The response of dependent communities to ontogenetic and genetic change in *Eucalyptus*: The case of the *Eucalyptus globulus* x *nitens* hybrid system

By

Rachel Lawrence B.Sc.

Submitted as a part requirement for the degree of
Bachelor of Science with Honours

School of Plant Science, University of Tasmania
(November 1998)

Declaration

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

Rachel Lawrence, 13th of November 1998

Authority of access to copying

Rachel Lawrence

Abstract

The abundance of 44 dependent taxa (insects and fungal pathogens) was censused on three year old trees of *Eucalyptus globulus*, *E. nitens* and their hybrids (F_1, F_2 and backcrosses) in a field trial, half of which had undergone the transition to adult foliage (heteroblastic) while the remainder still retained juvenile foliage (homoblastic). This ontogenetic change was shown to have an enormous impact on the composition of dependent communities, far exceeding the effect of genetic differences between the pure species and their hybrids.

The dependent communities on the adult foliage was distinct from the community on the juvenile foliage, regardless of whether the juvenile foliage occurred in the upper or lower canopy. Significant differences in the communities was demonstrated on the upper and lower canopy of the homoblastic trees, clearly emphasising the importance of removing positional effects *per se* in studies of ontogenetic responses by dependent species.

Eighty percent of the common taxa exhibited significant specialisation for either juvenile or adult foliage. Complex ontogenetic specialisation was shown by the *Chrysophtharta agricola* leaf eating beetle a significant pest species of eucalypt plantations in Tasmania. The adult beetle fed on the adult foliage of the eucalypts but the eggs were laid and the larvae predominantly fed on the juvenile foliage. This preferential response for adult foliage by the adult beetle was maintained in laboratory feeding trials clearly indicating a response to changing foliage characteristics.

The dependent communities on *E. globulus* and *E. nitens*, the two most important plantation eucalypts in Tasmania, were shown to be significantly different on juvenile foliage but not adult foliage. Of the 20 common taxa studied 35% showed significant specialisation to one or other eucalypt species.

Most species generalists exhibit either no response to F_I hybrids or tend to be more abundant on the hybrid (hybrid susceptibility). In contrast species specialists showed

either dominant or additive responses to the F_1 hybrids. On average trees *of E. globulus* and *E. nitens* supported equal numbers of dependent taxa, but all hybrid classes supported significantly greater numbers of dependent taxa than the pure species. This study is one of the few to remove confounding positional and genetic effects when studying the response of dependent taxa to ontogenetic change and is the first study to show a significant species response to ontogenetic variation at the community level. This is important in establishing a genetic basis to the increased dependent taxa reported previously on hybrids as it has not confounded responses with differences in heteroblasty between pure species and hybrids hosts.

Acknowledgements

I would firstly like to thank my supervisors, Brad Potts and Tom Whitham for the help which I received from them and also for the endless enthusiasm which they both showed for the project.

I was fortunate during the project to have the help of a variety of people. Amongst these were: Randy Swaty, Dave James, Sarah Scott and Sandra Paffe, who aided in the collection of leaves, the carrying of ladders and the retention of my sanity during the year; Jane Elek, Dick Bashford and Tim Wardlaw from Forestry Tasmania who generously donated their time for the identification of dependent taxa and Dr. Peter Minchin who helped with the use and understanding of statistical packages during his visit to The University of Tasmania.

The help I received from Andrew MacDonald and Paul Tilyard in overcoming the eccentricities of computers was much appreciated. I am also grateful for the tolerance Paul, Andrew and others who utilise the eucalypt lab showed towards me during the past few weeks of the project, when the lab seemed to be continually cluttered with my belongings.

Richard Eccleston, Hamish Jackson and Kathryn Redburn generously gave up their time to help during the final stages of this project and for this I am extremely grateful.

I would especially like to thank John Lawrence for his quiet encouragement throughout this degree.

"The complexity of the organic world is enormous and naturally frustrates the inquiring mind. So enormous is the number of organisms, so infinite their many and varied relationships, so subtle and far-reaching are the influences of their constant activity and evolving natures, as to laughingly belittle those persons who, usually lacking an extensive familiarity with the phenomenon, think that they will manufacture simple models of the system to facilitate their understanding. Physicists badger the fundamental material of the universe in their attempts to understand the complexities of the interaction of some fifty "elementary particles", an enormous task. Would you care to try to understand the possible interactions and relationships to other living things of 300,000 species of beetles?" .....

"Not only is the natural world complex beyond imagination, it also eludes understanding (not, I hope, appreciation) because it is constantly changing; the ecologist enters this shifting matrix at his own risk. Some delineate small aspects of the ecological theatre; others carefully investigate small parts of the evolutionary play. No man can encompass the entire performance."

Daniel C. Kozlovsky,
"An Ecological and Evolutionary Ethic"

Table of Contents

Table of Contents

Chapter 1

Introduction to experimental thesis

The topic of plant-herbivore and higher trophic level interactions is a complex and poorly understood issue due to the numerous interactions which occur in one system and the necessity of applying diverse disciplines to the underlying processes. For example, it is known that plants are highly heterogenous habitats for arthropods at the species, population, and even at individual levels (Suomela and Ayres 1994). There are a variety of physical and chemical traits in plants which may affect the distribution of dependent species (disease organisms, parasites, herbivores – mammalian, insectivorous; Linhart, 1989). Physical traits include leaf toughness, thickness, trichome density and wax thickness and quality. Chemical traits include compounds such as sugars and amino acids, as well as plant secondary chemicals such as terpenes and glycosides (Linhart 1989). Some of these traits may act as a defense for the plant against herbivores, while others may serve as attractants (Abrahamson 1989). Indeed one theory for the existence of plant secondary chemicals is that they have evolved as a defense mechanism against herbivory (New 1988; Abrahamson 1989).

Many adaptive traits in forest trees are under quantitative genetic control (Potts and Wiltshire 1997) and evidence suggests that this may also be true with plant defensive traits. For example, Raymond (1995) found extensive variation between families of *Eucalyptus regnans* and *E. nitens* in the amounts of herbivory by *Chrysoptharta bimaculata* and Farrow *et al.* (1994) found resistance to Autumn Gum Moth (*Mnesempala privata*) varied considerably between provenances of *E. globulus*. It is important to note that the nature of seed dispersal in eucalypts and in other forest trees can result in a patchy distribution of those genotypes which may differ in resistance traits (Linhart 1989). It is hypothesized that the distributions of dependent species will

reflect this patchy distribution of forest tree genotypes (Linhart 1989). Furthermore, it is known that dependent species can respond to variation in the host quality and resistance traits within a tree (Kearsley and Whitham 1989; Beckmann 1991; Suomela and Ayres 1994). Within tree variation can be due to physiological aging (Fontanier and Jonkers 1976), somatic mutations (Edwards *et al.* 1990), previous herbivory history (Haukioja 1980; Keane *et al.* 1981; Beckmann 1991), and the ontogenetic process (Bryant *et al.* 1985, Kearsley and Whitham 1989,1998; Waltz and Whitham 1997). Kearsly and Whitham (1989) suggest that variation in host quality both within and between trees is of great importance in ecological processes because of the heterogeneity in habitats it provides for dependent species.

Many studies of the distribution of dependent species have focussed on the effects of physiological aging within a tree (Koslowski 1971, Crawley 1985), but little attention has been given to the effects of the ontogenetic process. A review of the effects of this ontogenetic process in forest trees on the composition of dependent communities will be given here with reference to the response of dependent species to the process. Firstly, a general review of the relevant literature defining and describing ontogenetic changes in plants will be given, this review will then focus on the genus *Eucalyptus*, looking at specific traits and the possible ecological and adaptive significance of these ontogenetic changes within the genus. Secondly, an overview is given of the responses of individual dependent species to these changes, both from studies outside the genus *Eucalyptus*, and then specifically within the genus *Eucalyptus*. The examples presented mostly concentrate on the response of individual dependent species, as literature examining responses at the community level are scarce.

Ontogenetic changes and heteroblasty in plants

A significant source of within tree variation is ontogenetic change. During ontogeny a plant moves through different phases of development from seed to seedling through the various vegetative states to senescence (Zagory and Libby 1985). Ontogenetic change refers to changes in developmental state which occur through the life-cycle of a plant.

The changes are generally irreversible, programmed changes in gene expression that occur in the meristem which are stable to asexual propagation (Kozlowski 1971; Waltz and Whitham 1997; but see below). Within the genus *Eucalyptus* it has been shown that ontogenetic changes are genetically based (Wiltshire *et al.* 1998; Potts and Dukowski in press). It is important to note that ontogenetic changes are strictly distinct from changes which occur as a result of physiological aging (Waltz and Whitham 1997) - such as senescence, where a plant is unable to support all or some of its parts results from physiological aging.

During ontogenetic development the majority of plants pass through at least two major leaf phases. These two phases are the juvenile vegetative phase and the adult vegetative phase (Koslowski 1971; Poethig 1990). The juvenile phase of shoot development begins when the shoot meristem begins to initiate a stem, true leaves and axillary buds. This phase may last from a few days – such as in most herbaceous plants, to many years - as in eucalypts and other woody plants; and is followed by the transition to adult phase.

There are often large morphological, anatomical, physiological and chemical differences between the juvenile and adult vegetative phases (Johnson 1926; Wiltshire *et al.* 1998). Morphological characters such as changes in leaf size and shape and orientation (Johnson 1926), plant architecture (Barber and Jackson 1957), phyllotaxy (Schaffalitzky de Muckadell 1954) and thorniness (Koslowski 1971). Physiological changes may also occur which include differences in rooting ability (Zagory and Libby 1985; Paton 1991), photosynthetic efficiency (Beadle *et al.* 1989) and stomatal conductance (Beadle *et al.*1989; Poethig 1990; Donovan and Ehrlinger 1991). Differences in chemistry between the juvenile and adult phases have been tentatively shown by Li *et al.* (1997) and Edwards *et al.* (1993). Differences in disease susceptibility (Dungey *et al.* 1997) and insect resistance (de Little 1989; Farrow *et al.* 1994) have been noted in the two phases in *Eucalyptus.*

This change in leaf types along the shoot of a plant is termed "heteroblasty" (Pryor 1976; Poethig 1990) or "heterophylly" (Bell and Williams 1997), and is widespread among vascular plant species throughout the world.

Although such ontogenetic variation in juvenile and adult vegetative characteristics is common, in different taxonomic groupings, there is much variation in the degree of contrast between the two phases. In addition the transition from juvenile to adult foliage can be gradual, as in the *Populus* system (Waltz and Whitham 1998) or rapid as in the eucalypts (Cameron 1970; Pryor 1976), *Quercus* and *Robinia* (Koslowski 1971;Waltz and Whitham 1998), although Poethig (1990) suggests that the more usual condition is for a gradual change.

The case of *Eucalyptus*

Within the genus *Eucalyptus* there are many species which exhibit heteroblasty at some point in their lifecycle (Pryor 1976; Ohmart and Edwards 1991). Brooker and Kleinig (1990) consider that there are four leaf phases in the genus: the seedling; the juvenile; intermediate and adult leaf phases. The juvenile and adult phases are the two dominant phases and there are often striking morphological differences between them.

Eucalyptus globulus is possibly one of the most markedly heteroblastic species in the genus (Pryor 1976). The transition from juvenile to adult state (leaf transition) of *E. globulus* is shown in Figure 1.

Juvenile leaves in *E. globulus* are sessile, ovate, opposite and stem clasping, held transverse to the stem, are bluish green and glaucous and the stems are square and flanged in cross section (Brooker and Kleinig 1990). In contrast, the adult leaves are petiolate, lanceolate to narrow lanceolate, alternate, pendulous, approximately 30 x 3cm in size, glossy and green and normally non-glaucous (Brooker and Kleinig 1990). The anatomy and physiology of the leaves varies also with the adult leaves being isobilateral, with evenly distributed mesophyll and approximately equal numbers of stomata on both surfaces, whereas the juvenile leaves have more mesophyll and fewer stomata on the adaxial surface (Johnson 1926). *E. nitens* also exhibits this very marked heteroblasty and *E. nitens* leaves have a very similar morphology to the *E. globulus* leaves. It is important to note that the heteroblastic condition in eucalypts is mostly

only present in the early years of the trees life-cycle (there are exceptions eg. *E. risdonii*; Wiltshire *et al.* 1998).

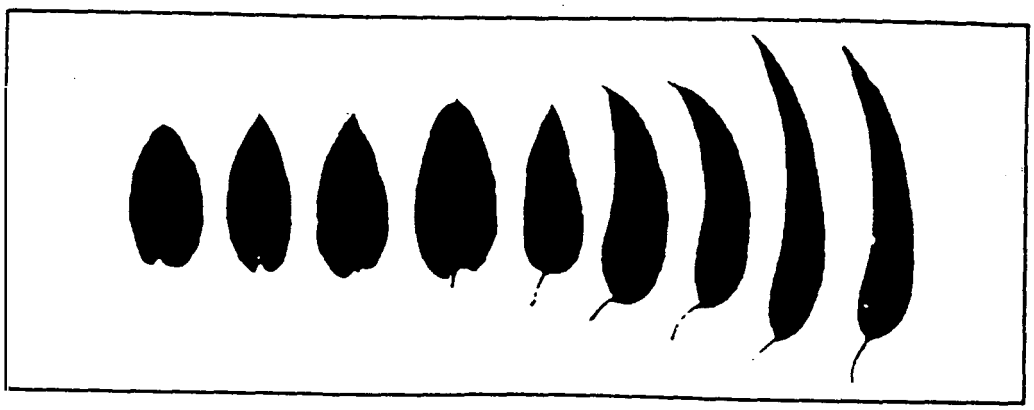


Fig. 1. Heteroblastic development in *E. globulus* showing the transition from juvenile (left) to adult leaves (right). Transition leaves are shown in between. (figure taken from Reid and Potts 1997).

The transition to adult foliage in eucalypts is not totally irreversible as is suggested by the definition of ontogenetic changes presented above "irreversible programmed changes in gene expression which occur in the meristem and are stable to asexual propagation". The growth of new shoots from epicormic buds often occurs as a response to damage by fire, wind, herbivory and other factors. This regrowth often involves varying degrees of reversion to juvenile foliage (Wiltshire and Reid 1992). Edwards (1982) suggests that this may impart extra protection to the young, vulnerable foliage such as protection from herbivory, frost, high insolation loads to the young, delicate tissue. Such reversion is also possible through a sequential series of grafts ('cascade grafting' - Cauvin 1981; Paton 1991), where adult foliage taken from the

stem apex of even a very old tree may be completely rejuvenated to juvenile type foliage.

Genetic variation in phase change

There is much variation in the timing of phase change both within and between populations of eucalypts (Brooker and Kleinig 1990). Common garden studies of the timing of phase change in the *E. risdonii/E. tenuiramis* complex (Wiltshire *et al.* 1998) and in *E. globulus* ssp. *globulus* (Jordan *et al.* unpub. data) show a high narrow-sense heritability; 0.46-0.67 and 0.67 respectively, for variation in the timing of the shift from juvenile to adult foliage within populations. There are also large differences in the timing of phase change between populations of both species complexes (Wiltshire *et al.* 1998; Dutkowski and Potts in press). These results clearly show that the timing of the phase change is under strong genetic control in eucalypts.

These studies have also shown that reproductive and vegetative phase change are genetically independent of one another (Wiltshire *et al.* 1998; Jordan *et al.* unpub. data). The genetic nature of these ontogenetic changes is such that selection can operate on these traits independently, thereby changing their relative timing. Large differences in the morphology of reproductive individuals may therefore occur with minimal genetic changes (Wiltshire *et al.* 1994; Potts and Wiltshire 1997) (this process is considered to be a rapid mechanism of evolution in an organism and is referred to as *heterochrony* (Wiltshire *et al.* 1994). The *Eucalyptus risdonii/E. tenuiramis* complex is a classic example of where a *heterochronic* process seems to have occurred (Wiltshire *et al.* 1992, 1998). *E. risdonii* precociously reproduces in the juvenile vegetative phase, which is in contrast to *E. tenuiramis* which reproduces (as is the ancestral condition) in the adult phase. It is thought that *E. risdonii* has recently evolved, and is still evolving from the *E. tenuiramis*, with a cline existing in southern Tasmania from the extreme form of *E risdonii* to the extreme form of *E. tenuiramis.*

The ecological and adaptive significance of heteroblasty within *Eucalyptus*

The ecological and adaptive significance of these ontogenetic changes is largely unknown (Bell and Williams 1997) but the strong genetic control of the phase change trait in eucalypts suggests that it is likely to be of some adaptive significance. Within the genus some studies have shown that the juvenile leaf phase is retained longer in extremes of altitude or drought (Barber and Jackson 1957; Pederick 1979; Potts and Reid 1985; Bell and Williams 1997; Wiltshire *et al.* 1998). However, this is inconsistent with a recent study of *E. globulus* growing in coastal situations where the extreme conditions were characteristically inhabited by trees which appeared to have an early change to adult foliage (Dutkowski and Potts, in press). These are apparently contradictory trends, however the environmental factors operating in each situation may be different.

Bell and Williams (1997), Beadle *et al.* (1989)and Pederick (1979) suggest that the leaf orientation of the juvenile foliage may maximise the capture of solar radiation, thereby increasing photosynthetic efficiency. Indeed in many cases individuals which retain the juvenile canopy the longest have a faster growth rate (Beadle *et al.* 1989; Farrow *et al.* 1994), which may be due to this increase in photosynthetic efficiency.

In some species of *Eucalyptus*, such as *E. urnigera*, the high glaucousness of the juvenile foliage has been suggested to be a mechanism of cold-hardiness (Pederick 1979), or of protection from high insolation loads (Ashton and Turner 1979) or drought stress (e.g. *E. tenuiramis/risdonii*, Potts and Wiltshire 1997).

It is also postulated that the susceptibility of eucalypts to herbivores might be a major selective force in eucalypts. Herbivory may significantly reduce the growth and reproductive success of a forest tree (Greaves 1966) even though the extent of the effect is highly variable across tree species (Carne *et al.* 1974). Therefore developmentally based resistance to herbivores should be under the same type of selective forces as other traits (Landsberg and Cork 1997; Kearsley and Whitham 1998) and it is possible that phase change is one means by which the impact of herbivory on a plant is dispersed thereby minimising the negative effects on carbon gain (Edwards 1982). Importantly,

the impact of more than one herbivore responding in opposite ways could complicate matters with adaptations possibly needing to circumvent two (or more) opposing effects.

From research on *Populus*, Waltz and Whitham (1997) consider that within tree variation due to ontogenetic changes may be influencing patterns of dependent species distributions in a genetically controlled manner and that this may have implications at the whole ecosystem level. It follows that an increase in habitat heterogeneity would result in an increase in the overall dependent species diversity.

The response of dependent communities to ontogenetic changes

Examples in forest trees

Recent work by Kearsely and Whitham (1989, 1998), Waltz and Whitham (1997), Bryant *et al.*(1985) and Zagory and Libby (1985) has shown the effects of differences in leaf quality, across the different ontogenetic phases of forest trees, on the distribution of insect, vertebrate and fungal pathogens respectively.

Insects

Studies of the response of dependent communities to developmental changes in the *Populus* system (Kearsley and Whitham 1989,1998; Dickson and Whitham 1996; Waltz and Whitham 1997) show how predictable changes in plant resistance traits can result in a differential response by insect herbivores.

In narrowleaf cottonwood, *Populus angustifolia*, Kearsley and Whitham (1989) were able to show that there was a direct influence on herbivore distributions with age due to phase change from the juvenile to the mature vegetative phase (the authors refer to the ontogenetic change in cottonwoods as the "developmental stream" - where the plant

expresses a gradient of increasingly mature phenotypes through its lifetime). The authors studied a site on the Weber River, Utah. In the genus *Populus,* plants reproduce vegetatively to form natural clones, the authors selected a series of these naturally occurring clones from across the Weber River site. Within a clone, ramets which differed in their juvenile and mature traits could be selected and for each clone they selected five different ramets of each different developmental class. This enabled an analysis of how insects might respond to juvenile and mature traits while at the same time holding plant genotype constant. On each ramet the abundance of the aphid, *Pemphigus betae*, and the Chrysomelid beetle *Chrysomela confluens* were examined. A part of the study involved artificially transplanting the insects to the juvenile and mature zones of ramets of the same genotype in order to quantify their performance on each foliage type. The methodology employed in this study also allowed the removal of physiological aging and environmental effects such as herbivore density, predation, soil and moisture regimes, mycorrhizal effects, and the effects of the flight paths of the colonising insects. It was shown that the two insects, *Chrysomela confluens* and *Pemphigus betae* responded in an opposing manner to the juvenile and adult foliage types in *Populus angustifolia* (Fig. 2); the survival of larvae of *Chrysomela confluens* was significantly higher on the shoots of juvenile trees (or juvenile foliage on heteroblastic trees) than on the shoots of adult foliage. In contrast, the aphid *Pemphigus betae* showed a significantly greater survival rate on adult trees (Kearsley and Whitham 1989).

In another study of the same system Kearsley and Whitham (1998) showed that the distribution of the surviving stem mothers of *P. betae* could be predicted using a quantitative measure of developmental distance. They found the distance from the shoot to the root crown to be a measaure of the degree of ontogenetic development and that the morphology (i.e. leaf characteristics and the proportion of reproductive buds to vegetative buds) could be predicted using this distance measure. Moreover it was shown quite unequivocably that the distribution and fitness of *Pemphigus betae* was positively related to this developmental distance (Kearsley and Whitham 1998).

