and phenyl ethyl esters in the leaf waxes of *E. nitens* and *E. globulus*.

Carbon No. of acid components	Phenyl ethyl esters			Benzyl esters		
	Compounds	*E. nitens*	*E. globulus*	Compounds	*E. nitens*	*E. globulus*
20	Phenyl ethyl eicosanoate	7.17	4.58	Benzyl eicosanoate	1.34	1.86
21	Phenyl ehtyl henicosanoate	t	t	Benzyl henicosanoate	-	-
22	Phenyl ethyl docosanoate	18.23	19.36	Benzyl docosanoate	8.17	7.96
23	Phenyl ethyl tricosanate	t	t	Benzyl tricosanate	t	t
24	Phenyl ethyl tetracosanoate	41.23	39.63	Benzyl tetracosanoate	38.9	21.25
25	Phenyl ethyl pantacosanoate	t	t	Benzyl pantacosanoate	t	t
26	Phenhyl ethyl hexacosanoate	15.98	28.58	Benzyl hexacosanoate	25	39.63
27	Phenyl ethyl heptacosanoate	t	t	Benzyl heptacosanoate	t	t
28	Phenyl ethyl octacosanoate	3.25	5.93	Benzyl octacosanoate	26.7	28.25
29	Phenyl ethyl nonacosanoate	-	-	Benzyl nonacosanoate	-	-
30	Phenyl ethyl triacontanoate	-	t	Benzyl triacontanoate	-	1.29

Appendix table 11.3-6. Homologue composition (%) of benzoate esters in the leaf waxes of *E. nitens* and *E. globulus*.

Carbon No. of acid components	Compounds	*E. nitens*	*E. globulus*
20	Eicosanoyl benoate	-	t
21	Henicosanoyl benoate	-	-
22	Docosanoyl benoate	1.09	3.25
23	Tricosanoyl benoate	-	t
24	Tetracosanoyl benoate	19.63	14.25
25	Pantacosanoyl benoate	t	t
26	Hexacosanoyl benoate	45.69	39.25
27	Heptacosanoyl benoate	t	t
28	Octacosanoyl benoate	33.58	40.12
29	Nonacosanoyl benoate	-	-
30	Triacontanoyl benoate	0.86	3.12