Oviposition site selection by the eucalypt herbivore *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae) and the implications for larval establishment

by

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Declaration

This thesis does not contain any material that has been accepted for the award of any other degree or diploma in any tertiary institution. To the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made in the text.

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Abstract

Chrysophtharta bimaculata (Olivier) is a chrysomelid folivore of several *Eucalyptus* species, including *E.* (*Monocalyptus*) *regnans*, *E.* (*M.*) *delegatensis* and *E.* (*Symphyomyrtus*) *nitens*. Both adults and larvae feed on the same tree species and eggs are laid directly onto host foliage. Adults feed and oviposit in aggregated swarms and, as a result, subsequent larval feeding may cause severe host tree defoliation and larval resource depletion. Further, previous research on a related chrysomelid has shown that factors associated with changing leaf age (eg variation in toughness and nitrogen concentration) can seriously impact on larval survival. Because oviposition site selection is likely to be of fundamental importance to larval survival in *C. bimaculata*, the factors affecting oviposition site selection and the impact that the selected site had on subsequent larval establishment were chosen as the primary foci of this thesis.

Research followed three main thrusts. In the first (Chapters 2-6), I documented exactly where *C. bimaculata* placed its eggs, both under natural and controlled conditions, from the individual leaf up to the level of tree species. Manipulative and correlative studies were used to determine what factors might affect site selection. *C. bimaculata* prefers to oviposit near the leaf tip and although there was no evidence that conspecific egg batches directly deter ovipositing beetles, leaves with egg batches on their tip are less preferred for oviposition. Other factors demonstrated to negatively influence oviposition site selection between leaves were increasing leaf toughness and conspecific beetle feeding damage. By altering leaf position it was demonstrated that leaf toughness, rather than leaf position, influenced *C. bimaculata* oviposition preference..

In chapter 5, I document the effects of egg batch placement on larval establishment. Wild, aggregated populations regularly deposit approximately one-third of egg batches on mature leaves unsuitable for neonate establishment. However, neonates had the ability to migrate to suitable leaves and establish with no increase in

mortality. This suggests a strong relationship between oviposition site selection and larval establishment within host trees.

Finally, I examined host plant phenology under natural conditions (Chapter 7). Significant differences in leaf toughness development, size and chemistry between and within host species were found. Leaf chemistry may influence host plant selection between species. However, the rate of leaf toughness development in current season leaves is likely to determine host vulnerability to defoliation under high egg batch densities.

This thesis indicates that *C. bimaculata* oviposition site selection is influenced by direct and indirect plant and conspecific factors. The interaction of these factors determine the egg distribution and often lead to high egg density within hosts. The strong relationship between *C. bimaculata* oviposition site selection and larval establishment increases the potential for high larval densities.

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Chapter 1

Introduction

1.1 Thesis aim and outline

Studies on paropsine-eucalypt host interactions to date, have concentrated on how plant characters influence adult and larval feeding choice and performance (see Table 8.1). Although these studies provide aspects required to understand host utilisation by these beetles, a proper perspective on these interactions cannot be gained without the examination of oviposition site selection and the influence this has on neonate establishment. A detailed examination of oviposition site selection within and between host species and the subsequent performance of neonates will not only provide a greater understanding of insect-host interactions for paropsines but also aid in understanding overall population dynamics of these beetles and host damage potential.

Using a native beetle *Chrysophtharta bimaculata* (Olivier) (Coleoptera: Chrysomelidae: Paropsinae), this thesis explores factors that influence oviposition choice, egg distribution and neonate larval establishment on three *Eucalyptus* (Myrtaceae) host species. The study focuses on young trees (less than 5 years old) in plantation and regeneration areas, which are prone to severe defoliation by this insect. Particular attention is given to the influence of plant physical and phenological traits on observed behaviours.

1.2 The interaction between ovipositing insects and their host plants - An introduction

Insects that have herbivorous larvae show a wide range of specificity in their oviposition site choice. At one extreme are insects such as the chrysomelid, *Disonycha plurigata* (Le Conte), that are nonspecific in their oviposition and rely on the larvae to find suitable leaves upon which to establish (Dodge & Price 1991; Marques et al. 1994). At the other extreme are species such as *Anthocharis cardamines* (L.) (orange-tip butterfly) which are highly specific in their oviposition selection, choosing sites where larvae can commence feeding (Dempster 1997). For insects that oviposit at sites unsuitable for the establishment of neonates, larvae

need to have attributes (e.g. a strong migration ability, an adaptability to food of various nutritional value) to overcome the disadvantages of not having a ready supply of the appropriate food source. In contrast, species in which females are highly specific in their oviposition site choice do not require their offspring to have strong migration ability or a need to feed on poor quality resources. Outlined below are some of the factors that may influence an insect's oviposition choice.

1.2.1 *Leaf chemistry/nutrition*

For insects that have sessile larvae this aspect must be of the highest importance to the selection of an oviposition site. Many insects, such as *Delia antiqua* (Meigen) (Onion Fly), can detect volatiles emitted by their host plants (Renwick 1989) which aid in their identification. On contact with the potential oviposition site a wide range of different chemicals, often in combination, are thought to act as oviposition cues for different insects. Leaf wax constituents (Udayagiri & Mason 1997), terpenoids (Wearing & Hutchins 1973; Shu et al. 1997), glucosinolates (Nair & McEwen 1976; Hughes et al. 1997; Hopkins et al. 1998), glycosides (Pereyra & Bowers 1988; Haribal & Renwick 1998), carbohydrates (Derridj et al. 1996) and alkaloids (Honda et al. 1997) are some of the leaf components that have been implicated in oviposition choice.

1.2.2 *Physical characteristics of the oviposition site*

Apart from chemical information, physical factors can influence an insects' oviposition choice. The presence of trichomes or other glandular structures on the surface of potential sites may deter ovipositing insects (Gregory et al. 1986; Kumar 1992). Leaf shape has also been shown to be important for some insects, along with leaf colour and surface texture (Degen & Städler 1997).

1.2.3 *The effect of conspecifics.*

Many insects can detect whether a potential oviposition site is already occupied by conspecific eggs or larvae. The ability of an ovipositing beetle to detect conspecific

eggs and/or larvae can act as either a mechanism for attraction or deterrence. As an attraction mechanism, it can minimise the insects search time through signalling suitable oviposition sites and may facilitate the formation of larval aggregations (Phillips & Strand 1994). Resulting larval aggregations may provide better defence against predators (Jolivet et al. 1990; Codella & Raffa 1995), reduce the individual risk to predation or parasitism (Grégoire 1988), increase larval development rate by increasing temperature (Phillips & Strand 1994) increase the efficiency of food utilisation (Storer et al. 1997), help overcome leaf toughness (Clark & Faeth 1997) and facilitate nutrient release from hosts (Phillips & Strand 1994). In contrast, chemicals associated with conspecific eggs can act as oviposition deterring pheromones (ODP's). These have been found for many insects species and have developed in several orders of insects (see Anderson 1988). ODP's may also act interspecifically as in the case of *Pieris brassicae* and *Pieris rapae* (L.) (Klijnstra 1985; Schoonhoven et al. 1990). They may also lead to a more uniform spread of eggs (Credland & Wright 1990). This effect reduces the potential of larval competition on limited resources (Kozlowski et al. 1983; Klijnstra 1985; Kozlowski 1989). Likewise, the presence of conspecific larvae may deter ovipositing insects for similar reasons (Mappes & Makela 1993; Heard 1995)

For some species, such as *Leptinotarsa decemlineata* Say (Colorado Potato Beetle) (Pelletier 1995) and *Pieris brassicae* L. (cabbage moth) (Rothschild & Schoonhoven 1977), conspecifics can be visually detected, particularly when eggs are brightly coloured (Schoonhoven et al. 1990). Some passion vine species even produce egg mimics to deter passion vine butterflies from ovipositing (Benson 1978).

The presence of eggs may alter plant chemistry influencing oviposition choice by conspecific insects. Oviposition deterrents are produced by cabbage leaves through the presence of *P. brassicae* eggs (Blaakmeer et al. 1994a).

Oviposition preference can be influenced by the presence of conspecific larvae. *Delia radicum* (L.) (cabbage root fly) larvae damage host plants which subsequently become more attractive to ovipositing females (Baur et al. 1996). Likewise, some

substrates from conspecific larvae may attract ovipositing females (Phillips & Strand 1994). In many cases plants become less attractive when conspecific larvae are present, either through changes in plant chemistry (Fitt 1984; Landolt 1993; Blaakmeer et al. 1994a), through the presence of larvae themselves (Hilker 1989; Hilker & Weitzel 1991), or through presence of larval frass (Renwick & Radke 1980; Williams et al. 1986; Weaver et al. 1990; Firempong & Zalucki 1991).

1.2.4 *Enemy free space*

Although there are obvious benefits for insects having an oviposition preference for sites which are suitable for larval establishment and growth, some studies have failed to find a strong correlation between these factors. For some insects, enemy free space has been used to explain this discrepancy. In these cases larval survival may be enhanced due to a reduced rate of attack by predators or parasitoids. Bjorkman et al. (1997) found that a pine sawfly (*Neodiprion sertifer*)(Geoffr.) had a preference for pine trees where larvae were less likely to be parasitised than trees where larval development was faster. Denno et al. (1990) found that for the leaf beetle, *Phratora vitellinae* (L.), larvae survived and developed well on both *Salix viminalis* (L.) and *Salix fragilis* (L.) in the absence of predators. However, females had a strong oviposition preference for *Salix fragilis* which was found to be correlated with larval ability to sequester a salicylate-based secretion from *Salix fragilis* that effectively deterred predators. The salicylate was absent in *Salix viminalis*.

1.2.5 *Abundance of preferred hosts.*

When the most favoured host is scarce in an insect's searching environment, compared to other lower ranked hosts, oviposition may predominantly occur on the more common hosts. This theory has been suggested to explain differences between laboratory and field studies for *Brachys tessellatus* (F.) on various *Quercus* species (Waddell & Mousseau 1996) and a trade-off between nutritional quality and resource availability for the butterfly *Euphydryas chalcedona* (Williams 1983). Similarly, for insects that have a preference for oviposition on young leaves over

older, a lowly ranked host with young leaves may be chosen over a higher ranked host with old leaves. In such cases leaf age could become more important than species selection (Thomas 1987; Steinbauer et al. 1998).

1.2.6 *Variation in individual ovipositing insects*

Insects within populations can vary significantly in their selectivity of hosts. Ng (1988) found that an *Euphydryas editha* population consisted of individuals which were consistently more specialised in their oviposition site choice than others. Factors that may influence the degree of specificity of an ovipositing insect include insect physiology, experience and genetics.

Physiological factors such as peripheral or central processes within the insect may affect receptor sensitivity and the response to host cues. Insects such as *Euphydryas editha* may have an increased readiness to oviposit if they are delayed of a suitable oviposition site for a long period of time, thus making the insect more generalised in its oviposition choice (Singer 1982).

Experience can affect whether an insect accepts or rejects a host. A young insect that is searching for a suitable oviposition site may not be as selective as older more experienced insects. Prokopy et al. (1982) found that first-time ovipositing *Rhagoletis pomonella* (Walsh) readily accepted *Crataegus mollis* (Hawthorn) in a binary choice test with apple. However, none of the females that had experience ovipositing on apple (*Malus pumila*) accepted *C. mollis.* Prokopy et al. (1986) suggest that experienced *R. pomonella* females are less likely to accept novel hosts for oviposition compared to naive females. Species from several insect orders have been found capable of modifying their behaviour through learning (Papaj & Prokopy 1989).

Genetic factors have been implicated in variation of oviposition site preference by individuals within a species. Variation in egg batch distribution between populations of the same species can be due to genotypic differences. This was found to be the case for the beetle, *Callosobruchus maculatus* (F.) (Messina 1989), the

butterfly *Euphydias editha* (Singer & Thomas 1996) and the moth *Heliothis virescens* (F.) (Waldvogel & Gould 1990). Genetic differences are thought to be accountable for the variation in specialisation found between ovipositing *Euphydias editha* within a population (Singer et al. 1988).

1.2.7 *Other factors affecting oviposition site choice*

Other factors may be important in influencing an insect oviposition site choice. These include the potential detrimental effect of leaf expansion on eggs (Johansen 1997), abiotic factors such as wind and rain (Despins et al. 1986) and avoiding potential egg damage caused by other feeding conspecific adults on the same hosts as the eggs (Raupp & Denno 1980).

Thompson (1988b) places all of the composite factors responsible for the survival of the egg, larval and pupal stages, growth rate of larvae, and the resulting fecundity and longevity of the adult, in the term 'performance'. Insects will vary greatly in the factors which are most important in their overall performance and combinations of factors may be additive or negative to the overall performance.

1.3 The interaction between egg distribution within host plants and the population dynamics of an insect

The degree of selectivity that an insect shows in its oviposition site choice, and the number of potential oviposition sites that a host plant offers, have important implications for the population dynamics of that insect and the degree of damage that a host plant may sustain. Price et al. (1990) and Price (1992; 1994) believe that the degree of oviposition site selectivity determines whether the species will have either latent or eruptive population dynamics. Price et al. (1990) defines an eruptive species as those that are capable of having an epidemic phase where populations become dense and damaging to their hosts. Eruptive species typically have populations that vary over a magnitude of three to five orders. In contrast latent species tend to have steady populations which rarely cause significant damage

to their hosts and vary in magnitude of one to two orders. Because latent insects are highly selective of their oviposition site choice it is the number of limited oviposition sites available that regulate the maximum population potential that can be achieved (Price 1992). Oviposition site choice does not regulate the population potential of eruptive species as their inability to assess suitable sites for offspring survival results in indiscriminate oviposition. Thus eruptive species have the potential to reach high densities and cause severe damage and even death to their host plants (Price et al. 1990).

Although latent insects have populations limited by the number of suitable oviposition sites, these species can increase their populations due to changes to their habitat. Any disturbance that increases the number of potential oviposition sites available to these types of insects will increase the potential population size. Examples where environmental changes have resulted in increased populations of such insects include: (i) forestry which tends to create large stands of even aged monocultures (Price et al. 1990; Landsberg & Cork 1997), (ii) host plant damage through fire (Viera et al. 1996) and mammal browsing (Hjältén 1996; Roininen et al. 1997) which produces young vigorous shoots more suitable for oviposition and larval development.

1.4 An introduction to the paropsines and *Chrysophtharta bimaculata*

The tribe Paropsini belongs to the subfamily Chrysomelinae of the family Chrysomelidae. The Paropsini contains the genera *Paropsis, Procris, Paropsisterna, Chrysoptharta, Trachymela* and *Pyrgo* (Cumpston 1939). Most species of paropsini feed on *Eucalyptus* species and other Myrtaceae. (de Little 1979; Selman 1994).

The diverse types of eucalypt feeding paropsines that have evolved in Australia are speculated to be closely linked to the factors leading to the evolution of the broad range of eucalypt species (Selman 1994). In Tasmania, the paropsines that feed on

Eucalyptus are represented by at least 36 species of which the majority are in the genus *Chrysopththarta* (de Little 1979).

Paropsine species (as addressed later in the Introduction) vary widely in factors such as fecundity and larval development time (de Little 1979). Larvae can vary greatly in their behaviour and appearance. Larvae in the genera *Chrysophtharta, Paropsis* and *Paropsisterna* predominantly have well developed defence glands, large bodies with short legs, feed during the daytime, do not move over large distances, have strong aggregative tendencies and are most often found during the summer (Selman 1994). In contrast, the genera *Trachymela* and *Sterromela* have larvae with poorly developed defensive glands, long legs with short bodies, are highly active and capable of travelling long distances, feed at night and hide under bark during the day, do not aggregate and can be found anytime during the year (Selman 1994).

Chrysophtharta bimaculata is one of the most studied of paropsine species. Adults are oval shaped and approximately 9mm in length and their colour ranges through green, grey or brown or combinations of these colours depending on their age and the time of year. This species can be distinguished from other *Chrysophtharta* by two distinct black maculae (spots) on the pronotum (hence bi-maculae) (de Little 1979). Like many of the other paropsines, *C. bimaculata* male beetles have expanded setae on their first tarsal segments, distinguishing them from females (de Little 1979).

C. bimaculata occurs throughout the state of Tasmania, predominantly where the eucalypt species *E. obliqua* (L' Hérit.), *E. regnans* (F. Mueller) and *E. delegatensis* (R. Barker) are found. More recently the beetle has been shown to utilise *E. nitens* (Dean & Maiden) successfully (de Little 1989) and may be commonly found in plantations of this tree species, particularly in the north of the state (de Little pers. comm.). It was originally thought to have a bivoltine life-cycle (Greaves 1966), but it is now widely accepted to be univoltine in Tasmania (de Little 1983). Both adult and larvae feed on the same eucalypt hosts (Patterson et al. 1996).

The life-cycle consists of an overwintering phase in which adult beetles shelter under bark, in cracks and crevices of old trees and stags (Greaves 1966) and in the leaf clumps of *Gahnia grandis* (Clarke et al. 1998b). During the overwintering stage, beetles are brown-red in colour. During warmer winter and spring days beetles may be seen on host trees (V. Patel pers. comm.) but will remain in their overwintering phase until mid to late spring.

On emergence from overwintering, adult beetles will begin feeding leaving a characteristic scalloping of leaf margins (Greaves 1966). Feeding occurs predominantly on the expanding soft leaves of their host trees. Beetles begin changing colour eventually becoming predominantly pale green during the summer (de Little 1983).

Oviposition occurs during late spring or early summer and appears to be dependent on temperature (Greaves 1966) with expanding or newly expanded young leaves of their host plants preferred for egg batch deposition (Steinbauer et al. 1998). Each egg is approximately 2.1 mm long by 0.8 mm wide (de Little 1979). The colour of egg batches range from pale yellow, pale brown to grey-green. They are deposited in rows that may contain more than 50 eggs, but generally between the range of 8 to 30 (Greaves 1966; de Little 1979). The time taken for eggs to hatch is temperature dependent but usually takes 9-11 days under field conditions (Greaves 1966).

Chrysophtharta bimaculata larvae hatch with the aid of an egg burster (as do other paropsines), a group of spines present on the back of larvae. This is used to split the chorion in two places from which the larvae can emerge (Greaves 1966). The larvae then feed on their chorion (Greaves 1966) before feeding on the expanding young leaves of their host plant. The larvae are olive green in colour and develop through four instars. Larval aggregation is the norm during the first three instars (Greaves 1966). The larvae *of C. bimaculata* contain dorsal abdominal defence glands which are thought to eject hydrogen cyanide and benzaldehyde (see Moore 1966). During the fourth instar larvae are commonly found as individuals (Patel pers. comm.) before they drop to the ground to pupate.

On completion of the pupal stage beetles are grey in colour with soft elytra which hardens and becomes pale green (Authors pers. obs.). Beetles will then feed on the young expanding foliage of their host trees before entering the overwintering phase.

C. bimaculata regularly causes major defoliaton to young (less than 10 metres in height) *Eucalyptus obliqua, E regnans* and *E.delegatensis* regeneration and plantation forests in Tasmania (Greaves 1966). Populations fluctuate from year to year, with weather, natural enemies and food quality most likely responsible (Greaves 1966; de Little 1979). However, previously collected data sets show no indication that population fluctuations vary any more than approximately 25 fold (Elek 1997, Howlett unpubl). Localised beetle and larval aggregations appear common every summer throughout the beetles range in Tasmania (Authors pers. obs.).

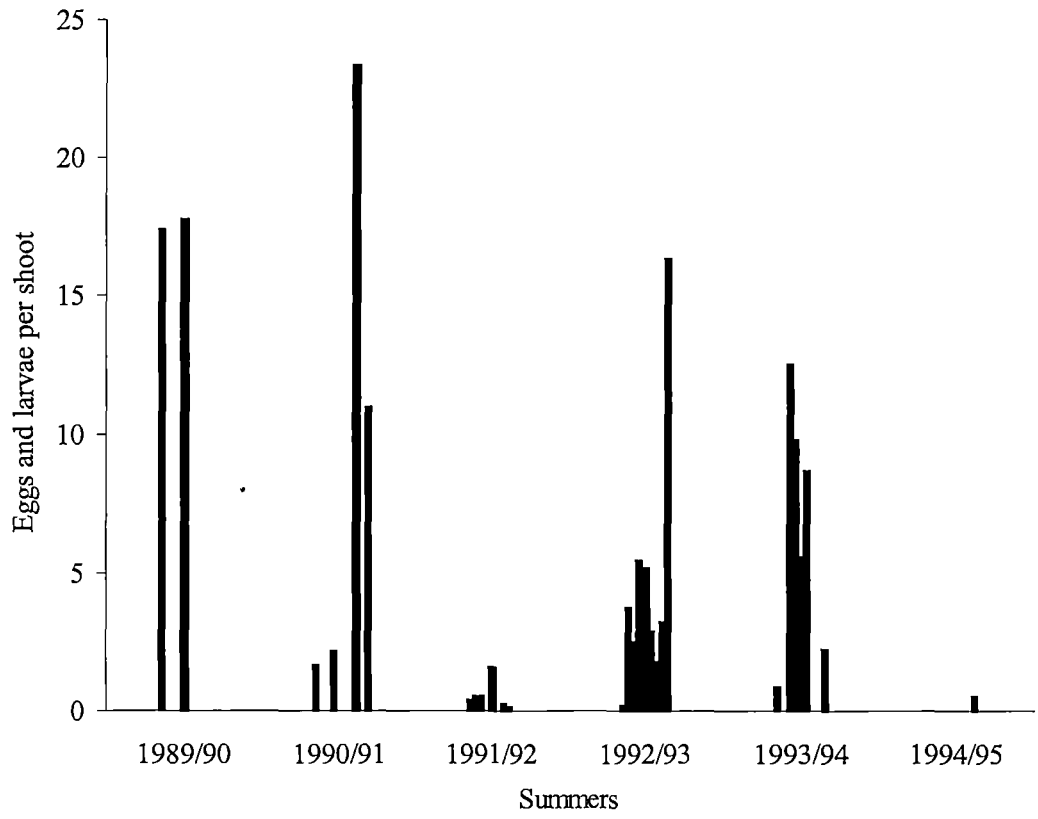


Figure 1.1 Estimates of populations of immature leaf beetles for six summers in an *E. regnans* plantation (mean number of eggs and larvae per shoot from sampling 10 shoots on 10 trees on each occasion). Reproduced from Elek (1997).

C. bimaculata utilises mature trees for feeding and oviposition (Kile 1974; Clarke et al. 1998a) but the economic impact of this damage has not been assessed. This insect has also become a major pest of the *Symphyomyrtus* species *Eucalyptus nitens* following planting of this species in Tasmania (de Little 1989). *C. bimaculata* is regarded as a major pest because it can repeatedly cause substantial reductions in the incremental growth of several economically important eucalypt species over a wide distribution of its range (Leon 1989; Elliott et al. 1991; Candy 2000). Both beetles and larvae are capable of causing significant feeding damage to their host trees (Fig. 1.2) and because both utilise the same species damage may be confounded.

Figure 1.2. *Chrysophtharta bimaculata* larval defoliation of a four year old *E. regnans* tree at Judbury Bluff, Southern Tasmania. The remaining foliage developed in previous seasons.

Sawlog and veneer production is currently restricted to old growth forests where *E. regnans*, *E. delegatensis* and *E. obliqua* are important species. Following clearfelling, the most common harvest technique (Anon. 1995a), the predominant method of forest regeneration involves burning then aerial sowing by planes or helicopters (Anon. 1995a).

The Eucalyptus (*Symphyomyrtus*) species *E. globulus* (Labill.) and the introduced *E. nitens* are currently the only trees grown in plantations. Up until recently the *monocalyptus* species *E. regnans* and *E. delegatensis* were also grown. The higher timber volumes obtained from small intensively managed plantation areas is resulting in an increasing rate in plantation establishment (Anon. 1995b).

1.5 Host utilisation in the eucalypt feeding paropsine genera - *Chrysophtharta* and *Paropsis*

Studies on the eucalypt feeding paropsines in the genera *Chrysophtharta* and *Paropsis* have concentrated on only a few species. The most notable are *Paropsis atomaria* (Olivier), *P. charybdis* (Stål), *Chrysophtharta bimaculata* and to a lesser extent *C. agricola* (Chapuis) and *C. hectica* (Boisduval). An outline of some key life-cycle traits for each of these species is provided in Table 1.1.

1.5.1 *Oviposition preferences in the genera Paropsis and Chrysophtharta*

Most species in these genera show host preferences for *Eucalyptus* species within a sub-genus, although some species such as *Chrysophtharta nobilitata* (Erichson), *Paropsis aegrota* (Boisduval), and *P. porosa* (Erichson) have been collected from the full taxonomic range of eucalypts (de Little 1979). Of the hosts utilised there is often a hierarchy of preferences. For example, in caged experiments using *C. bimaculata* it has been shown that *Eucalyptus regnans* is preferred for oviposition over *Eucalyptus nitens* (Steinbauer et al. 1998), even though larvae develop well on both species under laboratory conditions (Baker unpubl.).

A number of factors have been suggested as playing an important role in host selection for species in these genera. Li (1993) found a correlation between leaf oils and waxes and the acceptance or rejection of oviposition by hosts by *C. bimaculata* and *C. agricola*. For *C. bimaculata*, *Eucalyptus* species high in 1,8-cineole concentrations were avoided as hosts, while for *C. agricola*, host preferences appeared restricted to *Symphyomyrtus* species which had 1,8-cineole and β-diketones as major constituents in their essential oil (Li 1993). Also, like *P. charybdis* (Edwards 1982), *C. bimaculata* cannot utilise glaucous foliage due to its inability to grip the wax covered leaf surface (Li 1993).

Raymond (1995) and Strauss & Morrow (1988) have shown that paropsine species have preferences for particular trees within species. Raymond (1995) found significant differences between observed defoliation of different families of *E. regnans* and *E. nitens*. She also found that faster growing trees sustained a lower degree of defoliation. Strauss & Morrow (1988) found that larger trees, which had more foliage, supported higher densities of beetles. They suggest the amount of suitable foliage may be more important to beetles than foliar nutrient contents.

Table 1.1 A comparison of the life-cycle traits of five species from the genera *Chrysophtharta* and *Paropsis*.

BEHAVIOUR	*LIFE-CYCLE TRAIT*
Foliage preferred for larval feeding.	Soft expanding foliage is required for optimum larval development by early instars of *Chrysophtharta bimaculata* (Greaves 1966), *C. hectica* (Strauss & Morrow 1988), *Paropsis atomaria* (Ohmart et al 1985a, Ohmart et al. 1987, Larsson & Ohmart 1988) and *P charybis* (Murphy 1998). *C agricola* prefers young juvenile glaucous foliage (Ramsden & Elek 1998)
Foliage preferred for adult feeding	Soft expanding foliage is preferred for feeding by *Chrysophtharta bimaculata* (Greaves 1966), *C agricola, C hectica* (Strauss & Morrow 1988), and *P charybdis* (Murphy 1998). *P. atomaria* is more fecund when fed 'flush' foliage (Ohmart et al. 1985b; Ohmart et al 1987)
Oviposition site selection.	Soft expanding leaves are the preferred oviposition site for *C. bimaculata* (Steinbauer et al 1998). The tips of young juvenile, glaucous leaves are preferred for oviposition by *C agricola* (Ramsden & Elek 1998) *P charydbis* prefers to oviposit on the tips of old leaves, near expanding foliage (Murphy 1998) *C hectica* oviposits on stems, leaves and twigs of *Eucalyptus* trees (Strauss & Morrow 1988) *P atomaria* oviposits on stems of new shoots (Tanton & Khan 1978) (Ohmart et al 1987)
Oviposition and fecundity	*C bimaculata, C. agricola, P. atomaria and P charybis* females may deposit several hundred to several thousand eggs under laboratory conditions during their lifetime (Styles 1970; de Little 1983),(Ohmart et al. 1985b),(Ramsden & Elek 1998), and usually deposit eggs in batches of between 10-50 (Greaves 1966; Styles 1970; Ohmart, 1985b; Ramsden & Elek 1998, Murphy 1998) *C hectica* females can oviposit for several weeks and deposit eggs singularly or in small groups (Strauss & Morrow 1988).
Larval aggregation and defense.	Larval aggregation is present in the majority of instars for *C bimaculata* (Greaves 1966), *C agricola* (de Little 1979), and *P atomaria* (Carne 1966; Ohmart et al. 1985a) *P charybdis* larvae are only strongly gregarious for a short period in their development (de Little 1979; Murphy 1998) *C hectica* larvae feed singularly (Strauss & Morrow 1988) The larvae of *C bimaculata, C. agricola* (pers. obs), *P atomaria* (Carne 1966, Moore 1966) and *P charybdis* (Murphy 1998) have well developed defence vesicles Moore (1966) found that hydrogen cyanide is secreted from these vesicles for selected species within *Paropsis* and *Chrysophtharta* genera No information on defence mechanisms for *C. hectica* is available.
Larval development time on suitable hosts	Under laboratory conditions *C bimaculata* can complete its larval stage within 13 days (see (de Little 1983), *C agricola,* 14 days (Ramsden and Elek 1998) and *P atomaria* 14 days (see Carne 1966b). To develop from egg to adult stage, *P charybis* takes 7-9 weeks (Styles 1970), while *C hectica* takes about 35 days (Strauss and Morrow 1988)
Beetle Aggregation.	High, localised beetle densities occur due to beetle aggregation for *C. bimaculata* (Clarke et al. 1997), *C agricola* forms moderately dense aggregations (pers. obs). *P. charybdis* forms dense populations, but not strongly aggregated (Murphy, pers comm) *C hectica* populations show clumped distribution, but at relatively low densities (Strauss & Morrow 1988). *P. atomaria* has a clumped distribution (Carne 1966a)

Leaf age can be another important factor in oviposition preference and the availability of foliage from a given age class may be more important in influencing host choice than between species host preference. Steinbauer et al. (1998) found that when branches of *E. regnans* and *E. nitens* contained both expanding and fully mature foliage, *E. regnans* foliage was preferred for oviposition by *C. bimaculata*. However, young *E. nitens* leaves were significantly preferred for oviposition over mature, fully expanded leaves of *E. regnans*.

Most of the work conducted on the insect-host plant interaction for the genera *Paropsis* and *Chrysophtharta* have examined larval performance in relation to factors dealing with leaf quality. de Little (1979) describes the larvae from these genera as having a limited ability to migrate. Thus one would expect females to be able to assess oviposition site suitability based on qualities suitable for neonate establishment. Factors that have been studied in relation to the performance of larvae in these genera include: leaf toughness/leaf sclerophylly, nitrogen, tannins and essential oil concentrations and composition.

Several studies have examined the influence of nitrogen on paropsine performance. Carbon-Nitrogen ratios were found to influence the performance of *Chrysophtharta flaveola* (Chapuis) larvae although several foliar factors (e.g. nitrogen and water concentrations, specific mass, total phenolics and condensed tannins and carbohydrates) were also found to be correlated (Lawler 1993; Lawler et al. 1997). However, leaf nitrogen (correlated to C:N) has been shown to be important in the rate of development of paropsine larvae (Fox & Macauley 1977; Ohmart et al. 1985a). Ohmart et al. (1985a) found there to be a threshold were nitrogen concentrations somewhere below 1.7% resulted in increased larval development time and decreased pupal weights. For nitrogen concentrations above this percentage neither larval development time nor pupal weight was affected. However, nitrogen concentrations that are below 1.7% usually occur in old, very tough eucalypt leaves where leaf toughness is thought to be largely responsible for the poor performance of paropsine larvae (Ohmart et al. 1987; Larsson & Ohmart 1988; Ohmart 1991). Moreover, A reduction in nitrogen concentration may reduce the fecundity of paropsine beetles (Ohmart et al. 1985b; Ohmart 1991).

The effect of tannin and phenol concentrations in eucalypt leaves did not appear to influence the growth of *P. atomaria* larvae (Fox & Macauley 1977) even though the concentration of such compounds have been associated with plant defence (Feeny 1968; 1970; 1975; 1976; Reese 1978). Likewise, essential oil concentration in eucalypt leaves did not appear to influence the development of *P. atomaria* larvae (Morrow and Fox 1980). Instead, Ohmart & Larsson (1989) believe that *P. atomaria* larvae can metabolise essential oils. Also, variation in individual constituents of essential oils do not significantly influence the performance of *C. bimaculata* larvae (Patterson et al. 1996).

Apart from leaf chemistry, conspecifics and/or damage to hosts may influence beetle oviposition. For *P. atomaria*, Carne (1966a) reported that females prefer to oviposit on hosts that have not been damaged through larval feeding. When larvae had left the trees and new foliage had been produced these trees were again suitable for oviposition. Clarke et al. (1997) also found a negative correlation between damaged trees or presence of larvae and adult numbers of *C. bimaculata.* The presence of egg batches could also influence oviposition. Carne (1966a) reported that *P. atomaria* tended to lay egg batches near other conspecific egg batches.

1.5.2 *The population dynamics of Paropsis and Chrysophtharta*

The population dynamics of an insect species is dependent on both the characteristics of that insects life-cycle and life-cycle plasticity. Although many of the species studied within *Paropsis* and *Chrysophtharta* oviposit at a site suitable for neonate larval establishment, a characteristic which is typical of latent species (Price et al. 1990; Price 1992), a number of other factors can influence a species population dynamics. These include female fecundity, egg dispersion, adult dispersal, larval migration ability and selectivity of larval feeding sites (Price et al. 1990). All of the species studied in *Paropsis* and *Chrysophtharta* have larvae which in their early instars are restricted to soft expanding or newly expanded foliage (Table 1.1). and appear to have limited dispersal capabilities (de Little 1979). In other aspects species can differ widely. For example, *Chrysophtharta*

bimaculata beetles form dense aggregations (Clarke et al. 1997) which can deposit hundreds of egg batches (which can contain more than 50 eggs each) on its host plants. Greaves (1966) has reported mass starvation of third and fourth *C. bimaculata* instar larvae characteristic of resource depletion. In contrast, species such as *Chrysophtharta nobilitata* (Erichson) deposit small egg batches between 5 and 10 eggs (de Little 1979) with no record of beetles forming dense aggregations or causing significant damage to their hosts.

As outlined in section 1.2.5 there is evidence suggesting that natural enemies are important in population regulation (see Price 1987) and this appears the case for paropsine species. Although more than 75% of *C. bimaculata* egg batches may be predated in the field (de Little et al. 1990; Elliott & de Little undated), there are no major studies available on the effect of natural enemies on the population dynamics of paropsine species. For native paropsines, comparing egg mortality due to natural enemies between paropsine species can be misleading. Mo & Farrow (1993) found that populations of coexisting *P. atomaria* and *Chrysophtharta variicollis* (Chapuis) receive different predation and parasitism rates by different suites of natural enemies. Still, studies examining biological control suggests that natural enemies can have an important influence on paropsine populations. *P. charybis* populations were reduced for a few years with the introduction of *Enoggera nassaui* (Girault), a wasp, but populations of the beetle have since caused heavy defoliation (Murphy 1998).

Abiotic factors such as wind and rain may also affect the survival of paropsine larvae. Greaves (1966) reports that winds of high velocity, heavy rain and hail are capable of dislodging larvae and reducing larval populations. Low temperatures may also slow the development rate, affect larval weights and reduce survivorship of larvae (Carne 1966a; Ramsden & Elek 1998). Oviposition rates may also be influenced by climatic factors such as with oviposition timing in *P. atomaria* (Carne 1966a).

A few studies have examined aspects of the growth habit of host plants in relation to aspects of paropsine population dynamics. The growth habit within eucalypt

species, can be highly variable depending on factors such as tree age, stress or damage. Some eucalypt species, such as *E. nitens* and *E. globulus,* have juvenile foliage which differ markedly in size and shape from adult foliage (Kirkpatrick & Backhouse 1989; Duncan 1996) as well as having significant differences in essential oil quantity, essential oil composition and leaf wax composition (Li et al. 1994; Li et al. 1996; 1997). Moreover, the type of foliage that is dominant on the tree can influence the insect species utilising it as a host (Potts et al. 2000). *C. agricola,* for example, utilises *E. nitens* juvenile foliage, while *C. bimaculata* cannot utilise this host until it has developed adult foliage (de Little 1989). When trees suffer from mechanical or fire damage they often revert to their juvenile foliage (Brooker & Klenig 1990), thus markedly changing the populations of insects using them.

White's (1984) *Plant-Stress Hypothesis* predicts that any physiological stress on eucalypt trees will increase the amount of nitrogen available and thus make them more susceptible to insect damage. Although there is evidence that plant stress can increase shoot nitrogenous solutes, particularly some amino acids (Marsh & Adams 1995), Ohmart et al. (1985a; 1987) and Ohmart (1991) discovered that the performance of paropsine larvae is only affected at very low nitrogen concentrations (less than 1.7%).

Another hypothesis put forward by Price (1991), the *plant vigour hypothesis*, suggests that vigorously growing plant modules may be more susceptible to attack by some herbivores. The production of vigorous new shoots by many *Eucalyptus* species through fire or browsing can potentially increase phytophagous insect populations compared to less vigorous, undamaged plants (Landsberg & Cork 1997; Majer 1997; Steinbauer et. al 1998b). Likewise, the plant vigour hypothesis has applicability to disturbed ecosystems such as those caused through forestry. In young plantation or regeneration areas that consist of even aged single species stands of eucalypts, the canopy is undergoing vigorous expansion and is represented by a high proportion of young expanding foliage (Ohmart 1990). This not only increases the availability and predictability of young leaves to folivorous insect species, but may decrease the array of natural enemies present (Steinbauer 2000). In these situations, insect populations may increase above levels that would normally

be expected in natural ecosystems (Stone 1991). Simmul & de Little (1999) note that with the increased and intensive cultivation of various paropsine host plants, the number of paropsine species regarded as pests is increasing.

In a host plant-insect interactive system a feedback of repeated regrowth and defoliation can occur. This pattern has been termed 'resource regulation' by Craig et al. (1986) and is regarded as applicable in some eucalypt-insect interactions (Landsberg & Cork 1997; Steinbauer et. al 1998b).

The growth habit of different eucalypt species has been suggested as influencing their susceptibility to paropsine attack. Strauss & Morrow (1988) found that *C. hectica* beetles preferred to reside on *Eucalyptus stellulata* trees rather than *E. pauciflora*. They believe that this is due to the bushier canopies of *E. stellulata* which initially offer a larger amount of new foliage (suitable for feeding) and possibly better protection against weather, predators and parasites. However, for *C. bimaculata*, Clarke et al. (1997) failed to show a significant correlation between beetle abundance and an estimate of the amount of young foliage present on trees. However, in this case flush foliage was not limiting in the 3-year-old *E. obliqua* coupe (Clarke et al. 1997). Even so, the rate of development of flush foliage has been suggested as potentially influencing *C. bimaculata* oviposition preferences. Steinbauer et al. (1998) hypothesises that *E. regnans* may be more attractive to *C. bimaculata* than *E. nitens* for oviposition as it offers larger numbers of expanding leaves.

1.6 Thesis Outline

The aim of this thesis is to address the paucity of research on paropsine oviposition site selection and its influence on larval performance, a topic that is essential in better understanding host-plant insect interactions, population dynamics and subsequent host usage patterns. For insects that deposit on leaf surfaces, oviposition site selection can be influenced by several different cues that may determine preferences between trees, between leaves within trees and where oviposition occurs

on leaf surfaces. The following chapters examine the influence of physical and chemical leaf factors of host trees, and conspecific factors on *C. bimaculata* oviposition site selection and how the subsequent egg batch distribution affects larval performance and host tree defoliation. These chapters examine *C. bimaculata* oviposition preferences on individual leaves within trees through to between host *Eucalyptus* species preferences and the factors that influence preference. Additionally, the influence of oviposition site preference within trees is examined with regard to larval performance. Finally the foliage and leaf development of three different hosts of *C. bimaculata* is examined and compared in terms of their susceptibility to defoliation from spring through to autumn. Further detail for each chapter is provided below:

Chapter 1. Introduction

This chapter provides a review and introduction to factors that influence the population dynamics of phytophagous insects with particular reference to insect oviposition, egg distribution and subsequent larval performance. It also examines literature on the interaction of the *Paropsis* and *Chrysophtharta* with their eucalypt hosts.

Chapter 2. Factors affecting egg batch distribution upon *E. regnans* trees

This chapter examines the distribution of *C. bimaculata* egg batches on single leaves of a major host *E. regnans* and the factors that may influence that distribution. Factors assessed include leaf shape and colour, the presence of conspecific egg batches and the possibility of an oviposition deterring pheromone. Factors that influence egg batch distribution on the leaf surface may ultimately affect egg batch and subsequently larval densities.

Chapter 3. The influence of host development on *Chrysophtharta bimaculata* egg deposition

The effect of leaf number and size on egg batch distribution is examined in this chapter and whether these factors could ultimately affect larval densities. In addition the influence of larval defoliation on subsequent host leaf size and number is examined to determine whether *C. bimaculata* can affect plant architecture and the potential egg batch densities hosts receive.

Chapter 4. The relationship between *Chrysophtharta bimaculata* beetle feeding damage and subsequent egg batch densities on two eucalypt hosts

Both *C. bimaculata* adults and larvae feed on the expanding foliage of the same hosts and the aggregated nature of feeding adults decreases food resources available to offspring. The degree of correlation between adult feeding and oviposition thus has important implications for the population dynamics. Using regeneration and plantation areas containing mixed and single host species, adult defoliation and egg batch densities between individual trees are examined to determine if, and to what degree, a correlation exists. The influence of adult feeding damage on oviposition is further examined in the laboratory to determine whether beetle damaged foliage has a positive or a negative influence on oviposition and whether reduced leaf area and/or physical leaf scalloping influences oviposition preference.

Chapter 5. Oviposition site choice in relation to leaf toughness and neonate larval survival

This chapter examines the extent to which adult populations select leaves suitable for offspring establishment for oviposition by examining the egg batch distribution on three host species, *E. regnans, E. delegatensis* and *E. nitens* in the field. The ability of beetles to choose foliage suitable for neonate establishment is of fundamental importance in influencing the population dynamics of phytophagous insects (Price et al. 1990; Price 1992; 1994). In addition, the ability of neonates to migrate from unsuitable to suitable leaves for establishment is examined to determine whether larvae are capable of surviving, following hatching on leaves unsuitable for establishment.

Chapter 6 The effect of leaf position and beetle density on *Chrysophtharta bimaculata* egg batch distribution

This chapter examines whether leaf position is an important factor in influencing oviposition choice and if so, whether it is of greater importance compared to factors associated with leaf aging. In addition, oviposition site selection between suitable and unsuitable leaves for neonate establishment is examined for individual beetles and populations of beetles under various densities to determine (i) the degree to which individual beetles can access suitable oviposition sites and (ii) whether beetle density has any affect on oviposition site selection.

Chapter 7. A comparison of tree and leaf development between three host species of *C. bimaculata*

The importance of host plant development on the population dynamics of folivorous insects has been addressed in sections 1.2 and 1.5. This chapter examines factors associated with leaf and foliage development that have been implicated in the feeding and oviposition preferences of these insects and which may influence *C. bimaculata*. These include: leaf toughness development, leaf size and number, nitrogen concentrations, essential oil and surface wax composition. Comparisons are made between three host species, *E. regnans*, *E. delegatensis* and *E. nitens,* and between four families of *E. regnans* known to vary in their susceptibility to *C. bimaculata* defoliation.

Chapter 8 General discussion

The discussion will focus on:

(i) Plant and conspecific factors that influence *C. bimaculata* oviposition site selection on leaf surfaces and within tree hosts.

(ii) Factors that influence *C bimaculata* oviposition preference between hosts.

(iii) The correlation between oviposition site selection and the ability of larvae to establish following hatching.

The factors influencing *C. bimaculata* oviposition site selection will be compared with other insects that oviposit on plants and discussed in terms of larval defoliation of hosts.

Chapter 2

Factors affecting *Chrysophtharta bimaculata* egg batch distribution on leaves

2.1 Introduction

The ability to discriminate between oviposition sites can be of critical importance in determining the survival of an insects offspring, particularly if migration ability is poor. Physical and chemical cues are often used by phytophagous insects to detect suitable sites on and between leaves. Plant cues used and methods to detect them vary between insect species (Table 2.1). For those species that can discriminate between sites on a leaf surface, benefits may include reduced parasitism levels (Woods et al. 1996), reduced predation levels (Subinprasert & Svensson 1988), a reduction in larval establishment time and better nutrition (White 1970).

Factors affecting oviposition need not only be plant derived. Commonly the presence of conspecific eggs deters oviposition and one or more mechanisms may be involved. An oviposition deterring pheromone (ODP) may be deposited with the egg, or the egg may be visually apparent to other conspecific females (see 1.1.3). A deposited egg or the act of ovipositing may also change host plant chemistry, deterring other gravid conspecific insects. The leaf chemistry of *Brassica oleracea*, for example, has been shown to change through the deposition of a *Pieris brassicae* egg, acting as an oviposition deterrent to conspecific females (Blaakmeer et al. 1994).

Individuals within a species may respond differently to conspecific eggs. Some gravid insects may require prior experience with an ODP associated with the conspecific egg before they are deterred (Rothschild & Schoonhoven 1977) The physiological state of an insect, such as that caused through host deprivation, can also influence the deterrency of an ODP (Roitberg & Prokopy 1983). Likewise. conspecific egg batch deterrence may change with time. Ferguson & Williams (1991) found that the ODP for the cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) was active in deterrence for only 1-2 hours. In addition, rain may reduce the effectiveness of water soluble ODP's.

Table 2.1 Plant characters that impact on the oviposition of selected herbivorous insects.

Plant character	Insect	Detection method	Reference
Internal leaf chemistry	*Delia radicum* (Diptera)	Olfaction, palpation, proboscis & antennal tapping, drumming, running & possible ovipositor contact.	Städler & Roessingh 1990
	Acrolepiopsis assectella (Lepidoptera)	Chemo-tactile sensilla of ovipositor and antennomers.	Lecomte & Thibout 1981; Thibout & Auger 1996
Leaf wax	*Paropsis charybdis* (Coleoptera)	Deterrent as a physical barrier	Edwards 1982
	Chrysophtharta bimaculata (Coleoptera)	Deterrent as a physical barrier	Li 1993
Trichome density	*Heliothis armigera* *H. punctigera* (Lepidoptera)	Attractive for physically holding	Hassan et al. 1990
	Epiphyas postvittana (Lepidoptera)	Deterrent as a physical barrier	Tomkins et al. 1991
Leaf surface chemistry, waxes & increased trichome density	*Plutella xylostella* (Lepidoptera)	Possibly mechanoreceptors (chemistry)	Talekar et al. 1994; Spencer 1996; Hughes et al. 1997; Rigginbucci et al. 1998
Leaf edge	*Trioza eugenia* (Heteroptera)	Probably use physical cues	Luft & Paine 1997
Leaf veins and ridges	*Cacopsylla pyricola* (Heteroptera)	Mechanical cues	Horton 1990
Leaf colour	*Psila rosae* (Diptera)	Visual	Degen & Städler 1997

Factors influencing oviposition site selection upon leaves have not been studied in detail for paropsines such as *C. bimaculata*. For some of these beetles leaf age has been shown to influence oviposition between leaves within trees (Table 1.1). For *C. bimaculata*, young expanding leaves are preferred for oviposition (Steinbauer et al. 1998). Some paropsines also prefer to deposit egg batches at specific locations on leaf surfaces (de Little 1979; Table 1.1). Beckmann (1991) reported that *C. bimaculata* often deposit their egg batches near the colour change (from red to green) of developing eucalypt host leaves. Changing leaf colour for maturing *Eucalyptus* leaves is accompanied by changes in the surface wax coating and the internal biochemistry (Beckmann 1991). It should be noted, however, that Beckmann's report was simply reiterating the personal observations of a third person and were not based on hard data. To date, factors that influence paropsine oviposition choice on the leaf surface have not been specifically examined.

This chapter examines factors which may influence *C. bimaculata* oviposition on leaves and leaf surfaces. This was done through the following steps:

- The natural placement of eggs on leaves was quantified to see if specific level discrimination was occurring.

- Oviposition behaviour was observed to see if any behaviour patterns might give clues as to what factors were being used by the beetle or might otherwise be important.

- A series of manipulative experiments were run to test for factors that might be influencing egg placement. These manipulative experiments included studying the impact of leaf shape, leaf midrib and leaf colour, and the role of conspecific effects such as prior leaf damage, presence of eggs and presence of ovipositing deterring pheromones. Leaf surface chemicals were also measured to see if correlates could be found between egg placement and oviposition site.

2.2 Materials and Methods

General Materials and methods

All "laboratory" experiments in this study were conducted in a constant temperature 25°C glasshouse under natural light conditions. The cages used had the dimension 23 cm x 66 cm x 38 cm.

Unless otherwise stated, *E. regnans* foliage used in experiments (with and without *C. bimaculata* egg batches present) was collected from the Florentine Valley (42°39'S, 146°28'E). This was stored in a dark coolstore at 4°C until required. Each branch or shoot base was submerged in water to avoid leaf wilt. In all cases foliage was used in experiments within 4 days of collection.

When required, leaf area measurements were made using one of two methods. To avoid removing leaves from shoots, 'non-destructive leaf area measurement' was employed. In this case leaves were photocopied while still attached to their branch and then the leaf area measured from the images using a ΔT^{TM} (Delta-T Devices) Area Meter. When the removal of leaves from shoots was unimportant ('destructive leaf area measurement'), leaves were removed from their petioles and leaf area was measured from the actual leaf using the leaf area meter. Both techniques were deemed equivalent as the non-destructive method measured area from same size leaf images while the destructive method measured area from real leaves.

Leaf toughness was measured for many of the experiments using a hand held penetrometer (see Appendix 1). Three readings were made from the proximal portion of the leaf (at least 3mm from the leaf edge or midrib) and then averaged.

Laboratory experiments used wild populations of ovipositing *Chrysophtharta bimaculata* beetles. These were collected from the Florentine Valley and used in

experiments within 4 days of collection. They were stored in transparent plastic bags on *E. regnans* foliage in a dark coolstore at 4°C. When required, beetles were sexed a day before each experiment by examining for sexual dimorphism (ie different shape) in their tarsal segments (see de Little 1979).

When leaves containing conspecific egg batches were required, eggs were examined to ensure that egg bursters were not visible (indicating eggs were close to hatching). Egg batches collected were rarely at this stage as they were collected in areas where beetles were present and ovipositing.

2.2.1 Egg batch distribution upon individual leaves of *E. regnans* field trees

From each of 16 *E. regnans* ranging between 1.8 and 3 m in height, fifteen leaves with leaf length greater than 70 mm and width greater than 30 mm were randomly collected carrying *C. bimaculata* egg batches, from the Florentine Valley. Smaller leaves were excluded as egg batches tended to represent a large proportion of the leaf surface.

Leaves were placed on ice, brought back to the laboratory and stored in a refrigerator at 4°C until examined. Leaves were then divided into two groups based on leaf size. Egg batch position on the leaf surface was then examined in relation to classified zones on each side of the leaf. Leaves were not classified regarding abaxial and adaxial surfaces as leaf structure is similar on both sides of a leaf. Moreover, leaves tend to hang vertical rather than having clearly defineable upper and lower surfaces. Leaves with area less than 2000 mm^2 (one side) were divided into two zones. These were:

Proximal: The area of leaf between the petiole and the midpoint of the leaf length.

Distal: The area of leaf area between the tip and the midpoint of the leaf length.

For those leaves with area greater than 2000 mm^2 (one side). egg batches were classified as being in one of six zones depending on where the majority of the eggs were positioned. Each leaf was divided into six zones: upper left, upper right, lower left, lower right, tip and centre. If eggs were divided approximately 50% across two zones then the egg batch was considered to be half in both zones and given a value of half an egg batch in the data analysis. The zones were defined as follows:

Proximal Left: The upper portion of the leaf containing the area from the base of the petiole to half the leaf length within 6 millimetres of the leaf edge on the left side of the leaf.

Proximal Right: As above except encompassing the right side of the leaf.

Tip: That portion of the leaf within 25 millimetres of the tip end.

Distal Left: The area within 6 millimetres of the leaf edge encompassing the remaining leaf area between the upper left and the tip section.

Distal Right: As above except encompassing the right side of the leaf.

Middle: The remaining leaf area in the centre of the leaf.

Occasionally more than one egg batch would occur on each leaf. For this study an egg batch was regarded as a distinct entity if the eggs contained within it were separated at a distance greater than 2 cm from the closest egg in another batch. A pair-wise students t-test was conducted on the two zones for leaves with area less than 2000 mm^2 and

included leaves that contained more than one egg batch on the same as well as opposite sides of the leaf. For those leaves with area greater than 2000mm^2 Oneway ANOVA's were conducted on (i) all leaves with one or more egg batches (ii) leaves with only one egg batch and (iii) egg batches per mean zone area for leaves with one or more egg batches.

2.2.2 Oviposition behaviour of *Chrysophtharta bimaculata*

Four hundred field collected beetles were placed in a flight cage (2 m x 2 m x 1.8 m) with four potted *E. regnans* between 1.5 metres and 1.8 metres in height. The number of beetles were required to ensure each *E. regnans* was occupied by several beetles at any given time. Beetles were examined over two, three-hour periods for two days in a sitting position. The behaviour of five female beetles prior to oviposition was examined along with the behaviour of ten beetles during oviposition. Observations of beetles prior to oviposition began if abdominal expansions and contractions were observed. Rhythmic expansion and contraction of the abdomen occurs either prior to or following the deposition of an egg batch (pers. obs.) Beetles were then examined closely and behavioural sequences verbally recorded using a cassette recorder (used both prior to and during oviposition). Beetle movements recorded included resting, walking over leaf surfaces (including locations and direction) and between leaves, palpal, antennal, and abdominal contact with the leaf surface and conspecific egg batches. If a beetle had not deposited within twenty minutes of observation then the recording of behaviour ceased. The act of oviposition was examined prior to or during the deposition of an egg batch. Behavioural observations recorded included tarsal contact with leaf surface, direction and location of oviposition in relation to the leaf surface, movement (abdominal, tarsal, antennal and palpal) during and between egg deposition. Behavioural observations conducted following oviposition included movement away from the egg batch and abdominal movement. Observation were also conducted to determine whether any obvious egg batch marking occurred (ie the appearance and use of Oviposition Deterring

Pheromone glands). Specific time measurements were recorded for the cycle of egg deposition, interval between proceeding egg deposition and maximum time taken to leave an egg batch following deposition.

2.2.3 A comparison of leaf waxes and essential oils from the leaf tip and leaf centre of expanding leaves

Three *E. regnans* trees were selected for leaf sampling in the Plenty Valley (42°50'S, 146°53'E). Twenty shoots from around each tree were selected containing new leaves (predominantly red or orange in colour) less than 25 mm in length. Thin pieces of plastic tape were tied below each developing leaf (for identification) and the trees left for a two week period. Fifteen of the leaves were then sampled from each tree (due to the loss of marked leaves on one tree) and the leaves brought back to the laboratory on ice. All leaves sampled were predominantly orange or red in colour with the exception of the leaf tip area (within 25 mm of the leaf tip) which was green. Leaves were stored in a cool-room at 4°C overnight before leaf surface wax and internal essential oils were sampled.

To extract the surface waxes from the leaf tips for each tree replicate, the tips (below the colour change) were submerged in chloroform. The surface waxes from the leaf centre were extracted by holding the leaf tip and the base of the leaf petiole with tweezers and submerging the centre of the leaf in chloroform. For both methods 7.5 mls of chloroform was used and each leaf section submerged for 10 seconds. The wax extracts and solvent from each sample were then transferred to 5 ml glass vials for storage and analysis.

To separate the wax constituents a Hewlett Packard 5890 Gas Chromatograph connected to a 5970B Mass Selective Detector with an open split interface was utilised. A HP-1 column of length 25m and internal diameter of 0.32 mm was employed with 0.17 μm film. The carrier gas was Helium at 15 psi with an approximate flow rate of 3 ml/min.

The samples were injected at 1 μl spitless at 290°C while the detector temperature was at 300°C. The column temperature started at 50°C for 1 minute then increased at 30°C per minute to 200°C, then at 10°C per minute to 320°C. This temperature was held for 5 minutes on conclusion of each run. The data, recorded at an HP Dos Chem Station, included the areas of the major wax constituents, however some overlap of peaks occurred. This required manual identification and peak area corrections using the spectral information collected by the mass spectrometer.

To extract the essential oils from the tip and centre zones of collected leaves, the leaf tips were removed at the colour change zone. The tips (for each tree) were then submerged in a vial containing 10 ml of ethanol. To sample the leaf centre, two 5 mm^2 squares were cut on either side of the leaf midrib. All leaves were sampled and submerged in one of three vials corresponding to each represented tree. The samples were left for thirty hours before aliquots were removed to 5ml glass vials. These were stored at 4°C prior to analysis.

To examine essential oil components, the same Chromatograph-Mass Selective Detector system was used as for the waxes, however, a 25 m long column of 0.32 mm internal diameter with 0.52 μm film was employed. The carrier gas was again Helium at the same flow rate and samples were injected at 1 μl spitless at 260°C. The column temperature began at 40°C for 1 minute then increased at 6°C per minute to 230°C. The column was then heated to 290°C for approximately 5 minutes to burn off all extract before the next run was commenced. A HP Dos Chem Station recorded the data which was then used to determine the proportion of major essential oil constituents for each sample.

Both wax and essential oil compounds were identified through their recorded retention times and through examination of the abundance and mass of their ions using mass spectrometry. In addition, an in house library at the Central Science Laboratory (Hobart, Tasmania) and previous research by Li (1993) were used to aid compound identification.

The percentage data was examined for normality and subsequently an arcsin transformation conducted. The proportion of each oil and wax component recorded between leaf tip and centre samples were then statistically compared using t-tests.

2.2.4 Factors which may influence *C. bimaculata* oviposition

A series of laboratory experiments were designed to test for factors which may influence *C. bimaculata* oviposition. These factors and the sections under which they are discussed are:

2.2.4a Leaf shape.

2.2.4b Leaf colour.

2.2.4c The presence of conspecific egg batches and feeding damage to *E. regnans* leaves.

2.2.4d The presence of conspecific egg batches on *E. regnans* leaves.

2.2.4e Whether an Oviposition Deterring Pheromone (ODP) may be present.

2.2.4f Whether a water soluble ODP is responsible for conspecific egg batch deterrence.

2.2.4g Whether a hexane soluble ODP is responsible for conspecific egg batch deterrence.

2.2.4h Whether a ethanol soluble ODP is responsible for conspecific egg batch deterrence.

2.2.4i Whether the positioning of egg batches can act as physical barriers to further oviposition.

The experimental outlines listing the fundamental question posed, test, hypothesis, experimental design, materials and insects used, measured outcomes, number of replicates, run-time of the experiments and data analysis are described in Table 2.2.

Table 2.2 Experimental outlines for experiments 2.2.4a-j describing the fundamental question, test, hypothesis, design, materials, Insects used, measured outcomes, replicate number, experiment run time and analysis used.

Fundamental Question	Expmt No.	Test	Hypothesis	Design	Materials	Insects	Measured outcome	No reps	Run time	Analysis
Does leaf shape influence oviposition choice?	2 2 4a	Compare oviposition on plastic mimics modelled on *E. regnans* leaves versus oval tipless leaves	Ho: Tapered leaves receive the same no of egg batches as oval shaped leaves H1 Tapered leaves receive more egg batches	Binary test using mimic leaves on a bare branch	Plastic mimic leaves (12 each) knotted to bare *E. regnans* branches	30 female *C bimaculata*	Number of egg batches on mimic leaf types.	10	24 hrs	Students t-test
Does leaf colour affect oviposition choice?	2 2 4b	Compare oviposition on blue, yellow and red plastic leaves (shape modelled on *E regnans* leaves)	Ho· All coloured leaves receive the same No. of egg batches H1. One or two sets of coloured leaves will receive more egg batches.	As above Branch with three shoots.	As above and see figure 2 1.	30 female *C bimaculata*	As above with regards to colour.	10	24 hrs	Analysis of Variance
Do egg batches on damaged shoots affect oviposition choice?	2 2.4c	Compare oviposition on feeding damaged shoots with egg batches versus untouched shoots free from egg batches	Ho *C bimaculata* damaged shoots with egg batches will receive the same No. of egg batches compared to untouched shoots H1. Untouched shoots will receive more egg batches.	Binary test using bottled shoots.	Real leaves *E regnans*) on 30 cm terminal shoots.	10 female *C bimaculata*	Number of egg batches on test branches	10	8 hrs	Students t-test
Do shoots with egg batches affect oviposition choice?	2.2.4d	Compare oviposition on feeding damaged shoots with conspecific eggs versus feeding damaged shoots.	Ho· *C bimaculata* damaged shoots with egg batches will receive the same No of egg batches compared to damaged shoots with no batches present. H1: Damaged shoots with no conspecific egg batches will receive more egg batches	Binary test using bottled shoots.	Real leaves *E. regnans*) on 30 cm terminal shoots.	10 female *C. bimaculata*	Number of egg batches on test branches	20	8 hrs	Students t-test
Is an oviposition deterring pheromone used by *C bimaculata*?	2.2 4e	Compare shoots with conspecific egg batches removed versus shoots with unremoved egg batches	Ho Shoots with conspecific egg batches removed will receive the same No of egg batches as those with unremoved egg batches. H1: Both shoots with a egg batches and removed egg batches will receive the same No. of egg batches	Binary test using bottled shoots	Real leaves *E regnans*) on 30 cm terminal shoots	10 female *C bimaculata*	Number of egg batches on test branches	8	8 hrs	Students t-test
Is there a water soluble ODP?	2.2.4f	To test for the presence of a water soluble ODP influencing oviposition.	Ho Leaves with water washed tips and egg batches will receive the same No. of egg batches as water washed tips. H1: Leaves with water washed tips will receive more egg batches	Binary test using bottled shoots.	Real leaves *E. regnans*) on 30 cm terminal shoots	10 female *C. bimaculata*	Number of egg batches on test branches	12	8 hrs	Students t-test
Is there a hexane soluble ODP?	2 2 4g	As above with respect to hexane solubility.	As above with respect to hexane washed tips and egg batches.	Binary test using bottled shoots	Real leaves *E. regnans*) on 30 cm terminal shoots	10 female *C bimaculata*	Number of egg batches on test branches	10	8 hrs	Students t-test
Is there an alcohol soluble ODP?	2 2 4h	As for 2 2 4g with respect to ethanol solubility.	As for 2.2 4g with respect to 100% ethanol washed tips and egg batches	Binary test using bottled shoots	Real leaves *E regnans*) on 30 cm terminal shoots	10 female *C bimaculata*	Number of egg batches on test branches	10	8 hrs	Students t-test
Can the position of an egg batch influence further oviposition?	2.2 4i	To test whether the physical presence of an egg batch on the leaf tip influences oviposition differently to one present away from the tip.	Ho Mimic leaves with an artificial egg batch on their tip do not receive more egg batches than those with egg batches away from the tip. H1· Those leaves with egg batches on their tip will receive less egg batches.	As for 2.2 4a Artificial egg batch placed both sides on: (1) leaf tip, or (2) ½ leaf length 5mm from edge	As for 2 2 4a. Artificial egg batches made from 2 mm thick cardboard and stapled to leaves	30 female *C. bimaculata*	Number of egg batches on mimic leaf types	15	24 hrs	Students t-test

Figure 2.1 shows the design of each replicate used in experiment 2.2.4b.

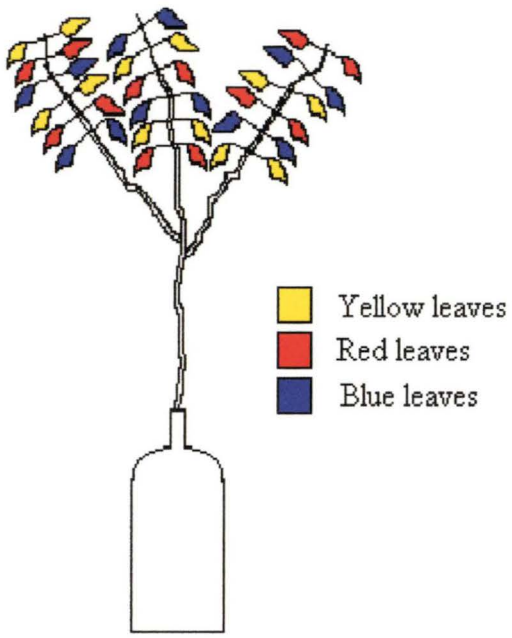


Figure 2.1 The arrangement of artificial leaves to test the role of leaf colour in *Chrysophtharta bimaculata* oviposition preference.

Experiments using artificial leaves

For experiments 2.2.4a, b and i, artificial plastic (polyvinyl-carbonate) leaves were constructed. A-type leaves modelled the shape of *E. regnans* leaves with a tapering tip present. B-type leaves were oval in shape lacking the tapered tip. Both leaf types had an area of 1350 mm^2 per side. Experiments 2.4b and 2.4i used A-type leaves while experiment 2.4a compared A-type with B-type leaves.

For experiment 2.2.4i artificial egg batches were constructed using 2 mm thick, light brown cardboard. The artificial egg batches were stapled on both sides of each leaf so that both were placed at the same location on each side. Two treatments were constructed based on the position the artificial egg batches were was stapled:

1. 5mm above the leaf tip and an equal distance from both left and right edges of the leaf.

2. Midway from the leaf tip and the start of the leaf petiole, 5mm from one of the leaf edges.

The locations chosen for stapling the mimic egg batches were specifically designed to test the influence of leaf tip blocking as opposed to the presence but non-blocking affect of mimic egg batch placement away from the leaf tip. For all experiments using artificial leaves, two leaves were constructed from one piece of plastic tape. These were separated by a strand of plastic 4 mm wide that was used both as a tie and 'petiole' for the two leaves. For experiment 2.4a, each tie consisted of one A-type and one B-type leaf; experiment 2.4b one leaf with an egg batch on the tip, the other away from the tip. This allowed for the different models to be equally distributed on the branches.

For all artificial leaf experiments, with exception of 2.2.4i, all replicates were conducted simultaneously. For 2.2.4i, eight were conducted initially, followed by a further seven five days later using newly collected beetles.

Experiments using real E. regnans leaves

Experiments 2.4c-h utilised real, current season *E. regnans* leaves with leaf area greater than 1200 mm^2/side. Leaves smaller than this were discarded due to the increased risk of abscission through beetle feeding. In all cases, two sets of shoots (treatments) were compared in binary choice tests. Leaves from one set were closely matched with those of the other based on size, toughness and position. For those experiments using leaves with egg batches, only those leaves containing batches between 10 and 20 eggs on one side within 20 mm of the leaf tip were utilised for consistency. Paired leaves were

marked with a fine tipped felt pen and excess foliage removed. The leaves from each shoot were then photocopied to estimate leaf area before the commencement of the experiment.

Binary choice experiments using real leaves were conducted by transferring the paired treatments to ten replicate cages. Each treatment was placed in diagonally opposing corners and alternated for each replicate. Only female beetles were used as experiments were concerned with data collected from egg deposition. Excluding males ensured minimal defoliation and thus reduced the risk of leaf abscission. Replicates for experiments 2.4c, e, g and h were conducted simultaneously. For experiment 2.4d, one set of ten replicates were initially conducted followed be a second set of ten seven days later. For experiment 2.4f, one set of eight replicates were initially conducted followed by a further set of four, five days later. Newly collected foliage and beetles were used for the second replicate sets for experiments 2.4 d and f..

For experiment 2.2.4c, leaves of one treatment had both *C. bimaculata* egg batches present, and more than five feeding scallops of diameter greater than 5 mm. The other treatment had leaves with no egg batches and no feeding damage evident. The replicates for experiments 2.2.4d-h used leaves from the same *E. regnans* trees for each treatment. For experiment 2.2.4d, only leaves containing more than 5 feeding scallops with diameter greater than 5 mm were utilised.

For experiment 2.2.4e, egg batches of one treatment where gently removed using a scalpel blade by breaking the cementing bonds connecting each egg to the leaf surface.

For experiments 2.2.4f-h, egg batches were washed with solvents to remove any ODP that may have been present. Experiment 2.2.4f used distilled water, 2.2.4h hexane and 2.2.4i 100% ethanol. In all experiments, leaf tips for both treatments (with and without egg batches) were submerged in their respective solvents and agitated. For experiment 2.2.4f, the leaf tip was agitated for one minute, then placed in a second bath for 30

seconds to remove any diluted ODP traces carried over from the first bath. For experiments 2.2.4g and h, leaves were agitated for 10 seconds then for 5 seconds in a second bath.

For all replicates and experiments, collected females from wild ovipositing populations were placed in each cage (Table 2.2). On conclusion of each set of replicates and experiments, leaves were removed and newly deposited egg batches and egg numbers counted. Leaf area was again measured directly from the leaves so that area loss through beetle feeding could be calculated.

For all the experiments, an additional *E. regnans* shoot containing 4-6 expanding leaves each with an area greater than 2000 mm^2/side was added to the middle of each cage. This was to allow beetles to feed during artificial leaf experiments and to minimise feeding damage in those experiments using real leaves. Egg batches deposited on these leaves were ignored.

2.3 Results

2.3.1 Egg batch distribution upon individual leaves of *E. regnans* field trees

Of the total leaves collected (n=240), 23.6% carried more than one egg batch and 7.3% had more than two egg batches. Of those leaves with two egg batches, 35.3% were on the same side. For those leaves collected with an area less than 2000 mm^2 (n=79), 79.7% of egg batches where found on the distal portion of the leaf, significantly more than in the proximal section [$t_{0.05\,(2),\,14} = 2.14$, $P(|t| \geq 12.36) < 0.001$].

Figure 2.2A shows the distribution of egg batches upon the leaf surface of all *E. regnans* leaves collected with area greater than 2000 mm^2 (n=161). The majority of egg batches (total n=216) were oviposited within the leaf tip zone (55.6%) while the leaf centre also received a high percentage of egg batches (22.0%) compared with the remaining zones. There were significant differences between zones ($F_{5,189} = 52.3$, $P < 0.001$) with the leaf tip receiving significantly more egg batches than all the other zones while the leaf centre had significantly more than the four other zones (Table 2.3)

Excluding those leaves with more than one egg batch, the leaf tip received 63.9% of egg batches (total n=122), the leaf centre 16.4%, lower left 6.1%, lower right 7.0%, upper right 2.5% and upper left 4.1% (see figure 2.2B). There were also significant zonal differences between egg batch numbers ($F_{5,189} = 88.20$, $P < 0.001$)(Table 2.3).

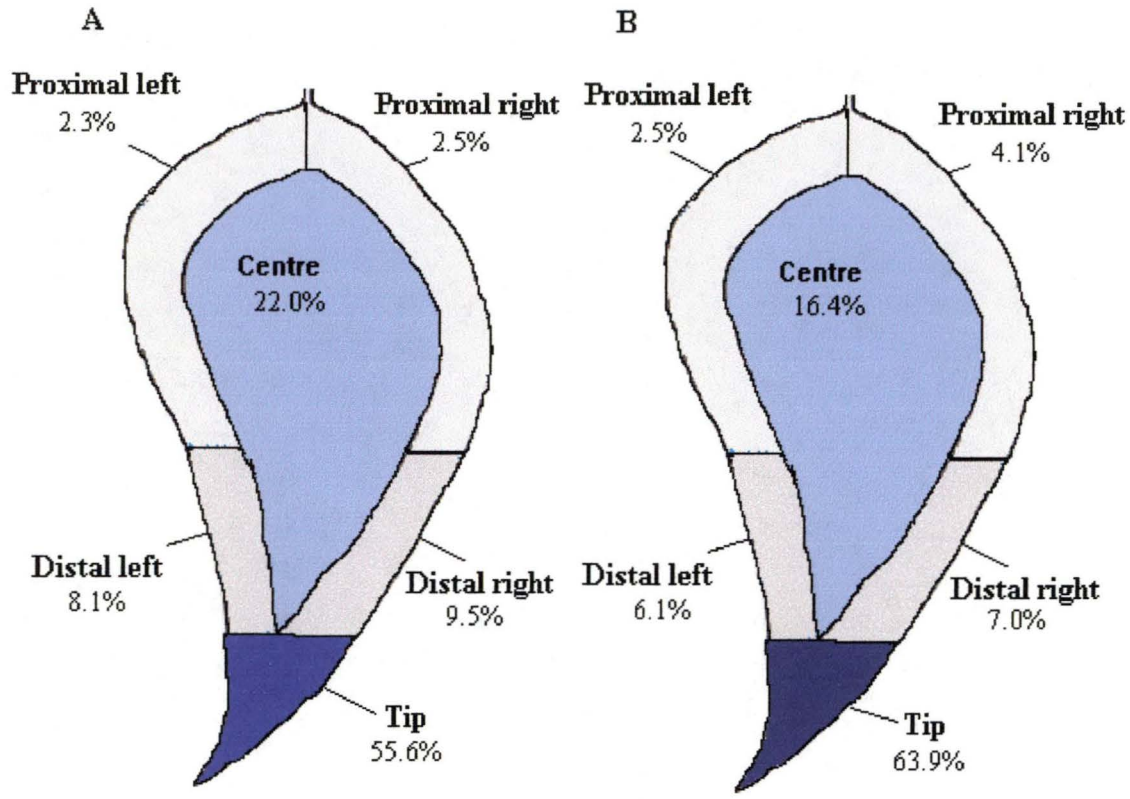


Figure 2.2 The percentage occurrence of egg batches in 6 *E. regnans* leaf zones (upper left, upper right, centre, lower left lower right and tip) when leaf area is greater than 2000 mm^2. **(A)** Field leaves bearing one or more egg batch and **(B)** Field leaves bearing only one egg batch.

Figure 2.3 takes into account the mean area of all leaf zones for all the leaves examined. The mean leaf area of leaves examined was 4483 ± 156 mm^2, with the centre zone having a mean area of 2282 ± 124 mm^2 representing more than half the leaf surface. When leaf area is taken into account the leaf tip receives significantly more egg batch per unit area (88.3%) compared to the other zones ($F_{5,210} = 82.01$, $P < 0.001$)(Table 2.3). Although the leaf centre received 22% of the egg batches, it only received 1.9% of egg batches per mm^2 of leaf surface.

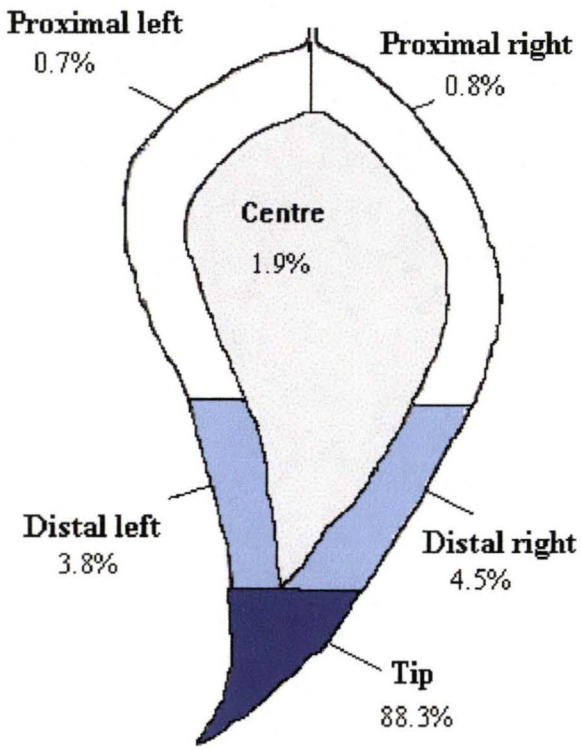


Figure 2.3 The percentage occurrence of egg batches per mm^2 of leaf surface area for 6 *E. regnans* leaf zones (upper left, upper right, centre, lower left lower right and tip) when leaf area is greater than 2000 mm^2.

Table 2.3 Mean *C. bimaculata* egg batch number per leaf zone for 16 leaves from each of 15 *E. regnans* trees (means in the same row followed by the same letter are not significantly different; Error values are ± S. E.).

	Leaf Zone					
Egg batch measurement	Tip	Centre	Lower left	Lower right	Upper left	Upper right
One or more egg batch present	8.0 ± 0.9a	3.2 ± 0.4b	1.2 ± 0.2c	1.4 ± 0.2c	0.3 ± 0.1c	0.4 ± 0.1c
One egg batch present	5.2 ± 0.4a	1.3 ± 0.2b	0.5 ± 0.1bc	0.6 ± 0.1bc	0.2 ± 0.1c	0.3 ± 0.1c
Egg batch/mm^2 of leaf blade	88.3 ± 9.4%a	1.9 ± 0.2%b	3.8 ± 0.7%b	4.5 ± 30.8%b	0.7 ± 0.3%b	0.8 ± 0.3%b

2.3.2 Oviposition behaviour of *Chrysophtharta bimaculata*

Behaviour leading up to oviposition.

Although the behaviours associated with oviposition site searching were not distinguished from other behaviours, rhythmic abdominal expansions and contractions (pulsations) were an indicator that oviposition was due to occur (abdominal pulsation was absent in other beetles). However, abdominal pulsations were also present in those beetles that had recently completed depositing an egg batch and only those that started ovipositing within 20 minutes of observation were included in the results. The frequency of the abdominal pulse increased as oviposition drew nearer (< 2 sec per pulse within one minute of oviposition as opposed to ≥ 2 sec at other times).

For those beetles that eventually deposited an egg batch, behaviour consisted of walking over several leaf surfaces (maximum number observed was fifteen) frequently to the tip of each one before settling. All beetles observed remained on the one branch and moved from leaf to leaf via shoots and petioles. Beetles moved onto leaves if they encountered the petiole while walking on a shoot. If they did not encounter the petiole (i.e walking on the opposite side of the shoot) they would walk past the leaf. After walking from a leaf they may return again without exploring other leaves (observed in 2 beetles, n=5), or return after the exploration of other leaves (observed in 4 beetles).

Movement to the leaf tip would not always occur and in all cases beetles were observed to walk off some leaves after examining less than half of one surface. Movement to the leaf tip varied with no obvious pattern. All beetles would regularly change direction while walking down or up from the leaf, from one edge to another and into the central parts of the leaf, or onto the opposite side of the leaf. All beetles would frequently change from walking behaviour (Figure 2.3 behavioural sequence 1. characterised by tarsal and leg movement, palpal extension for continual brushing over the leaf surface, circular antennal motion regularly brushing the leaf surface on the downward stroke and frequent abdominal contact through the expansion phase of the pulse) to pause behaviour

(2. beetle contact with the leaf surface through tarsi and abdomen (expansion phase of pulse), antennae held horizontal at an angle between each antenna of 90°-180° and may or may not be in contact with the leaf surface) (double headed white arrow, Figure 2.3). Both Pausal and walking time varied widely from <2 sec up to 10 mins approximately for pausal time and from <5 sec up to 5 mins approximately. There was no obvious pattern in the time spent within and between pausal and walking behaviours.

Four of the five beetles were observed manoeuvring their antennae to brush underneath the leaf from the leaf edge while walking. In no cases were beetles observed biting leaves. When a female came into contact with a conspecific egg batch her movements slowed and she often walked over it to the other side of the leaf, making contact with it through brushes involving the antennae and palps. Egg batches did not elicit any obvious behavioural deterrent.

Aspects on behaviour immediately proceeding and during the oviposition act

In all cases observed (n=5), one side of the leaf selected for oviposition was explored in an apparently random fashion, while four beetles were observed brushing their antennae on the opposite side of the leaf from near the leaf edge during their exploration (in all cases for periods < 3 secs). In 90% of actual ovipositions observed (n=10), beetles had contact with the edge of the leaf with at least one tarsi during oviposition and 70% had tarsi from both left and right sides in contact with the leaf edges. Seventy percent of the beetles also began ovipositing within 2 cm of the leaf tip. After exploring to the leaf tip, 90% (n=10) of beetles turned and faced away from the leaf tip when egg deposition began. There were no other discernible features of the leaf surface or regarding the beetle behaviour that appeared to influence oviposition site selection.