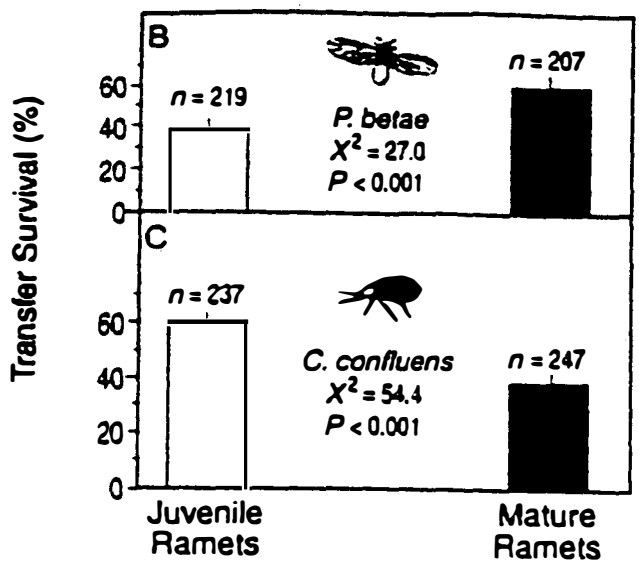


Fig. 2. *Pemphigus betae* and *Chrysomela confluens* survival on trees of different juvenile and mature developmental stages. (B) When aphids were experimentally transferred to both juvenile and mature ramets, survival was significantly lower on juvenile ramets. (C) Experimental transfers of beetles showed the opposite pattern: their survival was significantly higher on juvenile ramets than on mature ramets. (Figure from Kearsley and Whitham 1989).

Continuation of this study showed that the phase change in cottonwoods had a significant impact on the structure of the dependent community as a whole (Waltz and Whitham 1997). Because the distribution of aphids and beetles is dependent upon plant ontogeny, the aphids and beetles were experimentally removed to quantify the indirect impacts of plant ontogeny on the rest of the community. In their study they removed aphids through the use of a sticky barrier - Tanglefoot - and showed that there was a dramatic decrease in species richness and abundance (32% and 55% respectively; Fig. 3a) in the mature zones where aphids were excluded. The removal of the beetle resulted in an increased species richness and abundance in the juvenile foliage by 120% and 75% respectively (Fig. 3b). Thus, the ontogenetically determined distribution of these two insects influenced the community structure in opposing ways, with the presence of the aphid increasing biodiversity, while the presence of the beetle reduced biodiversity. In addition, a related study by Dickson and Whitham (1996) showed that fungi and vertebrates were also affected by the distribution of aphids.

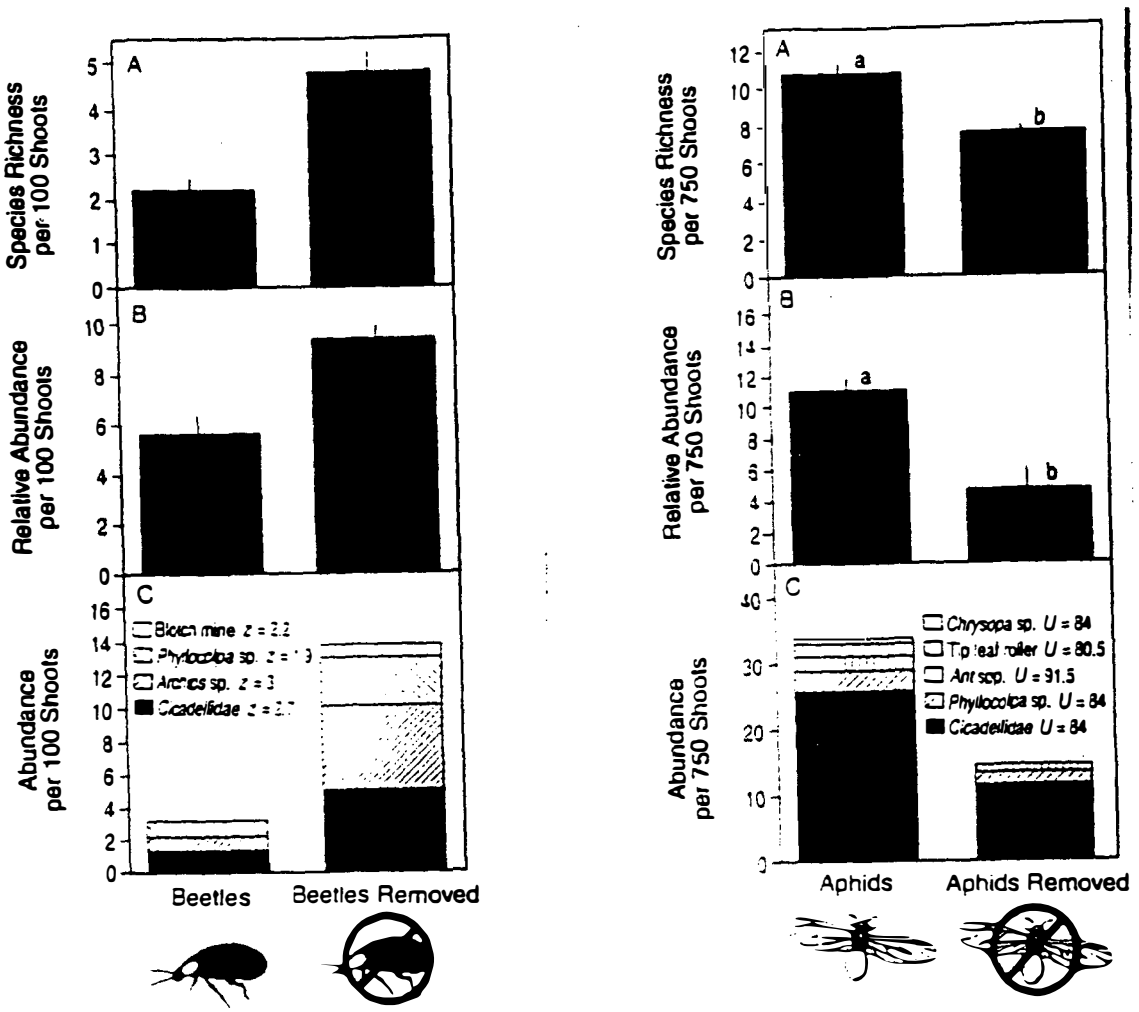


Fig. 3. (a) The experimental removal of the leaf galling aphid *Pemphigus betae*, whose distribution is determined by plant development, negatively affected mature zone biodiversity.

(b) The experimental removal of the leaf-feeding beetle, *Chrysomela confluens*, whose distribution is determined by plant development had a positive impact on the arthropod diversity of juvenile ramets. (Figure from Waltz and Whitham 1997).

This series of studies demonstrates that ontogenetic development in plants can directly influence the distribution of herbivores, and indirectly influence arthropod community structure. These findings are important for the idea that plant ontogeny can affect insect population and community structure in ways that rival or even exceed the traditionally accepted views of competition and predation (Kearsley and Whitham 1989).

Vertebrates

Phase changes in the willow, *Salix alaxensis*, have been shown to directly affect the population dynamics of the snowshoe hare by Bryant *et al.* (1985). Their study suggested that plant resistance traits in the willow *Salix alaxensis* may cause the well known 10 year snowshoe hare cycle (Curtis and Barnes 1989).

It is known that the juvenile form of woody plants can exhibit traits which are associated with low palatability and poor digestion for browsing; for example numerous shrubs that bear thorns or spines will be thorny or spiny only when juvenile (Koslowski 1971). In another example, the juvenile shoots may have secondary chemicals which are toxic to mammalian herbivores, such as catechol (Bryant *et al.* 1985).

Salix alaxensis is a highly preferred food of the snowshoe hare (*Lepas americanus*). In this willow the juvenile growth form differs both morphologically and physiologically from the adult form (Kozlowski 1971) in that the sprouts of the juvenile form are known to be significantly less palatable to snowshoe hares than the sprouts of adult form (Bryant *et al.* 1985). In addition, the digestibility (both dry matter and nitrogen) of the juvenile sprouts is lower than the adult twigs (Bryant *et al.* 1985). Bryant *et al.* (1985) studied two sites where the willow was grown, and at one of these the plants had been severely browsed back to snow level the previous year and had regenerated by resprouting the following summer. Through mechanical decapitation over a 5-year period the authors were able to produce a series of sprouts ranging in age from 1-5 years old. The hares were fed small twigs from each age class, and it was found that sprouts of juvenile form were significantly less palatable and less easily digested than sprouts of the adult form.

From the study it was predicted that as the abundance of adult form foliage increases so too does the snowshoe hare population. The consequence of this is that if there is severe winter browsing by the hares widespread reversion to the juvenile form will occur, as the juvenile form is low in palatability and nutritional quality a crash in the population of hares occurs as a consequence of the poor resource.

It is suggested that: 1) feeding deterrents in the juvenile growth form of the willow has developed in response to winter hare browsing and 2) that the dependent mammalian population is being influenced by the within plant variation found between the adult and juvenile forms of the willow. This is therefore an example of the influence of plant ontogenetic changes on the population dynamics of a dependent mammalian population.

Fungi

A study of Western Gall Rust (Zagory and Libby 1985) found that plant ontogenetic development in *Pinus radiata* affects the distribution of plant pathogens. The authors set out to separate environmental influences, particularly microclimate, from the influence of the developmental state in the susceptibility of the pine to western gall rust *(Endocronartium harknessii).* In the study, cuttings of *Pinus radiata* were taken from trees and hedges which were from the same clone. The hedges had been cut back to produce new growth which was juvenile in form so that between the hedged shoots and the shoots from the trees there was a difference in developmental state. As a result a pair of stecklings (grafted individual plants) which were of identical age and genotype, but in two different developmental states were produced. On the hedge origin stecklings (juvenile phenotype) the number of galls caused by the fungus under experimental conditions was double that which was found on the tree origin stecklings (mature phenotype).

Additionally, Zagory and Libby (1985) considered that the decrease noted by Patton (1961) in the susceptibility with age of the Eastern White Pine to infection by blister rust (*Cronartium ribicola*), was a response to the juvenile and adult foliage types.

Examples within the genus *Eucalyptus*

Fungi

Mycosphaerella species

Mycosphaerella leaf blight disease can be caused by several different species of *Mycosphaerella* fungus. *M. nubilosa* (syn: *M molleriana*) has been known to cause almost complete defoliation of juvenile and intermediate leaves in plantations of *E. globulus* importantly it is never found attacking mature leaves (Park and Keane 1982).

In a study of *Mycosphaerella* leaf diseases between families of *E. globulus* and *E. nitens* and their hybrids (Dungey *et al.* 1997) it was found that there was a greater severity of the disease on juvenile foliage. The trees in this study were affected by two species of *Mycosphaerella*; *M. cryptica* and *M. molleriana.* Dungey *et al.* (1997) noted that *M. cryptica* caused lesions on both juvenile and adult foliage, a fact which is supported by Park and Keane (1982), while the *M. molleriana* lesions were found only on the juvenile foliage. Parke and Keane (1982) found a feasible reason for the preference of *M. nubilosa* (Syn*: M. molleriana*) for juvenile foliage, the fungus could not penetrate into the palisade mesophyll which occurs on both sides of the mature leaf, but could penetrate the spongy mesophyll of the lower surface of the juvenile leaves.

The greater infestation of the juvenile leaves by the *Mycosphaerella* fungi may have been due to the ease with which *M. molleriana* can colonise juvenile leaves, however, the nature of this study did not exclude the possibility that the differences could have been the result of differences in micro-climate (greater humidity and temperature) between the upper and lower (juvenile) canopies, a possibility acknowledged by Dungey (1997).

The restriction *of M. nubilosa* to juvenile foliage meant that a further *Mycosphaerella* fungus, *M. parva*, which is a saprophyte of *M. nubilosa* was also restricted to the juvenile foliage.(Park and Keane 1982). This is interesting for the idea of a community

response by dependent species/detritovores to plant ontogenetic changes, as the fungus *M. parva* could only inhabit the plant after the previous infection by *M. nubilosa.*

Insects

Chrysomelid species

Numerous observations pertain to the differential colonisation of Chrysomelid beetles on juvenile and mature foliage of *Eucalyptus;* Ohmart and Edwards (1991) observed that the adult foliage of *E. nitens* is not attacked by *C. bimaculata* until after 5-6 years when the tree is predominantly adult foliage; de Little (1989) writes that *Chrysoptharta agricola* attacks the juvenile foliage of *E. nitens*, while *C. bimaculata* does not; Greaves (1966) notes that the 3rd and 4th instar larvae of *C. agricola* is a pest mainly of the juvenile foliage.

Edwards (1982) observed that adult foliage of *E. nitens*, and *E. globulus* in New Zealand had been intensely grazed by *Paropsis charybdis*, while juvenile foliage was left virtually untouched. This observation is also noted by de Little (1989) for *E. nitens* and *E. globulus* in Tasmania. Barber and Jackson (1957) suggest that the development of glaucous foliage may have, in part, been an adaptation to counter insect attack, a view which is supported by Edwards (1982). Laboratory experiments in the study by Edwards (1982) indicated that where beetles were placed on waxy juvenile foliage they were unable to grip and immediately fell off and on removal of the wax layer the beetles were able to cling to the foliage, an observation supported by Li (1993) (see below). Additionally, it was observed by Edwards, 1982) that in cages where the foliage was against the walls the beetles fed and oviposited on that juvenile foliage.

A study by Li (1993) showed a similar result for *C. bimaculata* as Edwards (1982) showed in *P. charybdis.* In the laboratory situation, *C. bimaculata* could not grip on the waxy juvenile foliage while *C. agricola* could. Li (1993) observed that the *C. bimaculata* had difficulty gripping onto juvenile foliage of *E. globulus* held at different slopes but that *C. agricola* in contrast could easily attach and crawl on the foliage irrespective of slope. An interesting result for *C. agricola* also came out of this study;

on examination of the beetles tarsi Li (1993) found that the structure in the tarsi of *C. agricola* differed from that of *C. bimaculata.* It was suggested that this may enable *C. agricola* to utilise a niche which is rarely inhabited by other chrysomelid species in Australia.

A potential reason for these differences in preference, by Chrysomelid beetles, for foliage of a different ontogenetic stage was explored by Li (1993) and Li *et al.* (1997). The examination of differences in secondary chemical compostions (which are thought to influence herbivory) showed that the chemical composition of the waxes in subgenus *Symphyomyrtus* did not differ significantly across juvenile and adult foliage, only the amounts of wax differed. In *E. globulus* the juvenile foliage wax yield was significantly higher than the adult foliage wax yield. These findings further support those of Li (1993) described above.

Autumn Gum Moth

The Autumn Gum Moth, *Mnesampela privata* is a pest of eucalypt trees in south eastern Australia (Elliot and Bashford 1978), and in western Australia (Abbott 1993), it is widely distributed in Tasmania in areas where blue gums are grown. While the larvae of the Autumn Gum Moth feed naturally on a wide range of eucalypt species (Elliot and Bashford 1978, Neumann and Collett 1997) they are significant pests of the economically important *E. globulus* and *E. nitens.*

Elliot and Bashford (1978) noted that the eggs of the moth were usually laid on the glaucous, juvenile foliage, and the larvae, in particular, often completely strip the juvenile foliage - where they are most commonly found. The moth is considered a problem in trees of up to four years old and Farrow *et al.* (1994) consider this to be a response to the juvenile foliage which is dominant in these young trees (i.e. once *E. globulus* produces adult foliage, attack by *M. privata* declines).

Neumann and Collett (1997) suggest controlling Autumn Gum Moth outbreaks through management practices which induce a rapid transition to adult foliage such as good site

conditions, fertilisation, irrigation, and selecting for genotypes which show an early transition, however such a regime may not be compatible with the selection of other favourable traits.

Although the above observations are common in the literature no controlled study has been undertaken, which eradicates possible confounding environmental factors, which shows this preference of Autumn Gum Moth for the juvenile foliage.

Leafblister sawfly

An examination of the study by Farrell and New (1980) of the Leafblister Sawfly *(Phylacteophaga froggatti*, - Hymenoptera:Pergidae) on *E. globulus* demonstrates the complicating factors which arise when looking at the response of a dependent community to the developmental phases in the natural situation (and the importance of complimentary studies in the absence of competitors and predators – see the following study). Farrell and New (1980) found that the Leafblister sawfly predominantly lays it's eggs on older mature foliage and that mines are rarely found on juvenile foliage. However, Farrow *et al.* (1994) found evidence to the contrary suggesting that as the Leafblister sawfly often appears in conjunction with the Autumn Gum Moth and as defoliation by the latter species is more dramatic, damage by the Leafblister sawfly will be masked. Farrow *et al.* (1994) also note that the damage from the Leafblister sawfly can be confused with damage by *Mycosphaerella* on juvenile leaves.

Conclusion

The idea that dependent species respond to variation in host plant ontogeny is relatively unexplored. It is of much importance as the variation present in forest trees due to these ontogenetic changes can be a major factor in determining the distribution of herbivores at higher trophic levels (insect predators, fungi and vertebrates) in a forest ecosystem. These studies of the responses of dependent species to host ontogenetic changes, with the exception of Waltz and Whitham (1997), have generally concentrated on a single dependent species. It is probable that the distribution of these herbivores

will have implications at the whole community level. It is suggested here that the impact of ontogeny on the distribution of these species rivals other important factors that are more commonly accepted as being important (e.g. plant resistance traits among trees in the population, predation, competition, etc.).

Within the genus *Eucalyptus* the degree of heteroblasty is so striking, and the genetic control of the timing to phase change from juvenile to adult foliage demonstrated to be so strong as to suggest that there is some adaptive significance for the trait. Some suggestions for the reason for the phase change are: frost resistance, tolerance to high insolation loads and insect and disease resistance.

If ontogeny is as important as these studies suggest, two applied implications are important to consider:

1) stands with mixed ontogenies will support the greatest biodiversity which may be important in conservation efforts.

2) selection for an ontogenetic stage that is least susceptible to insect attack may be important in commercial plantations to maximize plant growth and fibre production and in reducing the use of toxic pesticides.

Although the idea that dependent species respond to variation in host plant ontogeny is largely unexplored, it has the potential to represent a common mechanism that affects diverse taxa and trophic levels. The handful of studies conducted to date argue that this may be a very productive line of future research into understanding species distributions and community structure. The marked heteroblasty in eucalypts makes them ideal as a model system to study these effects.

The following study will specifically address this response of dependent communities and individual dependent species to changing plant ontogeny in the *Eucalyptus globulus* x *Eucalyptus nitens* hybrid system. It will be formulated in such a manner as to enable the direct comparison of the relative importance of these ontogenetic effects with genetic effects. The genetic effects are provided by a continuum of cross types between, and inclusive of, the *E. globulus* and *E. nitens* parental species. The response by the dependent communities will be examined from two separate, but related,

perspectives: Firstly, the extent to which the composition of dependent communities varies with changing ontogenetic or genetic type will be examined (Chapter 2). Secondly, the question of whether dependent species richness is influenced by changing genetic class or by an increase in effective habitats due the presence of a potentially heterogeneous habitat on the heteroblastic trees is investigated (Chapter 5). Additionally, detailed studies of the responses of individual species in the field (Chapter 3) and laboratory (Chapter 4) will be undertaken.

Experimental thesis:

Chapter 2

Community responses

Introduction

The following chapter assesses the effects of plant ontogenetic change on the structure of dependent communities. Dependent taxa include insect herbivores, fungal pathogens, predators and vertebrate herbivores (after Linhart 1989). Few studies have addressed this issue and none have quantified the importance of the ontogenetic component in comparison to traditional forms of genetic variation within an ontogenetic stage. There are reports of dependent taxa which exhibit a preference for one or the other ontogenetic leaf types (juvenile or adult) in *Eucalyptus* (Park and Keane 1982; de Little 1989; Farrow *et al.* 1994; Dungey *et al.* 1997). Results from these studies are not conclusive as they have not removed the confounding factors of changing genotype, age, canopy position (height), or changing environmental conditions. Moreover, none of these studies have quantified these relationships or demonstrated that they are a true response to variation in foliage quality as opposed to competition or predation. Additionally, only one study, in the genus *Populus*, has examined the overall dependent community response to changing ontogeny and in that study the effect of canopy position was a confounding factor (Waltz and Whitham 1998).

It has been suggested that the impact of ontogenetic change on community structure may be as important as other traditional studies of competition, predation, and resistance traits (see also Dickson and Whitham 1996; Waltz and Whitham 1997; Kearsley and Whitham 1998). However, more detailed research is necessary before any conclusion can be made. The present study uses ontogenetic and genetic variation within a *Eucalyptus globulus* x *E.*

nitens hybrid system to examine the relative influences of these two factors. As noted above, it is important to examine the influence of changing ontogeny independently of confounding environmental, genetic and positional effects and the use of a common trial in this study enables this to be achieved.

Eucalyptus globulus ssp. *globulus* (hereafter referred to as *E. globulus*) and *Eucalyptus nitens* are two species which exhibit very striking heteroblasty (Pryor 1976; Brooker and Kleinig 1990). Both species maintain the juvenile foliage from after the seedling phase until between 12-36 months of age, before switching to adult foliage. This subsequent phase, which can last up until five years of age, is described as the 'heteroblastic' phase, during which the trees exhibit juvenile and adult foliage simultaneously. The height (and corresponding age) to phase change in these species is highly variable (even within a population) and under strong genetic control (Dutkowski and Potts in press; Jordan *et al.* submitted). As a consequence of this, at a critical age (12 months to 5 years) there exists within a population trees which are heteroblastic and trees which are still homoblastic (supporting 100% juvenile foliage). The marked differences between the ontogenetic foliage types in *E. globulus* and *E. nitens,* as well as the variation in the height to phase change, provides an ideal system in which to examine the effects of ontogenetic change on the structure of dependent communities.