Figure 2.3 shows the behavioural steps for *C. bimaculata* during oviposition. Following a pause (2.) the first behaviour involved the lifting of the hind legs and abdomen as the egg protrudes vertically (3). The period taken for an entire egg to appear was

approximately 2 seconds (n=20). Beetles then moved their abdomen sideways until the egg was lying horizontal to the leaf surface (4.). The cycle of egg deposition (from when the egg first appeared until it was fully deposited) averaged 13.1 ± 0.2 secs, (n=20). Once an egg is deposited beetles would generally move one or two millimetres up the leaf (5.). Beetles would then manoeuvre their abdomens to make contact with the previously deposited egg, which is used as a positional guide for the next egg. The abdomen is then guided along the edge of the egg to its corner (6.) where the following egg is deposited (3.). Beetle only use their abdomen to locate the previous deposited egg. During the act of oviposition the antennae and palpae remained still except when tarsal and leg movement occurs (5.).

Most eggs (85%) were deposited within 3 minutes of one another although beetles may take much longer. The maximum time noted from one egg deposition to the next, in the same egg batch was 12:44 minutes, while the minimum was 1:21 minutes. Occasionally a beetle may turn around or move away from its egg batch for up to a centimetre (maybe more) and then return to its original position to deposit more eggs. This can result in longer periods between egg deposition. Excluding the three occasions when egg batch deposition took longer than 5 minutes, the mean period taken to deposit one egg to the next was 2:11 ± 0:06 minutes (n=20). Thus beetles may spend more than one hour depositing an egg batch in excess of 30 eggs.

Following oviposition, beetles may pause near the oviposited egg batch or immediately walk away. The maximum period taken for a beetle to move away from the deposited egg batch was 36:16 minutes. In all cases, beetle abdomens continued to pulse after the egg batch was abandoned. The period of abdominal pulsing following oviposition was not examined. There was no apparent behaviour indicating that an ODP is deposited although ODP glands could have been in the abdominal sternites.

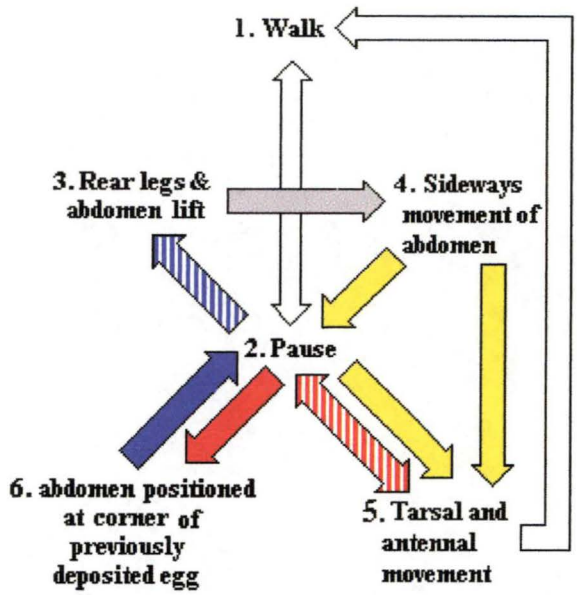


Figure 2.3 Diagram showing the steps between oviposition behaviour proceeding, during and immediately following oviposition. During the entire sequence the abdomen expands and contracts rhythmically (approximate cycle of 2 secs immediately proceeding and during oviposition). Walking consist of tarsal and leg movements, the continuous brushing of the leaf surface with extended palpae and circular antennal movement with frequent brushing of the leaf surface. White arrows are behaviours proceeding and following oviposition; the blue arrows, the production of an egg; the grey arrow, placement of egg on the leaf surface, the yellow arrows, movement up the leaf to facilitate the deposition of another egg; the red arrows, abdomen positioning for the deposition of another egg, the blue striped arrow is a behaviour that may occur during or proceeding the deposition of an egg batch while the red stripped is a behaviour that may occur during or following the deposition of an egg batch.

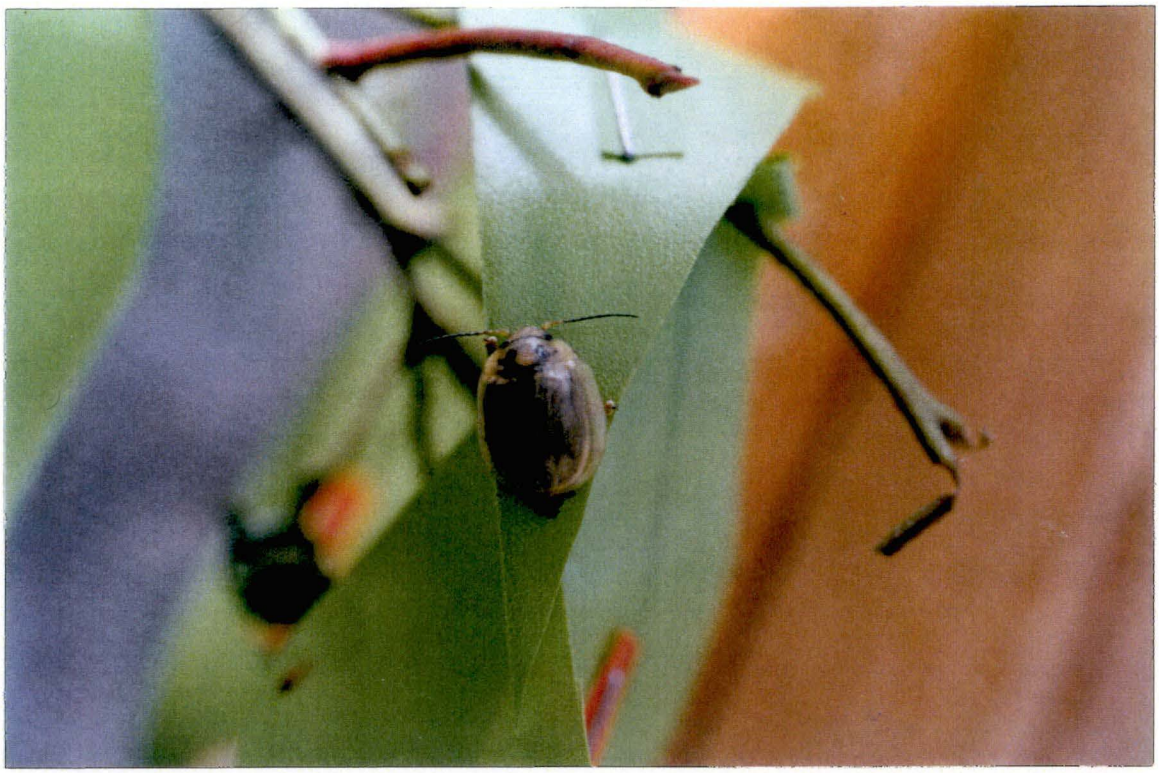


Figure 2.4 Ovipositing *Chrysophtharta bimaculata* beetle gripping both left and right edges of an artificial plastic leaf.

Figure 2.5 Ovipositing *Chrysophtharta bimaculata* beetle with abdomen extended about to deposit an egg.

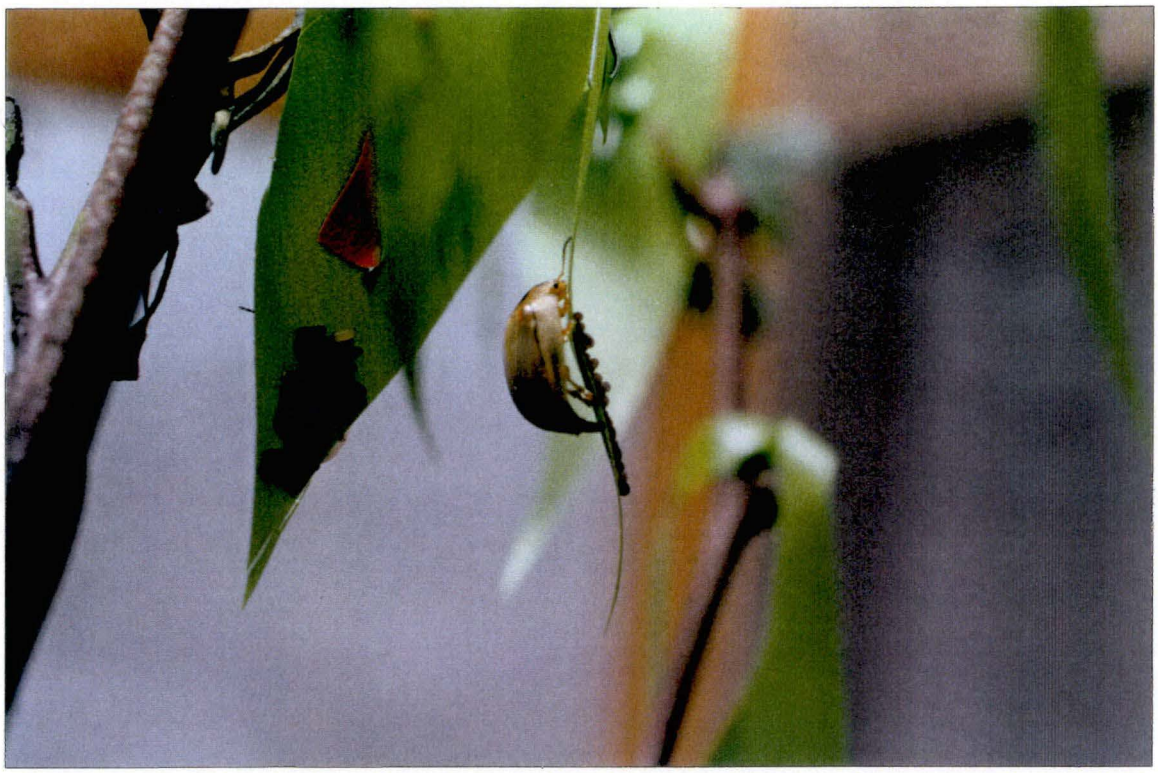


Figure 2.6 *Chrysophtharta bimaculata* beetle producing an egg batch. Beetles raises legs as the egg protrudes vertically from the abdomen.

Figure 2.7 *Chrysophtharta bimaculata* beetle moving her abdomen sideways so that the egg is deposited horizontally and adjacent to the previously deposited egg.

Figure 2.8 *Chrysophtharta bimaculata* beetles use their abdomens to locate the corner of the previously deposited egg prior to depositing the next egg.

2.3.3 A comparison of leaf waxes and essential oils from the leaf tip and leaf centre of expanding leaves

Table 2.4 compares 36 individual wax components recorded from the leaf centre and leaf tip. Three components [n-hexacosanal, benzyl hexacosanoate and an unidentified terpene (terpene 4)], were significantly different between the leaf tip and leaf centre samples. Benzyl hexacosanoate represented 4.20 ± 0.26% of the total wax for the leaf centre samples while representing 3.50 ± 0.12% of the leaf tip samples. Triterpene 4 also represented a relatively high proportion of the leaf wax obtained, being 2.70 ± 0.25% of total leaf centre wax compared to 3.40 ± 0.20 for the leaf tip. In contrast, n-hexacosanal made up less than 1% of the total leaf wax for both leaf tip and leaf centre samples.

Forty-two different compounds were recorded constituting the essential oil of *E. regnans* (Table 2.5). Three unidentified [55/70/41/126/97; 59/43/55/(205); 83/125/140/139/279] compounds plus bicycloelemene were found to vary significantly between the leaf tip and leaf centre samples. The three unidentified compounds represented a higher proportion and bicycloelemene a lower proportion of the leaf tip oils. However, in all cases proportions of these compounds comprised less than 1% of the total essential oil composition.

Table 2.4 Major wax components from centre and tip leaf samples of *E. regnans* expanding adult leaves. Values supplied are components mean (± SE) % of total wax composition (n=6, d.f.=4, t-crit=2.13). Triterpenes were not identified but were given a number

Wax Component	Centre	Tip	t-stat	Signif.
n-pentacosane	0.63 ± 0.07	0.50 ± 0.06	1.51	NS
n-tetracosanol	0.43 ± 0.26	0.77 ± 0.27	0.88	NS
n-heptacosane	1.97 ± 0.37	1.67 ± 0.43	0.53	NS
n-hexacosanal	0.50 ± 0.12	0.83 ± 0.07	2.50	<0.05
Phenyl ethyl octadecanoate	0.10 ± 0.00	0.10 ± 0.00	0.00	NS
n-hexacosanol	3.50 ± 0.65	4.73 ± 0.99	1.04	NS
n-nonacosane	1.07 ± 0.23	1.07 ± 0.09	0.00	NS
Desmethyl eucalyptin	8.67 ± 0.64	8.23 ± 1.21	0.32	NS
Eucalyptin	7.47 ± 1.26	7.73 ± 0.90	0.17	NS
n-octacosanal	0.90 ± 0.15	0.90 ± 0.15	0.00	NS
Phenyl ethyl eicosanoate	0.93 ± 0.28	1.17 ± 0.20	0.67	NS
n-alkanol	0.80 ± 0.60	0.77 ± 0.62	0.04	NS
triterpene (6)	1.53 ± 0.09	1.50 ± 0.21	0.15	NS
triterpene (1)	2.57 ± 0.60	1.87 ± 0.92	0.64	NS
triterpene (2)	4.40 ± 0.25	4.70 ± 1.17	0.25	NS
Phenyl ethyl docosanoate	1.10 ± 0.40	1.53 ± 0.15	1.01	NS
triterpene (3)	0.80 ± 0.25	0.47 ± 0.19	1.07	NS
triterpene (4)	2.70 ± 0.25	3.40 ± 0.20	2.18	<0.05
triterpene (5)	1.83 ± 0.29	2.33 ± 0.58	0.77	NS
Amyrin	0.67 ± 0.35	0.73 ± 0.37	0.13	NS
Benzyl tetracosanoate	1.33 ± 0.29	1.33 ± 0.22	0.00	NS
Methyl moronate	0.40 ± 0.26	0.77 ± 0.07	1.34	NS
Phenyl ethyl tetracosanoate	2.17 ± 0.74	3.13 ± 0.61	1.01	NS
triterpene (8)	7.90 ± 4.90	5.80 ± 2.32	0.39	NS
triterpene (7)	22.4 ± 4.22	17.2 ± 1.03	1.19	NS
n-triancontan-16,18-dione	3.17 ± 0.41	3.73 ± 0.73	0.68	NS
Phenyl ethyl pantacosanoate	0.17 ± 0.03	0.30 ± 0.10	1.26	NS
Benzyl hexacosanoate	4.20 ± 0.26	3.50 ± 0.12	2.42	<0.05
Hexasanoyl benzoate	2.00 ± 0.49	3.87 ± 1.23	1.41	NS
Benzyl heptacosanoate	0.10 ± 0.00	0.00 ± 0.00	0.00	NS
Phenyl ethyl hexacosanoate	3.33 ± 0.77	3.33 ± 0.77	0.00	NS
Benzyl octacosanoate	0.83 ± 0.34	0.53 ± 0.09	0.86	NS
Phenyl ethyl heptacosanoate	0.10 ± 0.00	0.10 ± 0.00	0.00	NS
Phenyl ethyl octocosanoate	0.77 ± 0.47	0.43 ± 0.12	0.69	NS

Table 2.5 Major essential oil components from centre and tip leaf samples of *E. regnans* expanding adult leaves. Values supplied are components mean (± SE) % of total essential oil composition (n=6, d.f.=4, t-crit=2.13). Unidentified compounds listed from the most abundant ions received from mass spectra, except where bracketed indicating more distinctive ions. TT = tasmanone type.

Essential Oil	Centre	Tip	t-stat	Signif.
Isobutyl isobutanoate	0.17 ± 0.17	0.30 ± 0.30	0.39	NS
Alpha thujene	0.47 ± 0.07	0.50 ± 0.06	0.38	NS
Alpha pinene	0.23 ± 0.12	0.23 ± 0.12	0.00	NS
Myrcene	0.10 ± 0.10	0.00 ± 0.00	1.00	NS
Alpha phellandrene	2.90 ± 0.45	2.20 ± 0.26	1.34	NS
alpha terpinene	0.30 ± 0.15	0.63 ± 0.07	2.00	NS
p-cymene	0.00 ± 0.00	0.00 ± 0.00	0.00	NS
Beta phellandrene	1.00 ± 0.38	0.87 ± 0.32	0.27	NS
Terpinolene	0.10 ± 0.10	0.00 ± 0.00	1.00	NS
Linalool	0.13 ± 0.13	0.13 ± 0.13	0.00	NS
Trans p-menth-2-en-1-ol	0.00 ± 0.00	0.23 ± 0.12	1.94	NS
Cis p-menth-2-en-1-ol	0.50 ± 0.10	0.70 ± 0.15	1.10	NS
Terpinene-4-ol	0.27 ± 0.15	0.50 ± 0.10	1.32	NS
Trans piperitol	7.47 ± 1.52	8.00 ± 1.56	0.25	NS
Piperitone	0.23 ± 0.23	0.67 ± 0.52	0.76	NS
55/70/41/126/97	0.23 ± 0.12	0.77 ± 0.20	2.26	<0.05
Bicycloelemene	0.43 ± 0.09	0.10 ± 0.10	2.50	<0.05
Alpha copaine	0.23 ± 0.12	0.23 ± 0.12	0.00	NS
Beta elemene	0.50 ± 0.06	0.47 ± 0.03	0.50	NS
Caryophylliene	0.33 ± 0.03	0.33 ± 0.03	0.00	NS
Humulene	0.20 ± 0.20	0.10 ± 0.10	0.45	NS
Alloaromadendrene	0.23 ± 0.12	0.20 ± 0.10	0.21	NS
Germacrene	0.10 ± 0.10	0.00 ± 0.00	1.00	NS
Bicyclogermacrene	6.50 ± 1.42	4.10 ± 0.57	1.57	NS
Elemol like compound	1.00 ± 0.06	0.83 ± 0.09	1.58	NS
Hedycaryol	42.73 ± 3.20	39.17 ± 3.26	0.79	NS
Gamma eudesmol	0.55 ± 0.20	0.47 ± 0.12	0.35	NS
Beta eudesmol	0.93 ± 0.49	1.53 ± 0.43	0.92	NS
Alpha eudesmol	2.47 ± 1.12	3.63 ± 1.17	0.72	NS
43/170/139/155/200	1.90 ± 1.11	1.47 ± 0.41	0.37	NS
221/41/(189)/(139)/(236) (TT)	2.10 ± 0.73	4.73 ± 2.21	1.13	NS
237/43/209/81/(252) (TT)	10.7 ± 4.08	11.4 ± 5.03	0.11	NS
83/125/171/139/(254) (TT)	3.00 ± 2.50	0.90 ± 0.26	0.84	NS
170/155/41/81	0.43 ± 0.26	0.80 ± 0.31	0.91	NS
251/266 (TT)	1.13 ± 0.15	1.17 ± 0.19	0.14	NS
241/43/(59)/(149)/(266) (TT)	0.83 ± 0.23	1.53 ± 0.32	1.77	NS
41/43/59/81/(209)	0.50 ± 0.10	0.97 ± 0.23	1.84	NS
59/43/55/(205)	0.60 ± 0.06	0.77 ± 0.03	2.50	<0.05
44/59/105/107/119	0.37 ± 0.07	0.33 ± 0.03	0.45	NS
83/125/140/139/279	0.10 ± 0.10	0.60 ± 0.20	2.24	<0.05
195/238 (TT)	1.13 ± 0.49	1.07 ± 0.35	0.11	NS
167/43/(70)/(55)/(210)	0.37 ± 0.19	0.87 ± 0.49	0.95	NS

2.3.4 Factors which may influence *C. bimaculata* oviposition

A brief summary of the results achieved for experiments 2.2.4a-j are provided in Table 2.5. More detailed information is provided below.

2.2.4 a The influence of different shaped artificial leaves on oviposition preference

Artificial leaves based on the shape of *E. regnans* (tapered shape) received an average of 6.8 ± 0.5 egg batches per replicate compared to 2.1 ± 0.7 egg batches for oval (tipless) artificial leaves. The difference between the means was significant [$t_{0\ 05\ (2),\ 9} = 2.26$, $P(|t| \geq 8.73) < 0.001$].

The mean distance of egg batches from the leaf tip (all measurements taking the nearest egg in the batch) was 11.1 ± 1.3 mm, significantly closer than the to the petiole base (51.2 ± 1.3 mm) [$t_{0.05\ (2),\ 134} = 1.98$, $P(|t| \geq 21.87) < 0.01$]. For the artificial oval leaves without tips, egg batches were placed significantly closer to the leaf tip (mean 13.3 ± 2.2 mm) compared to the petiole base (mean 40.3 ± 2.1 mm) [$t_{0.05\ (2),\ 44} = 2.01$, $P(|t| \geq 9.24) < 0.01$] demonstrating a preference to oviposit on the distal portion of the leaf disregarding leaf chemistry and whether or not a tip is present.

2.2.4 b The role of leaf colour in C. bimaculata oviposition choice

No significant difference was recorded for *C. bimaculata* egg batch deposition between replicates ($F_{9,18} = 0.52$, $P = 0.840$) or the three leaf colour treatments ($F_{2,18} = 0.57$, $P = 0.574$). Red leaves received 3.1 ± 0.6 egg batches on average per replicate compared to 2.7 ± 0.5 for yellow and 2.2 ± 0.6 for blue.

Figure 2.9 A *Chrysophtharta bimaculata* egg batch that has been deposited on the leaf tip of an artificial plastic leaf.

Table 2.5 A summary of experimental results for experiments 2.2.4a-j examining factors influencing *C. bimaculata* oviposition on *E. regnans* leaves.

Fundamental Question	Expmt. No.	Test	Summary Result
Does leaf shape influence oviposition choice?	2.2.4a	Compare oviposition on plastic mimics modelled on *E. regnans* leaves versus oval tipless leaves.	Mimic leaves of an *E. regnans* shape received significar more egg batches than oval leaves lacking tips.
Does leaf colour affect oviposition choice?	2.2.4b	Compare oviposition on blue, yellow and red plastic leaves.	There were no significant differences between the three colour treatments.
Do egg batches on damaged shoots affect oviposition choice?	2.2.4c	Compare oviposition on feeding damaged shoots with egg batches with untouched shoots free from egg batches.	Significantly more egg batches where deposited on untouched shoots compared to those with feeding damage and conspecific egg batches.
Do shoots with egg batches affect oviposition choice?	2.2.4d	Compare oviposition on shoots with conspecific eggs versus shoots without.	Significantly more egg batches where deposited on shoots without conspecific egg batches compared to those with conspecific egg batches
Is an oviposition deterring pheromone used by *C. bimaculata*?	2.2.4e	Compare shoots with conspecific egg batches removed versus shoots with unremoved egg batches.	Shoots with removed egg batches received significantly more egg batches than those with conspecific egg batches.
Is there a water soluble ODP?	2.2.4f	To test for the presence of a water soluble ODP influencing oviposition.	Leaves with water washed tips and conspecific egg batches received significantly less egg batches than leaves with water washed tips only.
Is there a hexane soluble ODP?	2.2.4g	To test for the presence of a hexane soluble ODP influencing oviposition.	Leaves with hexane washed tips and conspecific egg batches received significantly less egg batches than leaves with hexane washed tips only.
Is there an alcohol soluble ODP?	2.2.4h	To test for the presence of a ethanol soluble ODP influencing oviposition.	Leaves with ethanol washed tips and conspecific egg batches received significantly less egg batches than leaves with ethanol washed tips only.
Can the position of an egg batch influence further oviposition?	2.2.4i	To test whether the physical presence of an egg batch on the leaf tip influences oviposition differently to one present away from the tip.	Leaves with artificial egg batches on the leaf tip received significantly less egg batches than those with the egg batch positioned half way down leaf length 5mm from edge.

2.2.4 c The influence of conspecific beetle feeding damage and egg batches on C bimaculata oviposition choice

Undamaged leaves lacking conspecific egg batches received significantly more egg batches compared to leaves with conspecific egg batches present and feeding damage evident, although low numbers of egg batches were deposited in both treatments (Table 2.6). There was no significant difference in the leaf area variables measured (initial, available, final leaf areas and leaf toughness) between the two treatments suggesting that these were not influential on oviposition preference.

Table 2.6 Mean (± S. E.) number of *C. bimaculata* egg laid on, and leaf characteristics, of two *E. regnans* leaf treatments (n = 10, d.f. = 9, t-crit = 2.26).

	E. regnans leaf treatment			
	Eggs and Damage	No eggs or damage	t-stat.	P-value
Egg batch no.	0.4 ± 0.2	1.3 ± 0.3	3.25	0.009
Initial leaf area (cm^2)	429.6 ± 46.0	413.1 ± 43.0	1.56	0.154
Available leaf area (cm^2)	420.0 ± 45.9	413.1 ± 43.0	0.65	0.529
Final leaf area (cm^2)	323.4 ± 33.8	314.7 ± 32.1	1.20	0.262
Leaf toughness (g)	33.5 ± 0.5	33.1 ± 0.3	1.54	0.157

2.2.4 d A comparison of leaves damaged through C. bimaculata feeding, with and without conspecific egg batches

C. bimaculata prefers to deposit egg batches on leaves without conspecific egg batches, even when the foliage in both treatments had suffered conspecific feeding damage (Table 2.7). Other measured leaf variables were not significantly different between the two leaf treatments.

Table 2.7 Mean (± S. E.) number of *C. bimaculata* egg batches laid on, and leaf characteristics, of two *E. regnans* leaf treatments (n = 20, d.f. = 19, t-crit = 2.09).

	E. regnans leaf treatment			
	Eggs	No eggs	t-stat.	P-value
Egg batch No.	0.9 ± 0.2	2.3 ± 0.4	3.51	0.002
Initial leaf area (cm^2)	264.4 ± 33.6	263.0 ± 21.8	0.09	0.932
Available leaf area (cm^2)	254.5 ± 33.1	263.0 ± 43.0	0.54	0.598
Final leaf area (cm^2)	205.4 ± 27.7	207.5 ± 19.9	0.19	0.855
Leaf toughness (g)	33.2 ± 0.8	33.2 ± 0.8	0.19	0.850

2.2.4 e Does C. bimaculata use an oviposition pheromone to deter other females from depositing near conspecific egg batches?

There was no significant difference in *C. bimaculata* oviposition preference between leaves which had had egg batches removed (1.6 ± 0.4 egg batches per replicate) compared to those which had never contained egg batches (2.4 ± 0.3 egg batches per replicate)(Table 2.8). Thus removal of a conspecific egg batch results in the leaf becoming as attractive to oviposition as a leaf which had not been previously oviposited upon. Other measured leaf variables were not significantly different between the two leaf treatments.

Table 2.8 Mean (± S. E.) number of *C. bimaculata* egg laid on, and leaf characteristics, of two *E. regnans* leaf treatments (n = 8, d.f. = 7, t-crit = 2.36).

	E. regnans leaf treatment			
	Eggs Removed	No eggs	t-stat.	P-value
Egg batch No.	1.6 ± 0.4	2.4 ± 0.3	2.34	0.170
Initial leaf area (cm^2)	295.7 ± 20.6	293.9 ± 15.1	0.27	0.797
Final leaf area (cm^2)	247.2 ± 19.7	244.9 ± 13.3	0.30	0.777
Leaf toughness (g)	32.1 ± 0.3	32.1 ± 0.2	0.09	0.929

2.2.4 f,g and h Is an ODP that is soluble (g) in water, (h) hexane or (i) ethanol responsible for conspecific egg batch deterrence?

For all three solvent wash experiments there were significant oviposition preferences for the leaves without egg batches. The average number of egg batches received per replicate on leaves with a conspecific egg batch present were: water washed 1.3 ± 0.2; ethanol washed 0.9 ± 0.2 and hexane washed 0.5 ± 0.2; while the average per replicate for leaves without a conspecific egg batch present were: water washed 2.2 ± 0.4; ethanol washed 1.9 ± 0.4 and hexane washed 1.7 ± 0.3 (Table 2.9). Of the leaf variables measured for each experiment there were no significant differences between the two treatments in all three experiments (Table 2.9).

2.2.4 i The influence of egg batch position on C. bimaculata oviposition.

A comparison of the two artificial leaf and artificial egg batch treatments revealed that 'leaves' with egg batch placement on the tip receive significantly less *C. bimaculata* egg batches compared to leaves where the batch is placed an equal distance between the petiole base and leaf tip, 5mm from the leaf edge [$t_{0.05\ (2),\ 14} = 2.14$, $P(|t| \geq 5.55) < 0.001$]. The leaves with egg batches on their leaf tip received 0.8 ± 0.7 egg batches per replicate compared to 2.1 ± 0.8 for non-tip egg batch treatment.

Table 2.9 Mean (± S. E.) number of *C. bimaculata* egg batches laid on, and leaf characteristics of two *E. regnans* leaf treatments following one of three egg and leaf tip washes (either water, hexane or 100% ethanol).

			***E. regnans* leaf treatment**					
Experiment	Wash	Variable	Eggs	No eggs	d.f.	t-crit.	t-stat.	P-value
2.2.4f	water	Egg batch no.	1.3 ± 0.2	2.2 ± 0.4	11	2.20	2.73	0.020
2.2.4f	water	Initial lf. area (cm^2)	297.0 ± 16.8	295.6 ± 17.1	11	2.20	0.25	0.810
2.2.4f	water	Avail. lf. area (cm^2)	289.9 ± 16.3	395.6 ± 17.1	11	2.20	0.98	0.347
2.2.4f	water	Final lf. area (cm^2)	236.2 ± 14.7	233.2 ± 15.1	11	2.20	0.54	0.603
2.2.4f	water	Lf. toughness (g)	32.1 ± 0.3	32.3 ± 0.5	11	2.20	0.25	0.806
2.2.4g	hexane	Egg batch no.	0.5 ± 0.2	1.7 ± 0.3	9	2.26	3.67	0.003
2.2.4g	hexane	Initial lf. area (cm^2)	304.3 ± 21.4	304.1 ± 23.8	9	2.26	0.03	0.980
2.2.4g	hexane	Avail. lf. area (cm^2)	297.1 ± 21.3	304.1 ± 23.8	9	2.26	0.98	0.351
2.2.4g	hexane	Final lf. area (cm^2)	241.1 ± 15.3	237.8 ± 17.3	9	2.26	0.46	0.660
2.2.4g	hexane	Lf. toughness (g)	33.2 ± 0.5	33.4 ± 0.5	9	2.26	0.24	0.818
2.2.4h	ethanol	Egg batch no.	0.9 ± 0.2	1.9 ± 0.4	9	2.26	3.53	0.020
2.2.4h	ethanol	Initial lf. area (cm^2)	347.8 ± 19.6	341.4 ± 17.1	9	2.26	1.83	0.320
2.2.4h	ethanol	Avail. lf. area (cm^2)	340.8 ± 19.2	341.4 ± 17.1	9	2.26	0.09	0.924
2.2.4h	ethanol	Final lf. area (cm^2)	290.1 ± 19.2	285.2 ± 16.2	9	2.26	0.94	0.370
2.2.4h	ethanol	Lf. toughness (g)	31.5 ± 0.3	32.3 ± 0.5	9	2.26	0.03	0.975

2.4 Discussion

Like some other paropsine species, such as *C. agricola* (Ramsden & Elek 1998) and *P. charybdis* (Murphy 1998), *C. bimaculata* has a preference for ovipositing on leaf tips. The oviposition preference for expanding leaves (Steinbauer et al. 1998a) and leaf tips by *C. bimaculata*, results in the common placement of egg batches on or near a distinctive colour change within the leaf (green within 2 cm of the leaf tip versus red, orange or pale green through the centre of the leaf) (Beckmann 1991). However, results of this chapter suggest that placement is likely due to mechanical aspects of oviposition, rather than chemical or visual properties of individual leaves.

Observations of behaviour leading up to oviposition suggest that the insect may receive information on surface chemistry through possible receptors sites on the antennae, palpae or abdomen (these body parts regularly make contact with the leaf surface prior to oviposition). However, the potential importance of internal leaf chemistry is less likely to influence oviposition behaviour as there was no evidence that beetles test leaves by biting prior to oviposition.

Examination of essential oils and waxes collected from leaf material at the tip and in the centre of *E. regnans* leaves did show that the proportions of some compounds (4 essential oil and 3 surface wax) varied significantly. However, the four essential oil compounds each represented less than 1% of the total oil composition. In contrast, two wax components which varied significantly between the leaf tip and centre samples, benzyl tetracosanoate and an unidentified triterpene (triterpene 4), represented more sizeable proportions (3.5-4.2%, 2.7-3.4% respectively) of the total wax components. However, leaf chemistry or the colour change itself cannot explain *C. bimaculata* preference for depositing egg batches on the leaf tip since there was also a strong preference to deposit egg batches near the leaf tip of plastic leaves (made of uniform material) the same shape as *E. regnans* leaves.

Apart from being an important site for oviposition, the physical presence of a tip increases the chance of oviposition compared to no tip 'leaves', independent of other factors such as leaf chemistry (Section 2.3.4). One hypothesis for leaf tip preference is that beetles receive increased stability during oviposition compared to other locations. All but one beetle (n=10) had tarsal contact with at least one edge of the leaf during oviposition and all but three beetles gripped both edges. Holding the leaf edge may provide the beetle with more stability to deposit eggs against abiotic conditions such as wind and rain, particularly if egg batch deposition takes longer than 1 hour. Follow up experiments using treatments with and without air movement may be worth pursuing to test this hypothesis. However, a preference for the leaf tip based on increased stability does not explain the preference for depositing egg batches in the distal region of artificial tipless leaves (section 2.3.4) since both the distal and proximal regions have a similar amount of leaf edge.

Unlike leaf shape (most notably the presence of a leaf tip), leaf colour (at least as far as the colours tested) did not influence *C. bimaculata* oviposition preference. Leaf colour is known to influence the oviposition of other insects (see Table 2.1) while *C. bimaculata* is known to be more attracted to yellow and orange sticky traps as opposed to red (Leon 1988; Madden 1992). Thus leaf colour could possibly act as a visual attractant while beetles are airborne.

Although factors associated with the plant such as leaf aging (see Steinbauer 1998a), and leaf shape have been demonstrated to influence *C. bimaculata* oviposition preference within host plants, factors associated with conspecific insects may also influence egg batch distribution. Conspecific egg batches (independent of conspecific leaf feeding damage) were found to significantly decrease further egg batch deposition on the same leaf for *C. bimaculata*. This contrasts with another paropsine species *Paropsis atomaria* where females tend to deposit egg batches near to other conspecific egg batches. (Carne 1966a).

There was no evidence that the deterrent effect of conspecific egg batches used in the experiments was due to an oviposition deterring pheromone. The reasons being:

- There was no significant difference between leaves that had conspecific egg batches removed from their surface and those that did not have egg batches present. This indicates that if an ODP was present on the leaf surface, or with any remaining residue used to connect eggs to the leaf surface, then it would be active for only a short period of time (ie less than the time taken for eggs to hatch). Otherwise *C. bimaculata* does not appear to produce an ODP. This result also dismisses the possibility of oviposition deterrence associated with changes in plant chemistry which may have occurred through the presence of eggs.

- Leaves carrying eggs washed in water, ethanol and hexane still received significantly fewer eggs compared to similarly treated leaves without egg batches. If an oviposition deterring pheromone was present then it would have to be insoluble in the solvents used. Although ODP's of some insects such as *Rhagoletis indifferens* (Curan) (Mumtaz & Aliniazee 1983) can be extremely stable, most studies on ODP's, have demonstrated that they are extractable in either water or alcohol.

- Observations made on insects which deposit an ODP often reveal a specific behavioural sequence that is associated with its deposition. This usually involves touching or brushing the oviposition site with the ovipositor or structures on the abdomen (see (Prokopy 1972; Kozlowski et al. 1983; McNeil & Quiring 1983; Straw 1989). Although *C. bimaculata* touches the leaf surface with its abdomen in a pulsating manner prior to and following oviposition, there was no distinctive behavioural sequence suggesting an ODP was being deposited. Moreover, *C. bimaculata* females occasionally deposit eggs adjacent to other conspecific egg batches.

Although leaves with conspecific egg batches significantly reduce further *C. bimaculata* oviposition, deterrence due to beetle perception of conspecific eggs may not be responsible. Rather, conspecific egg batches may simply block the most preferred site from further oviposition. Beetles preferred to oviposit egg batches on artificial leaves where the mimic egg batch was placed in the middle of the leaf, close to the leaf edge, rather than on leaves where the mimic egg batch was placed on the leaf tip. The leaf tip surface being the favoured spot for oviposition is no longer available when an egg batch has been deposited there. In contrast, the most favoured spot for oviposition, the leaf tip, is still available when the egg batch is placed away from this location.

This study demonstrates that *C. bimaculata* oviposition site choice on a leaf surface is influenced by the presence and availability of a tapering structure (such as a leaf tip). The availability of a leaf tip for oviposition increases the likelihood of an egg batch being deposited when all other characteristics of the leaf are uniform. The preference for a region of the leaf which occupies a small portion of the total leaf area has implications not only for egg batch distribution on leaves, but also within trees. With regards to the leaf surface, the increased likelihood of egg batch placement on the leaf tip, rather than randomly on the leaf, effectively reduces the total leaf area (of a branch or tree) available for oviposition. Egg batch distribution within trees must then be partially dependent on the number of leaf tips available and thus reducing the likelihood (although not universally) of multiple egg batches occurring on the same surface of individual leaves. The non-random nature of oviposition site selection by *C. bimaculata* thus has important implications regarding host tree leaf development (explored in chapter 3), the population dynamics of *C. bimaculata* (explored in chapter 5) and egg batch distribution and density within and between hosts (explored in chapters 4 and 8).

Chapter 3

The Influence of Host Leaf Development on *Chrysophtharta bimaculata* Egg Deposition

3.1 Introduction

Examinations on plant susceptibility to phytophagous insect defoliation has largely focussed on the chemical and physical attributes of the host plant (as discussed in Chapter 1). For insect-eucalypt host interactions, several studies have found variable host susceptibility for trees within species to insect damage. For example, Raymond (1995) who found variable susceptibility in *E. regnans* families to *C. bimaculata* damage and Floyd et al. (1994) documented that *E. camaldulensis* provenances differed in susceptibility to *Cardiaspina albitextura*, *Cardiospina retator* and *Mnesampela privata* damage.

Several studies have suggested reasons as to why some genotypes within a *Eucalyptus* species show variable susceptibility to particular insect species. These include leaf waxes, the composition of which can vary widely between juvenile and adult leaves within the same species and trees. It is not known whether the glaucous wax covering juvenile leaves chemically influences some insects in their host selection. However, it has been found to impede physical attachment of some paropsine species (Edwards 1982; Edwards & Wanjura 1990; Li 1993), providing protection against feeding and oviposition. A correlation between cuticular wax and the susceptibility of *E. camaldulensis* to *Cardiaspina retator* has also been found, although to what degree, if any, it contributes to host suitability is unknown (Farrow & Floyd 1996).

Host leaf toughness has also been implicated with host susceptibility for some paropsines, by decreasing host suitability for larval development (Ohmart et al. 1987; Larsson & Ohmart 1988). Steinbauer et al. (1998) suggests that the rate of leaf sclerophylly development may have important implications in the susceptibility of eucalypt hosts to *Chrysophtharta bimaculata*.

Leaf chemistry has been implicated in influencing insect preference, host suitability and host susceptibility between eucalypt host species. The proportion of 1, 8 cineole in the terpenoid mixture of eucalypts leaves appears to be negatively correlated with *C. bimaculata* herbivory between eucalypt hosts (Li 1993) although

it is unknown whether this terpenoid actively deters feeding. Leaf chemistry is thought to influence eucalypt susceptibility to defoliation within species for some insects (Ohmart et al. 1984; Raymond 1998). Cineole has been implicated in host tree susceptibility within eucalypt species for *Anoplognathus* spp.(Edwards et al. 1993) and for the degree of insect herbivory received by *Eucalyptus camaldulensis* (Stone & Bacon 1994). Vertebrate studies indicate sideroxylonal, a diformylphloroglucinol) which has concentrations usually correlated with total terpenes, deters herbivory (Lawler 1999). Although no studies have yet been conducted on the relationship between invertebrate deterrence and this secondary plant metabolite, it may be important in host suitability and preference. Other invertebrate studies examining the influence of leaf chemistry on host susceptibility within species have failed to show any significant differences. These include *E. globulus* susceptibility to *Mnesampela privata* (Farrow et al. 1994) and *E. regnans* susceptibility to *C. bimaculata* (Patterson et al. 1996).

Foliar nutrient concentrations have been correlated with host susceptibility in eucalypt-insect interactions. Nitrogen content of *E. blakelyi* leaves is correlated with *C. albitextura* herbivory (Farrow & Floyd 1996). However, for paropsines, it is believed that nitrogen becomes limiting only when leaves are too tough for the insects to feed (Ohmart et al. 1987; Larsson & Ohmart 1988).

Changes in host plant leaf development may influence susceptibility to specific insects and induce changes in insect population dynamics. For example, mammal browsing (Hjältén & Price 1996; Roininen et al. 1997; Martinsen et al. 1998) and fire (Steinbauer 1998b) can produce young vigorous shoots more suitable for insect feeding and oviposition. In such cases, changes in leaf nutrition are often cited as reasons for the increased suitability (Martinsen et al. 1998; Steinbauer et al. 1998b). Once eucalypt defoliation has occurred, a positive feedback loop of continual insect defoliation can result (Ohmart 1991).

The influence of previous damage on host tree susceptibility to paropsine defoliation has not been examined. Besides food quality, leaf canopy characters (e.g tree bushiness, leaf development) could be altered through defoliation, influencing

insect host preference for feeding and oviposition and thus subsequent defoliation. Strauss & Morrow (1988) suggest that *Chrysophtharta hectica* may receive increased protection from abiotic factors and/or natural enemies in the bushier canopies of *Eucalyptus stellulata* compared to *E. pauciflora*.

Likewise, changes in leaf size, number or shape, caused through previous defoliation could potentially influence some eucalypt - paropsine interactions indirectly. Chapter 2 indicated that *C. bimaculata* has an oviposition preference for the leaf tip. This has potential ramifications to the susceptibility of an individual host tree as the number of leaf tips (determined by leaf number) available for oviposition may influence the number of eggs the tree will receive. This inturn may affect the population dynamics of the insect by influencing egg batch number and hence larval densities in host trees. Moreover, trees with larger leaves may receive less eggs per cm^2 of surface leaf area compared to smaller leaf trees, influencing defoliation.

In this study, leaf size and number were examined in the laboratory to see whether these factors influenced the number of *C. bimaculata* egg batches leaves received and thus potential larval defoliation. Secondly, field trees were examined to determine whether there are any relationships between mean leaf area and the number of eggs and eggs per cm^2 of surface leaf area. Finally, larval damaged and non damaged saplings were compared in the laboratory to determine whether leaf development is significantly altered by prior feeding and if so, whether there are significant differences between the number of egg batches or total eggs deposited by *C. bimaculata* on the two sapling treatments. Unless otherwise stated, leaves used and examined in all experiments were expanding or newly expanded.

3.2 Materials and Methods

3.2.1 Oviposition and the size and number of leaves

Shoots of *Eucalyptus regnans* were collected from forest regeneration in the Plenty Valley (42°50'S, 146°53'E) and transported back to the laboratory in buckets containing water. They were placed in a 4°C cool store overnight and utilised in experiments the following day, after at least an hour of acclimatisation to room temperature.

For experiment 1, twelve branches approximately 40 cm in length, each containing 2 shoots, were selected and all leaves removed. Two sets of mimic leaves were cut from polyester myler. Each 'leaf' in the first set had a leaf area of 15 cm^2/side while leaves in the second set had a leaf area of 45 cm^2/side. On each of six branches, twelve of the small mimic leaves were tied, six on each shoot. On the other six branches four of the larger leaves were tied, two on each shoot (thus each shoot had different numbers of "leaves", but the same total leaf area). For each shoot receiving small leaves a pair were tied two cm below each shoot tip. Pairs of small leaves were attached down the shoot at 4 cm intervals. For the large leaved shoots, only one pair of mimic leaves was tied 2 cm below the shoot tip.

From each of the small and large leaved groups one branch was placed into a bottle containing water and the top plugged with paper towelling. Bottles were then individually placed in the centre of a cage with dimensions 33 cm x 101 cm x 70 cm. This was repeated with the remaining branches to give six replicates. In each cage 20 female *Chrysophtharta* beetles were released. The beetles had been collected from an ovipositing population in the field two days before and stored in a cool room at 4°C. The beetles were left in the cages for 24 hours after which the experiment was concluded.

For experiment 2, shoots from *E. regnans* trees at a single site in the Florentine Valley (42°39'S, 146°28'E) were selected based on the predominant size of the

leaves they contained. One group of shoots collected contained expanding leaves predominantly in the leaf area range of 3.5 - 20 cm^2/side (small leaf shoots) while the other group contained expanding leaves predominantly in the range: 30 - 100 cm^2/side (large leaf shoots). The shoots were collected in early November from trees where the current seasons growth had yet to harden. For each replicate, three to four small leaf shoots carrying a total of approximately thirty leaves were combined with three to four large leaf shoots carrying approximately ten leaves. All shoots were randomly placed into a jar containing water, but positioned so that adjacent leaves were not in contact. The jars containing the shoots were then placed individually into the centre of cages (dimensions as for experiment 1) so that leaves were not touching the walls. Twenty female beetles (collected as for experiment 1) were placed in each cage and allowed to feed and oviposit for a period of six daylight hours. Six replicates were run concurrently. Counts of egg batches and egg numbers were made for both experiments while leaf area before and after the conclusion of experiments were measured using the 'non-destructive' technique outlined in General Materials and Methods (page 28). Data measuring percentage leaf area loss due to beetle feeding were arcsin transformed due to non normality before statistical analysis was conducted. Data was then analysed for both experiments 1 and 2 using paired student's t-tests.

3.2.2 The relationship between leaf size and the number of *C. bimaculata* eggs on *E. regnans* trees in the field

Foliage from twenty-five *E. regnans* between 2.5 and 4 metres high which had recently been oviposited upon by *C. bimaculata* were examined at a site in the Florentine Valley. Trees were marked and then all of the current season foliage was removed from five branches around each tree, so that no particular directional face was preferred. Current season foliage was easily distinguished from previous seasons due to the visually distinctive colour difference. The colour of current season foliage ranged from light green to red while previous seasons foliage was dark green.

The harvested leaves were taken back to the laboratory in a cool box and then placed in a store at 4°C until leaf area was determined and egg and egg batch numbers were counted. Leaf area was measured using a ΔT^{TM} (Delta-T Devices) Area Meter (Cambridge, UK.). The relationship between leaf numbers and areas of expanding leaves with the numbers of egg batches and eggs were analysed by regression.

3.2.3 The leaf development of saplings defoliated by *C. bimaculata* larvae and their influence on *C. bimaculata* oviposition

Twelve potted *E. regnans* saplings, approximately 50 cm high, were placed in a glasshouse at constant 23°C under natural light conditions. The saplings were spaced evenly at 20 cm between pots in three rows of four ensuring that no leaves between saplings touched. Approximately three-hundred second and third instar *C. bimaculata* larvae (collected from field trees in the Florentine Valley) were then placed on each alternate sapling so that six saplings were infested. Larvae were allowed to feed for the next three days until all expanding foliage had been removed. The remaining youngest leaves on each shoot for both larval damaged and undamaged treatments were then coded with a plastic tag placed around their petiole. The saplings were then placed in a shadehouse for three months to permit the development of new foliage.

Following this period the number of new leaves were counted and the leaf area of each estimated by measuring the length and width of each leaf and multiplying by a coefficient (0.70). For information on this relationship refer to Appendix 2. Data on leaf size, leaf numbers and total leaf area were analysed using a student's t-tests.

The stem of each sapling was then severed and the sapling material shortened so that the leaf and shoot material from each sapling was approximately 40 cm long. The base of each sapling was then placed in a bottle containing water and the opening plugged with paper towelling. All foliage that had developed prior to the larval treatment was then removed from both damaged and undamaged saplings.

Saplings that had received larval damage were matched with those which had not and each resulting branch pair placed in a cage (dimensions as for experiments 3.2.1). In each replicate a damaged sapling was placed in one corner and an undamaged diagonally opposite (corners were alternated for each replicate). Twenty female *C. bimaculata* collected from a wild ovipositing population in the Florentine Valley (stored overnight at 4°C) were then placed in each cage and left for six daylight hours after which eggs and egg batches were counted and final leaf area was measured. Data on actual and percentage (arcsin transformed due to non normality) leaf area lost due to beetle feeding, egg batch and egg numbers, egg batch size and egg batch per cm^2 of leaf area were analysed using paired student's t-tests.

3.3 Results

3.3.1 Oviposition and the size and number of leaves

The 12 small artificial leaves, as a group, received significantly more egg batches and eggs in total than the 4 large artificial leaves provided in each replicate, even though total leaf area was equal (Table 3.1). For the two leaf sizes, there were no significant differences between the mean number of egg batches or egg numbers per leaf (Table 3.1).

Table 3.1 Mean (± SE) *C. bimaculata* total egg batch number, total egg number, mean egg batches per leaf and mean egg numbers per leaf for 12 small (leaf area 15 cm^2) versus 4 big (leaf area 45 cm^2) artificial leaves (n = 6, d.f. = 5, t-crit = 2.57).

	Small leaves	Large leaves	t-stat	P-value
Total Egg batch No.	6.0 ± 1.8	1.8 ± 0.6	3.49	0.017
Total Egg No.	112.8 ± 21.2	40.0 ± 15.1	3.17	0.025
Mean Egg batch/leaf	0.5 ± 0.1	0.5 ± 0.2	0.26	0.807
Mean Egg No./leaf	10.0 ± 3.8	9.4 ± 1.8	0.16	0.880

Although carrying fewer leaves, the total leaf area of large leaf shoots was significantly greater than small leaf shoots in experiment 2 (Table 3.2). *C. bimaculata* beetles consumed a similar amount of leaf area on both small and large leaf shoots, resulting in small leaves losing a larger percentage of their total leaf area. Although the small leaves had significantly less total leaf area they still received significantly more egg batches and egg numbers (Table 3.2). There were no significant differences between egg batch size or the number of egg batches per leaf.

Table 3.2: Leaf characteristics of two shoot types exposed to *C. bimaculata* and subsequent levels of feeding and oviposition on those shoots. Error values are ± S.E. (n = 6, d.f. = 5, t-crit = 2.57).

	Shoot type			
	Small leaf shoots	Large leaf shoots	t-stat	P-value
Leaf characters				
Leaf size (cm^2)	11.1 ± 1.0	57.8 ± 3.2	13.35	<0.001
Leaf no.	32.0 ± 2.6	10.5 ± 0.8	6.03	0.002
Leaf area before (cm^2)	347.3 ± 15.3	609.3 ± 54.9	5.07	0.004
Feeding patterns				
Leaf area lost to feeding (cm^2)	39.0 ± 1.8	41.8 ± 3.2	0.88	0.417
Leaf area lost to feeding (%)	11.6 ± 0.9	7.2 ± 0.8	7.05	<0.001
Oviposition patterns				
Egg batch no.	11.5 ± 2.3	3.2 ± 0.7	3.63	0.015
Egg no.	232.8 ± 50.2	57.3 ± 14.5	3.39	0.019
Egg batch size	19.9 ± 1.0	18.0 ± 1.5	1.04	0.346
Egg batch/leaf	0.4 ± 0.1	0.3 ± 0.1	0.59	0.581

3.3.2 The relationship between leaf size and the number of *C. bimaculata* egg batches on field *E. regnans* trees

There was no significant correlation between egg batch number and the mean leaf area of expanding leaves for the 25 field selected *E. regnans* trees (r = 0.27, $F_{1,24}$ = 1.79, P = 0.194 and Figure 3.1A). However, there was a significant correlation between the number of egg batches and the number of expanding leaves present (r = 0.52, $F_{1,24}$ = 8.39, P = 0.008 and Figure 3.1B).

A Comparison of the leaf surface area available (cm^2) per egg with the mean leaf area gave a strong correlation (r = 0.90, $F_{1,24}$ = 95.73, P < 0.001 and Figure 3.1C). This correlation would have been influenced by the lack of correlation between egg batch size with leaf size (r = 0.14, $F_{1,24}$ = 0.43, P = 0.519). However, this result indicates that if all eggs were to hatch, trees with larger leaves would offer the greater leaf area (expanding leaves) for each individual larva.

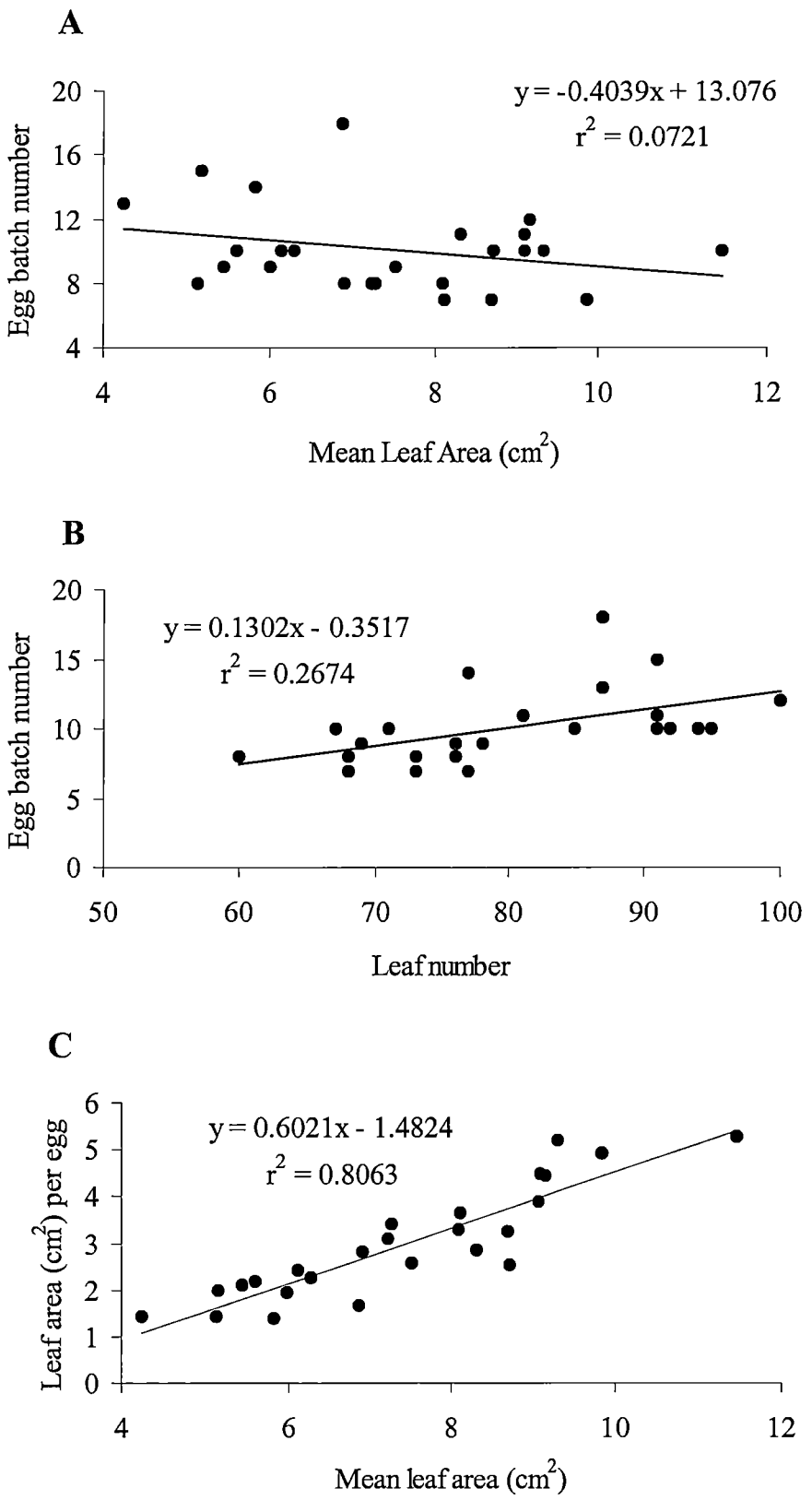


Figure 3.1. Regression of egg batch number versus **(A)** mean leaf area and **(B)** leaf number; and **(C)** cm^2 of surface leaf area per egg versus mean leaf area for 25 *E. regnans* field trees following oviposition by a wild population of *C. bimaculata*.

3.3.3 The leaf development of saplings defoliated by *C. bimaculata* larvae and their influence on *C. bimaculata* oviposition

After a 3 month period, saplings that had sustained severe larval damage produced significantly greater numbers of leaves that were smaller in size compared to saplings which had not been damaged (Table 3.3). Total leaf area developed three months following sapling defoliation was also significantly reduced compared to undamaged saplings (Table 3.3).

C. bimaculata deposited significantly more egg batches and total eggs on larval damaged saplings compared to undamaged saplings (Table 3.3). Egg batch size was not significantly different between undamaged and damaged saplings nor was the number of egg batches received per leaf. These results mirror those in 3.3.1 experiment 2.

There was no significant difference in leaf area loss due to beetle feeding during experiment 3.3.3, however, damaged saplings lost a higher percentage of total leaf area when compared to undamaged saplings (similar to experiment 3.3.1).

Table 3.3 Leaf characteristics of undamaged and larval damaged saplings exposed to *C. bimaculata* and subsequent levels of feeding and oviposition on those shoots. Error values are ± S.E. *Data analysed using student's t-tests assuming equal variances. The remaining data analysed by Paired student's t-tests.

	Sapling type					
	Damaged	Undamaged	d.f.	t-Crit.	t-stat	P-value
Leaf characters						
Leaf size (cm^2)*	9.4 ± 0.6	25.1 ± 1.7	10	2.23	8.92	<0.001
Leaf No.*	60.2 ± 9.0	36.7 ± 1.8	10	2.23	2.55	0.029
Total leaf area (cm^2)*	568 ± 115	912 ± 52	10	2.23	4.30	0.021
Feeding patterns						
Leaf area lost to feeding (cm^2)	44.0 ± 3.5	50.1 ± 3.2	5	2.57	1.13	0.311
Leaf area lost to feeding (%)	8.5 ± 0.9	5.6 ± 0.5	5	2.57	5.15	0.004
Oviposition pattern						
Egg batch No.	7.0 ± 1.0	3.8 ± 0.4	5	2.57	5.27	0.003
Egg No.	140 ± 21	74.7 ± 6.4	5	2.57	3.44	0.012
Egg batch size	19.8 ± 0.6	20.2 ± 1.8	5	2.57	0.16	0.876
Egg batch/leaf	0.13 ± 0.02	0.11 ± 0.01	5	2.57	1.31	0.248

3.4 Discussion

Field and laboratory results clearly demonstrate that the number of available leaves, rather than their size dictate *C. bimaculata* oviposition levels. However, leaf size influences the amount of leaf area per egg. This has important implications to the potential rate of defoliation and possibly overall damage of the host by *C. bimaculata* larvae.

Leaf size *per se* does not influence *C. bimaculata* oviposition choice. All laboratory and field results failed to show a significant difference or correlation between leaf size and egg batches received (Tables 3.1, 3.2, 3.3. results 3.3.2 and fig 3.1A). Although smaller leaves lost a higher percentage of their leaf area through beetle consumption in experiment 3.3.1, the percentage loss of leaf area through beetle feeding could not have been important in oviposition choice since these leaves still received more egg batches. The important factor is the number of leaves available for oviposition. Based on data from Chapter 2, I consider it is the presence of an available leaf tip that is the greatest factor explaining oviposition load on expanding and recently expanded leaves. A large expanding leaf is as equally attractive as a small expanding leaf, both having a single leaf tip. However, there is no significant difference between the egg batch size with regards to leaf size (Tables 3.2, 3.3). Thus large leaves have more surface leaf area per egg batch compared to small leaves (see results 3.3.2 and fig. 3.1C).

The number of egg batches (and hence egg numbers) a host plant receives is influenced by leaf number. The number of favourable oviposition sites, ie tip numbers, increases as leaf number increases. Thus shoots in experiments 3.1 a and b and 3.3, which contained a higher number of small leaves with total leaf area equivalent or less than the large leaf shoots, received significantly more egg batches than shoots with fewer, large leaves. In addition, a correlation between leaf number and the number of egg batches for *E. regnans* field trees was found (Fig. 3.1 B).

C. bimaculata populations may deposit hundreds of egg batches on a single host tree between the height range of three to six metres (pers obs.) Developing larvae

may completely defoliate their hosts of all expanding and fully expanded leaves leading to mass starvation (Greaves 1966). Severe larval defoliation by *C. bimaculata* can inturn, alter leaf development leading to an initial increase in leaf number and a reduction in leaf size. Trees which produce large numbers of small leaves suitable for neonate larval establishment (i.e. expanding leaves) are then more prone to receiving higher egg per cm^2 of surface leaf area ratios. This increases tree susceptibility by increasing the likelihood of severe larval defoliation if heavy egg batch deposition occurs.

Several other factors are known to affect the leaf development of eucalypts. Defoliation through fungal attack, herbivory, fire and drought may result in vigorous new growth through the activation of dormant buds (Williams & Brooker 1997). Likewise, the amount of water available to eucalypts may affect the rate of leaf production (Metcalfe et al. 1990; Stoneman et al. 1994) and leaf size (Stone & Bacon 1994;1995). These factors which can affect leaf development, may thus influence *C. bimaculata* population dynamics and the susceptibility of its hosts. Ohmart (1991) and Ohmart et al (1987) suggest that the population performance of *Paropsis atomaria* on *E. blakelyi* would be reduced in drought years due to the lower production of expanding leaves suitable for adult and larval feeding. Apart from these factors, the reduction in leaf number and thus leaf tips will also offer fewer preferable oviposition sites for *C. bimaculata.* However, if drought conditions lead to a reduction in leaf size then the number of eggs per cm^2 of surface leaf area may increase. The few expanding leaves could thus be more susceptible to severe larval defoliation.

Apart from *Chrysophtharta bimaculata,* some other paropsine species such *Chrysophtharta agricola* have an oviposition preference for the leaf tips of their eucalypt hosts (de Little 1979;Ramsden & Elek 1998). For these paropsines, host leaf size and leaf number may also influence the amount of foliage available for their offspring and thus influence both insect population dynamics and the level of host defoliation.

A

B

Figure 3.2 Plantation *E. regnans* trees located in the Plenty Valley having received **(A)** high larval defoliation, leading to the production of larger numbers of smaller leaves compared to **(B)** which has received a low level of larval defoliation.

With regards to *C. bimaculata*, factors such as leaf toughness (Steinbauer 1990 et al) and leaf chemistry (Li 1993; Patterson 1996) have been suggested as potential influences on host tree susceptibility. With the demonstration that: i. expanding and newly expanded leaf size can influence egg batches per cm^2 of surface leaf area and ii. leaf number can influence the number of eggs per tree, another dimension is added to the issue of host susceptibility and subsequently, *C. bimaculata* population dynamics. These issues will be discussed further in later chapters of this thesis.

Chapter 4

The relationship between adult *Chrysophtharta bimaculata* feeding damage and egg batch density on two *Eucalyptus* hosts

4.1 Introduction

Many adult phytophagous insects oviposit on the same host plants they use as their food source, including the paropsines *Chrysophtharta bimaculata* (Greaves 1966), *C. agricola* (Ramsden & Elek 1998), *Paropsis atomaria* (Carne 1966) and *P. charybdis* (Murphy 1998). This has the potential of promoting competition for resources between adults and larvae and/or damage to conspecific eggs. Damage to eggs may be caused by indirect predation (eggs eaten with leaf material), or by eggs dropping from the host on severed leaves. However, insects have different strategies to avoid or alleviate these problems. These include depositing eggs away from adult feeding sites, visual or chemical deterrence of hungry conspecific adults by conspecific larvae and eggs, and feeding preferences for different plant parts.

To avoid egg damage caused through adult feeding, insects may choose to deposit eggs away from adult feeding sites. For the willow leaf beetle, *Plagiodera versicolora*, adults feed on young foliage rather than old while females oviposit on old leaves. Raupp & Denno (1980) believe this may protect eggs and/or larvae from being disturbed or destroyed. The larvae also have the ability to consume both young and old foliage, thus reducing the risk of starvation if newly developed/developing foliage is depleted by feeding adults (Raupp & Denno 1980). Likewise, the paropsine *Paropsis charybdis* oviposits on the tips of old leaves while adults and larvae feed on young leaves (Murphy 1998). Another paropsine *P. atomaria* oviposits on the stems of terminal shoots, away from adult feeding sites (Carne 1966). This strategy may protect eggs from being damaged by feeding adults and larvae. Competition for limited resources may also be reduced through the use of chemical or visual deterrence of conspecific eggs and larvae to repel conspecific insects. The possible importance of some of these traits for *C. bimaculata* were explored in chapter 2.

For some insects, oviposition site selection may have a lower priority compared to factors such as maximising female fecundity and oviposition rate. This strategy may even be used by insects that have offspring with poor migration ability. For gall forming cecidomyiids, which have short life spans and poor migration ability, no advantage is gained discriminating between oviposition sites when there are a

large number of poor, relative to good, hosts (Larsson & Ekbom 1995). A similar result may arise for insects that have high fecundity and where adults and larvae feed on the same host substrates. Depositing eggs at the feeding site may produce as many or more surviving offspring than if insects spend more time (increasing the risk of death and a lower egg deposition rate) finding more suitable oviposition sites.

Chrysophtharta bimaculata adults feed and oviposit predominantly on the flush foliage of the monocalyptan species *Eucalyptus regnans*, *E. delegatensis* and *E. obliqua* and the symphyomyrtan species *E. nitens*. Likewise, larvae feed predominantly on the flush foliage (Greaves 1966; de Little 1983). The potential for competitive interactions between different life stages in this situation is high. The relationship, if any, between adult feeding and oviposition preference has not been explored for *C. bimaculata*, but it may have important implications for understanding host plant defoliation and successful larval development.

The aim of this chapter is to determine whether:

(i) Adult *C. bimaculata* beetle feeding damage and egg batch density are correlated in eucalypt stands containing mixed and single species hosts.

(ii) Foliage damaged through adult feeding (including physical edge damage) and leaf area loss through feeding influence *C. bimaculata* oviposition preference.

The implications of the experimental findings will then be discussed in terms of *C. bimaculata* host utilisation for adult and larval feeding and oviposition.

4.2 Materials and Methods

4.2.1 A comparison of adult *C. bimaculata* feeding damage and egg numbers in mixed stands of *E. delegatensis* and *E. regnans* at two locations

Mixed stands of *E. delegatensis* and *E. regnans* were examined during the first oviposition episode (of a season) in two separate years at Judbury Bluff (42°28'S, 146°23'E) and in one year at a site in the Florentine Valley (42°39'S, 146°28'E). The eucalypt regeneration site at Judbury Bluff, consisting of approximately 33% *E. regnans* and 67% *E. delegatensis* (P. Rowe, Forestry Tasmania pers. comm.), was monitored during November and December of 1994 and 1996 for populations of ovipositing *C. bimaculata*. During an oviposition event in 1994 (the first for the season), forty *E. regnans* and *E. delegatensis* tree pairs were selected within a 50 metre radius and tagged. Pairs consisted of trees visually assessed for similar height and amount of foliage and located within a 5 metres of each other. Selected trees were between 1.8 and 3 metres in height.

At the end of the oviposition period twelve shoots were removed from each tree, five of the top ten highest shoots and a further seven from around the tree. Shoots were taken from all sides of each tree so as not to favour any particular directional face. Leaves were transported back to the laboratory in a coolbox and stored at 4°C until examined.

Only leaves that had developed in the current season were used for analysis; old leaves receive significantly less egg batches (Steinbauer et al. 1998a; Chapter 5) and so were excluded. Current season leaves were easy to distinguish as leaves that had developed in earlier seasons were dark green in appearance and much tougher than new leaves. To estimate the original leaf area a transparency plastic sheet was placed over the leaf and a black felt pen used to fill in the lost area caused through *C. bimaculata* beetle feeding. The original estimated leaf area was then measured using an area meter (see section 2.2). If present, the number of egg batches on sampled leaves were counted.

In 1996, the same trees were sampled again during the first aggregation of ovipositing *C. bimaculata* beetles at the site in that season. Leaf area measurements and egg batch counts were made using the same methods as in 1994.

The second site consisting of a mixed stand of *E. delegatensis* and *E. regnans* was monitored in the Florentine Valley for *C. bimaculata* attack. This site was planted as a *E. regnans* plantation with some *E. delegatensis* regrowth (approximately 10% of trees). Twenty-four *E. delegatensis* trees were paired with *E. regnans* (pairs adjacent and within 5 m radius of each other) based on similar tree height and amount of foliage present. Trees were sampled over a radius of approximately 40 metres Leaves were sampled from the trees during the first aggregation of ovipositing *C. bimaculata* and from these leaf area measured and egg batch counts conducted using the same methods employed at Judbury Bluff.

For these experiments, egg batch counts were converted to egg batches per leaf and egg batches per mm^2 leaf area. Leaf area loss data was converted to percentage leaf area loss and arcsine transformed for analysis. These were then compared between the two eucalypt species using paired student's t-tests.

4.2.2 A comparison of adult *C. bimaculata* feeding damage and egg numbers in a pure stand of *E. regnans*

The correlation between oviposition and adult feeding damage was examined in a pure stand of *E. regnans* in the Plenty Valley (42°50'S, 146°53'E). *E. regnans* plantation trees were monitored beginning in November 1996 to record the earliest aggregation of ovipositing *C. bimaculata* beetles for that season. During this event, *E. regnans* tree pairs, each being adjacent and within five metres distance of each other, were selected based on similarities of tree size and amount of foliage present. Leaves were collected, area measured and egg batches counted using the same methods applied at Judbury Bluff. Thirty trees were sampled (15 pairs) within a radius of twenty metres. A comparison was made between pairs based on the level of defoliation the tree had received. Thus group 1 trees represented the tree in each pair with the higher defoliation and group 2 those in each pair with lower

defoliation (groups included those pairs showing marginal defoliation differences). Based on these groupings, comparisons were made between egg batches per leaf and egg batches per m^2 of surface leaf area using paired student's t-tests. Regressions were also conducted using all *E. regnans* trees sampled to determine whether there was a relationship between beetle egg batch per leaf and egg batch per m^2 of surface leaf area with beetle defoliation.

4.2.3 The influence of leaf scalloping on *C. bimaculata* oviposition preferences

Two artificial leaf types were constructed using plastic marking tape. The first model with a leaf area of 10 cm^2/side mimicked the shape of a typical *E. regnans* leaf. The second model, with the same final leaf area was scalloped along the leaf edge using a paper punch to mimic the shape of leaves damaged by adult *C. bimaculata* feeding. One of each leaf type was connected with the other by a plastic tie (see figure 4.1). This ensured that mimic leaf types where tied in equal numbers and in equivalent positions. Twelve of each mimic leaf types were tied to ten manually defoliated *E. regnans* branches.

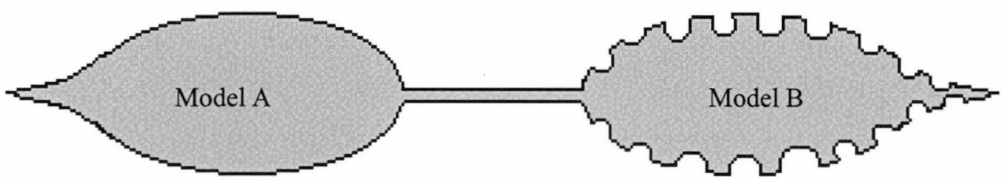


Figure 4.1 Diagram of artificial plastic leaves used in experiment 4.2.3. Model A represented the shape of an undamaged leaf while model B, the shape following *C. bimaculata* feeding damage. Both Model A and B 'leaves' were of equivalent area. Models were connected by a thin piece of plastic which was used to tie onto branches as well as acting as an artificial petiole.

Using these mimic leaf arrays, experiments were then conducted using field collected beetles under the same experimental conditions, cages and glasshouse as outlined in sections 2.2 and 2.2.4. Following the experiment the number of egg batches deposited were counted for each leaf type and statistically compared using a paired two-sample student's t-test.

4.2.4. The influence of manually reducing leaf area on *C. bimaculata* oviposition choice between *E. regnans* and *E. delegatensis*

Actively growing *E. regnans* and *E. delegatensis* shoots (no insect damage evident) were collected from trees at Judbury Bluff and stored as outlined in section 2.2 until required for experimental use. All previous seasons foliage was manually removed.

Three experiments testing *C. bimaculata* oviposition preferences were conducted:

i. A binary experiment comparing *E. regnans* leaves (area not reduced) with *E. delegatensis* leaves with reduced leaf area. The leaf area of *E. delegatensis* leaves was reduced using a paper hole punch so as to mimic the feeding damage caused by *C. bimaculata* beetles.

ii. Similar to experiment i. except *E. regnans* leaf area was reduced instead of *E. delegatensis*.

iii. Similar to the above experiments however there was no reduction in the leaf area of either eucalypt species.

Leaves with an initial leaf area less than 12 cm^2/side (before manual reduction of leaf area) were removed as these leaves were more likely to be severed from their shoots through beetle feeding. All replicates in the three experiments had the same number of leaves.

Binary choice experiments were conducted by transferring the paired treatments to replicate cages. Each treatment was placed at diagonally opposing corners and were

alternated for each replicate. Ten female and four male beetles were used in each replicate. Fifteen replicates were conducted for experiments i and ii and ten for experiment iii. For each replicate beetles were allowed to feed and oviposit for a period of 8 hours. The experiments were run under the same conditions as outlined in section 2.2 (General Materials and Methods). The numbers of egg batches deposited for each leaf type in all replicates were counted following the experiment and statistically compared using a paired student's t-test.

4.2.5 The influence of conspecific feeding damage on *C. bimaculata* oviposition choice

E. regnans and *E. delegatensis* foliage that had been damaged through *C. bimaculata* beetle feeding, along with undamaged foliage was collected from Judbury Bluff and stored as outlined in section 2.2 until required for experimental use. Damaged foliage was collected at a site where beetles were present and larvae absent. Previous seasons leaves were manually removed before experimentation commenced.

Two experiments testing *C. bimaculata* oviposition preferences were conducted:

i. A binary experiment comparing *E. regnans* leaves damaged through *C. bimaculata* beetle feeding with undamaged *E. regnans* leaves.

ii. A binary experiment comparing undamaged *E. regnans* leaves with *E. delegatensis* leaves damaged through *C. bimaculata* beetle feeding.

The same methods were used as those employed in 4.2.4, except that twenty replicates were run for each experiment. All replicates in both experiments had the same number of leaves. Leaf toughness was also measured (see 2.2). The number of egg batches deposited for each treatment were counted following the experiment and statistically compared using a paired student's t-test.

4.2.6 Larval development on expanding *E. regnans* and *E. delegatensis* foliage collected from Judbury Bluff.

C. bimaculata egg batches (approximately 150) were collected from *E. regnans* trees in the Florentine Valley and were allowed to hatch under natural light conditions and a constant temperature of 25°C. Larvae were used within six hours of hatching as follows: Eight groups of fifteen randomly selected first instar larvae were collected, weighed (as a group) and transferred by group to either a *E. regnans* shoot or a *E. delegatensis* shoot, giving four replicates of each eucalypt species. Undamaged shoots containing current season foliage were collected from trees at Judbury Bluff.

The base of each shoot was placed in a 100 ml vial filled with water and the top plugged with paper towelling. Vaseline was smeared around the shoot base, just above the plug of each bottle to stop larvae from migrating off the shoot. Shoots were replaced daily and larval weight measured every one to three days (larvae were measured as a group per replicate up until day 6, and from then on individually). After ten days shoots were placed in closed plastic containers as pre-pupation approached.

In addition to larval weight, the percentage of larvae surviving to pupation (data arcsine transformed prior to analysis), the time taken to reach pre-pupation (measured in days for each larva) and pupal weight were also measured. Comparisons between the *E. regnans* and *E. delegatensis* replicates were made using a two sample student's t-test.

4.3 Results

4.3.1 A comparison of adult *C. bimaculata* feeding damage and egg numbers in mixed stands of *E. delegatensis* and *E. regnans* at two locations

In the Florentine Valley, *E. regnans* lost a significantly higher percentage of expanding leaf material through adult *C. bimaculata* feeding (13.1%) compared to *E. delegatensis* (10.7%). In contrast, *E. delegatensis* received significantly more egg batches per leaf (0.23) and egg batches per leaf area (134.8 egg batches/m^2) compared to *E. regnans* (0.10 eggs per leaf and 60.2 egg batches/m^2) (Table 4.1).

Similar results were obtained over two consecutive seasons from the mixed stand at Judbury Bluff. *E. regnans* lost a significantly higher mean percentage of expanding leaf material (12.8%) through *C. bimaculata* beetle defoliation in 1994 compared to *E. delegatensis* (8.0%)(Table 4.1). *E. delegatensis* received higher numbers of egg batches per leaf (0.19) and egg batches/m^2 of surface leaf area (154.0 egg batches/m^2) compared to *E. regnans* (0.12 egg batches/leaf and (82.3 egg batches/m^2) (see Table 4.1). In 1996, *E. regnans* lost 12.0% of expanding leaf material, significantly more than *E. delegatensis* 5.9% (Table 4.1). *E. delegatensis* again received significantly more egg batches per leaf (0.05) and egg batches per leaf area (14.3 egg batches/m^2) compared to *E. regnans* (0.02 egg batches/leaf and 4.2 egg batches/m^2) (Table 4.1). There were no significant differences between the number of leaves sampled at either location or year sampled (Table 4.1).

Table 4.1 Feeding and oviposition patterns by *C. bimaculata* on two tree species in the Florentine Valley in 1996 (n=24, d.f. =23, t-crit = 2.07), and at Judbury Bluff in 1994 and 1996 (n=40, d.f. =39, t-crit = 2.02). Error values are ± S.E.

		Tree species			
Feeding or oviposition pattern	Site and date	*E. regnans*	*E. delegatensis*	. t. stat	P-value
Leaf Number	Florentine Valley 1996	26.5 ± 0.7	27.4 ± 0.9	0.88	0.387
Initial leaf area (cm^2)	Florentine Valley 1996	42.0 ± 2.0	40.3 ± 1.7	0.60	0.553
Final leaf area (cm^2)	Florentine Valley 1996	36.5 ± 1.8	36.1 ± 1.6	0.18	0.859
% Leaf lost	Florentine Valley 1996	13.1 ± 0.5	10.7 ± 0.4	4.58	<0.001
Egg batch/leaf	Florentine Valley 1996	0.10 ± 0.03	0.23 ± 0.03	3.52	0.002
Egg batch/m^2 surface leaf area	Florentine Valley 1996	60.2 ± 19.9	134.8 ± 18.3	2.61	0.016
Leaf Number	Judbury Bluff 1994	29.0 ± 0.7	27.7 ± 0.8	1.68	0.192
Initial leaf area (cm^2)	Judbury Bluff 1994	43.2 ± 1.5	41.0 ± 1.2	1.07	0.289
Final leaf area (cm^2)	Judbury Bluff 1994	38.4 ± 1.4	38.0 ± 1.1	0.23	0.817
% Leaf lost	Judbury Bluff 1994	12.8 ± 0.5	8.0 ± 0.6	6.73	<0.001
Egg batch/leaf	Judbury Bluff 1994	0.12 ± 0.02	0.19 ± 0.02	3.08	0.003
Egg batch/m^2 surface leaf area	Judbury Bluff 1994	82.3 ± 11.2	154.0 ± 16.4	3.89	<0.001
Leaf Number	Judbury Bluff 1996	31.0 ± 1.8	31.3 ± 1.2	0.17	0.865
Initial leaf area (cm^2)	Judbury Bluff 1996	44.2 ± 2.0	33.5 ± 1.7	4.65	<0.001
Final leaf area (cm^2)	Judbury Bluff 1996	38.8 ± 1.9	31.5 ± 1.5	3.33	0.002
% Leaf lost	Judbury Bluff 1996	12.0 ± 1.1	5.9 ± 0.4	6.75	<0.001
Egg batch/leaf	Judbury Bluff 1996	0.02 ± 0.01	0.05 ± 0.0	2.90	0.006
Egg batch/m^2 surface leaf area	Judbury Bluff 1996	4.2 ± 0.0	14.3 ± 0.4	3.34	0.002

A

B

Figure 4.2 Adult *Chrysophtharta bimaculata* feeding damage at Judbury Bluff on **(A)** *E. regnans* left and *E. delegatensis* right and **(B)** *E. delegatensis* left and *E. regnans* right.