Eucalyptus globulus and *E. nitens* are two of the most important plantation eucalypts in temperate Australia (Tibbits 1986; Eldridge *et al.* 1994). The two species are taxonomically close, both belonging to the subgenus *Symphyomyrtus* series *Viminales*, and they are also morphologically similar in vegetative traits, but differ in reproductive traits (Brooker and Kleinig 1990). *Eucalyptus globulus* naturally occurs in Tasmania, south eastern coastal regions of Victoria and the Bass Strait islands (Jordan *et al.* 1993). On the other hand, *Eucalyptus nitens* naturally occurs in south eastern mainland Australia, but not in Tasmania. However, *E nitens* has been introduced to Tasmania and is extensively used as a plantation species in the state. There has been considerable interest in the potential use

of *E. globulus* x *nitens* in plantations as the fast-growing high pulp-yield qualities of *E. globulus* may combine with the greater frost resistance of *E. nitens* (Potts *et al.* 1992).

In the present study, the impact of ontogenetic variation is compared with the impact of genetic variation generated by the genetic differences between *E. globulus* and *E. nitens* and their hybrids. Hybrid systems can be used as a tool with which to examine the effects of genetic variation on dependent community structure (Whitham *et al.* 1997). Hybrid systems are a focus for a wide range of responses by dependent species (Fritz *et al.* 1994; Strauss 1994). While the most common response is that of hybrid susceptibility (reviewed in Whitham *et al.* 1997), other recognised patterns of herbivory in hybrid zones include patterns of intermediate susceptibility (Gange 1995), resistance (Boecklen and Spellenberg 1990) and susceptibility equal to one of the parental types (Messina *et al.* 1996). The majority of studies on hybrids have been undertaken in natural hybrid zones which will have confounded environmental with genetic influences (Whitham *et al.* 1994). It is only in common environmental field trials using pedigreed genetic material that these responses can be conclusively attributed to variation in plant genetics (with changing hybrid class). Using such an approach with plants of known pedigree, Dungey (1996) was able to show a genetic basis to the responses exhibited by dependent taxa in the natural hybrid zone between *E. risdonii* x *E. amygdalina* (studied by Whitham *et al.* 1994). However, to date the mechanisms underlying these differential genetic responses to hybrids are poorly understood. In the *E. risdonii* x *amygdalina* system, the response of dependent taxa to the hybrid genotypes may have been confounded by the differing degree of heteroblasty in the hybrid phenotypes. *Eucalyptus amygdalina* switches very early to adult foliage and is effectively homoblastic adult for most of its life, while *E. risdonii* rarely or never switches to adult foliage and is effectively homoblastic juvenile for most or all of its life (Wiltshire *et al.* 1998). However, the hybrids between the two species are heteroblastic for a considerable portion of their life. If as suggested above, dependent taxa exhibit preferences for different ontogenetic foliage types, it is possible that the differential responses by dependent species across that hybrid system were confounded with the (inherited) heteroblastic nature of the hybrids. A different pattern of heteroblasty is seen in the *E.*

globulus x *nitens* hybrid system where the hybrids are heteroblastic to the same degree as the parental types. Any change in dependent taxa responses to the hybrid classes will therefore be independent of changing patterns of ontogeny.

This study of the *E. globulus* and *E. nitens* hybrid system was an opportunity to examine the distributions of dependent species in response to both ontogenetic and genetic variation. This will be the first time that these two factors have been examined simultaneously and the study will quantify how ontogenetic and genetic variation in forest trees affects the distribution of dependent species at the individual and community level. The age of the trees used in the study, and the genetic framework provided by the *E. globulus* x *nitens* system provided a unique opportunity to examine these responses independent of environmental effects.

Materials and methods

Tyenna *Eucalyptus globulus* x *nitens* hybrid trial

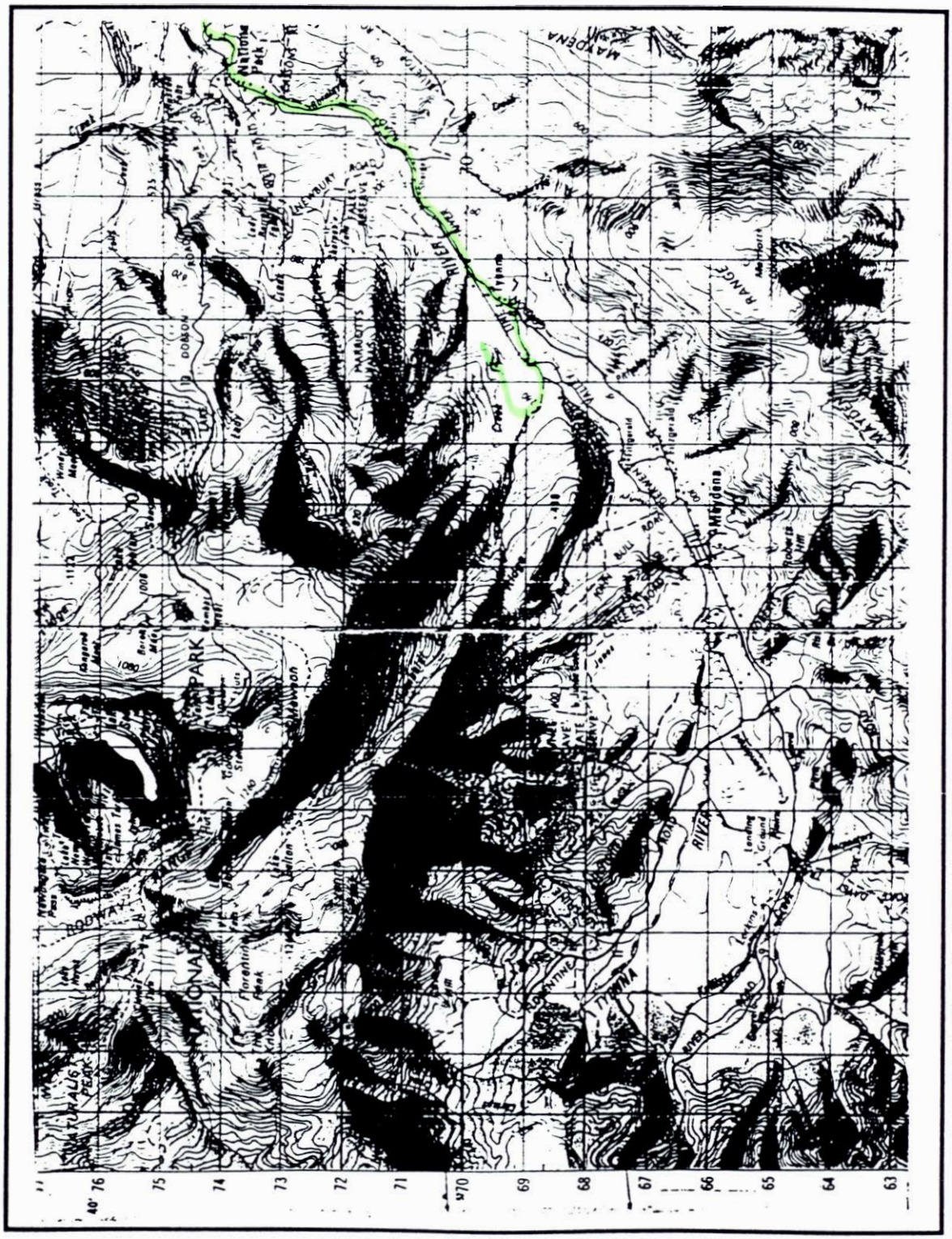
The *Eucalyptus globulus* x *nitens* hybrid trial used in the study was located at Tyenna in southern Tasmania, approximately 7km south west of Mt Field National Park (Tasmap "Tyenna" 1:100 000 Sheet 8212 Edition 1. Grid Reference DN705696; Fig. 2.1).

The trial was a randomised, complete block design consisting of 126 families. There were seven replicates in the trial, with each replicate containing 132 plants (11 rows x 12 individuals) (Fig. 2.2). Individual plants within a family were randomly allocated to a replicate and, within a replicate, individual trees were randomly allocated positions.

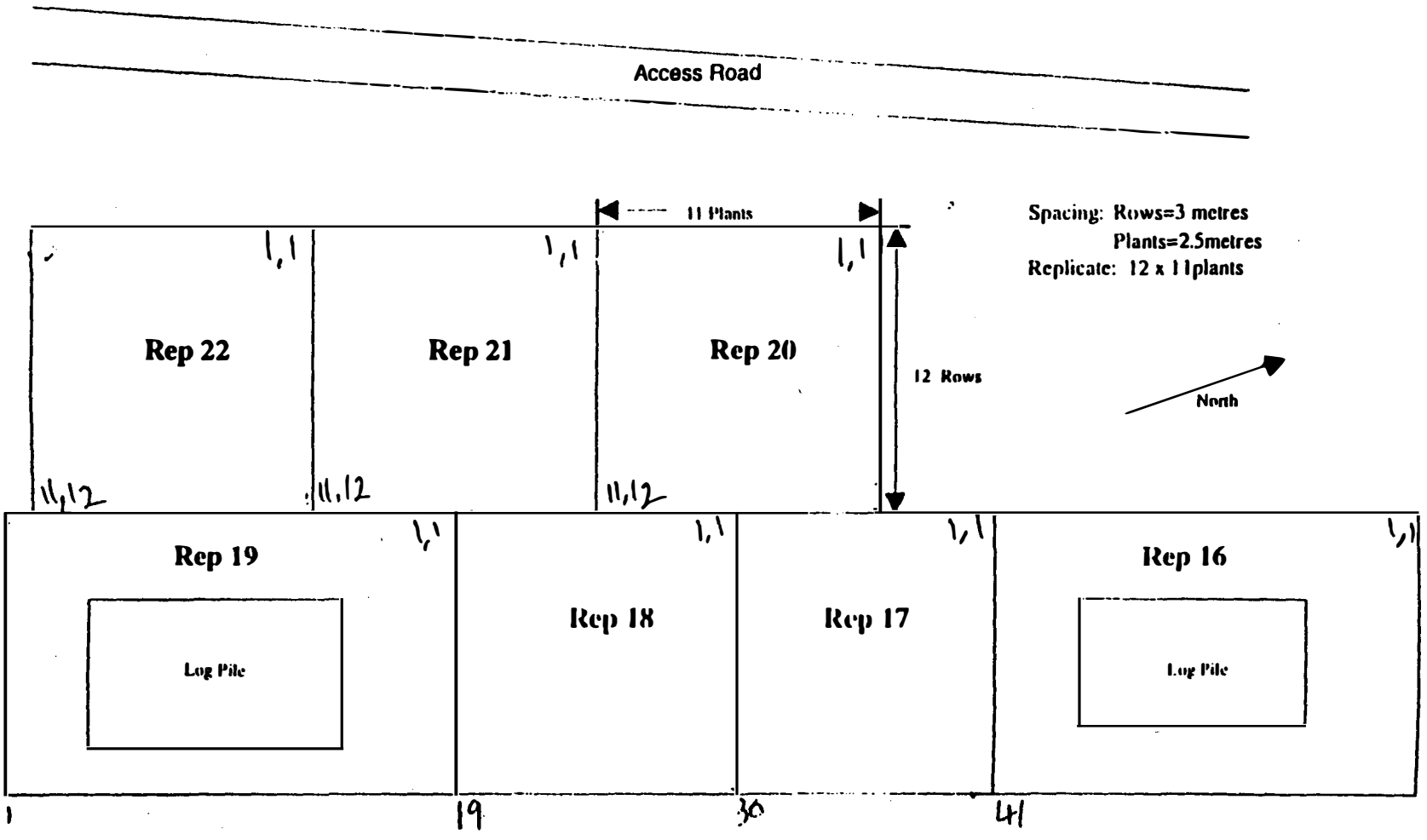
Phase change in both *E. globulus* and *E. nitens* generally occurs when trees are between 12 months to five years of age. At the beginning of 1998 the trees in the Tyenna trial were 30 months old and in the process of changing from the juvenile vegetative phase to the adult

Fig. 2.1. (below) The location of the *Eucalyptus globulus* x *nitens* hybrid trial in southern Tasmania. (Source: Tasmap "Tyenna" 1:100 000 Sheet 8212 Edition 1; Grid Reference DN705696).

Fig. 2.2. (overleaf) The randomised complete block design of the Tyenna trial. The trial is comprised of seven replicates, each of 132 trees (11 x 12 rows).



Tyenna Advanced Generation Hybrid Trial CRC95_5 (1995)
Landowner: Australian Newsprint Mills



The plants used in the field trial were derived from controlled crossing of *E. globulus* from King Island and Taranna provenances with *E. nitens* from the Toorongo provenance. Six different cross types were represented in the trial (Fig. 2.4): the two parental species, *Eucalyptus globulus* and *Eucalyptus nitens*; the F_1 hybrid; F_2s (F_1 x F_1- outcrossed); backcrosses to *E. nitens* (BCnitens – *E. nitens* x F_1 or F_1 x *E. nitens*) and backcross to *E. globulus* (BCglob – *E. globulus* x F_1 of F_1 x *E. globulus*). These six genetic classes provided a complete genetic continuum between the two parental types.

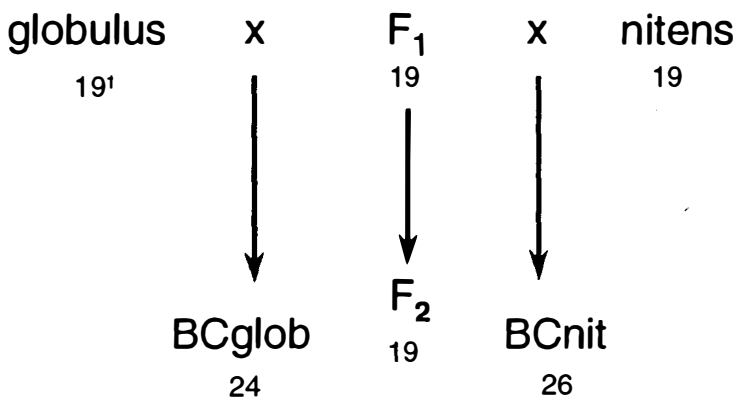


Fig. 2.4 The crossing scheme used in the *Eucalyptus globulus* x *nitens* hybrid trial. Numbers in italics refer to the numbers of families of that type present in the trial at Tyenna. The cross types correspond to *E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens* and *E. nitens*.

Experimental design

The collection of data on the composition of dependent communities involved the selection of equal numbers of trees of the two ontomorphic types (heteroblastic and homoblastic; Fig. 2.3) and of each of the six genetic classes (Fig. 2.4) for detailed scoring. Six replicates in the trial were sampled and within each replicate a heteroblastic and a homoblastic ontomorph (tree) from each of the six cross types were chosen, resulting in a total of 72

trees. Trees were chosen which had new growth in the lower as well as upper canopy thus enabling a comparison of foliage of the same physiological age from the upper and lower canopies. The dependent communities in the upper and lower canopy of each tree, potentially 144 sampling units, were assessed. Ten trees did not have sufficient growth in the lower canopy to be assessed and the lower canopies of these trees were excluded from the analysis resulting in 134 sampling units. All of the trees selected were approximately three-four metres in height and had a normal phenotype (i.e. were not abnormally small and had normal foliage characteristics). The allocation of trees to an experimental replicate closely followed the replicated design of the trial shown in Fig. 2.2. This replicated design allowed the removal of any environmental effects due to heterogeneous growing conditions or patchy colonisation habits by dependent species.

	globulus	BC*glob*	F1	F2	BC*nit*	*nitens*
• **Heteroblastic**						
– high	6	6	6	6	6	6
– low	3	5	6	5	6	6
• **Homoblastic**						
– high	6	6	6	6	6	6
– low	5	4	4	6	6	6

Fig. 2.5 Summary of the experimental design for censusing dependent communities in the Tyenna field trial. The cross types are detailed in Fig. 2.4. and the foliage types are shown in Fig. 2.3. The numbers indicate the numbers of trees censused in each category.

The selection of these trees enabled the simultaneous examination of three independent gradients: the ontogenetic gradient; the genetic gradient and the positional gradient. The experimental design is summarised in Figure 2.5. The effect of changing ontogeny on dependent communities was examined by comparing juvenile and adult foliage at the top of the homoblastic and heteroblastic trees (1 vs 2; Fig. 2.3). This comparison was independent of any confounding positional effects. The genetic gradient was derived from the six different genetic classes (Fig. 2.4). Responses to this genetic variation could be examined independently of ontogenetic and position effects by comparing the response of dependent communities on one or the other ontogenetic foliage type at a comparable height. The positional gradient was supplied by the high and low juvenile foliage on the homoblastic ontomorphs (2 vs 3; Fig. 2.3). It therefore allows responses to changing canopy position to be examined independent of confounding ontogenetic factors associated with changing foliage type. In addition, the comparison of low juvenile foliage from the homoblastic and heteroblastic trees of each genetic class (3 vs 4; Fig. 2.3) provided the opportunity to test, firstly, whether there were inherent genetic differences in susceptibility to dependent species between trees in the homoblastic and heteroblastic populations, or alternatively, if the presence of adult foliage influenced the communities on the lower juvenile foliage.

Collection of community data

Forty four dependent taxa were studied. Dependent taxa are classified following Whitham *et al.* (1994) as unique damage types and/or live species found on the foliage. Additionally, damage resulting from different ontogenetic stages (i.e. immature and mature specimens) were classified as separate taxa. A full list and photographic record of these dependent taxa is given in Table 2.1 and Appendix 1 and hereafter taxa are given with their corresponding numeric codes in parentheses..

The distributions of dependent taxa were censused on the 134 sampling units. The collection of data was undertaken by replicate. Within a replicate the trees from each cross

type and each ontomorphic type were sampled in a random order. However, where live insects were being sampled, the lower canopies were sampled before the upper canopies to avoid dislodging insects by the ladder. All data was collected with the worker in the midst of the canopy, this was achieved in the upper canopy using 4m orchard ladders (Fig. 2.6). Where two workers were involved in the collection of data, each person censused opposite sides of alternating trees and diagonally opposite sides of upper and lower foliage of the trees.

Fig 2.6. The collection of community data from the upper canopy involved the use of 4m orchard ladders to enable the worker to census in the midst of the canopy.

Foliage was collected from the current growing season in order to sample dependent taxa on foliage of a standard physiological age and which had a consistent time for colonisation by dependent taxa. To achieve this the first node on the stem of the present season's growth was determined prior to the collection of data. In these eucalypt species the node at

which the previous seasons growth ended is markedly shorter than the first node on the current seasons growth, and consequently the current seasons growth is readily identifiable.

Three different censusing methods were used for the collection of this data: 1) proportions of leaves (pre-determined number) with evidence of a given taxa (sampled in the field); 2) proportions of shoots (pre-determined number) with evidence of a given taxa; 3) the proportion of leaves collected from the field and examined closely in the laboratory for damage by a given taxa. The sampling method used for each taxa is given in Table 2.1. Two of the methods involved the collection of data on relatively common taxa in the field: Chysomelid #2 beetles (family Chrysomeline) (2); Chrysomelid #1 beetles *Chrysophtharta agricola* – both adult (1) and larval (4) damage; Psyllidae damage #1 (3). The third method involved the collection of information on the distribution of rarer taxa and was done on collected leaves in the laboratory.

Method 1 (common taxa)

Chrysomeline Beetle (Chrysomelid #2)

Data was collected on the abundance of a species of the small brown Chrysomeline beetle (taxa #2). The abundance of this beetle was scored on 20 shoots in the upper and lower canopy of every tree. A shoot was classified as the leaves on one stem which extended back to a maximum of ten nodes, this standardised the age and amount of foliage by looking at a constant amount of the current season's growth. Data was collected on this beetle on 11th March 1998.

Chrysophtharta agricola larvae

The proportion of shoots with damage caused by the larval stage of *C. agricola* (taxa #4) was scored on a total of 14 shoots on each sampling unit. This damage was attributed to *C. agricola* for three reasons: 1) in the early stages of the study (early February) this

Chrysomelid beetle was found in large numbers in the trial and the eggs and larval stage of this beetle were common; 2) the larvae and fresh eggs of *C. agricola* were the only chrysomelid larvae and eggs found in the trial and additionally the proportion of *C. agricola* eggs present in the trial was far greater than its sister species *C. bimaculata* (no eggscars from other Chrysomelid species were found – the eggs are considered an effective means of identification of Chrysomelid species. This evidence suggested almost conclusively that the damage being scored was due to *C. agricola* larvae. This damage was scored at the same time as the collection of data on the rarer taxa (17^{th} – 30^{th} May, 1998).

Method 2 (common taxa)

Chrysophtharta agricola beetles

Chrysomelid beetles leave characteristic chewing 'scalloping' damage on the edges of leaves (Dungey, 1996) (Appendix 1 – species 1). The numbers of leaves of the current growing season with and without this scalloping damage by adult beetles was scored on a total of 50 leaves on each sampling unit. From this data the percentage of leaves exhibiting scalloping damage on each sampling unit was calculated.

As with the larval damage the scalloping damage by the adult beetle had mostly occurred prior to the commencement of the study. The conclusion that this damage was due to *C. agricola* is: 1) that this was by far the most abundant beetle in the trial for the duration of the study; 2) the scalloping damage was observed actually happening in the field; 3) the scalloping damage in laboratory experiments which were undertaken (Chapter 4) was identical to the damage observed in the field and 4) the laboratory experiments showed a similar preference by the *C. agricola* beetle as that found in the field. This damage was scored on the 11^{th} February, 1998.

Pysllid damage *(Ctenarytaina eucalypti).*

The distinctive leaf lesion damage caused by the adults of the psyllid species, *Ctenarytaina eucalypti* (3) was censused as the proportion of 200 leaves (100 on the eastern side, 100 on the western side of the tree) within each sampling unit which exhibited this damage. This data was then converted into a percentage score. The collection of this data was undertaken at the same time as the sampling of leaves for censusing the rarer taxa (17^{th} – 30^{th} May, 1998).

Method 3 (rarer taxa) – leaf collection

The third method involved the collection of data pertaining to the distributions of the rarer dependent taxa. This involved the detailed examination in the laboratory of individual leaves collected from the field for distinctive damage types.

Leaves were removed from each sampling unit and taken back to the laboratory for examination. From each tree, leaves from a constant number of nodes were removed, this was necessary to keep the amount of foliage used for the analysis constant. The canopies on some of the trees were less dense than others, and consequently fewer leaves were taken from these canopies. The amount of foliage of the current seasons growth which could be taken from the adult canopy was limited for two reasons, firstly, the alternate habit of the adult leaves on the stem reduced the canopy density and, secondly, the fact that the trees had only recently phase-changed meant that the adult foliage was not highly abundant.

The collection of this foliage was done in a relatively short time (14 days; 17^{th} – 30^{th} May, 1998) with the help of three co-workers. This was necessary in order to keep the time for colonisation by dependent species constant. The foliage was stored in labelled, zip-lock bags and transported to the University where it was stored in a cool room (4^{o} C) until inspection of the leaves could be undertaken.

In the laboratory, each leaf was carefully inspected for individual distinctive damage types and the proportion of leaves with the presence of a given damage type was recorded. Damage types caused by both biotic (insect, fungal, spiders) and abiotic factors (wind, frosts, nutritional deficiencies) were sorted and scored. By consulting with insect and fungal taxonomists (Dick Bashford, Jane Elek, Tim Wardlaw and Yuan C. Mohammed) the biotic types were identified as accurately as possible. In some cases they could be identified to species level, where this was not possible it was confirmed that the types were truly distinct and any taxon which was doubtful was not included in the analysis. During the collection of the data, damage types which were even slightly different were kept separate, if it was decided at the end of the leaf examinations that two or more groups were of the same type they were merged at that later stage. This treatment ensured that data was not lost through the inaccurate grouping of two apparently similar, but in fact distinct, damage types.

Statistical Analyses

Community compositions on the different foliage types

Analysis was performed to examine the change in community composition (based on 44 dependent taxa) across the three different gradients: 1) ontogenetic (adult and high juvenile), 2) positional (high and low homoblastic) and 3) genetic (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens* and *E. nitens*). An ordination on the community data was performed in order to summarise compositional dissimilarity of the samples and relate this to the design features of the experiment (ontogenetic variation, positional variation, genetic variation). Following Minchin (1987) the samples were ordinated using the non-linear technique of GNMDS (Global non-metric multi-dimensional scaling). This technique was applied to the dissimilarity matrix produced using the Bray-Curtis dissimilarity co-efficient; i.e. Czekanowski coefficient, (Greig-Smith 1983). The dissimilarities were calculated using abundance data, standardised to a unit maxima, such that all taxa contributed equally to the dissimilarity between a given pair of samples. This

standardisation prevented the data from being swamped by particularly abundant taxa. Non-metric multi-dimensional scaling (NMDS) is considered to be a robust and reliable technique of indirect gradient analysis (Minchin 1987). The 'global' variant derives an ordination of the samples, in a specified number of dimensions, such that the distances between all pairs of samples have the best possible monotonic (rank order) fit with their compositional dissimilarities across all samples, rather than samples within a group. Details of the approach are summarised in Bowman and Minchin (1987).

GNMDS ordinations were performed in one to six dimensions from ten random starting configurations. The configuration with the minimum stress level was retained for each dimension. The solution with the minimum stress level in four dimensions was retained for further analysis as the stress levels increased dramatically in the lower dimensions.

The same ordination and significance tests (see below) were repeated using presence/absence, as opposed to abundance data, of dependent taxa distribution.

Factors associated with community variation

The compositional trends revealed in the ordination are often oblique to the axis (Dargie 1984) and the search for associations of independent gradients (e.g. ontogeny, position and genetic) with the positions of samples in the ordination space was achieved using a technique called 'vector fitting'. This technique finds the direction in the ordination space which is such that the projections (scores) of samples on that vector are maximally correlated with the value of the given environmental variable (Bowman and Minchin 1987). The correlation between the fitted vector and the samples indicates the goodness-of-fit of that independent variable in the ordination space. The significance of the correlation was tested based on 1000 randomisations of the data. The directions of the vectors were determined from the cosines of the angles with the axes defining the configuration and other vectors. Vectors were standardised to a unit length such that in a two-dimensional plot the length of the vector indicates the extent to which the vector dips into other dimensions (Potts 1989).

For the present study, three major vectors were fitted into the ordination space: 1) the ontogenetic vector using samples from the high (juvenile) foliage of the homoblastic trees and the high (adult) foliage of the heteroblastic trees (the fitting of this vector therefore ignored cross-type effects and did not compound any effects due to changing height in the canopy); 2) the positional vector using samples from the high and low canopies on the homoblastic trees (the selection of these samples removed any ontogenetic effects and ignored cross type effects) and 3) the genetic vector using the samples on low juvenile foliage from the heteroblastic and the homoblastic trees. The genetic vector was fitted using the samples from all of the genetic classes ranked from *E. globulus*, backcross *globulus*, F_1 and F_2, backcross *nitens* and *E. nitens*. The samples from the F_1 and F_2 samples were treated as one class for the fitting of this vector (as vector fitting requires linear trends). The selection of these samples removed any effects due to changing ontogenetic foliage type and to changing position in the canopy.

The scores of all samples and species were calculated along the ontogenetic, position and genetic vectors and samples were ranked according to these scores.

Responses of individual species

Samples were ranked in order of their positions along the ontogenetic, position and genetic vectors. Based on these rankings ordered tables were produced in which the key species which were contributing to the differences between particular groups could be determined. Further, the mean scores of each of the six genetic classes were calculated along the genetic vector to quantify more precisely the inheritance of dependent community characteristics.

Testing group differences

One way bootstrap tests were performed with ANOSIM (Analysis Of SIMilarities) to determine whether the differences in the defined groups (adult vs high juvenile, low vs high juvenile foliage, cross types on the low juvenile foliage) were significant. These tests

were based on the same dissimilarity matrix as used in the GNMDS. The test specifically addresses whether the dissimilarity in the communities of dependent taxa between the defined groups (see above) is greater than the dissimilarities within the groups. An R value is calculated as:

$$R = \frac{rb - rw}{n(n-1)/4} \qquad \textbf{Equation 2.1}$$

where 'R' is the test statistic, 'rb' is the rank of the 'between' group dissimilarities and 'rw' is the mean rank of the 'within' group differences. A value which is close to one indicates that there is a very big difference, conversely a value close to zero indicates that there is virtually no difference. The tests were based on 1000 random reassignments of the data to groups and determined whether the R values obtained from the group structure in the dissimilarity matrix were significantly different from group structures obtained through chance alone (Warwick *et al.* 1990).

Computing programs

Data analysis (ordinations, vector fitting, ordered tables) was performed with DECODA (Database for Ecological Community Data) and MDS (Multi-dimensional scaling) written by Dr. P. Minchin (1998). Data was imported into the database from an excel data file comprising a sample x species matrix of the abundance data.

Results

Of the 44 dependent taxa were used for the analysis (Table 2.1; Appendix 1), 11 were common to all foliage types: Adult *Chrysophtharta agricola* damage (1); Psyllid damage (3); *Chrysophtharta agricola* larval damage (4); Microlepidopteran #1 (genus

Table 2.1. The 44 dependent taxa used in the analysis of dependent community structure. The species are identified by a number (#) which also corresponds to the reference number in Appendix 1. Through consultation with taxonomists some of the species were identified to species level. However where this was not possible it was confirmed that taxa were distinct. The collection methods (Coll. method) are detailed in the methods section of this chapter. The frequency of rare taxa (<0.06) are indicated in italics, these rare taxa were excluded from the analysis in Chapter 3.

#	Species	frequency	% abund	Coll. method
1	*Chrysophtharta agricola* (adult)	127	94.8	2
2	Chrysomelid beetle #2	38	28.4	1
3	*Ctenarytaina eucalypti* Psyllidae #1	127	94.8	3
4	*Chrysophtharta agricola (larval)*	94	70.1	1
5	*Chrysophtharta agricola (eggscar)*	62	46.3	4
6	Psyllidae #2	3	*2.2*	4
7	*Mnesempala privata* (Autumn Gum Moth)	6	*4.5*	4
8	*Chrysophtharta bimaculata (eggscar)*	14	10.4	4
9	Microlepidopteran #1 (Genus *Acrocercops*)	103	76.9	4
10	Hymenopteran #1	1	*0.7*	4
11	Hymenopteran #2	1	*0.7*	4
12	Hymenopteran #3	2	*1.5*	4
13	Homoptera #1 *Erriococcus sp.*	8	*5.8*	4
14	Hymenopteran #4	3	*2.2*	4
15	*Heteronyx sp.*	74	55.2	4
16	Psyllidae #3	25	18.7	4
17	Psyllidae #4 (Genus *Hyalinaspis*)	24	17.9	4
18	Mite gall	7	*5.2*	4
19	Psyllidae # 5	59	44.0	4
20	Psyllid #6	3	*2.2*	4
21	Lepidopteran #2	12	9.0	4
22	Aranae #1	8	6.0	4
23	Leaf miner #3	1	*0.7*	4
24	Hymenopteran #5	90	67.2	4
25	Microlepidopteran #2	27	20.1	4
26	Hymenopteran #6	1	*0.7*	4
27	Hymenopteran #7	9	6.7	4
28	Aranae #2	68	50.7	4
29	Leaf miner #4	5	*3.7*	4
30	Homoptera #2 (Coccoidae)	71	53.0	4
31	Lepidopteran #2	84	62.7	4
32	Microlepidopteran #3	11	8.2	4
33	Hymenopteran #8	16	11.9	4
34	Microlepidopteran #4	4	*3.0*	4
35	Dipteran gall	1	*0.7*	4
36	weevil damage (*Gonipterus spp.*)	3	*2.2*	4
37	fungal type A	21	15.7	4
38	fungal type B	11	8.2	4
39	fungal type C	1	0.7	4
40	*Cylindrosporium samueli* (Fungal type D)	17	12.7	4
41	fungal type E	8	*5.7*	4
42	Fungal type G	3	*2.2*	4
43	Fungal type H	6	*4.5*	4
44	Fungal type I	118	88.1	4

Acrocercops) (9); *Heteronyx* sp. (15); Hymenopteran #5 (midrib galler) (24); Aranae #2 (28); Homopteran #2 (superfamily Coccoidea - scale) (30); Fungal type A (37).

Community ordinations

The community ordinations based on standardised abundance data and presence/absence data are shown in Figures 2.7a,b and 2.8 respectively. The results of vector fitting for both of these ordinations are given in Table 2.2 and the results of the ANOSIM analysis are given in Table 2.3.

The 4-dimensional GNMDS solutions shown in Figure 2.7 and 2.8, using standardised abundance data and presence/absence data respectively, were accepted as adequate summaries of the variation in community composition among the different foliage samples. These solutions both had stress levels of 0.12. Three major trends were seen in the ordination based on standardised abundance data. These were associated with ontogenetic variation, variation in canopy position and genetic variation amongst the different cross types. These trends were most closely aligned with axis one (ONTOGENETIC 7° [180-173°] POSITION 41°) and axis three (GENETIC 38° [180-142°]) (Table 2.2 standardised abundance data). A fourth level of variation was retained (axis 4) which could not be explained. The three vectors associated with the three major trends, ONTOGENETIC, POSITION and GENETIC, ran in different directions (Fig. 2.7; Table 2.2) indicating that the direction of change (i.e. associated taxa) in community composition along these gradients were relatively independent. Effectively, the same trends were found in the ordination using presence/absence data (Fig. 2.8; Table 2.2).

It is clear from this ordination that the samples on adult foliage have a community composition which is distinct from that on juvenile foliage (regardless of position). The difference, tested in ANOSIM, between the group of samples from adult versus high juvenile foliage samples was highly significant ($p<0.001$; Table 2.3). The greatest amount of variation in community composition was found between these two foliage types

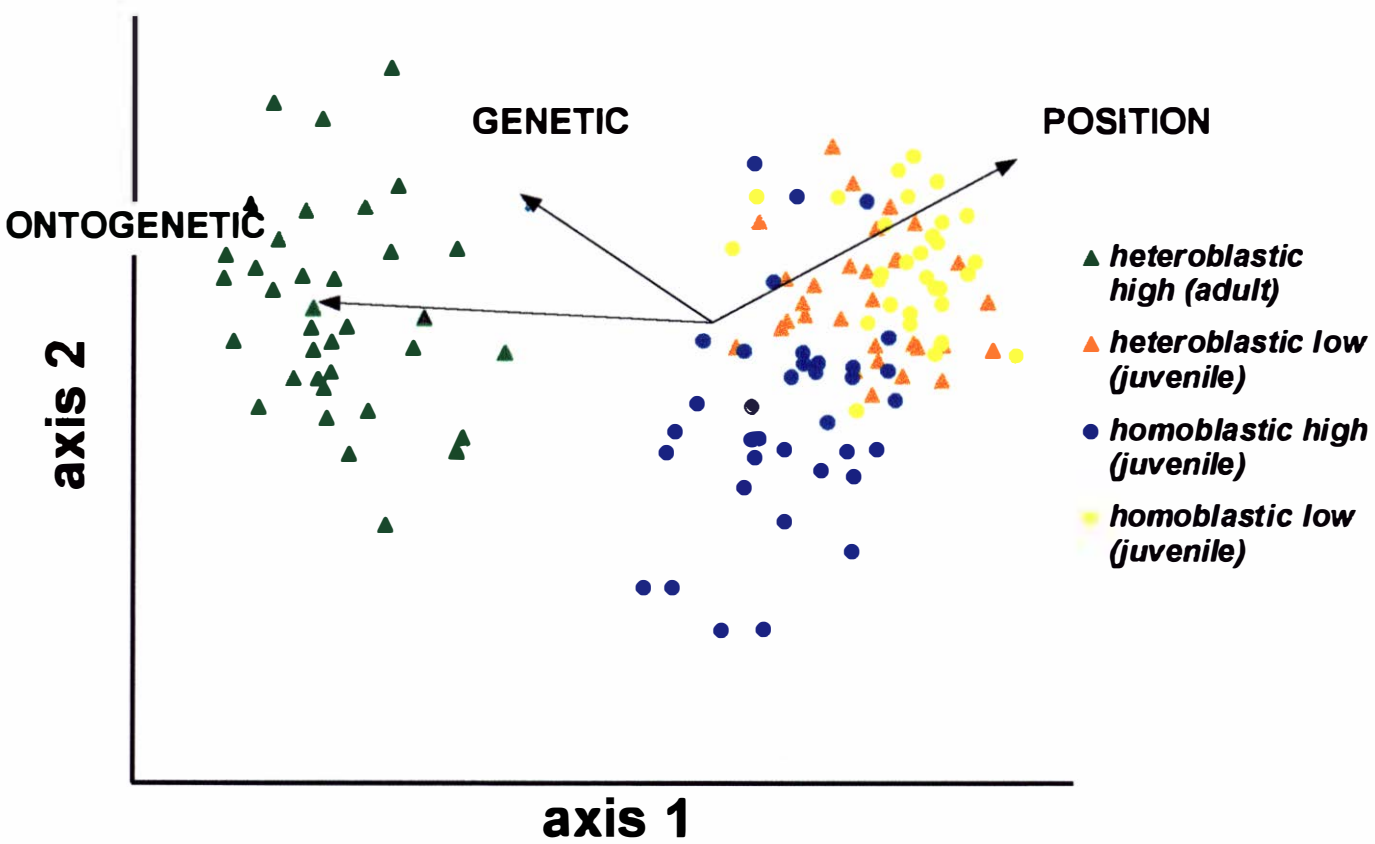


Fig. 2.7a. The distribution of samples in the first two dimensions of the 4-dimensional ordination space, based on the abundances of dependent species (standardised to a unit maxima). The arrows indicate the direction of the fitted vectors. The correlation value and significance of these vectors are: ONTOGENETIC $r=0.96$ ($p<0.001$); POSITION $r=0.81$ ($p<0.001$); GENETIC $r=0.66$ ($p<0.001$). Completely distinct communities are present on the adult foliage compared to the juvenile foliage. The communities on the high juvenile foliage are different from the communities on the low juvenile foliage but this change is continuous.

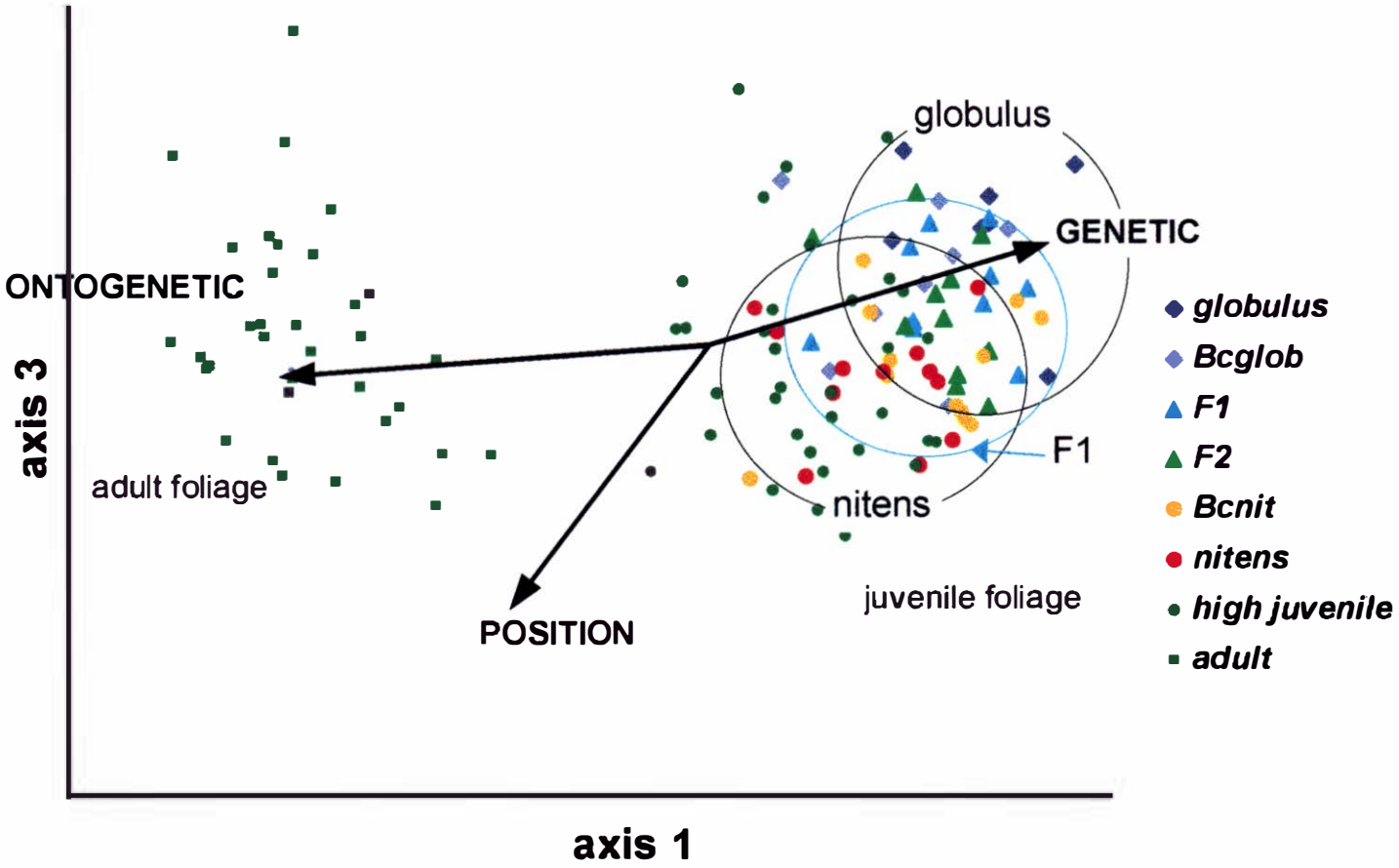


Fig. 2.7b The distributions of samples in the first and third dimensions of the 4-D ordination space based on the abundances of dependent species (standardised to a unit maxima). The arrows indicate the direction of the fitted vectors. The correlation value and significance of the vectors are: ONTOGENETIC r=0.96 ($p<0.001$); POSITION r=0.81 ($p<0.001$); GENETIC r=0.66 ($p<0.001$). The GENETIC vector describes the change in community composition among low samples associated with the transition from *E. nitens* to *E. globulus* (*E. nitens*, backcross nitens, F_1 and F_2 combined, backcross globulus, *E. globulus*).