4.3.2. A comparison of adult *C. bimaculata* feeding damage and egg numbers in a pure stand of *E. regnans*

The mean number of leaves sampled from the lesser defoliated tree in each pair (29.7 ± 0.18) was not significantly different compared from the greater defoliated trees (29.5 ± 0.42) [$t_{0\,05\,(2),\,14}$ = 2.14, P(|t| ≥ 0.468) = 0.647]. There were no significant differences between *C. bimaculata* egg batch numbers per leaf or egg batches/m^2 of surface leaf area of relative to the percentage of expanding leaf material defoliated by beetles. The trees in each *E. regnans* pair that received the higher percentage of leaf area loss through beetle feeding received 0.42 egg batches/leaf and 328.9 egg batches/m^2 of leaf surface area. This compared to 0.36 egg batches/leaf and 271.8 egg batches/m^2 of leaf surface area received for those *E. regnans* trees (in each pair) which had received the lower defoliation level (Table 4.2).

Table 4.2 Oviposition patterns by *C. bimaculata* on paired *E. regnans* trees which had received (1) a higher level of defoliation and (2) a lower level of defoliation. The mean percentage of leaf area lost through adult feeding damage is also given. Error values are ± S.E. (n=15, d.f. =14, t-crit = 2.14).

	E. regnans (1)	*E. regnans* (2)	t. stat	P-value
Oviposition patterns				
Egg batch/leaf	0.42 ± 0.04	0.36 ± 0.06	0.72	0.486
Egg batch/m^2 surface leaf area	328.9 ± 30.7	271.8 ± 45.4	1.00	0.385
% Leaf area lost	11.7 ± 0.8	8.7 ± 0.8	4.16	<0.001

There was a weak but significant correlation between the percentage current seasons defoliation and egg batches per leaf (r^2 = 0.23, $F_{2,27}$ = 4.01, P = 0.030) for the *E. regnans* trees sampled in the Plenty Valley using polynomial regression. The correlation was greater between current seasons defoliation and egg batches/m^2 of surface leaf area using the same method of regression (r^2 = 0.31, $F_{1,29}$ = 5.90, P < 0.001) (Fig. 4.2). Thus egg batches per leaf and per m^2 of leaf surface area initially increase with increasing levels of defoliation but beyond 0.43 egg batches/leaf and 375 egg batches/m^2 of leaf surface area the relationship becomes negative.

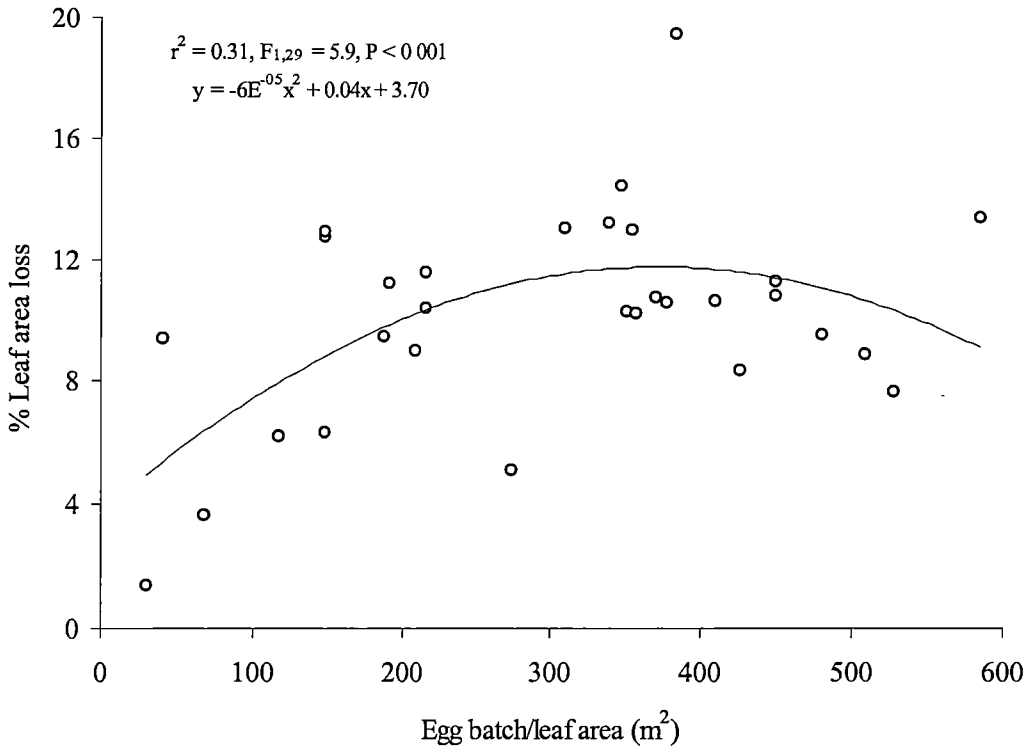


Figure 4.3 Leaf area loss through *Chrysophtharta bimaculata* adult feeding versus *C. bimaculata* egg batches per leaf area for *Eucalyptus regnans* trees in the Florentine Valley.

4.3.3 The influence of leaf scalloping on *C. bimaculata* oviposition preferences

There was no significant difference between egg batch deposition between scalloped or non-scalloped artificial leaves [$t_{0.05\ (2),\ 9} = 2.26$, $P(|t| \geq 1.22) = 0.252$]. The scalloped leaves averaged 2.7 ± 0.8 egg batches per replicate compared to 1.7 ± 0.4 egg batches for non-scalloped leaves.

4.3.4. The influence of manually reducing leaf area on *C. bimaculata* oviposition choice between *E. regnans* and *E. delegatensis*

C. bimaculata preferentially oviposited on *E. delegatensis* over *E. regnans* leaves despite the reduced leaf area in experiment (i) (Table 4.3). *E. regnans* lost significantly more leaf area through beetle feeding, however *E. delegatensis* lost a significantly higher percentage of leaf area compared to *E. regnans* (Table 4.3).

Table 4.3 Adult *C. bimaculata* feeding and oviposition patterns on two *Eucalyptus* species. The leaf area of treatments in the shaded boxes was manually reduced. Error values are ± S. E.

Experiment	Feeding & Oviposition patterns	Tree species		No. of reps	d.f.	t-crit.	t. stat	P-value
		E. regnans	*E. delegatensis*					
i	Initial leaf area (cm^2)	493.4 ± 32.9	232.0 ± 15.5	15	14	1.76	7.49	<0.001
i	% Leaf area lost	11.7 ± 0.9	18.3 ± 1.2	15	14	1.76	4.53	<0.001
i	Leaf area lost (cm^2)	57.7 ± 5.1	42.5 ± 2.7	15	14	1.76	4.44	<0.001
i	Egg batches	1.1 ± 0.1	2.0 ± 0.1	15	14	1.76	2.61	0.021
ii	Initial leaf area (cm^2)	230.8 ± 15.4	531.9 ± 35.4	15	14	1.76	8.06	<0.001
ii	% Leaf area lost	20.0 ± 1.3	11.3 ± 0.8	15	14	1.76	5.63	<0.001
ii	Leaf area lost (cm^2)	46.2 ± 4.0	60.3 ± 7.4	15	14	1.76	1.68	0.120
ii	Egg batches	0.6 ± 0.0	1.7 ± 0.1	15	14	1.76	3.76	0.002
iii	Initial leaf area (cm^2)	319.8 ± 24.2	326.4 ± 70.8	10	9	2.26	8.06	0.786
iii	% Leaf area lost	19.2 ± 1.7	14.3 ± 1.9	10	9	2.26	1.69	0.128
iii	Leaf area lost (cm^2)	61.1 ± 6.1	46.7 ± 6.5	10	9	2.26	1.50	0.168
iii	Egg batches	1.6 ± 0.4	3.6 ± 0.5	10	9	2.26	3.46	0.007

When *E. regnans* foliage was reduced in area (experiment ii), *E. delegatensis* leaves still received significantly more egg batches while *E. regnans* lost a significantly higher percentage of leaf area. However, there was no significant difference between actual leaf area loss (Table 4.3).

When neither *Eucalyptus* species had their leaf area manually reduced (experiment iii), *E. delegatensis* again received significantly more egg batches compared to *E. regnans* leaves (Table 4.3). However, there were no significant differences in percentage leaf area lost between the two eucalypt species or actual leaf area lost through beetle feeding. (Table 4.3).

4.3.5 The influence of conspecific feeding damage on *C. bimaculata* oviposition choice

E. regnans leaves which had not received conspecific damage were significantly favoured for oviposition over *E. regnans* leaves which had received previous conspecific feeding damage. There was no significant difference between the percentage leaf loss or actual leaf loss through beetle feeding between the two treatments (Table 4.4).

Table 4.4 Adult *C. bimaculata* feeding and oviposition patterns on *E. regnans* foliage damaged through adult feeding versus undamaged foliage. Leaf toughness is also compared. Error values are ± S. E. (n=20, d.f.=19, t-crit=2.0).

	E. regnans treatment			
	damaged	not damaged	t. stat.	P-value
Initial leaf area (cm^2)	335.1 ± 8.8	333.9 ± 7.8	0.64	0.529
% Leaf area lost	6.6 ± 0.4	7.6 ± 0.4	1.85	0.080
Leaf area lost (cm^2)	22.1 ± 1.6	25.6 ± 0.8	1.82	0.084
Egg batches	1.0 ± 0.2	1.9 ± 0.3	2.49	0.022
Leaf toughness (g)	31.4 ± 0.1	31.4 ± 0.1	0.18	0.860

In experiment ii, there was no significant difference in oviposition preference between *E. delegatensis* leaves which had received previous conspecific feeding damage leaves and undamaged *E. regnans* leaves. However, there was a significant

difference between percentage leaf area loss and actual leaf area loss between the two treatments (Table 4.5).

Table 4.5 Adult *C. bimaculata* feeding and oviposition patterns on *E. delegatensis* foliage damaged through adult feeding versus undamaged *E. regnans* foliage. Leaf toughness is also compared. Error values are ± S. E. (n=20, d.f.=19, t-crit=2.0).

	Treatment			
	E. delegatensis damaged	*E. regnans*	t. stat.	P-value
Initial leaf area (cm^2)	305.4 ± 7.3	305.0 ± 6.2	0.20	0.842
% Leaf area lost	5.0 ± 0.4	6.4 ± 0.4	3.76	0.001
Leaf area lost (cm^2)	15.1 ± 1.0	19.2 ± 0.9	3.69	0.002
Egg batches	1.4 ± 0.2	1.2 ± 0.2	0.45	0.659
Leaf toughness (g)	31.2 ± 0.1	31.1 ± 0.0	1.39	0.179

4.3.6 Larval development on expanding *E. regnans* and *E. delegatensis* foliage

C. bimaculata larvae gained weight more quickly feeding on *E. delegatensis* foliage compared to *E. regnans* (Figure 4.2). Larvae also reached pre-pupation (cease feeding and drop from foliage) significantly faster on *E. delegatensis* (12.0 ± 0.2 days) than on *E. regnans* (13.9 ± 0.3) [$t_{0.05\,(2),\,6} = 2.45$, $P(|t| \geq 6.49) = 0.001$] and had significantly greater pupal weights: *E. delegatensis* fed (45.7 ± 0.8 mg); *E. regnans* fed (39.6 ± 1.0 mg) [$t_{0\,05\,(2),\,6} = 2.45$, $P(|t| \geq 4.55) = 0.002$]. A higher percentage of larvae also survived on *E. delegatensis* (88%) compared to *E. regnans* (77%) however the difference was not significant [$t_{0.05\,(2),\,6} = 2.45$, $P(|t| \geq 2.18) = 0.072$].

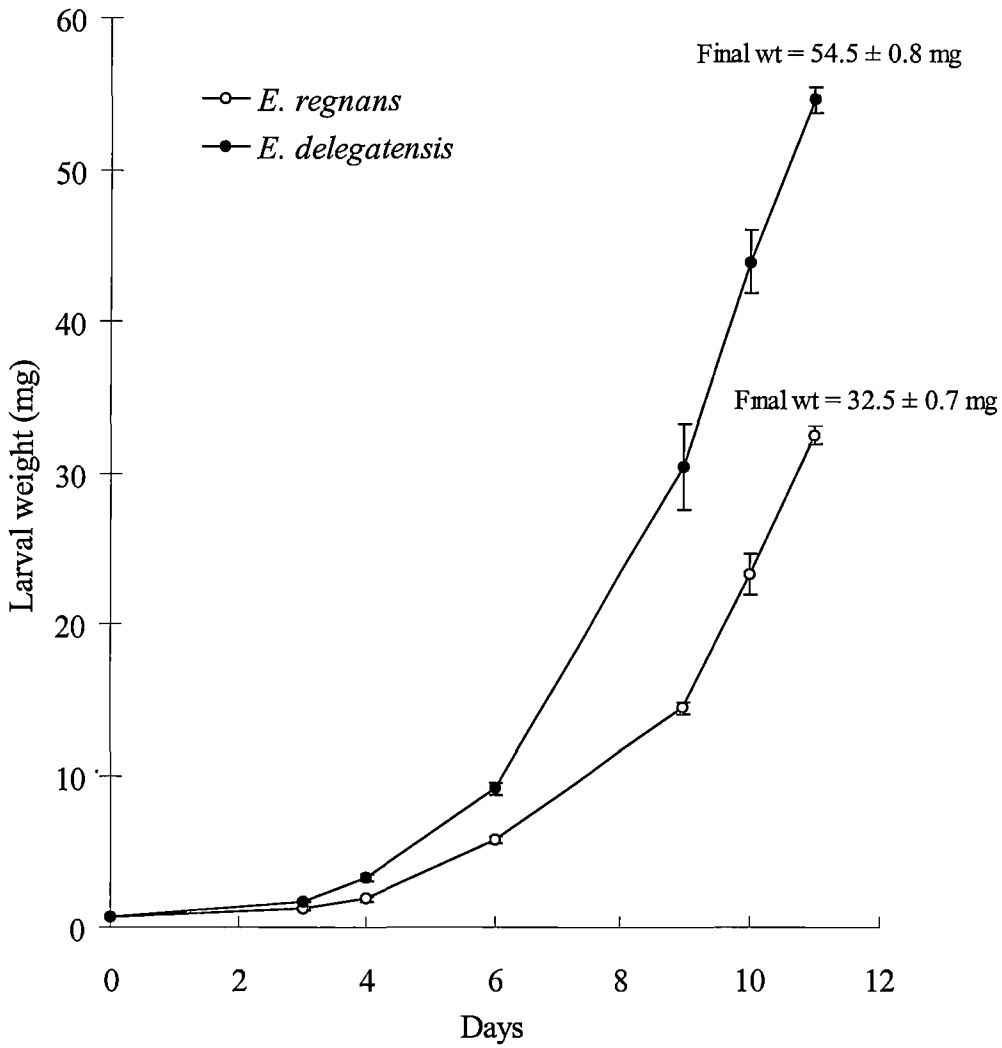


Figure 4.4 Mean (± S. E.) weight increase over time for *C. bimaculata* larvae developing on *E. delegatensis* and *E. regnans* leaves (n=8).

4.4 Discussion

For mixed stands of *E. delegatensis* and *E. regnans*, *C. bimaculata* adults appear to utilise the two host tree species in different ways. *E. regnans* loses a higher percentage of leaf area through adult feeding, however *E. delegatensis* receives more egg batches. This phenomenon occurred at one site over two seasons and at two different sites where trees were examined, independent of which tree species was more abundant.

Laboratory experiments found a similar oviposition preference for *E. delegatensis* over *E. regnans*, even when the leaf area of *E. delegatensis* was manually reduced. Given that leaf numbers were the same in each replicate, a reduction of leaf area through beetle feeding does not appear to influence beetle oviposition choice. This finding is in agreement with the those of chapter 3, which indicated that increasing leaf number (of expanding and newly expanded leaves), not leaf area, increases the likelihood of a higher egg batch count.

The oviposition preference for *E. delegatensis* over *E. regnans* observed at Judbury Bluff appeared beneficial for larval development under laboratory conditions. Larvae developing on *E. delegatensis* gained weight faster, reached pre-pupation earlier and had significantly greater pupal weights compared to those developing on *E. regnans* foliage from Judbury Bluff. These results agree with Li (1993), who found that *C. bimaculata* larvae feeding on Tasmanian *E. delegatensis* provenances consumed more leaf material, produced more frass, had greater larval weights and greater moulting frequencies compared to those feeding on *E. regnans* provenances. However, the importance of abiotic (e.g. exposure) and biotic factors (e.g. predation and parasitism) on egg and larval mortality for different host species has not been examined and may differ between host species.

Comparisons within a stand consisting entirely of *E. regnans* did not show a strong correlation between feeding damage and egg batch numbers. There was a weak correlation between egg batches per m^2 of surface leaf area and feeding damage, however this relationship was probably due to the aggregative nature of the insect

(see Clarke et al. 1997), with beetle density varying greatly over small areas. When trees were paired and compared based on percentage leaf loss, those with higher defoliation did not contain significantly more egg batches. However, a polynomial regression of these trees did reveal a correlation between feeding damage and egg batch number and egg batch/m^2 of surface leaf area. This correlation suggested there was a positive relationship between oviposition and feeding patterns when feeding damage was low. However, the relationship becomes negative as egg batch number and egg batch/m^2 increase.

The laboratory experiments also found that *C. bimaculata* oviposition preference appeared to be influenced by foliage previously damaged through conspecific beetle feeding. Undamaged *E. regnans* foliage was significantly preferred for oviposition over foliage that had suffered feeding damage (Table 4.4). Likewise, when *E. delegatensis* foliage is damaged through beetle feeding, *C. bimaculata* oviposition preference for this species appears nullified when compared to undamaged *E. regnans* foliage (Table 4.5). The cause of the oviposition deterrence to beetle damaged foliage was not examined. but may be due to factors such as changes in plant chemistry and/or beetle products such as faecal material.

Several factors may be important in influencing the differences between feeding damage and egg batch distribution for *C. bimaculata.* Beetle feeding and/or oviposition may be influenced by conspecific egg batches, presence of other beetles, faecal material or plant damage. In Chapter 2 conspecific egg batches on leaf tips were found to reduce the chance of further oviposition. However, there was no evidence that beetles responded to an oviposition pheromone or through visual perception of conspecific egg batches. Laboratory experiments also failed to show any significant differences in feeding preference for female beetles on leaves with or without conspecific egg batches (Chapter 2).

Chapter 2 also demonstrated that the leaf tip is the most favoured position for egg batch deposition. While depositing eggs on a tip, nearly all beetles hold the leaf edge which may provide stability. Physical deformation of the leaf edge through beetle feeding damage could thus influence oviposition. The results using artificial

leaves show that this is probably not the case with no significant differences in egg batch numbers between scalloped or plain edged leaves.

The tendency of *C. bimaculata* beetles to aggregate (Clarke et al. 1997) and the ability of females to deposit batches of up to 50 eggs, may explain why major localised defoliation of host plants occurs. For *C. bimaculata*, which utilises the same host trees for adult and larval feeding, it is beneficial for the insect to avoid high levels of adult defoliation of trees that also have large numbers of egg batches deposited upon them. Greaves (1966) observed *C. bimaculata* larvae removing all current seasons foliage from trees and concluded that a large proportion are likely to have died through starvation (also observed by myself & V. Patel, CRC Forestry). The frequency of larval starvation is likely to be increased if adult beetles cause heavy defoliation of trees that are to be used for larval development. A strong correlation between adult beetle feeding and oviposition would also leave conspecific egg batches more vulnerable to beetle feeding damage. Damaged egg batches believed to have been caused by *C. bimaculata* beetle feeding have been observed in the field (Fig. 4.5).

For mixed stands of *E. delegatensis* and *E. regnans* the evolution of a distinct feeding preference for *E. regnans* and oviposition preference for *E. delegatensis* would help reduce conspecific damage to egg batches and larval starvation. However, under natural conditions, *Eucalyptus regnans* is most often found in pure stands (Ashton 1981) and only occasionally exists ecotonally with *E. delegatensis* (Williams & Potts 1996). The fact that these two eucalypt species only occasionally form mixed stands suggests that there is little pressure on *C. bimaculata* to evolve distinct feeding and oviposition preferences based on a competition arguement. A more likely explanation is that *E. delegatensis* simply offers a better larval resource than *E. regnans* (as demonstrated in Fig. 4.2), but that *E. regnans* is a better food resource for adults (not tested). Such differences in adult/larval host suitability for insects which repeatedly use the same hosts in the same way has been reported (Velasco & Walter 1992).

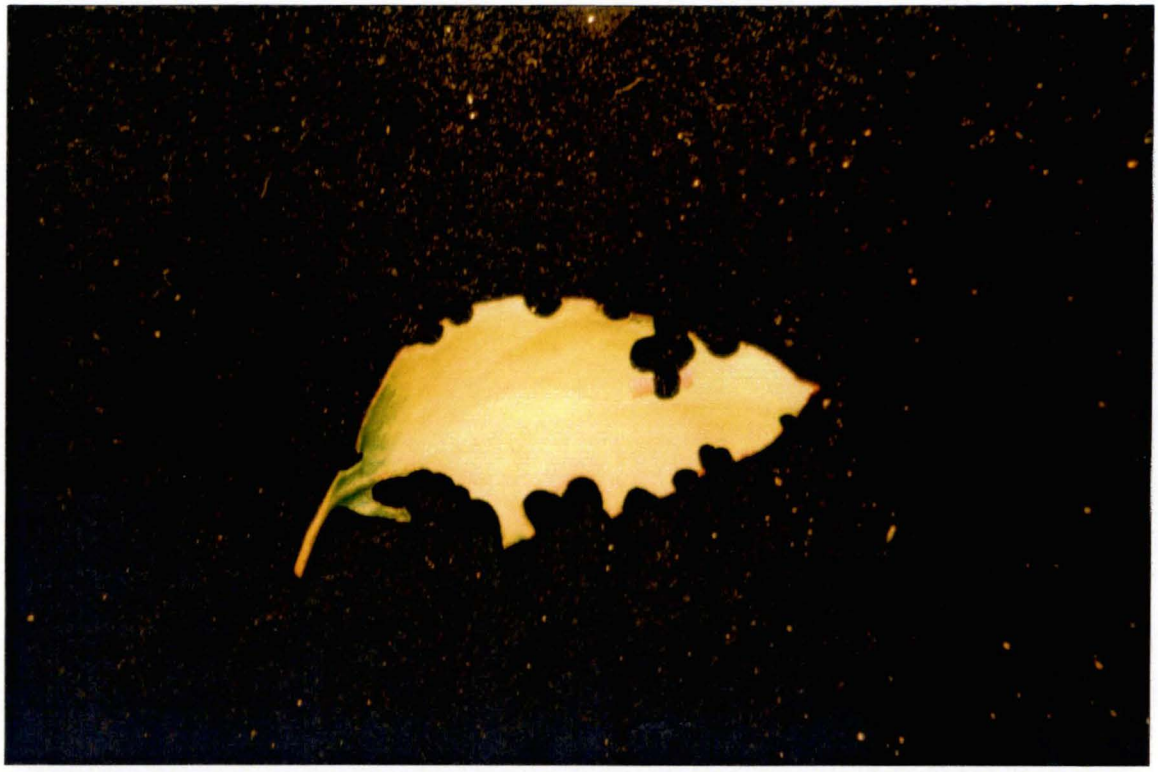


Figure 4.5 *C. bimaculata* egg batch on a *E. regnans* leaf collected from Judbury Bluff and believed to have been damaged through adult conspecific feeding.

Although this study has shown that there is no strong relationship between beetle feeding damage and oviposition for beetles in natural aggregations, the factors responsible for this low level of correlation have not been determined. Females are more reluctant to deposit eggs on foliage that has been damaged by conspecific feeding adults indicating that the associated factors (i.e. changes in plant chemistry, substances emitted from beetles) may play a role. However, other factors may be responsible such as differences in oviposition and feeding cues and physical interference by conspecifics.

Chapter 5

Chrysophtharta bimaculata oviposition site choice in relation to leaf toughness and neonate larval survival

The work from this chapter can be found in: Howlett, B. G., Clarke, A. R. & Madden, J. L. (2001) The influence of leaf age on the oviposition preference of *Chrysophtharta bimaculata* (Olivier) and the establishment of neonates. *Agricultural and Forest Entomology* (in press).

It has been modified here to aid thesis continuity.

5.1 Introduction

Phytophagous insect populations interact with other species at three trophic levels: the host plant, other folivores and natural enemies (Faeth 1987). Each of these trophic levels is, inturn, influenced by weather, habitat and heritable traits (Wallner 1987; Chapter 1). These interactions, along with the phylogenetical constraints of the insects lifecycle, influence the population dynamics of an insect.

Price (1994) hypothesised that phylogenetic constraints influence the nature of phytophagous insect populations by setting limits to the evolution of life history patterns and behaviours. Species evolve adaptive syndromes to minimise the limitations of phylogentic constraints which inturn determine the emergent properties exhibited by their population dynamics (Price 1994). Price et al. (1990) and Price (1992) describe two extreme types of herbivore population dynamics. At one end of the spectrum are eruptive species, which can contain an epidemic phase in their life-cycle where populations can fluctuate over three to five magnitudes order. Latent species, in contrast, have populations that only fluctuate in the range of one to two orders of magnitude (Price et al. 1990).

The most distinctive feature that separates eruptive species from non-eruptive species is the ability of females to assess oviposition sites based on the suitability for their offsprings' survival (Price et al. 1990; Dodge & Price 1991; Price 1992). Eruptive insects such as *Disonycha plurigata* (LeConte), a chrysomelid beetle, show unspecific ovipositional choice regardless of the oviposition site's suitability for larval survival (Dodge & Price 1991; Marques et al. 1994). In contrast, latent species such as galling tenthredinid sawflies, *Euura* sp. and *Pontania* sp. (Stein & Price 1995) and the orange-tip butterfly, *Anthocharis cardamines* (L.) (Dempster 1997), show a strong positive relationship between ovipositional choice and larval survival.

The specificity of female oviposition choice has major implications for population dynamics. Larvae of indiscriminate species have the ability to feed on foliage of

variable quality, with the ultimate controlling factor on their populations being larval starvation (Price 1994). For latent species, competition by females for limited oviposition sites is likely to be the controlling factor on population size (Price 1992;1994).

There are other traits that are likely to be associated with eruptive species. These include high fecundity, clumping of egg batches, low adult female dispersal and the ability of larvae to disperse from the oviposition site (Price et al. 1990). However, eruptive species may not show all these characteristics which are of secondary importance to oviposition preference (Price et al. 1990).

Chrysophtharta bimaculata (Olivier), causes major defoliation to several *Eucalyptus* species from the subgenus *E. Monocalyptus* in Tasmania, Australia. *Eucalyptus (Symphomyrtus) nitens*, an introduced plantation species, is also utilised as a host (de Little 1989). Both native and plantation stands are attacked (Clarke et al. 1998) and, on young trees all current seasons growth may be removed through larval feeding (Greaves 1966). In the most severe cases, tree death may occur (Elliot et al. 1992). Field observations in the Florentine Valley (42°39'S, 146°28'E) on *E. regnans* indicate that there is a high degree of fluctuation (up to 25 fold) in *C. bimaculata* populations from year to year (Elek 1997).

The life-cycle of *C. bimaculata* has a number of traits that are similar to those of eruptive insects. Adults form large aggregations (Clarke et al. 1997), often containing thousands of individuals that feed and oviposit heavily within patches of its host. Eggs are deposited in batches, usually in the range of 10-50 eggs (Greaves 1966) and larvae in the first three instars (of four) are gregarious. Females can also produce a large number of eggs, with de Little (1983) reporting that individual females may lay over 1500 eggs (mean production of 674 eggs/female) during their lifetime in the laboratory.

Chrysophtharta bimaculata, however, is unlike those species Price (1990) classes as eruptive. Females in the laboratory show a strong preference for oviposition on soft,

expanding leaves over tough, mature leaves (Steinbauer et al. 1998). This suggests oviposition discrimination, as young leaves are utilised by neonate paropsine larvae (Greaves 1966; Larsson & Ohmart 1988). This preference is not complete, however, as eggs are regularly deposited on mature foliage in the field (Greaves 1966).

Like *C. bimaculata*, many insects that feed on woody plants have larvae that can only feed on newly developed, expanding leaves. Older leaves tend to be tougher, have lower moisture and nitrogen contents, and higher secondary compound levels, than young leaves (Morrow 1980; Lowman & Box 1983). This makes it difficult for younger larvae to establish on old leaves. Leaf toughness has been suggested as a major factor influencing the establishment of paropsine larvae (Ohmart et al. 1987; Larsson & Ohmart 1988) and is the factor focussed on in this chapter. In other taxa, neonate larvae may find older leaves difficult to establish upon as they have comparatively lower chewing capacity than later instars (Jordano & Gomariz 1994).

In order to determine the strength of the oviposition preference - neonate performance linkage in *C. bimaculata*, the following investigations were undertaken: (i) the critical leaf toughness for neonate larval survival was quantified in the laboratory; (ii) with respect to that critical leaf toughness, the distribution of egg batches on three host species was surveyed in the field and; (iii) the ability of neonate larvae to migrate from leaves unsuitable for survival and establish on leaves that are suitable was examined on cut shoots. The results of these experiments are then discussed in terms of *C. bimaculata*'s potential population dynamics, i.e latent versus eruptive.

5.2 Materials and Methods

5.2.1 Determining leaf toughness critical for neonate larval establishment on *Eucalyptus regnans, E. delegatensis* and *E. nitens*

Foliage of *E. nitens, E. regnans* and *E. delegatensis* was collected from trees within the Florentine Valley (42°39'S, 146°28'E). In the laboratory, the toughness of individual leaves was measured (2.2. General Materials and Methods). Individual leaves with a portion of connecting shoot stem were then placed individually into water filled vials. The leaf blade was left protruding at least 1 cm from the rim of the vial and Tanglefoot® (The Tanglefoot Company, Grand Rapids, Michigan, 49504, USA) was then placed around the vial-rim to stop migrating larvae.

Egg batches of approximately 15-20 eggs/batch were obtained from laboratory cultures of field collected adults. One egg batch was attached to each test leaf by firstly cutting two small (~ 5mm wide) slits in the test leaf then threading the egg batch (on a cut section of leaf) through the slits. The distance between slits was approximately 1 cm less than the length of the leaf section with eggs allowing for secure attachment. The small piece of leaf on which eggs had been deposited, dried prior to eggs hatching and was rejected as a larval food source. Eggs were then allowed to hatch and neonates to commence feeding. At the end of 72 hours the number of larvae surviving was counted. Any leaves that showed signs of desiccation, or those where eggs had failed to hatch, were excluded from the results.

The experiment was conducted in a 25°C constant temperature glasshouse. In the first run of the experiment, 25 vials were allocated per tree species, containing leaves ranging in toughness from 22.5 - 69.0 g. The initial toughness range of leaves chosen represented the full suite of leaves expected within a tree from expanding to previous season leaves. Subsequent replicates used leaves with a narrower range of leaf

toughness, concentrating around the critical leaf toughness. In all, there were 100 replicates of *E. regnans*, 94 of *E. delegatensis* and 95 of *E. nitens*.

Because a number of different experimental factors other than leaf toughness (eg genetic variability of larvae and variability in leaf chemistry), may have caused partial mortality within cohorts, a conservative approach was taken to determining whether leaves were "suitable" or "unsuitable" for larval establishment. Only leaves where no larvae established were considered unsuitable based on leaf toughness. Leaves with at least one larva surviving were deemed suitable. This may have biased results in that the true maximum leaf toughness might be indicated as very large, but this was deemed less of a fault, than categorising unsuitable leaves as suitable.

5.2.2 The toughness of leaves which neonate larvae establish on in the field

In the field, five trees each of *E. regnans, E. delegatensis* and *E. nitens* were monitored in the Southern Forests (43°03'S, 146°53'E) to determine the maximum leaf toughness on which neonate *C. bimaculata* would establish. Trees were selected that had been naturally oviposited on by wild *C. bimaculata* and these trees were then examined every two days until larvae had hatched and become established.

For each tree a single branch with approximately 20-40 leaves was selected. The oldest leaf, nearest to the trunk, was then examined for newly established larvae. If first instar larvae were present and less than 75% (by visual estimation) of the leaf area had been consumed, then the number of larvae present and the leaf toughness were recorded. A minimum of 75% leaf area remaining was required to ensure good penetrometer readings. The next leaf, second nearest to the trunk, was then assessed and so on until the youngest leaf (furthest from the tree trunk) had been examined. Only leaves along the primary shoot (the primary shoot has a diameter larger than all secondary shoots) were assessed. By examining only leaves on the primary shoot a larger range of leaf ages (and thus toughnesses) were sampled. This technique was repeated until 10 leaves

had been sampled per tree bearing feeding neonates. By initiating sampling near the base of a shoot, I ensured the oldest (=toughest) leaves with neonates were sampled.

5.2.3 Egg batch occurrence on *E. regnans*, *E. delegatensis* and *E. nitens* field trees

Field sites in southern Tasmania containing stands of *E. regnans*, *E. delegatensis* and *E. nitens*, which had been oviposited upon by wild *C. bimaculata* over a two day period, were selected. The *E. regnans* site was located in the Plenty Valley (42°50'S, 146°53'E), the *E. nitens* site near Judbury (42°50'S, 146°53'E) and the *E. delegatensis* site in the Southern Forests. Most trees within each site were between three and five metres in height.

From 25 trees of each species, five branches were selected which were estimated to contain between 60 and 100 leaves. The branches were selected from around the tree, i.e. no particular directional face was preferred, from a height of 100 to 300 cm. Using penetrometer readings as a guide, leaves were placed into three classes: (1) Suitable for neonate feeding (leaf toughness $\leq$ 58.5 g) (see Results); (2) unsuitable for neonate larval establishment (leaf toughness greater than $>$ 58.5 g), but within 20 cm shoot distance of suitable foliage; and (3) unsuitable for neonate larval establishment and $>$ 20 cm shoot distance away from suitable foliage. Preliminary observations indicated that very few egg batches were deposited further than 20 cm from suitable foliage. Therefore, unsuitable foliage was divided into two classes based on this distance. Thus, class 2 and class 3 leaves were distinguished only by the distance that neonate larvae had to migrate to reach suitable leaves. At all sites, data pertaining to leaves was collected before egg batches had hatched. The number of egg batches in each foliage class were also counted.

5.2.4 The ability of neonate larvae to migrate from unsuitable to suitable feeding foliage

Individual shoots of *E. delegatensis* and *E. regnans* were placed in water filled vials in a 25°C constant temperature glasshouse. Each shoot was trimmed so that it carried five leaves suitable for larval establishment and three unsuitable leaves, that were at least 20 cm from the nearest suitable leaf. An egg batch of between 14-20 eggs was placed on one of the unsuitable leaves and the eggs allowed to hatch. Larval cohorts were then monitored until two days after hatching when all surviving larvae had begun feeding (always on the terminal, suitable leaves). At this point the number of larvae surviving migration and the distance of larval migration was recorded. Concurrent to this experiment a control (non migrating) was established, in which egg batches were placed on suitable foliage of similarly treated shoots. The data records taken for the controls were the same as that for the treatments. Sixteen replicates of each treatment and control were run for each of the two eucalypt species.

5.2.5 Data analysis

Unless otherwise stated, Oneway Analysis of Variance was used to analyse data. When the results of an ANOVA were significant and more than one factor was involved, a post-hoc Tukey's test was used to determine which factors were significantly different. Percentage data on leaf toughness critical for neonate larval establishment (5.2.1) was arcsine transformed prior to analysis.

5.3 Results

5.3.1 Neonate larval establishment versus leaf toughness on *Eucalyptus regnans, E. delegatensis* and *E. nitens*

Neonate feeding always commenced along the leaf margin. On hosts where larvae failed to become established, the majority (92%) of leaves showed scalloping less than 2 mm deep where feeding had been started but not continued.

For all three host species, there was a distinct pattern of larval survivorship with increasing leaf toughness. Below a certain leaf toughness, all replicates had some survivors, while above a narrow critical range, establishment always failed. This pattern is shown in Figure 5.1 for *E. delegatensis* only, but was essentially identical for the other two species. Figure 5.2 combines the slopes of the three host species for all the leaf toughness values where neonate establishment occurred in at least some replicates, but beyond the toughness level where 100% establishment consistently occurred.

A comparison of the regression lines between the three host species was made using a General Linear Model and it was found that there were no significant differences in slopes ($F_{2,22} = 0.45$, $P = 0.64$) or elevations ($F_{2,24} = 1.71$, $P = 0.20$). The combined regression of the data (Fig. 5.2) predicts that larvae will not be able to establish beyond a leaf toughness of 59.1 g, while below 46.9 g some neonates should always establish.

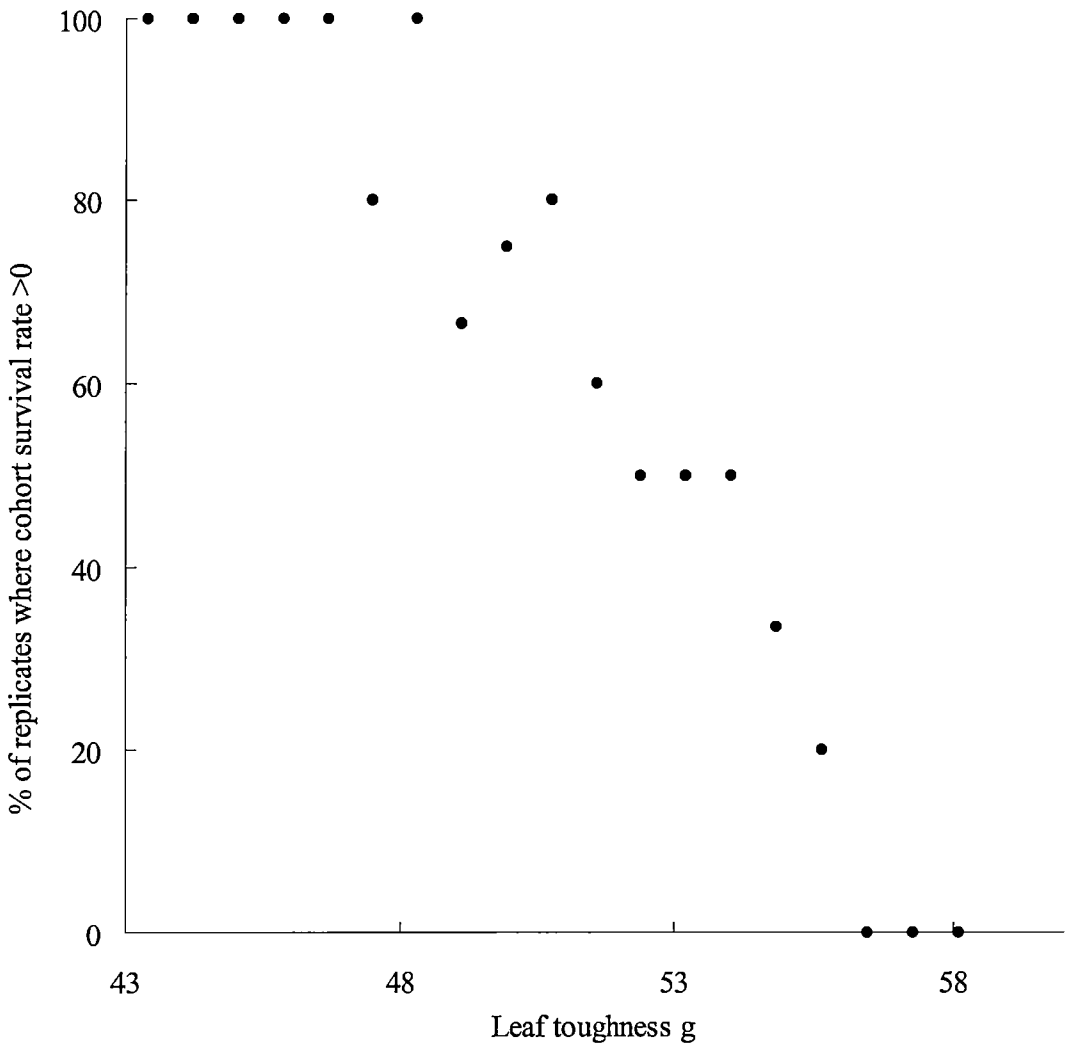


Figure 5.1 Establishment of *Chrysophtharta bimaculata* neonate larvae at different leaf toughness values for *Eucalyptus delegatensis* foliage. (Each data point is the percentage of replicates at a particular toughness value that had at least two established larvae from the initial egg batch. Minimum number of replicates per data point = 3.0, max number = 6.0, average number = 4.2).

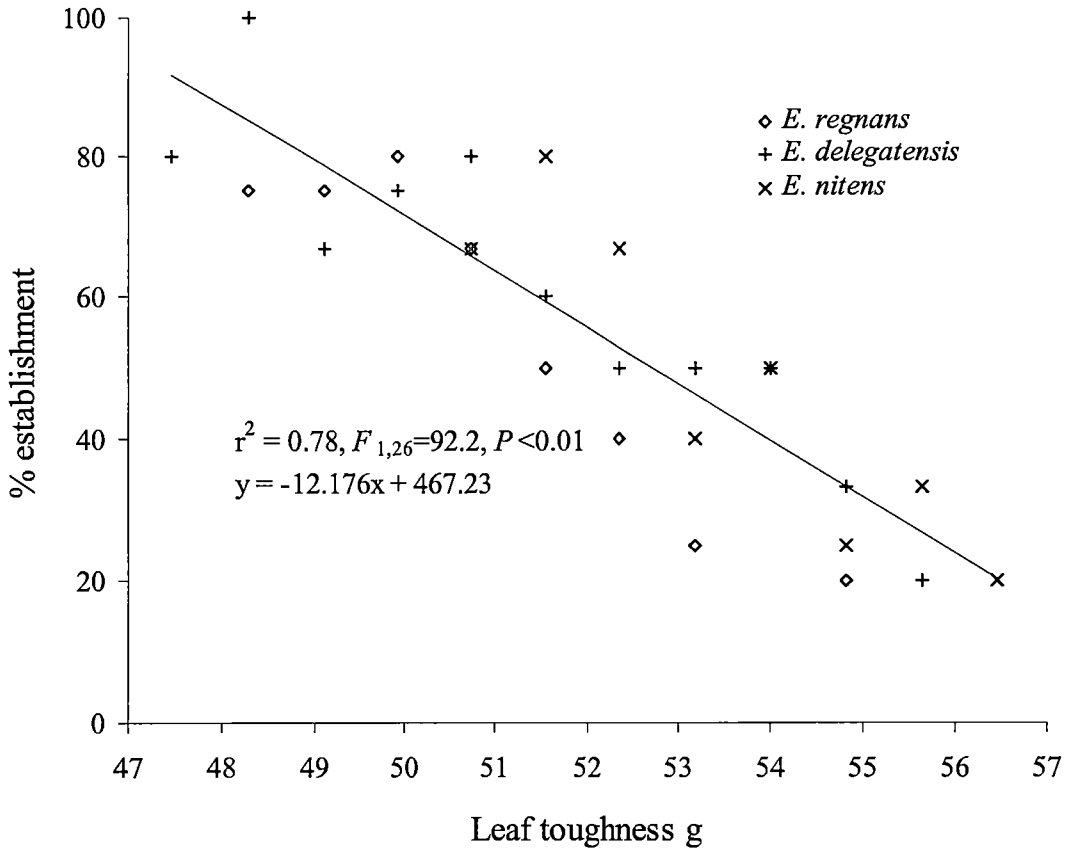


Figure 5.2. Establishment of *Chrysophtharta bimaculata* neonate larvae at different leaf toughness values for three host species, *Eucalyptus regnans*, *E. delegatensis* and *E. nitens*. (Each data point is the percentage of replicates at a particular toughness value that had at least two established larvae from the initial egg batch. Minimum number of replicates per data point = 3.0, max number = 6.0, average number = 4.9). Leaves tested with toughness below 46 g and above 60 g are excluded from this analysis as larval establishment was at 100% for leaves below 46 g, while on leaves above 37 g, no larval establishment occurred. The fitted regression line is for the combined data set.

5.3.2 The leaf toughness at which neonates establish in the field

There was a significant difference ($F_{2,14}$ =5.84, P=0.017) at the tree species level for the mean toughness of leaves on which first instar larvae were established (Table 5.1). Post-hoc Tukey tests revealed that for those leaves with larvae, *E. nitens* leaves were significantly tougher than *E. delegatensis* leaves, while *E. regnans* leaves were not significantly different from the other two *Eucalyptus* species. However, there was no

significant difference between the toughest leaf on which first instar larvae were established for the three *Eucalyptus* species ($F_{2,14}$ =1.83, P=0.203) (Table 5.1).

There was a larger mean number of larvae per leaf on *E. regnans* (with a post-hoc Tukeys test suggesting marginal significance with *E. nitens*)($F_{2,14}$ =3.65, P=0.058). However, larval numbers may be a reflection of different densities in the tree due to original egg batches deposited rather than an effect of tree on larval survival.

Table 5.1. The mean leaf toughness, toughest leaf and number of *Chrysophtharta bimaculata* larvae found on leaves of three *Eucalyptus* species in the field (means in the same column followed by the same letter are not significantly different, *post-hoc Tukey test marginal. Error values are ± S. E.).

Host Species	Mean leaf toughness (g)	Toughest leaf larvae established on (g)	Mean number of larvae
E. nitens	36.8 ± 1.8 a	48.1 ± 1.1 a	12.5 ± 0.9 a*
E. regnans	34.8 ± 2.1 a b	46.2 ± 1.5 a	17.7 ± 1.9 b*
E. delegatensis	32.6 ± 2.0 b	44.8 ± 1.2 a	14.4 ± 2.7 a b

5.3.3 Egg batch occurrence on *E. regnans*, *E. delegatensis* and *E. nitens* field trees

On the three *Eucalyptus* species, there were significant differences between numbers of leaves in each class and the proportion of class 1 to class 2 leaves (for between *Eucalyptus* spp. comparisons of class 1, 2, 3 and the proportion 1-2 leaves, df = 2,72, respectively, F = 33.12, 6.47, 11.78, 16.38; p<0.01 for all classes) (Table 5.2). The different number of leaves in each leaf class may have influenced the within tree egg batch distribution across tree species. Consequently, the data on egg batch deposition in relation to leaf class is not pooled across species.

Table 5.2. Mean number of leaves from three leaf classes on 5 branches of three *Eucalyptus* species. (n = 25 trees/species. Class 1 leaves have a leaf toughness ≤ 58.5 g; class 2 leaves have a leaf toughness > 58.5 g and are within 20 cm shoot distance of class 1 foliage; class 3 leaves have a leaf toughness > 58.5 g and are > than 20 cm shoot distance of class 1 foliage. Means within columns followed by the same letter are not significantly different at $p<0.05$ Error values are ± S. E.).

***Eucalyptus* species**	**Class 1 leaves**	**Class 2 leaves**	**Class 3 leaves**	**Class 1/Class 2 leaves**
E. regnans	163.5 ± 9.5 a	103.0 ± 6.4 a	139.6 ± 11.1 a	1.7 ± 0.1 a
E. delegatensis	102.4 ± 3.6 b	91.4 ± 3.0 ab	91.5 ± 3.7 b	1.1 ± 0.0 b
E. nitens	101.2 ± 3.4 b	81.6 ± 1.6 b	152.2 ± 11.1 a	1.2 ± 0.0 b

Figures 5.3a, b and c indicate that although egg batches are more frequently found on class 1 leaves, they are also common on class 2 leaves. For each species there was a strong linear relationship between the percentage of egg batches found on class 1 and class 2 leaves (Figure 5.3).

Linear regressions predict that when egg batches occupy only a small percentage of class 1 leaves (less than 3%), egg batches will be found on class 2 leaves, for all three host species. As predicted by the slope of the regression lines (Figure 5.3), the ratio of egg batches on suitable foliage (class 1) versus unsuitable foliage (class 2) varied between host species, e.g. 2:1 for *E. regnans*, 2.2:1 for *E. nitens* and 3:1 for *E. delegatensis*.

Although egg batches are common on foliage unsuitable for neonate feeding, very few of the occupied leaves occurred further than 20 cm away from leaves which were suitable (i.e. class 3 leaves). For each of the 25 trees per host species examined, *E. regnans* had only 7 occupied class 3 leaves (1.05% of all occupied leaves), *E. nitens* five (0.76%) and *E. delegatensis* one (0.19%). This was despite class 3 leaves constituting approximately one-third or greater of all leaves on a shoot (Table 5.2).

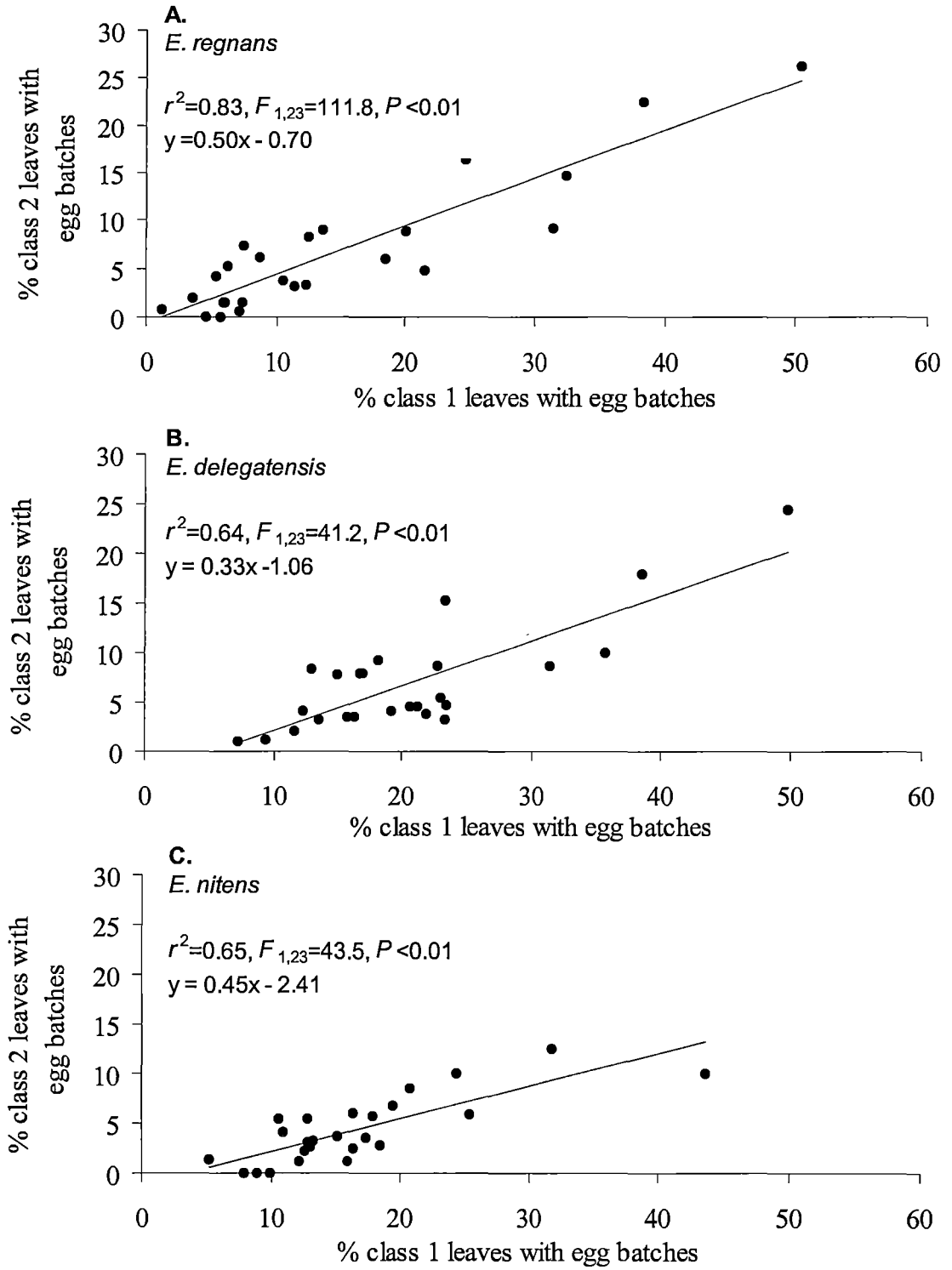


Figure 5.3. Percentage of leaves carrying *Chrysophtharta bimaculata* egg batches in two leaf classes for 25 trees of (a) *Eucalyptus regnans* (b) *Eucalyptus delegatensis* and (c) *Eucalyptus nitens*. Class 1 leaves are suitable for neonate larval establishment (leaf toughness less than 58.5 g) and class 2 leaves are unsuitable for neonate larval establishment (leaf toughness greater than 58.5 g, within 20 cm of class 1 leaves). The slopes of the regressions are not significantly different between *E regnans* & *E. nitens* ($F_{1,46}$ = 0.37, P = 0.34) and *E. delegatensis* & *E. nitens* ($F_{1,46}$ = 2.04, P = 0.16), but are significantly different between *E. regnans* & *E. delegatensis* ($F_{1,46}$ = 5.61, P = 0.02).

5.3.4 The ability of neonate larvae to migrate from class 3 leaves to class 1 leaves

Neonate establishment on *E. regnans* and *E. delegatensis* was high in both control and migration treatments. No significant differences in larval establishment were found using balanced ANOVA, between host species ($F_{1,60}$ =0.93, P=0.338), treatments, (migrating, non migrating) ($F_{1,60}$ =2.38, P=0.128), or treatment x species interaction ($F_{1,60}$ =0.11, P=0.745).

In the migration treatments, larvae did not always migrate to the same expanding leaf. For *E. delegatensis*, only four replicates (from 16) contained all of the established larvae on the one leaf, while *E. regnans* had six replicates (from 16). The maximum number of leaves with established larvae in a replicate was five for *E. delegatensis* and four for *E. regnans*. The mean maximum and minimum distances travelled by individuals from a cohort was 44.8 ± 0.73 cm and 20.6 ± 0.27 cm for *E. delegatensis* and 56.4 ± 0.25 cm and 20.0 ± 0 cm for *E. regnans*. There was no significant correlation between larval survival and mean cohort migration distance for *E. delegatensis* (r = 0.16, $F_{1,14}$ = 0.38, P = 0.55) or *E. regnans* (r = 0.07, $F_{1,14}$ = 0.0.08, P = 0.79).

Figure 5.4 *Chrysophtharta bimaculata* larvae feeding on the chorion following hatching. In this example, egg batches were dyed blue using food colouring. After feeding on the chorion most larvae will migrate to the leaf edge to feed if the leaf is suitable. If the leaf is too tough for establishment larvae will migrate to other leaves.

5.4 Discussion

Oviposition choice and larval migration

Results from this study indicate that in the wild *C. bimaculata* deposit the majority of their egg batches on leaves that are suitable for neonate larval establishment (class 1 leaves). This supports Steinbauer et al.'s (1998) laboratory findings that expanding leaves are preferred for oviposition. However, a proportion of egg batches are also deposited on fully expanded leaves unsuitable for neonate larval establishment (class 2 leaves). Egg batches occur on class 2 leaves even when there is a low egg batch saturation of class 1 leaves, indicating that *C. bimaculata* shows some, but not complete selectivity in its oviposition site location when populations are aggregated.

However, laboratory experiments indicated that neonate larvae are able to successfully migrate more than 20 cm from unsuitable leaves to suitable leaves and become established. In these experiments mortality of migratory larvae was not greater than the control larvae, which did not need to migrate. A minimum migratory ability of 20 cm appears sufficient for most larvae, as eggs are rarely laid more than 20 cm away from the nearest suitable leaf. Thus, while *C. bimaculata* may not oviposit all their eggs directly on suitable leaves, eggs are positioned sufficiently close to suitable leaves to minimise neonate mortality.

By depositing a proportion of egg batches on fully mature leaves, *C. bimaculata* may effectively be 'spreading the risk'. Adult beetles utilise similar *Eucalyptus* species for feeding and oviposition and prefer to feed on expanding foliage. This could potentially lead to damage of conspecific egg batches (see figure 4.5). Thus, egg batches deposited on old foliage should be at less risk to feeding damage than those on expanding foliage.

In addition to migration from unsuitable to suitable leaves, movement by neonate *C. bimaculata* must also occur between apparently suitable leaves. Firstly, the field results indicate that most first instar larvae establish on leaves with a toughness around 31-37 g, well below the estimated critical value of 58.5 g. This suggests larval selectivity for feeding sites in the field. Secondly, it was found in glass-house trials, that groups of *C. bimaculata* neonates may have some or all members move off leaves suitable for establishment. The dispersal of some larvae may be advantageous by making more, and/or better, host resources available, or by spreading the risk in event of catastrophe to the natal leaf (Myers & Campbell 1976). Migration of neonate larvae may also facilitate the formation of larger larval aggregations. *Chrysophtharta bimaculata* larvae have well developed defence glands and pooling them may provide better defence against predators (Jolivet et al. 1990). Larger aggregations can also reduce the individual risk of each larvae to predation or parasitism (Grégoire 1988).

The population dynamics of C.bimaculata

The egg batch distributions from aggregated populations of *C. bimaculata* show a strong selection for oviposition sites suitable for larval establishment. Thus, according to Price's (1994) phylogenetic constraints hypothesis, *C. bimaculata* more closely resembles a latent rather than an eruptive species in this aspect. Likewise, *C. bimaculata* populations are not known to go through fluctuations of several orders of magnitude difference typical of eruptive insects. Rather than being a "boom or bust" insect, *C. bimaculata* is regarded by workers as having high years or low years, possibly driven by the weather (Greaves 1966). The only data available (Greaves 1966; Elek 1997) suggests populations vary as little as 25 fold, rather than the 1,000-100,000 fold variations which Price et al. (1990) consider typical of eruptive species.

Price et al. (1990) and Price (1992) argue that latent species are capable of becoming pests when management of trees, through forest practices, creates young, vigorous even

aged stands (also see Price 1991). In these situations, latent species typically do not kill trees, however, relatively low populations can cause damage to leading shoots and so reduce tree growth. This is the situation in which *C. bimaculata* has been observed causing severe defoliation. During the first 10 years of growth, eucalypt trees offer numerous growing points for attack by herbivorous insects as their canopies undergo vigorous expansion (Ohmart 1990). In particular, regeneration or plantation trees less than 10 m in height will have a high number of class 1 leaves compared to class 2 and 3 leaves. This will offer an increased larval resource to *C. bimaculata* and may lead to an elevation of the population equilibrium density (*sensu* Berryman 1991).

Price et al. (1990) and Price (1992;1994) fail to describe whether the emergent properties of latent species can commonly result in larval competition for resources when the habitat is altered through forestry practices. For *C. bimaculata*, Greaves (1966) has observed mass larval starvation indicating that at high densities larval competition does occur. Complete defoliation of current seasons foliage by larvae can also occur over consecutive seasons (authors pers. obs.) indicating that larval competition for food resources is probably common at high beetle densities.

This study demonstrates that grouping phytophagous insects into latent or eruptive categories based on the phylogenetic constraints is simplistic. Price (1990) states that 'eruptive and noneruptive species are only two ends of a continuum of variation in natural insect herbivore populations' yet the definitions he uses for latent and eruptive species limit the ability to categorise species beyond these two groups. As a consequence subsequent authors group insects as having one or the other population dynamics type (e.g. Landsberg & Cork 1997).

Using Price's (1994) phylogenetic constraints hypothesis *C. bimaculata* would most closely resemble a latent species. However, the population dynamics of *C. bimaculata* are likely to contrast with some other insects in the same category. For example, the orange-tip butterfly, *Anthocharis cardamines* (L.) which is highly selective of its

oviposition site, avoiding conspecific eggs through an oviposition-deterring pheromone (Dempster 1997). If by chance another egg is deposited on an occupied flower head then larval cannibilism occurs ensuring that competition between larvae for limited food resources is highly unlikely (Dempster 1997). Although a latent species, this butterfly clearly will have different factors influencing its population dynamics to the highly aggregated *C. bimaculata.*

Chapter 6

The effect of leaf position and adult density on *Chrysophtharta bimaculata* egg batch distribution

6.1 Introduction

The oviposition behaviour of phytophagous insects within and between host plants may be influenced by a number of factors related to the plant (e.g. chemistry and physical factors such as apparency, colour) and the insect (e.g. ability to discriminate between sites, density of conspecific adults, larvae and eggs and insect phenotypic factors)(Courtney & Kibota 1990). The cues that may or may not be used by ovipositing insects to discriminate between oviposition sites within host plants will influence egg distribution and thus, potentially influence offspring competition for resources and the degree of host defoliation (Price et al. 1990; Price 1992).

Phyophagous insects that are highly specialised in selecting oviposition sites based on suitability for offspring development such as *Disonycha plurigata* (Dempster 1997), are likely to utilise various plant stimuli when choosing a site. When airborne, these insects are likely to use both chemical and visual stimuli to locate oviposition sites (Renwick 1989 and see Courtney & Kibota 1990). Chemical and physical information through contact with the potential site may then be obtained to determine whether the site is suitable for oviposition (Renwick & Chew 1994). In contrast, some insects such as *Disonycha plurigata* (Dodge & Price 1991; Marques et al. 1994) and *Epirrita autumnata* (Tammaru et al. 1995) show indiscriminate oviposition site selection and may use few, if any, plant cues.

However, the degree of discrimination an insect shows in selecting an oviposition site may be influenced by other factors besides the inherent ability to discriminate based on the sites suitability for larval performance. Increased insect density can result in some insect's oviposition behaviour becoming more generalised (e.g. White 1970; Rossiter 1987; Bigger & Fox 1997). Although oak species are the favoured host for oviposition by gypsy moth (*Lymantria dispar* L.), a greater range of plant species are chosen under population outbreaks (Rossiter 1987). Changes in oviposition behaviour through increased density are most likely a reflection of other

factors such as the lack of suitable oviposition sites, insect interference and insect physiological state.

Differencess in phenotype have also been shown to influence oviposition site selectivity. An insect's experience (Prokopy et al. 1982; 1986), physiological state (e.g. host deprivation Roitberg & Prokopy 1983; Messina et al 1992 and age Stanek 1987 et al.), and genetics (Courtney & Chen 1988; Thompson 1988b; Scriber 1991; Singer & Thomas 1996) may influence the degree of oviposition site discrimination and thus can lead to wide individual variation within a species population.

The apparency of particular plant parts may be an important influence on egg distribution. Some insects are known to choose less preferred hosts for oviposition if preferred hosts are less apparent (see Williams 1983; Waddell & Mousseau 1996). Similarly, the apparency of particular plant tissues within a plant such as newly developed/developing leaves on the outside of host plants could receive more eggs than older leaves deeper within the canopy. Likewise, if ovipositing females examine the nearest leaf of a host plant while flying then it will more likely be a leaf on the outside of the canopy. Thus plant architecture could influence egg distribution.

In the wild, *Chrysophtharta bimaculata* oviposits predominantly on soft expanding leaves, although up to one third of egg batches can occur on fully mature foliage (Chapter 5). What factors, if any, influence *C. bimaculata* oviposition site selection have not previously been examined.

To determine the importance of some of these factors on *C. bimaculata* egg batch distribution within its host *Eucalyptus regnans*, several experiments were conducted as outlined below:

- The ability of single ovipositing female beetles to select leaves suitable for neonate larval establishment under laboratory conditions.

- *C. bimaculata* egg batch distribution on current and previous season leaves under various beetle densities in the glasshouse and in outdoor flight cages.

- Leaf apparency in young *E. regnans* trees and the influence of leaf position in field trees on beetle landing frequency.

- The movement of walking beetles from leaves within the host canopy.

- The effect of leaf position on oviposition preference in the glasshouse.

The potential influence of each of these factors on *C. bimaculata* egg batch distribution are then discussed.

6.2 Materials and Methods

6.2.1 Leaf selection for oviposition by single female *C. bimaculata*

Wild *C. bimaculata* were collected from the Florentine Valley and stored in a 4°C dark store room overnight on *E. regnans* foliage. Beetles were sexed the following day, again stored overnight and then the females were used in experiments.

Eucalyptus regnans branches, with no recent insect damage evident were also collected from the Florentine Valley. Fifty shoots containing three expanding leaves and 50 shoots containing three leaves from the previous season were removed from the branches. Only leaves with a surface area greater than 24 cm^2 (measurement of area on both sides of the leaf) were used to reduce the chance of leaf abscission occurring through beetle feeding in the experiments. The two shoot treatments were then matched based on approximate leaf area (area measured using the non-destructive technique, see 2.2 General Materials and Methods), then each set was placed in a bottle containing water and the bottle neck sealed.

Mesh bags (35 x 45 cm), containing a single female *C. bimulata* beetle, were then placed over the shoots and top half of each bottle and sealed. Each bottle replicate was then transferred to a constant 25°C glasshouse under natural light conditions and left for 24 hours.

The experiment was repeated five days later using newly collected beetles and foliage to give a total of 100 replicates. Following the experiment each replicate was examined for egg batches and if present, the leaf age treatment noted. Data was analysed using a chi-squared test.

6.2.2 Oviposition leaf selection by *C. bimaculata* under various beetle densities in glasshouse experiments

C. bimaculata beetles were collected from wild populations in the Florentine Valley along with *E. regnans* branches containing current and previous seasons foliage. There was no insect feeding damage evident on the current season foliage collected. Both beetles and foliage were stored as for 6.2.1.

Shoots containing current seasons foliage and previous seasons foliage were severed from the branches and all leaves with leaf area less than 12 cm^2 (one side) were removed. Sets of shoots, each containing 20 developing and 20 previous seasons leaves had their bases submerged in bottles containing water. The two treatments for each replicate were matched based on leaf area measured using the non-destructive technique outlined in 2.2 General Materials and Methods.

Four experiments were constructed based on beetle density: experiment (i) utilised 20 females, no males, (ii) 20 females, 20 males, (iii) 20 females, 40 males and experiment (iv), 40 females, no males. For each replicate, beetles and foliage were placed in cages with the dimensions 23 cm x 66 cm x 38 cm and left in a constant 25°C glasshouse under natural light conditions. Beetles were exposed to the foliage for a period of eight hours. Six replicates were conducted for each experiment.

Following the experiments, egg batches were counted on each leaf treatment for each replicate. Two-way analysis of variance was then used to compare the effect of treatment, the effect of leaf age and the treatment x leaf age interaction.

6.2.3 Oviposition leaf selection by a population of adult *C. bimaculata* in flight cages

C. bimaculata were collected from wild populations in the Florentine Valley and were stored in a dark 4°C room overnight. A sample of the beetle population (75 individuals) was sexed and the ratio of male to female beetles was found to be 1.08:1. Two flight cages were erected on pasture in South-East Tasmania with dimensions 2m x 2m x 1.8 m. Callico sheets were placed on the floor to prevent beetles escaping. In each cage, four potted *E. regnans* saplings (approximately 1.6 metres in height) were positioned, one in each corner. In the first flight cage, three hundred beetles were released while in the second cage, six hundred beetles were released.

During the experiment the toughness of leaves chosen for oviposition by *C. bimaculata* was measured (see 2.2 Materials and Methods). Accumulated egg batches were tallied after 2, 4.5, 6.5, 8.5 and 11 hours. Leaves with egg batches present were divided into two classes: (1) those with toughness greater than 58.5 g and (2) those with toughness less than 58.5 g. *C. bimaculata* neonate larvae are unable to survive on leaves tougher than 58.5 g (see Chapter 5).

The number of egg batches on class 1 & 2 leaves accumulating over time were then compared using paired student t-tests. Also, the proportion of egg batches deposited on class 1, relative to class 2 leaves for the two beetle density experiments were regressed to determine if egg batch distribution showed any significant change over time.

6.2.4 True leaf area versus leaf area visually exposed with regards to leaf position on *regnans* trees

Sheets of paper (red, yellow and purple) were cut into triangles of eight different sizes ranging from 2 cm^2 to 82.50 cm^2. The shapes and sizes were used to approximately represent the range of expanding and fully expanded *E. regnans* leaves present on trees.

Three *E. regnans* trees were selected between 1.8 and 2.1 m tall. For each tree, I faced the plant from a distance of 3 metres and all leaves on branches and shoots that were visible from my location were included in the experiment. On every shoot the first two leaves (youngest leaves) had red paper triangles of the closest comparable size, stapled to them. These were classified as class 1 leaves. Using the same method, yellow triangles were then stapled to every third and fourth leaf on every shoot (class 2 leaves) while purple triangles were stapled to every fifth and sixth leaf (class 3 leaves) (Fig. 6.1). Following this a photograph was taken, at a distance of 3 metres and a height of 1.75 metres. The distance chosen was approximately the distance of the nearest tree neighbours, thus representing the farthest unobstructed view. Previous studies have also shown that the majority of ovipositing beetles fly at or below tree height (Madden & Paterson unpubl.) where tree growth is most active. Thus the photograph position represents the farthest unobstructed view that the majority of ovipositing beetles will receive when approaching a host tree in plantations.

From the photograph, a count was then made of the triangles, their colour and size. From this data, an estimate of the total leaf area of each leaf class was made.

To determine the comparative area of leaves visible, the leaf area of each class was measured by placing transparency plastic over each photograph. For the first leaf class (the two youngest leaves on each shoot), a fine point felt pen was used to colour out each red triangle or portion of triangle visible. The size of the transparency was then doubled by photocopying the image. The area of the final

image was then measured using a ΔT^{TM} (Delta-T Devices) Area Meter (Cambridge, U. K.). The method of visual area measurement was the same for the other two leaf classes. Using this method it was possible to compare the proportion of leaves of each leaf class visually exposed with the true area of each leaf class. A comparison between leaf classes was made using Oneway ANOVA. This was done for each class by dividing the proportion of visual leaf area by the proportion of total leaf area that each class represented.

A further leaf class (class 4) was developed for leaves situated more than six leaves from a shoot tip. For these leaves only a visual estimate of leaf area was made. This was achieved using the same method as the other classes, however, the visual area of the true leaf was used, rather than the area estimated by the paper triangles.

6.2.5 Observations of adult landings relative to leaf position on *E. regnans* trees

A wild *C. bimaculata* beetle population was observed over a two day period in a plantation of *E. regnans* (tree height approximately 1.5-3 metres). Beetle landings on *E. regnans* leaves were noted along with the position of the leaf relative to all others on the primary shoot. Following landing beetles were captured, placed in plastic containers and transported back to the laboratory so that they could be sexed. A comparison was then made between leaf position and frequency of beetle landings. Leaf position was categorised into the 4 classes defined as for 6.2.4. The counts of both male and female beetle landings on class 1-3 leaves were then analysed using Chi-squared tests in relation to the proportional leaf area (% beetle landings/% leaf class area) and leaf number (% beetle landings/% leaf class number) obtained for these leaf classes in 6.2.4.

Figure 6.1 *E. regnans* tree in the Plenty Valley with coloured paper triangles stapled to the first six leaves on each shoot. Each triangle was approximate the size of the leaf it was stapled. Red triangles were stapled to every first and second leaf on each shoot (i.e. youngest); purple triangles to every third and fourth leaves and yellow triangles to every fifth and sixth leaves.

6.2.6 Observations of beetle walking direction from leaves on *E. regnans* plantation trees

The walking direction of *C. bimaculata* beetles from wild ovipositing populations were observed on *E. regnans* shoots at four different locations (3 in the Florentine Valley and 1 in the Plenty Valley) under field conditions. The direction when leaving a leaf was noted to determine whether beetles had a preference to either move toward the younger expanding leaves nearer the shoot tip or towards older leaves further from the shoot tip. Beetles on shoots were randomly observed, however, beetles on the youngest leaves closest to leaf tip were excluded from the study as they were unable to move to younger foliage. Data was analysed using a paired student's t-test.

6.2.7 The effect of leaf position on *C. bimaculata* egg batch distribution under laboratory conditions

Two experiments were set up to examine the effect of leaf position on egg batch distribution. In experiment (i), the egg batch distributions on *E. regnans* shoots were noted based on leaf position and a gradient of increasing age from leaf 1 (youngest leaf) to leaf 5 (oldest leaf). In experiment (ii), old leaves were positioned above young leaves. By altering the position of leaves based on age, the aim was to determine whether leaf position influenced *C. bimaculata* oviposition choice.

Approximately 1000 beetles were collected from *E. regnans* plantation trees in the Florentine Valley. Shoots of *E. regnans* containing both expanding and previous season foliage were also collected from the Florentine Valley. There was no insect damage on current season foliage. Beetles and foliage were then stored in the dark at 4^0C. The beetles were removed the following day and placed in 5 cages (dimensions 33 x 101 x 70 cm) containing *E. regnans* foliage. The cages were placed in a glasshouse at constant 25^0C, under natural light conditions and beetles

removed when required for experiments. Beetles were provided with fresh *E. regnans* foliage every day.

Beetles were sexed and twenty females were used in each replicate for both experiments. Males were excluded to limit damage to food resources. Three test shoots were used in each experimental replicate, each approximately 50 cm in length. The bases of each set of three shoots were then placed in water filled bottles. The neck of each bottle was then sealed.

Experiment (i): Shoots were selected based on appropriate leaf size (>12 cm^2 both sides) and adequate spacing (>15 mm) between leaves (petiole to petiole). The distance between leaf 1 and leaf 5 was in excess of 110 mm in all cases. For each shoot all but five leaves were removed. The leaves remaining represented a gradient from young and expanding (leaf 1) to fully mature (leaf 5). Leaf area was determined using the non-destructive technique outlined in 2.2 (General Materials and Methods). The shoot distance between each leaf was also measured using a ruler. Shoots were then placed in a coolstore at 4°C until experiments were conducted.

Experiment (ii): On one set of shoots all but three previous season leaves were removed, while on a second set all but three current seasons leaves were removed. Shoots from each set were matched based on leaf size (leaf area measured as for experiment (i) and the two shoots tied together with the previous season leaves positioned above the current season leaves. The two combined shoots were then regarded as one experimental shoot. The experimental set up was then identical to experiment (i).

Along with the test shoots, two *E. regnans* shoots were supplied per replicate in both experiments (i) and (ii) to provide additional food for the beetles during the test period. These were also approximately 50 cm in length. All but four leaves with leaf area >30 cm (both sides) were removed from each. These shoots were placed in water filled bottles in all experimental replicates.

Both the bottles containing the test shoots and the shoots provided as a food source for each replicate were placed in the middle of cages with dimensions 33 cm x 101 cm x 70 cm for all three experiments. Care was taken to ensure that all experimental leaves were not in contact with either cage walls, other leaves or shoots.

Experiments were run over an 8 hour period and were conducted in a glasshouse at constant 25^0C and under natural light conditions. Eight replicates of each experiment were run.

Following both experiments, egg batches were counted and leaves removed from the test shoots. Each leaf was weighed and then placed in a labelled paper bag. They were then pressed and dried for one week at 40°C. Following drying, leaves were weighed once again and their individual areas measured using a ΔT^{TM} (Delta-T Devices) Area Meter (Cambridge, U. K.). Specific leaf weight (g/cm^2) was then calculated using the oven dried weight.

Paired student's t-tests were used to analyse egg batch counts (a comparison between the two highest and two lowest leaves on each shoot for experiment (i), specific leaf weight and % moisture for the two leaf treatments in experiment (ii) while one-way analysis of variance was used to compare specific leaf weight and % leaf moisture (arcsine transformed) between leaves for experiment (i).

Egg batch data (egg batch numbers and eggs per leaf area) in relation to leaf position was then analysed using a paired student t-test for each experiment, while one-way Analysis of variance was used to compare specific leaf weight and % leaf moisture (arcsine transformed) between leaves.

6.3 Results

6.3.1 Leaf selection for oviposition by single female *C. bimaculata*

From the 100 single females tested there was a significant preference to oviposit egg batch on leaves that had developed in the current season over previous season leaves ($\chi^2 = 16.94$, $df = 1$, $P < 0.001$), Twenty-nine beetles oviposited egg batches on the current seasons leaves while five deposited egg batches on previous seasons leaves. The remainder either oviposited on the cage or did not oviposit.

6.3.2 Oviposition leaf selection by *C. bimaculata* under various beetle densities in glasshouse experiments

For all experiments significantly more egg batches were deposited on current season leaves ($F_{1,47} = 152.11$, $P < 0.001$). The mean number of egg batches (± SE) laid on previous season's versus current season's foliage for experiments (i-iv) were:

Experiment (i)	1.2 ± 0.3	vs	6.3 ± 0.7
Experiment (ii)	0.8 ± 0.3	vs	5.2 ± 0.4
Experiment (iii)	1.3 ± 0.3	vs	5.8 ± 0.5
Experiment (iv)	2.3 ± 0.5	vs	11.5 ± 1.1

There was also a significant difference between experiments ($F_{3,47} = 10.02$, $P < 0.001$) and the experiment x age interaction ($F_{3,47} = 4.82$, $P = 0.006$) reflecting the higher numbers of egg batches received in experiment (iv) which had 40 females as opposed to 20 in the other three experiments. Excluding experiment (iv), the experiment effect ($F_{2,35} = 1.55$, $P = 0.229$) and the experiment x age interaction are not significant ($F_{2,35} = 0.49$, $P = 0.620$) between experiments. The ratio of egg batches between current and previous season leaves did not vary greatly between experiments, ranging from 4.4 for experiment (iii) to 6.5 for experiment (ii).

6.3.3 Oviposition leaf selection by a population of *C. bimaculata* in flight cages

At both beetle densities, egg batches had appeared on *E. regnans* leaves with toughness less than and greater than 58.5 g after two hours exposure (Figure 6.2). However, at all counts leaves with toughness < 58.5 g contained significantly more egg batches per leaf compared to leaves with toughness > 58.5 g (Figure 6.2).

There was no significant trend over time in the egg batch/leaf ratio between leaves of the two toughness classes for the 300 beetle regime ($r^2 = 0.47$, $F_{1,5} = 2.67$, $P = 0.201$), although the 600 beetle regime almost reached significance ($r^2 = 0.69$, $F_{1,5} = 6.56$, $P = 0.083$).

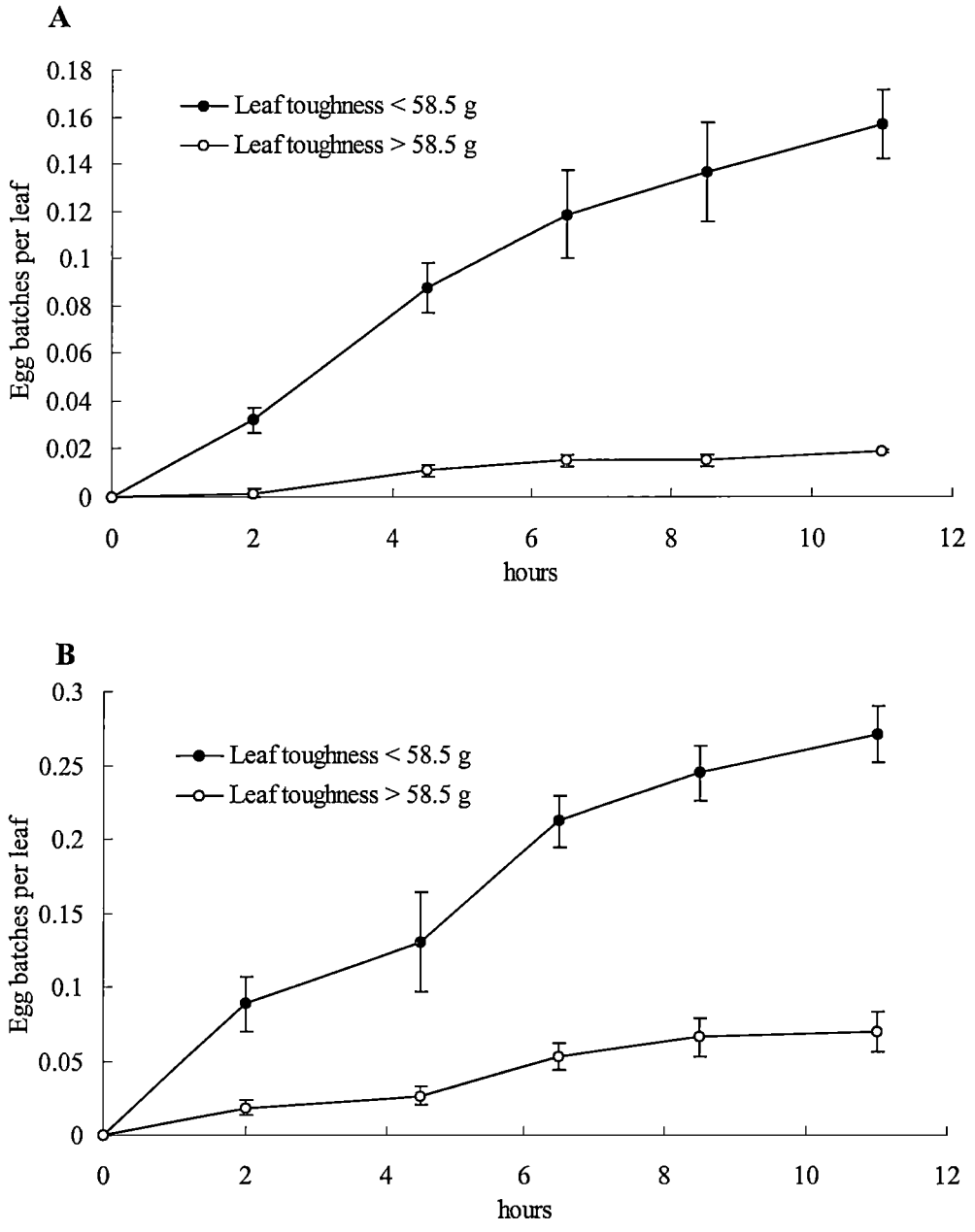


Figure 6.2 Cumulative *C. bimaculata* egg batches/leaf during the duration of the experiment for leaves with toughness less than 58.5 g and greater than 58.5 g for **A**: 300 beetles and **B**: 600 beetles. All pair-wise time comparisons are significantly different at $p < 0.05$.

6.3.4 True leaf area versus total leaf area visually exposed with regards to leaf position on *Eucalyptus regnans* trees

Examining only the first six leaves of each shoot, leaves that occupied the first two positions (class 1 leaves), represented 30.2 ± 1.9 % of the total leaf area, but represented 44.2 ± 3.0 % of the total visual area. In contrast, leaves that occupied positions 5 and 6, (class 3 leaves) represented 32.2 ± 1.5 % of the total leaf area while visually represented only 21.4 ± 1.4 % of the leaf area. Leaf class 2 made up similar total and visual leaf areas (Figure 6.3). There were significant differences between the three classes regarding the proportion of actual leaf area ($F_{2,6}$ =7.39, P = 0.024), visual leaf area ($F_{2,6}$ = 27.15, $P < 0.001$) and visual area relative to total leaf area ($F_{2,6}$ = 112.38, $P < 0.001$). Thus older leaves, deeper in the tree canopy of *E. regnans* trees will be visually less apparent when compared to younger leaves located at the end of shoots.

There was a significant difference between leaf classes regarding leaf number ($F_{2,6}$ =15.45, P = 0.004), with class 1 leaves representing 43.0 ± 2.2%, class 2, 32.7 ± 0.7% and class 3, 24.3 ± 1.6% of the leaves in all three classes combined. Leaves in leaf class 4 (remaining leaves within the tree canopy) only represented 15.8 ± 1.7 % of the total visual leaf area for the trees examined.

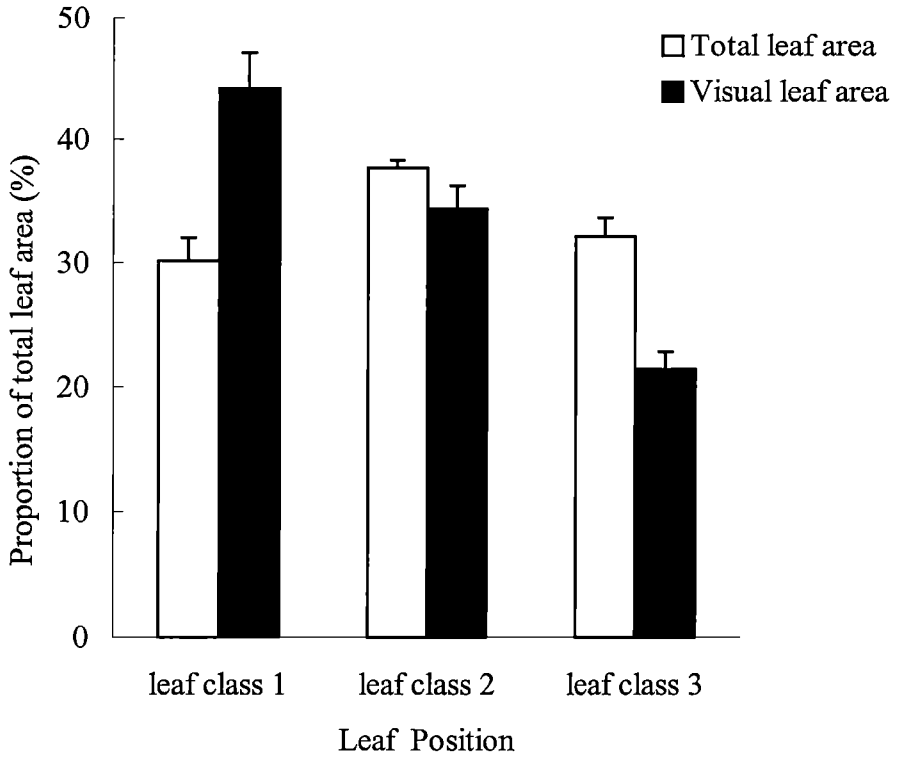


Figure 6.3 A comparison of the total leaf area and visual leaf area (of the first six leaves) for three leaf classes on three *Eucalyptus regnans* saplings. Class 1 leaves occupy the first two positions on each shoot; class 2 leaves, the third and fourth and class 3 leaves the fifth and sixth.

6.3.5 Observations of adult landings relative to leaf position on *E. regnans* trees

Based on the same leaf classes and data collected in 6.3.4, leaf classes 1 and 2 received the bulk of beetle landings compared to leaf classes 3 and 4 (Figure 6.4). Removing leaf class 4 from the analysis (this class received very few beetle landings), class 1 leaves received more landings by both males and females than was expected with respect to the predicted proportion of total area of this class (section 6.3.4), while leaf class 3 received fewer. This bias was significant [females $\chi^2 = 6.38$, $df = 2$, $P < 0.041$; males $\chi^2 = 7.05$, $df = 2$, $P < 0.030$]. However, the younger the leaf class, the larger the numbers of leaves (class 1: 220.0 ± 18.9, class 2: 167.0 ± 6.1, class 3: 124.3 ± 9.7) and the smaller the leaf size (class

1: 11.8 ± 0.4 cm, class 2: 19.3 ± 0.6 cm, class 3: 22.2 ± 1.5 cm). There was no significant difference in landing frequencies [females $\chi^2 = 4.57$, $df = 2$, $P = 0.102$; males $\chi^2 = 1.51$, $df = 2$, $P = 0.460$] with regard to the predicted numbers of leaves in each of class 1 to 3, beetle landings were in direct proportion to leaf numbers.

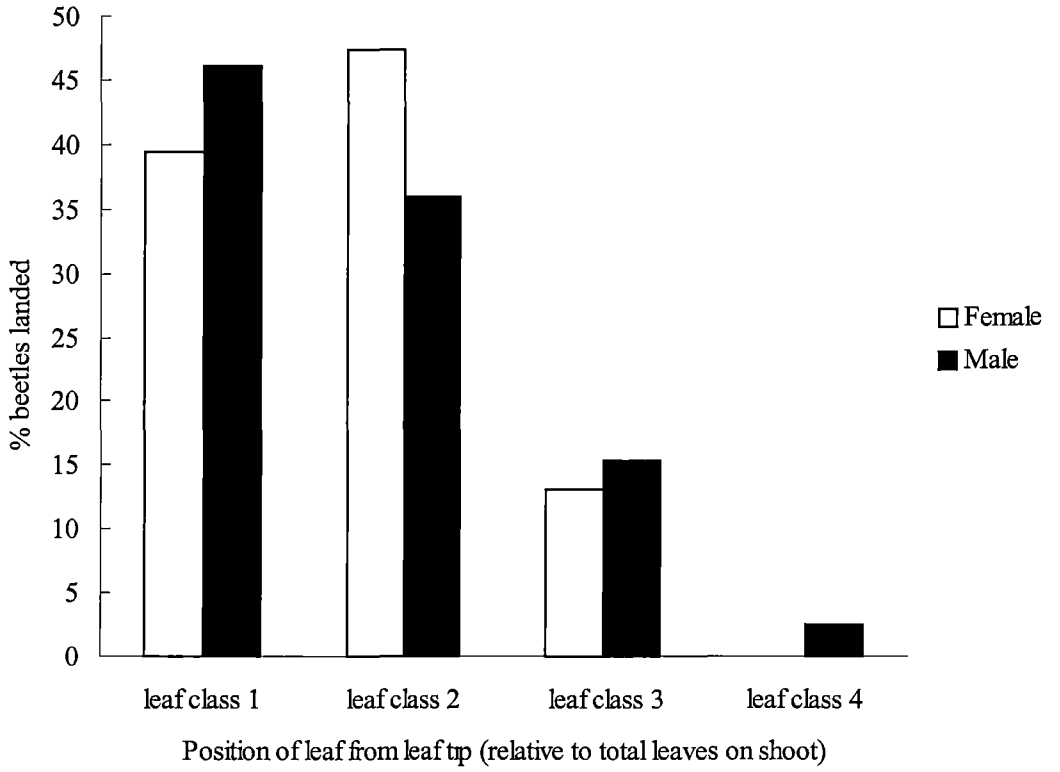


Figure 6.4 The percentage of flying male and female beetles landing on four leaf classes on *E. regnans* plantation trees. (Leaf class 1: first two youngest leaves; class 2: 3rd and 4th youngest, class 3: 5th and 6th youngest, class 4: all other leaves).

6.3.6 Observations of beetle walking direction from leaves on *E. regnans* plantation trees

For both female and male beetles there was a significant preference to move from a leaf towards leaves that were younger {females [$t_{0\,05\,(2),\,3} = 3.18$, $P(|t| \geq 3.81) = 0.032$]; males [$t_{0.05\,(2),\,3} = 3.18$, $P(|t| \geq 4.31) = 0.023$]}. For females a mean of 31.3 ±

6.0 beetles moved toward younger leaves compared to 20.8 ± 3.7 toward older leaves, while for males a mean of 34.3 ± 6.7 beetles moved towards younger leaves compared to 21.5 ± 3.9 towards older leaves.

6.3.7 The effect of leaf position on *C. bimaculata* egg batch distribution under laboratory conditions

Experiments (i).

C. bimaculata deposited significantly more egg batches/leaf on the first two leaves (youngest) (0.8 ± 0.1) compared to the two leaves lowest and oldest on the shoots (0.3 ± 0.1) [$t_{0.05\,(2),\,7} = 2.36$, $P(|t| \geq 3.81) < 0.001$].

Specific leaf weight [a parameter used by Steinbauer et al. (1998a) to indicate leaf sclerophylly] increased from leaf 2 to 5, while % leaf moisture decreased (Table 6.1). A strong negative correlation was found between egg batch numbers and both specific leaf weight ($r = 0.99$, $F_{1,3} = 346.6$, $P < 0.001$) and decreasing % leaf moisture ($r = 0.98$, $F_{1,3} = 86.5$, $P = 0.003$) in this experiment.

Table 6.1 Mean (± S. E.) specific weight and mean % leaf moisture of leaves from *E. regnans* shoots used in experiment (i). Leaves increase in age from leaf 1 (highest on each shoot) to 5 (lowest on each shoot). Means in the same column followed by the same letter are not significantly different.

Leaf Position	Egg batch/leaf	Specific leaf weight (g/cm^2)	% Leaf Moisture
Leaf 1	0.71 ± 0.11	0.021 ± 0.004 a	64.0 ± 0.4 a b
Leaf 2	0.92 ± 0.12	0.020 ± 0.004 a	64.5 ± 0.4 a
Leaf 3	0.63 ± 0.15	0.022 ± 0.004 a	62.7 ± 0.4 b
Leaf 4	0.33 ± 0.10	0.024 ± 0.005 b	60.5 ± 0.4 c
Leaf 5	0.17 ± 0.08	0.025 ± 0.005 b	58.8 ± 0.3 d

Experiment (ii)

When leaf arrangement was altered, so that previous season leaves were terminal in each treatment, and current season leaves basal, current season leaves still received

significantly more egg batches per leaf (0.5 ± 0.2) compared to previous season leaves (0.1 ± 0.0)[$t_{0\,05\,(2),\,7} = 2.36$, $P(|t| \geq 7.52) < 0.001$]. This was despite current season leaves becoming significantly smaller (21.2 ± 0.5 cm^2) by the conclusion of the experiment due to adult feeding compared to previous seasons leaves (25.8 ± 1.0 cm^2)[$t_{0\,05\,(2),\,7} = 2.36$, $P(|t| \geq 5.20) = 0.001$]. The specific leaf weight for the current season leaves (leaf position 4-6 on each shoot) compared to previous season leaves (positioned 1-3) was significantly lower while percentage leaf moisture was significantly higher (Table 6.2).

Table 6.2 Mean (± S. E.) specific weight and mean % leaf moisture of leaves from *E. regnans* shoots used in experiment (ii). Leaves 1-3 are previous season leaves (highest on each combined shoot) while leaves 4-6 are current season leaves. Means in the same column followed by the same letter are not significantly different.

Leaf Position	Specific leaf weight (g/cm^2)	% Leaf Moisture
Leaf 1	0.026 ± 0.006 a	58.3 ± 0.3 a
Leaf 2	0.026 ± 0.005 a	58.0 ± 0.3 a
Leaf 3	0.027 ± 0.006 a	58.0 ± 0.3 a
Leaf 4	0.019 ± 0.003 b	65.5 ± 0.4 b
Leaf 5	0.020 ± 0.003 b	64.8 ± 0.4 b
Leaf 6	0.021 ± 0.004 b	64.7 ± 0.3 b

6.4 Discussion

Chapter 5 demonstrated that aggregated populations of *C. bimaculata* do not always oviposit on leaves suitable for neonate larval establishment. Although individual *C. bimaculata* females most commonly chose current season leaves, some still oviposited on previous season leaves. The reason as to why some beetles oviposited on leaves unsuitable for neonate larval establishment was not explored but may be influenced by phenotype variation between beetles, particularly genetic variation between individuals and the motivation of individuals to oviposit (see introduction).

Although beetle density may have potentially influenced egg batch distribution by creating increased competition for limited oviposition sites and increased conspecific beetle interference, there was no evidence that changing beetle densities between 20 and 60 individuals per replicate in glasshouse experiments greatly influenced the ratio of eggs on current season to previous season leaves. It was not possible to test beyond this range due to the increased risk of leaf abscission caused through beetle feeding and it remains a possibility that very dense beetle aggregations could influence oviposition choice. Likewise, there was no indication that the ratio of eggs between suitable and unsuitable leaves for neonate larval establishment changed with time under two density treatments. Thus, egg batches appear on tough leaves unsuitable for neonate larval establishment soon after the foliage was exposed to the beetles and when numerous leaves with tips (see Chapter 2) suitable for neonate establishment were still available for oviposition. These results indicate that the discrimination between soft and tough leaves for at least some of the beetles is not strong enough to prevent oviposition occurring on tough leaves.

The degree of discrimination ovipositing beetles show between developing leaves versus fully developed leaves may also be influenced by leaf apparency. Although *C. bimaculata* is known to be influenced by coloured sticky traps (Leon 1989; Madden 1992), the ability of *C. bimaculata* to use visual and chemical cues to

locate oviposition sites within hosts has not been examined. The results of this study demonstrate that younger leaves on the outside of three year old *E. regnans* canopies are more apparent regarding area visually exposed than older leaves more obscured deeper within the canopy. Likewise, younger leaves will tend to be in closer proximity as beetles approach a tree during flight. Thus, younger leaves on the outside of the canopy are likely to have an increased probability of being alighted upon whether or not *C. bimaculata* uses visual or chemical stimuli to orientate towards them. The results of this study demonstrate that younger leaves were significantly more likely to be alighted upon in terms of total leaf area compared to leaves deeper within the canopy. Walking movement of beetles from leaves also significantly favoured the movement towards younger leaves. These results suggest beetles may spend more time on younger leaves compared to older, thus increasing the likelihood of oviposition occurring on them.

However, factors associated with leaf age appear more influential in oviposition choice. When leaves are in their normal orientation (expanding leaves are positioned above developed leaves), significantly more egg batches were deposited on the expanding leaves. However, current season leaves again received more egg batches when the order of leaves on a shoot were reversed so that previous season leaves were placed above current season's, (i.e. in the top three positions on a shoot). This chapter has shown that specific leaf weight and leaf moisture which are correlated with leaf age, are highly correlated with *C. bimaculata* egg batch placement. These along with other leaf age correlated factors [e.g. essential oil composition (Li 1993)] may influence *C. bimaculata* oviposition site selection.

In conclusion, *C. bimaculata* egg batch distribution on *E. regnans* appears to be at least partially due to variation in individual beetle oviposition site discrimination. Although beetle density could still possibly influence egg batch distribution, under the densities tested there was no evidence of its influence with egg batches readily occurring on old foliage at low densities and soon after foliage exposure. Although oviposition site discrimination appear to vary between beetles it appears that the majority of beetles can discriminate between leaves based on factors related to leaf

age. This seems more important than the effect of leaf position. Apart from the factors examined in this chapter, egg batch distribution may be further complicated by other deterrent factors such as conspecific larvae and residues produced by beetles such as frass (see Chapters 2 & 4). Although more than one-third of egg batches may be deposited on foliage unsuitable for neonate larval establishment, the ability of larvae to migrate and establish on suitable foliage reduces the importance of oviposition site selection based on the ability of neonates to establish (Chapter 5).

Chapter 7

A comparison of tree and leaf development between and within three host species of *Chrysophtharta bimaculata*

7.1 Introduction

Insects respond to plant phenotypes (Weis & Campbell 1991), hence the plant phenotype can be vitally important to the population dynamics of phytophagous insects as well as its susceptibility to defoliation. A plant's susceptibility to insect defoliation can be influenced by its genetics (Paige & Capman 1993), age (Roininen et al. 1993; Spiegel & Price 1996), vigour (Roininen et al. 1997; Cronin & Abrahamson 1999) and stress (Brodbeck & Strong 1987). These factors can influence leaf chemistry (Martinsen et al. 1998), nutrient concentration (Marsh 1995) and plant architecture (Steinbauer et al 1998b) which in turn, may directly influence host plant - insect interactions.

For the genera *Eucalyptus* and *Corymbia*, trees tend to be evergreen with leaf age varying widely (Williams & Brooker 1997). Although the average leaf survival age has been estimated at 18 months for eucalypts (Boland et al. 1991), abiotic factors have been shown to directly influence the longevity of leaves (Madden & Turnbull 1984; Pook 1984). While eucalypt leaves can be relatively long lived, fully developed leaves tend to be of low nutritional quality compared to the developed leaves of deciduous trees (Bell & Williams 1997). Mature eucalypt leaves also tend to have a high degree of lignification of cell walls (Cork 1984) and high concentration of phenolics (Fox & Macauley 1977).

For eucalypt - insect interactions, a number of plant physical and chemical attributes have been suspected of, influencing plant susceptibility to phytophagous insects. Many of these have been outlined earlier in chapter 2 and include leaf waxes (Edwards 1982; Edwards & Wanjura 1990; Li 1993), leaf toughness (Ohmart et al. 1987; Larsson & Ohmart 1988), essential oils (Edwards et al. 1993; Li 1993; Stone & Bacon 1994), and foliar nutrient concentrations (Farrow & Floyd 1996; Steinbauer et al. 1998b). Nitrogen concentration in particular has been implicated in the performance of paropsine larvae (Ohmart et al. 1985a). Likewise, tree damage may be influenced by

nitrogen concentration. Low nitrogen concentration has been implicated in increased ingestion of dry matter by *Paropsis atomaria* larvae on *Eucalyptus* species (Fox & Macauley 1977; Ohmart et al. 1985a).

Most phytophagous insects, including many paropsine species, which utilise *Eucalyptus spp.* as hosts, preferentially feed on, or are restricted to, the developing and newly developed foliage (Strauss & Morrow 1988; Landsberg & Cork 1997). Such foliage is less tough (Larsson & Ohmart 1988) and often higher in nitrogen concentration than older leaves (Steinbauer et al. 1998a). The concentration of secondary metabolites, including essential oils, phenolics and tannins are often associated with poor performance of grazing animals (see Feeny 1968; Meyer & Montgomery 1987; Appel 1993), but may not show any significant variation in concentration with *Eucalyptus* leaf age (Macauley & Fox 1980; Boland et al. 1991; Li 1993) or may even be higher in newly developed foliage (Larsson & Ohmart 1988). However, paropsine larvae do not appear to be affected by the concentration of essential oils and tannins in leaves and are able to absorb and metabolise terpenoids (Ohmart & Larsson 1989). Still, some eucalypt feeding insects appear to be influenced by the composition of oils or by individual components within the oil. Edwards et al. (1993) found that eucalypt susceptibility to *Anoplognathus* (Coleoptera: Scarabaeidae) was correlated with the percentage of eucalyptol in the terpenoid mixture. However, it was not possible for them to determine which components were responsible for the defoliation due to autocorrelation between some of the terpenoid components. Likewise, general insect herbivory and *Chrysophtharta bimaculata* herbivory has been negatively correlated with 1,8 cineole in eucalypts (Li 1993, Stone & Bacon 1994) although other components autocorrelated with this oil component such as sideroxylonal (Lawler 1999) may be responsible for deterrence and/or poor performance of herbivores.

The preference for developing and newly developed leaves by many phytophagous insects for feeding and/or oviposition may make trees with a large amount of such foliage more vulnerable to increased insect damage. The production of young vigorous

shoots and foliage following plant damage through mammal browsing and fire can increase damage by phytophagous insects (Seyffarth et al. 1996, Martinsen et al. 1998). Findings in Chapter 3 also demonstrated that following defoliation by *C. bimaculata* larvae, the production of larger numbers of leaves with reduced area can subsequently result in an increased number of eggs per unit leaf area from future oviposition events. Likewise, young and vigorously growing plantation trees with a high proportion of current season foliage may be more vulnerable to insect damage (Price 1991). In these circumstances, trees that have a faster rate of leaf maturation reduce the time individual leaves are vulnerable to insects opportunistic on developing foliage. Steinbauer et al. (1998a) (and see chapter 5) found that *E. regnans* trees under field conditions contained a larger proportion of expanding leaves compared to *E. nitens*. They proposed that the rate of leaf development may be important in influencing the oviposition preference of *C. bimaculata.*

Few studies have examined the leaf development rate of *Eucalyptus* species under field conditions, particularly between species. Metcalfe et al. (1990) found that leaf expansion rates for *E. globulus* Labill. were influenced by weather conditions, particularly availability of water. It was found that although previously wilted seedlings developed leaves of similar leaf area, they took twice as long to expand compared to non-wilted seedlings. Beadle & Turnbull (1986) also demonstrated that leaf expansion rate can vary between species, with *E. nitens* having greater leaf expansion rates than *E. delegatensis* in a mixed species trial plot. Apart from leaf expansion rate, the rate of change in other leaf factors which are known to influence paropsine herbivory (in particular leaf toughness) have not been examined.

There are only a few studies that have examined eucalypt susceptibility to paropsine damage based on changes in leaf and tree characters with time. Raymond (1998) examined the development of leaf numbers, leaf area and colour change over time for various families of *E. regnans*, finding that trees which developed a higher proportion of red leaves were more vulnerable to *C. bimaculata* damage. A once-off study on leaf

nutrition and the terpenoid composition of young leaves of these same trees failed to reveal any significant differences between these leaf characters and tree susceptibility (Patterson et al. 1996). The only other significant correlations found between the susceptibility of *E. regnans* families to *C. bimaculata* related to early growth rate, tree height at end of season and relative growth rate based on tree height (Raymond 1995; Patterson et al. 1996; Raymond 1998).

This study adds to the studies conducted by Patterson et al. (1996), Raymond (1995, 1998) and Steinbauer et al. (1998a), by further examining factors related to leaf development which may be important in influencing host tree susceptibility to *C. bimaculata* larval damage. The factors measured are:

- The number and size of leaves produced over a six month period prior to insect attack

- Time taken for such leaves to reach a toughness unsuitable for neonate larval establishment.

- Total leaf area and toughness of leaves produced over the six month period.

- The ratio of carbon to nitrogen with leaf age.

- Essential oil and leaf wax composition relative to leaf age.

These factors are examined between three *C. bimaculata* eucalypt host species: *E. regnans*, *E. delegatensis* and *E. nitens*, as well as between families of *E. regnans*. *C. bimaculata* egg batch counts are also be related to these trees to help assess tree susceptibility to attack

7.2 Materials and Methods.

7.2.1 Monitoring sites and tree species examined

Franklin-14: A comparison within a host species.

At a site in southern Tasmania (Franklin 14, 43°04'S, 146°53'E, altitude 360 m), four *E. regnans* families were selected from a 5 year old *Eucalyptus regnans* family trial. In a previous study the families at this site were shown to vary significantly in their damage to *C. bimaculata* larval feeding (Raymond 1995). Raymond (1998) classed these families into two classes: "susceptible", those receiving a great deal of defoliation, and "non susceptible", those receiving significantly less defoliation. For this study the term "non susceptible" is replaced with "less susceptible" to reflect that *C. bimaculata* damage still occurs on these trees. Two susceptible families were chosen for examination along with two less susceptible families. At the time of planting the trial was designed as a randomised complete block design consisting of 9 families in 6 replicates, 120 of the original 160 trees planted were alive on commencement of this study. In this study, five of the blocks containing one tree of each family were utilised.

Plenty Valley: A Comparison between three Eucalyptus *host species.*

Three sites in the Plenty Valley (42°50'S, 146°53'E) were chosen for this study and at each site 5 trees of two species were chosen for monitoring. At all sites trees were in their fourth year of growth. Site 1 consisted of *Eucalyptus regnans* and *E. nitens* plantation trees. Trees labelled for monitoring were chosen in a zone where the two species overlapped in a small mixed stand. Site 2 consisted of *E. delegatensis* and *E. regnans* in a regeneration stand, while site three consisted of *E. nitens* plantation trees interspersed with regrowth *E. delegatensis*.

Tree monitoring

For each tree monitored, three branches were chosen and from each, one shoot was selected and marked. Due to the height of the taller trees at Franklin-14, all branches chosen for monitoring were approximately one third the height of total tree height. At the sites in the Plenty Valley, the branches chosen were at a height of approximately two thirds that of tree height. At both locations branches were selected so that no particular directional aspect was preferred. All new leaves initiated by the shoot were marked and remarked every four to six weeks using a waterproof felt pen. Data on leaf expansion and leaf toughness (collected every one to two weeks) was collected from the 10th of October 1996 until the 5th of March 1997 for the Plenty Valley trees, while the Franklin 14 trees were monitored from the 5th of November 1996 until the 11th of March 1997. Material for essential oil and wax analysis was collected on the 18th of December 1997 at the Plenty Valley sites and on the 16th of December 1997 at the Franklin-14 site. Material for leaf carbon to nitrogen ratios was collected on the 15th of December 1996 at Plenty Valley and 16th December 1996 at Franklin-14.

7.2.2 Leaf number

The number of new leaves that developed over the season for each tagged shoot were counted throughout the monitoring period. The numbers of leaves initiated along with the number of leaves surviving at the end of the monitoring period were compared between species and within species at different sites in the Plenty Valley using student t-tests and between *E. regnans* families using one-way Analysis of Variance (ANOVA).

7.2.3 Leaf size

Using the collected data, a comparison was made between mean leaf size of fully expanded leaves for species at each site and within species at different sites in the Plenty Valley and also between families and susceptibility class for *E. regnans* at

Franklin-14. Leaf area was estimated by measuring leaf length and width and multiplying by a coefficient (*E. regnans* 0.71; *E. delegatensis* 0.70 and *E. nitens* 0.64, see Appendix 2). The data used was from the leaves monitored over the six month period. Data was analysed using the same methods as in 7.2.2.

7.2.4 Time taken for leaves to reach a toughness of 58.5 g

For newly initiated leaves, toughness was measured using a leaf penetrometer (see Chapter 2 General Material and Methods) at each monitoring occasion, up until leaf toughness exceeded 92.1 g (toughness value equivalent to previous season *E. regnans* leaves)(Appendix 9). For each leaf, two readings were taken near the widest point of each leaf. The average of these was used in the data analysis. The units were later converted to grams. Leaf toughness was only measured for those leaves with a width exceeding 7 mm as smaller leaves lacked sufficient leaf area.

To estimate the time taken for a leaf to reach a toughness of 58.5 g the following records and calculations were made:

1. The days from leaf initiation to the first sampling occasion when the recorded leaf toughness was equal to or greater than 58.5 g ($d_{t>58.5}$) was recorded.

2. The days from leaf initiation to the sampling occasion immediately preceding leaf toughness reaching 58.5 g ($d_{t<58.5}$) was noted.

3. The leaf toughness measures made on these two sampling occasions ($t_{t<58.5}$ and $t_{t>58.5}$) were noted.

4. The following calculation was made

$(d_{t>58.5} - d_{t<58.5}) / (t_{t>58.5} - t_{t<58.5}) = C$

Where C represents the change in time relative to the change in toughness.

5. The calculation: $t_{t>58\,5} - 58.5 = T$, where T represents the increase in toughness between $d_{t<58.5}$ and $d_{t=58\,5}$ was then made, so that $(T \times C) + d_{t<58\,5} = d_{t=58.5}$.

For example: A leaf on day 65 had a toughness of 56 g and then on day 73 had a toughness of 62 g. Thus: $d_{t<58.5} = 65$; $d_{t>58.5} = 73$; $t_{t<58\,5} = 56$; $t_{t>58.5} = 62$

$C = (73 - 65)/(62 - 56)$

$C = 1.33$

$T = 62 - 58.5$

$T = 3.5$

$d_{t=58\,5} = (3.5 \times 1.33) + 65$

$d_{t=58.5} = 69.7$ days

The estimated time for leaves to reach a toughness of 58.5 g was calculated for all initiated leaves where possible along with the month this value was achieved. Monthly comparisons were then made between the two species at the same site and within species at different sites in the Plenty Valley using student t-tests. Similar comparisons were made between families and defoliation susceptibility class for *E. regnans* at Franklin-14 using oneway (ANOVA).

7.2.5 Total leaf area and leaf toughness development

Using the collected data on leaf expansion, it was possible to estimate the cumulative total leaf area per branch over the entire period for each monitored tree. Leaf toughness data was also used to determine:

1. The cumulative total leaf area of foliage with toughness less than 58.5 g. Above this value first instar larvae are unable to establish (Chapter 5).

2. The cumulative total leaf area of foliage with toughness greater than 92.1 g. This value is equivalent to the toughness of mature previous season *E. regnans* leaves which were tested in a preliminary examination. Previous season leaves are resistant to all larval instars of *C. bimaculata*.

Comparisons were then made between species within sites and within species between sites for the trees in the Plenty Valley by analysing: (i.) consecutive monitoring contrasts (average between) to determine treatment effect (i.e between sites or species), and (ii.) change between consecutive monitoring contrasts (difference between) to determine the treatment x time interaction using Z-tests. The same approach was also used to analyse *E. regnans* families and their susceptibility class at Franklin-14 using oneway ANOVA.

7.2.6 Change in Carbon to Nitrogen ratio with leaf age

Six of the monitored leaves from one shoot were sampled from three of the five trees of each species (randomly selected) at each site in the Plenty Valley and from three of the five trees from each *E. regnans* family at Franklin-14. Sampling was conducted using a hole punch, with the sample taken from midway down the length of the leaf. Samples taken were free from toughness measurement wounds and observable leaf veins.

Samples were dried at 40°C for one week and then total percentages of N and C was measured using a Carlo Erba, CHNS-O, EA 1108-Elemental Analyzer®.

Comparisons between the changes in Carbon to Nitrogen ratios with leaf age were made by linear regression analysis between species and within species at different sites for Plenty Valley trees and between *E. regnans* families at Franklin-14.

7.2.7 Essential oil and leaf wax composition

During December, two trees from each species at each Plenty Valley site and three of each *E. regnans* family at Franklin-14 were selected. Fifteen to twenty-five newly emerged leaves (length less than 30 mm long) were individually marked on the petiole and twenty-one days later three leaf classes were collected from the marked leaves.

(1) Old leaves - leaves from the previous season

(2) Leaves approaching full expansion - those leaves marked three weeks earlier. All were still below a toughness of 50 g and therefore regarded as suitable for *C. bimaculata* neonate larval establishment

(3) newly emerged leaves. Leaves that were between 25 and 40 mm in length and had just emerged on shoots.

Five to fifteen leaves of each class were collected from each tree, placed in sealed plastic bags and transported back to the laboratory on ice. Leaf area and fresh weights were then measured for each sample. Samples were then stored in a cool room at 4°C overnight and specimens prepared for analysis the following day.

The materials and methodology adopted to extract and determine leaf waxes and essential oils was similar to Chapter 2 (2.2.3). To collect the leaf waxes present on the leaf surface, between 5 ml and 15 ml of chloroform for each sample was used, depending on the leaf area of the sample. The chloroform was pipetted into a rectangular glass box with a surface area of 40 cm^2. Whole leaves were submerged in the chloroform using tweezers, gently agitated for 10 seconds then removed. Aliquots of the chloroform containing the wax from each sample were then transferred to 5 ml glass vials.

Following the chloroform treatment, the leaf specimens for each tree sample were chopped and placed in ethanol for 30 hours to collect the essential oils before aliquots were removed to 5ml glass vials. These were refrigerated at 4°C until analysis.

Section 2.2.3, Chapter 2 describes wax and essential oil compound identification. Any unidentified compounds were listed from the most abundant ions received from the mass spectra, except where bracketed indicating more distinctive ions. Unidentified triterpenes are described numerically.

Balanced ANOVA was used to examine the variation between individual oil and leaf wax components between the *E. regnans* families and tree susceptibility class at Franklin-14. Differences in oil and leaf wax components were tested at the family, susceptibility and age levels along with the family x age and susceptibility x age interactions. Individual compounds from both the oils and leaf wax were then examined using student t-tests (least significant difference) to determine if there were any significant difference in their percentage of total composition between susceptibility classes at specific leaf ages.

One-way ANOVA followed up by post-hoc Tukeys tests were used to determine which individual compounds of both oils and leaf waxes varied significantly in percentage composition with leaf age at Franklin-14. One-way ANOVA and where appropriate,

post-hoc Tukeys were also used to determine whether the percentage change in composition of individual compounds from the oil and leaf wax samples significantly varied from young to medium aged leaves (both leaf classes suitable for establishment of *C. bimaculata* larvae) at the family and susceptibility level.

For the Plenty Valley data, extensive analysis of individual compounds in the oil and wax samples between species was not persued due to the degree of dissimilarity of the compounds present and the wide variation in percentage composition of those in common between species. Rather, the most common components are listed and compared.

Oil and leaf wax dissimilarity based on tree species, leaf age and site location were compared within and between Plenty Valley sites while at Franklin-14, similar comparisons were made between *E. regnans* families and leaf age. This was achieved using incremental sum of squares with standardised euclidean metric (Taxon 1.0 beta 3). Dendrograms were then constructed based on the similarity of samples.

7.2.8 *C. bimaculata* egg batch occurrence

Over the monitoring period at both Plenty Valley and Franklin-14 sites, *C. bimaculata* oviposition events were observed. At both sites, data on the frequency of egg batches for each monitored tree was collected once by counting and measuring the leaf area (see method used in 7.2.3) of class 1 leaves (leaves with toughness less than 58.5 g) along with egg batches present on four branches. Branches were selected from all sides of the tree at approximately one third tree height at Franklin-14 and at approximately half tree height for the Plenty Valley sites. Egg batches on other leaves were not counted as these tended to be correlated with numbers on class 1 leaves (see chapter 5).

Data analysis was conducted on the number of egg batches present per leaf and the number of egg batches present per unit leaf area (unit resource available). For the Plenty Valley data, student t-tests were used to determine whether results between species at each site were significantly different. For the Franklin-14 data, one-way ANOVA was used to determine whether there were any significant differences at the family and susceptibility levels.

7.3 Results

7.3.1 Leaf number

Plenty Valley

At site 1 there was no significant difference in the number of leaves initiated over the six month period between *E. regnans* (44.6 ± 7.2) and *E. nitens* (39.0 ± 3.4) [$t_{0\,05\,(2),\,6}$ = 2.45, P(|t| ≥ 0.51) = 0.707] and likewise between *E. delegatensis* (42.2 ± 5.31) and *E. nitens* (62.6 ± 8.5) at site 3 [$t_{0\,05\,(2),\,7}$ = 2.36, P(|t| ≥ 2.04) = 0.080]. However, there was a significant difference between *E. regnans* (46.6 ± 3.3) and *E. delegatensis* (32.0 ± 1.7) at site 2 [$t_{0.05\,(2),\,6}$ = 2.45, P(|t| ≥ 3.95) = 0.007]. A comparison between species at different sites did not reveal any significant differences: *E. regnans* [$t_{0\,05\,(2),\,8}$ = 2.31, P(|t| ≥ 1.61) = 0.147], *E. delegatensis* [$t_{0\,05\,(2),\,5}$ = 2.57, P(|t| ≥ 1.82) = 0.128] and *E. nitens* [$t_{0.05\,(2),\,8}$ = 2.31, P(|t| ≥ 1.86) = 0.142].

Regarding number of leaves initiated in the current season and surviving through to the final monitoring date, there was no significant difference in leaf survival between *E. regnans* (19.2 ± 2.2) and *E. nitens* (33.2 ± 6.4) at site 1 [$t_{0\,05\,(2),\,5}$ = 2.57, P(|t| ≥ 2.05) = 0.095], and likewise between *E. delegatensis* (17.6 ± 0.51) and *E. regnans* (27.2 ± 3.9) at site 2 [$t_{0\,05\,(2),\,4}$ = 2.78, P(|t| ≥ 2.44) = 0.070]. However, there was a significant difference between *E. nitens* (42.0 ± 4.1) and *E. delegatensis* (23.2 ± 1.6) at site 2 [$t_{0\,05\,(2),\,5}$ = 2.57, P(|t| ≥ 4.29) = 0.008]. A comparison between species at different sites revealed a significant difference in the number of leaves produced by *E. delegatensis* between sites 2 and 3 [$t_{0.05\,(2),\,5}$ = 2.57, P(|t| ≥ 3.41) = 0.019]. For the other two species no significant differences were observed: *E. regnans* [$t_{0\,05\,(2),\,6}$ = 2.45, P(|t| ≥ 1.78) = 0.126], and *E. nitens* [$t_{0.05\,(2),\,7}$ = 2.36, P(|t| ≥ 1.15) = 0.287].

It should be noted that although several of the results above (i.e. survival of leaves between species at site 1 and 3) were not statistically different, the actual differences were large and may represent real biological differences.

Franklin-14

There was no significant difference between *E. regnans* families regarding the number of leaves initiated throughout the year (family 1, 22.8 ± 2.1; family 2, 21.8 ± 1.5; family 3, 22.4 ± 2.7; family 4, 21.8 ± 1.0)($F_{3,19}$ = 0.06, P = 0.978). Likewise, there was no significant difference between families when grouped based on their susceptibility to *C. bimaculata* damage (susceptible, 22.3 ± 1.7; less susceptible 22.1 ± 1.9)($F_{1,19}$ = 0.01, P = 0.914).

There was also no significant difference between *E. regnans* trees for the number of current season leaves surviving at the end of the monitoring period at the family level (family 1, 13.0 ± 1.5; family 2, 12.8 ± 2.1; family 3, 14.4 ± 1.6; family 4 13.4 ± 1.3($F_{3,19}$ = 0.19, P = 0.902), or tree susceptibility level (susceptible, 12.9 ± 1.7; less susceptible, 13.9 ± 1.4)($F_{1,19}$ = 0.42, P = 0.527).

7.3.2 Leaf size

Plenty Valley

Although there was no significant difference in leaf size between *E. regnans* (34.5 ± 3.3 cm) and *E. nitens* (40.3 ± 2.4 cm) at site 1[$t_{0.05\ (2),\ 7}$ = 2.36, P(|t| ≥ 1.41) = 0.202], the removal from the analysis of one *E. regnans* tree that had noticeably larger leaves (mean 47.0 ± 7.5) reduced the *E. regnans* mean to (31.3 ± 1.4 cm) and resulted in a significant difference [$t_{0\ 05\ (2),\ 6}$ = 2.45, P(|t| ≥ 3.15) = 0.020]. At site 2 there was no

significant difference between leaf size between *E. regnans* (44.2 ± 2.1cm) and *E. delegatensis* (37.1 ± 2.9) [$t_{0\,05\,(2),\,7} = 2.36$, $P(|t| \geq 1.99) = 0.087$], while at site 3 there was also no significant difference between the leaf size of *E. nitens* (61.8 ± 3.3 cm) and *E. delegatensis* (52.8 ± 3.1 cm)[$t_{0.05\,(2),\,8} = 2.31$, $P(|t| \geq 1.67) = 0.134$].

A comparison between the same species at different sites revealed that for each pairwise comparison, leaf size differed with site. Site 2 *E. regnans* leaves were significantly larger than site 1 trees [$t_{0\,05\,(2),\,7} = 2.36$, $P(|t| \geq 2.47) = 0.043$]; site 3 *E. delegatensis* had significantly larger leaves than site 2 trees [$t_{0.05\,(2),\,7} = 2.36$, $P(|t| \geq 3.05) = 0.019$]; and for *E. nitens* site 1 leaves were larger than site 3 leaves [$t_{0.05\,(2),\,7} = 2.36$, $P(|t| \geq 5.25) = 0.001$].

Franklin-14

There was a significant difference between the leaf size of *E. regnans* at the family level ($F_{3,19} = 3.67$, $P = 0.035$) with a post-hoc Tukeys test revealing that family 2 (33.5 ± 8.2 cm) had significantly smaller leaves compared to family 4 (62.3 ± 8.3). The leaf size for families 1 (36.9 ± 4.7 cm) and 3 (38.4 ± 5.4 cm) were insignificant from the other families. At the susceptibility level, the leaf size of the less susceptible families was larger (50.3 ± 8.7) than susceptible trees (35.2 ± 6.4) but fell short of significance ($F_{1,19} = 3.94$, $P = 0.063$).

7.3.3 Time taken for initiated leaves to reach a toughness of 58.5 g

Plenty Valley

At site 1, initiated leaves of *E. nitens* took significantly less time to reach a toughness of 58.5 g compared to *E. regnans*, while at site 3 *E. nitens* also took significantly less

time than *E. delegatensis* in November, December and January. At site 2, *E. regnans* and *E. delegatensis* leaves generally took a similar time to reach a toughness of 58.5 g, except in December when *E. regnans* took significantly longer.

A comparison within species between sites did not reveal any significant difference between *E. regnans* and *E. delegatensis* trees in any month. However there were significant differences between *E. nitens* at site 2 and 3 in November [$t_{0.05\ (2),\ 5} = 2.31$, $P(|t| \geq 3.58) = 0.007$], December [$t_{0.05\ (2),\ 5} = 2.31$, $P(|t| \geq 7.95) < 0.001$] and January [$t_{0\ 05\ (2),\ 5} = 2.31$, $P(|t| \geq 5.35) < 0.001$].

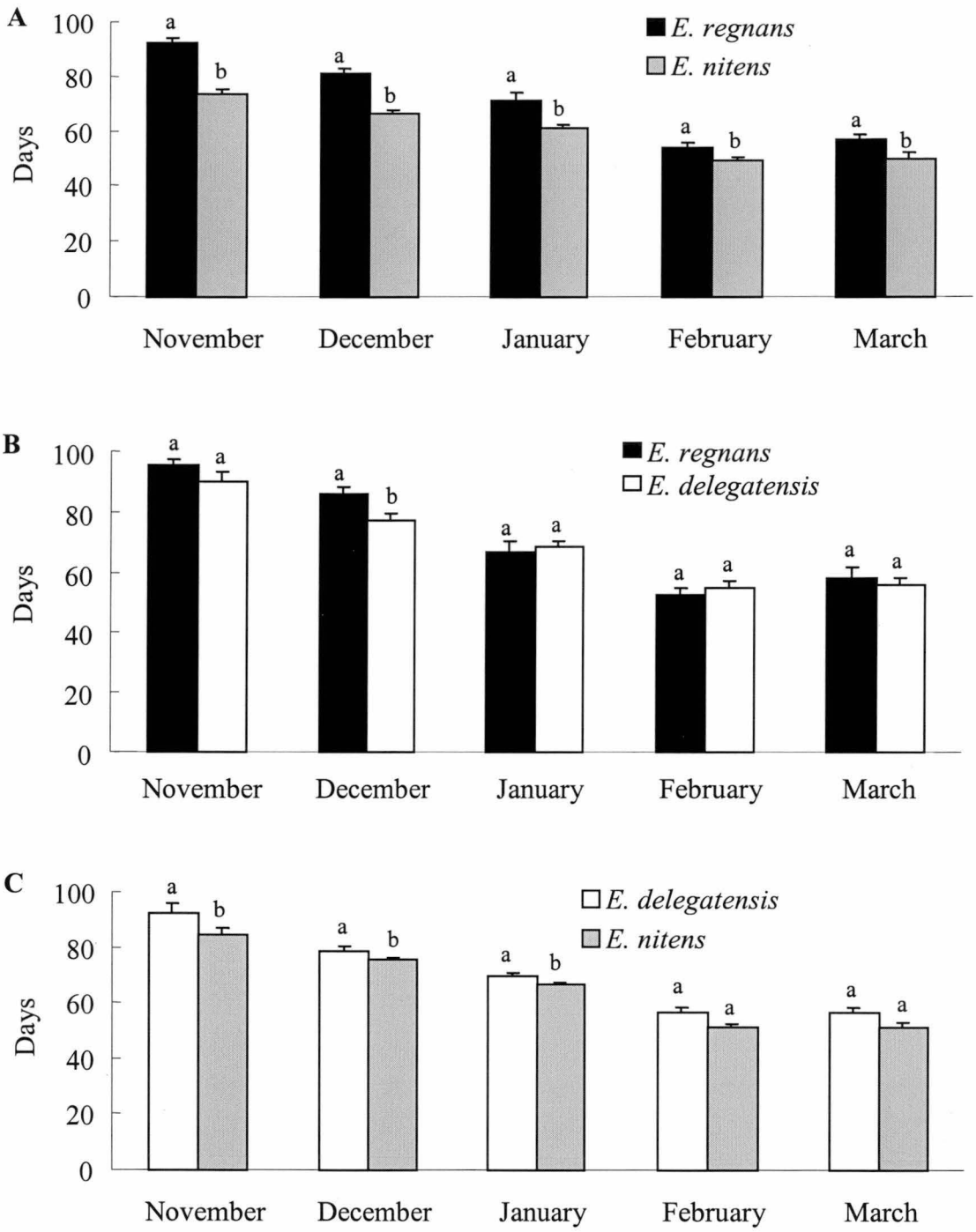


Figure 7.1A, B and **C** Estimated time taken for initiated leaves to reach a toughness value of 58.5 g by month. **(7.1)** *E. regnans* and *E. nitens* at site 1, **(7.2)** *E. regnans* and *E. delegatensis* at site 2 and **(7.3)** *E. delegatensis* and *E. nitens* at site 3. Pairs of columns surmounted with the same letter are not significantly different.

There were no significant difference between *E. regnans* families or tree susceptibility class for any of the five months regarding the time taken for initiated leaves to reach a toughness of 58.5 g (Fig. 7.2).

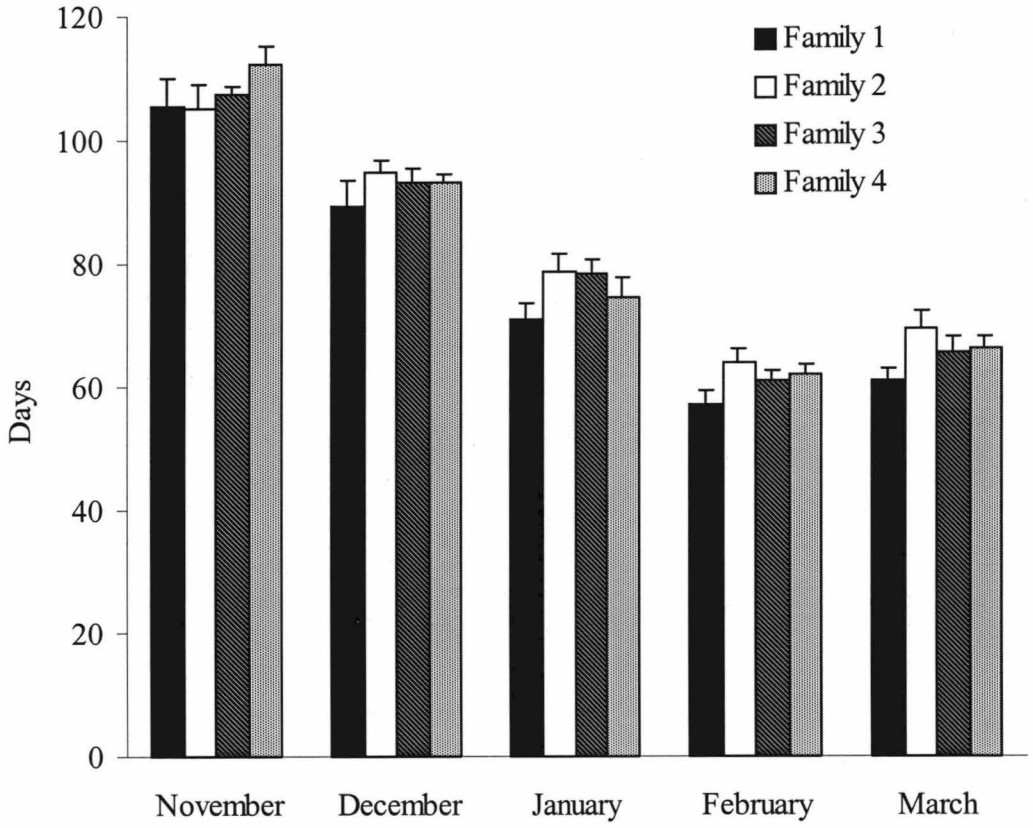


Figure 7.2 Estimated time taken for initiated leaves to reach a toughness value of 58.5 g by month for four *E. regnans* families. Families 1 & 2 are classed as susceptible to *C. bimaculata* defoliation while families 3 & 4 are classed as less susceptible.

7.3.4 Total leaf area and toughness development

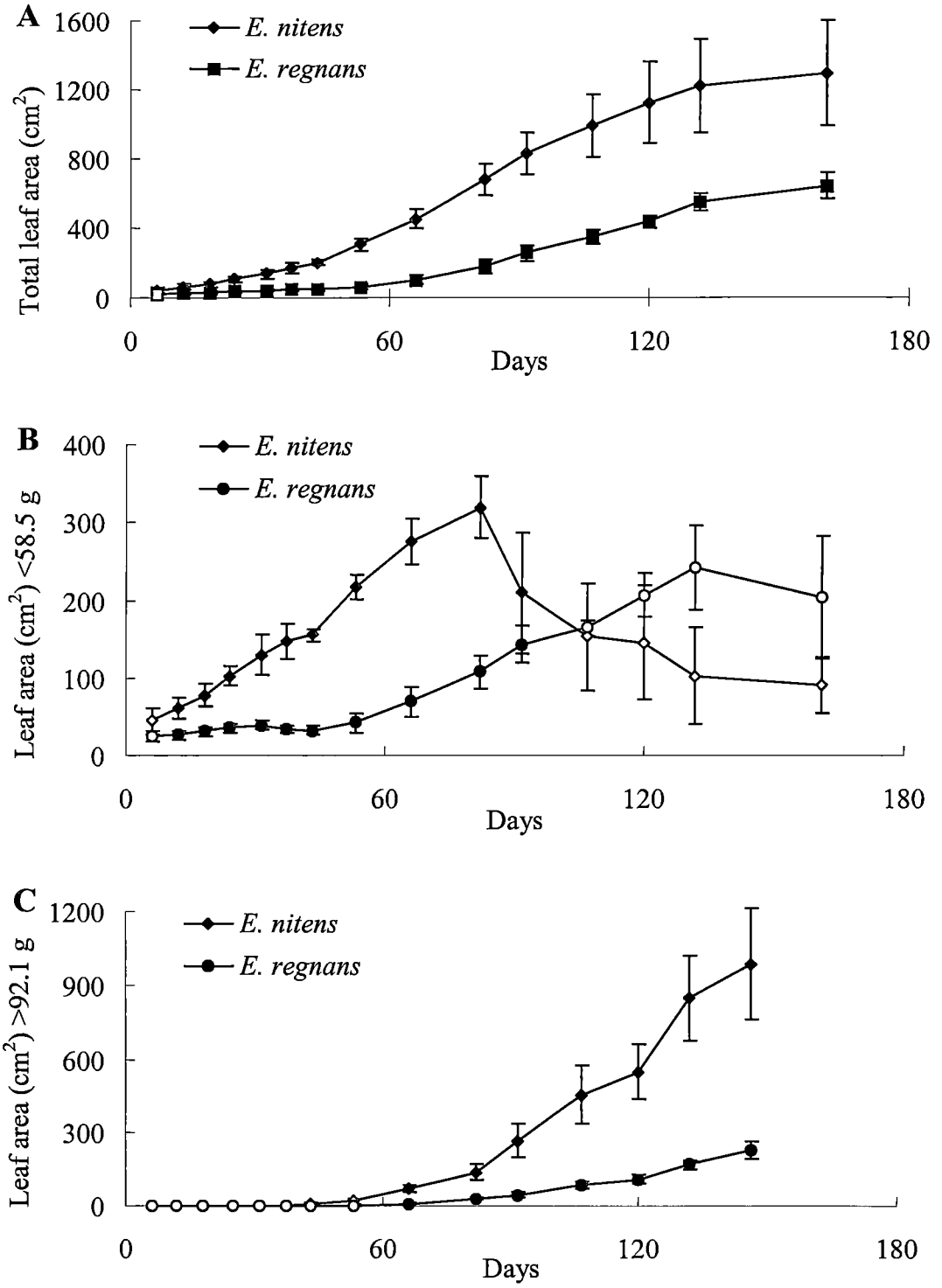
Plenty Valley

The results comparing total leaf area, leaf area less than 58.5 g and leaf area greater than 92.1 g for *E. nitens*, *E. regnans* and *E. delegatensis* over time between species and within species at different sites are listed in Tables A3.1-A3.6 in Appendix 3. The results presented here summarise the important trends recorded in the analyses.

At site 1 *E. nitens* current season's total leaf area was higher than that of *E. regnans* throughout the monitoring period (all contrasts significant apart from the first and last) (Figure 7.3A). *E. nitens* also had significantly greater leaf area increase compared to *E. regnans* between several contrasts occurring between October to the end of December.

E. nitens initially had greater leaf area with toughness less than 58.5 g than to *E. regnans* (significant over several contrasts). However, for *E. nitens*, the area of this leaf class began declining from early January onwards. From late January until monitoring was concluded, there were no significant differences between the leaf area for this leaf class between the two eucalypt species.

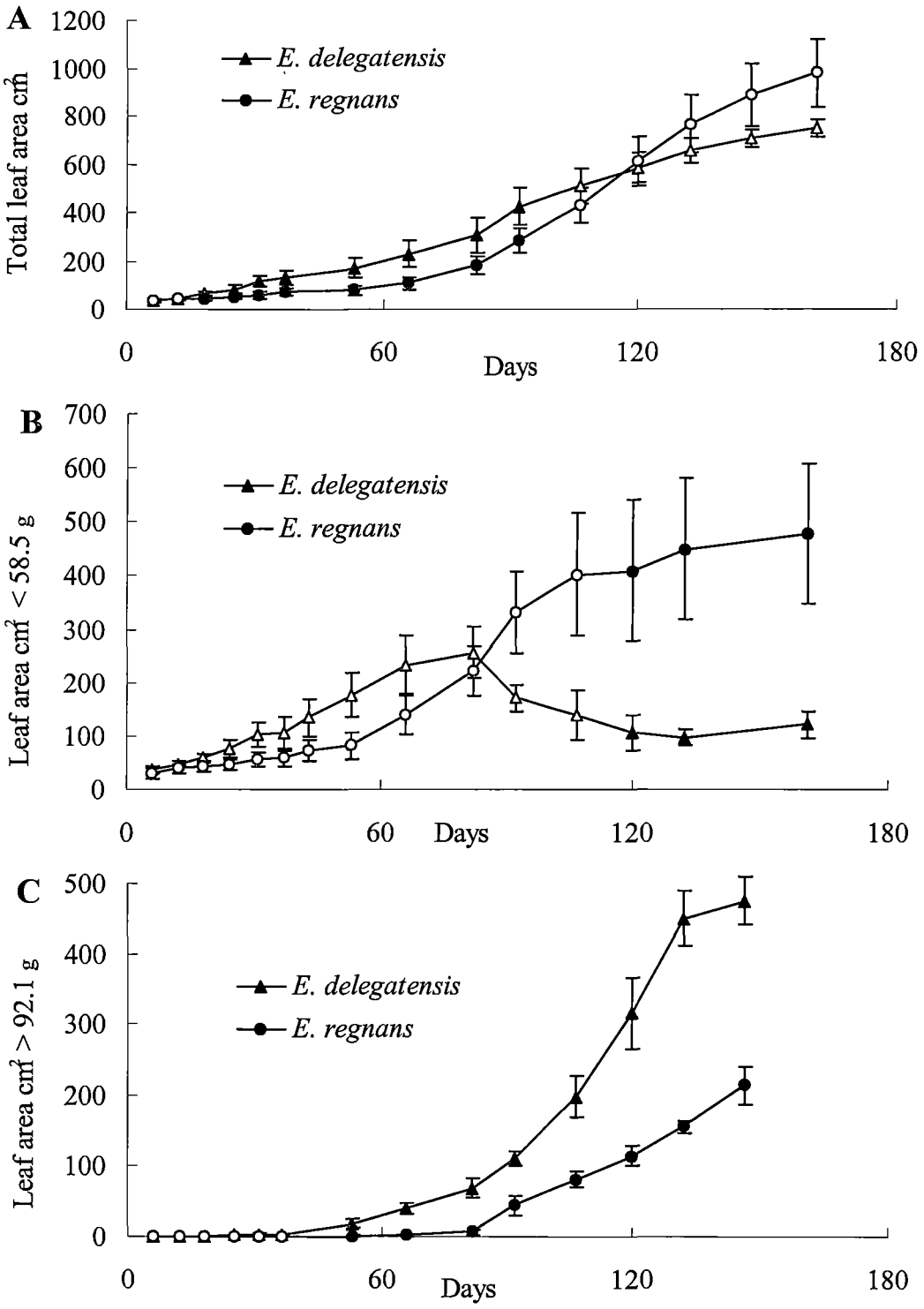
For current season's leaves with toughness greater than 92.1 g, *E. nitens* showed significantly greater leaf area increases between several contrasts. Subsequently, by early December, *E. nitens* had a significantly greater area of these leaves compared to *E. regnans* (Table 7.3, Figure 7.3C).



Figs. 7.3 A, B and C. Mean leaf development over time (monitoring began 10th Oct. 1996), for three shoots from five *E. regnans* and five *E. nitens* trees at Site 1 in the Plenty Valley for; **(7.3A)** Total leaf area; **(7.3B)** Leaf area with toughness < 58.5 g; **(7.3C)** leaf area with toughness > 92.1 g. Contrasts between closed shapes are significant between *E. regnans* and *E. nitens*, open shapes non-significant.

Apart from October, *E. delegatensis* had a significantly greater total leaf area compared to *E. regnans* at site 2 until the end of December (Figure 7.4A). However, from the last week in January onwards, *E. regnans* had a greater but not a significantly different total leaf area compared to *E. delegatensis*.

E. delegatensis initially had greater (although not significant) leaf area with toughness under 58.5 g compared to *E. regnans*. However, the area of this leaf class increased at a greater rate for *E. regnans* (significantly so in early January) from early December onwards. By early January the area of *E. regnans* leaves with toughness less than 58.5 g had surpassed that of *E. delegatensis* and by mid March had reached significance (Figure 7.4B). From late November onwards *E. delegatensis* had a significantly greater area of current season leaves with toughness greater than 92.1 g compared to *E. regnans,* with three contrasts showing a significantly greater rate of increase (Figure 7.4C).



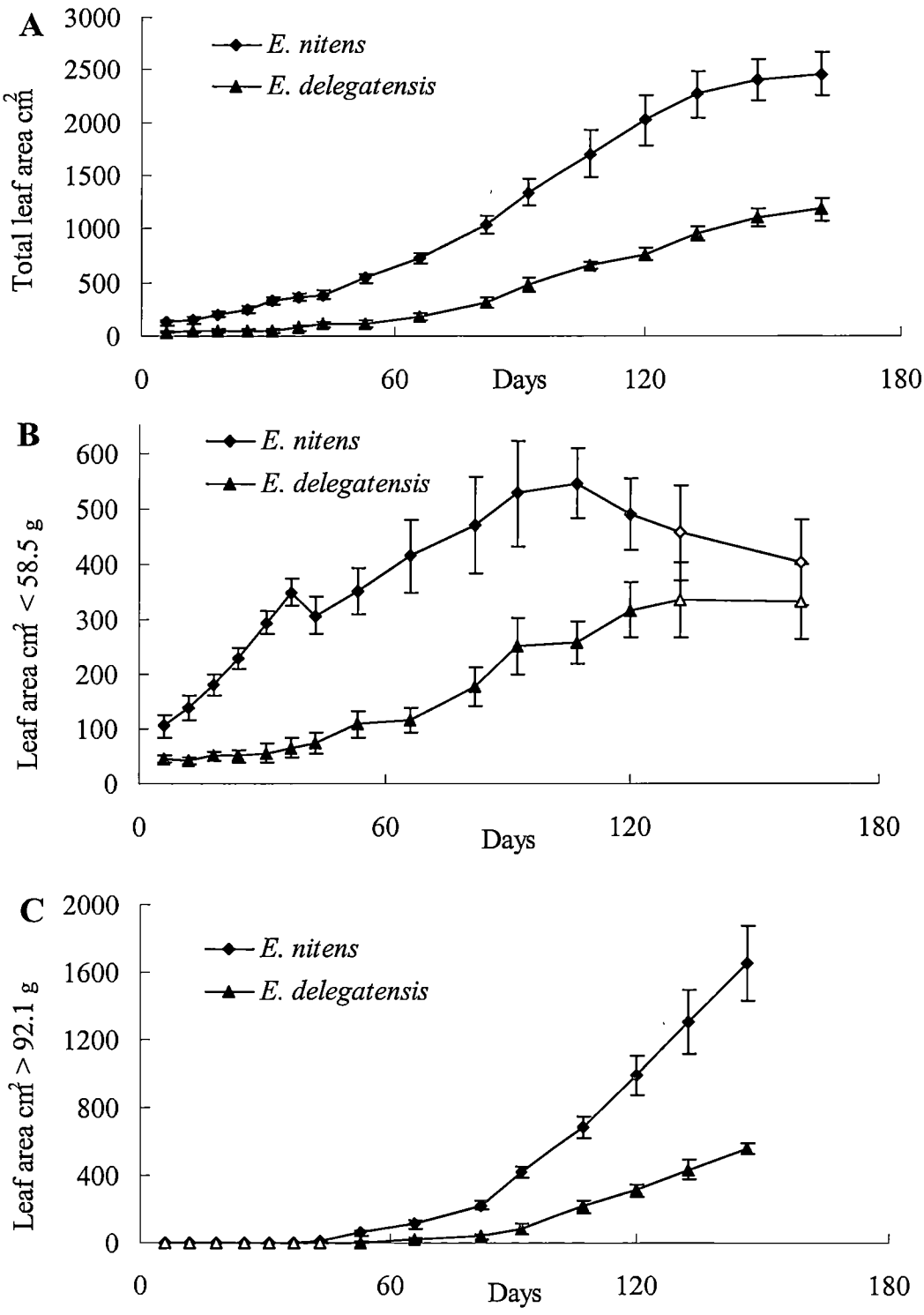
Figs. 7.4A,B and C. Mean leaf development over time (monitoring began 10th Oct. 1996), for three shoots from five *E. regnans* and five *E. delegatensis* trees at Site 2 in the Plenty Valley for; **(7.4A)** Total leaf area; **(7.4B)** Leaf area with toughness < 58.5 g; **(7.4C)** leaf area with toughness > 92.1 g. Contrasts between closed shapes are significant between *E. regnans* and *E. nitens*, between open shapes non-significant.

At site 3 *E. nitens* had a significantly greater total leaf area compared to *E. delegatensis* over the entire monitoring period. The rate of total leaf area increase was also significantly greater for *E. nitens* compared to *E. delegatensis* for much of the period between October and (Table 7.5, Figure 7.4A).

During the entire monitoring period *E. nitens* contained a larger mean leaf area with toughness less than 58.5 g, although the difference was not significant from mid February onwards. *E. nitens* initially gained significantly more area for this class of leaves compared to *E. delegatensis* but by late November there were no significant differences (Figure 7.4B). The rate of mean leaf area development for leaves with toughness greater than 92.1g was always greater for *E. nitens* compared to *E. delegatensis*, significantly so on several occasions. As a result, from late November *E. nitens* had a significantly greater area of this class of leaves compared to *E. delegatensis* (Figure 7.4C).

A comparison between *E. regnans* at site 1 and site 2 revealed no significant differences between the total area of current season leaves developed or the area of current season leaves with toughness greater than 92.1 g for any contrasts. There was also only one contrast in early January where site 2 trees had significantly greater current season leaf area with toughness less than 58.5 g.

There were site differences for *E. delegatensis,* with site 3 trees having developed leaf area significantly faster than site 2 trees during two October contrasts. This led to trees at site 3 having a significantly greater total leaf area of current season's leaves from mid November until early January. However, during February, site 2 trees had a significantly greater rate of total leaf area development compared to site 3 trees and by early March site 2 trees had significantly greater total leaf area. The greater rate of total leaf area development during early February also correlated with a significantly greater area of leaves with toughness less than 58.5 g.



Figs. 7.4A,B and C. Mean leaf development over time (monitoring began 10th Oct. 1996), for three shoots from five *E. nitens* and five *E. delegatensis* trees at Site 2 in the Plenty Valley for; **(7.4A)** Total leaf area; **(7.4B)** Leaf area with toughness < 58.5 g; **(7.4C)** leaf area with toughness > 92.1 g. Contrasts between closed shapes are significant between *E. regnans* and *E. nitens*, between open shapes non-significant.

Following the development of leaves with toughness greater than 92.1 g, site 3 trees always had a greater area of this leaf class (significantly so from late November to early January) compared to site 2 trees.

For *E. nitens* the mean total leaf area developed for the current season was always greater (significantly so for most of the time) for trees at site 3 trees compared to site 1. A similar trend was also observed for leaf area with toughness below 58.5 g with site 3 trees having more area for this leaf class, although significance was lost from mid December to early January. There was no significant site difference between leaf area of current seasons foliage with toughness greater than 92.1 g for *E. nitens* at the two sites.

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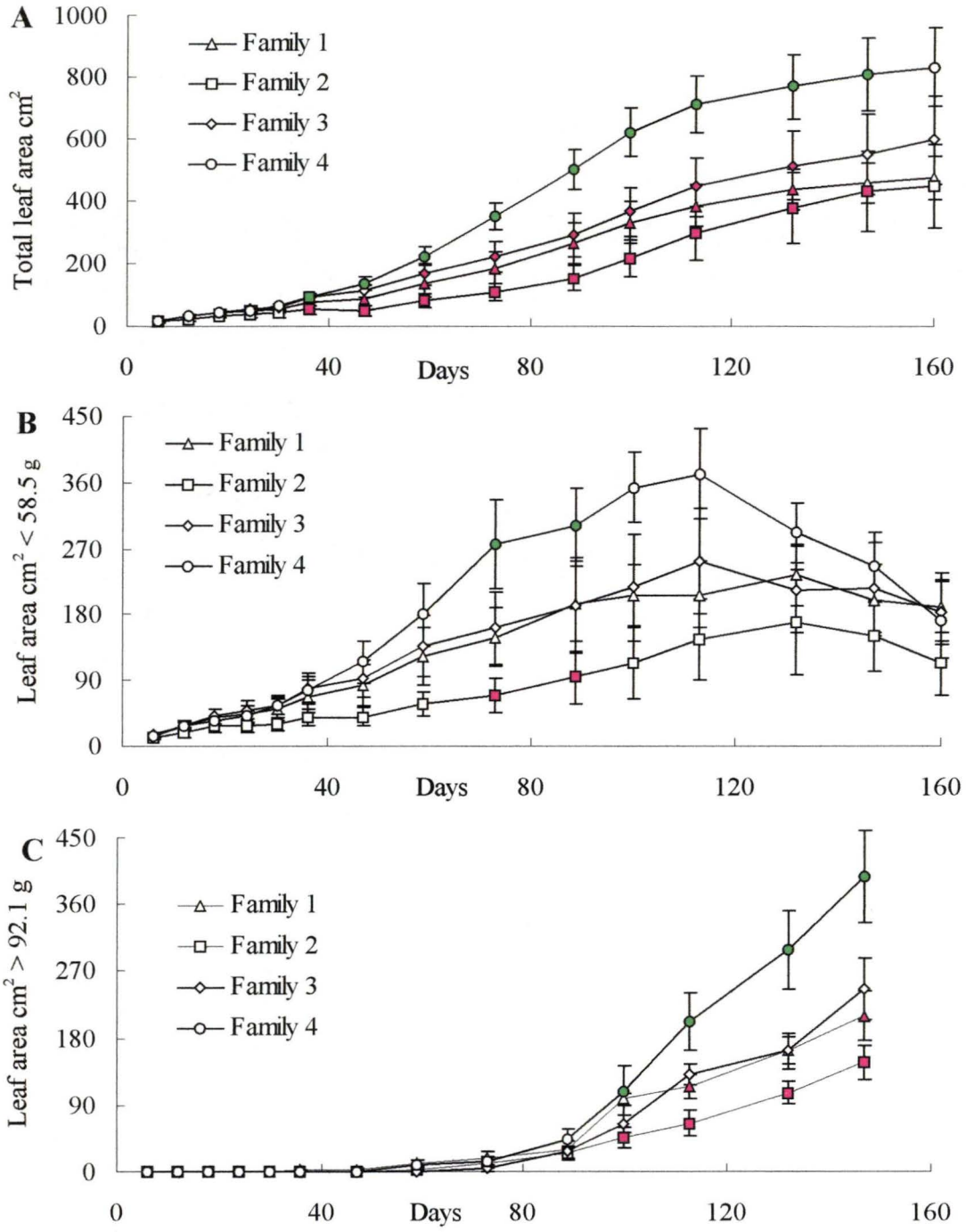
The results comparing total leaf area, leaf area less than 58.5 g and leaf area greater than 92.1 g over time for the four different *E. regnans* families and for two susceptibility classes are listed in Table A4.1 in Appendix 4. A summary of the important trends are provided below.

For most of the time between late November and early January family 4 had a significantly greater rate of increase in current season leaf area compared to the other three families. Family 4 also had consistently greater area of current seasons foliage compared to the other families during the summer months (Figure 7.5A). For much of the time there were also significant differences at the susceptibility level with less susceptible trees having a greater rate of leaf area increase from mid November through to early January, leading to significantly greater total leaf area from early December onwards.

No significant differences were found between families for the rate of area change and leaf area over time for leaves with toughness less than 58.5 g with exception of family 4 versus family 2 which did show significant differences between rate of area increase and total leaf area for a single time contrast. (Figure 7.5B). At the susceptibility level, less susceptible trees had greater leaf area than susceptible trees but this was only significant for two contrasts.

For those leaves with toughness greater than 92.1 g, family 4 had developed significantly greater leaf area from the beginning of February onwards compared to families 1 & 2 (Figure 7.5C). At the susceptibility level, less susceptible trees had a significantly greater area of these leaves from the beginning of February onwards.



Figs. 8.5A, B and **C.** Total leaf area development over time (monitoring began 30th Oct. 1996) for four *E. regnans* families (families 1 & 2 more susceptible to *C. bimaculata* damage than families 3 & 4) at Franklin 14: **(8.5A)** total leaf area; **(8.5B)** leaf area with toughness < 58.5 g; **(8.5C)** leaf area with toughness > 92.1 g. Contrasts between different coloured shapes are significant from one another. White and same coloured shapes are non significant.

7.3.5 Change in Carbon-Nitrogen Ratio with leaf age

Plenty Valley

For all trees examined at the three Plenty Valley sites, Carbon:Nitrogen (C:N) increased with leaf age (Figures 7.7 A,B and C). At site 1 there was no significant differences between *E. regnans* and *E. nitens* for the change in the C:N with leaf age ($F_{1,32} = 1.79$, $P = 0.190$) or between the two species leaves at a given age ($F_{1,33} = 2.48$, $P = 0.125$) (Figure 7.6A). At site 2 there was a significant difference between *E. regnans* and *E. delegatensis* for the change in C:N with leaf age ($F_{1,32} = 4.20$, $P = 0.049$), with *E. regnans* having an increasingly greater C:N compared to *E. delegatensis* at a given age beyond the 17th day (Figure 7.6B). At site 3 there were no significant differences between *E. delegatensis* and *E. nitens* for the change in the C:N with leaf age ($F_{1,32} = 0.20$, $P = 0.654$) or between the two species at a given age ($F_{1,33} = 2.53$, $P = 0.121$) (see fig 7.6C).

A comparison between the same species at different sites revealed no significant differences between the change in C:N with leaf age for *E. regnans* at site 1 and site 2 ($F_{1,32} = 0.00$, $P = 0.963$) or for *E. delegatensis* at sites 2 and 3 ($F_{1,32} = 0.09$, $P = 0.769$). There was also no significant difference between the C:N ratio at any given age for the within species comparison at their two sites (*E. regnans*, $F_{1,33} = 3.21$, $P = 0.083$; *E. delegatensis* $F_{1,33} = 2.91$ $P = 0.097$). However, site 1 *E. nitens* had a significantly greater increase in C:N with leaf age compared to site 3 *E. nitens* ($F_{1,32} = 7.00$, $P = 0.013$).

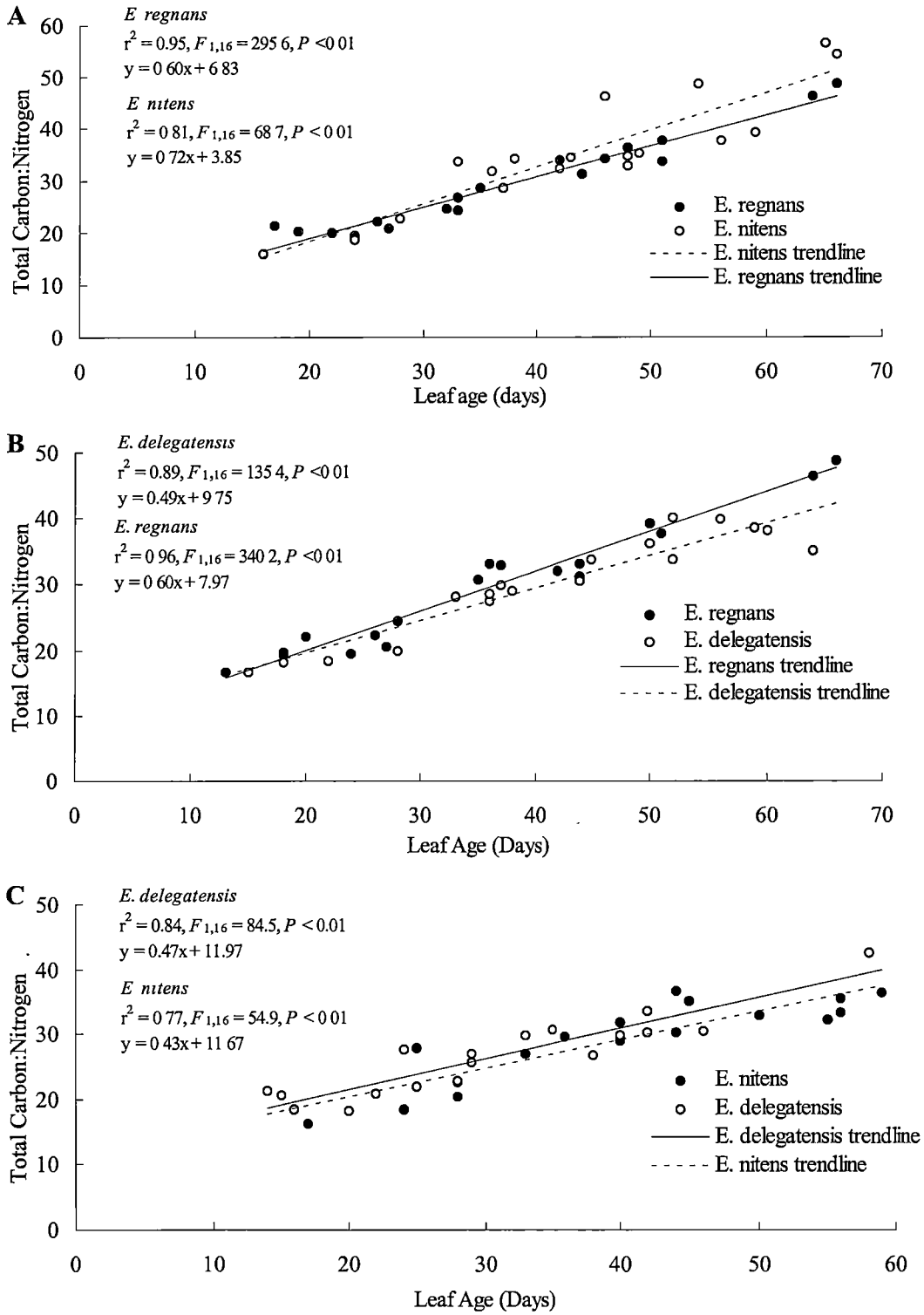


Figure 7.7 Total Carbon:Nitrogen versus leaf age for **A**; Site 1; *Eucalyptus regnans* versus *E. nitens* **B** Site 2; *E. regnans* versus *E. delegatensis* and **C**; Site 3; *E. nitens* versus *E. delegatensis*.