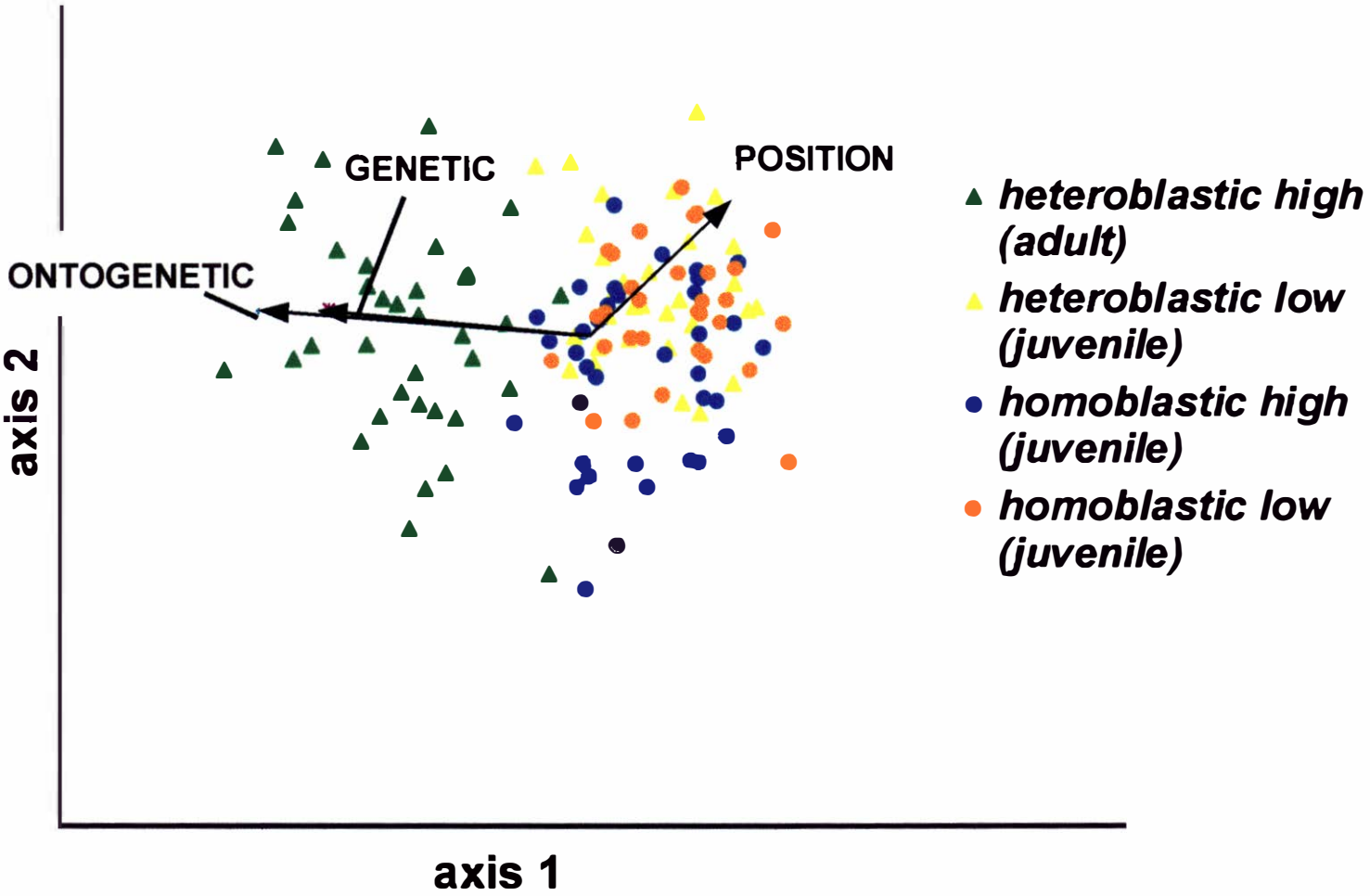


Fig. 2.8. The distributions of samples in the first two dimensions of the 4-dimensional ordination space, based on the presence/absence of dependent species. The arrows indicate the direction of the fitted vectors. The correlation value and significance of each vector are: ONTOGENETIC r=0.88 ($p<0.001$); POSITION 0.66 ($p<0.001$); GENETIC r=0.47 ($p<0.01$). There is only a small amount of overlap between the communities present on the adult foliage and the communities on the juvenile foliage.

Table 2.2 The fit of the vectors in the four-dimensional space, their significance and the angles between vectors. The vectors are shown for the ordinations based on standardised abundance data and on presence/absence data. The independent variables are detailed in the methods; b) the angles between significant ($p<0.05$) vectors fitted in the four dimensional ordination derived from both standardised abundance and presence/absence data.

a)

Standardised abundance ordination				Angle of fitted vector from axis				**Presence/absence ordination**							
variable	n	r	Prob	axis 1	axis 2	axis 3	axis 4	n	r	Prob	axis 1	axis 2	axis 3	axis 4
ONTOGENETIC	72	0.96	0.000***	173	86	96	89	72	0.88	0.000***	159	87	70	94
POSITION	67	0.81	0.000***	41	59	73	107	67	0.66	0.000***	68	70	53	128
GENETIC (low juvenile)	62	0.66	0.000***	119	68	142	91	62	0.43	0.021*	137	87	48	83
HIGH JUVENILE	36	0.60	0.005**	144	87	124	80							
HIGH ADULT	36	0.32	0.506 ns	15	76	94	91							

b)

Standardised abundance ordination					**Presence/absence ordination**	
	ONTOGENETIC	POSITION	GENETIC	JUVENILEHIGH	ONTOGENETIC	POSITION
POSITION	**138**				**114**	
GENETIC	**54**	**113**			**64**	**53**
JUVENILEHIGH	**30**	**143**	**32**			
ADULTHIGH	**158**	**33**	**108**	**137**		

Table 2.3. Results of the one-way analysis using ANOSIM (Analysis Of SIMilarities) showing differences (ANOSIM R) and their corresponding significance between groups using the Bray-Curtis dissimilarities derived from standardised abundance and presence/absence data.

	Standardised abundance data		Presence/absence data	
Effect	**ANOSIM R**	**P**	**ANOSIM R**	**Prob**
ontogenetic				
adult vs high juvenile	0.84	0.000***	0.54	0.000***
ontomorphic				
low juvenile(heteroblastic vs homoblastic)	0.02	0.2 00ns	0.04	0.300 *
position				
low vs high (homoblastic juvenile)	0.33	0.000***	0.16	0.000***
genetic - low juvenile	0.14	0.000***	0.08	0.004**
E. globulus vs *E. nitens*	0.41	0.000***	0.38	0.000***
E. globulus vs F1	0.25	0.009**	0.06	0.221 ns
E. nitens vs F1	0.26	0.000***	0.28	0.000***
E. globulus vs BC*globulus*	0.09	0.144 ns	0.05	0.251 ns
E. nitens vs BC*nitens*	0.03	0.77 ns	0.05	0.186 ns
E. globulus vs F2	0.17	0.042*	0.12	0.081 ns
E. nitens vs F2	0.11	0.040*	0.17	0.003 **
genetic - high juvenile	0.11	0.025 *	not tested	
genetic - adult	0.03	0.717 ns	not tested	

(R=0.84; Table 2.3). Effectively no difference was found between the communities on the low foliage of the heteroblastic and homoblastic ontomorphs (Fig. 2.7a; Table 2.3) indicating that the changing community responses to adult and juvenile foliage was not due to gross genetic differences between the heteroblastic and homoblastic populations. Additionally, the change in community composition with ontogenetic variation (change from juvenile to adult foliage) was effectively the same in all of the cross types examined (Fig. 2.9). The change along the ONTOGENETIC vector (gradient) arises mainly from a compositional change from those taxa associated with juvenile foliage, such as *Chrysophtharta agricola* larval and egg scars (4,5), Microlepidopteran (32), Psyllidae #1 (3), Psyllidae #4 (17), Psyllidae #5 (19), *Heteronyx* sp. (15), Homopteran #2 (30), *Gonipterus* sp. (36) and Aranae #2 (28), to those mainly associated with adult foliage, such as *Chrysophtharta agricola* beetle damage (1), Hymenopteran galler #4 (14), Mite galler (18), Chrysomelid sp. #2 (2) and Hymenopteran #7 (27) (Table 2.4a).

A highly significant ($p<0.001$) difference was found in the dependent community compositions on the high and low foliage of the homoblastic ontomorph. The POSITION vector associated with this change ran at 42° from the ONTOGENETIC vector (Table 2.2) indicating that the community change in response to position was due to a different suite of species than the community change in response to the ontogenetic transition. The amount of variation in community structure in response to positional change was large (ANOSIM $R=0.33$; $p<0.001$), but not as large as the change in community composition due to ontogenetic variation (Table 2.3). This positional gradient reflects the compositional change resulting from those species mainly associated with low juvenile foliage, such as Homopteran #2 (30), Psyllidae #4 (17), Fungal type A (37), Psyllidae #3 (16) and *Mnesempala privata* (7), to those species mainly associated with high juvenile foliage, such as the Microlepidopteran #2 (25), Fungal type I (42) and Fungal type H (43) (Table 2.4b).

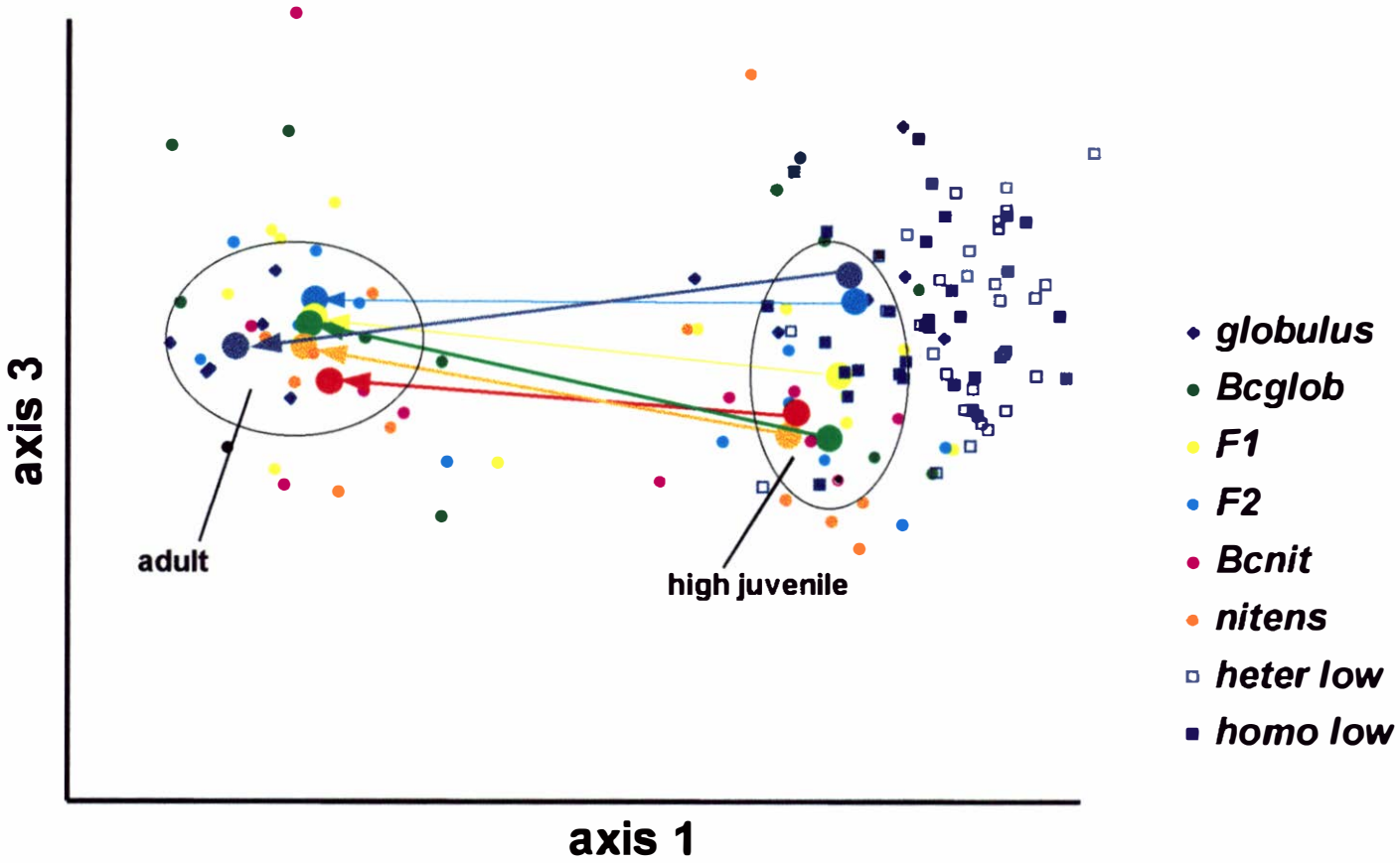


Fig. 2.9. The average positions of samples from high juvenile and high adult foliage for each cross type in the first and third dimension of the 4-dimensional GNMDS ordination based on the abundances of dependent species (standardised to a unit maxima). The arrows indicate the direction of change from the juvenile to the adult foliage based on the average positions of each genetic class. ANOSIM differences between the genetic classes on the high juvenile foliage are significant ($p<0.05$) while there is no significant differentiation between genetic classes on the adult foliage ($p>0.05$).

Table 2.4a. The ordering of samples and dependent taxa by their position along the ONTOGENETIC vector (fitted using samples from the high juvenile foliage and adult foliage. The numbers refer to the sample numbers, juvenile samples are an even number, adult samples are an odd number. The standardised abundance range for each taxa is partitioned into 10 equal width classes; low (1) to high (9). T is total abundance on one sample.

juvenile foliage // adult foliage

```
                111 11 11111 1 111111111  1 1     1     1   1111 111 11111111 11 1 11 1
               813293181441370904203302288291997780899880989331373418123211007247292070
               288880462200448024042662608466426840971573539575133331999517715717331995

36 Gonip. sp   ---1---T-------------------------------------------------------------------
11 Hyme #2     -------T-------------------------------------------------------------------
10 Hymen #1    ----------T-----------------------------------------------------------------
19 Psyll. #5   T2-12523--1255-12--22-375--1--221341-------------------------------------
32 M.lepi #3   -----T-------------5----5-----5-----------------------------------------
 5 Cagr eggs   2-2T---T2-22-3---2-----53225----52-22-----------------------------------
17 Psyll. #4   ----------55---T-------5---------5-----------------------------------------
15 Hete sp.    2-31-426-121-11---12--1T2-5--2-22--1--1----------11------1-------1------
 3 Psyll. #1   2T54359T453334277323375434-5384313221112311-111111111-11111111-11-1111--
 4 Cagr larv   323356395136-35655-263-2836958-13-T-3-21---8-----3---------1--1---1-----
28 Aranae #3   -4-4--42-442-222--2--22T-----6-2-4-----4--2---2-2----------------------
 7 Mpri chew   ---------------------------T-----------------------------------------
30 Homop. #2   -T-82-2--2-92-8562-5532-2-2-326-------------52----2----2----23--2-------
 9 M. lepi #1  -2363225T15--2435313221354-1111327--11--212-2-22--12111-2---2------1---1
25 M. lepi #2  4----T--22--66----4----2422---8---2------2---2-2--2-2-------------------
13 Fung  D     ----------------------T-5--5--------5-----------------------------------
13 Homop. #1   -----------T----TT-----------------------T-------------------------------
33 Hymen #8    --------5--------5-----T-----------T-------------------------5--------
21 lepi #2     ---------5----5--------------------T---------5-------------------------
41 fung   E    ---5-----------------T--------------T-------------------------5--------
43 Fung  I     -525134532362T2542-3126244326291254472635 35-3-143-132134732225-33112-23-
22 Aranae #1   -------T-------------5-----------------------------------------5------5---
44 Fung  H     ----------4--7------4-----------------------------T4--------------------
38 Fung   B    --------------------------4-----T--4-----------------4----4---------4---
37 fung   A    T-------------------------------T----------TT-----------T--------------
29 Miner #4    ----------------------------T-----------------------------------T--------
 8 Cbim eggs   -------T-------------------5-5---------55---5----5----------5-5--5----5-
31 lepi  #3    ---1--1-1-2---1----132-------2--1--21--21-T-21221-----2-6-1--2-----1--21
16 Psyll. #6   ---------------------2-----------------2------2--T-2----2---------------
12 Hyme  #3    ---------------------------------------5---------T----------------------
24 midr hyme   1----1--111---1--11111-2-1-1---7-1--141252554-11T11514122221323322 3T2136
 1 Cag adult   11-111121111111221--22234111122133426138746797873T67748486378T2787896889
14 Hyme # 4    ---------------------------------------T--------T------------------T-----
18 Mite gall   -------------------------4---------------4--------4--T----------4---7-4-
 2 Chrys. #2   ----------------------1-----4---1----5----T--9--3533--2933TT127-1122161
27 Hymen #7    ---------------------------------------4-44T---------4-----4----4---
23 Miner #3    ------------------------------------------------------------T----------
38 M. lepi     ---------------------------------------------4-----74-----------T--
20 Psyll. #6   ------------------------------------------------5-----------T------
26 hymen # 6   ------------------------------------------------------------T------
35 Dipt gall   -------------------------------------------------------------T----
39 Fung   C    --------------------------------------------------------------T-
```

Table 2.4b. The ordering of samples and dependent taxa by their position along the POSITION vector (fitted using samples from the high and low canopies on the homoblastic trees). The standardised abundance range is 1 (low) to 9 (high) and T (total abundance on one particular sample). Numbers above 72 are high foliage samples, numbers 72 and below are low foliage samples.

```
                    high foliage            //          low foliage
                  1     1   11111111 1 1 11 11 111 11 1 1
                  0988973877203102149382900944232211321312681643663 7 3543744555 25 16
                  0448664048062046242024882002882824606484066886242048860240264860606

18 Mite gall      -------T-------------------------------------------------------------
25 M.lepi #2      -822--626-4----42--T4------2--2----2----------2-----2--------2----2
43 Fungi H        --------T---5-------------5-----5-----------------------------------
38 Fung B         4--4-T-------------------------T----------------------4--4-------
44 Fung I         49436224T5-616343213-21245322225T42123522521122212212-112112-12---1
12 Lepi #2        T----------------------5---5--------------------T-5--5-------------
10 Hymen #1       -------------------------T-----------------------------------------
 1 Cagr adult     45T3271137-452-T2113131355119-81914-4311441-112-2132-512-322323731-
20 Psyll. #5      124--15-53-32225--25T12-211-2-7112---72--3----5-1-1-53-----43--3-4-
24 Hyme #5        -------1-11-1-1-12T111-1--113-3-1-15---1--11111-21-11--1----11-1--1
33 Hymen #8     1---------------11----------T-1---------1--2---------------1---2---
13 Homop #1       -------------T---T------T---T-----------------------------T----T---
 4 Cagr larv      --T653-33---662855163955563133236338 8-259953338778435238758175275-2
32 M.lepi #3      -1------------11---2-------------------------T---1-------11--------
 9 M.lepi #1      -1--12-427112-35T332-134535133363224122535134 3-53253-27274146T23724
22 Aranae #1      ---------------------------------5-5------T-------------------------
 8 Cbim eggs      ---------------------5----------5---5----T--------5-----5----------
41 Fung   E       ---------------------------------5--T-----------------------------5---
40 Fung   D       -----------1---1-----1-------------3------T----2--------1--1-------
15 Hete sp.       1--5-2--1-11-122--232--1--21-391-2-221-155823--1322--2-3315-5-172-T
 2 Chrys. #2      ---------3---------------------3-----T-------------3-------------3-3
 5 Cagr eggs      2--2-5-232---2-322--25----2--25T2--3---25T32-28-8-5-33632-68532T283
 3 Psyll. #1      232-3134321523223324243266356434T8677996796686T89T978TT99T9TTTT8989
11 Hyme #2        ------------------------------------------T-------------------------
31 Lepi #3        ----1------3-1-1------1--2-T--1111-1--67-3134-5119111421531311 31-2
36 Gonip sp.      -------------------------------1---------T---------------------1----
30 Homo #2        -2-11-1----12321-1----1222-11--2-1111-3-2-13212535-511258332T2-3213
17 Psyll. #4      ---------2---2-----------42---2----2-2-------2----2---2--4--2---T22
37 Fung   A       -----3--------------3-----------------------------838--8--355--T---
16 Psyll. #3      ---------------------------------5-----------T---5T5--T-T5--T-TT-5
29 Miner #4       -------------------------------------5-----------------------TT----
 7 Mpri chew      -------------------------------------5-------------------T---------T
42 Fung G         ------------------------------------------------------------T----T---
 6 Psyll. #2      ------------------------------------------------------------------T--
```

Table 2.4c. The ordering of samples and dependent taxa by their position along the GENETIC vector (fitted using samples from the low juvenile foliage of five genetic classes: *E. globulus*, backcross *globulus*, F_1 and F_2 combined, backcross *nitens*, *E. nitens*). The standardised abundance range is 1 (low) to 9 (high) and T (total abundance on one particular sample).