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As with the eucalypts in the Plenty Valley, C:N significantly increased with leaf age for the four *E. regnans* families (Family 1, $r^2 = 0.89$, $F_{1,16} = 125.82$, $P < 0.001$; Family 2, $r^2 = 0.81$, $F_{1,16} = 67.53$, $P < 0.001$; Family 3, $r^2 = 0.81$, $F_{1,16} = 68.75$, $P < 0.001$; Family 4, $r^2 = 0.79$, $F_{1,16} = 61.01$ $P < 0.001$).

The change in C:N with leaf age varied significantly between families 1 & 2; 3 & 4; 1 & 4 and 2 & 3, while no significant differences were recorded for families 2 & 4 or 1 & 3 (Table 7.2). However, the C:N was significantly less for any given leaf age for family 2 compared to family 4 ($F_{1,33} = 38.40$, $P < 0.001$). No significant difference between C:N for any given leaf age was recorded between families 1 & 3 ($F_{1,33} = 0.68$, $P = 0.480$).

Table 7.2 Analysis of variance results comparing the change in Carbon-Nitrogen ratio with leaf age between four *E. regnans* families located at Franklin-14 (n = 36, d.f. 2,33), * are significantly different.

E. regnans family comparison	*F*-statistic	*P*-value
Family 1 versus Family 2	4.95	0.033*
Family 3 versus Family 4	10.35	0.003*
Family 1 versus Family 4	12.42	0.001*
Family 2 versus Family 3	6.36	0.017*
Family 1 versus Family 3	1.57	0.219
Family 2 versus Family 4	1.56	0.220

7.3.6 Essential oil composition

Franklin-14

Of the 59 individual compounds isolated from the oil of the four *E. regnans* families (see Appendix 5, Table A5.1 for analyses of all compounds), 15 varied significantly in their proportion of total oil at the family level, 41 with leaf age and 9 for the family x

leaf age interaction. At the susceptibility level (see Table A5.1 for analyses of all compounds), 7 differed significantly [alpha terpinene, caryophylliene, humulene, an unidentified elemol related compound, hedycaryol and an unidentified compound "161/119/41/(105)/(204)", while 6 varied at the susceptibility x age interaction [alpha terpineol, beta elemene, spathulenol and three unidentified compounds "121/138/39/(161)"; "43/139/125/200" (Compound 1) and "43/139/125/200" (Compound 2) (Table 7.3).

Table 7.3 Analysis of variance results for the proportion of those individual components present in essential oils taken from two *E. regnans* susceptibility classes that are either significant at the susceptibility or susceptibility x leaf age interaction. The degrees of freedom were: family 3, age 2, susceptibility x age 6 and total 35, M.S. indicates Mean Square, * indicates $P < 0.05$, ** indicates $P < 0.01$, n.s. indicates $P > 0.05$.

ESSENTIAL OIL	SUSCEPT. M.S.	AGE M.S.	SUSXAGE M.S.
ALPHA TERPINENE	0 204 *	0.017 n.s.	0.030 n.s
ALPHA TERPINEOL	0.080 n s.	0.080*	0.080*
BETA ELEMENE	0.018 n s.	2.371**	0.088*
CARYOPHYLLIENE	1.725**	0.226*	0 044 n s.
HUMULENE	0.097*	0 237**	0.048 n.s.
121/138/39/(161)	0.285**	1.003**	0.285**
ELEMOL RELATED	5.808*	6.118**	1.461 n.s.
161/119/41/(105)/(204)	1.000**	0.007 n.s.	0 007 n.s.
HEDYCARYOL	308.50*	2986 76**	22.00 n.s.
SPATHULENOL	0.754 n.s	3.291**	1.314**
43/139/125/200 (C1)	0.303 n.s.	3.180**	0.303*
43/139/125/200 (C2)	0.632 n.s.	2.918**	0.632*

Only one compound, caryophylliene was found to be significant at the *E. regnans* susceptibility level for a leaf age class preferred for *C. bimaculata* oviposition. This occurred in medium-aged leaves averaging $0.80 \pm 0.09\%$ of total oil composition in less susceptible trees compared to $0.31 \pm 0.06\%$ for susceptible trees [$t_{0\,05\,(2),\,5} = 2.57$, $P(|t| \geq 3.86) = 0.012$].

Of the 41 compounds that varied significantly with leaf age, 10 [trans piperitol, bicycloelemene, pyrogallol, beta elemene, humulene, alloaromadendrene, bicyclogermacrene and three unidentified compounds "84/105/41/126/97"; "121/138/39/(161)" and "43/139/125/200"] were significantly different between the

young and medium leaf age classes (Table 7.4). A comparison between the young and old leaf samples indicated that 39 varied significantly and that 37 varied significantly between the medium and old aged leaves (Table 7.4).

Table 7.4 Mean percentage of individual components present in essential oils from three leaf age classes, young, medium and old, from 4 *E. regnans* families at Franklin-14. Components listed in the table are those (i) recorded as being significantly different with regards to age (Table A8.1) and (ii) excluding those that were exclusively recorded (ignoring traces) in the leaf samples of old leaves. Unidentified compounds are listed from the most abundant ions received from the mass spectra, except where bracketed indicating more distinctive ions. Means in the same row followed by the same letter are not significantly different. Error values are ± S. E. (n = 36, d.f. 2,33).

Oil Component	Young	Medium	Old
ALPHA PHELANDRENE	3.05 ± 0.28a	2.36 ± 0.25a	1.09 ± 0.18b
P-CYMENE	0.42 ± 0.04a	0.61 ± 0.12a	1.97 ± 0.24b
TRANS P-MENTH-2-EN-1-0L	0.00 ± 0.00a	0.15 ± 0.08a	1.69 ± 0.20b
CIS P-MENTH-2-EN-1-0L	0.49 ± 0.03a	0.52 ± 0.07a	2.10 ± 0.25b
81/43/167/77	0.00 ± 0.00a	0.02 ± 0.02a	0.30 ± 0.08b
TRANS PIPERITOL	6.20 ± 0.46a	4.66 ± 0.52b	6.20 ± 0.46c
84/105/41/126/97	0.45 ± 0.06a	0.00 ± 0.00b	0.00 ± 0.00b
BICYCLOELEMENE	0.54 ± 0.04a	0.20 ± 0.06b	0.00 ± 0.00c
PYROGALLOL	0.73 ± 0.29a	3.17 ± 0.43b	1.29 ± 0.26a
ALPHA COPAINE	0.34 ± 0.08a	0.32 ± 0.06a	0.00 ± 0.00b
BETA ELEMENE	0.88 ± 0.05a	0.53 ± 0.06b	0.00 ± 0.00c
HUMULENE	0.00 ± 0.00a	0.21 ± 0.06b	0.27 ± 0.06b
ALLOAROMADENDRENE	0.61 ± 0.08a	0.22 ± 0.06b	0.29 ± 0.08b
121/138/39/(161)	0.00 ± 0.00a	0.50 ± 0.17b	0.00 ± 0.00a
BICYCLOGERMACRENE	11.29 ± 1.08a	5.53 ± 0.82b	1.28 ± 0.26c
ELEMOL RELATED	1.29 ± 0.36a	1.17 ± 0.34a	0.00 ± 0.00b
HEDYCARYOL	35.13 ± 2.56a	34.57 ± 2.80a	7.53 ± 1.65b
GAMMA EUDESMOL	0.84 ± 0.16a	1.09 ± 0.35a	2.54 ± 0.41b
BETA EUDESMOL	1.69 ± 0.23a	3.03 ± 0.96a	8.89 ± 0.98b
ALPHA EUDESMOL	2.89 ± 0.32a	3.83 ± 0.56a	5.81 ± 0.65b
43/139/125/200	0.89 ± 0.16a	0.00 ± 0.00b	0.00 ± 0.00b
43/139/182/125	0.00 ± 0.00a	0.32 ± 0.23a	1.87 ± 0.37b

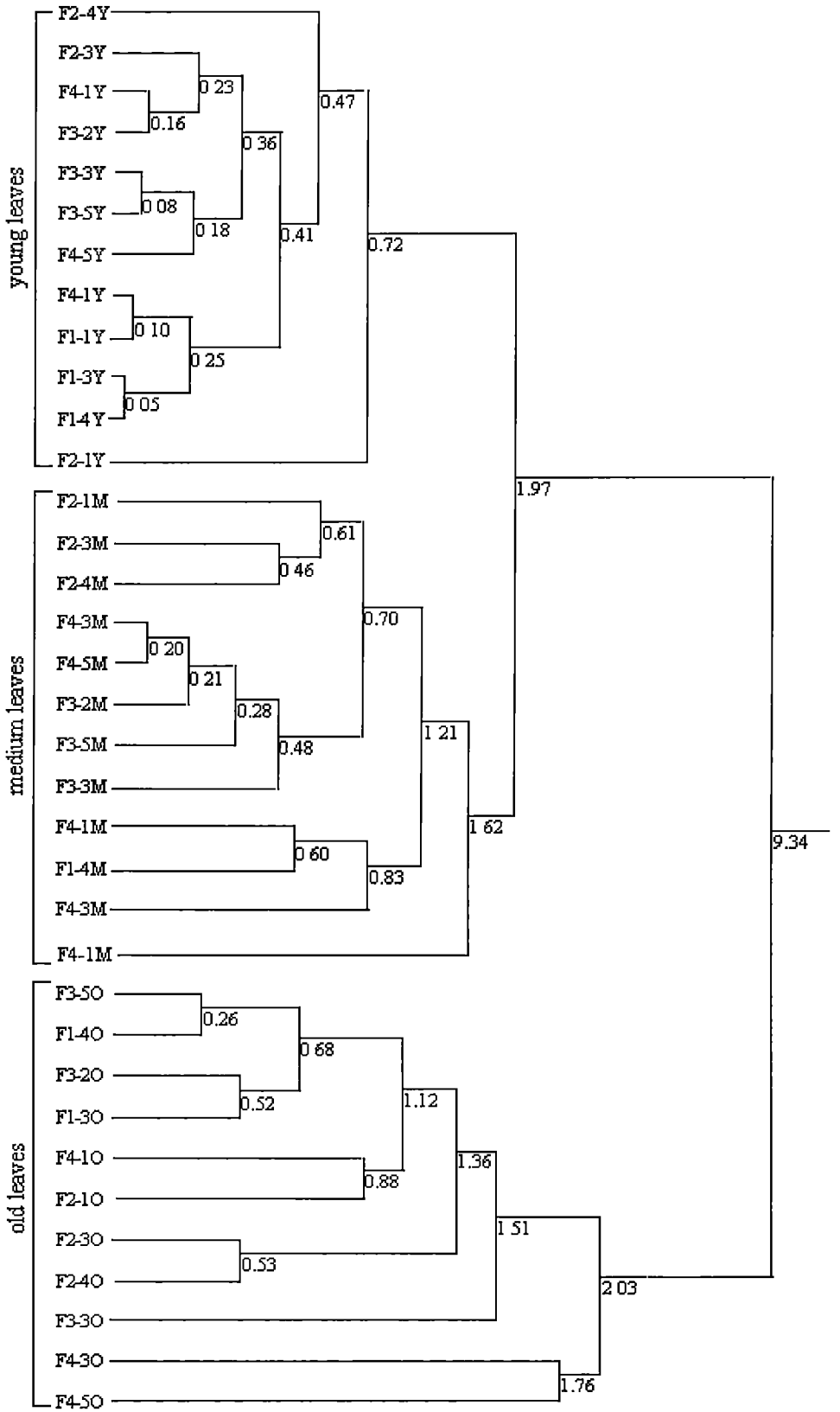
Statistical examination of the percentage composition change of individual oil compounds from young to medium aged leaves indicates that four compounds varied significantly at the family level. These were: beta elemene ($F_{3,8}$ = 6.09, P = 0.018) which increased in family 1 by 0.77 ± 0.34% compared to 0.10 ± 0.2% for family 3; "121/138/39/(161)"($F_{3,8}$ = 6.51, P = 0.015) which was not recorded in family 2 but

decreased by 1.08 ± 0.60% in family 4; "43/139/182/125" ($F_{3,8}$ = 5.22, P = 0.027) which increased by 1.63 ± 0.06% compared to 0.50 ± 0.26% in family 3; and "223/195/42/(163)" ($F_{3,8}$ = 9.76, P = 0.005) which was absent in family 1 but increased in family 2 by 0.67 ± 0.23%.

There were no individual compounds that changed significantly in their proportional composition of the total essential oil mixture at the tree susceptibility level between young and medium aged leaves and there were only four below $P \leq 0.10$. These were beta elemene ($F_{1,10}$ = 4.54, P = 0.059) which increased by 0.19 ± 0.07% for less susceptible trees compared to 0.52 ± 0.14% for susceptible trees; bicyclogermacrene ($F_{1,10}$ = 3.74, P = 0.082) which increased by 4.05 ± 1.03% increase for less susceptible trees versus 7.46 ± 1.44% for susceptible; the "43/139/182/125" ($F_{1,10}$ = 3.54, P = 0.089) which increased by 0.62 ± 0.19% for less susceptible trees versus 1.67 ± 0.54% for susceptible; and "43/139/125/200") ($F_{1,10}$ = 4.00, P = 0.074) which decreased 0.46 ± 0.78% for less susceptible versus 1.13 ± 0.58% for susceptible.

The dissimilarity of oil composition as seen in Figure 7.7 revealed that the leaf samples taken from the four *E. regnans* families were clearly definable based on leaf age class. The young and middle aged leaves were more similar than the old aged leaves. However, the variation in oil composition was not significant enough to separate leaf samples at the family level or at the susceptibility level with regards to *C. bimaculata* damage.

Figure 7.7 Dendrogram showing dissimilarity of oil composition for three leaf age classes (Y=young, M=medium, O=Old) for four *E. regnans* families (F1 & F2 susceptible, F3 & F4 non-susceptible to C. bimaculata damage). at Franklin-14. Values at each connection are incremental sum of squares.



Plenty Valley

A total of 151 different compounds (see Table A6.1 in Appendix 6) were found in the *Eucalyptus* oil (*E. nitens, E. regnans* and *E. delegatensis*) examined at the three sites. For *E. regnans*, 101 compounds were detected, compared to 49 in *E. delegatensis* and 33 in *E. nitens*. *E. regnans* contained 80 compounds unique to its oil while *E. delegatensis* had 20 and *E. nitens* 20. For the monocalypt species (*E. delegatensis* and *E. regnans*), 18 compounds were present in both but not found in *E. nitens*. Two compounds were found in both *E. nitens* and *E. regnans* but not in *E. delegatensis*. Only 11 compounds were common to all three species.

Table 7.5 lists the most dominant compounds found in the oil of each species with regards to leaf age. For *Eucalyptus regnans*, hedycaryol dominates the three age classes but represents a lower percentage for young leaves (young 10.7 ± 3.8; medium 27.3 ± 11.3; old 34.0 ± 11.5%). The large standard error associated with the mean percentage of total oil composition for Hedycaryol reflects one tree at site 1 which was completely lacking in this compound for all leaf age classes. For *E. delegatensis*, bicyclogermacrene was the most dominant in the young and middle aged leaf classes and also represent a large percentage of the total oil in old leaves (young 17.2 ± 2.8; medium 18.3 ± 3.2; old 15.5 ± 1.8%) but an unidentified compound, "110/64/39/81/(126), making up 20.3 ± 4.7%, was the most plentiful in the oil of old leaves. For *E. nitens*, pyrogallol (young 37.8 ± 6.5; medium 20.4 ± 3.0; old 23.5 ± 3.1%) and 1, 8 cineole (young 9.8 ± 0.8; medium 14.0 ± 2.9; old 13.6 ± 3.7%) dominated the oil of each age class. A comparison between the oil composition of medium and young leaves of *E. delegatensis* and *E. regnans* show that Hedycaryol, bicyclogermacrene, and alpha phellandrene represent high proportions of the oil. Bicyclogermacrene was also a dominant compound in *E. nitens*.

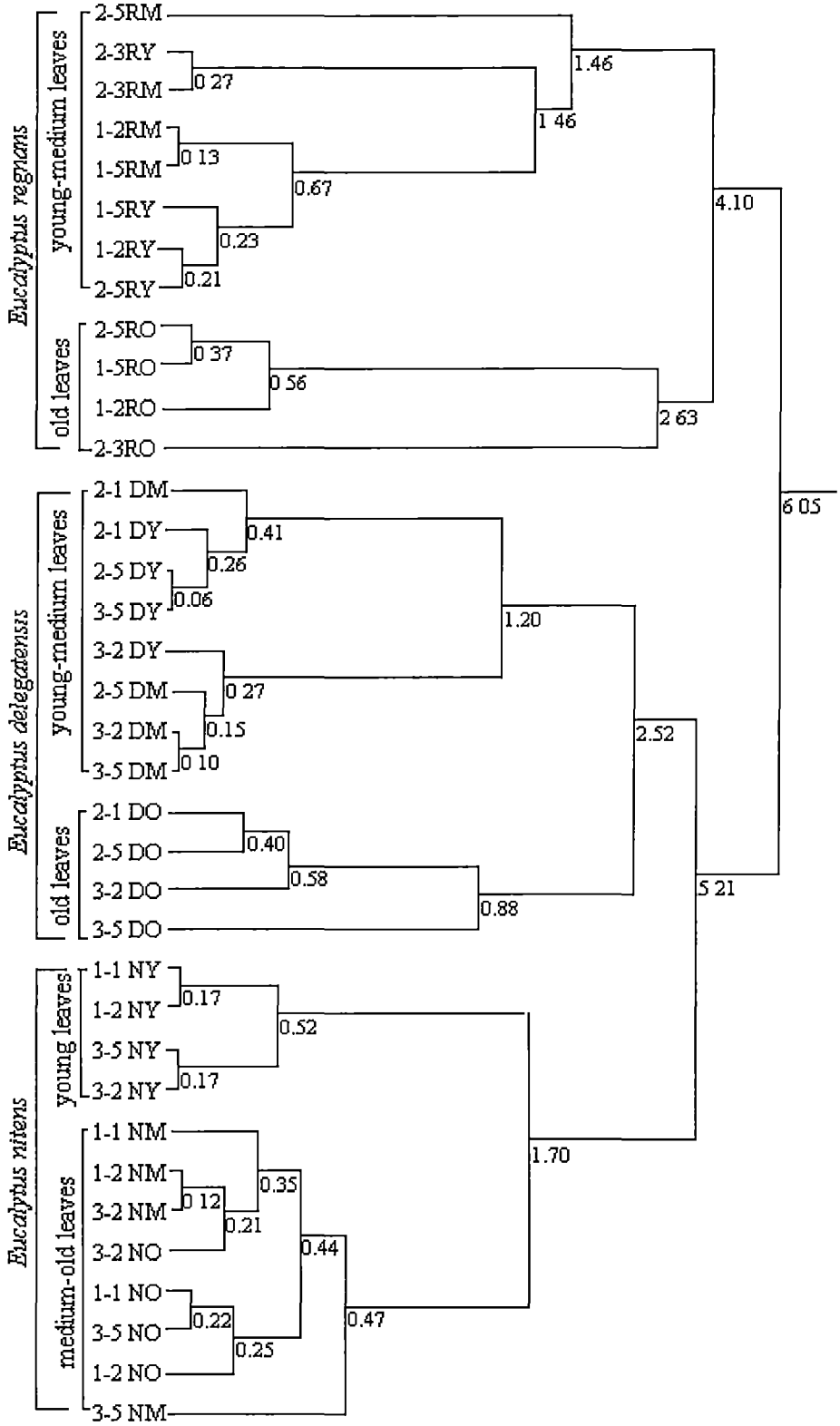
Unlike the Franklin-14 data, the dissimilarity of oil composition, as seen in Figure 7.8, revealed those leaf samples taken from within the same species were not clearly

definable. Fusions occurred between young and medium aged leaves before complete intrafusion of each age group for *E. regnans* and *E. delegatensis* trees, while for *E. nitens* this occurred between medium and old aged leaves. However, the three eucalypt species were clearly defined with fusions occurring within species samples prior to interspecies linkages. The linkage of *E. delegatensis* (Monocalyptus) with *E.* (*Symphyomyrtus*) *nitens* prior to *E.* (*Monocalyptus*) *regnans* reflects the much higher number of compounds uniquely found in the oil of *E. regnans*.

Table 7.5 The dominant essential oil compounds, by percentage composition (±S.E.), found in newly emerged leaves (young), three to four week old leaves (medium) and previous season (old) leaves of *E. regnans*, *E. delegatensis* and *E. nitens* trees in the Plenty Valley. Unidentified compounds are listed from the most abundant ions received from the mass spectra, except where bracketed indicating more distinctive ions.

Species	Leaf age	Rank	Compound	% of total composition
E. regnans	young	1	HEDYCARYOL	10.74 ± 3.77
E. regnans	young	2	BETA EUDESMOL	10.04 ± 2.61
E. regnans	young	3	215/83/209/(266)	7.30 ± 3.75
E. regnans	young	4	237/43/209/81/(252)	6.42 ± 3.70
E. regnans	young	5	ALPHA EUDESMOL	4.34 ± 2.51
E. regnans	medium	1	HEDYCARYOL	27.27 ± 11.34
E. regnans	medium	2	237/43/209/81/(252)	9.48 ± 5.19
E. regnans	medium	3	BICYCLOGERMACRENE	8.45 ± 4.00
E. regnans	medium	4	TRANS PIPERITOL	5.49 ± 3.00
E. regnans	medium	5	ALPHA PHELLANDRENE	4.46 ± 3.03
E. regnans	old	1	HEDYCARYOL	34.01 ± 11.46
E. regnans	old	2	BICYCLOGERMACRENE	16.83 ± 5.31
E. regnans	old	3	237/43/209/81/(252)	7.90 ± 3.25
E. regnans	old	4	ALPHA EUDESMOL	2.91 ± 0.23
E. regnans	old	5	ALPHA PHELLANDRENE	2.32 ± 0.18
E. delegatensis	young	1	BICYCLOGERMACRENE	17.17 ± 2.76
E. delegatensis	young	2	ALPHA PHELLANDRENE	13.55 ± 1.37
E. delegatensis	young	3	PYROGALLOL	7.88 ± 0.90
E. delegatensis	young	4	BETA PHELLANDRENE	4.04 ± 0.65
E. delegatensis	young	5	HEDYCARYOL	3.88 ± 0.78
E. delegatensis	medium	1	BICYCLOGERMACRENE	18.31 ± 3.16
E. delegatensis	medium	2	ALPHA PHELLANDRENE	12.82 ± 0.37
E. delegatensis	medium	3	HEDYCARYOL	8.24 ± 0.94
E. delegatensis	medium	4	93/121/105/67/(161)/(204)	5.11 ± 0.51
E. delegatensis	medium	5	110/64/39/81/(126)	3.69 ± 0.58
E. delegatensis	old	1	110/64/39/81/(126)	20.34 ± 4.73
E. delegatensis	old	2	BICYCLOGERMACRENE	15.50 ± 1.81
E. delegatensis	old	3	71/41/57/81/(312)	10.31 ± 3.05
E. delegatensis	old	4	ALPHA PHELLANDRENE	8.73 ± 5.12
E. delegatensis	old	5	(81)/(71)/(91)/(116)	4.38 ± 1.78
E. nitens	young	1	PYROGALLOL	37.82 ± 6.51
E. nitens	young	2	1/8 CINEOLE	9.77 ± 0.76
E. nitens	young	3	BICYCLOGERMACRENE	7.80 ± 1.98
E. nitens	young	4	ALPHA THUJENE	6.51 ± 0.55
E. nitens	young	5	P-(3OXYBUTYL) PHENYLACETATE	5.13 ± 1.96
E. nitens	medium	1	PYROGALLOL	20.41 ± 3.02
E. nitens	medium	2	1/8 CINEOLE	14.03 ± 2.88
E. nitens	medium	3	ALPHA THUJENE	7.31 ± 0.62
E. nitens	medium	4	BICYCLOGERMACRENE	6.69 ± 2.57
E. nitens	medium	5	CATCHETOL	5.43 ± 1.09
E. nitens	old	1	PYROGALLOL	23.47 ± 3.07
E. nitens	old	2	1/8 CINEOLE	13.62 ± 3.67
E. nitens	old	3	ALPHA THUJENE	5.59 ± 1.65
E. nitens	old	4	CATCHETOL	4.37 ± 1.75
E. nitens	old	5	71/41/57/81/(312)	4.31 ± 1.11

Figure 7.8 Dendrogram showing dissimilarity of oil composition for three leaf age classes (Y=young, M=medium, O=Old) for three *Eucalyptus* species (R=*regnans*, D=*delegatensis*, N=*nitens*) at three sites (1-site 1, 2=site 2, 3=site 3). Values at each connection are incremental sum of squares.



7.3.7 Leaf wax composition

Franklin-14

Of the 42 compounds analysed from the leaf wax of the four *E. regnans* families, 15 varied significantly in their proportion of total wax at the family level, 38 with leaf age and 16 for the family x leaf age interaction (Table 7.6). At the susceptibility level, 6 differed significantly [desmethyl eucalyptin, triterpene (4), triterpene (8), phenyl ethyl hexacosanoate, benzyl octacosanoate and 11,12 dehydroursolic lactone acetate], while 3 varied at the susceptibility x age interaction level (n-hexacosanol, benzyl octacosanoate and phenyl ethyl octacosanoate) (Table 7.15). At the susceptibility level, and excluding old leaves, two compounds were found to differ significantly in their proportion of total oil. For medium aged leaves triterpene (8) represented a higher proportion (8.58 ± 1.01%) in the susceptible trees compared to the less susceptible trees (5.69 ± 0.87%) [$t_{0.05\ (2),\ 5} = 2.57$, $P(|t| \geq 3.17) = 0.025$], while in medium leaves, 11,12 dehydroursolic lactone acetate was only present in the wax of susceptible trees 0.73 ± 0.21% [$t_{0\ 05\ (2),\ 5} = 2.57$, $P(|t| \geq 3.48) = 0.018$].

Table 7.6 Analysis of variance results, for the proportion of individual compounds present in leaf wax taken from four *E. regnans* families and two susceptibility classes with regard to leaf age and leaf age x family interaction. Undetermined triterpenes are listed numerically. The degrees of freedom were: family 3, age 2, family x age 6 and total 35, M.S. indicates Mean Square, * indicates $P < 0.05$, ** indicates $P < 0.01$, n.s. indicates $P > 0.01$. Phenyl ethyl is abbreviated to P. E, $^{+}$ is 11,12 dehydroursolic lactone acetate.

Wax	Fam. M.S.	Age M. S.	FamXAge M. S.	Susc. M. S.	Age M. S.	SuscXAge M. S.
c25 n-pentacosane	0.182.n.s.	1.722**	0 114 n s	0 007 n.s.	1.722**	0.008 n.s.
c27 n-heptacosane	0 275 n s	4.566**	0.212 n.s.	0.590 n.s.	4.566**	0.295 n.s.
c26 n-hexacosanal	0.183 n.s	4.712**	0.548 n.s.	0.340 n.s.	4.742**	1.280**
c26 n-hexacosanol	0.727*	0.547 n.s.	0.094 n.s.	0.002 n.s.	0.556 n.s.	0.063 n s.
c29 n-nonacosane	0.338 n.s.	1.851**	0.158 n.s.	0.024 n s.	2.068**	0.215 n.s
Desmethyl eucalyptin	14.773*	66.236**	7.279 n.s	24 290*	60 100**	7.315 n.s.
Eucalyptin	1.474 n s	4.839 n s.	4 015 n.s.	2.300 n.s.	4.895 n.s.	5.724 n.s.
c28 n-octacosanal	0.304 n.s.	2.399**	0.192 n s.	0.619 n s.	2 332**	0 171 n.s
P. E. eicosanoate	0 320*	1.360**	0.323**	0.097 n.s.	1.598**	0.010 n.s.
c31 n-henicontane	0.046 n.s.	0.117*	0.048 n.s	0.077 n.s	0.105*	0.001 n s
Triterpene (6)	0.177 n.s	2 073**	0.845*	0.139 n.s.	2.299**	0.028 n.s.
Triterpene (11)	0.575 n.s.	115.584**	0.594 n s.	0.214 n.s.	116.187**	0.216 n.s.
Triterpene (1)	4 095 n.s.	69.325**	2.372 n.s.	0 003 n s	71.341**	1.687 n.s.
Triterpene (2)	4.751*	93.162**	3.062*	1.257 n.s.	98.310**	0.520 n.s.
Triterpene (17)	1.974 n.s.	151.866**	1.989 n.s.	0.198 n.s.	152.659**	0.199 n.s.
Triterpene (3)	1.354 n.s	9.430**	0.957 n.s.	0 053 n.s	10.199**	0.059 n.s.
Triterpene (4)	15 146**	20.159**	14.572**	25 886*	18.774*	10.855 n.s.
Triterpene (5)	3 185 n s.	25.930**	6.439**	0.169 n.s.	25.486**	6.824 n.s.
P. E. docosanoate	0.099*	0.565**	0.071*	0.037 n.s.	0.658**	0.009 n.s.
Amyrin	1.802 n s	23 889**	2.028 n.s.	5 038 n s	24 155**	0.635 n.s.
Benzyl tetracosanoate	0.747*	4.118**	0.715**	0 241 n.s	4.959**	0.231 n.s.
Hentriacontan-14,16-dione	0 039 n.s.	0.048 n.s.	0.035 n s	0.039 n.s.	0.048 n.s.	0.036 n s.
Triterpene (32)	16.500 n.s.	103.87*	16 57 n.s.	12.07 n.s.	104.41*	12.150 n.s.
Methyl moronate	25.52 n s	2276.47**	45.63*	34 810 n s.	2294.43**	52.880 n.s.
Triterpene (18)	4.945*	17.970**	3.503*	0.184 n s	17 138**	0.425 n.s.
Triterpene (21)	2.482*	20.310**	2.525*	0 811 n.s.	18.726**	0.845 n.s.
P. E. tetracosanoate	0.949 n.s.	4.768**	0 200 n.s.	1.149 n.s	5.285**	0.078 n.s.
Triterpene (8)	9.519 n.s.	170.434*	3.827 n s	24.986**	167.874*	7 584 n.s.
Triterpene (33)	0 700 n s.	63.943**	0.706 n s.	0.020 n.s.	64.276**	0.020 n.s.
Triterpene (7)	60.57**	1459.04**	29.20*	16.08 n.s.	2971.27**	26.02 n.s.
Triancontan-16,18-dione	3.909 n.s.	14.939*	1.519 n.s.	1.962 n s	17.200*	2.187 n.s.
P. E. pantacosanoate	0.003 n s.	0.046**	0.003 n.s.	0.007 n.s.	0.047**	0.006 n.s.
Benzyl hexacosanoate	1.289*	17.572**	1.123*	0.755 n.s.	19.722**	0.720 n.s.
Triterpene (14)	0.668 n.s.	14.306**	0.670 n.s.	0.523 n.s.	14.381**	0.527 n.s.
Triterpene (15)	3.236*	23.352**	3.431**	0.048 n.s.	21.100**	0 050 n.s.
Hexasanoyl benzoate	0.544*	2.301**	0 430*	0.114 n.s.	1.917**	0 073 n.s.
P. E. hexacosanoate	1.124 n.s.	5.588**	0 821 n s.	2.761*	5.949**	1.460 n.s.
Triterpene (9)	0 558 n.s.	19.028**	0 586 n.s.	0.053 n s.	18.144**	0.055 n.s.
Benzyl octacosanoate	0 022 n.s.	0.059 n.s.	0 021 n.s.	0.063*	0.060 n.s.	0.060*
Octasanoyl benzoate	0 143**	0.315*	0 016 n s.	0.070 n s.	0.665*	0.073 n.s.
P. E. octocosanoate	0.020 n s.	0.035*	0.016 n s	0.039 n.s.	0.035*	0.032 n s
11,12 dehyd. lact. acetate^{+}	0 605**	0.674**	0.247**	1.267**	0 674**	0.316 n s.

Of the 38 compounds in the leaf wax that varied significantly with leaf age, 22 were between young and medium leaf age classes, 31 between young and old and 21 between medium and old (Table 7.7).

The percentage composition of three individual wax compounds changed significantly from young to medium aged leaves at the family level. These were: phenyl ethyl eicosanoate ($F_{3,8} = 11.41$, $P = 0.003$)(significant differences between families 1 & 2, 2 & 3 and 1& 4) which decreased by 1.14 ± 0.33% for family 1, 0.97 ± 0.66% for family 3 and 0.07 ± 0.21% for family 4, but increased by 0.33 ± 1.16% for family 2; benzyl tetracosanoate ($F_{3,8} = 4.83$, $P = 0.033$) which decreased by 2.12 ± 0.72% for family 1, significantly greater than family 2 (0.19 ± 0.18%); and octocosanoyl benzoate ($F_{3,8} = 6.29$, $P = 0.017$) where family 4 decreased by 0.81 ± 0.28%, significantly more than family 3 (0.17 ± 0.02%), while this compound was not recorded in family 1.

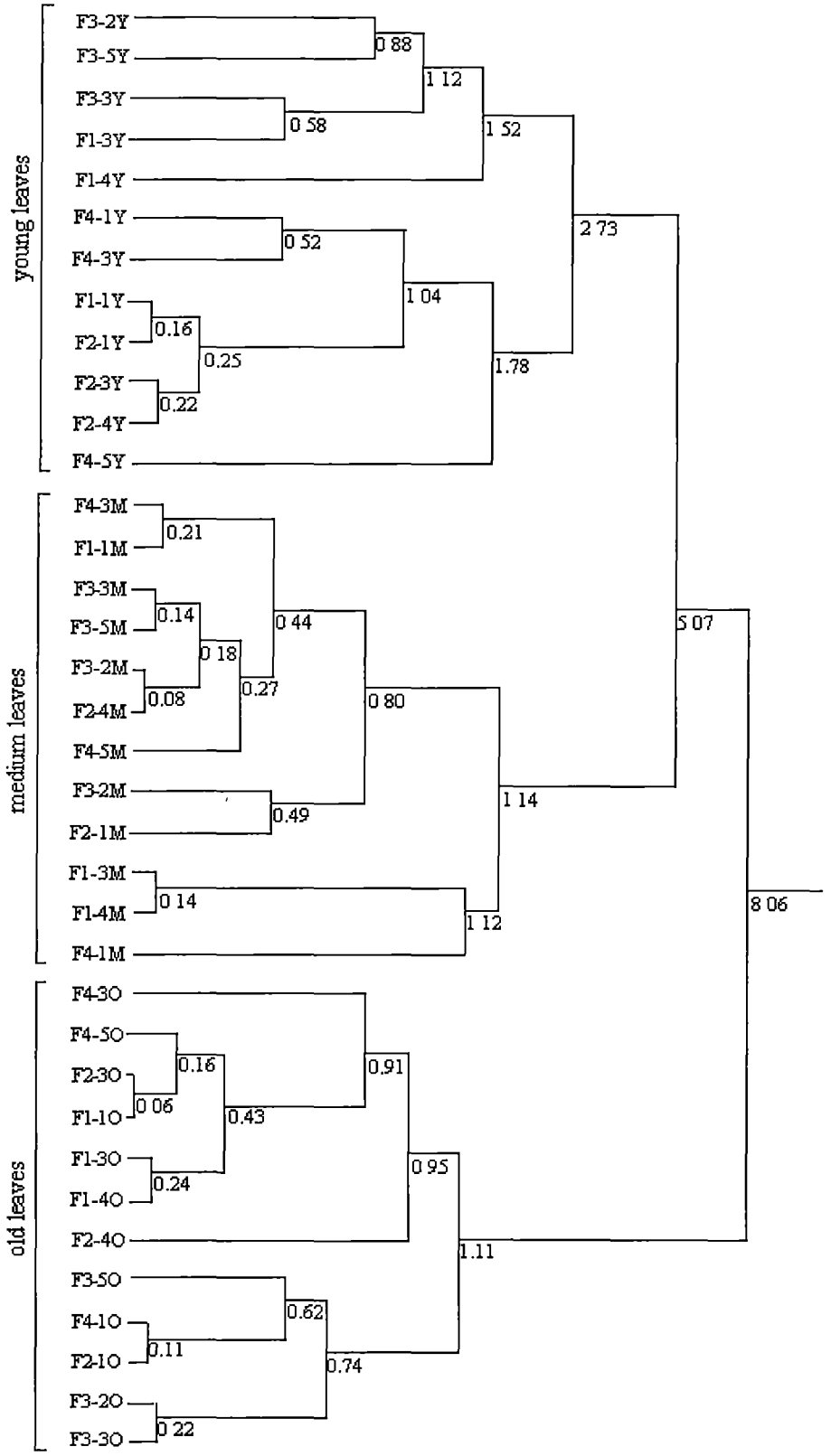
There were no compounds that showed a significant change ($P \leq 0.05$) in proportion at the susceptibility level and only one, n-Triancontane-16,18-dione, was below $P \leq 0.10$ ($F_{1,10} = 4.51$, $P = 0.060$). For this compound the less-susceptible trees increased by 3.15 ± 1.31% compared to an 0.73 ± 0.09% increase in susceptible trees.

As with the oil composition, the dissimilarity of the wax composition (Figure 7.9) revealed that the leaf samples taken from the four *E. regnans* families were clearly definable based on leaf age class. The young and middle aged leaves were also more similar than the old aged leaves. However, like the oil composition, the variation in wax composition was not significant enough to separate leaf samples at the family level or at the susceptibility level with regards to *C. bimaculata* damage.

Table 7.7 Mean percentage (± S. E.) of individual compounds present in leaf waxes from three leaf age classes, young, medium and old from 4 *E. regnans* families at Franklin-14. Undetermined triterpenes are listed numerically. Means in the same row followed by the same letter are not significantly different. (n = 36, d.f. 2,33). $^{+}$ 11,12 dehydroursolic lactone acetate

Wax	Young		Medium		Old nnnnnnnn	
c25 n-pentacosane	0.87 ± 0.19	a	0.34 ± 0.07	b	0.18 ± 0.04	b
c27 n-heptacosane	1.71 ± 0.21	a	0.97 ± 0.11	b	0.48 ± 0.04	c
c26 n-hexacosanal	0.25 ± 0.08	a	1.59 ± 0.18	b	0.74 ± 0.11	a
c26 n-hexacosanol	0.91 ± 0.18	a	1.19 ± 0.13	a	0.78 ± 0.16	a
c29 n-nonacosane	1.43 ± 0.18	a	1.15 ± 0.12	a	0.70 ± 0.07	b
Desmethyl eucalyptin	7.61 ± 0.87	a	4.21 ± 0.68	b	3.31 ± 0.40	b
Eucalyptin	5.06 ± 0.48		3.88 ± 0.29		4.59 ± 0.70	
c28 n-octacosanal	0.66 ± 0.12	a	1.47 ± 0.18	b	0.65 ± 0.06	a
Phenyl ethyl eicosanoate	0.77 ± 0.17	a	0.32 ± 0.09	b	0.13 ± 0.02	b
c31 n-henicontane	0.22 ± 0.06	a	0.13 ± 0.05	a	0.05 ± 0.03	b
Triterpene (6)	1.18 ± 0.22	a	1.03 ± 0.18	a	0.32 ± 0.13	b
Triterpene (11)	0.00 ± 0.00	a	0.00 ± 0.00	a	5.51 ± 0.59	b
Triterpene (1)	4.10 ± 0.50	a	4.29 ± 0.65	a	0.00 ± 0.00	b
Triterpene (2)	5.34 ± 0.56	a	4.33 ± 0.40	a	0.00 ± 0.00	b
Triterpene (17)	0.00 ± 0.00	a	0.00 ± 0.00	a	6.25 ± 0.86	b
Triterpene (3)	1.83 ± 0.43	a	0.86 ± 0.11	b	0.00 ± 0.00	c
Triterpene (4)	2.75 ± 0.28	a	4.58 ± 0.39	ab	4.79 ± 1.07	b
Triterpene (5)	2.05 ± 0.24	a	2.57 ± 0.28	a	5.41 ± 0.78	b
Phenyl ethyl docosanoate	0.41 ± 0.10	a	0.00 ± 0.00	b	0.08 ± 0.02	b
Amyrin	0.88 ± 0.16	a	3.48 ± 0.44	b	3.30 ± 0.29	b
Benzyl tetracosanoate	1.03 ± 0.29	a	0.00 ± 0.00	b	0.00 ± 0.00	b
n-Hentriacontan-14,16-dione	0.00 ± 0.00		0.11 ± 0.10		0.00 ± 0.00	
Triterpene (32)	0.00 ± 0.00	a	0.00 ± 0.00	a	5.18 ± 2.16	b
Methyl moronate	1.05 ± 0.33	a	2.17 ± 0.62	a	26.09 ± 2.28	b
Triterpene (18)	0.00 ± 0.00	a	2.25 ± 0.58	b	2.26 ± 0.38	b
Triterpene (21)	0.00 ± 0.00	a	2.34 ± 0.52	b	0.00 ± 0.00	a
Phenyl ethyl tetracosanoate	1.35 ± 0.27	a	0.12 ± 0.12	b	0.64 ± 0.23	b
Triterpene (8)	5.22 ± 0.77	a	7.13 ± 0.77	b	0.00 ± 0.00	c
Triterpene (33)	0.00 ± 0.00	a	0.00 ± 0.00	a	3.95 ± 0.45	b
Triterpene (7)	28.00 ± 0.99	a	24.03 ± 1.79	a	6.71 ± 0.46	b
n-Triancontan-16,18-dione	1.54 ± 0.43	a	3.48 ± 0.53	b	2.21 ± 0.36	b
Phenyl ethyl pantacosanoate	0.10 ± 0.04	a	0.00 ± 0.00	b	0.00 ± 0.00	b
Benzyl hexacosanoate	2.13 ± 0.43	a	0.01 ± 0.01	b	0.00 ± 0.00	b
Triterpene (14)	0.00 ± 0.00	a	0.00 ± 0.00	a	1.79 ± 0.52	b
Triterpene (15)	0.00 ± 0.00	a	2.44 ± 0.59	b	0.00 ± 0.00	a
Hexasanoyl benzoate	0.67 ± 0.24	a	0.00 ± 0.00	b	0.00 ± 0.00	b
Phenyl ethyl hexacosanoate	1.44 ± 0.41	a	0.32 ± 0.09	b	0.20 ± 0.05	b
Triterpene (9)	0.00 ± 0.00	a	2.28 ± 0.52	b	0.00 ± 0.00	a
Benzyl octacosanoate	0.13 ± 0.07		0.00 ± 0.00		0.00 ± 0.00	
Octasanoyl benzoate	0.27 ± 0.12	a	0.00 ± 0.00	b	0.00 ± 0.00	b
Phenyl ethyl octocosanoate	0.11 ± 0.05		0.02 ± 0.01		0.02 ± 0.01	
11,12 dehyd. lact. acetate^{+}	0.00 ± 0.00	a	0.36 ± 0.15	ab	0.57 ± 0.15	b

Figure 7.9 Dendrogram showing dissimilarity of wax composition for three leaf age classes (Y=young, M=medium, O=Old) for four *E. regnans* families (F1 & F2 susceptible, F3 & F4 non-susceptible to C. bimaculata damage)at Franklin-14. Values at each connection are incremental sum of squares.



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A total of 105 different compounds were found (96 are listed in Table A6.2, Appendix 6) in the *Eucalyptus* leaf waxes (*E. nitens, E. regnans* and *E. delegatensis*). For *E. regnans*, 40 compounds were detected compared to 54 in *E. delegatensis* and 58 in *E. nitens*. *E. regnans* contained 16 compounds unique to its leaf wax while *E. delegatensis* had 27 and *E. nitens* 29. For the monocalypts, only 6 compounds were present in both and absent in *E. nitens*. Six compounds were found in both *E. nitens* and *E. regnans* but not in *E. delegatensis* while 9 compounds were in both *E. regnans* and *E. nitens* but not in *E. delegatensis*. Only 14 compounds were common to all three species.

Table 7.8 lists the most dominant compounds found in the leaf wax of each eucalypt species with regards to leaf age. Eucalyptin represented a high proportion of the leaf wax for all three species. For *E. nitens* it represented a much higher proportion of the wax for old leaves (15.22 ± 1.55%) compared to medium aged (2.26 ± 0.27%) and young leaves (4.11 ± 0.63%). For *E. delegatensis* there was a gradual increase in proportion of this compound (young 5.12 ± 0.69; medium 7.43 ± 1.02; old 10.73 ± 0.76), while for *E. regnans* the trend was in the opposite direction (young 9.42 ± 1.01; medium 8.04 ± 0.32; old 5.82 ± 1.01). Desmethyl eucalyptin was also a major component of the leaf wax of *E. regnans* young leaves (14.82 ± 2.32) and medium aged leaves (7.22 ± 0.60) and in *E. nitens* young leaves (5.43 ± 1.55).

The most common β-diketone for *E. delegatensis* was n-hentriacontan-14,16-dione, being a major component of *E. delegatensis* leaf wax (young 9.75 ± 1.63; medium 11.36 ± 1.33; old 6.76 ± 0.68), while for *E. nitens* n-triancontan-14,16-dione was a major component (young 10.68 ± 1.61%; medium 18.21 ± 3.45%; old 14.64 ± 2.33%). N-triancontan-14,16-dione was also the major β-diketone in *E. regnans* oil but represented a lower proportion of the total leaf wax (young 3.30 ± 0.80%; medium 3.18 ± 1.18%; old 2.31 ± 0.97%) compared to the β-diketones in the other species.

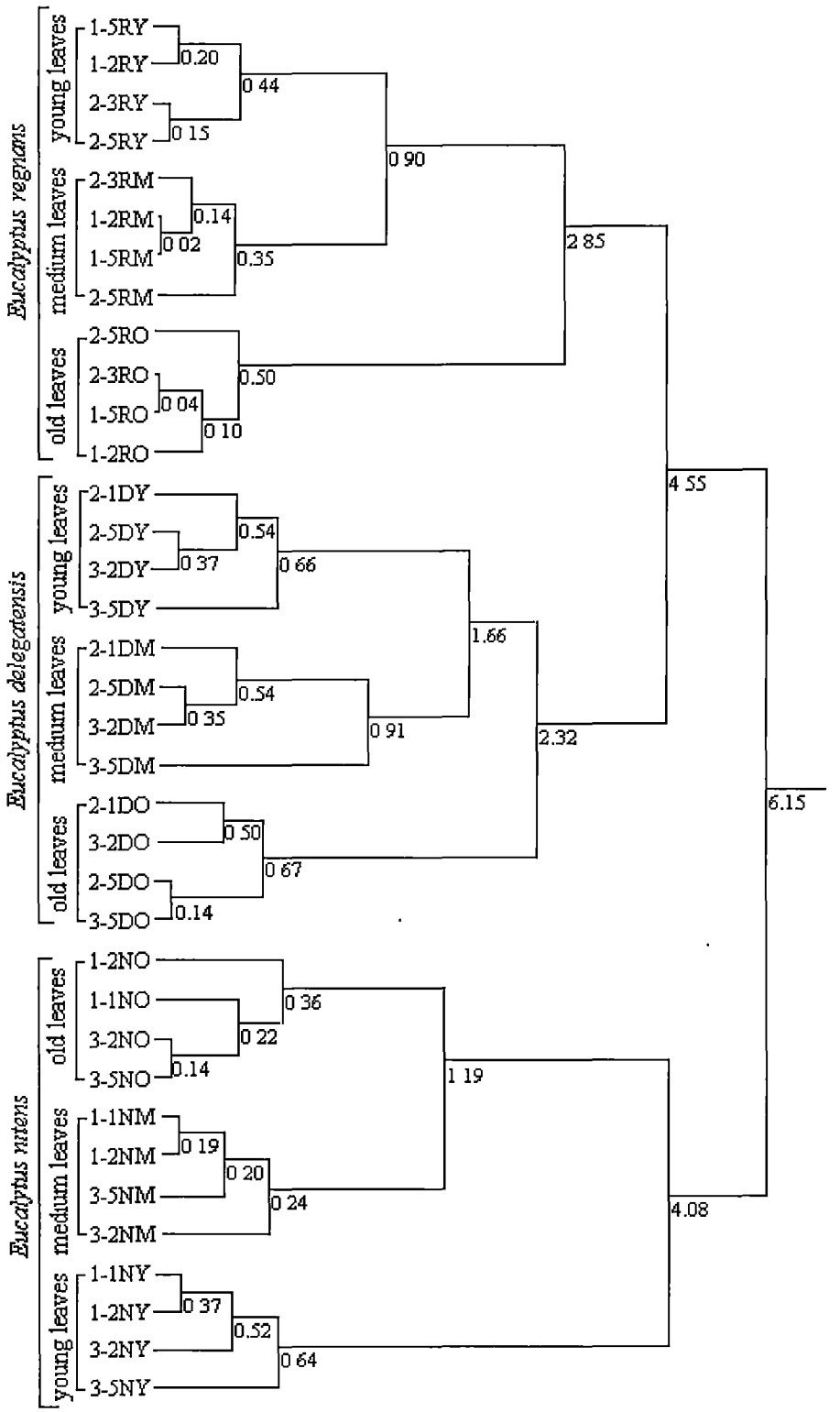
The aldehyde n-tetracosanal (young 9.75 ± 1.63%; medium 11.36 ± 1.33%; old 6.76 ± 0.68%) was also a major constituent of the wax of *E. delegatensis* at all leaf ages, while for *E. nitens* n-triacontanal was the most common (young 2.22 ± 0.35%; medium 6.93 ± 0.70%; old 1.99 ± 0.32%). For *E. regnans* aldehydes were not a major constituent.

Unlike the Plenty Valley oil composition data, the dissimilarity of leaf wax composition (Figure 7.10) indicates that leaf samples were clearly definable based on eucalypt species and with regards to leaf age. However, like the oil data, the young and middle aged leaf classes for both *E. regnans* and *E. delegatensis* were more similar to each other than to old aged leaves. For *E. nitens,* the middle and old aged leaves were again more similar to each other than to the young leaves. In contrast to the oil composition data, *E. regnans* and *E. delegatensis* were more similar to each other than to *E. nitens.*

Table 7.8 The dominant leaf wax compounds found in newly emerged leaves (young), three to four week old leaves (medium) and previous season (old) leaves of *E. regnans*, *E. delegatensis* and *E. nitens* trees in the Plenty Valley. Undetermined triterpenes are listed numerically followed by most abundant ion received from the mass spectra.

Species	Leaf age	Rank	Compound	% of total composition
E. regnans	young	1	Triterpene (7) 203	23.26 ± 1.26
E. regnans	young	2	desmethyl eucalyptin	14.82 ± 2.32
E. regnans	young	3	eucalyptin	9.42 ± 1.01
E. regnans	young	4	n-Triancontan-16,18-dione	3.30 ± 0.80
E. regnans	young	5	Triterpene (2) 408	2.89 ± 0.69
E. regnans	medium	1	Triterpene (7) 203	20.47 ± 3.37
E. regnans	medium	2	eucalyptin	8.04 ± 0.32
E. regnans	medium	3	desmethyl eucalyptin	7.22 ± 0.60
E. regnans	medium	4	Triterpene (8) 203	6.03 ± 0.51
E. regnans	medium	5	Triterpene (2) 408	3.98 ± 0.16
E. regnans	old	1	Methyl moronate	19.06 ± 2.98
E. regnans	old	2	Triterpene (4) 204	9.88 ± 1.92
E. regnans	old	3	Triterpene (7) 203	6.65 ± 1.78
E. regnans	old	4	eucalyptin	5.82 ± 1.01
E. regnans	old	5	Triterpene (11) 163,191	5.25 ± 0.75
E. delegatensis	young	1	n-Hentriacontan-14,16-dione	13.30 ± 3.23
E. delegatensis	young	2	Tetracosanal	9.75 ± 1.63
E. delegatensis	young	3	c26 alkanal (n-hexcosanal)	9.73 ± 0.47
E. delegatensis	young	4	Phenyl ethyl docodanoate	6.33 ± 1.36
E. delegatensis	young	5	eucalyptin	5.12 ± 0.69
E. delegatensis	medium	1	Tetracosanal	11.36 ± 1.33
E. delegatensis	medium	2	Triterpene (D20) 408	10.09 ± 2.25
E. delegatensis	medium	3	eucalyptin	7.42 ± 1.02
E. delegatensis	medium	4	n-Hentriacontan-14,16-dione	7.07 ± 1.11
E. delegatensis	medium	5	c24 alkanol (n-tetracosan-1-ol)	5.96 ± 0.66
E. delegatensis	old	1	Methyl moronate	14.17 ± 3.05
E. delegatensis	old	2	eucalyptin	10.73 ± 0.76
E. delegatensis	old	3	Tetracosanal	6.76 ± 0.68
E. delegatensis	old	4	Triterpene (D12) 410	6.68 ± 2.11
E. delegatensis	old	5	n-Hentriacontan-14,16-dione	5.74 ± 1.64
E. nitens	young	1	n-Triancontan-16,18-dione	10.68 ± 1.61
E. nitens	young	2	Phenyl ethyl docodanoate	5.96 ± 0.97
E. nitens	young	3	Triterpene (D20) 408	5.56 ± 0.11
E. nitens	young	4	desmethyl eucalyptin	5.43 ± 0.58
E. nitens	young	5	Phenyl ethyl tetracosanoate	5.17 ± 0.83
E. nitens	medium	1	n-Triancontan-16,18-dione	18.21 ± 3.45
E. nitens	medium	2	Triterpene (N10) 450	13.38 ± 2.30
E. nitens	medium	3	c29 alkane (n-nonacosane)	9.10 ± 1.44
E. nitens	medium	4	n-c30 alkanal (n-triacontanal)	6.93 ± 0.70
E. nitens	medium	5	11,12 dehydroursolic lactone acetate	6.43 ± 1.18
E. nitens	old	1	eucalyptin	15.22 ± 1.55
E. nitens	old	2	n-Triancontan-16,18-dione	14.64 ± 2.33
E. nitens	old	3	Triterpene (N10) 450	13.26 ± 2.10
E. nitens	old	4	c29 alkane (n-nonacosane)	6.54 ± 0.82
E. nitens	old	5	desmethyl eucalyptin	4.65 ± 1.02

Figure 7.10 Dendrogram showing dissimilarity of leaf wax composition for three leaf age classes (Y=young, M=medium, O=Old) for three *Eucalyptus* species (R=*regnans*, D=*delegatensis*, N=*nitens*) at three sites (1=site 1, 2=site 2, 3=site 3). Values at each connection are incremental sum of squares.



7.3.8 *C. bimaculata* egg batch occurrence

Plenty Valley

A patchy *C. bimaculata* oviposition event occurred at site 1 on the 31st of December 1996, however, the monitored trees at site 1 and 2 both received higher numbers of egg batches on the 25th of January and leaves were collected from these sites the following day. At site 3, monitored trees received egg batches on the 7th of February and leaves were collected on the same day.

At site 1, 24.0 ± 4.2% of *E. regnans* leaves with toughness less than 58.5 g received egg batches, significantly more than *E. nitens* leaves of equivalent toughness of which 0.8 ± 0.2% received eggs [$t_{0.05\,(2),\,8} = 2.31$, $P(|t| \geq 5.49) < 0.001$]. At site 2 there was no significant difference between *E. delegatensis* (13.6 ± 2.5) and *E. regnans* (8.8 ± 3.0) [$t_{0\,05\,(2),\,8} = 2.31$, $P(|t| \geq 1.23) = 0.253$], while at site 3 *E. delegatensis* (38.8 ± 2.9%) received significantly more egg batches compared to *E. nitens* (19.6 ± 3.9%) [$t_{0.05\,(2),\,8} = 2.31$, $P(|t| \geq 3.95) = 0.004$].

With regards to leaf area, *E. regnans* received significantly more egg batches (1567.0 ± 165.1 egg batches/m^2) compared to *E. nitens* (39.2 ± 25.4 egg batches/m^2) at site 1 [$t_{0\,05\,(2),\,8} = 2.31$, $P(|t| \geq 9.15) < 0.001$]. At site 2, *E. delegatensis* (934.1 ± 158.2 egg batches/m^2) received significantly more egg batches per unit leaf area compared to *E. regnans* (412.1 ± 147.3 egg batches/m^2) [$t_{0\,05\,(2),\,8} = 2.31$, $P(|t| \geq 2.41) = 0.042$], while at site 3 *E. delegatensis* (1730.8 ± 332.7 egg batches/m^2) received significantly more eggs than *E. nitens* (499.9 ± 99.1 egg batches/m^2)[$t_{0\,05\,(2),\,8} = 2.31$, $P(|t| \geq 3.55) = 0.008$].

Franklin 14

At Franklin 14 there were two distinct periods of egg batch deposition by *C. bimaculata*. Egg batches were present but patchy on the 16th December 1996 with many trees failing to receive eggs. Egg batches were again present on the 20th of February 1997 and although the distribution was patchy the egg batches were distributed more widely. The latter data was analysed.

At the family level no significant difference was found with regard to the percentage of egg batches present on leaves with toughness less than 58.5 g ($F_{3,19} = 2.05$, $P = 0.148$). Family 1 had 14.8 ± 2.8% of leaves with egg batches, family 2, 26.40 ± 7.1% family 3, 12.8 ± 4.6% and family 4, 12.4 ± 2.6%. Likewise, no significant difference was recorded at the susceptibility to *C. bimaculata* level ($F_{1,19} = 2.80$, $P = 0.111$) with the susceptible trees receiving 20.6 ± 5.8% compared to less susceptible trees 12.6 ± 3.5%.

With regards to egg batches per unit leaf area there was a significant difference between families ($F_{3,19} = 5.85$, $P = 0.007$) with a post-hoc Tukeys test revealing significant differences between family 2 (2187.1 ± 621.7 egg batches/m^2) versus family 3 (550.5 ± 215.1 egg batches/m^2) and family 4 (291.0 ± 56.9 egg batches/m^2). Family 1 (796.4 ± 23.15 egg batches/m^2) was not significantly different from the others. There was also a significance at the susceptibility level ($F_{1,19} = 6.98$, $P = 0.017$) with susceptible trees receiving 1491.8 ± 55.05 egg batches/m^2 compared to less susceptible trees 420.8 ± 16.0 egg batches/m^2.

7.4 Discussion

Plenty Valley

Previous studies have shown that the *Monocalyptus* species *E. regnans* and *E. delegatensis* and the *Symphyomyrtus* species *E. nitens* can all receive a great deal of damage from feeding *C. bimaculata* (Greaves 1966; de Little & Madden 1975; de Little 1989). However, this study has shown that this insect has an oviposition preference hierarchy between these three species with more eggs oviposited per leaf number and unit leaf area on *E. regnans* compared to *E. nitens*, on *E. delegatensis* compared to *E. nitens* and more on *E. delegatensis* per unit leaf area than on *E. regnans* (also see Chapter 4).

The amount of nitrogen in the foliage does not appear to influence *C. bimaculata* oviposition choice between these eucalypt species. Steinbauer et al. (1998a) found that there was no significant difference between oviposition preference for *E. regnans* expanding leaves compared to *E. nitens* expanding leaves in the laboratory, even though they recorded *E. nitens* as having a marginally higher total nitrogen content compared to *E. regnans* for these leaf types. Likewise, this study failed to show any significant difference in the carbon-nitrogen ratio between these two species or between *E. delegatensis* and *E. nitens* with leaf age. However, the influence of total leaf nitrogen on oviposition choice and tree susceptibility to larval defoliation between *E. regnans* and *E. delegatensis* cannot be ruled out since *E. regnans* had a significantly greater increase in the C-N ratio with leaf age while *E. delegatensis* received significantly more egg batches per unit leaf area compared to *E. regnans*. It is important to note that the change in the C-N ratio with leaf age can vary significantly within tree species with location as was noted between *E. nitens* at sites 1 and 3. Such variations caused through site effect could potentially influence insect herbivory.

The presence of egg batches on *E. nitens*, *E. delegatensis* and *E. regnans* demonstrates that *C. bimaculata* will oviposit on tree species which vary greatly in their leaf essential

oil and wax composition. *E. regnans* contains a much greater richness of oil compounds compared to *E. delegatensis* and *E. nitens,* while all contained a large proportion of compounds unique to their species. There was a greater degree of similarity between the more dominant compounds in *E. delegatensis* and *E. regnans* as opposed to *E. nitens*, particularly in the essential oil composition.

However, in all cases essential oil and leaf wax composition within species remains more similar with changing leaf age compared to between species differences. Although Steinbauer et. al (1998a) found no significant preference for *C. bimaculata* oviposition between expanding *E. regnans* and *E. nitens* leaves, it was shown that *E. nitens* expanding leaves were preferred over fully mature *E. regnans* leaves and vice versa. As Steinbauer et al. (1998a) suggest, this implies that leaf age effects are more influential in oviposition preference than essential oil composition. It also appears that essential oil yield is unlikely to be involved in these preferences, since Li (1993) found little variation as leaves aged for the *Eucalytpus* species (including *E. nitens* and *E. delegatensis*) he examined. However, volatile essential oils may influence the pre-landing behaviour of *C. bimaculata* and could account for the hierarchical preferences between host species.

The production of current season leaves, both initiated and surviving through to the end of the season, does not appear to significantly influence the egg batch density between the three species. Although *E. regnans* initiated significantly more leaves compared to *E. delegatensis* at site 2, there was no significant difference between egg batches per leaf between the two species. At site 3, significantly more leaves initiated by *E. nitens* during the monitoring period survived through to the end of the season compared to *E. delegatensis*, however, *E. delegatensis* received significantly more egg batches per leaf. In all other cases there were no significant differences between species regarding leaf numbers initiated or initiated and surviving at the end of the season, even though there were significant differences between egg batches per leaf number (apart from between *E. delegatensis* and *E. regnans* at site 2).

Although mean leaf size has the potential to influence the density of egg batches per unit leaf area (see Chapter 3), it does not account for the significant differences between egg batch numbers/leaf recorded for *E. nitens* versus the two monocalypt host species at site 1 and site 3. At both sites the monocalypt species received significantly more egg batches per leaf and unit leaf area compared to *E. nitens*. At site 1 in particular, *C. bimaculata* egg batches were rare in the *E. nitens* foliage but common in the foliage of all *E. regnans* trees monitored. However, site effects may affect leaf size within species and thus egg batch density per leaf area.

The rate of leaf maturation (in this thesis measured as leaf toughness) may influence the degree of oviposition a tree receives since *C. bimaculata* prefers to oviposit on expanding leaves rather than fully mature leaves (Steinbauer et al. 1998a &Chapter 5). There were significant differences in leaf maturation rates between *E. regnans* and *E. nitens* at site 1 for all six months and between *E. nitens* and *E. delegatensis* for November, December and January. Both monocalypt species leaves matured more slowly than *E. nitens*. However, the egg batch data examined the same number of leaves with toughness lower than 58.5 g and so the difference between egg batch density between host species cannot directly be explained by leaf toughness. The proportional abundance of expanding leaves (usually with toughness less than 58.5 g) to mature leaves, which can vary between species (see Steinbauer et al. 1998a) may have indirectly influenced egg batch density on this leaf class (e.g. time insect is willing to search a host for suitable leaves). This could then support the hypothesis proposed by Steinbauer et al. (1998a) that the relative amount of expanding foliage may influence oviposition choice.

The rate of leaf maturation with regards to leaf area produced in the current season can potentially influence the degree of larval herbivory and the carrying capacity of the tree for *C. bimaculata*. At all times, and most of the time significantly so, *E. nitens* had developed a greater leaf area than *E. regnans* at site 1. Earlier in the season (up until mid January) *E. nitens* had a greater area of leaves under 58.5 g, suitable for neonate larval establishment and thus in this respect would have a greater carrying capacity for

this larval type compared to *E. regnans*. However, late in the season *E. regnans* had a greater area of this foliage compared to *E. nitens*. By the time leaves had reached toughness levels in excess of 92.1 g (unable to be utilised by larvae for development), *E. nitens* always had greater leaf area. Thus early in the season (until mid November) both species appear vulnerable to losing all current season foliage through larval feeding following the deposition of a high density of egg batches. However, from early December onward *E. nitens* is increasingly likely to retain more current season foliage than *E. regnans*, thus reducing the severity of defoliation. Intense oviposition by *C. bimaculata* rarely begins before mid November (Greaves 1966).

The same trend in leaf maturation was also observed between *E. nitens* and *E. delegatensis* at site 3, although *E. nitens* retained a significantly larger area of foliage with toughness less than 58.5 g for all but the last time contrast measurement in March. *E. nitens* would thus support a higher carrying capacity for first instar larvae with regards to leaf toughness. However, *E. nitens* also had significantly more current season foliage with toughness greater than 92.1 g from mid December onwards compared to *E. delegatensis*. This suggests that beyond mid December *E. nitens* trees are more likely to retain a larger area of current season foliage under severe *C. bimaculata* larval defoliation.

At site 2, *E. delegatensis* initially developed greater leaf area than *E. regnans*, although there was no significant difference between leaf area with toughness less than 58.5 g. This suggests little difference between tree carrying capacity and vulnerability to larval defoliation. However, nearing the end of the monitoring period in February and March the total leaf area of *E. regnans* surpassed *E. delegatensis* along with an increasingly significant increase in the area of leaves with toughness less than 58.5 g. During this period *E. regnans* would have the higher carrying capacity for neonate larvae based on toughness. From mid November onwards *E. delegatensis* had a significantly greater total leaf area with toughness greater than 92.1 g. This suggests *E. regnans* is more likely to lose a greater area of current season foliage to severe larval defoliation than would *E. delegatensis* based on leaf toughness.

The data also shows that site may affect *C. bimaculata* larval carrying capacity and tree vulnerability to defoliation. For *E. delegatensis* the significantly larger area of foliage with toughness less than 58.5 g for trees at site 2 may increase the carrying capacity of neonate larvae in February and March. Also, site 3 *E. delegatensis* had a larger area of foliage with a toughness greater than 92.1 g and was more likely to resist *C. bimaculata* larval damage. There were also differences between *E. nitens* at site 1 and 3 with *E. nitens* at site 3 potentially having the higher carrying capacity for neonate larvae for much of the monitoring period.

To determine whether the area of expanding leaves with toughness less than 58.5 g may have significantly influenced *C. bimaculata* oviposition, a comparison between species at site 1 and 2 needs to be made when the oviposition event occurred. At site 1, there was no significant difference between the leaf area of foliage with toughness less than 58.5 g (i.e expanding and suitable for neonate larval establishment) for *E. nitens* and *E. regnans*. In this respect, both species still had abundant leaves suitable for oviposition. However, only 0.8% of *E. nitens* expanding leaves received egg batches compared to 24% for *E. regnans*. Although leaf size can influence egg batch number per unit leaf area, the difference between these two species would not be great enough to explain this difference. At site 3, a similar conclusion can be drawn since the area of foliage with toughness less than 58.5 g, although larger for *E. nitens*, only bordered on significant compared to *E. delegatensis,* while the latter species received significantly more egg batches. Thus, although Steinbauer et. al (1998a) hypothesised that trees with larger numbers of expanding leaves may be preferred for oviposition, this study provides no clear evidence of this.

Franklin-14

Raymond (1995) found significant differences in the defoliation caused by *C. bimaculata* between *E. regnans* families at Franklin-14. There was also a tendency for susceptible families to receive more egg batches than less susceptible families

(Raymond 1998). Although this study failed to show a significant difference between families regarding percentage egg batches per leaf, family 2 (susceptible) had significantly more egg batches per unit leaf area (reflected by smaller mean leaf size) compared to the two less susceptible families. There was also a significant difference between egg batches per leaf area unit at the susceptibility level. Thus, trees which Raymond (1995) described as more susceptible to *C. bimaculata* damage have less leaf area per egg batch suitable for neonate larval establishment compared to those listed as less susceptible.

Although levels of nitrogen could exacerbate feeding damage (see Chapter 1), there is no evidence from this study that it could influence tree damage based on tree susceptibility class. The ratio between carbon and nitrogen varied significantly between some families with leaf age and for leaves of a given age. Much of this variation was between families within a given susceptibility class. Hence, this variable is unlikely to account for the significant variation in tree damage recorded by Raymond (1995).

Seven essential oil and six leaf wax compounds were found to vary significantly at the tree susceptibility level. This contrasts with the study conducted by Patterson et al. (1996) who found that only two essential oil compounds, alpha-phellandrene and trans piperitol, varied significantly at the family level. Their study incorporated more susceptible and less susceptible families and examined more trees within families. Only one essential oil compound (caryophylliene) and two leaf wax compounds (triterpene 8 and 11, 12 dehydroursolic lactone acetate) varied between trees at the susceptibility level and between leaves of equivalent age classes that are preferred for oviposition (i.e. expanding current season leaves). Of these, only triterpene 8 represented a sizeable proportion (between 5 and 9% of wax yield) of the total yield. The other compounds represented less than 1%. It is debateable whether these individual oil and wax compounds within the same species and that represent a small proportion of the total mix could influence *C. bimaculata* oviposition choice, particularly when oil composition can vary significantly with sampling time (Li 1993).

Also, waxes such as triterpene 8 are only likely to influence beetles on landing as they have low volatility. Studies by Li (1993) and Steinbauer et al. (1998a) indicated that in the laboratory, *C. bimaculata* shows no obvious preference between expanding *E. nitens* and expanding *E. regnans* leaves, even though there is a great variation in leaf wax composition. The proportional change in oil and wax compounds from young to medium aged leaves (both readily fed and oviposited upon) did not reveal any significant differences between susceptibility and is considered unlikely to influence tree susceptibility to oviposition.

Likewise, there was no evidence that the leaf essential oil and wax composition of tree leaves influenced *C. bimaculata* oviposition. The data indicated that leaves of a given age were more similar than leaves of different ages independent of the tree from which the leaf samples came. However, within age classes there were many cases of trees from different families and susceptibility class being more similar in their essential oil and wax composition than trees within the same family.

The leaf development characters of total leaf area, leaf area with toughness less than 58.5 g and leaf area toughness greater than 92.1 g varied significantly between some families (particularly families 2 and 4) and at the susceptibility level at various times. The less susceptible trees had significantly greater leaf area for much of the time over the susceptible trees, but more importantly, developed a greater leaf area with toughness greater than 92.1 g, significantly so by mid February. This should leave less susceptible trees less vulnerable to losing as much current season leaf area, caused by severe *C. bimaculata* larval feeding later in the season. However, less susceptible trees tended to have a greater leaf area of foliage with toughness less than 58.5 g, particularly in January and February, which would support a greater number of early instar larvae. There was no significant difference in the time taken for leaves in both categories to reach a toughness of 58.5 g at the family level or susceptibility level.

At the time oviposition data was collected, less susceptible trees had developed significantly more current seasons foliage, significantly more area of foliage with

toughness greater than 92.1 g, and more foliage (bordering on significance) with toughness less than 58.5 g. Thus less susceptible trees potentially have a higher carrying capacity for early instar *C. bimaculata* larvae along with a greater area of the more resistant current seasons foliage (based on leaf toughness). However, less susceptible trees also tended to have larger leaves (just short of significant) than susceptible trees. Leaf size is known to influence the number of egg batches *C. bimaculata* oviposits per unit leaf area (Chapter 3) due to a preference to oviposit on leaf tips (Chapter 2). This resulted in less susceptible trees having less egg batches per unit leaf area compared to susceptible trees. This would exacerbate tree damage on susceptible trees.

In conclusion, the development of a significantly greater area of current seasons foliage with toughness equivalent to previous seasons, within families that Raymond (1995) describes as less susceptible to *C. bimaculata* damage, should reduce the risk of complete larval defoliation of current season leaves. Also, the significant difference at the tree susceptibility level in egg batches per unit leaf area, as opposed to egg batches per leaf number, indicates that susceptible trees have less leaf area per developing larvae compared to less susceptible trees. These two factors would contribute to the observed differences in defoliation levels observed between susceptible and less susceptible *E. regnans* families. As with Patterson et al. (1996), this study failed to show any conclusive evidence that leaf chemistry (in terms of leaf essential oils, leaf waxes and the carbon-nitrogen ratio) could influence the different defoliation levels as observed by Raymond (1995) in these *E. regnans* families.

Chapter 8

General Discussion

8.1 Introduction

Oviposition site selection will influence both larval establishment and larval distribution within hosts. Thus, factors influencing oviposition site selection are fundamental in determining larval densities and hence defoliation of host plants. Although there have been several studies examining aspects of the interaction between the paropsine genera and their eucalypt hosts (Table 8.1), this thesis has aimed at addressing the paucity of information relating to factors influencing oviposition site selection in these beetles by using a native Tasmanian paropsine species, *Chrysophtharta bimaculata* (Olivier). Three aspects were examined: i. oviposition site selection and egg batch distribution within eucalypt hosts, ii. the influence of oviposition site selection on larval establishment and iii. the influence of tree phenology on oviposition. These issues were then related to subsequent host use by larvae. The examination of *C. bimaculata* oviposition site selection not only provides a vital aspect in understanding host plant usage by this species, but may also relate to some other paropsine species and invertebrate herbivores with similar life histories.

8.2 Factors influencing *C. bimaculata* egg batch distribution within host eucalypts.

The distribution of eggs by phytophagous insects that oviposit on leaves within host trees may be influenced at two levels. Firstly, egg distribution within individual leaves (i.e. a preferred, specific location on a leaf) and secondly, egg distribution between leaves (e.g. discrimination between leaves based on age or shape). The ability to discriminate at one or both the within leaves and between leaves level has important implications for egg batch distributions and hence larval densities within hosts (see Price et al. 1990). *Chyrsophtharta bimaculata* discriminates at both levels within its eucalypt hosts.

Table 8.1 The topics and findings/conclusions of studies that have examined various aspects of paropsine-eucalypt host interactions.

Author and Date	Paropsine species	Topic	Findings and/or Conclusions
Fox & Macauley (1977)	*Paropsis atomaria*	Influence of tannin and phenol concentration on larval performance	Growth rate and nitrogen utilisation of larvae not influenced tannin and phenol concentration
Larsson & Ohmart (1988)	*Paropsis atomaria*	Influence of leaf age on larval performance	Failure to feed most responsible for poor survival of larvae. With leaf age, toughness is likely to be more important in larval performance than oil, tannin and N concentration.
Lawler et al. (1997)	*Chrysophtharta flaveola (Chapuis)*	Influence of CO_2 on nutritional quality of leaves.	Increased C N and decreased N reduced digestibility of diets.
Li (1993)	*Chrysophtharta bimaculata* (Olivier)	Influence of host species and leaf chemistry on oviposition and larval performance.	Adults show oviposition preference between species which may be related to differences in the composition of essential oils. Larvae performance was influenced by eucalypt species with those high in 1,8 cineole and alpha pinene consistently rejected.
Morrow & Fox (1980)	*Chrysophtharta m-fuscum (Boh)* *Paropsis atomaria* (Olivier)	Influence of essential oil yield on growth and feeding damage.	Essential oil yield had no significant effect on larval growth and plant damage.
Ohmart et al. (1985a)	*Paropsis atomaria* (Olivier)	Effect of leaf nitrogen and toughness on larval performance.	Larval performance negatively affected when N levels are below 1.7% and leaves are tough.
Ohmart et al (1985b)	*Paropsis atomaria* (Olivier)	Effect of leaf nitrogen and on fecundity.	Foliage low in N reduced fecundity.
Ohmart et al. (1987)	*Paropsis atomaria* (Olivier)	Effect of leaf nitrogen and toughness on larval performance.	Larval survival more likely to be influenced by leaf toughness than limiting nitrogen concentration.
Ohmart & Larsson (1989)	*Paropsis atomaria* (Olivier)	Ability of larvae to absorb essential oils.	Larvae thought to metabolise leaf terpenoids
Ohmart (1991)	*Paropsis atomaria* (Olivier)	Effect of leaf toughness, nitrogen, tannin and oil concentrations on larval performance and adult fecundity.	The intercorrelation of N and leaf toughness, (no influence of changing tannin and oil concentrations) were probably responsible in influencing larval performance. Adult fecundity directly related to N concentration.
Patterson et al. (1996)	*Chrysophtharta bimaculata* (Olivier)	Plant factors influencing larval performance.	Larvae not influenced by herbivore resistant mechanisms in *E. regnans*
Raymond (1995)	*Chrysophtharta bimaculata* (Olivier)	Influence of genetic variation on defoliation.	Strong correlation between host genetics, growth rate and defoliation
Raymond (1998)	*Chrysophtharta bimaculata* (Olivier)	Influence of leaf development and colour change and defoliation.	Trees with a higher proportion of red expanding leaves were more prone to defoliation. Leaf development was not correlated.
Steinbauer et al. (1998a)	*Chrysophtharta bimaculata* (Olivier)	Oviposition preference and influence of leaf age on oviposition	*E. regnans* preferred for oviposition over *E nitens* when branches contained expanding to fully mature leaves. Expanding leaves of both species preferred for oviposition over fully mature.
Strauss & Morrow (1988)	*Chrysophtharta hectica* (Boisduval)	Adult distribution within and between hosts.	Tree height and foliage production are correlated with beetle numbers within host species.

Oviposition discrimination between locations on a leaf

Factors, that influence oviposition site location on the leaf surface, have been much less studied compared to those that influence oviposition site selection between leaves within hosts and between hosts. Factors that are known to influence oviposition site discrimination within leaves of phytophagous insects are mainly physical and include trichomes (Hassan et al. 1990; Kumar 1992; Talekar et al. 1994), venation (Horton 1990; Barker & Maczka 1996; Woods et al. 1996) and leaf edges (Barker & Maczka 1996; Luft & Paine 1997). A number of paropsine species also deposit egg batches at specific locations on leaves. *Paropsis tasmanica* Baly deposit egg batches next to the leaf edge or petiole, while *P. dilatata* Erichson deposit eggs near the leaf margin (de Little 1979). Some species also deposit egg batches near the leaf tip including *Paropsis charybdis* (Murphy 1998) and *Chrysophtharta agricola* (de Little 1979). The latter species apparently shows a universal preference for this location (pers. obs.).

C. bimaculata shows oviposition site discrimination at the leaf surface level as the majority of egg batches are deposited on leaf tips. Using artificial leaves, it was demonstrated that this preference is not influenced by leaf chemistry, as previously hypothesised by Beckmann (1991). Observations of ovipositing beetles suggest the leaf tip may be favoured as it provides increased stability. At this location, as opposed to other locations on the leaf, beetles can hold both right and left leaf edges with their tarsi (Chapter 2). Other insects are thought to discriminate between sites based on improved structural conditions for oviposition (Luft & Paine 1997).

Oviposition site discrimination at the leaf surface level may have important implications for larval resource depletion and the level of host defoliation. This thesis demonstrated that discrimination at the leaf surface level can influence the numbers of eggs received per unit of leaf area. The oviposition preference shown for leaf tips by *C. bimaculata* (with egg number per batch not influenced by leaf size) over other locations on the leaf surface leads to trees with larger average leaf size having a greater unit of leaf area per individual egg (Chapter 3). Thus, plants

with larger leaves will have more leaf area per developing larvae, reducing the chance of larval resource depletion (Chapter 3).

Oviposition discrimination between leaves within hosts

C. bimaculata oviposition discrimination between leaves was found to be correlated with leaf toughness (or leaf aging) (Steinbauer et al. 1998a; Chapters 5&6), whether a conspecific egg batch was present on the leaf tip (Chapter 2) and leaf shape (Chapter 2).

The influence of conspecifics eggs on oviposition

There are numerous examples of discrimination between leaves based on the presence of conspecific egg batches (see Roitberg & Prokopy 1987; Anderson 1988; Thompson & Pellmyr 1991). This discrimination is usually based on either chemical deterrence, through the presence of an oviposition deterring pheromone (Roitberg & Prokopy 1987), or visual deterrence (Hayes 1985; Pelletier 1995).

For *C. bimaculata*, there was no evidence that egg batches directly deterred ovipositing beetles (i.e. no oviposition deterring pheromone was found and beetles occasionally deposited egg batches adjacent to conspecific egg batches). Rather, the presence of egg batches appeared to indirectly deter ovipositing beetles by blocking the most favoured position for oviposition, the leaf tip. Direct egg batch deterrence (visual and/or chemical) has been argued as a method that insects use to space eggs and reduce larval density (Thompson & Pellmyr 1991). An oviposition preference for a particular position on a leaf and the subsequent blocking of further oviposition may have some influence on egg spacing for *C. bimaculata*. However, this effect is unlikely to reduce egg batch density as substantially as direct deterrence because: (i) eggs are deposit in batches that often exceed 20 eggs and (ii) there is no adverse reaction to conspecific eggs by adults that result in a significant change in behaviour, as witnessed in some insect species (Roitberg et al. 1982; Roitberg & Prokopy 1984; Boller et al. 1987; Hurter et al. 1987; Klijnstra & Schoonhoven 1987).

The preference for depositing egg batches on leaf tips and the subsequent blocking affect of conspecific egg batches can influence egg batch density and subsequent larval density per unit leaf area, depending on host leaf size. In chapter 3, the egg batch density was not influenced by leaf size and subsequently smaller leaves offered less resource per unit of area for each larvae than larger leaves. Thus, factors which influence leaf size such as previous larval damage (Chapter 3), moisture stress (Stone & Bacon 1994, 1995), altitudinal variation (Williams & Potts 1996) and genetics could influence *C. bimaculata* egg batch density per unit of surface leaf area, independent of specific host cues.

The influence of leaf shape on oviposition

C. bimaculata preferred to oviposit on artificial leaves mimicking the shape of *E. regnans* leaves, rather than oval (non host shaped) leaves. Other insects are known to discriminate between leaves based on their physical shape including *Delia antiqua* (Meigen)(onion fly) and *Delia radicum* (L.)(carrot fly) (Degen & Städler 1996; 1997). However, unlike these species that appear to visually discriminate between leaf shapes (Degen & Städler 1997), *C. bimaculata* appears to be indirectly influenced by the presence or absence of a leaf tip.

The influence of leaf age on oviposition

Like many other insects, including *Chrysomela scripta* (F.) (Coleoptera: Chrysomelidae) (Bingaman & Hart 1992), *Entheus priassus* L. (Hesperiidae)(Aide & Londono 1989), *Heliothis zea* (Lepidoptera: Noctuidae)(Wiesenborn & Trumble 1989), *C. bimaculata* has a preference to oviposit on softer expanding leaves rather than fully mature leaves, within a host. This preference has been demonstrated both in the laboratory (Steinbauer et al. 1998a) and in the field (Chapter 5). Although this correlation is strong, approximately one-third of egg batches were still deposited on fully mature foliage in the field (Chapter 5).

The methods used by *C. bimaculata* and other paropsine species to discriminate between mature and expanding leaves with regards to oviposition are still not fully understood. Prior to this thesis, no examination had been conducted to determine whether *C. bimaculata* really showed oviposition discrimination between leaves. *C. bimaculata* egg batch distribution may have simply been determined by beetle location, the greater apparency and/or closer proximity of expanding/newly expanded leaves on the outside of the canopy to flying females.

Patterson et al. (1996) suggested that *C. bimaculata* oviposition could simply occur on the same substrate where beetle feeding occurs. That is, feeding preference largely determines egg batch distribution. This may explain why egg batches are most commonly found on expanding leaves. This has been suggested for other insects, including *Chrysomela scripta* (Bingaman & Hart 1992). However, Chapter 4 demonstrated that adult feeding damage and egg batch distribution are not always strongly correlated. Moreover, foliage damaged by adult feeding was less preferred for oviposition than undamaged foliage. These findings suggest that factors independent of adult feeding preference can influence *C. bimaculata* oviposition.

The influence of leaf position as a possible explanation for egg batch distribution between expanding and fully mature leaves was also examined. The apparency of young leaves has been suggested as a possible explanation for egg distribution for *Heliothis zea* (Wiesenborn & Trumble 1989), while the apparency of foliage at the top of host plants is known to influence the feeding preference of *Popillia japonica* (Newman)(Coleoptera: Scarabaeidae)(Rowe & Potter 1996). For *C. bimaculata*, beetles in the field were more likely to land on the foliage on the outside of the canopy, which is dominated by expanding leaves. After alighting they will also walk up towards younger leaves. These findings suggest *C. bimaculata* is more likely to encounter and spend time on young expanding leaves. However, laboratory studies found that beetles preferred to oviposit on expanding leaves regardless of leaf position, indicating that factors specific to the leaf influence oviposition choice (Chapter 6).

The influence of adult conspecific density on oviposition between expanding and fully mature leaves was also explored. Conspecific insect density is known to influence some insects' oviposition site selection (e.g. Rossiter 1987). For *C. bimaculata*, changing beetle density did not alter the ratio between egg batches deposited on expanding and mature leaves in the laboratory (Chapter 6). Nor did egg batch distribution between mature and expanding leaves change with time as leaves received increasing numbers of egg batches. In addition, laboratory studies using isolated females indicated that when given choices of expanding and mature *E. regnans* leaves, a small but significant proportion of females (5/34) deposit egg batches on fully matures leaves. This indicates that close range discrimination with respect to leaf age is not always consistent. There are many factors that may apply to *C. bimaculata* that may reduce oviposition discrimination. These include: egg load, previous experience, insect age and genetic variation between adults (see Courtney & Kibota 1990). It is important to note that the experimental set up ignored factors that may influence oviposition site discrimination prior to alightment. However, observations made on beetle behaviour prior to egg batch deposition indicate that beetles regularly walk from one leaf to another prior to oviposition (Chapter 2). Thus oviposition site discrimination between leaves within a host is more likely to occur after the beetle has made contact with the leaf surface.

The influence of leaf age on *C. bimaculata* oviposition preference is likely to have implications for egg batch distribution within hosts. Hosts that produce a greater number of expanding leaves are more likely to receive greater numbers of *C. bimaculata* egg batches (under a given beetle density) as these leaves are preferred for oviposition. Abiotic and biotic factors such as weather, fire and herbivory are known to influence the development of flush foliage in *Eucalyptus* (Ohmart 1991; Landsberg & Cork 1997; Chapter 3) and could thus influence *C. bimaculata* egg batch distribution.

8.3 Factors influencing *C. bimaculata* egg batch distribution between eucalypts

A great deal of research has been conducted examining the factors that influence insect oviposition host selection. Many researchers have examined the influence of leaf chemistry in oviposition preference, with many examples of correlations (e.g. Malcolm & Brower 1986; Oyeyele & Zalucki 1990) but fewer examples identifying active compounds which illicit oviposition responses (e.g. Pereyra & Bowers 1988; Renwick 1989). Renwick & Radke (1989) emphasise the importance of chemical cues in guiding insects to host plants and their influence on oviposition site acceptance or rejection. However, later reviews by Courtney & Kibota (1990) and Renwick & Chew (1994) provide greater acknowledgment to the importance that physical cues can have on oviposition site preference.

Oviposition preference between hosts can be determined by chemical and/or visual cues prior to landing. Many gravid insects are believed to be specialised in choosing hosts through early stimuli received prior to landing (Courtney & Kibota 1990). Factors such as plant apparency (Courtney 1982; Chew & Courtney 1991), and plant volatiles (Phelan & Baker 1987) may attract a gravid insect to a host. Plant architecture is known to influence the aggregation of the paropsine *Chrysophtharta hectica* (Strauss & Morrow 1988). Factors that influence gravid *C. bimaculata* prior to landing have still to be determined. Flying *C. bimaculata* adults show preferences for yellow and green sticky traps over other colours ((Leon 1988; Madden 1992), but the role of colour per se does not appear to be a major influence on the outcome of oviposition site selection (Raymond 1998; Chapter 2). However, factors influencing the attraction of flying gravid *C. bimaculata* could explain discrepancies between laboratory and field findings comparing oviposition preferences between *E. regnans* and *E. nitens*. When beetles were given a choice between *E. regnans* and *E. nitens* under caged conditions, egg batches were commonly found on both species (Li 1993; Steinbauer 1998a). However, when these eucalypt species were in close proximity under field conditions, *E. regnans* received large numbers of egg batches while *E. nitens* received very few (Chapter 7). Any sensory information used by airborne *C. bimaculata* to discriminate

between oviposition sites prior to landing may have been impaired with the use of restrictive cages.

On making contact with the leaf surface, the decision whether to oviposit by insects can be influenced by both physical and chemical factors (Renwick & Chew 1994). Surfaces that are hairy and rough are preferred by many moth species (Ramaswamy 1988), while oviposition stimulants on the leaf surfaces of hosts appear common (see Renwick 1989). With leaf surface contact, *C. bimaculata* will readily utilise *E.* (*symphomyrtus*) *nitens* expanding foliage for oviposition (Li 1993; Steinbauer 1998a), along with its traditional *Monocalyptus* hosts from the series *Obliquae* i.e. *E. delegatensis*, *E. regnans* and *E. obliqua*. The essential oil and wax components of *E. nitens* leaves are distinctly different from those of monocalypts (Li 1993; Chapter 7) and variation in these components with leaf age is only minor compared to inter-species differences (Chapter 7). However, in the laboratory, there was no oviposition preference between the expanding leaves of *E. nitens* and *E. regnans*, but there was a strong, negative influence of increasing leaf age within species (Steinbauer et al. 1998a). Thus, for *C. bimaculata*, factors associated with leaf aging (probably physical) appear more important than differences between essential oil and wax composition for oviposition preference (also see Steinbauer 1998a).

For insects that prefer to deposit eggs on expanding foliage, the development of host foliage may potentially influence oviposition. Plant vigour is known to influence insect defoliation and/or oviposition rates (Spiegel & Price 1996; Roininen et al. 1997; Steinbauer et al. 1998b; Cronin & Abrahamson 1999), however, the relevance of the plant vigour hypothesis (Price 1991) to *C. bimaculata* host plant preferences and defoliation is difficult to assign. Firstly, leaf toughness has a major influence on *C. bimaculata* oviposition preference and larval performance. Plant vigour is not necessarily associated with this. For example, *E. nitens* may be described as more vigorous than *E. delegatensis* judging by leaf development characteristics (Chapter 7). Yet due to the faster rate of leaf toughness development, *E. nitens* is less prone to complete defoliation by the time *C. bimaculata* oviposition usually occurs. Secondly, the susceptibility to *C. bimaculata* defoliation for various *E. regnans* families was negatively correlated with tree growth rate (Patterson et al. 1996).

Steinbauer (1998a) hypothesised that observed differences in *C. bimaculata* host preference between *E. regnans* and *E. nitens* may be influenced by the amount of expanding foliage present on the tree. However, field studies suggest that other factors, possibly pre-landing stimuli, are more important to *C. bimaculata* oviposition discrimination between these two species (see section 7.4).

8.4 *Chrysophtharta bimaculata* oviposition site selection and larval establishment

Many studies have found a strong correlation between an insect's oviposition choice and the growth and survival of offspring (Barker & Maczka 1996). For those insects that have sessile larvae, it is a necessity that oviposition occurs at a site suitable for neonate larval establishment. However, for species that have neonates that can migrate, the suitability of an oviposition site for larval establishment may be unimportant in relation to other factors [e.g. reduced viral infection rate (Rossiter 1987)].

The larvae of *C. bimaculata*, like several other paropsine species, require expanding/newly-expanded leaves to establish and develop. Expanding leaves are more nutritious and less tough than mature leaves (Landsberg & Cork 1997; Chapter 7). The greater toughness of mature eucalypt leaves is regarded as the main inhibiting factor to paropsine larval development (Ohmart et al. 1987; Larsson & Ohmart 1988). For *C. bimaculata*, neonate larvae can only establish on *E. regnans*, *E. delegatensis* and *E. nitens* leaves that are below a uniform toughness threshold (Chapter 5).

C. bimaculata aggregations in the field were found to deposit approximately one-third of egg batches on leaves above the toughness threshold for neonate larval establishment (Chapter 5). However, virtually all egg batches were deposited within 20 cm of leaves soft enough for neonate larval establishment. It was found that following hatching and consumption of the chorion, *C. bimaculata* neonates are

capable of migrating from leaves too tough for establishment and establish on softer leaves. Moreover, under laboratory conditions, the necessity to migrate (over a distance of at least 20 cm) did not significantly increase larval mortality compared to larvae hatching on leaves suitable for establishment. A proportion of *C. bimaculata* larvae hatching on leaves suitable for establishment also commonly migrated (Chapter 5). The ability of neonates to migrate without any obvious deleterious effect suggests that *C. bimaculata* oviposition site selection does not impede larval performance within hosts.

The similar larval morphology of other species in the genera *Paropsis*, *Chrysophtharta* and *Paropsisterna* [i.e large bodies and short legs (Selman 1994)] suggest that these species may show similar behaviour. The few studies examining neonate larval behaviour suggest this, as *Paropsis atomaria* (Carne 1966) and *P. charybdis* (Murphy 1998) also feed on the chorion before migrating to foliage suitable for larval establishment.

The placement of approximately one-third of egg batches on leaves unsuitable for neonate establishment and the ability of hatching larvae to migrate successfully to suitable foliage has important implications for the population dynamics of *C. bimaculata.* Although *C. bimaculata* shows oviposition site discrimination, the deposition of large numbers of egg batches on leaves both unsuitable and suitable for larval establishment (more than 50% of current season leaves in host trees may contain an egg batch) can lead to high larval densities per tree (Chapter 5). This probably accounts for the complete defoliation of expanding and newly expanded foliage commonly observed along with potential larval starvation (Greaves 1966; Authors pers. obs.). However, the aggregated nature of *C. bimaculata* adult populations and the limited ability of paropsine larvae to migrate over large distances (Selman 1994) will result in patchy resource depletion restricted to those individual trees that have received the largest numbers of egg batches.

Although *C. bimaculata* appears to show a strong correlation between oviposition preference and larval performance within host trees, it is interesting to note that oviposition preferences between hosts do not necessarily correspond with *C.*

bimaculata feeding performance in the laboratory. *C. bimaculata* shows a strong oviposition preference for *E. regnans* over *E. nitens* in the field (Chapter 7) yet Baker et al. (In Press) found that larval feeding performance and survival was stronger for *E. nitens* over *E. regnans*. Whether feeding performance in the laboratory corresponds with actual overall larvae performance in the field is an area that still requires further study. Many other factors can influence larval performance besides larval development rate (see chapter 1), and host breadth may be reflected by this (e.g. Bernays & Graham 1988; (Denno et al. 1990).

8.5 Conclusion

Although the influence of plant chemistry can be of major importance for many insects (Courtney & Kibota 1990; Renwick 1989; Renwick & Chew 1994) its influence on the oviposition preference of *C. bimaculata* and other eucalypt utilising paropsines, is still not understood. Although Li (1993) has noted that *C. bimaculata* oviposition preference negatively correlates with the essential oil 1,8 cineole, any direct role it may have in oviposition site selection in nature has not been determined.

Factors that influence an insects' oviposition site selection usually involve a sequence of orientation, landing and surface evaluation, and these all depend on a variety of sensory cues (Renwick & Chew 1994). Thus, oviposition preference and site selection decisions may involve any number of physical and chemical cues that determine whether and where an insect will oviposit. This thesis has demonstrated the importance of host plant physical factors in oviposition site selection following alightment on a host. The influence of the leaf tip, leaf shape, the presence of conspecific egg batches and leaf toughness (autocorrelated with age) have all been shown to influence egg batch placement and thus egg batch distribution within hosts.

Although *C. bimaculata* discriminates between oviposition sites based on several host factors, egg density within hosts can be high enough to subsequently cause

complete larval defoliation of expanding/newly expanding leaves. Thus, the potential severity of defoliation will be dependent on the amount of these softer leaves. This study has shown that this varies at the host species level (comparisons between *E. regnans*, *E. delegatensis* and *E. nitens*) with *E. regnans* most likely to lose the greatest area of current season leaves in the summer and autumn months and *E. nitens* the least.

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Appendix 1

Conversion of penetrometer units into grams

Where measurements of leaf toughness were required a hand held penetrometer was used consisting of a Chatillon AG 50 (John Chatillon and Sons Inc. New York) dial tension gauge with removal probes. A description of this gauge and its use as a penetrometer are provided by Sands & Brancatini (1991). For all experiments measuring leaf toughness, a probe with a radius of 0.455 mm^2 and area of 0.650 mm^2 was used. Penetrometer units were converted into grams by clamping the gauge (with probe) into a retort stand and adjusting it so the probe applied force on a balance. The retort stand was continually adjusted giving a range of penetrometer readings and corresponding grams from the balance. The balance readings were noted to the 100^{th} decimal place. The penetrometer units were then regressed against the balance readings to give a linear equation (see figure A1.1).

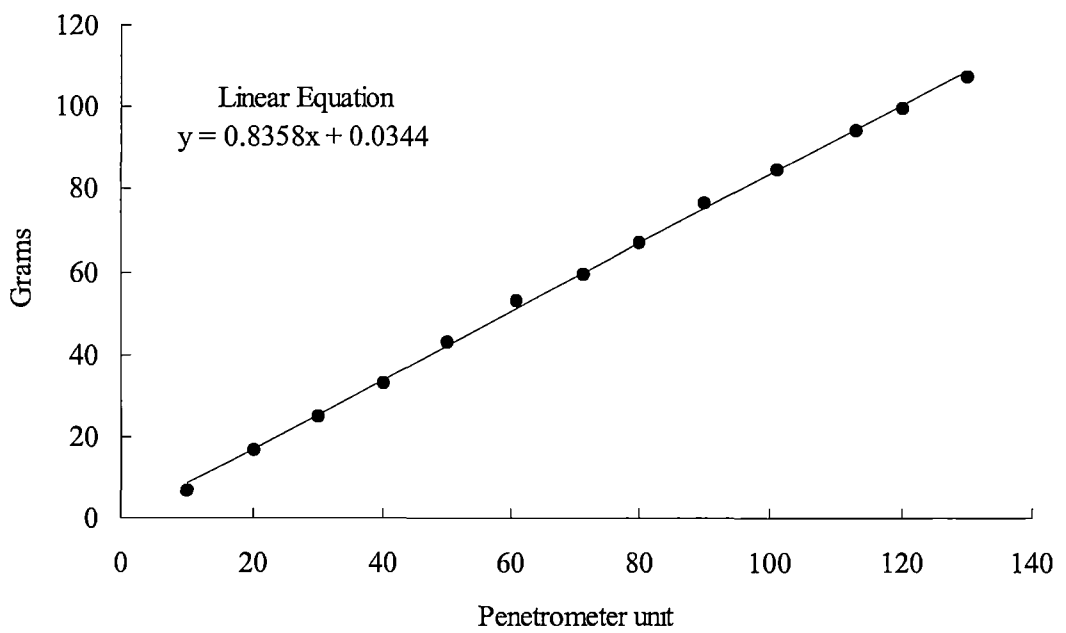

Figure A1.1 Linear equation between units of a Chatillon AG 50 gauge with a 0.455 mm^2 probe and grams from a balance when equal force is applied to both.

Appendix 2

The relationship between leaf length and width and leaf area for *E. regnans*, *E. delegatensis* and *E. nitens*

Undamaged leaves of *E. regnans*, *E. delegatensis* and the adult leaves of *E. nitens* varying in size and age were collected from regeneration and plantation trees aged between 2-4 years from several locations in the Florentine Valley (42°39'S, 146°28'E) and the Plenty Valley (42°50'S, 146°53'E). Leaves were brought back to the laboratory on ice.

The length and width of each leaf was measured with a ruler and the values multiplied. The leaf area for each leaf was also measured using a ΔT^{TM} (Delta-T Devices) area meter. The leaf length x width values were then regressed against the leaf area measurements (see Fig. A2.1) to obtain a coefficient for each tree species. An estimate of leaf area was then obtained by measuring leaf length and width for *E. regnans*, *E. delegatensis* and *E. nitens* in the field without removing leaves from trees.

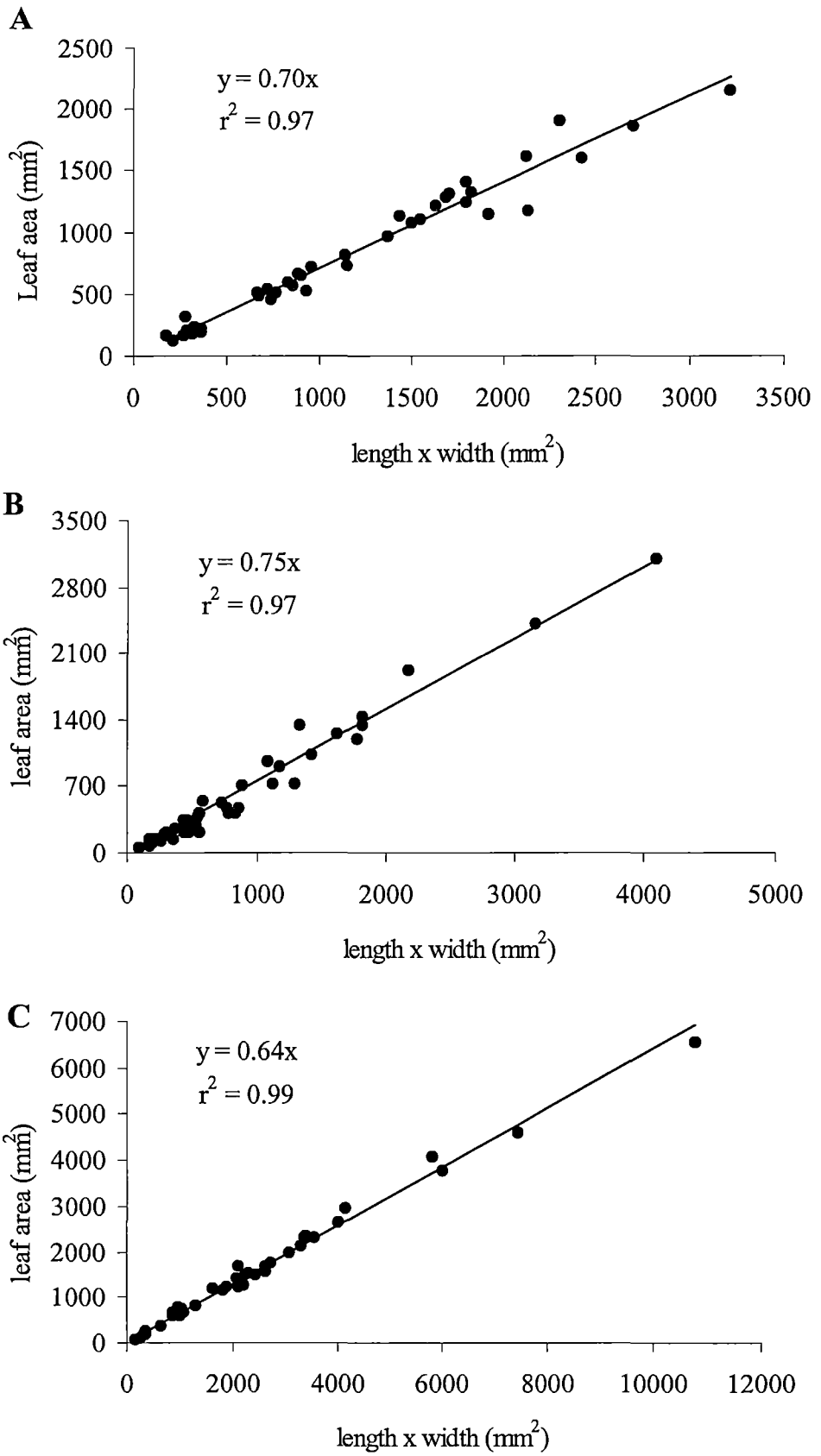

Figure A2.1 Leaf length multiplied by width versus leaf area for leaves of **(A)** *E. delegatensis*, **(B)** *E. regnans* and **(C)** *E. nitens*.

Appendix 3

Tables A3.1 - A3.3 Compare total leaf area development, total leaf area with toughness less than 58.5 g and total leaf area with toughness greater than 58.5 g for current season foliage from three branches per tree for three eucalypt species (*E. regnans*, *E. delegatensis* and *E. nitens*) at three sites in the Plenty Valley (two species per site). Measurements were taken at intervals of 1 to 2 weeks over a period of 161 days beginning on the 10th of October 1996. Contrasts between monitoring periods were compared between species along with the species x time interaction (change in leaf area between contrasts) using Z-tests. Tables A3.4 - 37.6 compare site differences within species using the same data and analyses.

Table A3.1 Z-values for a comparison between species (*E. regnans* versus *E. nitens*) and species x time interaction at different time contrasts for total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g (monitoring began 10th of October 1996) at site 1 in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per species = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Spp. x time	Species	Spp. x time	Species	Spp. x time	Species
6 to 12	-2.98 n.s.	-1.67 n.s.	-2.98 *	-1.67 n.s.	0.00 n.s.	0.00 n.s.
12 to 18	-4.53 **	-2.58 *	-4.53 **	-2.58 *	0.00 n.s.	0.00 n.s.
18 to 24	-4.32 **	-3.64 **	-2.33 n.s.	-3.85 **	0.00 n.s.	0.00 n.s.
24 to 31	-1.66 n.s.	-3.82 **	-1.74 n.s.	-4.03 **	0.00 n.s.	0.00 n.s.
31 to 37	-3.19 *	-3.99 **	-1.85 n.s.	-4.38 **	0.00 n.s.	0.00 n.s.
37 to 43	-2.13 n.s.	-6.44 **	-0.40 n.s.	-8.88 **	-1.40 n.s.	-1.40 n.s.
43 to 53	-3.83 **	-7.67 **	-3.50 **	-10.86 **	-1.67 n.s.	-1.58 n.s.
53 to 66	-3.92 **	-6.22 **	-1.40 n.s.	-7.27 **	-7.49 **	-3.27 *
66 to 82	-2.68 *	-5.72 **	-0.15 n.s.	-5.74 **	-2.21 n.s.	-3.70 **
82 to 92	-1.41 n.s.	-4.84 **	2.23 n.s.	-2.42 *	-2.71 *	-3.47 **
92 to 107	-0.98 n.s.	-3.93 **	1.44 n.s.	-0.38 n.s.	-2.54 *	-3.19 *
107 to 120	-0.57 n.s.	-3.19 *	1.61 n.s.	0.50 n.s.	-2.28 n.s.	-3.49 **
120 to 132	0.31 n.s.	-2.64 *	1.93 n.s.	1.29 n.s.	-3.42 *	-3.93 **
132 to 161	0.41 n.s.	-2.22 n.s.	-0.54 n.s.	1.55 n.s.	-0.79 n.s.	-3.66 **

Table A3.2 Z-values for a comparison between species (*E. regnans* versus *E. delegatensis*) and species x time interaction at different time contrasts for total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g (monitoring began 10th of October 1996) at site 2 in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per species = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Spp. x time	Species	Spp. x time	Species	Spp. x time	Species
6 to 12	-1.49 n.s.	-2.12 n.s.	-0.51 n.s.	-0.72 n.s.	0 n.s.	0 n.s.
12 to 18	-1.28 n.s.	-2.17 n.s.	-2.43 *	-1.23 n.s.	0 n.s.	0 n.s.
18 to 24	-4.24 n.s.	-2.55 *	-1.08 n.s.	-1.62 n.s.	0 n.s.	0 n.s.
24 to 31	-0.48 n.s.	-2.69 *	-1.70 n.s.	-1.69 n.s.	1 n.s.	-1 n.s.
31 to 37	-1.98 n.s.	-2.55 *	-0.13 n.s.	-1.62 n.s.	1 n.s.	-1 n.s.
37 to 43	-2.27 n.s.	-2.88 *	-0.92 n.s.	-1.51 n.s.	1 n.s.	-1 n.s.
43 to 53	-1.99 n.s.	-3.03 *	-1.21 n.s.	-1.85 n.s.	-2.58 *	-2.82 *
53 to 66	-0.82 n.s.	-3.04 *	0.09 n.s.	-1.70 n.s.	-3.13 *	-4.12 **
66 to 82	0.90 n.s.	-2.64 *	1.41 n.s.	-1.00 n.s.	-1.76 n.s.	-5.27 **
82 to 92	1.62 n.s.	-1.59 n.s.	2.89 *	0.98 n.s.	-0.21 n.s.	-4.58 **
92 to 107	2.29 n.s.	-0.51 n.s.	1.71 n.s.	2.14 n.s.	-1.99 n.s.	-4.11 **
107 to 120	1.88 n.s.	0.11 n.s.	0.85 n.s.	2.22 n.s.	-1.77 n.s.	-4.46 **
120 to 132	2.91 *	0.60 n.s.	1.17 n.s.	2.47 *	-2.86 *	-5.71 **
132 to 161	1.47 n.s.	1.05 n.s.	0.12 n.s.	2.71 *	0.86 n.s.	-7.48 **

Table A3.3 Z-values for a comparison between species (*E. nitens* versus *E. delegatensis*) and species x time interaction at different time contrasts for total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g (monitoring began 10th of October 1996) at site 3 in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per species = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Spp. x time	Species	Spp. x time	Species	Spp. x time	Species
6 to 12	-4.11 **	-3.48 *	-4.11 **	-3.48 *	0 n.s.	0 n.s.
12 to 18	-3.28 **	-5.27 **	-3.28 **	-5.27 **	0 n.s.	0 n.s.
18 to 24	-5.28 **	-7.49 **	-5.28 **	-7.49 **	0 n.s.	0 n.s.
24 to 31	-4.00 **	-8.56 **	-4.00 **	-8.56 **	0 n.s.	0 n.s.
31 to 37	-2.51 *	-8.92 **	-2.57 *	-9.25 **	0 n.s.	0 n.s.
37 to 43	-0.28 n.s.	-8.16 **	2.56 *	-7.68 **	-1.23 n.s.	-1.23 n.s.
43 to 53	-1.66 n.s.	-8.50 **	-0.32 n.s.	-5.97 **	-2.77 *	-3.01 *
53 to 66	-3.66 **	-7.37 **	-1.21 n.s.	-4.79 **	-0.89 n.s.	-5.17 **
66 to 82	-2.92 *	-6.18 **	0.05 n.s.	-3.69 **	-3.63 **	-5.49 **
82 to 92	-1.47 n.s.	-5.55 **	0.30 n.s.	-2.97 *	-7.45 **	-7.28 **
92 to 107	-1.73 n.s.	-4.64 **	-0.22 n.s.	-3.23 *	-2.02 n.s.	-7.53 **
107 to 120	-2.03 n.s.	-4.45 **	1.96 n.s.	-3.21 *	-3.38 *	-5.91 **
120 to 132	-1.48 n.s.	-5.10 **	1.42 n.s.	-1.57 n.s.	-2.31 n.s.	-4.87 **
132 to 161	-1.58 n.s.	-5.79 **	0.78 n.s.	-0.96 n.s.	-3.64 **	-4.69 **

Table A3.4 Z-values for a comparison of total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g at different time contrasts for *E. regnans* at two sites and site x time interaction (monitoring began 10th of October 1996) in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per site = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Site X time	Site	Site x time Z-value	Site	Site x time	Site
6 to 12	2.12 n.s.	0.73 n.s.	2.55 *	0.81 n.s.	0 n.s.	0 n.s.
12 to 18	-0.32 n.s.	1.07 n.s.	-1.87 n.s.	1.11 n.s.	0 n.s.	0 n.s.
18 to 24	-0.67 n.s.	0.97 n.s.	0.15 n.s.	1.03 n.s.	0 n.s.	0 n.s.
24 to 31	0.81 n.s.	0.99 n.s.	1.27 n.s.	1.14 n.s.	0 n.s.	0 n.s.
31 to 37	0.77 n.s.	1.11 n.s.	1.91 n.s.	1.43 n.s.	0 n.s.	0 n.s.
37 to 43	2.06 n.s.	1.47 n.s.	1.71 n.s.	1.76 n.s.	0 n.s.	0 n.s.
43 to 53	1.39 n.s.	1.78 n.s.	-0.05 n.s.	1.66 n.s.	0.25 n.s.	0.25 n.s.
53 to 66	1.39 n.s.	1.86 n.s.	1.71 n.s.	1.66 n.s.	-0.83 n.s.	-0.34 n.s.
66 to 82	1.11 n.s.	1.72 n.s.	1.31 n.s.	2.17 n.s.	-0.44 n.s.	-0.70 n.s.
82 to 92	1.80 n.s.	1.78 n.s.	2.06 n.s.	2.38 *	1.33 n.s.	0.77 n.s.
92 to 107	1.88 n.s.	1.88 n.s.	0.89 n.s.	2.26 n.s.	1.99 n.s.	1.83 n.s.
107 to 120	1.00 n.s.	2.01 n.s.	-0.72 n.s.	1.80 n.s.	-0.40 n.s.	1.91 n.s.
120 to 132	0.39 n.s.	2.07 n.s.	0.15 n.s.	1.50 n.s.	1.40 n.s.	1.93 n.s.
132 to 161	-0.51 n.s.	2.03 n.s.	1.56 n.s.	1.66 n.s.	-0.05 n.s.	1.97 n.s.

Table A3.5 Z-values for a comparison of total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g at different time contrasts for *E. delegatensis* at two sites and site x time interaction (monitoring began 10th of October 1996) in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per site = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Site x time	Site	Site x time	Site	Site x time	Site
6 to 12	-3.03 *	-1.26 n.s.	-2.87 *	0.24 *	0 n.s.	0 n.s.
12 to 18	-0.84 n.s.	-1.82 n.s.	-0.53 n.s.	-0.82 n.s.	0 n.s.	0 n.s.
18 to 24	-3.89 **	-2.16 n.s.	-1.44 n.s.	-1.19 n.s.	0 n.s.	0 n.s.
24 to 31	-0.46 n.s.	-2.32 n.s.	-1.31 n.s.	-1.51 n.s.	-1 n.s.	-1 n.s.
31 to 37	-1.36 n.s.	-2.26 n.s.	0.30 n.s.	-1.43 n.s.	-1 n.s.	-1 n.s.
37 to 43	-1.93 n.s.	-2.69 *	-0.98 n.s.	-1.42 n.s.	1 n.s.	-1 n.s.
43 to 53	-1.66 n.s.	-2.82 *	-0.29 n.s.	-1.54 n.s.	-2.39 *	-2.63 *
53 to 66	-1.52 n.s.	-2.95 *	-1.59 n.s.	-1.77 n.s.	-2.71 *	-3.82 **
66 to 82	0.28 n.s.	-2.93 *	1.13 n.s.	-1.70 n.s.	-0.59 n.s.	-3.63 *
82 to 92	1.83 n.s.	-2.43 *	1.94 n.s.	0.00 n.s.	-3.02 *	-3.37 *
92 to 107	1.66 n.s.	-1.53 n.s.	0.78 n.s.	1.89 n.s.	-1.39 n.s.	-3.17 *
107 to 120	2.54 *	0.13 n.s.	2.54 *	2.84 *	0.30 n.s.	-2.20 n.s.
120 to 132	2.53 *	1.82 n.s.	0.57 n.s.	3.67 **	-0.95 n.s.	-2.16 n.s.
132 to 161	2.17 n.s.	2.37 *	-1.55 n.s.	3.22 *	2.40 *	-1.58 n.s.

Table A3.6 Z-values for a comparison of total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g at different time contrasts for *E. nitens* at two sites and site x time interaction (monitoring began 9th of November 1996) in the Plenty Valley. No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom = 7, number of trees per site = 5.

Contrast	Total Leaf area		Leaf area < 58.5 g		Leaf area > 92.1 g	
(days)	Site x time	Site	Site x time	Site	Site x time	Site
6 to 12	2.50 *	2.63 *	1.76 n.s.	2.63 *	0 n.s.	0 n.s.
12 to 18	4.10 *	3.54 **	2.98 *	3.54 **	0 n.s.	0 n.s.
18 to 24	3.47 n.s.	4.65 **	2.09 n.s.	4.98 **	0 n.s.	0 n.s.
24 to 31	1.86 n.s.	4.80 **	2.35 n.s.	5.31 **	0 n.s.	0 n.s.
31 to 37	-1.15 n.s.	4.73 **	2.39 *	5.68 **	0 n.s.	0 n.s.
37 to 43	1.86 n.s.	4.77 **	-2.05 n.s.	5.55 **	-0.24 n.s.	-0.24 n.s.
43 to 53	2.55 *	4.51 **	-0.41 n.s.	4.07 **	2.35 n.s.	1.46 n.s.
53 to 66	1.80 n.s.	3.06 *	0.04 n.s.	2.46 *	-0.06 n.s.	2.22 n.s.
66 to 82	0.88 n.s.	2.17 n.s.	0.25 n.s.	1.78 n.s.	1.43 n.s.	1.92 n.s.
82 to 92	1.78 n.s.	1.98 n.s.	2.40 *	2.23 n.s.	1.64 n.s.	2.17 n.s.
92 to 107	1.75 n.s.	2.03 n.s.	1.10 n.s.	3.41 *	0.86 n.s.	1.88 n.s.
107 to 120	1.48 n.s.	2.15 n.s.	-0.91 n.s.	4.03 **	3.47 **	2.28 n.s.
120 to 132	1.62 n.s.	2.51 *	0.22 n.s.	3.52 **	0.12 n.s.	2.15 n.s.
132 to 161	-1.90 n.s.	2.84 *	-0.56 n.s.	3.77 **	2.03 n.s.	1.98 n.s.

Appendix 4

Table A4.1 Analysis of variance results for a comparison of total leaf area, leaf area with toughness less than 58.5 g and leaf area with toughness greater than 92.1 g at different time contrasts for four *E. regnans* families and two susceptibility classes to *Chrysophtharta bimaculata* damage at Franklin-14 (monitoring began 30th of October 1996). M.S. indicates Mean Square, No significance = n.s., $0.05<P<0.01$ = *, $P<0.01$ = **, degrees of freedom at family level = 3,19 and susceptibility level = 1,19. Numbers in brackets indicate families with significant difference.

Contrast	Familyxtime M. S.	Family M.S.	Susceptxtime M. S.	Susceptibility M. S.
For total leaf area				
6 to 12	8137 n.s.	32784 *(4&2)	1190 n.s.	52598 *
12 to 18	4284 n.s.	28720 n.s.	2731 n.s.	48605 n.s.
18 to 24	12138 n.s.	38327 n.s.	26144 *	75725 n.s.
24 to 30	55243 **(4&1;4&2;4&3)	125882 n.s.	53541 n.s.	222521 n.s.
30 to 36	102113 n.s.	385517 *(4&2)	248266 *	699810 *
36 to 47	757217 **(4&1;4&2;4&3)	1446883 **(4&1;4&2)	1100884 **	2593044 **
47 to 59	987222 **(4&1;4&2;4&3)	4535255 **(4&1;4&2;4&3)	1527884 **	7578713 **
59 to 73	1472201 **(4&1;4&2;4&3)	10430000 **(4&1;4&2;4&3)	1963012 *	16580000 **
73 to 89	542426 n.s.	17540000 **(4&1;4&2;4&3)	614847 n.s.	26670000 **
89 to 100	76742 n.s.	21850000 **(4&1;4&2;4&3)	185224 n.s.	33310000 *
100 to 113	118147 n.s.	22150000 **(4&1;4&2;4&3)	2 n.s.	35830000 *
113 to 132	151347 n.s.	19820000 *(4&2)	77588 n.s.	34170000 *
132 to 147	119653 n.s.	17550000 n.s.	49521 n.s.	31310000 *
For leaf area less than 58.5 g				
6 to 12	7853 n.s.	4227 n.s.	1354 n.s.	7021 n.s.
12 to 18	2558 n.s.	15238 n.s.	682 n.s.	13282 n.s.
18 to 24	4008 n.s.	30033 n.s.	8586 n.s.	30497 n.s.
24 to 30	10759 n.s.	51053 n.s.	8597 n.s.	71462 n.s.
30 to 36	26655 n.s.	113399 n.s.	58699 n.s.	189071 n.s.
36 to 47	115645 n.s.	304009 n.s.	150579 n.s.	562476 n.s.
47 to 59	189847 n.s.	842050 n.s.	341064 n.s.	1527721 n.s.
59 to 73	707527 **(2&4)	2265992 n.s.	840336 *	3945637 n.s.
73 to 89	39760 n.s.	3587132 *(2&4)	19603 n.s.	5639233 *
89 to 100	138757 n.s.	4178146 n.s.	234832 n.s.	6487206 n.s.
100 to 113	117866 n.s.	4674039 n.s.	60472 n.s.	8481214 *
113 to 132	1359525 n.s.	2583667 n.s.	3626436 *	4339087 n.s.
132 to 147	220150 n.s.	976085 n.s.	15280 n.s.	1422520 n.s.
For leaf area greater than 92.1 g				
30 to 36	198 n.s.	50 n.s.	198 n.s.	50 n.s.
36 to 47	406 n.s.	584 n.s.	406 n.s.	584 n.s.
47 to 59	12678 n.s.	6136 n.s.	10 n.s.	1068 n.s.
59 to 73	6019 n.s.	16093 n.s.	14569 n.s.	8367 n.s.
73 to 89	48638 n.s.	17212 n.s.	125198 n.s.	630 n.s.
89 to 100	250758 n.s.	168942 n.s.	16620 n.s.	71007 n.s.
100 to 113	699666 n.s.	839904 *(4&2)	1919206 *	1047774 *
113 to 132	425048 *(4&2)	2365149 **(4&1;4&2)	172665 n.s.	3701970 *
132 to 147	401330 n.s.	4391071 **(4&1;4&2)	1140399 *	7106291 *

Appendix 5

A comparison between the proportions of individual compounds present in the essential oil and wax of four *E. regnans* families.

Table A5.1 compares individual essential oil components and Table A5.2 individual leaf wax components between four *E. regnans* families at Franklin-14 based on leaf age, family and age x family interactions. Analysis of variance was used to determine the level of significance.

Table A5.1 Analysis of variance results, for the proportion of individual compounds present in essential oils taken for four *E. regnans* families with regard to leaf age and leaf age x family. The degrees of freedom were: family 3, age 2, family x age 6 and total 35, M.S. indicates Mean Square, * indicates $P < 0.05$, ** indicates $P < 0.01$, n.s. indicates $P > 0.01$.

ESSENTIAL OIL	FAMILY M.S.	AGE M.S.	FAMXAGE M.S.	ESSENTIAL OIL	FAMILY M.S.	AGE M.S.	FAMXAGE M.S.
ISOBUTYL ISOBUTANOATE	0.413 n.s.	0 303 n.s.	0.641 n.s.	HUMULENE	0.097*	0.237**	0.048 n.s.
ALPHA THUJENE	0.029 n.s.	0.056 n.s.	0.007 n.s	ALLOAROMADENDRENE	0 163 n.s.	0.508**	0.030 n.s.
68/40/(84)/(94)/(112)	0.020*	0.020 n.s	0.020*	121/138/39/(161)	0 285**	1.003**	0.285**
ALPHA PHELLANDRENE	1.869 n.s	11.897**	0.022 n.s.	BICYCLOGERMACRENE	19.800*	303.245**	8 837 n s
ALPHA TERPINENE	0.104 n.s.	0.017 n s.	0.028 n.s.	43/161/207/105	0.413 n s.	1.013 n.s.	0.413 n.s.
P-CYMENE	0.420 n s	8.600**	0.347 n.s.	ELEMOL RELATED	4.279**	6.118**	1.105 n.s.
LIMONENE	0.015 n.s.	0.015 n s.	0.015 n.s.	161/119/41/(105)/(204)	0.893**	0.007 n.s.	0.010 n.s.
BETA PHELLANDRENE	0.452 n.s.	0.199 n.s.	0.113 n.s.	HEDYCARYOL	228.05*	2986 76**	67.40 n.s.
TERPINOLENE	0.007 n.s.	0 040*	0.007 n.s.	SPATHULENOL	0.503 n.s.	3.291**	0.641**
LINALOOL	0.003 n.s.	0 003 n.s.	0.003 n.s.	GLOBULOL	0.058 n.s.	0.100*	0.058 n s.
TRANS P-MENTH-2-EN-1-OL	0.027 n.s.	10.473 **	0.176 n s.	41/91/119/(205)	0.067 n.s.	0 201 n s	0 067 n.s.
CIS P-MENTH-2-EN-1-OL	0.020 n.s	10.126**	0.192 n s.	GAMMA EUDESMOL	0 888 n s.	10.050**	0.709 n.s
TERPINENE-4-OL	0.026 n.s	0.146*	0.022 n s	43/145/165/157/119	1.00 n.s	0.023 n.s.	1.00 n.s.
ALPHA TERPINEOL	0 080*	0.080*	0.080**	BETA EUDESMOL	5.724 n.s.	176.009**	7.938 n.s.
81/43/167/77	0.052 n s.	0.344**	0.047 n.s.	ALPHA EUDESMOL	0.761 n.s.	26.700**	0.884 n.s.
CIS PIPERITOL	0 004 n s	0.957**	0 004 n.s.	43/139/125/200	0.255**	3.180**	0.255**
41/97/126/69	0 046**	0.126**	0.046**	43/91/119/105	0.237 n.s.	6.200 n.s	0 237 n s
TRANS PIPERITOL	2.501 n s	48 297**	1.400 n.s.	43/139/125/200	0.345 n.s.	2.918**	0.344 n.s.
82/(39)/(110)/(95)/(55)	0.158 n.s.	0.509*	0.158 n.s	221/41/(189)/(139)/(236)	4 160 n s.	6.993 n.s.	2.401 n.s.
55/70/41/126/97	0.084 n.s	0.113 n.s.	0.012 n.s.	43/(55)/(91)/(221)	0.183 n.s.	2.000**	0.183 n.s.
43/111/126/71	0.010 n.s.	0.288**	0.010 n.s.	237/43/(81)/(209)/(252)	147.130 n.s.	48.440 n s	4.310 n.s.
84/105/41/126/97	0.022 n.s.	0.810**	0.022 n.s	83/125/171/139/(254)	2.144 n.s.	3.328 n.s.	0.259 n.s.
91/44/104/150	0.003 n.s.	0.003 n.s.	0.003 n.s.	170/155/43/57/81	1.171*	4.491**	0.333 n s.
BICYCLOELEMENE	0.043 n.s.	0.899**	0.028 n.s.	43/59/149/109/(164)	0.322 n.s.	0 431 n.s.	0.322 n s.
PYROGALLOL	2.131 n.s.	19.609**	1.343 n.s.	251/266 TASMANONE	1.824**	0.320 n s	0 113 n.s
ALPHA COPAINE	0.072 n.s.	0.438**	0 067 n.s.	59/41/221/142	0 143**	0.598**	0.145**
43/67/(95)/(126)/(182)	0 010 n s.	1.383**	0.010 n.s.	43/139/182/125	0.642 n.s.	11.927**	0.326 n.s.
BETA ELEMENE	0.040 n.s.	2.371**	0.065*	EUDESMOL RELATED 1	0.048 n.s.	16 538**	0.048 n.s.
107/138/79/78	0.068 n.s.	3.759**	0.160 n.s.	EUDESMOL RELATED 2	1.150 n.s	599.980**	1.150 n.s.
CARYOPHYLLIENE	0.899**	0.226*	0.021 n.s.				

Table A5.2 Analysis of variance results, for the proportion of individual components present in essential oils taken for two *E. regnans* susceptibility classes with regard to leaf age and leaf age x susceptibility interaction. The degrees of freedom were: family 3, age 2, susceptibility x age 6 and total 35, M.S. indicates Mean Square, * indicates $P < 0.05$, ** indicates $P < 0.01$, n.s. indicates $P > 0.01$.

ESSENTIAL OIL	SUSCEPT. M.S.	AGE M.S.	SUSXAGE M.S.	ESSENTIAL OIL	SUSCEPT. M.S.	AGE M.S.	SUSXAGE M.S.
ISOBUTYL ISOBUTANOATE	0.000 n.s.	0.303 n.s.	0.063 n.s.	HUMULENE	0.097*	0.237**	0.048 n.s.
ALPHA THUJENE	0.011 n.s.	0.056 n.s.	0.004 n.s.	ALLOAROMADENDRENE	0.163 n.s.	0.508**	0.030 n s.
68/40/(84)/(94)/(112)	0.020 n.s.	0.020 n.s.	0.020 n.s	121/138/39/(161)	0.285**	1.003**	0.285**
ALPHA PHELLANDRENE	1.814 n.s.	11.897**	0.016 n.s	BICYCLOGERMACRENE	2.013 n.s.	303.245**	9.830 n.s.
ALPHA TERPINENE	0.204 *	0.017 n.s.	0.030 n.s.	43/161/207/105	0.113 n.s.	1.013 n s.	0 113 n s.
P-CYMENE	0.304 n.s.	8.600**	0.382 n.s.	ELEMOL RELATED	5.808*	6.118**	1.461 n.s.
LIMONENE	0.015 n.s.	0.015 n.s.	0 015 n.s.	161/119/41/(105)/(204)	1.000**	0.007 n.s.	0.007 n.s.
BETA PHELLANDRENE	0.616 n.s.	0.199 n.s.	0.508 n.s	HEDYCARYOL	308.50*	2986.76**	22.00 n.s.
TERPINOLENE	0.010 n s.	0.040*	0.010 n.s	SPATHULENOL	0 754 n.s.	3.291**	1 314**
LINALOOL	0.003 n.s.	0.003 n.s.	0 003 n s	GLOBULOL	0 037 n.s.	0.100*	0.037 n.s.
TRANS P-MENTH-2-EN-1-OL	0.031 n.s.	10.473 **	0.040 n.s.	41/91/119/(205)	0.000 n.s.	0.201 n.s.	0.000 n.s.
CIS P-MENTH-2-EN-1-OL	0.000 n s	10.126**	0.199 n.s.	GAMMA EUDESMOL	0.837 n.s.	10.050**	0.041 n.s.
TERPINENE-4-OL	0.080 n.s.	0.146*	0.014 n s	43/145/165/157/119	0 023 n.s	0.023 n.s.	0.023 n.s
ALPHA TERPINEOL	0.080 n s	0 080*	0.080*	BETA EUDESMOL	1.181 n.s	176.009**	11.412 n.s.
81/43/167/77	0.089 n.s.	0.344**	0.054 n s	ALPHA EUDESMOL	1.408 n.s	26.700**	0.920 n.s.
CIS PIPERITOL	0.001 n s	0.957**	0.001 n s	43/139/125/200 (C1)	0.303 n.s	3.180**	0.303*
41/97/126/69	0.018 n s	0.126**	0.018 n.s.	43/91/119/105	0.230 n.s.	6 200 n.s	0.230 n.s.
TRANS PIPERITOL	4.306 n.s.	48.297**	1.264 n s	43/139/125/200 (C2)	0.632 n.s.	2.918**	0.632*
82/(39)/(110)/(95)/(55)	0.188 n.s.	0.509*	0.188 n.s.	221/41/(189)/(139)/(236)	7.209 n.s.	6.993 n s	3 120 n s.
55/70/41/126/97	0.020 n.s.	0.113 n.s	0.027 n s.	43/(55)/(91)/(221)	0.183 n.s.	2.000**	0.183 n.s.
43/111/126/71	0.003 n.s	0.288**	0.003 n.s.	237/43/(81)/(209)/(252)	3.400 n.s.	48.440 n.s.	0 890 n.s.
84/105/41/126/97	0.028 n.s.	0.810**	0.028 n.s.	83/125/171/139/(254)	2.366 n.s.	3.328 n.s.	0.197 n.s.
91/44/104/150	0 003 n.s.	0.003 n.s.	0.003 n.s.	170/155/43/57/81	0.130 n.s	4.491**	0.104 n.s.
BICYCLOELEMENE	0.000 n.s.	0.899**	0.028 n.s.	43/59/149/109/(164)	0.431 n.s.	0.431 n.s.	0.431 n.s.
PYROGALLOL	0.022 n s	19.609**	1.327 n.s.	251/266 TASMANONE	0.606 n.s.	0.320 n.s.	0.038 n.s.
ALPHA COPAINE	0 121 n.s.	0.438**	0.036 n.s.	59/41/221/142	0.078 n.s	0.598**	0.078 n.s.
43/67/(95)/(126)/(182)	0.002 n.s.	1.383**	0.003 n.s.	43/139/182/125	0.629 n.s.	11.927**	0.266 n.s.
BETA ELEMENE	0.018 n s	2.371**	0.088*	EUDESMOL RELATED 1	0.105 n.s.	16 538**	0.105 n.s.
107/138/79/78	0.037 n.s.	3.759**	0.117 n.s.	EUDESMOL RELATED 2	3.000 n.s.	599.980**	3.000 n.s.
CARYOPHYLLIENE	1.725**	0.226*	0.044 n.s.				

Appendix 6

Essential oil and surface wax components from the leaves of *Eucalyptus regnans*, *E. delegatensis* and *E. nitens*.

The following tables list the essential oil and leaf wax components recorded from the leaves of *E. regnans*, *E. delegatensis* and *E. nitens* leaves using gas chromotography. Leaves examined ranged from newly expanding leaves to previous season leaves (for a description of leaf age classes refer to chapter 7, section 7.2.7). Table A6.1 lists essential oils while Table A6.2 lists leaf waxes.

Table A6.1 Mean percentage composition (±SE) and retention times recorded from GLC of leaf essential oils recorded from *Eucalyptus regnans*, *E. delegatensis* and *E. nitens* from three leaf age classes: young - newly emerged leaves, 25 - 40 mm in length, medium - leaves with toughness less than 50 g.

time	wax	*E regnans* young	*E. regnans* medium	*E. regnans* old	*E deleg.* young	*E deleg.* medium	*E deleg* old	*E nitens* young	*E nitens* medium	*E nitens* old
5 13	isobutyl isobutanoate	0.10 ± 0 10	0 40 ± 0.40	0 30 ± 0 30	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
5.65	alpha thujene	0.23 ± 0 05	0 08 ± 0.08	0.00 ± 0.00	1 40 ± 0.15	1.41 ± 0 02	1.05 ± 0.63	6 51 ± 0.55	7.31 ± 0.62	5.59 ± 1 65
6.04	sabinene	0 02 ± 0 02	0.07 ± 0 07	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
6 13	68/40/44/(84)	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.34 ± 0.14	0 78 ± 0 04	2.69 ± 1.09	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
6 56	55/112/(83)/(70)	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.78 ± 0.14	0 62 ± 0 21	1 64 ± 1.64	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6 66	94/66/40/(110)	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 46 ± 0.12	0.64 ± 0.24	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00
6.81	68/40/(84)/(94)/(112)	0.03 ± 0.03	0.18 ± 0 11	0 00 ± 0.00	0 85 ± 0 18	1.10 ± 0.12	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
6.96	myrcene	0 05 ± 0 05	0 20 ± 0 20	0 00 ± 0.00	0 95 ± 0 13	1.02 ± 0.12	0 37 ± 0 37	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00
7.29	alpha phellandrene	2.52 ± 0 25	4.46 ± 3 03	0.75 ± 0.07	13 55 ± 1.37	12.82 ± 0.37	8 73 ± 5 12	2.45 ± 0 77	3 67 ± 1 03	0 00 ± 0.00
7 61	93/121/77/(136)	0 00 ± 0 00	0 00 ± 0 00	0.39 ± 0 03	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00
7.61	91/39/65/120	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 14 ± 0 08	0 00 ± 0 00	0 00 ± 0.00
7.62	alpha terpinene	0 14 ± 0 08	0 58 ± 0 29	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
7 63	93/121/(77)/(136)	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	2 95 ± 0 48	1 43 ± 0.08	2.70 ± 1 19	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00
7.68	p-cymene	0 29 ± 0 11	0 57 ± 0.46	1 65 ± 0 19	1 81 ± 0 31	1 36 ± 0 14	2.15 ± 1.40	0 34 ± 0 12	0 69 ± 0 33	2 97 ± 0.66
7 86	beta phellandrene	1 40 ± 0.20	2 48 ± 1.93	0 79 ± 0 14	4 04 ± 0 65	2 67 ± 0.37	3.19 ± 1 33	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
7 90	1, 8 cineole	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	9 77 ± 0.76	14 03 ± 2 88	13 62 ± 3 67
8.34	trans beta ocimene	0 02 ± 0.02	0 08 ± 0.08	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
8 40	93/77/(39)/(65)/(128)	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	1.94 ± 0.35	1.07 ± 0.24	0 69 ± 0 69	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
8.56	gamma terpinene	0 02 ± 0 02	0 09 ± 0.09	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	1.87 ± 0.63	4 07 ± 3 01	0 75 ± 0 75
8 63	43/93/128/77	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 73 ± 0 08	0.18 ± 0.11	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00
8.66	trans sabinene hydrate	0.02 ± 0 02	0 07 ± 0 07	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00
9 31	terpinolene	0 19 ± 0 07	0 18 ± 0 18	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.70 ± 0.09	0.00 ± 0 00	0 00 ± 0 00
9 38	93/121/(136)/(77)	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	1.00 ± 0.10	0 84 ± 0 03	0 34 ± 0 34	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
9.40	cis sabinene hydrate	0 03 ± 0 03	0 13 ± 0 13	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
9 51	71/41/(93)/(55)	0.05 ± 0 05	0.21 ± 0 21	0.00 ± 0 00	0 42 ± 0 02	0 11 ± 0 11	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00

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Table A6.1 *continued*

time	oil	*E. regnans* young	*E. regnans* medium	*E regnans* old	*E deleg.* young	*E. deleg.* medium	*E. deleg* old	*E. nitens* young	*E. nitens* medium	*E. nitens* old
9.74	isopentyl isopentanoate	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 89 ± 0.11	1.25 ± 0.26	1.24 ± 0.55
10.06	trans p-menth-2-en-1-ol	0 00 ± 0.00	0.66 ± 0.22	0.50 ± 0 07	1.38 ± 0.53	1.31 ± 0.74	1 84 ± 1.09	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
10.17	43,(101),(55),(72),(144)	0 01 ± 0.01	0.37 ± 0 05	0 00 ± 0 00	0.51 ± 0.04	0.90 ± 0.12	2 43 ± 0 97	0 20 ± 0.12	1 16 ± 0 27	0.85 ± 0.31
10 47	cis p-menth-2-en-1-ol	0.33 ± 0.11	0.54 ± 0.45	0.72 ± 0 13	1.65 ± 0.19	1.89 ± 0.33	0 51 ± 0.51	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
11 03	alpha phellandrene like	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	1 03 ± 0.35	0.84 ± 0 61	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00
11 33	93,(77),(121),(136)	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.87 ± 0.12	0 91 ± 0 18	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00
11.41	terpinene-4-ol	0.22 ± 0 07	0.28 ± 0 28	0 16 ± 0 10	0.85 ± 0.31	0 56 ± 0 33	2 25 ± 1 03	0 00 ± 0 00	0.32 ± 0.32	0 17 ± 0 17
11.74	81,43,167,77	0 03 ± 0 03	0.10 ± 0 10	0.00 ± 0 00	0 47 ± 0.18	0 39 ± 0.26	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
11.74	alpha terpineol	0 07 ± 0 07	0.29 ± 0 29	0.12 ± 0 12	0.57 ± 0 03	0 43 ± 0.15	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
11.96	catchetol	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	1 74 ± 0 37	5.43 ± 1 09	4.37 ± 1.75
11 98	110,64,39,81,(126)	0 05 ± 0.05	0 34 ± 0 21	0 00 ± 0.00	0 82 ± 0 07	3.69 ± 0.58	20 34 ± 4.73	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
11 99	41,97,126,69	0 00 ± 0 00	0 00 ± 0.00	1 78 ± 0.19	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00
12 11	trans piperitol	0 75 ± 0.75	5.49 ± 3.00	0 00 ± 0.00	1.45 ± 1 45	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
12 44	43,(167),(93),(121)	0.03 ± 0 03	0 10 ± 0 10	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
12 52	93,(77),(121),(136)	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	1.73 ± 0 29	1 67 ± 0 85	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
12.81	93,(77),(121),(136)	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.88 ± 0.30	0 70 ± 0.49	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
12.94	82,110 39 95,(137)	0.12 ± 0 12	0 47 ± 0 47	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00
12.98	44,112,83,55	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 39 ± 0.13	0 13 ± 0 13	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00
12.99	82,(39),(110),(95),(55)	0.00 ± 0 00	0 00 ± 0 00	0.71 ± 0.41	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
14 14	135,150,91,(224)	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	1.16 ± 0 10	0.17 ± 0.10	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00
14 39	55,70,41,126,97	0 04 ± 0.04	0.18 ± 0 18	0.00 ± 0 00	0.15 ± 0 15	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
14 56	39,(57),(87),(144)	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	1.55 ± 0 44	0.27 ± 0 27	0.26 ± 0 26
14.70	84,105,41,126,97	0.19 ± 0 11	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
15 12	43,(97),(55),(85),(110)	0 02 ± 0 02	0.08 ± 0 08	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00
15.47	bicycloelemene	0.70 ± 0 26	0 57 ± 0 26	0.00 ± 0 00	1 05 ± 0 16	1.24 ± 0.20	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00

Continued overleaf

Table A6.1 *continued*

time	oil	*E. regnans* young	*E. regnans* med.	*E regnans* old	*E. deleg* young	*E. deleg.* med	*E deleg.* old	*E nitens* young	*E nitens* med	*E nitens* old
16 11	PYROGALLOL	0.86 ± 0 39	3.17 ± 0.45	3.47 ± 0 58	7 88 ± 0.90	3 00 ± 0 84	4.19 ± 2 27	37 82 ± 6.51	20 41 ± 3 02	23.47 ± 3 07
16 17	43,71,111,100	0 15 ± 0.12	0 08 ± 0 08	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
16 32	43,(71),(84),(111),(150)	0.03 ± 0 03	0 11 ± 0 11	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
16 35	43,99,81,(290)	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 27 ± 0 18	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00
16 41	ALPHA COPAINE	0.77 ± 0 48	0.93 ± 0 65	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
16.46	105,(161),(91),(43)	0.00 ± 0 00	0.00 ± 0 00	1.08 ± 0.93	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16 71	41,93,126,(167)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0 07 ± 0 07	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
16 71	BETA ELEMENE	1.04 ± 0 33	1.06 ± 0 57	0.00 ± 0.00	0.39 ± 0.13	0.43 ± 0.16	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00
16 75	43,67,(95),(126),(182)	0.00 ± 0 00	0.00 ± 0 00	0.25 ± 0.16	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
16 87	107,138,79,78	0 00 ± 0 00	0.00 ± 0 00	1.50 ± 0.29	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
17 37	CARYOPHYLLIENE	1.59 ± 0.71	1.71 ± 1 12	1.32 ± 1 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
17 81	41,105,133,(204)	0 00 ± 0 00	0 00 ± 0 00	0.20 ± 0.20	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
17 82	41,77,(53),(189),(204)	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.79 ± 0.32	2.18 ± 0 57	1 48 ± 0 36
17.82	60,(73),(91),(79),(119),(161)	0 55 ± 0.07	0 44 ± 0.38	0.99 ± 0 99	0.98 ± 0.17	0.56 ± 0 37	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
17.90	43,71,60,(108),(147)	0 00 ± 0 00	0.00 ± 0 00	0 25 ± 0 15	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
18.02	HUMULENE	0 52 ± 0 13	0 56 ± 0 34	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00
18 09	93,41,80,(105),(121)	0 00 ± 0.00	0 00 ± 0 00	0 55 ± 0.55	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
18 25	ALLOAROMADENDRENE	0.76 ± 0 31	0 47 ± 0.29	0 71 ± 0 51	1.89 ± 0.47	1 18 ± 0.28	2 45 ± 1 80	1 57 ± 0.62	1.78 ± 1.03	1 95 ± 0 74
18.50	104,57,43,(121)	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	1 78 ± 0.31	2.61 ± 1.54	2 80 ± 1 42
18.57	121,138,93,65,(189)	0.00 ± 0.00	0.00 ± 0.00	0 84 ± 0.09	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
18.57	121,138,39,(161)	0 20 ± 0 07	1.16 ± 0 25	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00
18.76	93,121,105,67,(161,(204)	0 00 ± 0 00	0.00 ± 0.00	0 39 ± 0 23	3.21 ± 0.51	5 11 ± 0.51	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00
18 86	43,207,161,123	0 00 ± 0 00	0.00 ± 0 00	0 25 ± 0.16	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
18.98	BICYCLOGERMACRENE	13 52 ± 6 18	8 45 ± 4.00	1.23 ± 0 43	17.17 ± 2 76	18 31 ± 3 16	15 50 ± 1.81	7 80 ± 1.98	6 69 ± 2.57	3.58 ± 1 50
19 13	105,133,91,159	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.42 ± 0 14	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00

Continued overleaf

Table A6.1 *continued*

time	oil	*E regnans* young	*E regnans* med.	*E. regnans* old	*E. deleg.* young	*E. deleg* med.	*E. deleg.* old	*E nitens* young	*E nitens* med	*E nitens* old
19.20	60,45,(105),(69),(204)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	1.97 ± 0 80	0 00 ± 0 00	0 00 ± 0 00
19.30	ELEMOL LIKE	0 70 ± 0.19	0 80 ± 0 17	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
19.41	159,129,143,(202)	0.00 ± 0.00	0 00 ± 0 00	0.11 ± 0.11	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
19 51	P-(3OXYBUTYL) PHENYLACETATE	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	5.13 ± 1 96	4 14 ± 0 30	1.17 ± 1 17
19.52	161,119,41,(105),(204)	0 61 ± 0.30	0 50 ± 0 42	0.43 ± 0.43	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
19 78	107,138,77,148	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	1.88 ± 0 99
19 93	HEDYCARYOL	26.64 ± 12.34	27 27 ± 11.34	10.74 ± 3 77	3 88 ± 0.78	8.24 ± 0 94	3.08 ± 1 24	0 46 ± 0 30	0 86 ± 0 64	0 00 ± 0.00
20 42	43 91,119,105,(205)	0 05 ± 0.05	0.35 ± 0.20	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
20.50	43,91,119,105,(205)	0 17 ± 0.17	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
20 50	SPATHULENOL	0 00 ± 0.00	0.00 ± 0.00	1 94 ± 1 76	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
20.59	41,(69),(93),((79),(87)	0.00 ± 0 00	0.00 ± 0 00	0 33 ± 0 33	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
20 70	GLOBULOL	0.00 ± 0 00	0.00 ± 0 00	0 36 ± 0 36	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
20 84	VIRIDIFLOROL	0.00 ± 0 00	0.00 ± 0.00	0 55 ± 0 55	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
21 00	43,79,91,109,(161)	0.02 ± 0 02	0.08 ± 0 08	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
21 09	41,91,119,(205)	0 00 ± 0 00	0 00 ± 0 00	0.24 ± 0.20	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
21.12	69,41,93,57,(156)	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	1.33 ± 0.26	0 42 ± 0.42	0 00 ± 0 00
21.32	137,43,194,(119)	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	1.19 ± 0.69	0 00 ± 0 00	0.57 ± 0 33
21.33	43,67,(105),(161)	0 02 ± 0 02	0.08 ± 0.08	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00
21 41	137,70,95,(182),(371)	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.58 ± 0 34	0.00 ± 0 00	0.00 ± 0 00
21 43	209,43,77,55	0.02 ± 0 02	0 08 ± 0.08	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
21.44	59,149,43,107	0.00 ± 0 00	0 00 ± 0.00	0 38 ± 0 38	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
21 60	91,119,43,107,(162)	0 04 ± 0 04	0 14 ± 0.14	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
21 61	189,59,41,161,(204)	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 54 ± 0.23	0 18 ± 0.18	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
21.62	GAMMA EUDESMOL	0.75 ± 0 46	0 61 ± 0.22	1.76 ± 1 03	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
21 67	119,43,93,(205),(220)	0.00 ± 0 00	0.00 ± 0.00	0.37 ± 0 25	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00

Continued overleaf

Table A6.1 *continued*

time oil	*E regnans* young	*E. regnans* med.	*E regnans* old	*E. deleg.* young	*E deleg* med	*E deleg.* old	*E nitens* young	*E nitens* med.	*E nitens* old
21 82 43,145,165,157,119	0 00 ± 0.00	0.00 ± 0.00	3.82 ± 3.55	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
21 96 BETA EUDESMOL	1 14 ± 0 51	1 58 ± 0.55	10.04 ± 2.61	1.12 ± 0 24	0.82 ± 0 03	0.00 ± 0 00	0.31 ± 0.18	0.00 ± 0.00	0.00 ± 0 00
22 03 43,60,55,70,(163)	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.08 ± 0.08	0 00 ± 0.00	0 00 ± 0 00
22 06 ALPHA EUDESMOL	2 27 ± 0.74	3.97 ± 0.16	4 34 ± 2.51	1.17 ± 0 26	1 03 ± 0.09	0.00 ± 0 00	0.35 ± 0.20	0 22 ± 0.22	0 00 ± 0 00
22 47 43 139.125,200	0 17 ± 0.17	2.39 ± 0.70	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22 50 43,139,125,200	1 45 ± 0 57	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00
22.53 43,91,119,105	0 00 ± 0.00	0.00 ± 0.00	1.22 ± 0 45	0.24 ± 0 24	0.26 ± 0.13	0.72 ± 0.72	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00
22.54 43,170,139,155	0 00 ± 0 00	0.00 ± 0.00	2 12 ± 2.12	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00
22 76 223,(43),(165),(266)	0 07 ± 0.07	0.27 ± 0 27	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
22 83 221,41,(189),(139),(236)	1 86 ± 0 58	3 48 ± 0 47	2 29 ± 0 43	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
23 09 43,(55),(91),(221)	0 00 ± 0 00	0 00 ± 0.00	2.54 ± 1 85	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00
23 21 237,43,209,81,(252)	8 09 ± 3 18	9 48 ± 5.19	6.42 ± 3.70	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00
23 30 69,41,81,55,(121)	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 16 ± 0 10	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00
23.44 83,125,171,139,(254)	1.11 ± 0.31	1.16 ± 0 61	1 34 ± 1 03	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00
23 55 170,155,43,57,81	0.20 ± 0 15	0 40 ± 0.17	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00
23 61 170,155,41,81	0 00 ± 0 00	0 00 ± 0.00	0 86 ± 0.45	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
23 63 43,40,(75),(121),(91)	0 05 ± 0 05	0.36 ± 0.21	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00
24 12 43,59,149,109,(164)	0 00 ± 0 00	0 00 ± 0.00	1.99 ± 0 50	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
24 48 43,59,81,112,(182)	0 02 ± 0.02	0.26 ± 0.09	0.95 ± 0.10	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
24 57 251,266	0 86 ± 0 33	0.90 ± 0.26	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
24.66 215,83,209,(266)	0 00 ± 0 00	0.00 ± 0 00	0 92 ± 0.50	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
24 70 251,43,(59),(149),(266)	0.75 ± 0 21	1.53 ± 0 14	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
24.77 215,83,209,(266)	0 00 ± 0 00	0.00 ± 0 00	7.30 ± 3 75	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
24 84 43,59,149,71,(109),(240)	0 00 ± 0 00	0 00 ± 0.00	3.56 ± 3 56	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
25 07 43,93,159,195,(238)	0 03 ± 0.03	0 33 ± 0.12	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00

Continued overleaf

Table A6.1 *continued*

time oil	*E. regnans* young	*E. regnans* medium	*E. regnans* old	*E. deleg.* young	*E. deleg.* medium	*E. deleg* old	*E. nitens* young	*E nitens* medium	*E. nitens* old
25.08 43,59,105,220,159	0 00 ± 0.00	0 00 ± 0.00	0 62 ± 0 45	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
25.09 41,43,59,81,(209)	0 10 ± 0.10	0 69 ± 0.40	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
25 18 43,55,(82),(123)	0.00 ± 0.00	0 00 ± 0 00	0 24 ± 0.24	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00
25 24 43,59,(92),(109),(134)	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 24 ± 0 15	0 00 ± 0 00	0 00 ± 0 00
25.31 41,43,59,81,(209)	0.29 ± 0 11	0 84 ± 0.49	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
25 38 43,139,182,125	0.00 ± 0.00	0 00 ± 0 00	1.77 ± 0 65	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
25 51 170,155,41,97	0 03 ± 0 03	0 14 ± 0 14	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
25 66 59,43,41,55,(205)	0 05 ± 0 05	0 38 ± 0.22	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
25 81 43,55,(82),(123)	0.00 ± 0 00	0 00 ± 0.00	0.11 ± 0 11	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
25.82 43,49,105,220,159	0 00 ± 0.00	0 00 ± 0 00	0 82 ± 0 39	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
26 05 59,43,(162),(220)	0 00 ± 0.00	0.00 ± 0 00	1 72 ± 0 72	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
26 31 195,238,43,152	1.35 ± 0.86	0 47 ± 0 21	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
26 33 43,83,140,125,(279)	0.36 ± 0.22	1 19 ± 0 87	1 89 ± 0.63	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
27 07 195,238,43,152	0.49 ± 0.15	0 08 ± 0 08	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00
27 58 60,43,73,(115),(103)	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 41 ± 0.08	0.00 ± 0 00	0 00 ± 0.00
27.60 43,59,111,81,170	0 00 ± 0 00	0 00 ± 0.00	0 40 ± 0 13	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
27 65 55,43,73,60,(129)	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 56 ± 0 19	0.13 ± 0 13	0 17 ± 0.17
27.66 73,60,41,(129),(256)	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0.60 ± 0 08	0.62 ± 0 32	1 51 ± 1.51	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
27 70 167,43,(70),(55),(210)	0 49 ± 0 15	0.11 ± 0 11	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
30 14 43,71,55,123,126	0.23 ± 0.05	0.66 ± 0 08	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00
30 22 71,41,57,81,(312)	0.00 ± 0 00	0.00 ± 0 00	2 29 ± 0.51	0 98 ± 0.12	2 11 ± 0 42	10.31 ± 3.05	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
30.22 71,41,57,81,(312)	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0.21 ± 0.13	2 54 ± 0 93	4.31 ± 1.11
30.33 79,41,95,67,(108)	0 00 ± 0 00	0.00 ± 0.00	0 25 ± 0.15	0.00 ± 0.00	0 18 ± 0.18	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
30.39 (81),(71),(91),(116)	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.65 ± 0.05	0 99 ± 0 51	4 38 ± 1.78	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
30.39 79,108,93,(181)	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 87 ± 0 15	1 08 ± 0 45	1 94 ± 0.48
31 45 91,(57),(73),(196)	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	1.16 ± 0 54	1.07 ± 0 41

Table A6.2 Mean percentage composition (±SE) and retention times recorded from GLC of wax compounds recorded from *Eucalyptus regnans*, *E. delegatensis* and *E. nitens* from three leaf age classes: young - newly emerged leaves, 25 - 40 mm in length, medium - leaves with toughness less than 50 g.

time	wax	*E reg.* young	*E reg* medium	*E. reg* old	*E del* young	*E. del.* medium	*E del.* old	*E. nit* young	*E nit* medium	*E nit* old
8.81	Tricosane	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.22 ± 0 13	0 30 ± 0 14	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00
9.28	Heptanoyl hexadecanoate	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.84 ± 0.27	0 66 ± 0 32	0 36 ± 0 36	0.39 ± 0 23	0 00 ± 0 00	0 00 ± 0 00
10.04	n-pentacosane	0 67 ± 0 12	0.54 ± 0.09	0.00 ± 0 00	0.61 ± 0.11	0.38 ± 0 13	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
10 61	Heptanoyl octadecanoate	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 71 ± 0.36	0.77 ± 0.32	0 33 ± 0.33	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
10 88	Tetracosanal	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	9 75 ± 1 63	11 36 ± 1 33	6 76 ± 0 68	0 21 ± 0 21	0 00 ± 0 00	0.00 ± 0 00
11 30	n-tetracosan-1-ol	0 60 ± 0 25	0 00 ± 0.00	0 00 ± 0.00	4 39 ± 0 28	5 96 ± 0 66	4 00 ± 0 51	1 43 ± 0.06	0 00 ± 0 00	0.00 ± 0 00
11.39	n-heptacosane	2.39 ± 0 24	1 85 ± 0.41	0 59 ± 0.10	1 00 ± 0.35	0.85 ± 0 15	0 57 ± 0 06	1.21 ± 0.29	0 84 ± 0.08	0.98 ± 0 26
11.55	n-pentacosanal	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 94 ± 0 13	0.56 ± 0 09	0.11 ± 0 11	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
11.81	Nonanoyl hexadecanoate	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 08 ± 0.08	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
11.96	Heptanoyl eicosanoate	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 18 ± 0.18	0 00 ± 0 00	0.21 ± 0.18	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
12.20	Benzyl octadecanoate	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.10 ± 0.00	0 08 ± 0 03	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00
12 16	n-hexcosanal	0.61 ± 0 13	1 65 ± 0 22	0 24 ± 0 14	9.73 ± 0.47	5 56 ± 0.55	2 56 ± 0.44	0 15 ± 0 15	0.00 ± 0 00	0 00 ± 0.00
12 40	Phenyl ethyl octadecanoate	0.14 ± 0 04	0 03 ± 0 03	0 00 ± 0 00	0 20 ± 0 03	0 15 ± 0 05	0 05 ± 0.03	0 15 ± 0 09	0.00 ± 0.00	0 00 ± 0 00
12 58	n-hexacosanol	1.52 ± 0 16	1.53 ± 0.36	0 00 ± 0.00	1 17 ± 0 41	0 90 ± 0 23	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00
12 72	n-nonacosane	1 56 ± 0 31	1.75 ± 0.42	0 92 ± 0.31	1 43 ± 0 40	0 76 ± 0 12	0 56 ± 0 07	3 15 ± 0 80	9.10 ± 1.44	6 54 ± 0.82
13 10	Benzyl eicosanoate	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 43 ± 0 18	0.44 ± 0 12	0 08 ± 0 03	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
13 05	Desmethyl eucalyptin	14 82 ± 2 32	7.22 ± 0 60	3 98 ± 0 88	2 67 ± 0.30	3.01 ± 0 48	2.18 ± 0 25	5 43 ± 0 58	1.41 ± 0 21	4 65 ± 1 02
13 25	Eucalyptin	9.42 ± 1 01	8 04 ± 0 32	5.82 ± 1 01	5.12 ± 0.69	7.42 ± 1 02	10 73 ± 0 76	4 11 ± 0 63	2.56 ± 0.27	15 22 ± 1.55
13.36	eicosanoyl benzoate	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	1 28 ± 0 29	0 66 ± 0.24	0 23 ± 0 23
13.50	n-octacosanal	1 00 ± 0.29	2 58 ± 0.29	1 01 ± 0.35	2.73 ± 0 30	1.30 ± 0 12	0 80 ± 0.23	0 77 ± 0 14	2 66 ± 0 42	1 31 ± 0 34
13 75	Phenyl ethyl eicosanoate	0 83 ± 0 07	0.17 ± 0.06	0 00 ± 0.00	3 04 ± 0.78	1 88 ± 0.56	0.68 ± 0 41	4 63 ± 1.17	0 73 ± 0.32	0 08 ± 0.05
13 80	Benzyl henicosanoate	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 08 ± 0 03	0 05 ± 0.03	0 00 ± 0 00	0 16 ± 0 03	0 03 ± 0 03	0 00 ± 0.00

Continued overleaf

Table A6.2 *continued*

time	wax	*E. reg.* young	*E reg.* medium	*E. reg.* old	*E del.* young	*E. del.* medium	*E. del* old	*E nit* young	*E. nit.* medium	*E. nit.* old
14.03	henicontane	1.06 ± 0.17	0.14 ± 0.14	0.00 ± 0 00	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0 00	1 10 ± 0 56	0 31 ± 0.11	0.10 ± 0.10
14.46	Benzyl docosanoate	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.89 ± 0 11	0 29 ± 0 10	0.08 ± 0 08	2 09 ± 0 39	0 66 ± 0.31	0.05 ± 0.03
14.40	Phenyl ethyl henicosanoate	0.08 ± 0.03	0 06 ± 0 06	0.00 ± 0.00	0 09 ± 0 03	0.00 ± 0 00	0 03 ± 0 03	0 29 ± 0 06	0.00 ± 0.00	0.00 ± 0 00
14.72	Triterpene (N21)	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	1.24 ± 0.07	0 00 ± 0 00	0 00 ± 0.00
14.79	n-triacontanal	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	2.22 ± 0.35	6.93 ± 0 70	1 99 ± 0.32
14 85	Triterpene (D18)	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	2 22 ± 0 15	0.00 ± 0.00	0.26 ± 0 26	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
14.87	Triterpene (6)	1 32 ± 0.46	0.30 ± 0 18	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
14 93	Triterpene (N4)	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 84 ± 0 14
15.01	Triterpene (D20)	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	10 09 ± 2 25	3 53 ± 0 40	5 56 ± 0 11	4.68 ± 1.09	0 00 ± 0 00
15.00	Triterpene (N5)	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	3 48 ± 0.72
14.98	Triterpene (1)	2 82 ± 0 79	3.11 ± 0.50	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
15.05	Triterpene (D21)	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	2.06 ± 2 06	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
14 98	Triterpene (11)	0.00 ± 0 00	0 00 ± 0.00	5 25 ± 0 75	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
15.11	Triterpene (D10)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	1.38 ± 1.38	1.92 ± 1 92	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
15 03	Triterpene (17)	0.00 ± 0.00	0.00 ± 0.00	3 89 ± 0.51	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00
15.03	Triterpene (2)	2.89 ± 0.69	3 98 ± 0 16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00
15.05	Triterpene (3)	0 89 ± 0.34	0 63 ± 0.24	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
15.07	Triterpene (4)	2 75 ± 0.44	3 60 ± 0 09	9.88 ± 1 92	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
15.09	Triterpene (5)	1.92 ± 0 47	1.82 ± 0 26	1.55 ± 0 89	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
15.10	Phenyl ethyl docodanoate	0 92 ± 0.30	0.18 ± 0 06	0 10 ± 0 00	6.33 ± 1.36	0.19 ± 0 05	0.09 ± 0 03	5 96 ± 0 97	1 78 ± 0 61	0 54 ± 0 38
15.15	Triterpene (D23)	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	1.28 ± 1.28	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00
15.14	Triterpene (N1)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	1 16 ± 0 40	0 00 ± 0 00	0 55 ± 0.34
15.21	Triterpene (N8)	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	1.73 ± 0.21	2.57 ± 0 61	2 81 ± 0 31
15.36	Triterpene (N9)	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	1.55 ± 0.24	3.04 ± 0.56	2 82 ± 0.36

Continued overleaf

Table A6.2 *continued*

time	wax	*E. reg* young	*E. reg* medium	*E. reg.* old	*E. del* young	*E. del.* medium	*E. del* old	*E nit.* young	*E. nit.* medium	*E nit* old
15.21	Triterpene (D19)	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	3 57 ± 1 39	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00
15 21	Triterpene (D7)	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	1 59 ± 0 92	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
15.33	Amyrin	0 61 ± 0.12	2 40 ± 0.29	4.59 ± 1 37	1 10 ± 0 72	2 33 ± 0 39	3 80 ± 1.35	0 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15 60	Phenyl ethyl tricosanoate	0.08 ± 0.03	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.23 ± 0 05	0.10 ± 0.00	0.04 ± 0 04
15 70	Benzyl tetracosanoate	0.61 ± 0.23	0.00 ± 0 00	0 00 ± 0.00	0 03 ± 0 03	0 00 ± 0 00	0 00 ± 0 00	3.78 ± 0 55	1.36 ± 0 56	0.05 ± 0 03
15 69	Hentriacontan-14,16-dione	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	13.30 ± 3 23	7.07 ± 1 11	5.74 ± 1 64	1.12 ± 0 28	0.68 ± 0 22	0.53 ± 0 08
16.06	Methyl moronate	0 33 ± 0 16	0 00 ± 0 00	19.06 ± 2 98	1.11 ± 0 70	4.41 ± 1.81	14.17 ± 3 05	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
16 00	Benzoate ester	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	1 00 ± 0 22	0 87 ± 0 38	2 17 ± 0 30	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00
15 60	Triterpene (N10)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	4 60 ± 0.91	13 38 ± 2 30	13 26 ± 2 10
15 65	Triterpene (N11)	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 38 ± 0.23	1 32 ± 0 18	1 61 ± 0 27
15.86	Triterpene (N20)	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	1 13 ± 1.13
15.93	tetracosanoyl benzoate	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.83 ± 0.22	0 24 ± 0 14	0.00 ± 0.00
16.15	Triterpene (N15)	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0 22 ± 0 22	0.00 ± 0 00	0 00 ± 0 00
16 14	Triterpene (12)	0 00 ± 0 00	0 00 ± 0.00	1 19 ± 1 19	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
16 00	Triterpene (32)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0.00	1 45 ± 1 29	1 93 ± 1 93	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
16.25	Triterpene (D8)	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	1 89 ± 0.66	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
16 18	Triterpene (D6)	0 00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	4.97 ± 0 22	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00
16 30	Triterpene (18)	0 00 ± 0 00	0 00 ± 0.00	5 03 ± 0 35	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00
16.40	Phenyl ethyl tetracosanoate	1.95 ± 0.52	0.32 ± 0 13	0.09 ± 0.03	0 72 ± 0 72	0.03 ± 0 03	0 05 ± 0.03	5 17 ± 0 83	1.35 ± 0.27	0.64 ± 0 09
16.30	Benzyl pantacosanoate	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 25 ± 0 08	0 08 ± 0.03	0.00 ± 0 00
16.36	Triterpene (N12)	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	2 61 ± 0 79
16 44	Triterpene (D9)	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0.00	2 04 ± 0.12	0 00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00
16 40	Triterpene (8)	2 88 ± 0 36	6.03 ± 0.51	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00
16.40	Triterpene (D1)	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	3.53 ± 0 62	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00
16.62	Triterpene (7)	23.26 ± 1.26	20.47 ± 3 37	6 65 ± 1.78	1 86 ± 0 35	3.37 ± 0 82	2.48 ± 0 64	0 75 ± 0.10	0 00 ± 0 00	0 00 ± 0.00

Continued overleaf

Table A6.2 *continued*

time	wax	*E reg* young	*E. reg.* medium	*E. reg.* old	*E. del* young	*E. del.* medium	*E del.* old	*E nit.* young	*E nit* medium	*E. nit* old
16 78	n-Triancontan-16,18-dione	3 30 ± 0.80	3.18 ± 1.18	2.31 ± 0.97	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	10 68 ± 1.61	18 21 ± 3 45	14 64 ± 2 33
16 87	Triterpene (20)	0 00 ± 0.00	0.00 ± 0 00	4 10 ± 0.20	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00
16 86	Benzyl hexacosanoate	2.15 ± 0.76	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	1 75 ± 0 30	1 78 ± 0 29	1.20 ± 0 17
16 90	Phenyl ethyl pantacosanoate	0 11 ± 0 01	0.03 ± 0 03	0 00 ± 0.00	1.21 ± 0 48	0 00 ± 0.00	0.00 ± 0 00	0.18 ± 0 03	0 10 ± 0 00	0.08 ± 0 03
17 40	Benzyl heptacosanoate	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0.00	0.12 ± 0 02	0 00 ± 0 00	0 00 ± 0 00
16 80	Triterpene (D15)	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.43 ± 0 43	0.00 ± 0 00	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00
16 86	n-Triancontane-16,18-dione	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0.37 ± 0.14	0 13 ± 0 08	0.13 ± 0 06	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00
16.86	Triterpene (D14)	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	4.24 ± 1.09	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
17.12	Triterpene (14)	0 00 ± 0.00	0.00 ± 0.00	4 55 ± 1.04	0 00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00
17.12	Triterpene (D13)	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	2.06 ± 2.06	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
17.12	Triterpene (15)	0.00 ± 0.00	3 14 ± 0.35	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00
17.19	Triterpene (D2)	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	4 97 ± 1 89	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
17.50	Phenyl ethyl hexacosanoate	2.22 ± 0.49	2 03 ± 1.72	0 37 ± 0.30	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0.87 ± 0 24	0 35 ± 0 09	0 13 ± 0 08
17.12	Benzoate ester	0.00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0 73 ± 0.73	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
17 92	n-Pentatriacontan-16,20-dione	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	1.53 ± 0 32	1 23 ± 0.30	0 71 ± 0 17
18.00	Benzyl octacosanoate	0.16 ± 0.06	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	1.30 ± 0 35	1 34 ± 0.53	0 23 ± 0.13
18.47	Triterpene (D12)	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0 00 ± 0.00	0 23 ± 0 23	6 68 ± 2.11	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0 00
18.10	Phenyl ethyl heptacosanoate	0 09 ± 0 03	0.00 ± 0.00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0 00	0 00 ± 0 00	0.07 ± 0 02	0 03 ± 0 03	0.00 ± 0.00
18.40	Triterpene (N13)	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0 00 ± 0.00	1.32 ± 0.08	0 00 ± 0 00	0 00 ± 0 00
18.36	Triterpene (9)	0.00 ± 0 00	3.83 ± 0 58	1.94 ± 0 47	0.00 ± 0.00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0 00	0.00 ± 0 00
18.60	Phenyl ethyl octocosanoate	0.05 ± 0 03	0.19 ± 0 13	0 31 ± 0 12	0.00 ± 0.00	0 00 ± 0.00	0 00 ± 0 00	1.05 ± 0 28	0 26 ± 0.11	0 18 ± 0.10
18 60	Benzyl nonacosanoate	0 00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.10 ± 0 00	0 03 ± 0.03	0.00 ± 0.00
18 97	11,12 dehyd. lact acetate	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	2.26 ± 0 69	6 43 ± 1 18	4 61 ± 0 85
19 10	Benzyl triacontane	0.00 ± 0 00	0 00 ± 0 00	0 00 ± 0.00	0.00 ± 0 00	0.00 ± 0 00	0 00 ± 0.00	1.02 ± 0 43	0 51 ± 0 28	0 05 ± 0 03
20.03	Phenyl ethyl triacontanoate	0.00 ± 0.00	0.00 ± 0.00	0 00 ± 0 00	0.00 ± 0 00	0.00 ± 0 00	0.00 ± 0.00	0.37 ± 0.13	0 00 ± 0 00	0 00 ± 0 00

Appendix 7

The major components in the leaf essential oil and surface wax of *E. regnans*, *E. delegatensis* and *E. nitens* for three different age classes.