E. globulus **//** **hybrids** **//** ***E. nitens***

```
                15  543655662556 312 475463 22371 3646136452 5417436313241 242
                089776416326904351951522172806705608001958174937195486634234 78

20 Psyll. #6    ---------T-------------------------------------------------------
25 M.lepi #2    -3-53-----33------T-3------33-----------------------------------
19 Psyll. #5    6892-5T8658-455523-32---311---2-3-----212--11-1--12----1-1---2
37 Fung   A     -8-55---55---T5-33----83------3-------------83------3--5------
42 Fung   G     ------------4T---------T-----------------------------------------
21 Lepid #2     -----4-T-------4-4---4----7-----------------44-------------------
38 Fung   B     ----T---3-------3-----------------------------------------38----
 5 Cagr eggs    63-433-86-63-84732-2--353263323-2252-4-T3-674--3244--32322-2--
16 Psyll. #3    -4--7--4--74-7--4---T-74-T-7-----7--7-7-7-4-4-------7---------
28 Aranae #2    2422322763453T367-3-5-5237-3262427-224--433-333-2-2--43-2633--
17 Psyll #4     2---2-2----2--2-7---72--24--2-2-2T2-4-------2---------2---2---
15 Hete sp.     --416111-1-8T54-13221134222-211221-21411121211-51--1-6-13-312-
29 Miner #4     --------------------T------T-T-----------------------------------
 2 Chrys #2     --44-------4----4-7-4------------4--------4-4--4-T------------
 3 Psyll. #1    879T8T9T95T988T8T87T7989899T6T8969T5T754T8T588T7877876649TT656
36 Gonip sp.    ----------------------------T-----------------------------------
41 Fung E       -------5-----5-------------------------5------T---------------
30 Homop #2     11-121--232323T2-3--2-53-2321-151223321-2212--3-21-5511-8---11
40 Fung   D     --------15--3---51------12--3-------------2----2---2-T--1-----
 9 M.lepi #1    2-12222342-4636484-22-21233T4222-77343139452522-24453135733513
 4 Cagr larv    -52--2-61282-7754-T12688527582389533591763234 7T613673539765553
 7 Mpri chew    -----------T------4-------------------------2---------T-4---
 1 Cagr ad.     1--11523212-4721416-T-223322-351231-333-31433226327-2115-96128
44 Fungi I      -2122-312-212---1323-211321112124-11122241112125121212242T2232
27 Hymen #7     ------------------------------------5---T--------------------
24 Hyme #5      -1-12----111-211-1-22-1-1143T-11---1--2--241-2-1212-1211-11226
13 Homop #1     -------------5---------5------------------T-------------------5
31 M.lepi #3    ------------------------11------11---1----------1------T-------
35 lepi #2      -11-11-111-11223111111121111-11111213511222254 11T1631224116417
 6 Psyll #2     ------------------------3--------3-----------------------T---
 8 Cbim eggs    ------------------------------------------T----------TT-----
22 Arach #1     ----------------------------------------T-----T----------T--T-
43 Fung H       ------------------------------------------------------------T----
33 Hymen #8     ----1---1----2--------------------2-1--1-6---1-12-----------T
```

There was a highly significant difference in the community composition amongst the six genetic classes on the low foliage in the ordination using standardised abundance data which was also evident in the ordination using presence/absence data ($p<0.001$; Table 2.3; Fig 2.7b). On the low juvenile foliage, the changes associated with genetic class were in a different direction to the changes associated with changing ontogeny or changing position in the canopy. In the ordination space, the GENETIC vector and the ONTOGENETIC vector were separated by 54° and the GENETIC vector and POSITION vector were separated by 67° (180°-113°; Fig. 2.7a,b; Table 2.2). The differences of the average positions of each of the genetic classes on the GENETIC vector can be seen in Figure 2.10. and in the ordination in Figure 2.9. The communities on the *E. globulus* and *E. nitens* foliage were separated in the ordination space with only a small degree of overlap (Fig. 2.7b) and the differences between the two were highly significant ($p<0.001$; Table 2.3).

The communities on the F_1 hybrids lie effectively intermediate in the ordination space between the parental samples (Fig 2.7b and 2.9), as would be expected under an additive genetic model. These differences between the F_1 communities and the *E. globulus* and *E. nitens* were significant ($p>0.01$) and highly significant ($P<0.001$), respectively (Table 2.3). As a population, the four hybrid classes exhibit a continuum between the two parental types with the positions occupied by the samples from the backcross hybrids being close to their respective parental samples (Fig. 2.7b). There were significant differences in community composition among the advanced generation hybrids and their respective parental types (Table 2.3). Further, the backcross *globulus* had a community more similar to the F_1 hybrid than *E. globulus* (Fig 2.10). The change in community composition along the genetic gradient was characterised by a change from those taxa clearly associated with *E. globulus,* such as Microlepidopteran #2 (25), Psyllidae #5 (19) and Psyllidae #6 (20), to only one species which was clearly associated with *E. nitens, Chrysophtharta bimaculata* eggscars (8) (Table 2.4c).

The communities on the different genetic classes were compared on the low juvenile foliage as this did not compound community variation due to changing canopy position and changing ontogeny. However, the community compositions were also looked at on the high juvenile and adult foliage samples separately. There was found to be a significant response to genetic variation in the high juvenile foliage but not in the adult foliage (Tables 2.2a and 2.3).

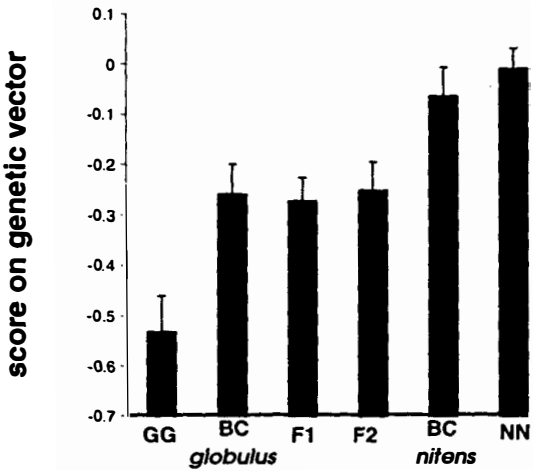


Fig. 2.10. The average position of each cross type on the genetic vector (GENETIC) fitted for samples from the low homoblastic and low heteroblastic trees.

Discussion

The impact of ontogeny

The comparison of adult and juvenile foliage in the same position in the tree canopy in the present study clearly shows that ontogenetic variation in the *Eucalyptus globulus* x *nitens* hybrid system has a major influence on the composition of dependent communities. This effect was shown to be even greater than the influence of genetic variation between these two species and their hybrids and the response to ontogenetic variation was effectively the

same for each cross type examined. In addition, the study demonstrated the large impact that changing position in the canopy can have on the structure of dependent communities. The absence of significant differences in the dependent communities on the low juvenile foliage of the heteroblastic and homoblastic morphs indicates that the changing response to the juvenile and adult foliage could not be due to gross genetic differences between these heteroblastic and homoblastic ontomorphs, or being influenced by the communities on the adult foliage in those trees which had phase changed.

Differences between the adult and juvenile foliage in *Eucalyptus* in susceptibility to attack by dependent species have previously been reported. The fungus *Mycosphaerella nubilosa* is found on the juvenile foliage of *E. nitens* and *E. globulus* (Dungey *et al.* 1997); there are differences in susceptibility to the major insect pests. For example, *Mnesampela privata* (Autumn Gum Moth) larval feeding is reported on juvenile foliage of *E. globulus* (Farrow *et al.* 1994) and the paropsine chrysomelids *Chrysophtharta bimaculata* and *C. agricola* are considered to be pests of adult and juvenile foliage, respectively (de Little 1989; Li 1995). Two important factors separate the study undertaken here from the above mentioned studies. Firstly, these studies have concentrated on responses at the individual species level to ontogenetic change, and secondly, they have invariably confounded differences in foliage types with variation in other factors such as canopy position, physiological age, growth conditions and genetics in the plants examined. However, responses by individual species to ontogenetic change have been shown independently of these confounding factors in other genera. Bryant *et al.* (1985) looked at the differential response by the Snowshoe hare (*Lepas americanus*) to foliage of juvenile and adult type in Feltleaf willow (*Salix alaxensis*) and Kearsley and Whitham (1989) showed opposing responses by two species of insects, the chrysomelid beetle *Chrysomela confluens* and the aphid *Pemphigus betae,* to ontogenetic change in *Populus angustifolia.* In addition, while detailed studies of these responses of individual species to ontogenetic change are rare, studies of the responses of dependent species at the community level are even rarer. There is only one case, in the genus *Populus* (Waltz and Whitham 1997) and this study appears to have unavoidably confounded positional and ontogenetic effects. This present study is the

first to show a response by dependent communities to ontogenetic change at the community level independent of canopy position.

The importance of looking at changing ontogeny independently of changing position in the canopy is demonstrated here in the comparison of communities on the same foliage (ontogenetic and genetic) from the high and low positions in the canopy. A significant change in the composition of dependent communities was found in response to this positional effect. With changing height in the canopy may come corresponding changes in availability of nutrients. Recher *et al.* (1996) found that there were differential flushes in nutrients towards the growing tips of the tree and one of these nutrients, nitrogen, is widely considered to be a limiting factor in insect growth and reproductive capacity (Mattson 1980). There are also micro-climatic factors which will change with position in the canopy such as light, frost and wind exposure. The methods employed in this study, using juvenile and adult foliage from the tops of two trees with similar genotypes and differing degrees of phase change, enabled this important effect of changing position in the canopy to be removed from the analysis and therefore enabled the examination of the response to changing ontogeny independent of changing height.

The exact factors which are causing this marked change in dependent community composition are difficult to define. Several authors have noted the striking morphological differences between the juvenile and adult foliage types in *Eucalyptus* (Johnson 1926; Cameron 1970; Pryor 1976; Ashton and Turner 1979; Pederick 1979; Beadle *et al.* 1989) In addition to these morphological differences which might affect dependent species distributions, there are also chemical differences such as in wax yield and composition (Li *et al.* 1997), which are thought to affect species colonisation (Edwards 1982; Li 1993). There are also differences in the habits of the juvenile versus the adult foliage, the former are horizontal, sessile, and arranged opposite on the stem, while the latter are petiolate, alternately arranged and vertically orientated. These differences result in a markedly different canopy structure and density which, by themselves, may affect species

distributions. These are all factors which could potentially alter the responses of dependent species, both by direct responses and by responses at higher trophic levels.

It was shown that genetic variation across the hybrid system had a significant effect on dependent community composition, although this effect was subtle in comparison to ontogenetic variation. Significantly different communities of dependent species were found on the *E. globulus* and *E. nitens* parental species. Community differences between the different genetic classes was examined on the low juvenile foliage as this effectively removed any effects due to changing position or ontogeny. There was also significant differentiation between these genetic classes on high juvenile foliage but not on the adult foliage. A possible explanation for this response is the lack of a source of colonisation on the adult foliage, while the juvenile foliage had a source of dependent species from the lower canopy. Thus there would not have been sufficient species present on the adult foliage to show a significant differential response. The close taxonomic and morphological relationship of the two eucalypt species might suggest that traits which attract or deter insects would also be quite similar, however it is seen here that this is not the case, and that the dependent taxa used in this study do effectively discriminate between the two species.

The communities on the hybrid classes were generally intermediate between the communities on the parental types and there was no evidence of marked changes in variability between the different genetic classes. More particularly, the F_1 hybrids supported a community which was effectively intermediate between the parental types. The communities on the F_2 hybrids were very similar to the communities on the F_1 hybrids. The advanced generation backcross hybrids supported communities which were more similar to, and intermediate between the F_1s and their corresponding parental type. This is the first study of the response of dependent taxa to advanced generation hybrids (pedigreed) in a common garden. Previously, common garden experiments looking at the responses of dependent species have looked only at the first generation hybrids (Messina *et al.* 1996).

To date, most studies of dependent species responses to hybrid plants have been conducted in natural stands of hybrids (Whitham *et al.* 1994; Morrow *et al.* 1994; Aguilar and Boecklen 1992; Christensen *et al.* 1995; Floate *et al.* 1993). These studies inevitably confound environmental variation (e.g. due to micro-site differences and associated species [see Whitham *et al.* 1994]) with genetic effects. It is only through the use of common garden trials, as in the present case, that environmental and genetically induced response can be separated. The present study clearly demonstrates a true genetic effect on the composition of dependent communities.

Genetic variation is considered to be a major source of variation in determining the distributions of dependent species in *Eucalyptus* (Farrow *et al.* 1994; Morrow *et al.* 1994; Raymond 1995) and in other genera (Floate *et al.* 1993; Fritz *et al.* 1997). Variation in host plant quality which occurs in a forest ecosystem most commonly refers to the variation which arises between plants due to changing genotypes and/or environment (Linhart 1989; Kearsley and Whitham 1989). This study has shown that community level responses by dependent species occur in response to genetic variation between two taxonomically and morphologically similar species. Moreover, it has been shown in the present study that a further type of genetic variation, variation within a plant through time (ontogenetic variation), can have an influence on the structure of dependent communities which far surpasses the significant response to genetic variation in the traditional sense.

This study demonstrates that variation with changing ontogeny in a single eucalypt species is likely to play a major role in the determination of dependent community structure within a population of trees. The ecology of eucalypts is such that after major disturbance events (e.g. fire) regeneration occurs, often with a parallel reversion to juvenile foliage of large expanses of forest. The present study suggests that the reversion to juvenile foliage, associated with such events will also cause major changes in the structure of dependent communities associated with the eucalypts. The implications of this for the conservation of biodiversity in natural and managed stands and for pest management in commercial plantations are considerable and have barely been considered to date.

Chapter 3

Individual taxa responses

Introduction

Dependent taxa exhibit differing degrees of specialisation to different host plants, with insects and fungal pathogens classified as monophages, oligophages or polyphages depending on their degree of specialisation (Schoonhaven 1998). Co-evolution may occur between plants and their dependent species with the subsequent ecological linking of a dependent species with its host plant (Ehrlich and Raven 1964, Rhoades 1979). Dependent species may also respond to ontogenetic variation within plants (Kearsley and Whitham 1989;1998; Waltz and Whitham 1997; Chapter 2) and specialise in feeding on a specific ontogenetic stage of the host plant (Waltz and Whitham 1997). This concept has not been explored in detail and this chapter examines the response by individual dependent taxa to ontogenetic variation in the *E. globulus* x *nitens* hybrid system.

The chapter examines the response of individual species to both ontogenetic variation and genetic variation between the parental eucalypt species and the hybrid classes of known pedigree. Dependent taxa exhibit variable responses to hybrids (Strauss 1994; Fritz *et al.* 1994, 1997; Whitham *et al.* 1997) which have been classified as susceptible, dominant, additive and resistant responses (Fritz *et al.* 1994). With the use of a common environment field trial using progeny of known pedigree the genetic basis of these responses can be determined (Dungey 1996; Messina *et al.* 1996). Four questions are explored:

1) Do individual dependent taxa exhibit preferences for one or the other ontogenetic types?

2) Do dependent taxa exhibit preferences for one or the other parental host species?

3) How do dependent taxa respond to the hybrid classes?

4) What is the degree of specialisation to ontogenetic variation when compared with genetic variation in this hybrid system?

Due to the significant pest status of the chrysomelid leaf eating beetle this taxa will be dealt with in the most detail.

Methods

The responses of individual taxa were analysed using the abundance data presented in Chapter 2. Analysis was undertaken using the GLM and MIXED procedures in SAS (SAS 6.12, 1997). The abundance of a given taxa was examined using the mixed model:

$$\text{abundance} = \text{mean} + \textit{replicate} + \text{pctype} + \text{ctype} + \text{position} + \text{pctype*ctype} + \text{ctype*position} + \text{pctype*position} + \text{pctype*position*ctype} + \textit{error} \qquad \textbf{Equation 3.1}$$

where 'replicate' is the replicate number, 'pctype' is the phase change type (heteroblastic, homoblastic), 'ctype' is the cross type (*E. globulus*, BC*globulus*, F_1, F_2, BC*nitens*, *E. nitens*), 'position' is position in the canopy (upper or lower). Replicate and error were treated as random effects and pctype, ctype, position and their interactions were treated as fixed.

The transformation of the data which optimised the normality and homogeneity of the residual variances was initially determined (Table 3.1) by fitting the model in PROC GLM in SAS. The same model was subsequently fitted as a repeated measures mixed model using PROC MIXED with trees treated as subjects. Specific contrasts were undertaken with the CONTRAST statement in PROC MIXED to test for foliage type (homoblastic low, homoblastic high, heteroblastic low, heteroblastic high), ontogenetic (high juvenile versus adult), positional (high juvenile versus low juvenile on the homoblastic trees), ontomorphic (heteroblastic low juvenile versus homoblastic low juvenile) and various genetic (*E. globulus* versus *E. nitens*, F_1 vs F_2, F_1 versus mid-parental value, F_2 versus mid-parental value, *E. globulus* vs backcross *globulus*, *E.*

nitens versus backcross *nitens*) effects. This analysis was only undertaken for dependent taxa which occurred on 6% or more of the samples (see Table 2.1).

#	Dependent taxa	Transformation
1	*C. agricola* (adult)	arcsin
2	Chrysomelid beetle #2	log10
3	Psyllidae #1	untransformed
4	*C. agricola* (larval)	*square root*
5	*C. agricola* (eggscar)	*arcsin*
7	*Mnesempala privata*	*arcsin*
8	*C. bimaculata* (eggscar)	untransformed
9	Microlepidopteran #1	arcsin
15	*Heteronyx sp.*	*arcsin*
17	Psyllidae #4	arcsin
19	Psyllidae # 5	log10
21	Lepidopteran #2	untransformed
22	Arachnae #1	arcsin
24	Hymenopteran #5	log10
25	Microlepidopteran #2	log10
27	Hymenopteran #7	untransformed
28	Aranae #3	arcsin
30	Homoptera #2	log10
32	Microlepidopteran #3	untransformed
40	*Cylindrosporium samueli*	untransformed

Table 3.1. The 20 dependent taxa for which detailed analysis of their distributions were undertaken. The taxa number and names are detailed in Table 2.1. The transformations of the abundance score which was used in the analysis are indicated.

Dependent taxa were classified as: 1) 'ontogenetic specialists' if they showed a significant ($p<0.05$) ontogenetic effect and 2) 'species specialists' if their abundance on *E. nitens* and *E. globulus* differed significantly ($p<0.05$). Taxa were classified as generalists in both cases if they did not show a significant response ($p>0.05$). Further the responses of dependent taxa to F_1 hybrids were classified following Fritz *et al.* (1994) and Dungey (1996) as: 1) 'susceptible' if their abundance on the F_1 was greater than the most susceptible pure species; 2) 'additive' if the mean abundance on the F_1 hybrid class was intermediate to the pure species and not significantly different ($p>0.05$) from the mid-parent value; 3) 'resistant' if the mean abundance on the F_1 was significantly ($p>0.05$) lower than the most resistant pure species and 4) 'dominant' if the mean abundance on the F_1 hybrids was at least as high as the most susceptible pure species and not significantly different ($p>0.05$) from that species.

Results

The results of the mixed model analysis of the responses by individual dependent taxa are given in Table 3.2 and least squares means of the ontogenetic and genetic main effects are plotted in Fig. 3.1 –3.20.

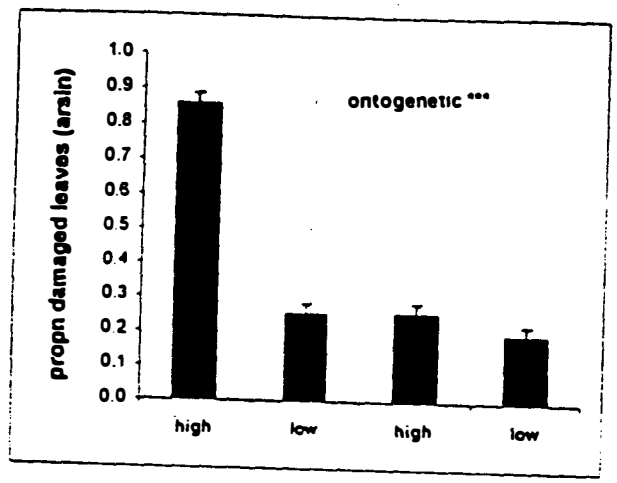
Of the taxa used in the community analysis 20 were present in sufficient numbers to allow a detailed individual analysis of their distributions across the different foliage types (ontogenetic and position) and genetic classes. Eighteen of these taxa were insects, 15 (90%) of which showed a significant ($p<0.05$) preference for either adult or juvenile (high) foliage and were thus ontogenetic specialists. Ten (56%) of these were highly significant ($p<0.001$). Dependent taxa which exhibited a highly significant ($p<0.001$) preference for juvenile foliage were: *Ctenarytaina eucalypti* (3); *Chrysophtharta agricola* larval damage (4) and eggscars (5); Microlepidopteran #1 (9); *Heteronyx* sp. (15), Psyllidae #5 (19), Microlepidopteran #2 (25) and Homoptera #2 (30). Taxa which exhibited a highly significant ($p>0.001$) preference for adult foliage were: *Chrysophtharta agricola* adult (1); Chrysomelid beetle #2 (2) and Hymenopteran #5 (24).

All of the lepidopteran taxa exhibited preferences for the juvenile foliage type as did all of the Psyllids (sapsuckers). In contrast, both hymenopteran species with a significant ontogenetic preference were found on the adult foliage type. Two spider morphs and one fungal type were present in sufficient numbers to examine their distribution but did not show a significant preference for adult or juvenile foliage. The ontogenetic preferences were tested by comparing the adult and high juvenile foliage, a comparison which removed the effects of position in the canopy. This effect of changing height in the canopy on homoblastic trees was shown to be significant in 40% (eight) of the taxa examined. However, only four taxa showed a highly significant positional effect (Table 3.2).

Table 3.2. Responses by individual species (abundance>6%) to ontogenetic, genetic and positional change. The values in the table are the F values and their significance for contrasts: 1) between genetic classes (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). 2) amongst foliage types (homoblastic low juvenile, homoblastic high juvenile, heteroblastic low juvenile, heteroblastic high adult); 3) between ontogenetic types (high juvenile and high adult); 4) between ontomorphic types (homoblastic low juvenile and heteroblastic low juvenile) and 5) between canopy positions (homoblastic low and homoblastic high).

Dependent taxa	genetic	foliage	G vs N	sp vs h	F1 vs F2	F1 vs mp	F2 vs mp	ontogenet	ontomorpl	position
df.	5	3	1	1	1			1	1	1
1 ***Chrysoptharta agricola*** (adult)	1 ns	119 ***	1 ns	0 ns	4 ns	1 ns	1 ns	158 ***	3 ns	3 ns
2 Chrysomelid beetle #2	1 ns	17 ***	0 ns	2 ns	0 ns	4 *	3 ns	34 ***	4 ns	0 ns
3 Psyllidae #1	3 **	206 ***	9 **	2 ns	1 ns	4 *	1 ns	74 ***	2 ns	178 ***
4 ***Chrysoptharta agricola*** (larval)	2 ns	29 ***	9 **	2 ns	0 ns	1 ns	1 ns	47 ***	2 ns	3 ns
5 ***Chrysoptharta agricola*** (eggscar)	2 ns	23 ***	0 ns	9 **	0 ns	2 ns	3 ns	24 ***	1 ns	13 **
7 ***Mnesempala privata***	2 ns	2 ns	0 ns	2 ns	1 ns	1 ns	7 **	1 ns	0 ns	3 ns
8 ***Chrysoptharta bimaculata*** (eggscar)	1 ns	5 **	1 ns	3 ns	0 ns	0 ns	1 ns	5 *	3 ns	0 ns
9 Microlepidopteran #1	3 *	11 ***	4 **	5 **	0 ns	8 **	6 *	13 ***	3 ns	5 *
15 ***Heteronyx sp.***	1 ns	18 ***	1 ns	1 ns	1 ns	3 ns	1 ns	17 ***	1 ns	4 ns
17 Psyllidae #4	3 *	8 ***	3 ns	2 ns	6 *	8 **	0 ns	6 *	0 ns	5 *
19 Psyllidae # 5	4 ***	49 ***	9 **	0 ns	1 ns	0 ns	1 ns	102 ***	3 ns	12 ***
21 Lepidopteran #2	1 ns	1 ns	1 ns	0 ns	1 ns	1 ns	0 ns	1 ns	1 ns	0 ns
22 Arachnae #1	0 ns	1 ns	1 ns	0 ns	0 ns	0 ns	0 ns	0 ns	1 ns	0
24 Hymenopteran #5	*	59 ***	4 *	1 ns	7 **	7 **	0 ns	125 ***	0 ns	0 ns
25 Microlepidopteran #2	2 ns	13 ***	8 *	0 ns	3 ns	0 ns	1 ns	33 ***	0 ns	23 *
27 Hymenopteran #7	1 ns	3 *	0 ns	2 ns	1 ns	1 ns	3 ns	6 *	1 ns	0 ***
28 Aranae #3	*	4 ns	10 **	4 *	0 ns	2 ns	4 ns	4 ns	4 *	4 ns
30 Homoptera #2	ns	15 ***	0 ns	1 ns	0 ns	1 ns	2 ns	9 **	8 *	17 *
32 Microlepidopteran #3	1 ns	2 ns	1 ns	1 ns	0 ns	1 ns	1 ns	4 *	1 ns	1 ***
40 fungal type D	ns	2 ns	0 ns	3 ns	1 ns	4 *	1 ns	1 ns	0 ns	2 ns

Fig. 3. The mean abundance and standard errors of individual dependent taxa on a) the homoblastic low, homoblastic high, heteroblastic low and heteroblastic high foliage. The ontogenetic effect is compared on the high juvenile and high adult foliage; (b) the different genetic (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens* and *E. nitens*).



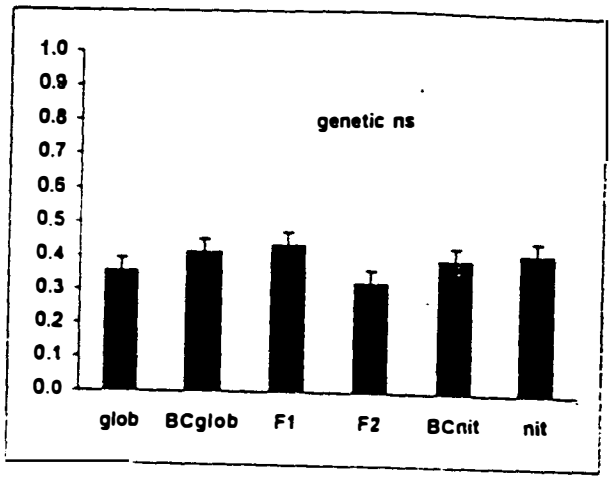
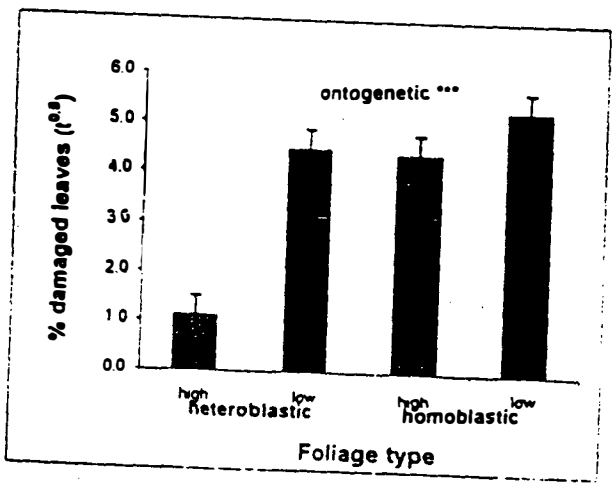
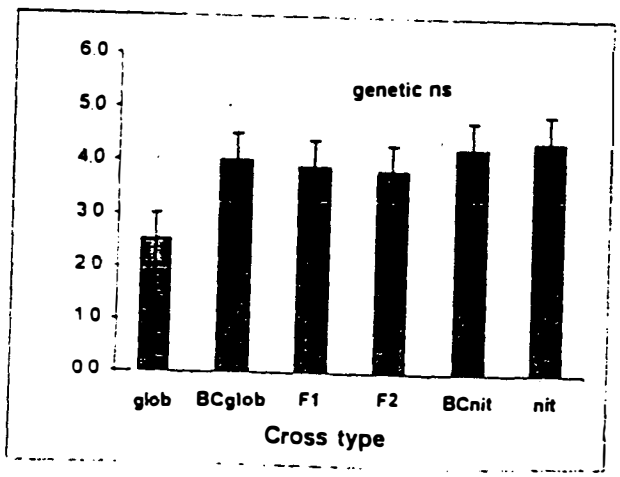


Fig. 3.1. *Chrysoptharta agricola* adult damage (1)
a)

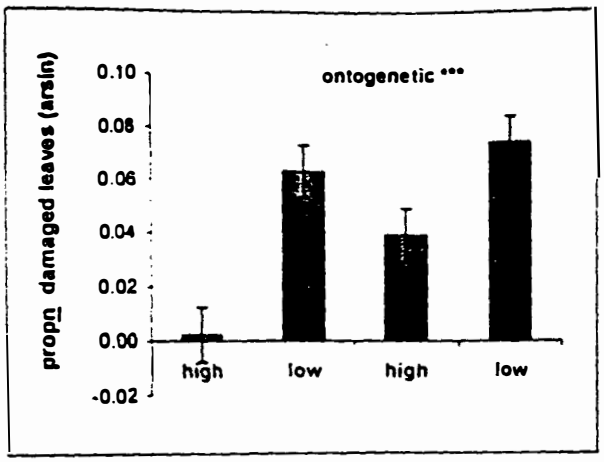
b)

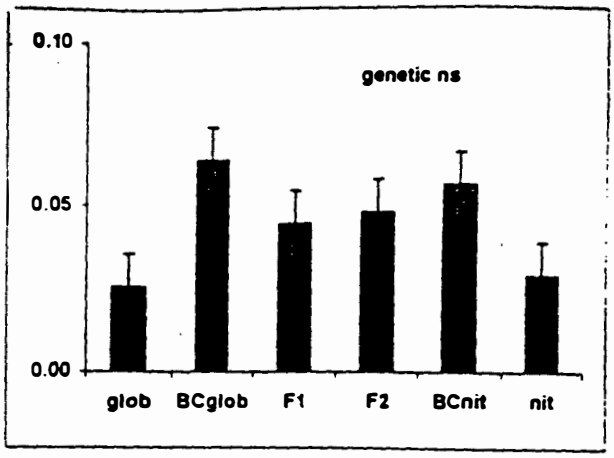




a)
Fig. 3.2 *Chrysoptharta agricola* larval damage (4)

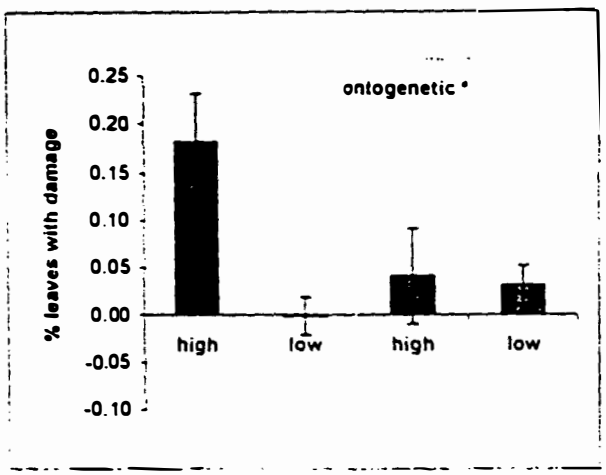
b)

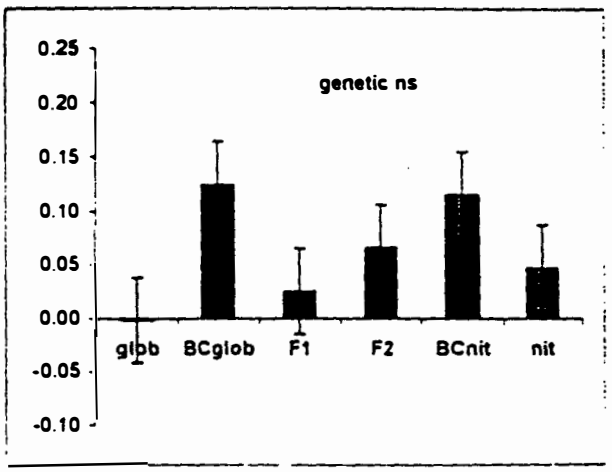




a) b)

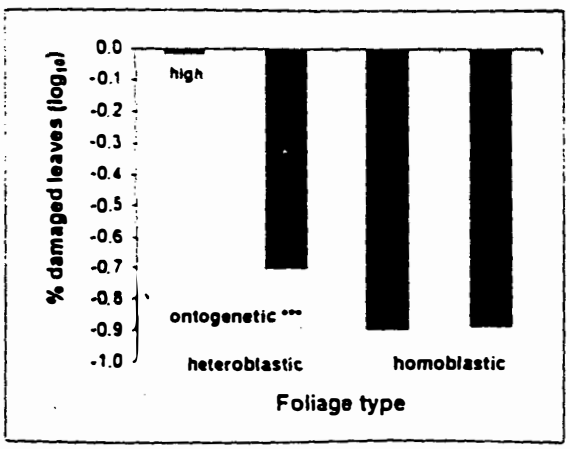
Fig. 3.3 *Chrysoptharta agricola* eggscars (5)

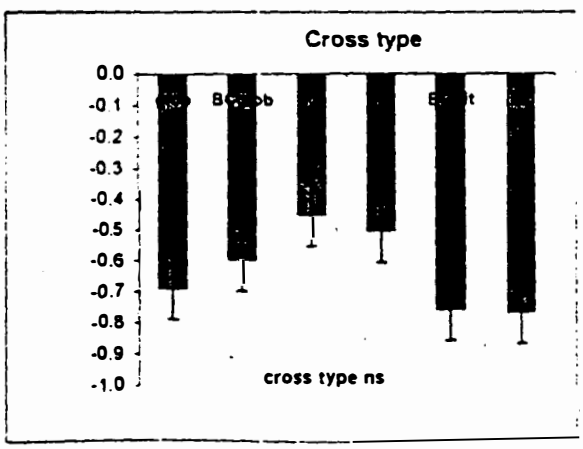




a) *b)*

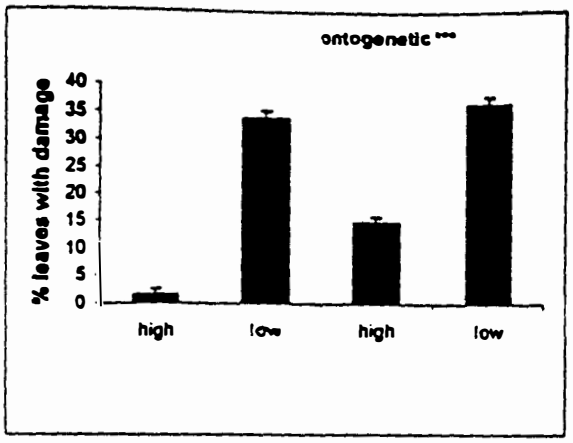
Fig. 3.4. *Chrysoptharta bimaculata eggscars* (8)

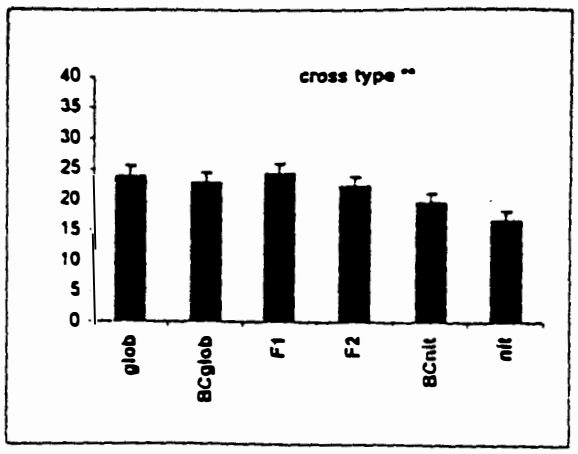




a) *b)*

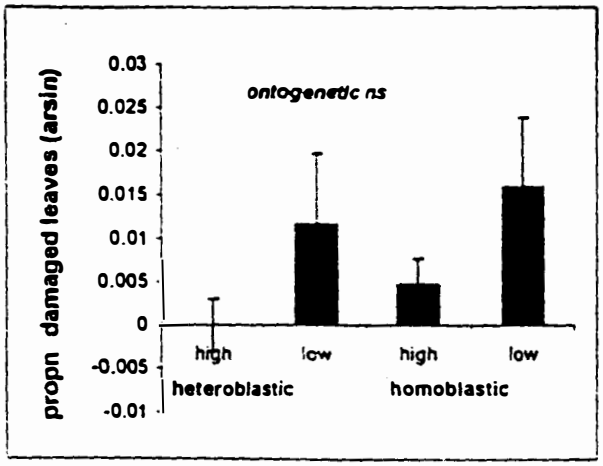
Fig. 3.5. Chrysomelid beetle #2 (2)

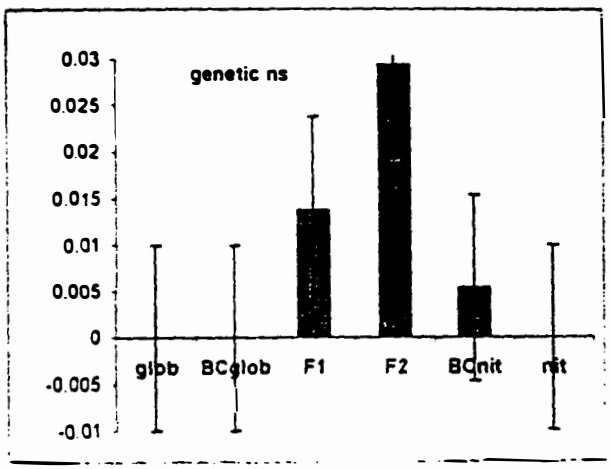




a) b)

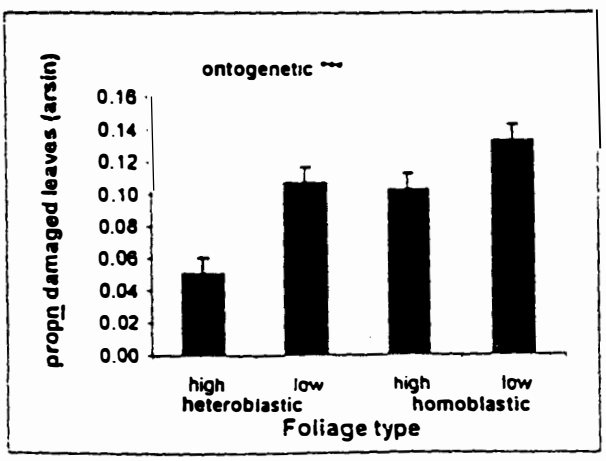
Fig. 3.6 *Ctenarytaina eucalypti* (3)

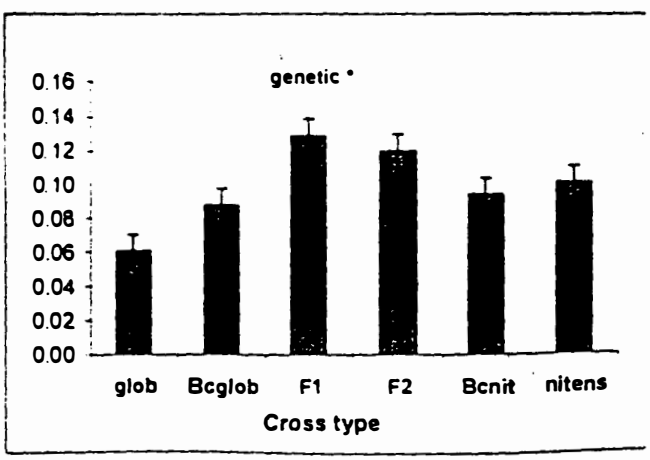




a) *b)*

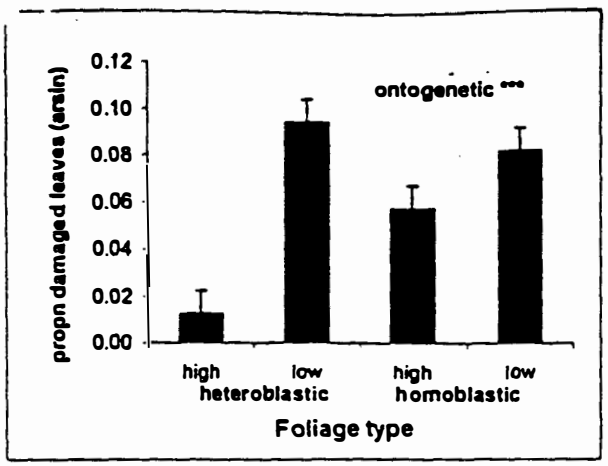
Fig. 3.7. *Mnesampela privata* (7)



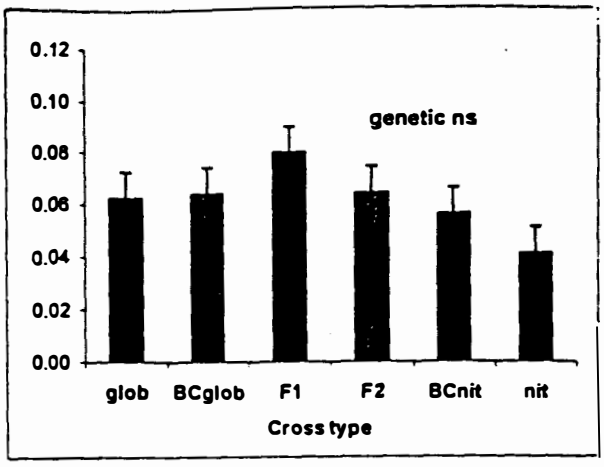


a) *b)*

Fig. 3.8. Microlepidopteran #1 (9)

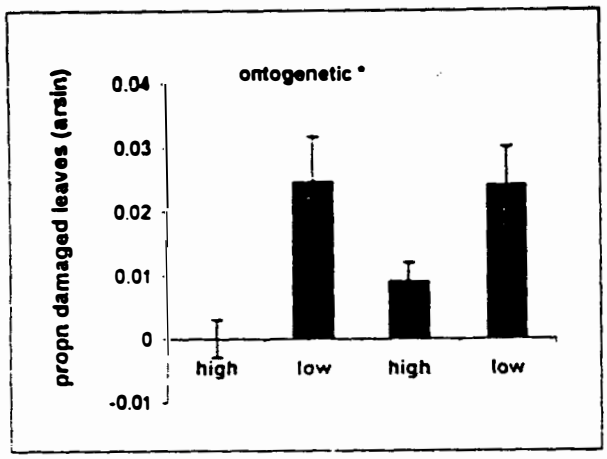


a)

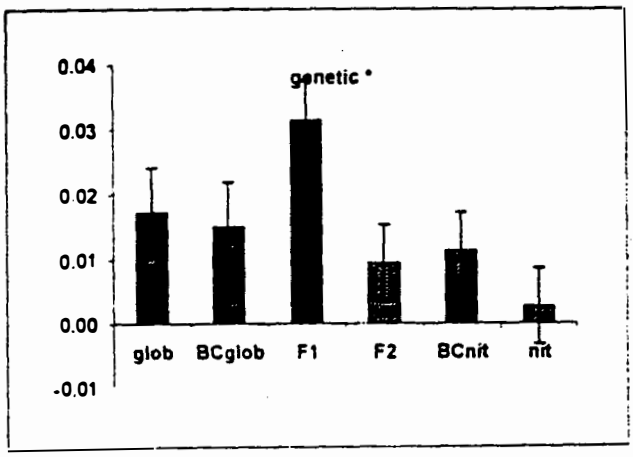


b)

Fig. 3.9 *Heteronyx* sp. (15)

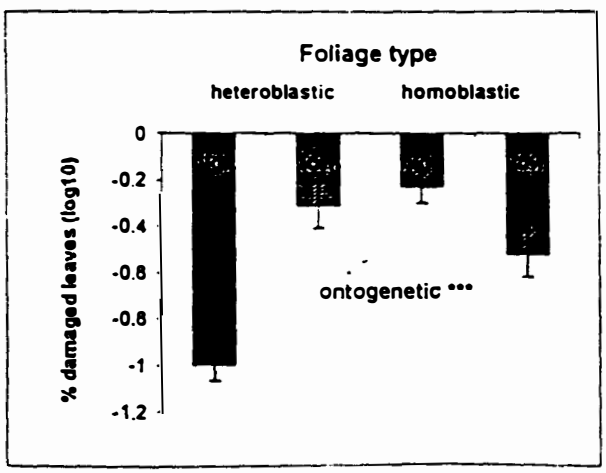


a)