Separation of components using GLC for the leaf essential oil and surface was for *E. regnans*, *E. delegatensis* and *E. nitens* for three different age classes:

young: Newly emerged leaves between 25 and 40 mm in length and younger than three weeks of age.

medium: Current season leaves older than three weeks but with toughness less than 50 g.

old: Leaves from the previous season.

In the following figures major peaks are numbered and their identity given. Unidentified triterpenes are given a number while other unidentified compounds are listed by their most abundant ions (determined by mass spectroscopy) unless bracketed indicating distinctive ions. The y-axes indicates component abundance while the x-axes is the retention time.

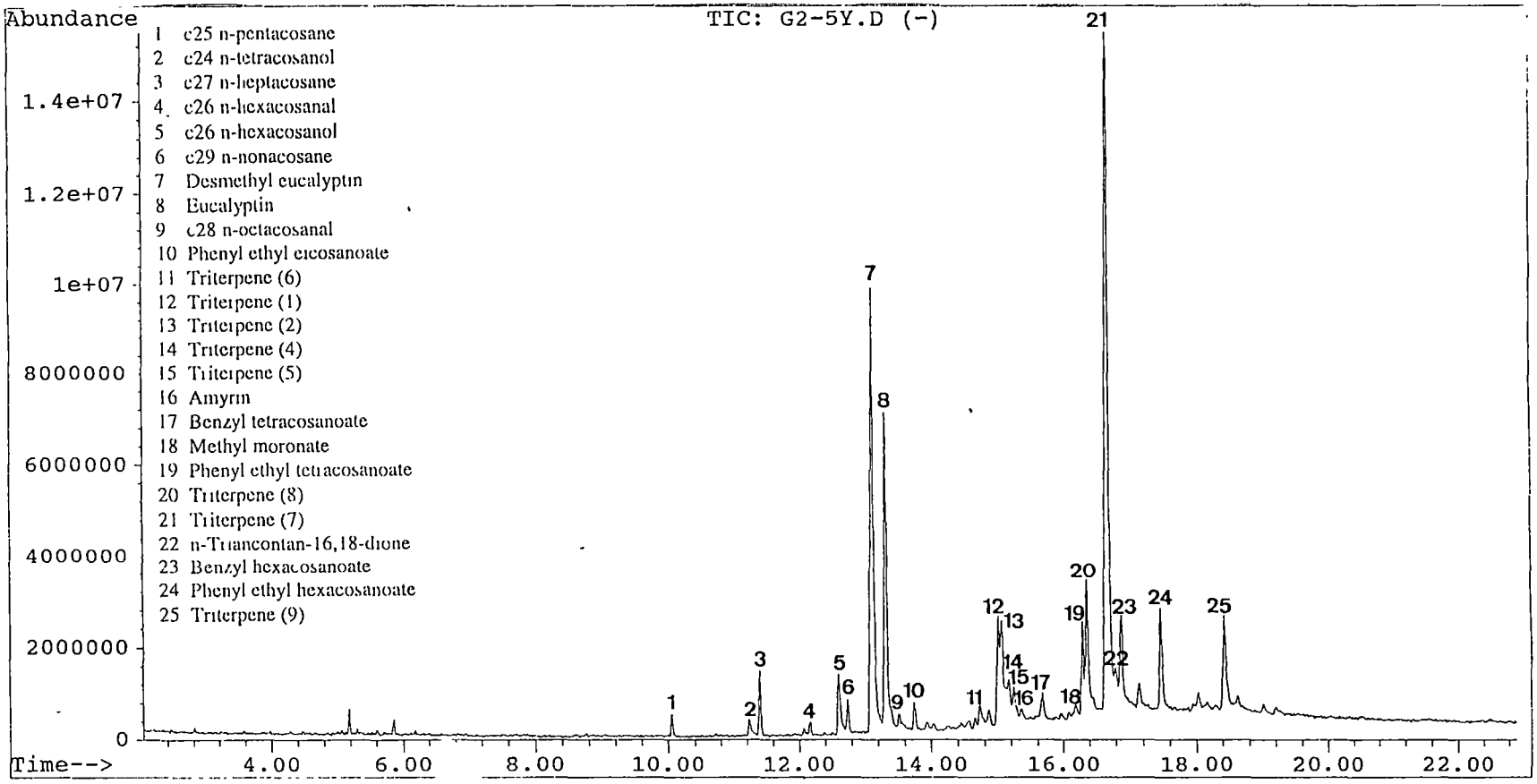

Figure A7. 1 GLC separation of leaf surface wax components for young *E. regnans* leaves. See the start of the appendix section for further details.

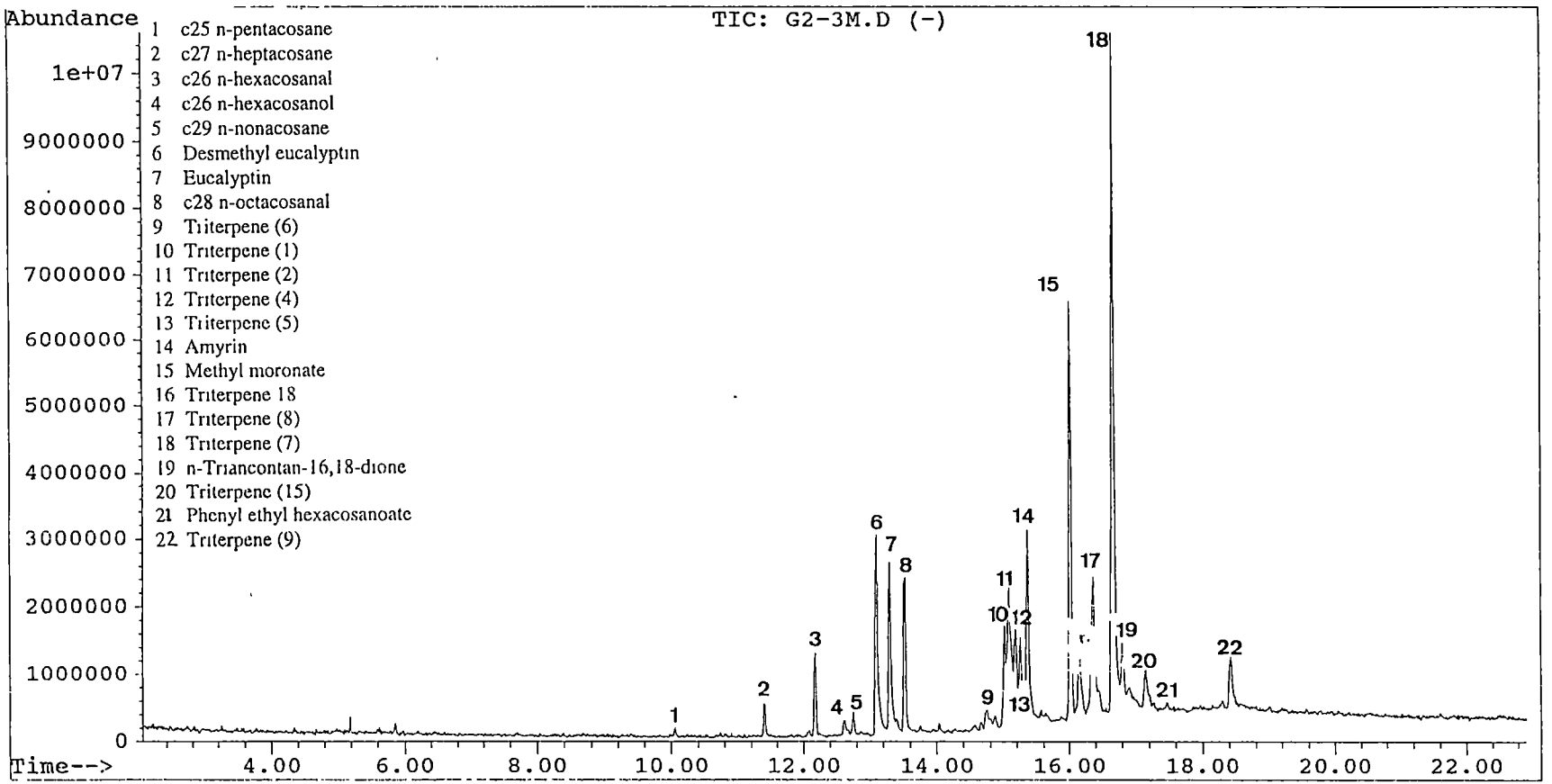

Figure A7. 2 GLC separation of leaf surface wax components for medium aged *E. regnans* leaves. See the start of the appendix section for further details.

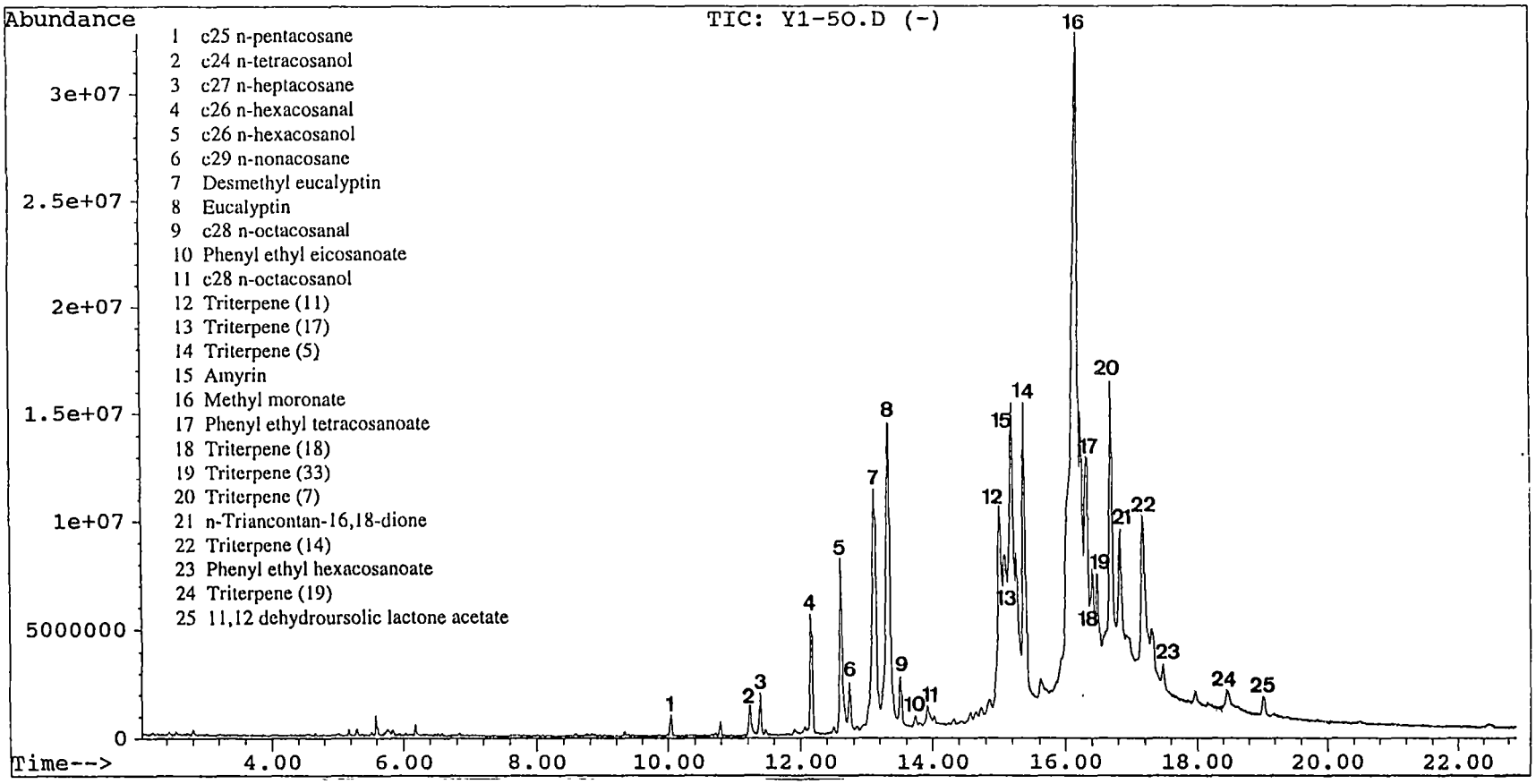

Figure A7. 3 GLC separation of leaf surface wax components for old aged *E. regnans* leaves. See the start of the appendix section for further details.

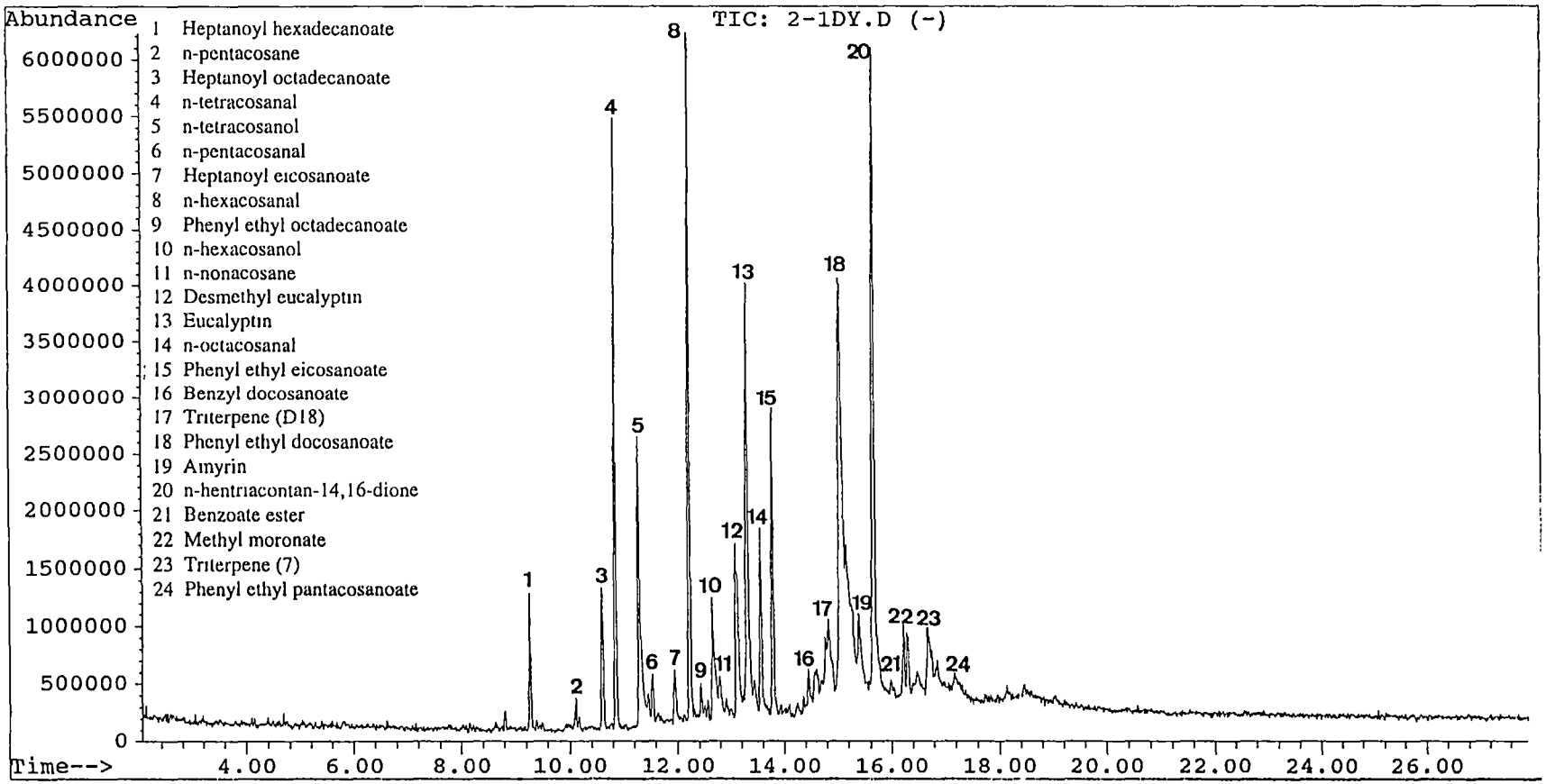

Figure A7. 4 GLC separation of leaf surface wax components for young *E. delegatensis* leaves. See the start of the appendix section for further details.

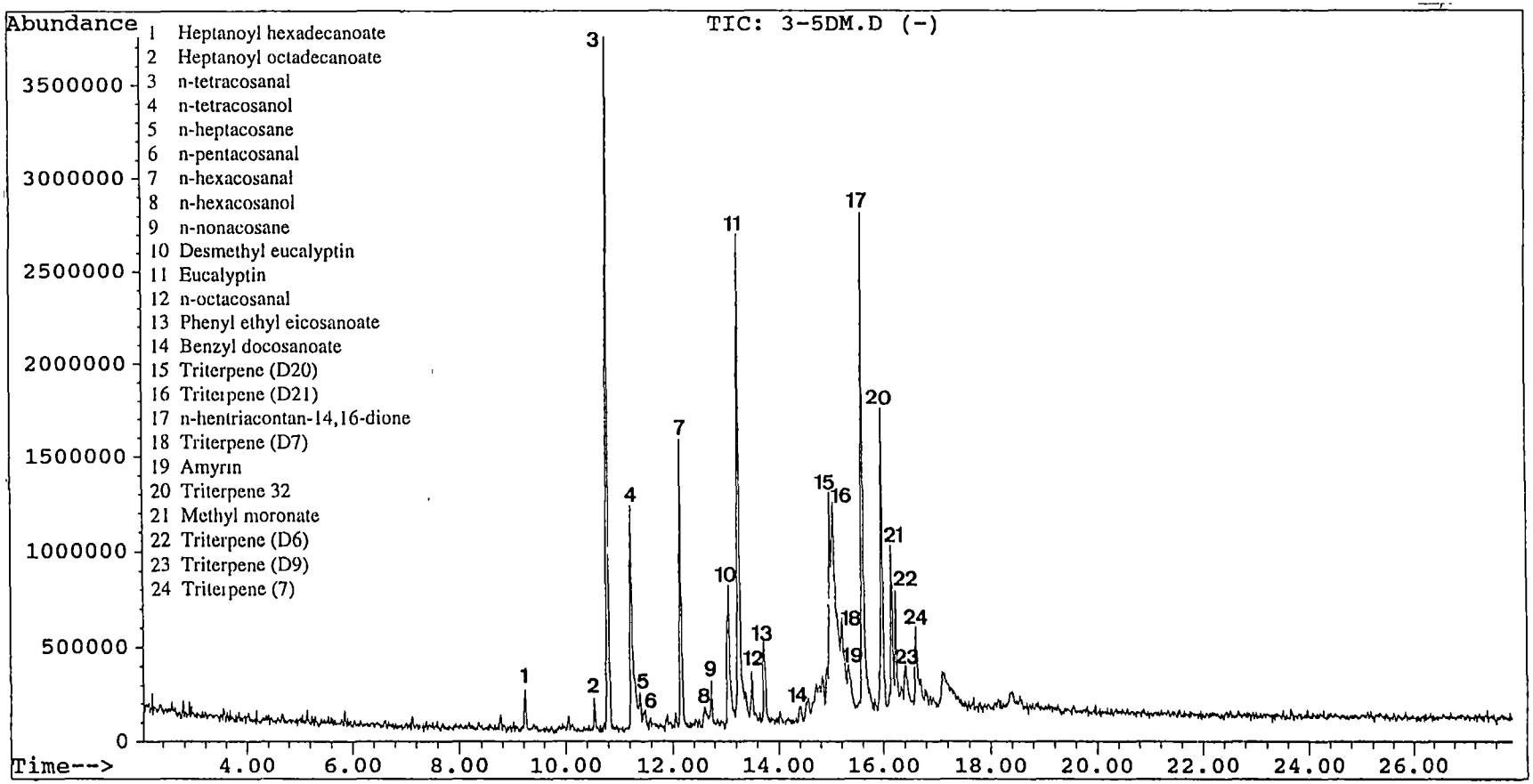

Figure A7. 5 GLC separation of leaf surface wax components for medium aged *E. delegatensis* leaves. See the start of the appendix section for further details.

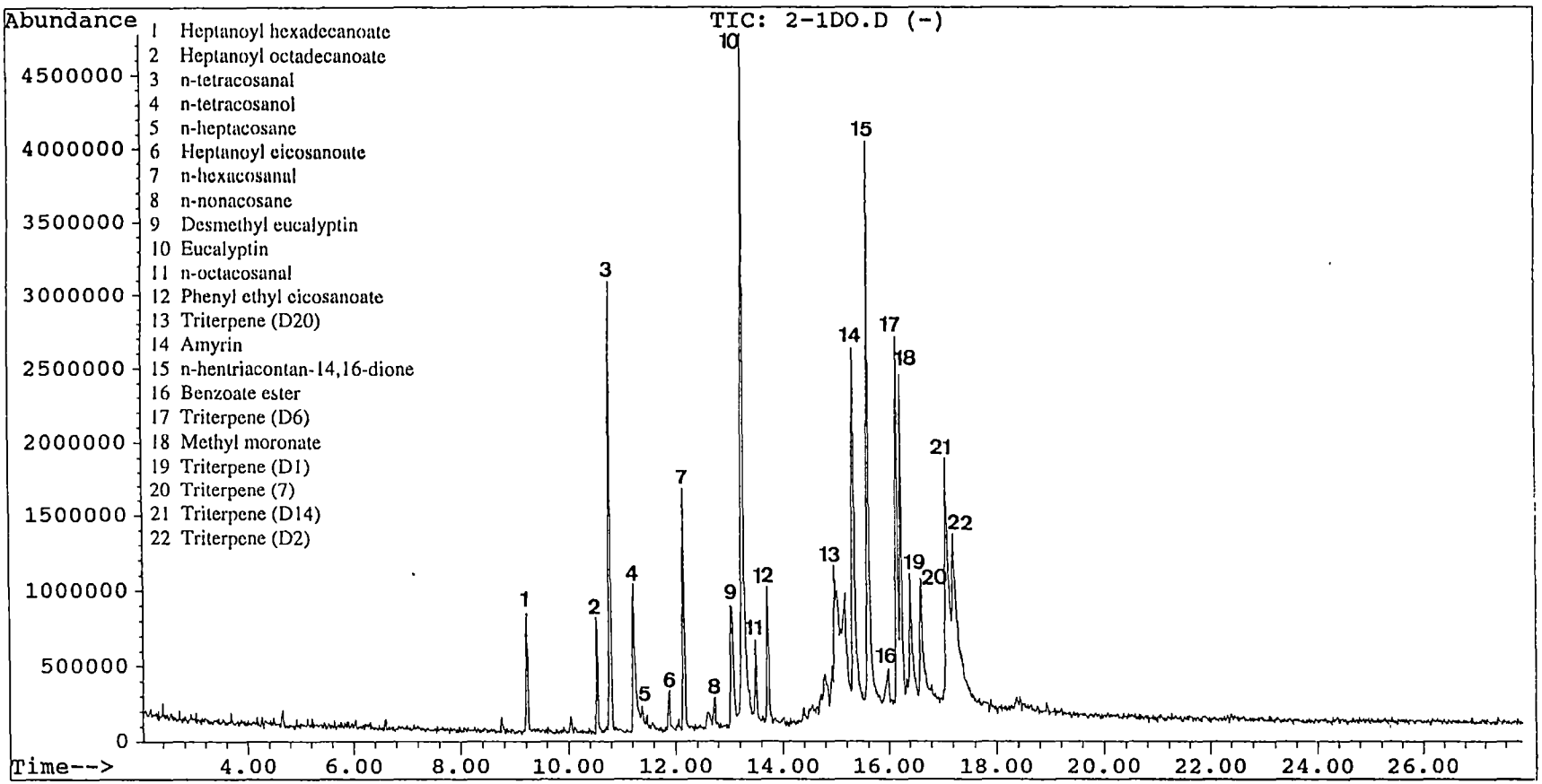

Figure A7. 6 GLC separation of leaf surface wax components for old aged *E. delegatensis* leaves. See the start of the appendix section for further details.

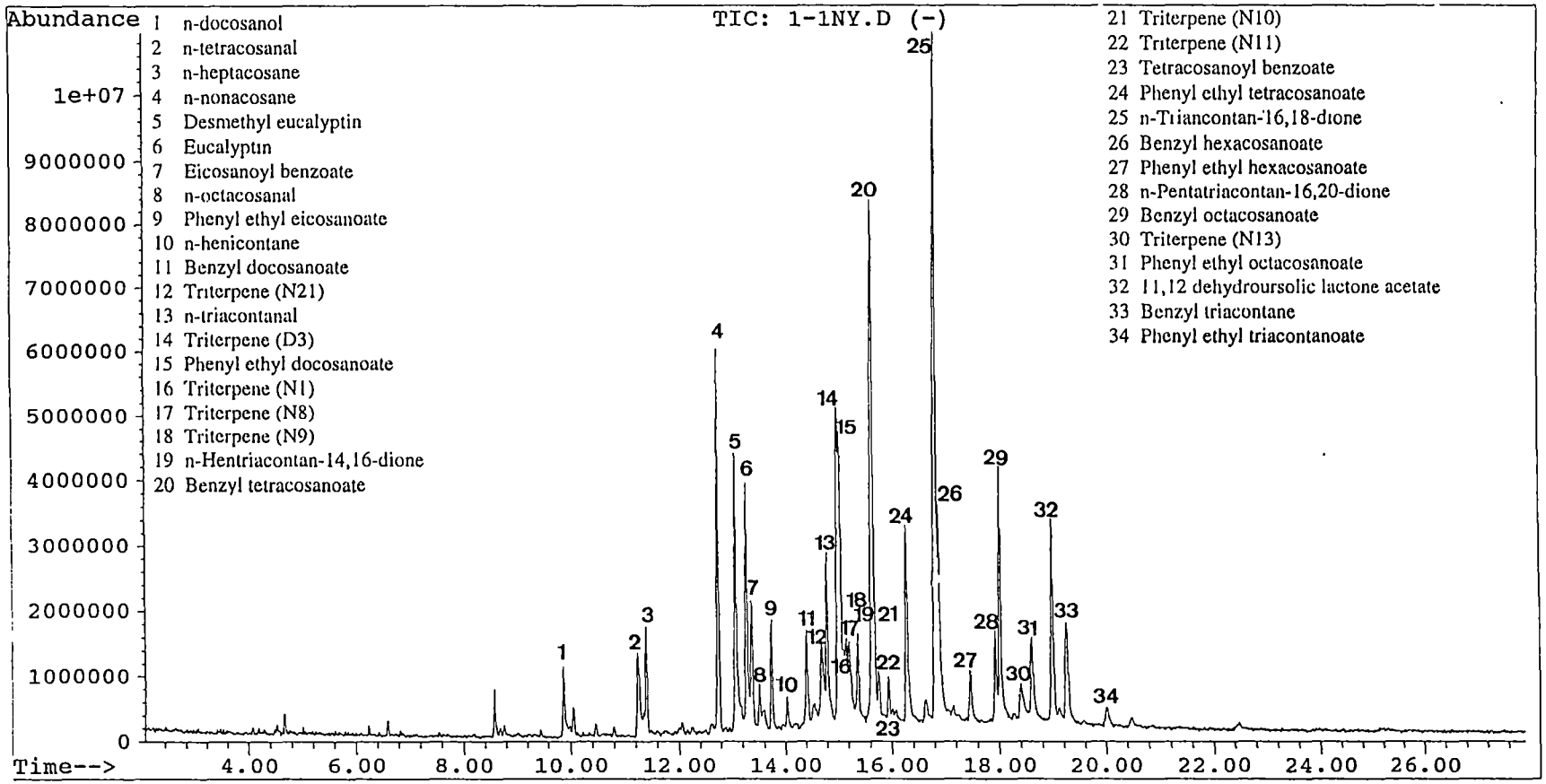

Figure A7. 7 GLC separation of leaf surface wax components for young *E. nitens* leaves. See the start of the appendix section for further details.

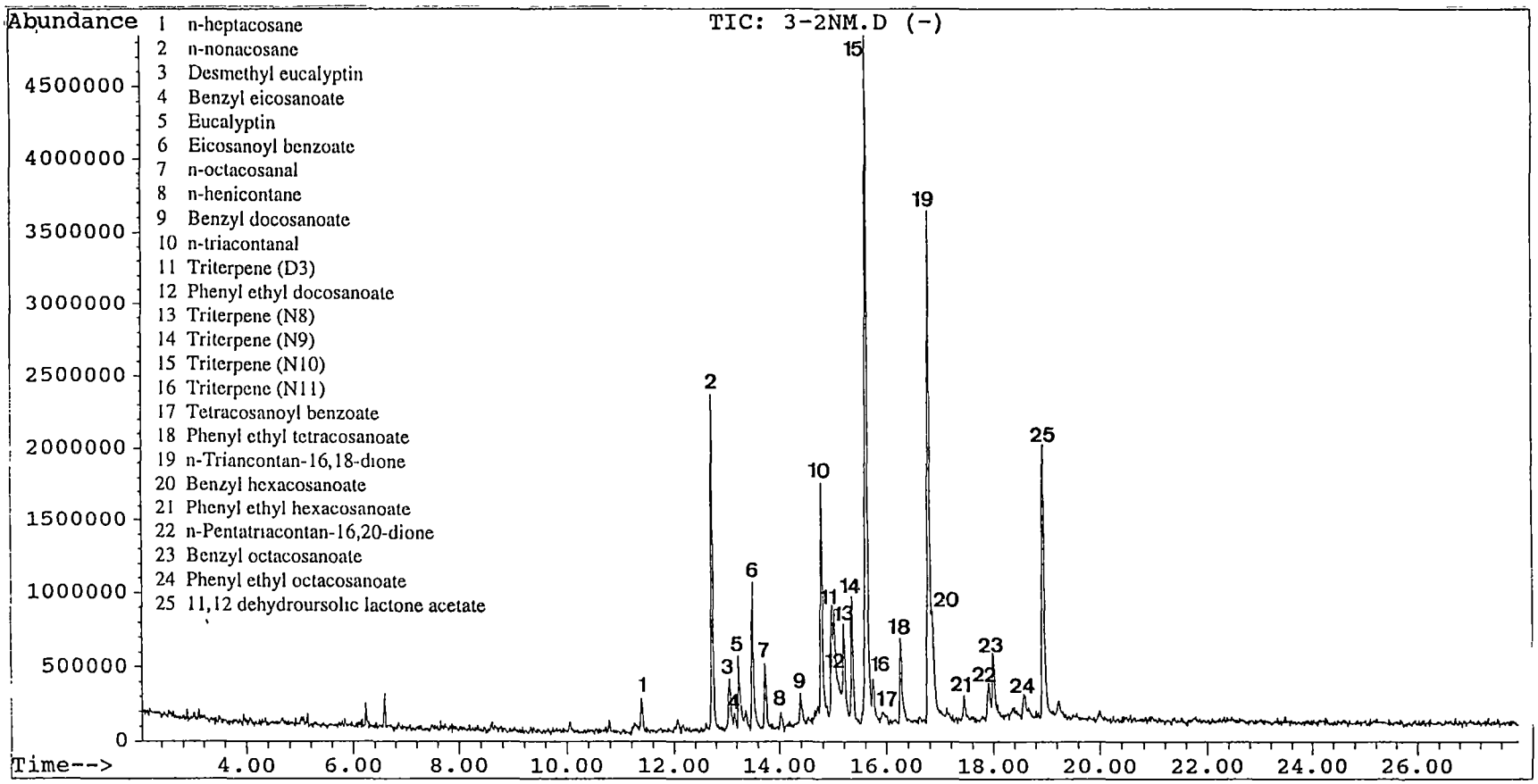

Figure A7. 8 GLC separation of leaf surface wax components for medium aged *E. nitens* leaves. See the start of the appendix section for further details.

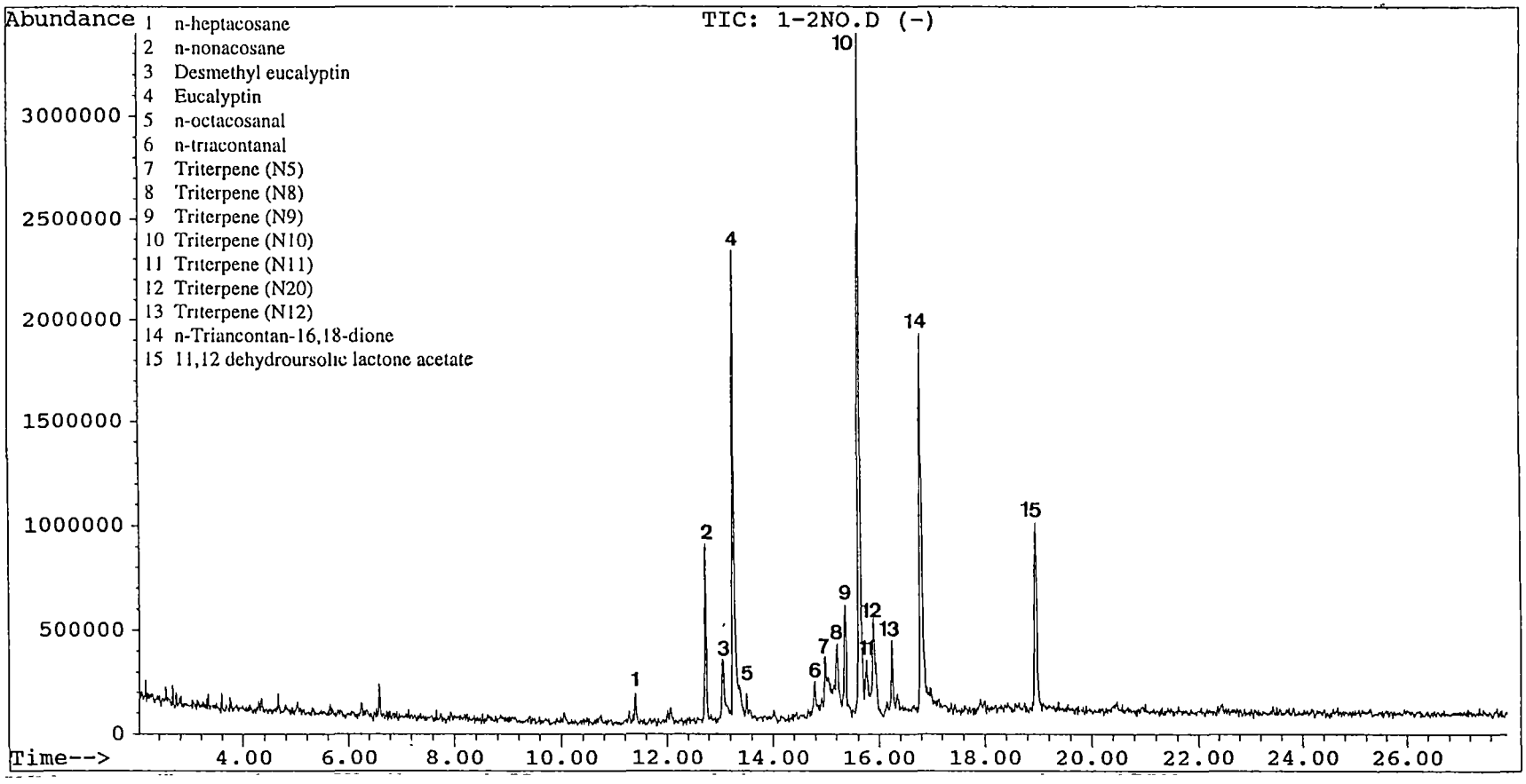

Figure A7. 9 GLC separation of leaf surface wax components for old aged *E. nitens* leaves. See the start of the appendix section for further details.

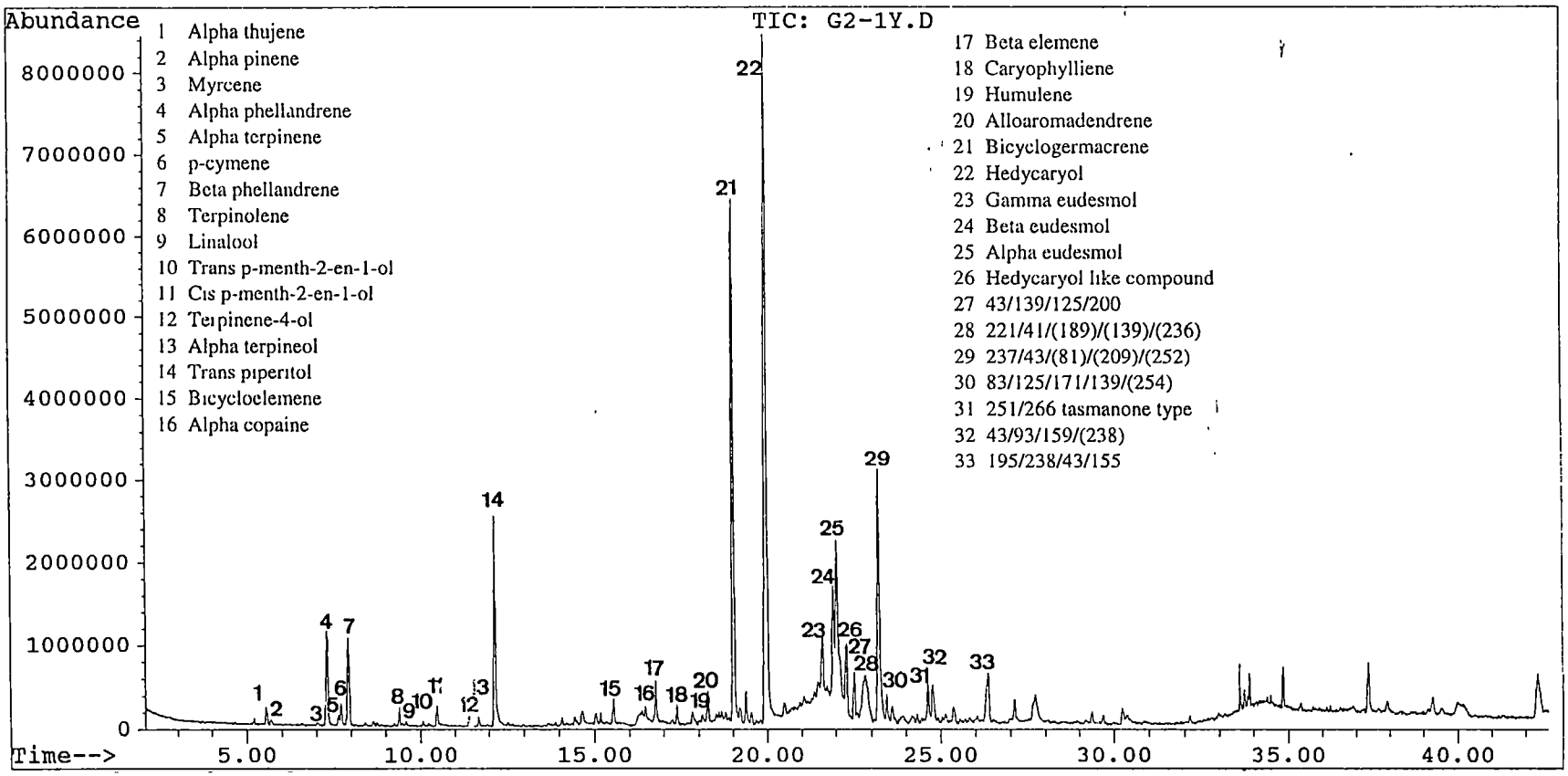

Figure A7. 10 GLC separation of leaf essential oil components for young *E. regnans* leaves. See the start of the appendix section for further details.

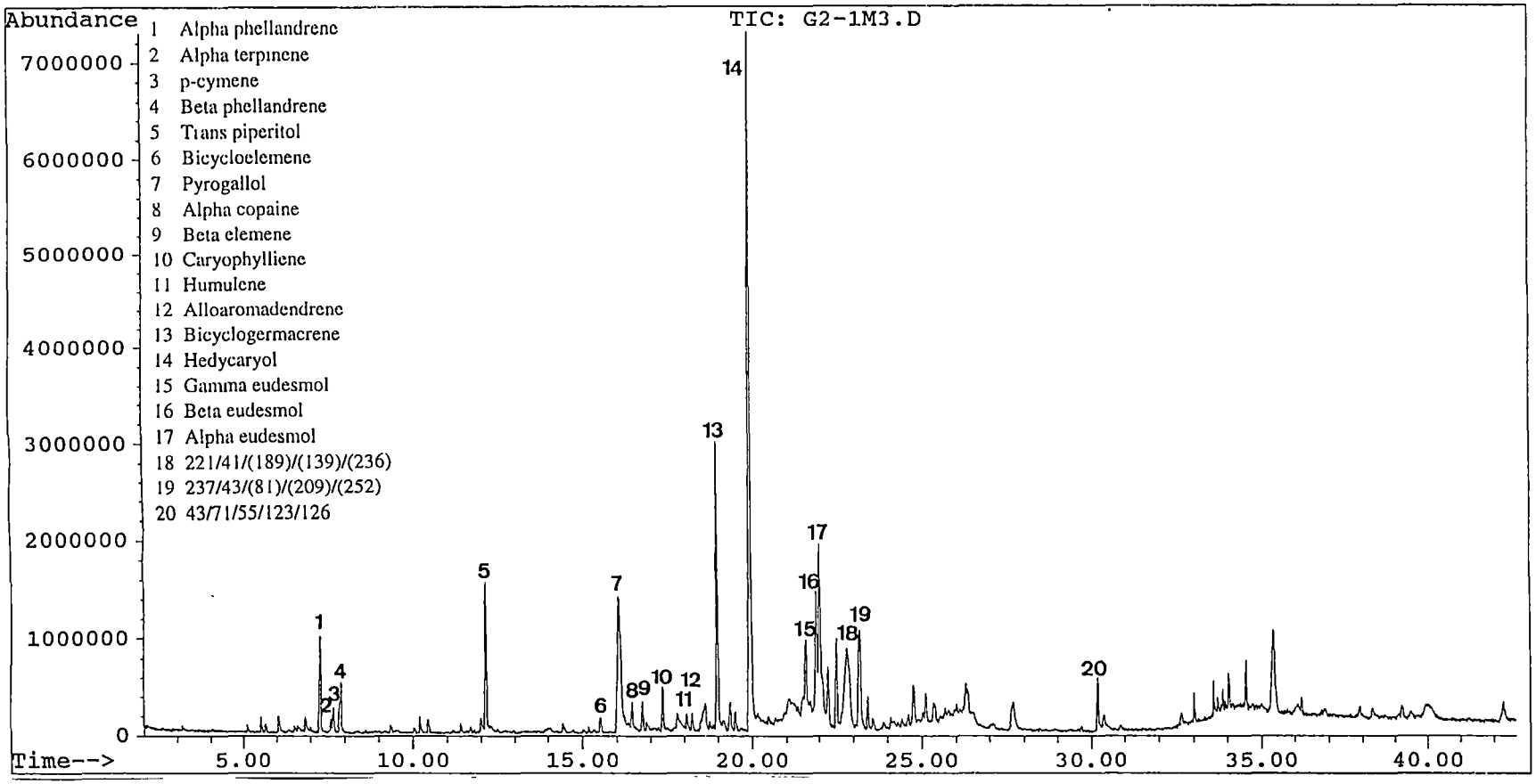

Figure A7. 11 GLC separation of leaf essential oil components for medium aged *E. regnans* leaves. See the start of the appendix section for further details.

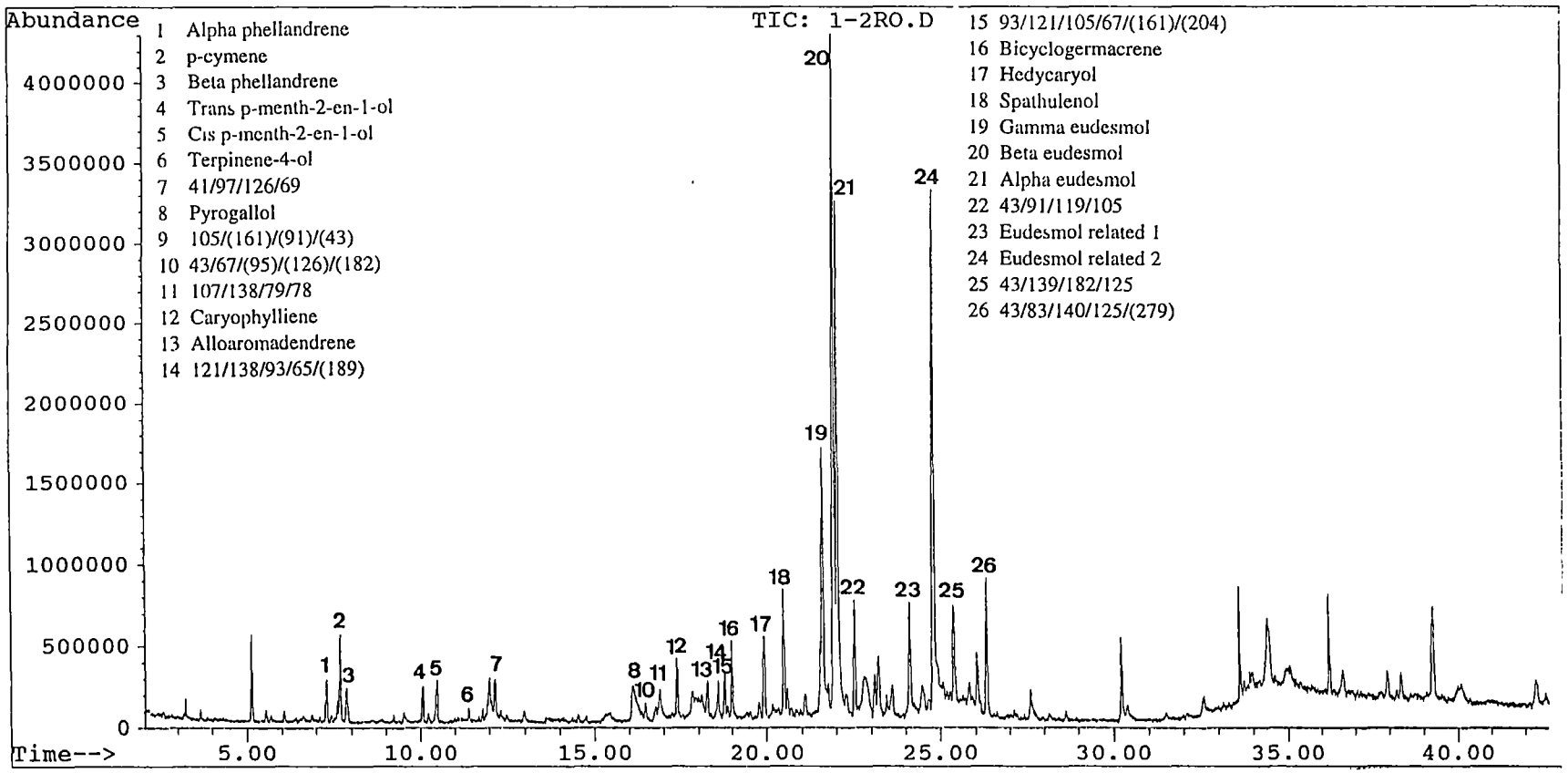

Figure A7. 12 GLC separation of leaf essential oil components for old aged *E. regnans* leaves. See the start of the appendix section for further details.

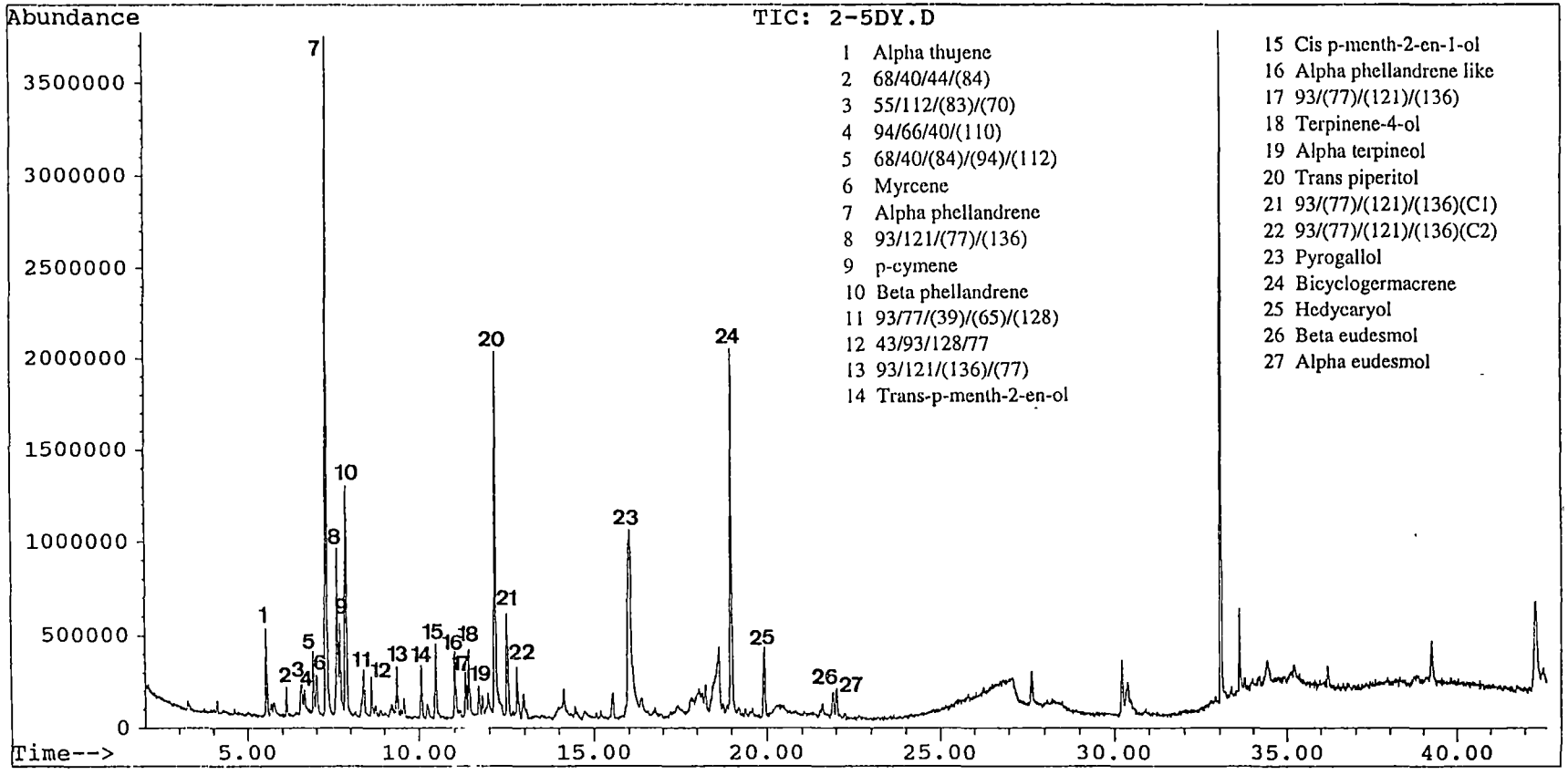

Figure A7. 13 GLC separation of leaf essential oil components for young *E. delegatensis* leaves. See the start of the appendix section for further details.

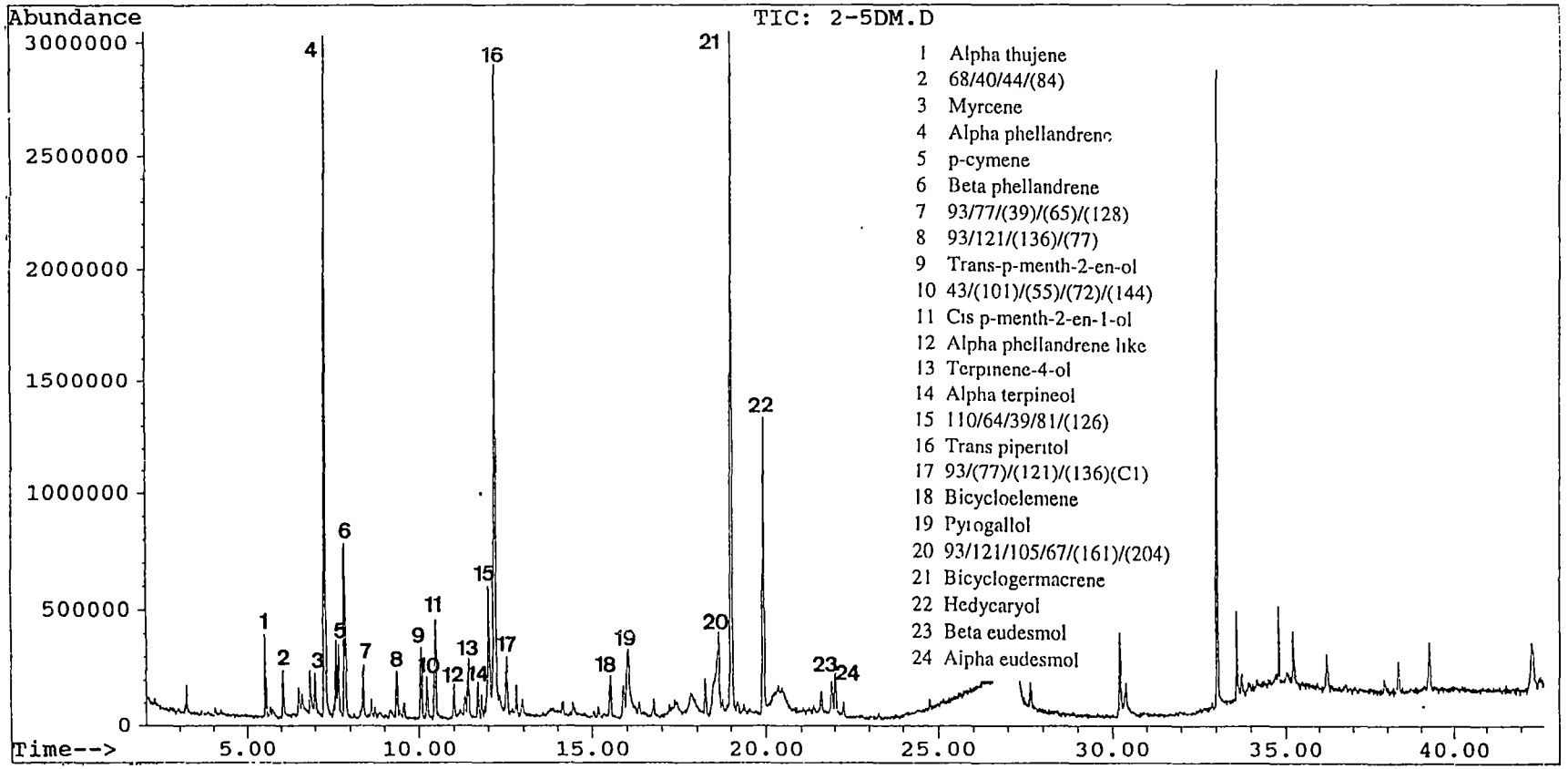

Figure A7. 14 GLC separation of leaf essential oil components for medium aged *E. delegatensis* leaves. See the start of the appendix section for further details.

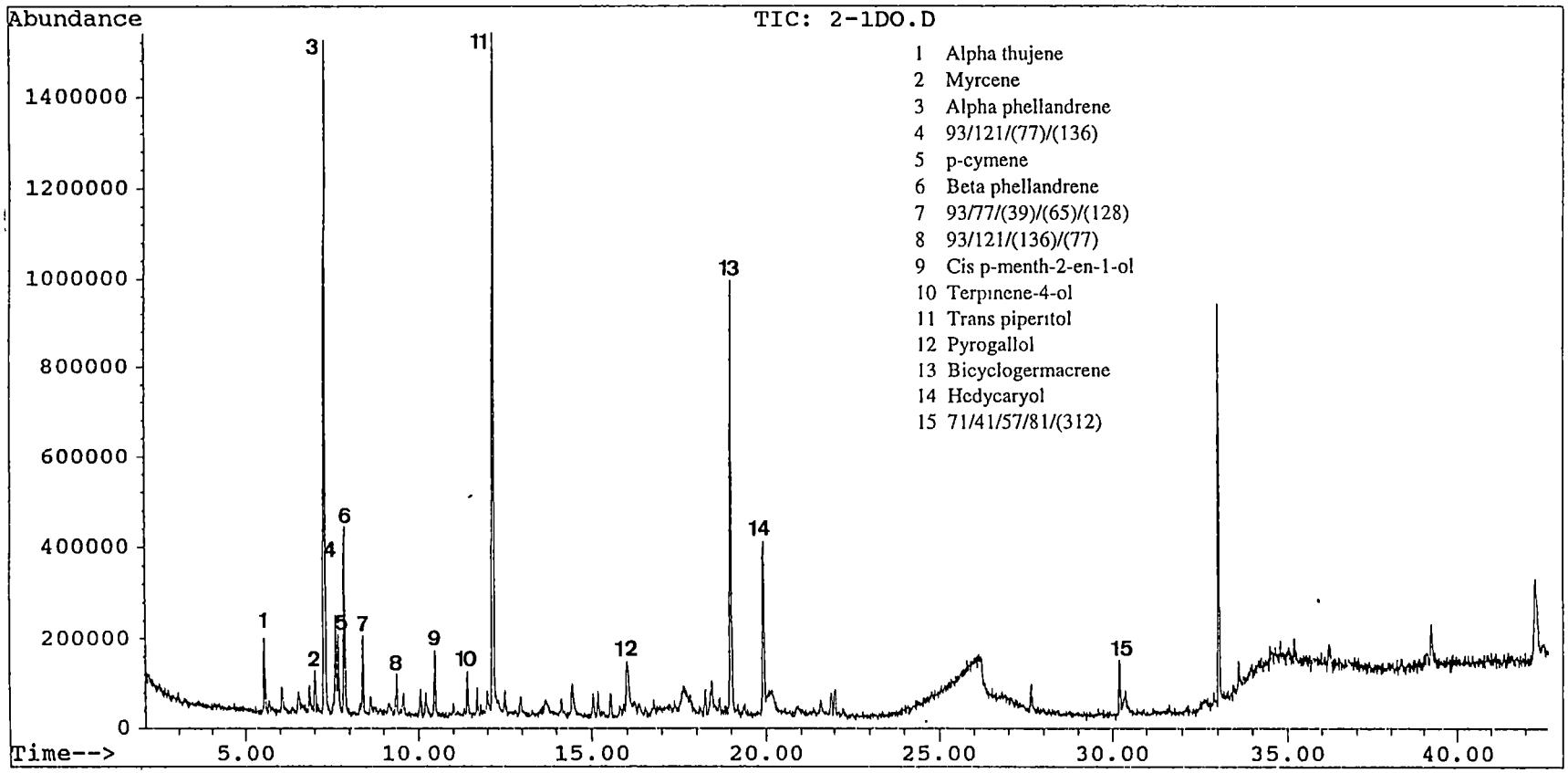

Figure A7. 15 GLC separation of leaf essential oil components for old aged *E. delegatensis* leaves. See the start of the appendix section for further details.

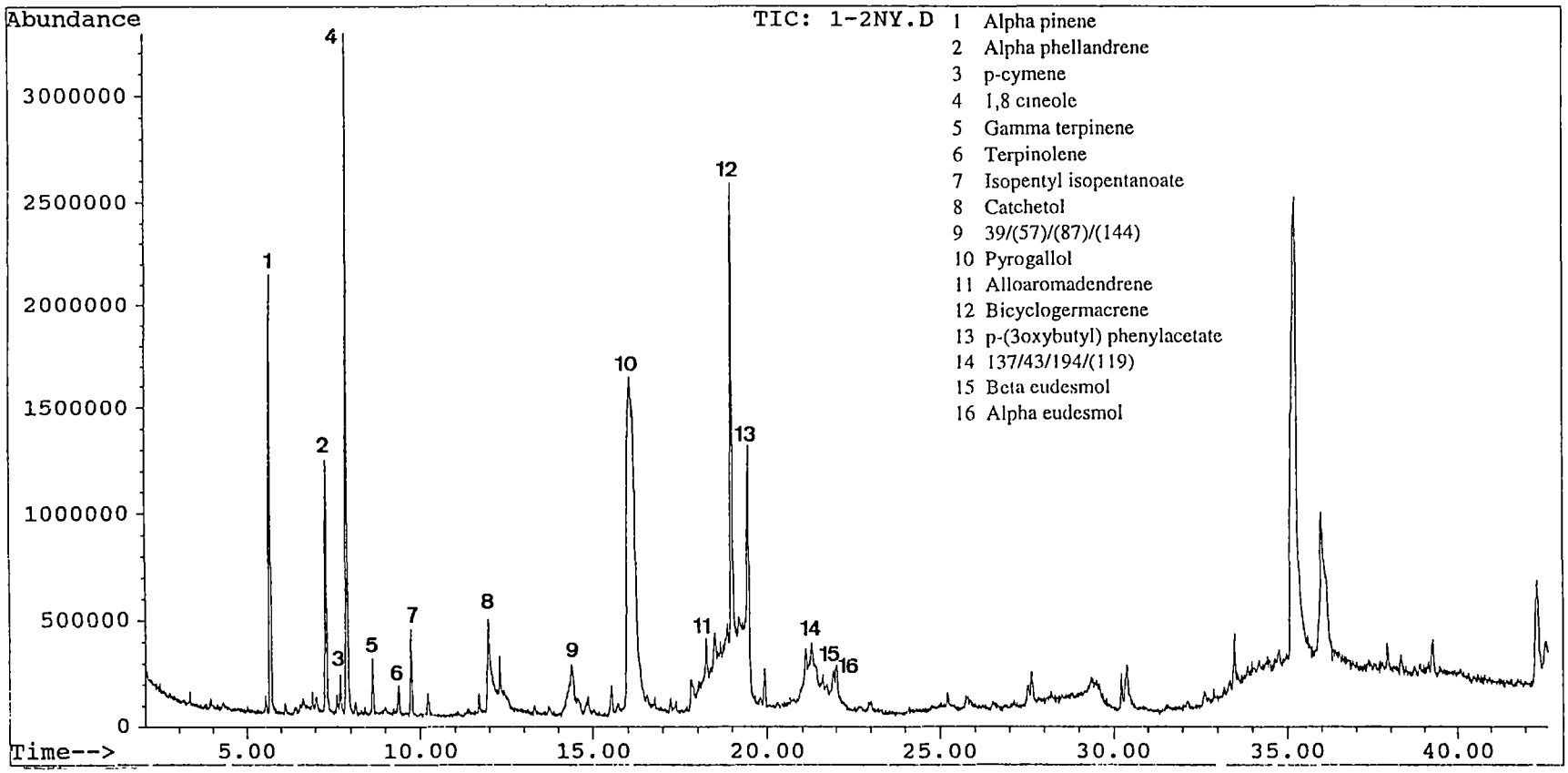

Figure A7. 16 GLC separation of leaf essential oil components for young *E. nitens* leaves. See the start of the appendix section for further details.

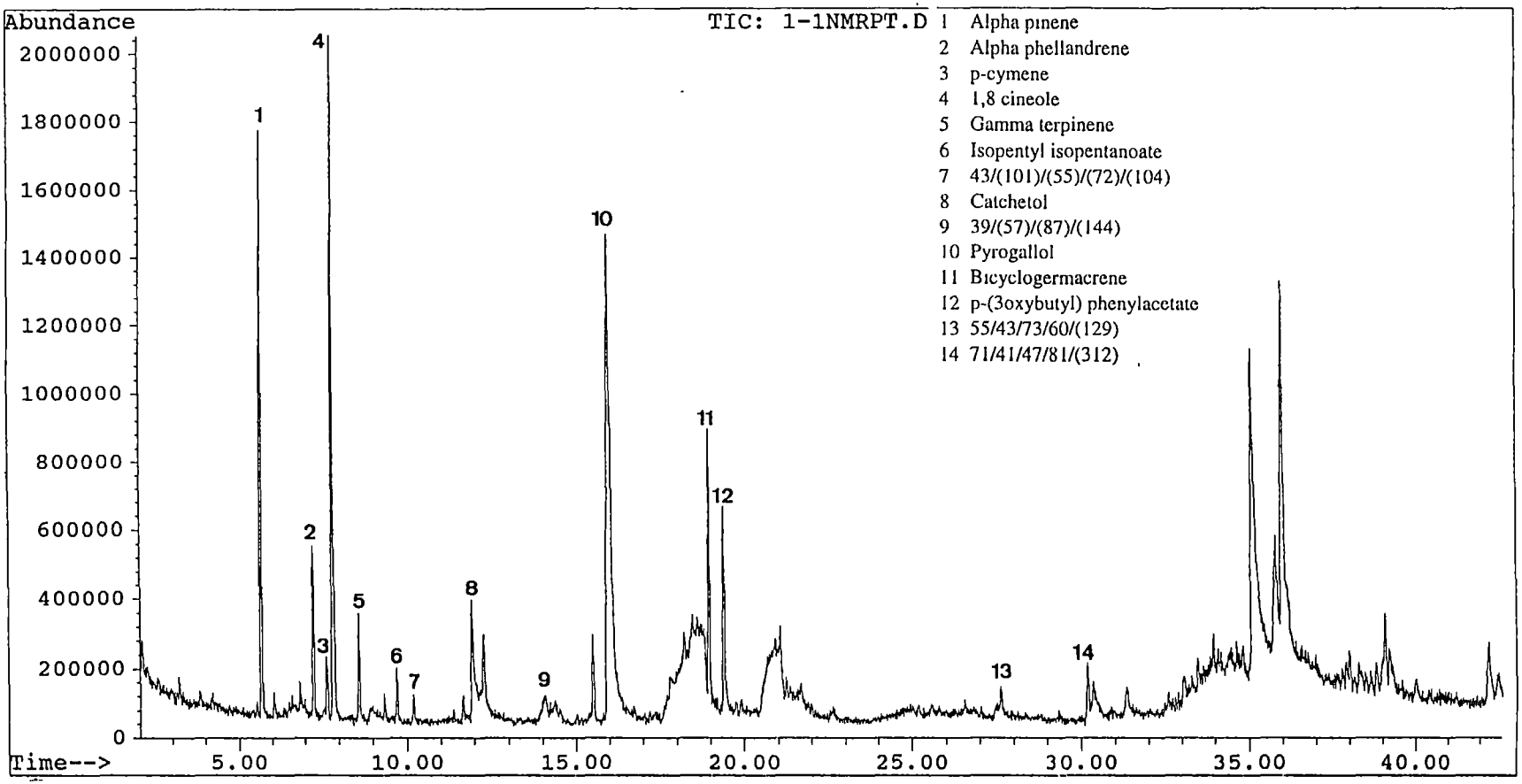

Figure A7. 17 GLC separation of leaf essential oil components for medium aged *E. nitens* leaves. See the start of the appendix section for further details.

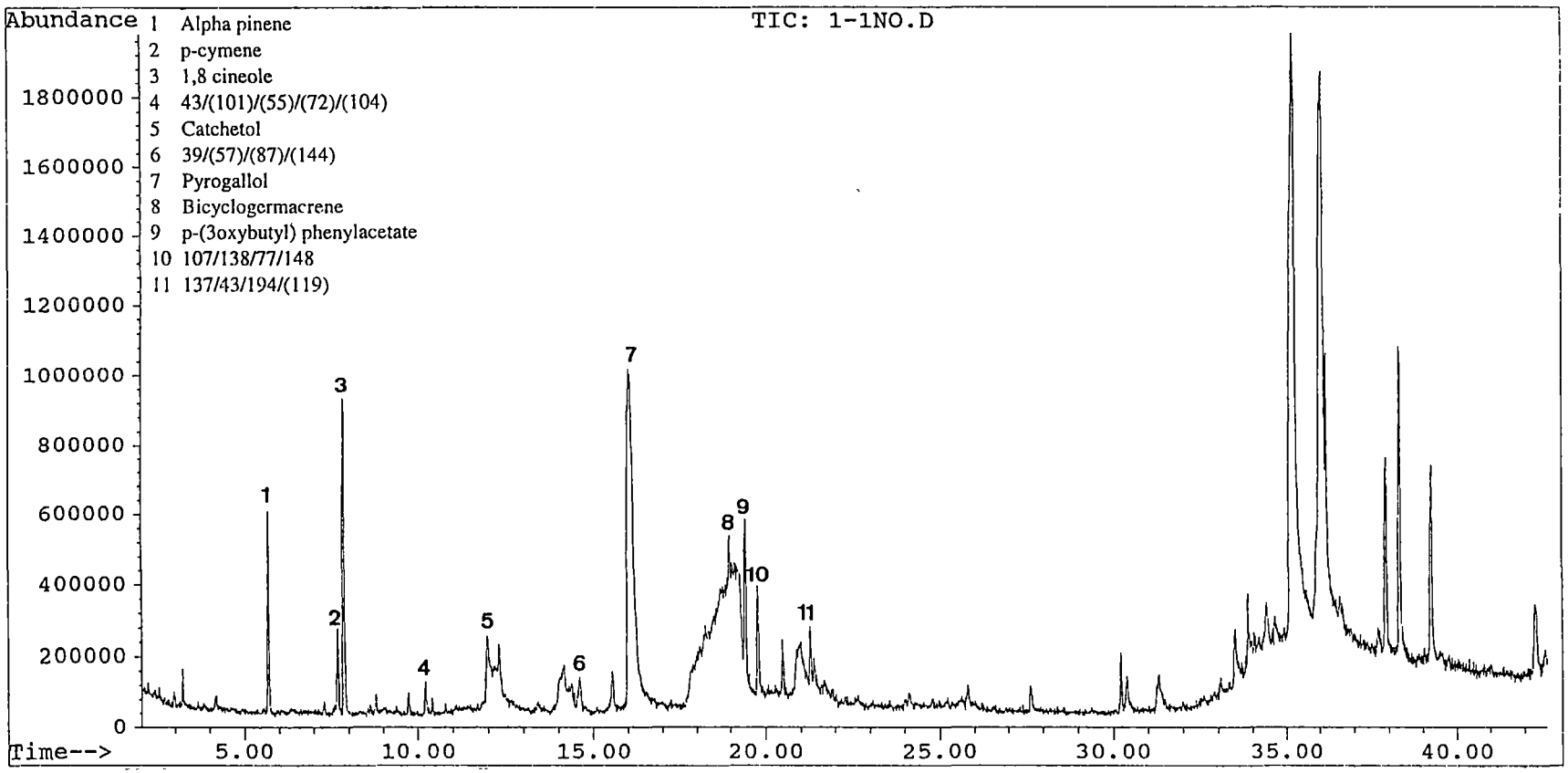

Figure A7. 18 GLC separation of leaf essential oil components for old aged *E. nitens* leaves. See the start of the appendix section for further details.

Appendix 8

The figures in the following appendix show the mass spectra (detected ions) of common triterpene compounds along with typical spectra of major compound classes found in the leaf wax of *E. regnans*, *E. delegatensis* and *E. nitens* leaves. Figures A8.1 - A8.17 show the mass spectra of triterpenes found in the leaf wax of *E. regnans* leaves, Figure A8.18 the spectra of triterpene (D3) from the wax of *E. delegatensis* leaves and Figures A8.19-A8.32 the spectra of triterpenes found in the wax of *E. nitens* leaves. The mass spectra of eucalyptin and desmethyl eucalyptin are shown in figures A8.33 and A8.34 while the spectra of major compound classes found in the leaf wax of the eucalypts are shown in figures A8.35-A8.41.

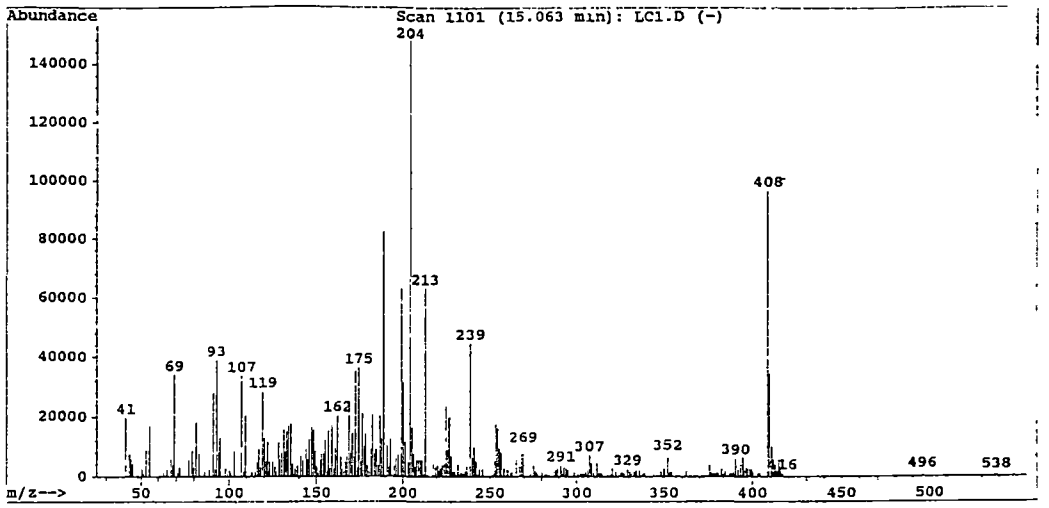

Figure A8.1 Mass spectra of triterpene (1) from the wax of *E. regnans* leaves.

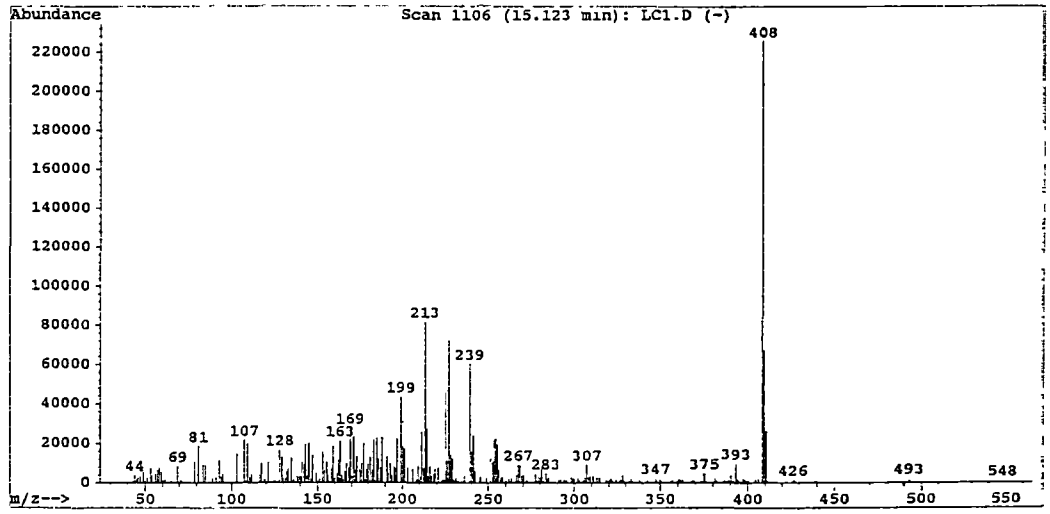

Figure A8.2 Mass spectra of triterpene (2) from the wax of *E. regnans* leaves.

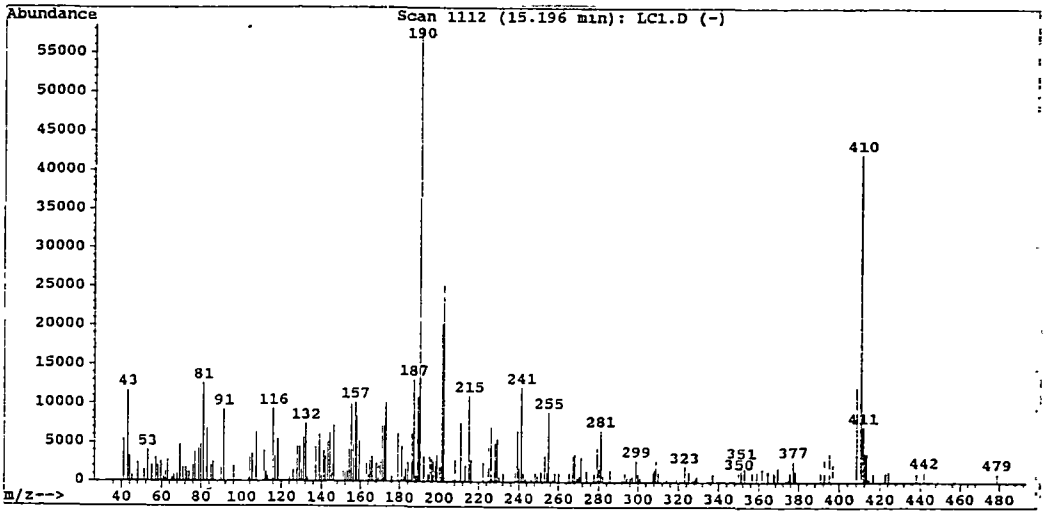

Figure A8.3 Mass spectra of triterpene (3) from the wax of *E. regnans* leaves.

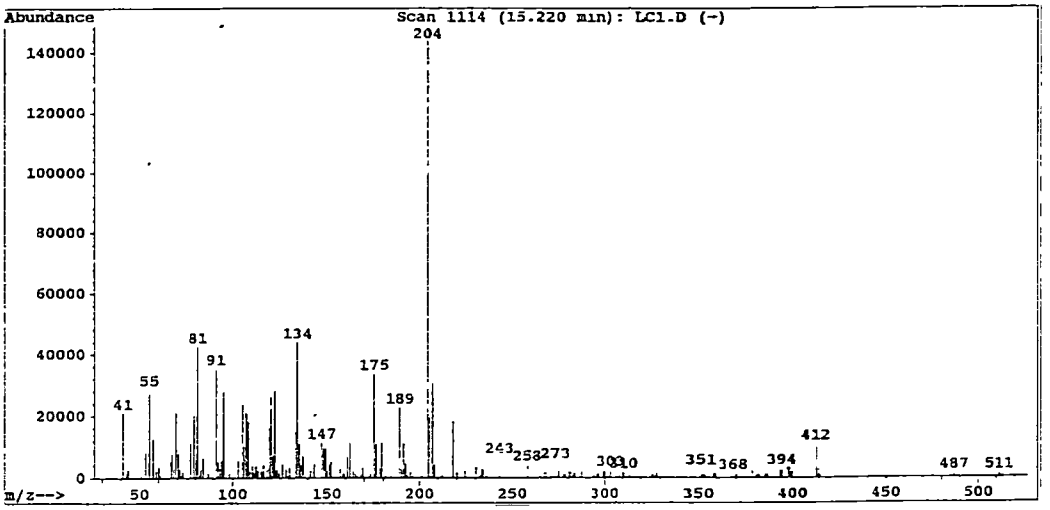

Figure A8.4 Mass spectra of triterpene (4) from the wax of *E. regnans* leaves.

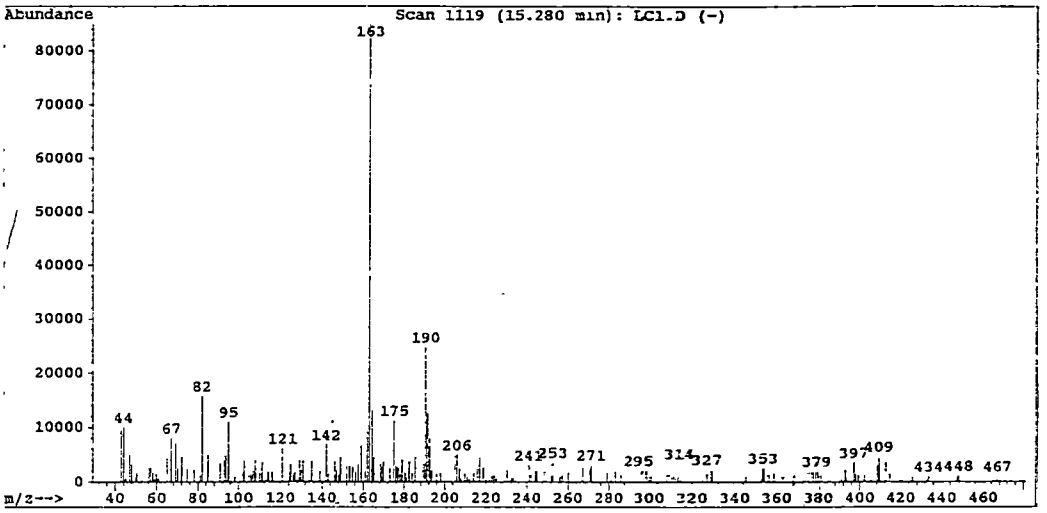

Figure A8.5 Mass spectra of triterpene (5) from the wax of *E. regnans* leaves.

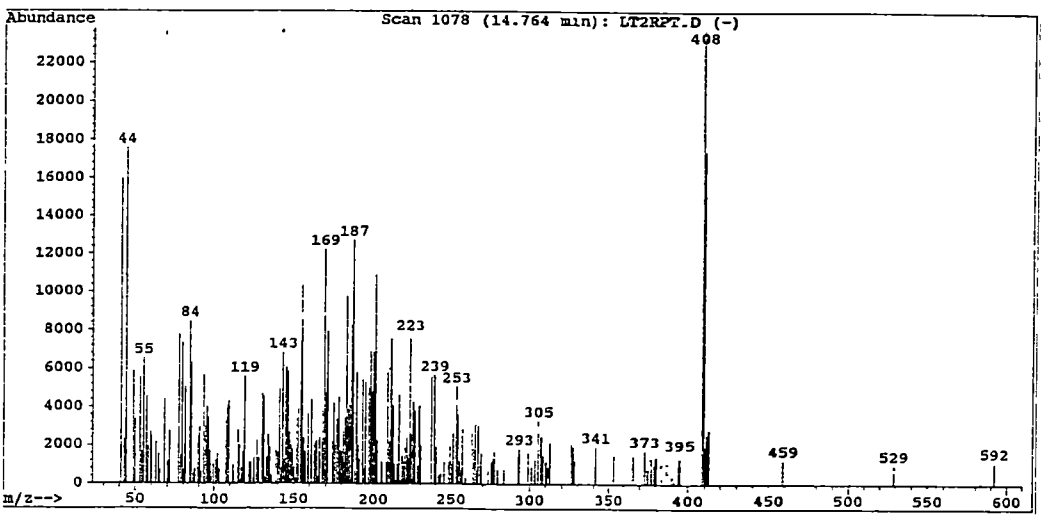

Figure A8.6 Mass spectra of triterpene (6) from the wax of *E. regnans* leaves.

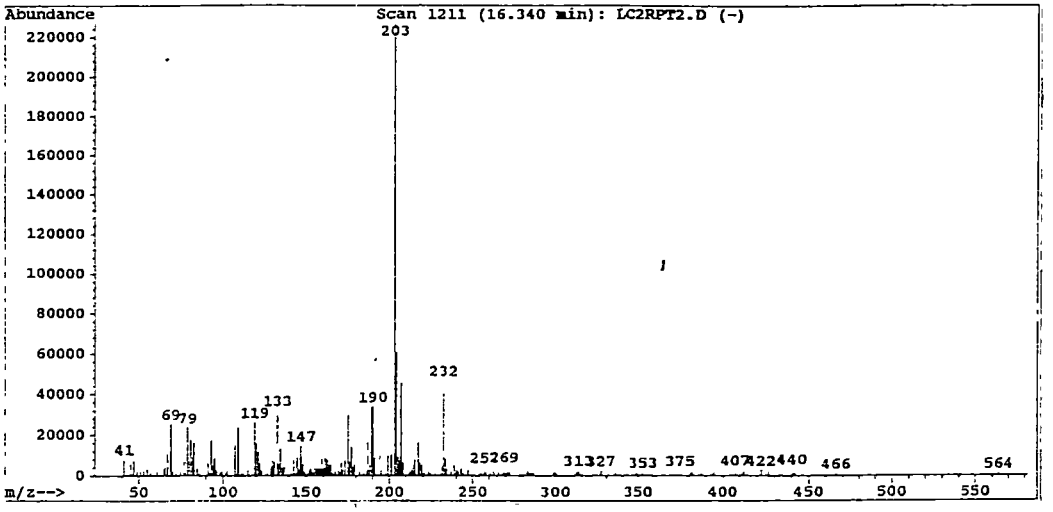

Figure A8.7 Mass spectra of triterpene (7) from the wax of *E. regnans* leaves.

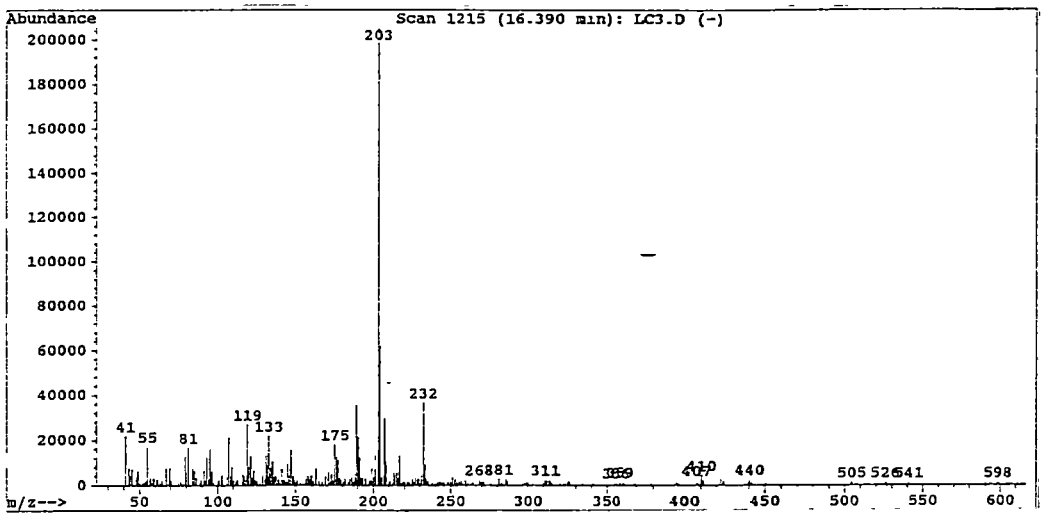

Figure A8.8 Mass spectra of triterpene (8) from the wax of *E. regnans* leaves.

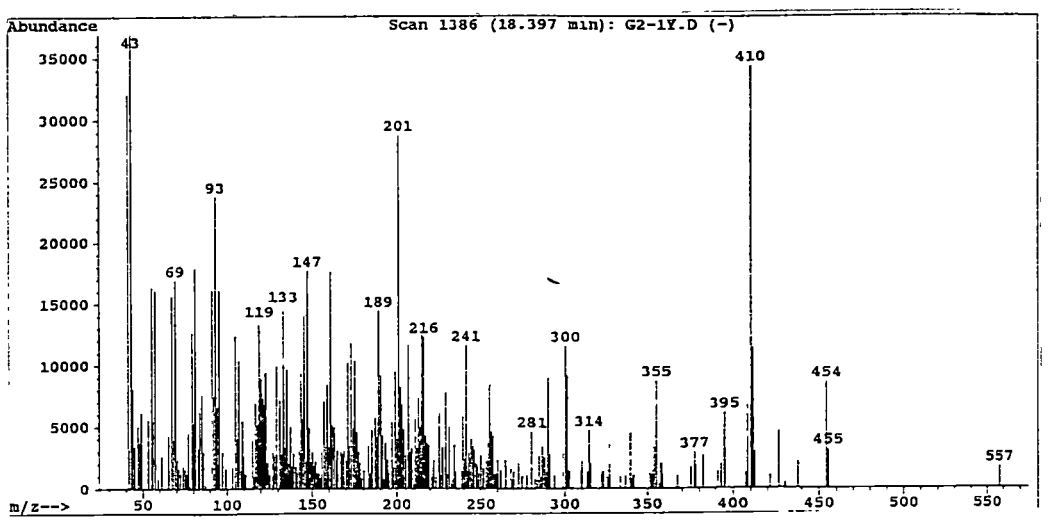

Figure A8.9 Mass spectra of triterpene (9) from the wax of *E. regnans* leaves.

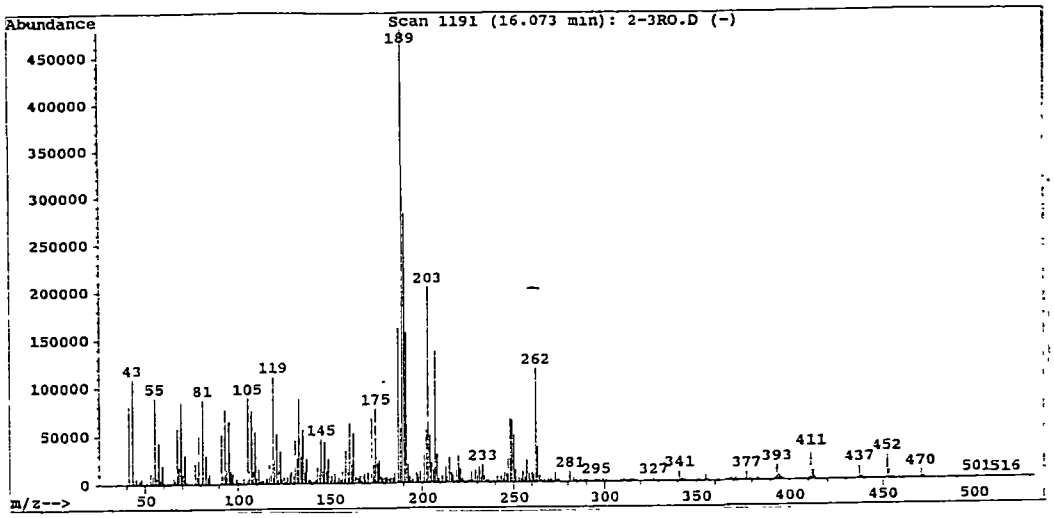

Figure A8.10 Mass spectra of triterpene (10) from the wax of *E. regnans* leaves.

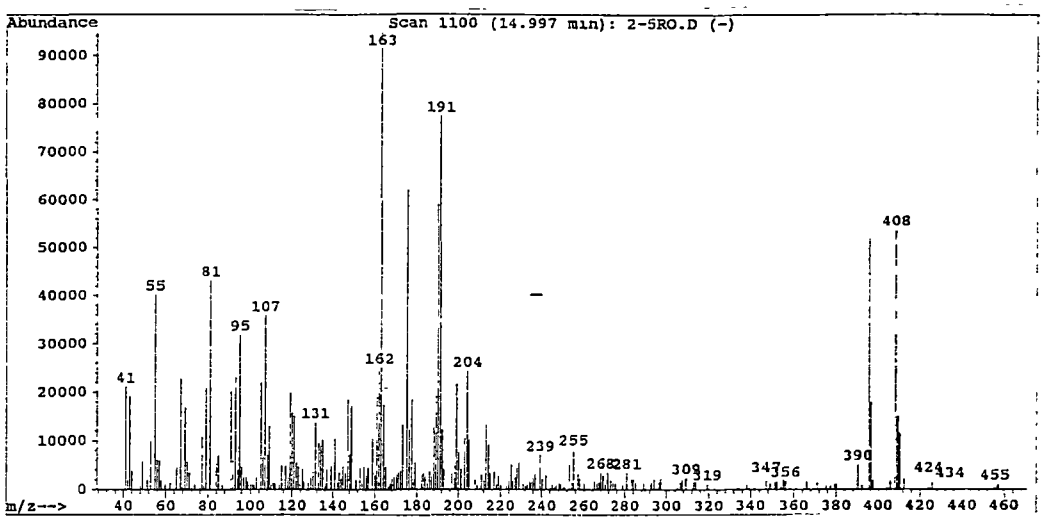

Figure A8.11 Mass spectra of triterpene (11) from the wax of *E. regnans* leaves.

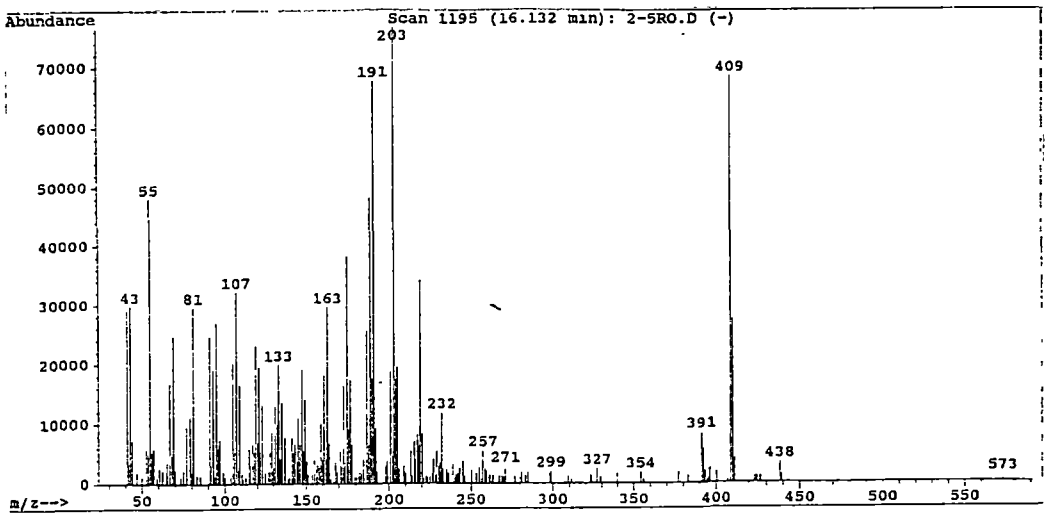

Figure A8.12 Mass spectra of triterpene (12) from the wax of *E. regnans* leaves.

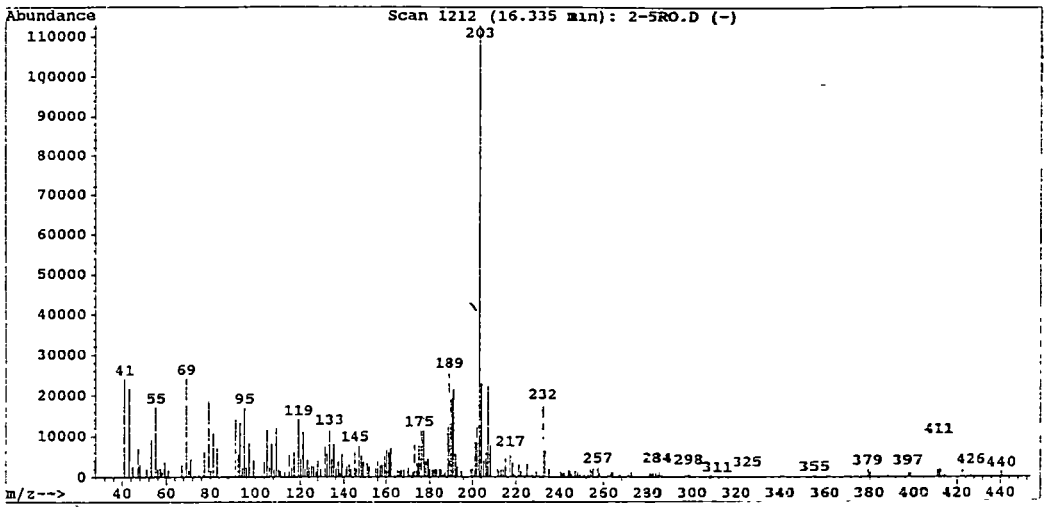

Figure A8.13 Mass spectra of triterpene (13) from the wax of *E. regnans* leaves.

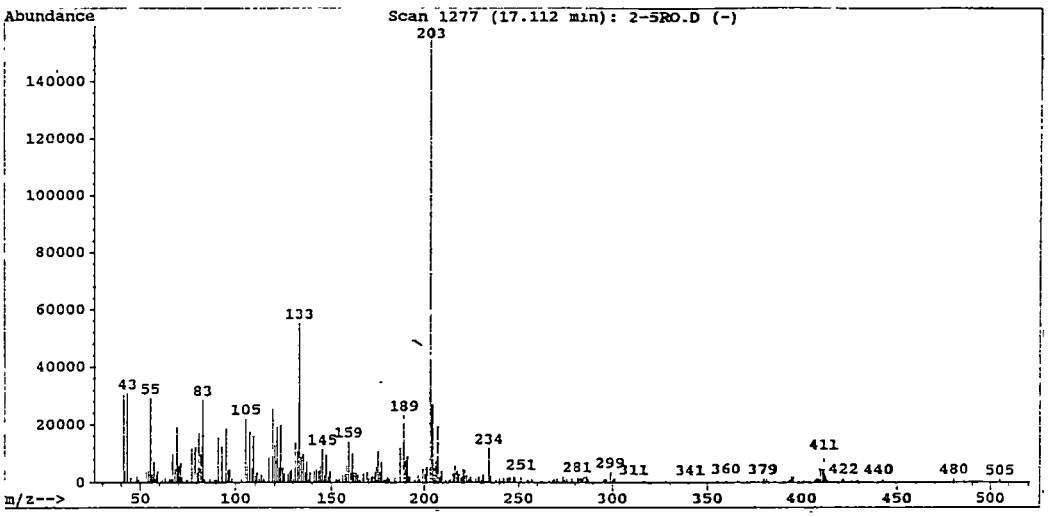

Figure A8.14 Mass spectra of triterpene (14) from the wax of *E. regnans* leaves.

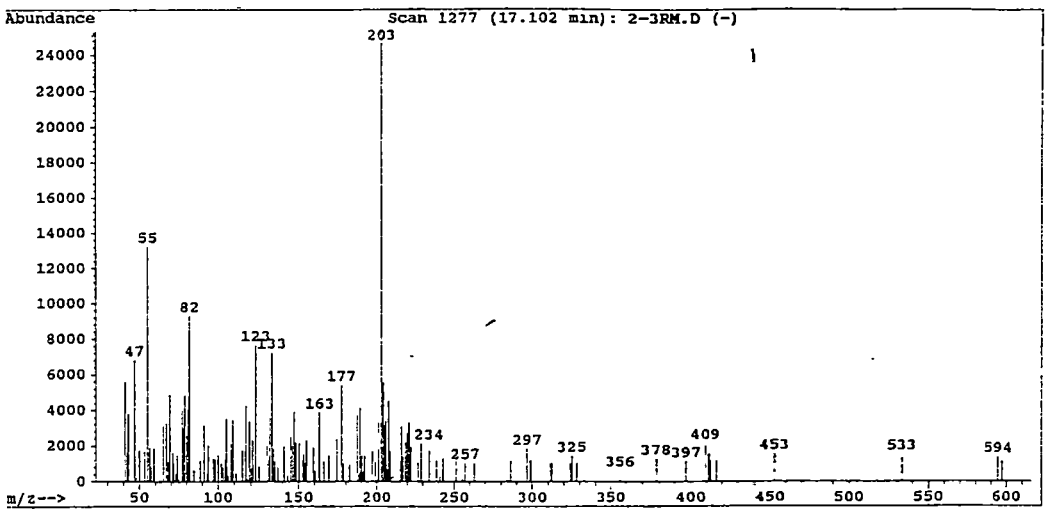

Figure A8.15 Mass spectra of triterpene (15) from the wax of *E. regnans* leaves.

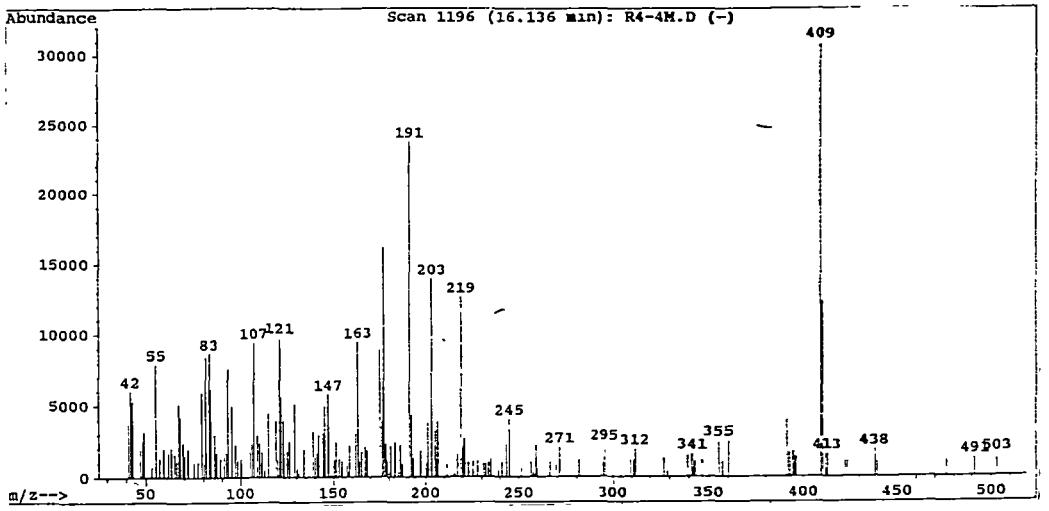

Figure A8.16 Mass spectra of triterpene (21) from the wax of *E. regnans* leaves.

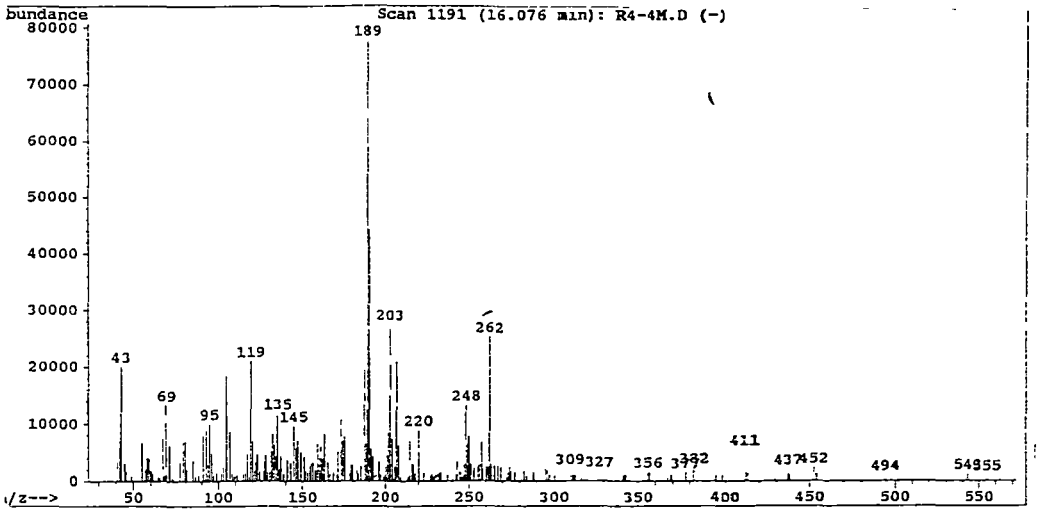

Figure A8.17 Mass spectra of triterpene (22) from the wax of *E. regnans* leaves.

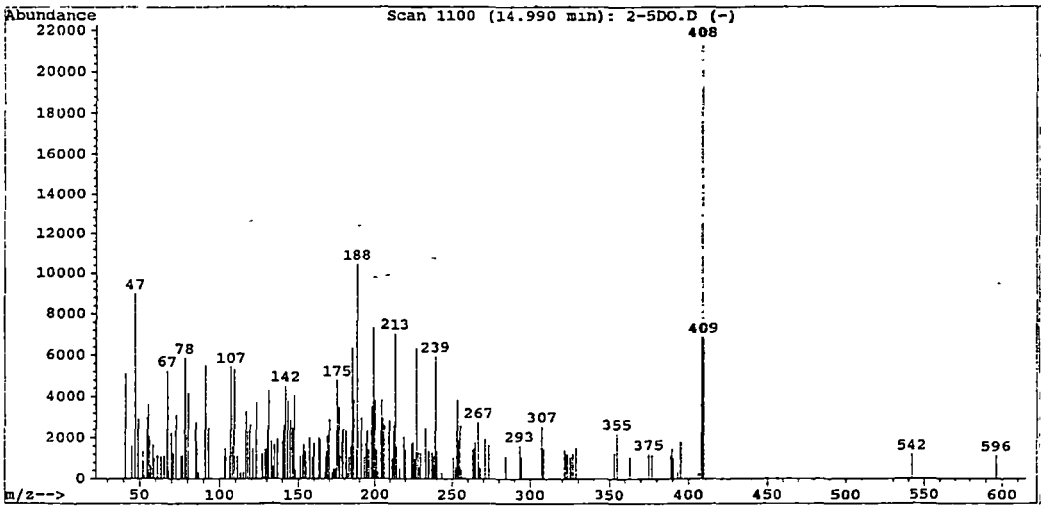

Figure A8.18 Mass spectra of triterpene (D3) from the wax of *E. delegatensis* leaves.

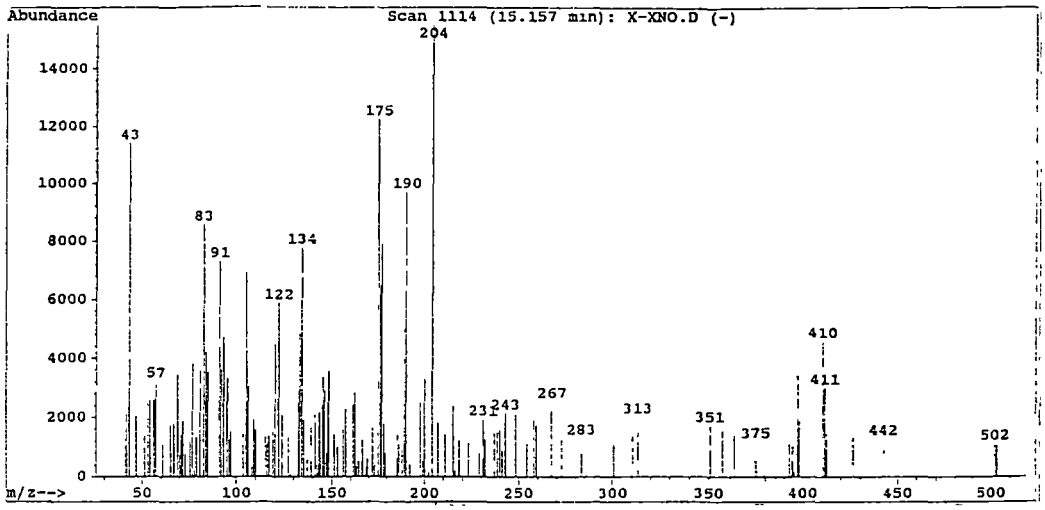

Figure A8.19 Mass spectra of triterpene (N1) from the wax of *E. nitens* leaves.

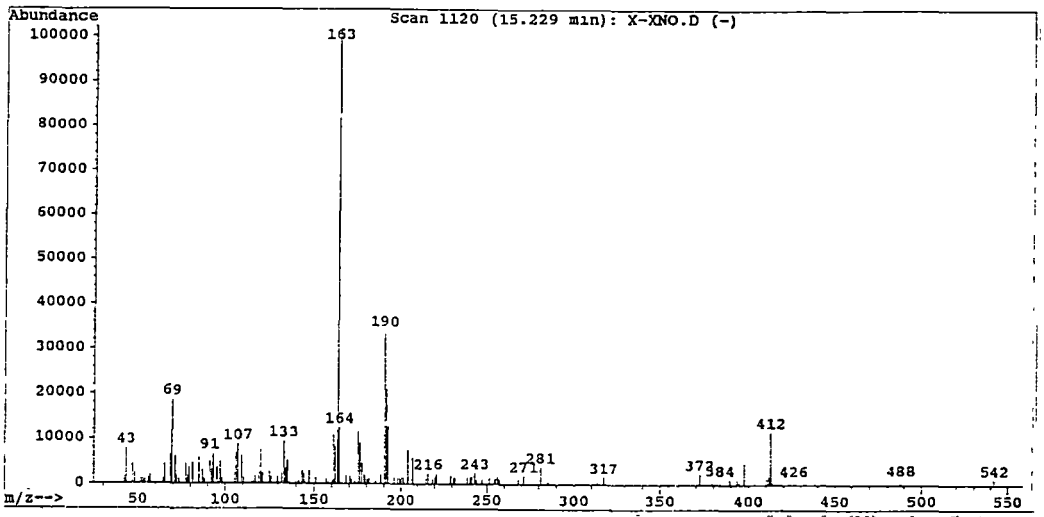

Figure A8.20 Mass spectra of triterpene (N2) from the wax of *E. nitens* leaves.

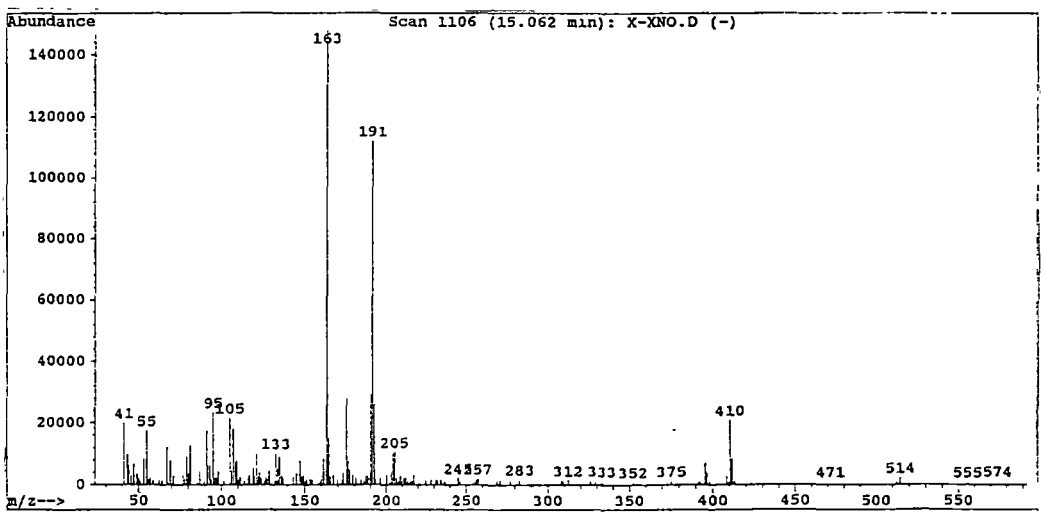

Figure A8.21 Mass spectra of triterpene (N3) from the wax of *E. nitens* leaves.

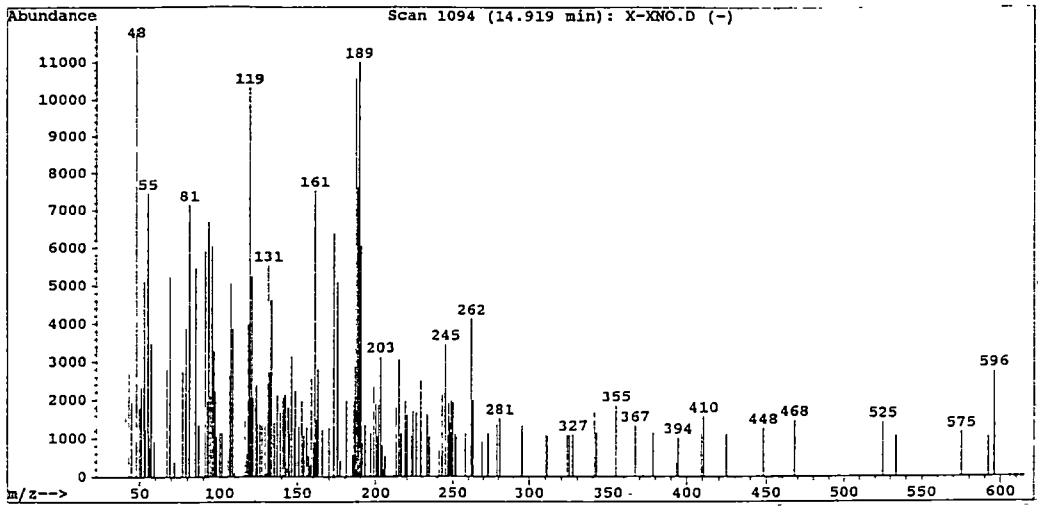

Figure A8.22 Mass spectra of triterpene (N4) from the wax of *E. nitens* leaves.

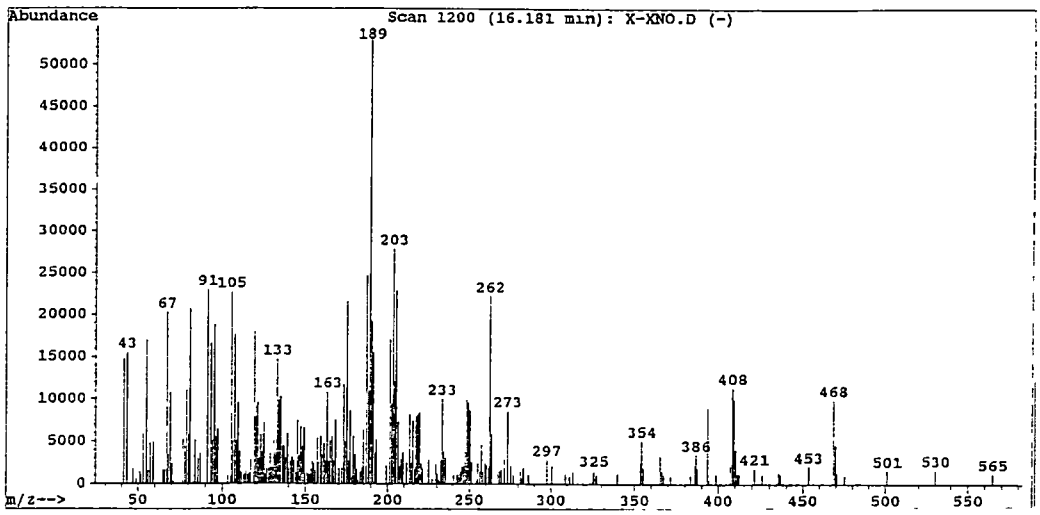

Figure A8.23 Mass spectra of triterpene (N5) from the wax of *E. nitens* leaves.

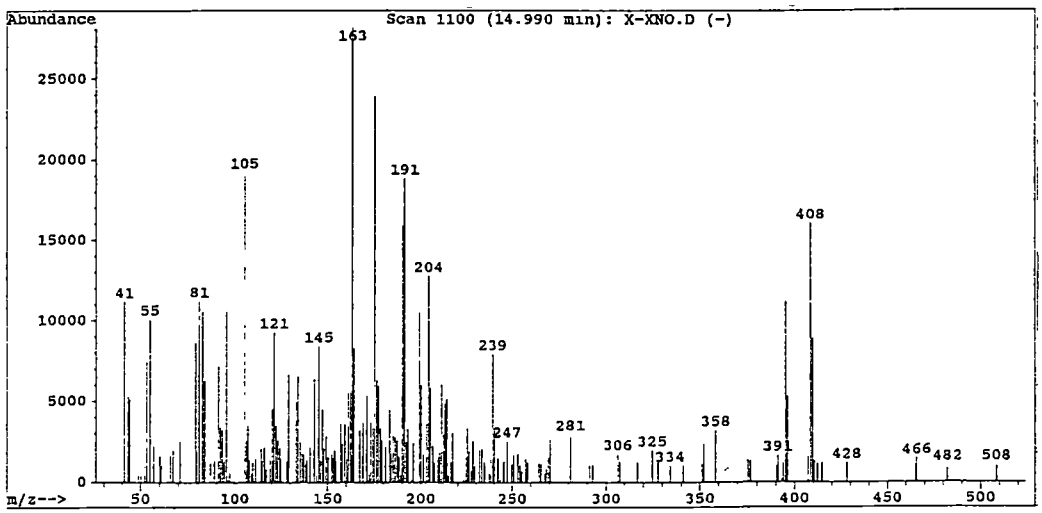

Figure A8.24 Mass spectra of triterpene (N6) from the wax of *E. nitens* leaves.

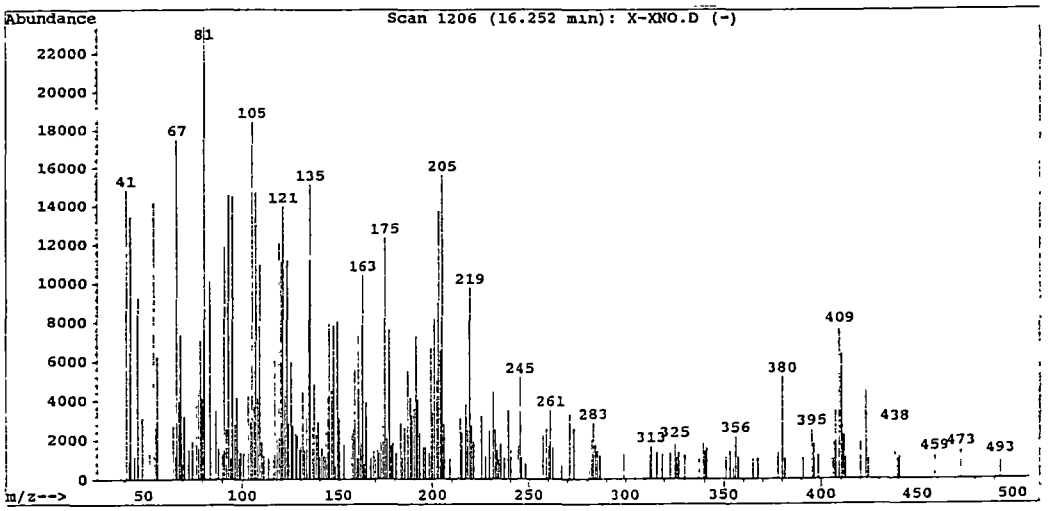

Figure A8.25 Mass spectra of triterpene (N7) from the wax of *E. nitens* leaves.

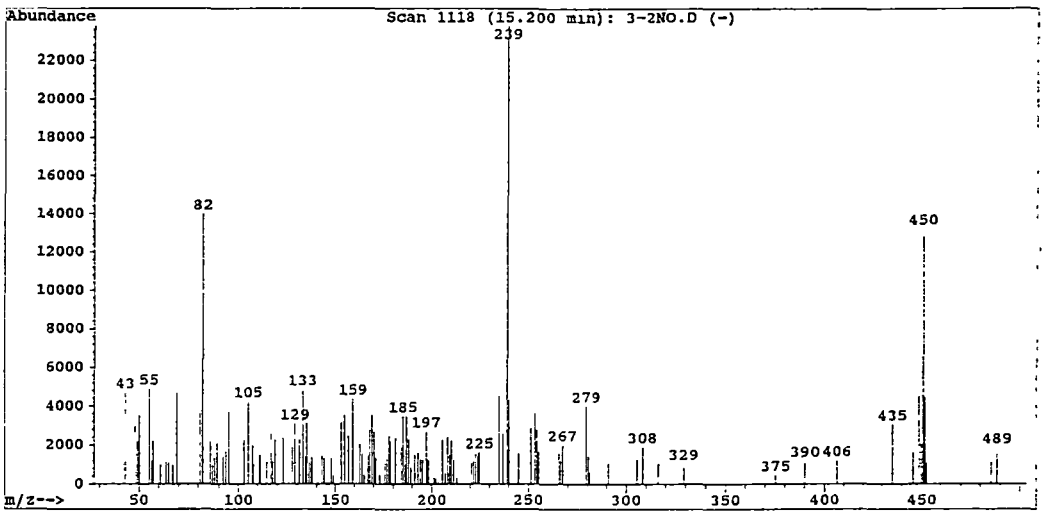

Figure A8.26 Mass spectra of triterpene (N8) from the wax of *E. nitens* leaves.

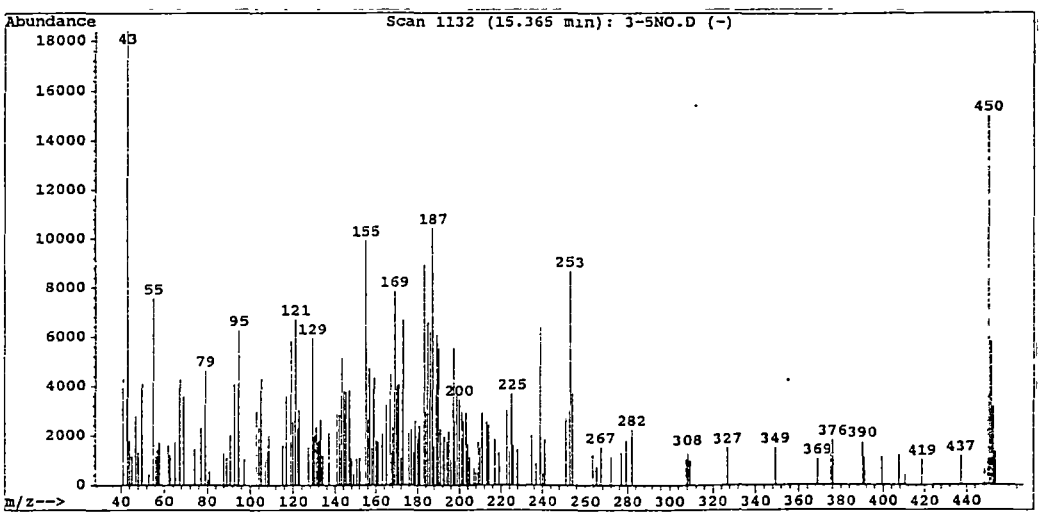

Figure A8.27 Mass spectra of triterpene (N9) from the wax of *E. nitens* leaves.

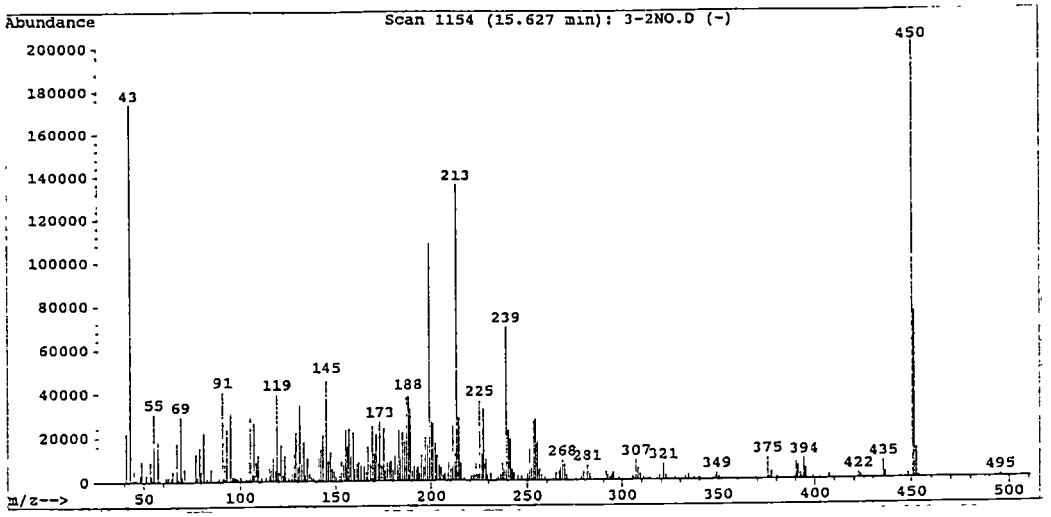

Figure A8.28 Mass spectra of triterpene (N10) from the wax of *E. nitens* leaves.

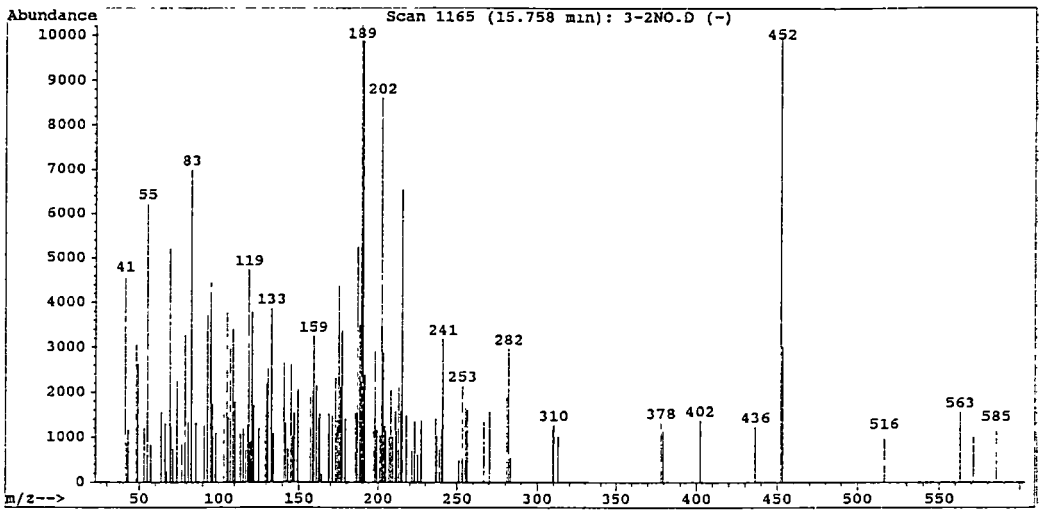

Figure A8.29 Mass spectra of triterpene (N11) from the wax of *E. nitens* leaves.

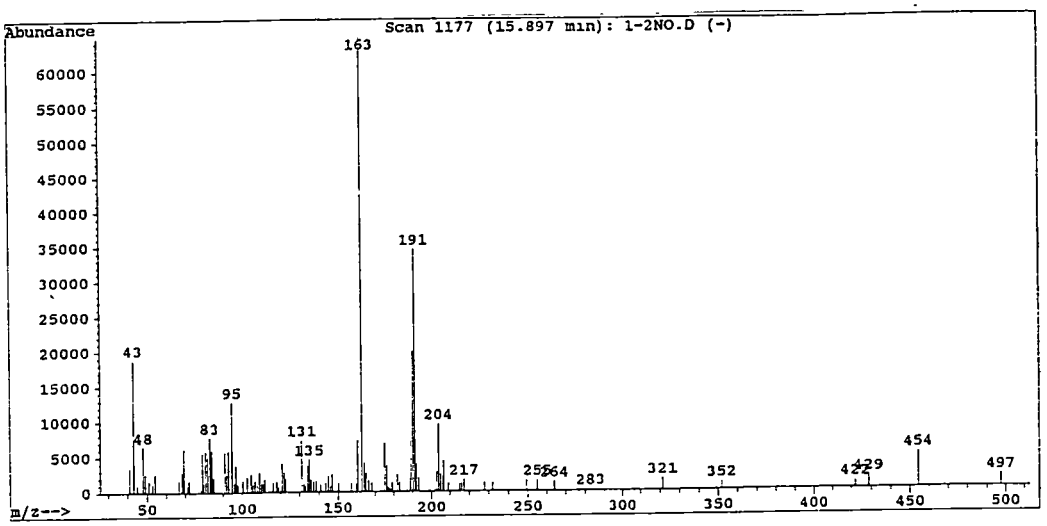

Figure A8.30 Mass spectra of triterpene (N12) from the wax of *E. nitens* leaves.

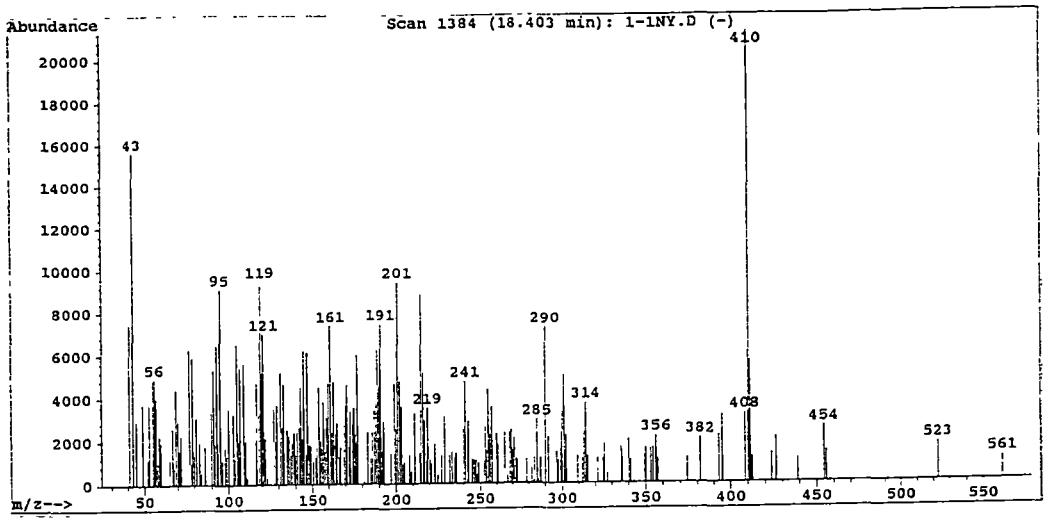

Figure A8.31 Mass spectra of triterpene (N13) from the wax of *E. nitens* leaves.

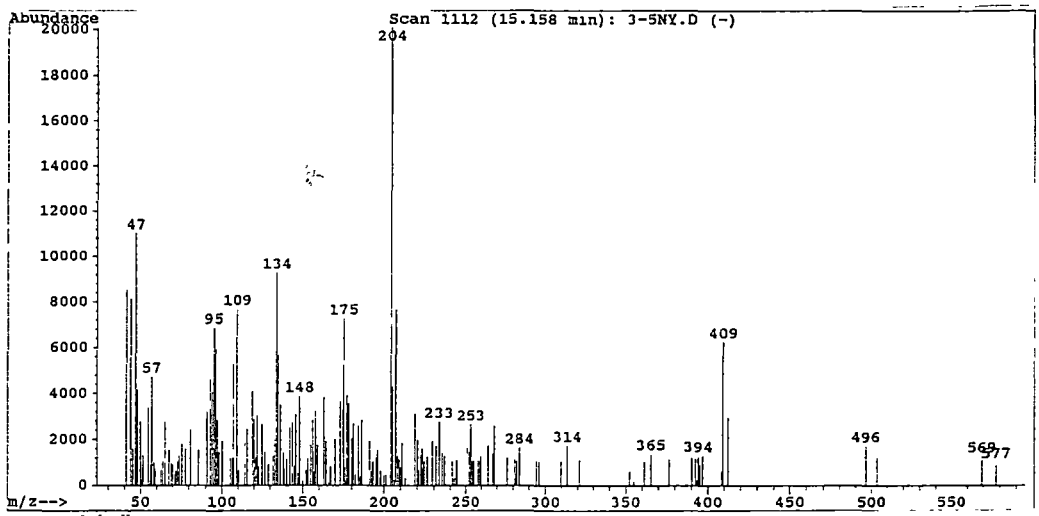

Figure A8.32 Mass spectra of triterpene (N14) from the wax of *E. nitens* leaves.

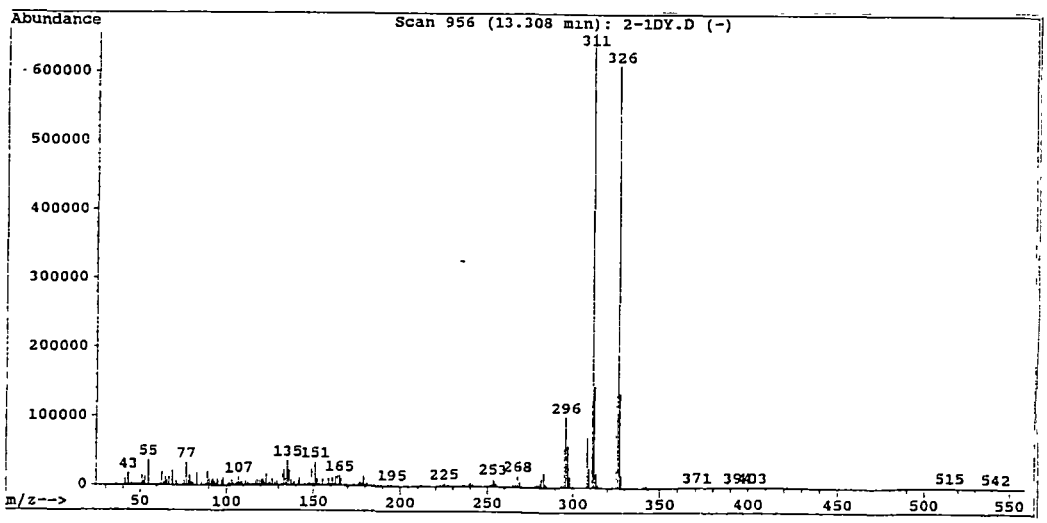

Figure A8.33 Mass spectra of eucalyptin.

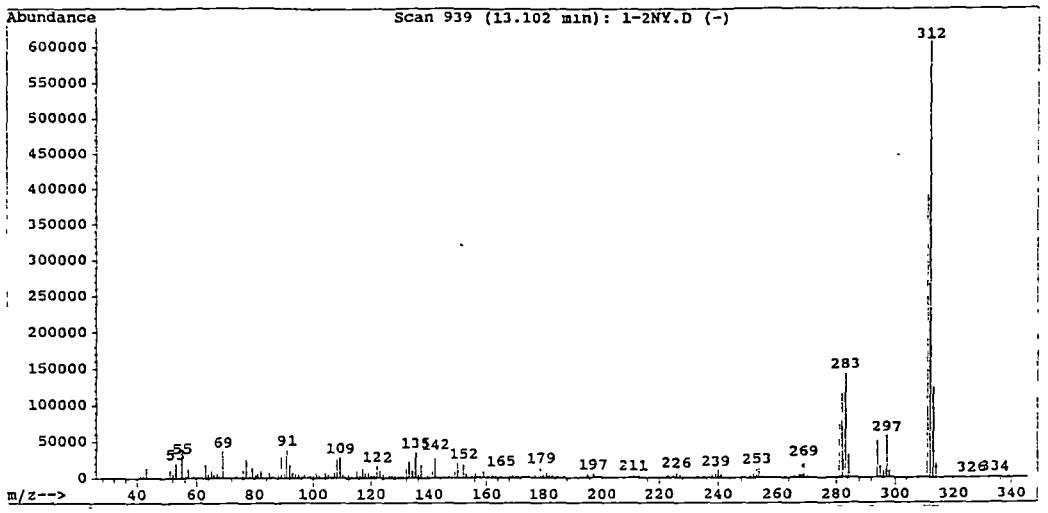

Figure A8.34 Mass spectra of desmethyl eucalyptin.

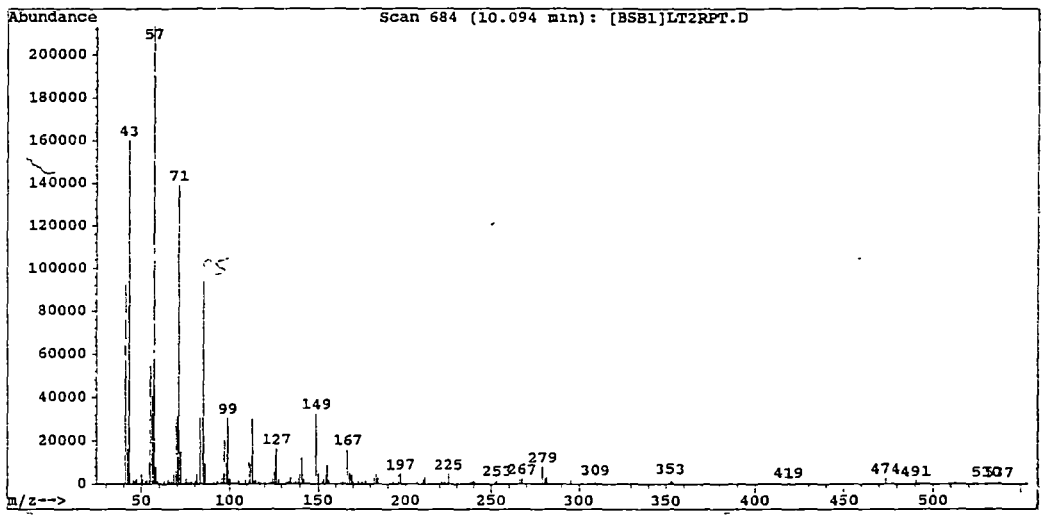

Figure A8.35 Typical mass spectra of an n-alkane type compound.

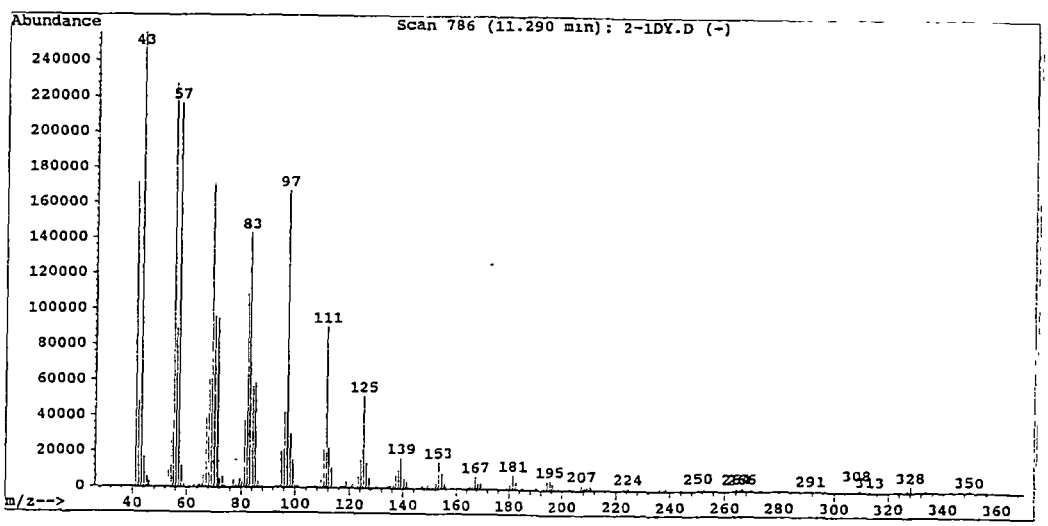

Figure A8.36 Typical mass spectra of an n-alkanol type compound.

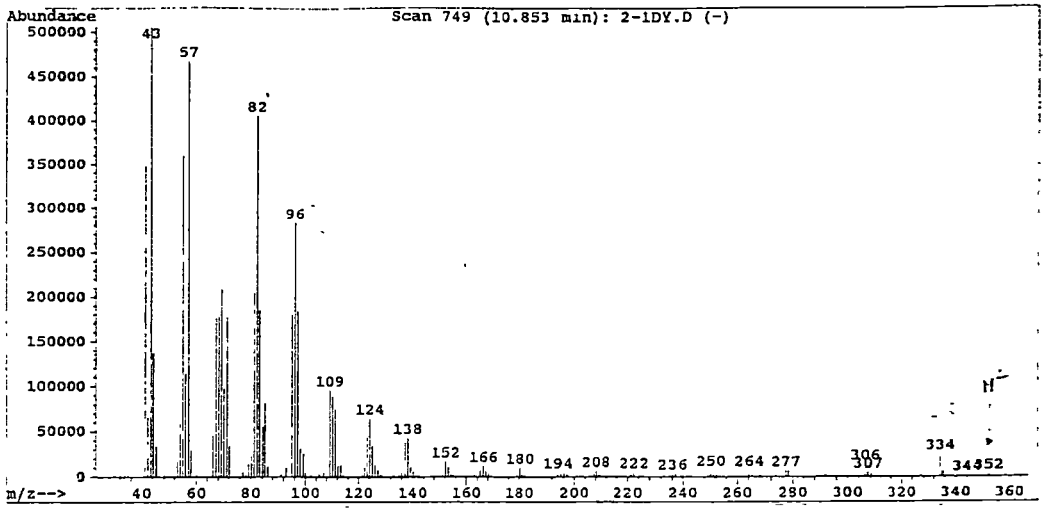

Figure A8.37 Typical mass spectra of an n-alkanal type compound.

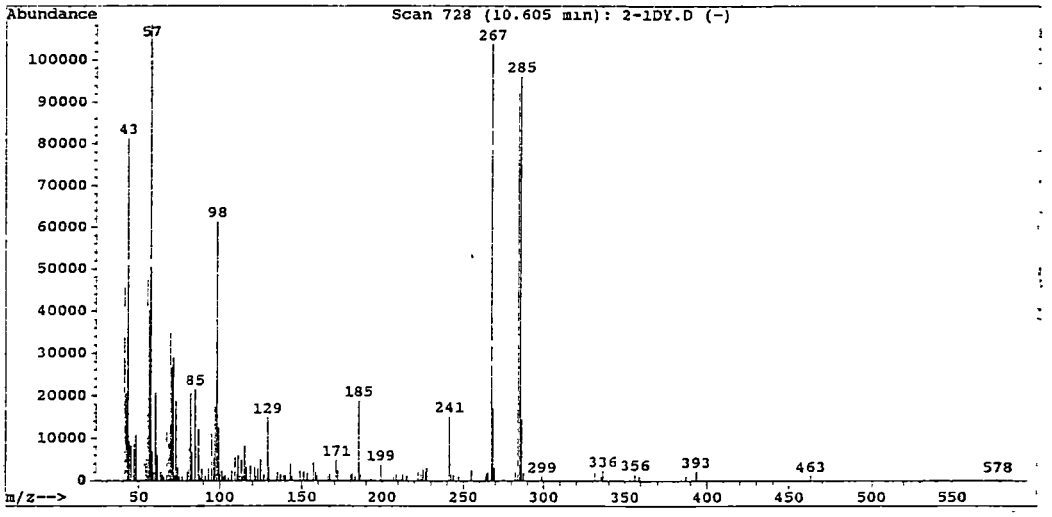

Figure A8.38 Typical mass spectra of an n-alkan-2-yl ester type compound.

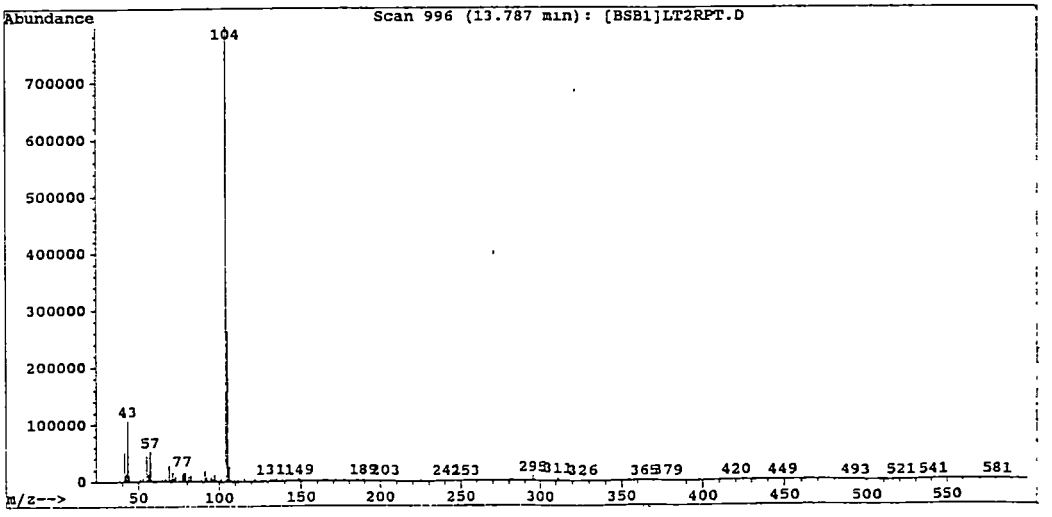

Figure A8.39 Typical mass spectra of a phenyl ethyl ester type compound.

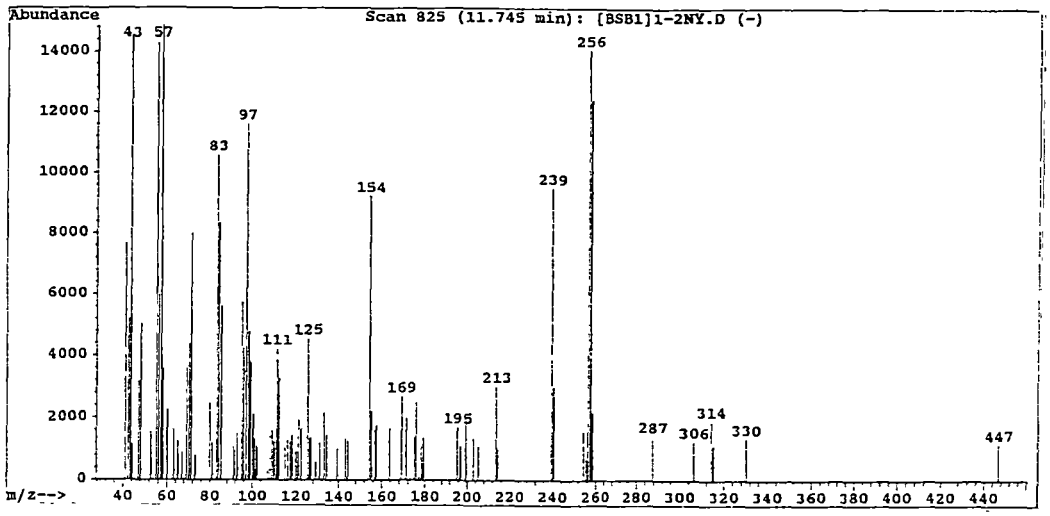

Figure A8.40 Typical mass spectra of a secondary alcohol ester type compound.

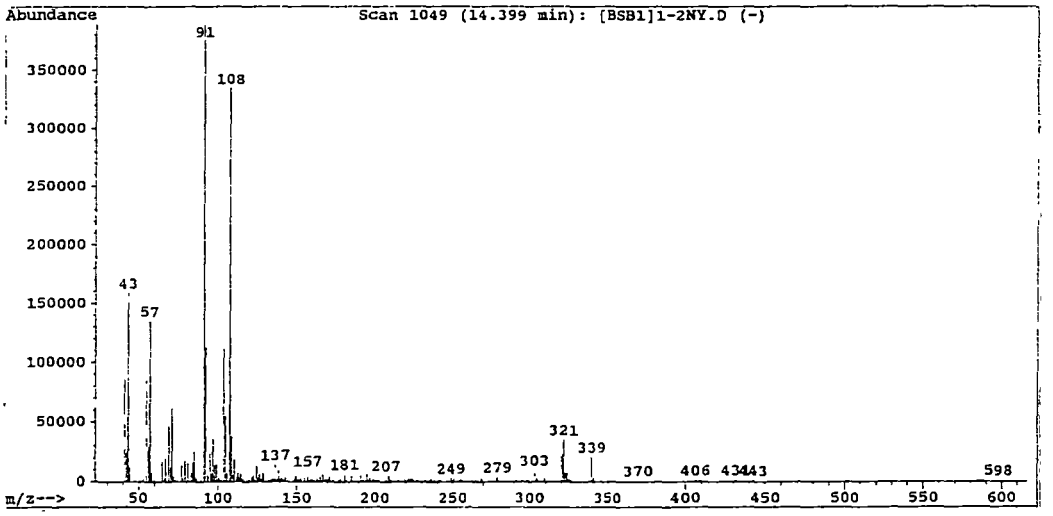

Figure A8.41 Typical mass spectra of a benzyl ester of fatty acids type compound.

Appendix 9

Chrysophtharta bimaculata third and fourth instar larval survival on previous season *Eucalyptus regnans* leaves.

Introduction

Chrysophtharta bimaculata larvae are known to prefer expanding leaves for consumption over fully mature leaves (Li 1993) and in the field, larval defoliation appears to be restricted to current season leaves (Authors pers. obs.). For the closely related *Paropsis atomaria*, larvae also develop best on expanding leaves with only the final instar capable of utilising, albeit poorly, fully mature *Eucalyptus blakelyi* leaves (Larsson & Ohmart 1988). This study examines whether *C. bimaculata* third and fourth instar are capable of utilising any of the fully developed previous season *Eucalyptus regnans* leaves.

Materials and Methods

Chrysophtharta bimaculata egg batches were haphazardly collected from ten trees in the Florentine Valley (42°39'S, 146°28'). Ten egg batches along with current season *E. regnans* foliage (collected from the Florentine Valley) were placed in each of ten 600 ml clear plastic containers (with a gauze covered hole in the lid for ventilation). In five containers, the larvae were reared through to their third instar while those in the remaining containers were reared through to fourth instar. New foliage was provided every second day during larval rearing up until the third instar stage. For those containers where larvae were reared through to fourth instar foliage was changed daily after larvae had developed into their third instar.

From *E. regnans* branches collected in the Florentine Valley, the leaf toughness of previous season leaves was measured (mean of three readings). The softer previous season leaves that ranged between 92.1 g (softest previous season leaf measured) and

93.5 g were removed from their shoots and for each, the petiole submerged in a vial (method used as described in 5.2.1). Five third instar larvae (one from each rearing container) were then transferred to the leaf. For larvae reared through to third instar, five larvae were removed. Ten replicates were conducted.

A similar method was used for fourth instar larvae except Tanglefoot® was not placed around the rim vial and each leaf and vial were placed into the same type of containers as used in rearing. This was to ensure larvae (that are prone to fall from leaves as prepupation approaches) were kept in close proximity to leaves if they were became detached. Leaf toughness again ranged from 92.1 to 93.5 g. Ten replicates were conducted.

The containers were then placed in a constant 25°C glasshouse and the third and fourth instar larvae were then monitored over the next four days to determine whether larvae utilised the leaves for feeding. The status of the larvae (dead, alive or pre-pupating) was then examined.

Results and Discussion

In all cases, third instar larvae died without feeding having commenced. However, there was evidence of small scallops (all less than 2mm wide) were larvae appeared to have made a feeding attempt. For the fourth instar larvae, five had reached the pre-pupation stage and all the rest had died. In all cases larvae had not fed upon the mature leaves although like the third-instar larvae, attempts had been made as observed through the presence of small scallops (again less than 2mm wide). Rather than utilising the fully mature foliage, those fourth instar larvae that had reached pre-pupation must have received enough food during the rearing phase. These results indicate that *C. bimaculata* larvae are unable to develop on previous season foliage, probably because it is too tough.