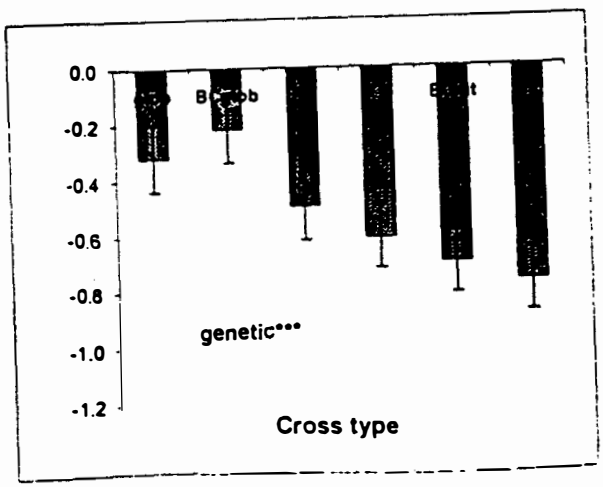


b)

Fig. 3.10. *Psyllidae* #4 (17)

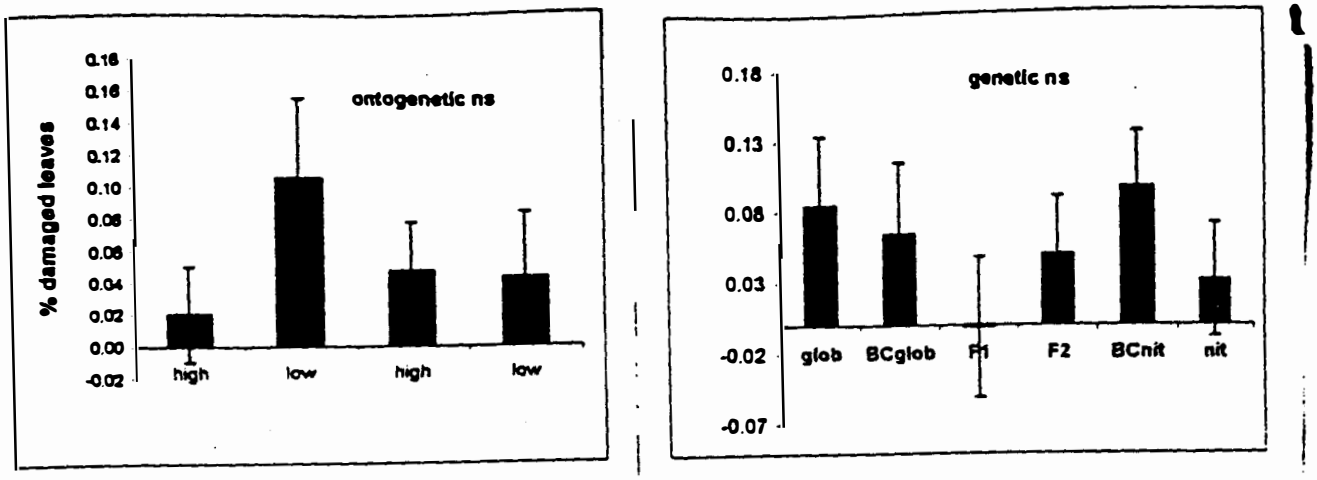


a)



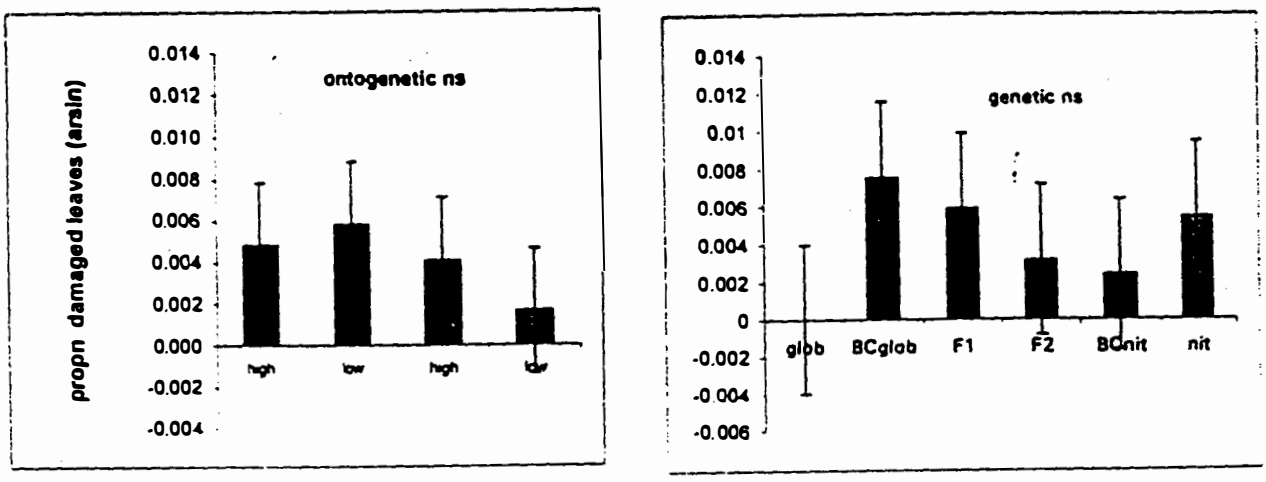
b)

Fig. 3.11. Psyllidae #5 (19)



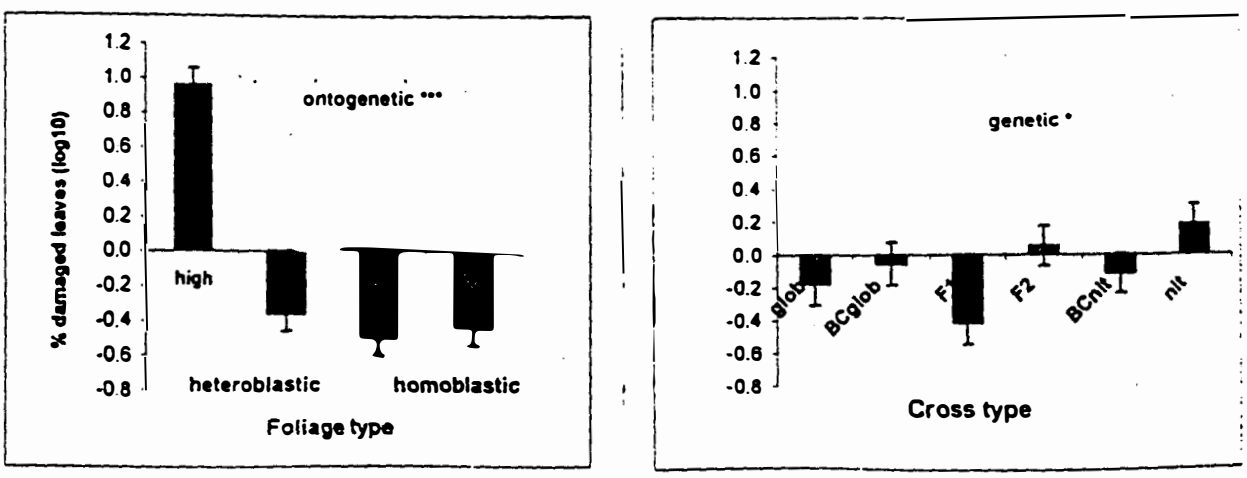
a) b)

Fig. 3.12 Lepidopteran #2 (21)



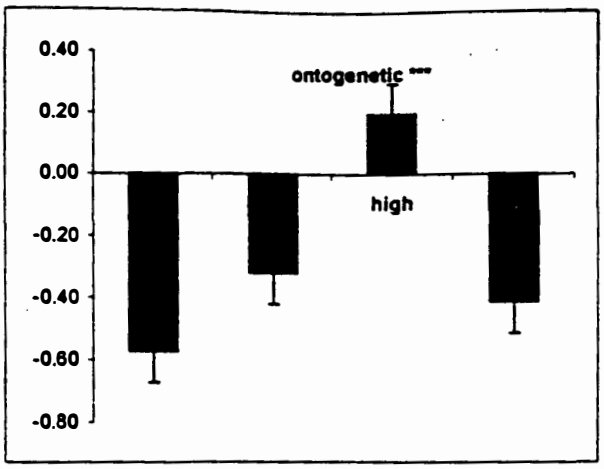
a) *b)*

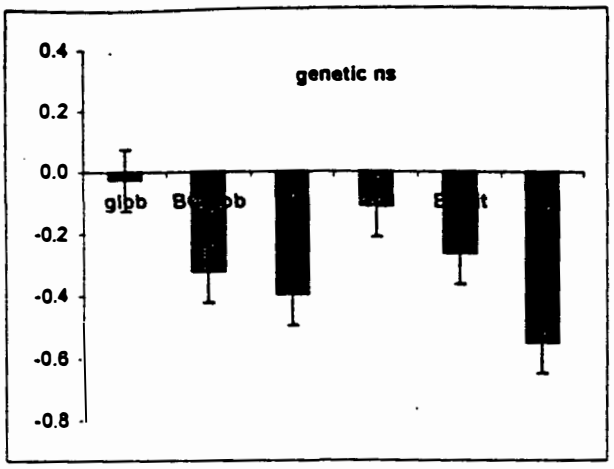
Fig. 3.13. Aranae #1 (22)



a) *b)*

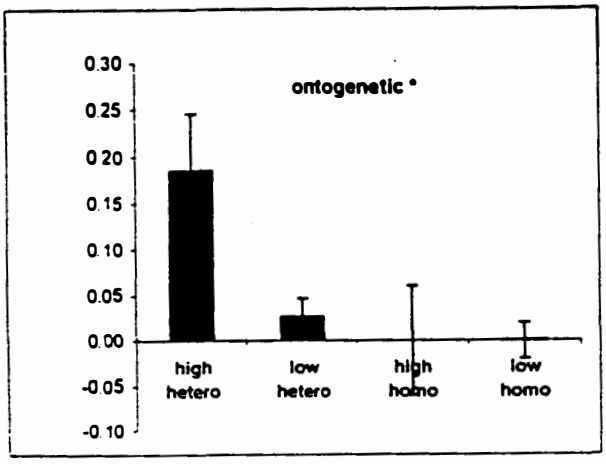
Fig. 3.14. Hymenopteran #5 (24)

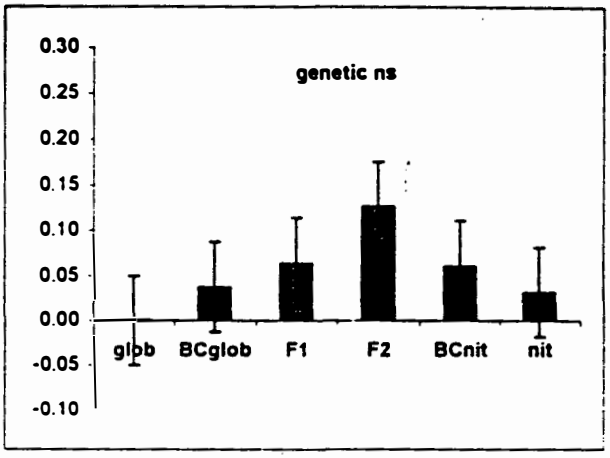




a) b)

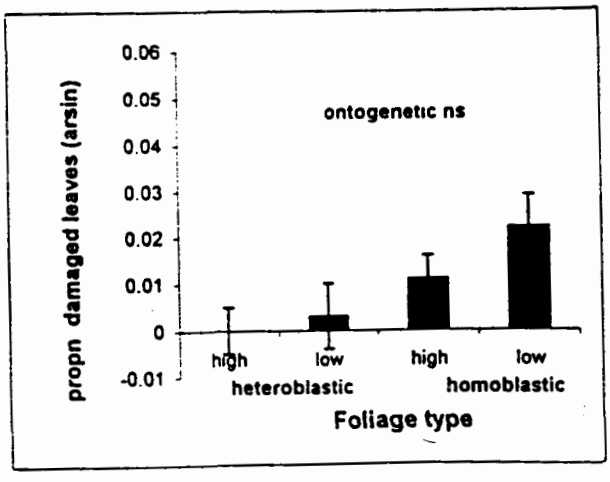
Fig. 3.15 Microlepidopteran #2 (25)

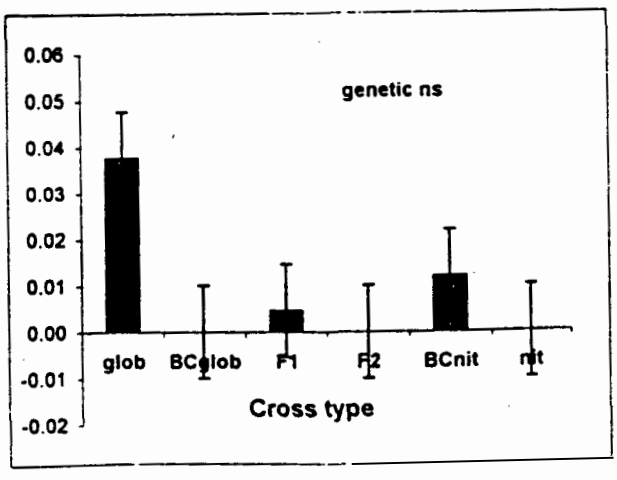




a) *b)*

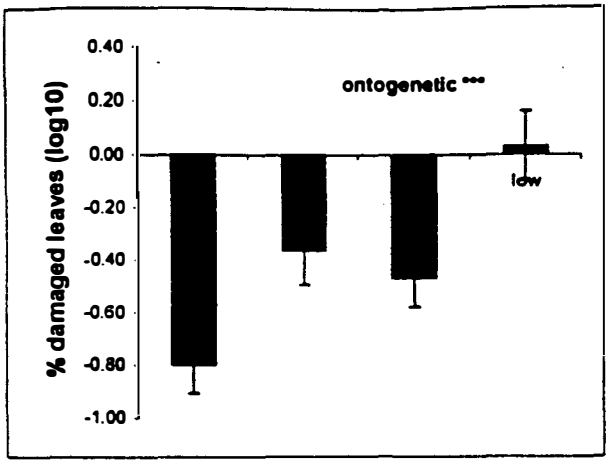
Fig. 3.16. Hymenopteran #7 (27)

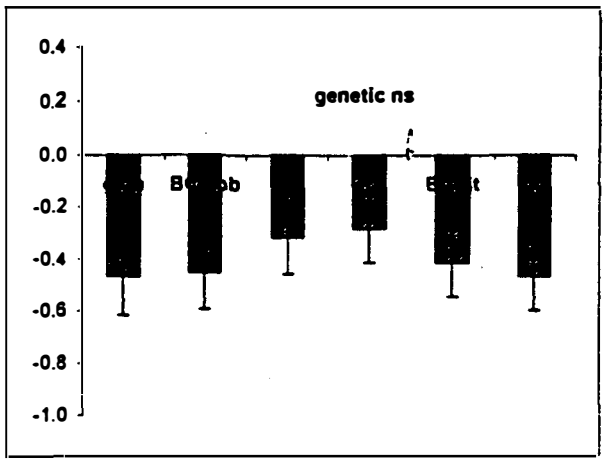




a) *b)*

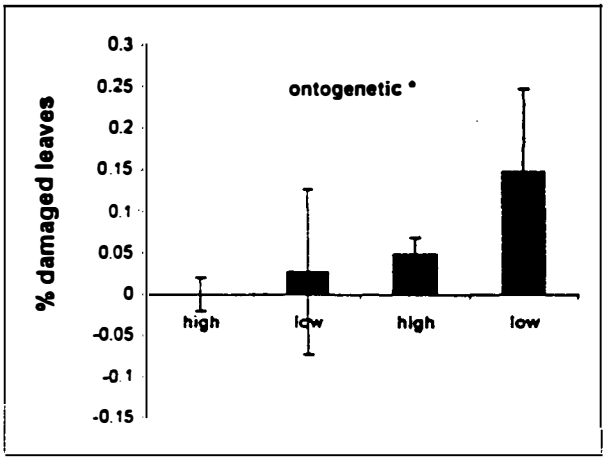
Fig. 3.17. Aranae #2 (28)

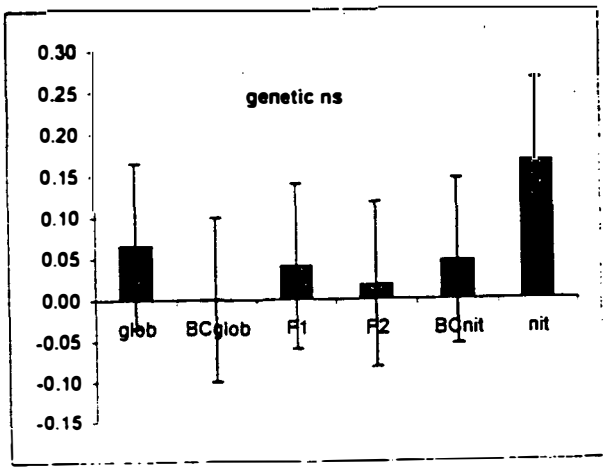




a) b)

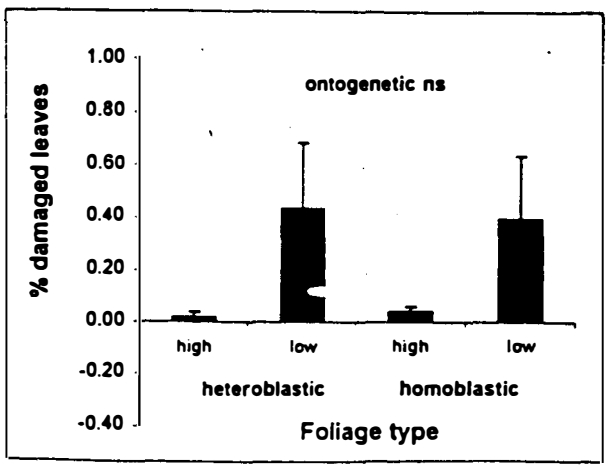
Fig. 3.18. Homoptera #2 (30)

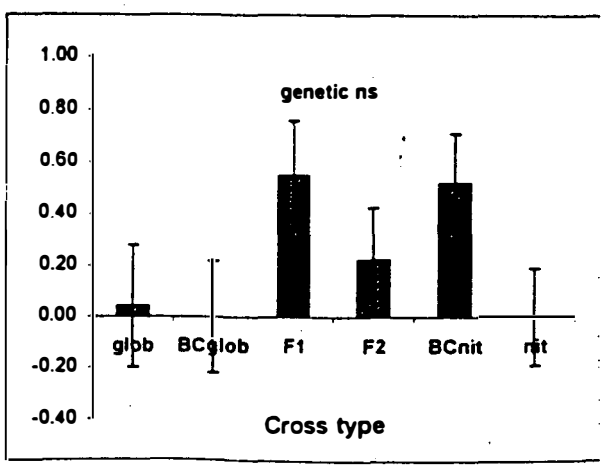




a) *b)*

Fig. 3.19 Microlepidopteran #3 **(32)**





a) *b)*

Fig. 3.20. *Cylindosporium samueli* (40)

Only seven (35%) of the taxa showed a significant preference for one of the pure eucalypt species. Psyllidae #5 ($p<0.01$) (19), Microlepidopteran #2 ($p<0.05$) (25), Aranae #2 ($p<0.01$) (28) and *Ctenarytaina eucalypti* ($p<0.01$) (3) showed a preference for *E. globulus*. *Chrysophtharta agricola* (larval) ($p<0.01$) (4), Microlepidopteran #1 ($p<0.01$) (9) and Hymenopteran #5 ($p<0.05$) (24) showed a preference for *E. nitens* (Fig. 3). In contrast to the preferences for ontogenetic types, none of these preferences for either pure species were highly significant. Further, the magnitude of the of the genetic differences are relatively low when compared to the magnitude of the ontogenetic responses (F values for 'genetic' versus 'ontogenetic' effects; Table 3.2).

The majority (6/20 or 70%) of the dependent taxa did not exhibit a significant response to the hybrid classes. Of the six taxa which did, three exhibited dominant responses; *Ctenarytaina eucalypti (3);* Microlepidopteran #1 (9) and Hymenopteran #5 (24). All of these dependent taxa were species specialists. There were two cases of intermediate susceptibility involving a species specialist, Psyllidae #5 (19) and a species generalist, Microlepidopteran #2(25). The only clear case of a susceptibility response involved Psyllid #4 (17) and this was a species generalist. Thus of the seven species specialists, a dominant response to the F_1 was exhibited in four cases (one of which involved resistance), the remaining responses being additive. Of the 13 dependent taxa which classified as species generalists, eight (62%) also exhibited no response to the hybrids, whereas the others all appeared to exhibit a susceptibility response, although the F_1 was only significantly greater than the more susceptible parent in the case of Chrysomelid beetle #2 (2). Nevertheless, significant susceptibility was exhibited in the overall hybrid response (across all classes); pure versus hybrid Table 3.2) in the case of *C. agricola* egg scars (5).

In 90% (eighteen) of the cases there was no significant difference in the susceptibility of the F_1 and F_2 hybrids. Of the other two cases one taxon, Homopteran #5 (24) showed greater abundance on the F_2 than the F_1 ($p<0.01$) and the other taxon, Psyllidae # 4 (17), showed a greater abundance on the F_1 than the F_2 ($p<0.05$) (Table 3.2). Eighty percent of the taxon showed responses to the back cross hybrids which were intermediate between the F_1 and the parental types as would be expected under an additive genetic model.

Chrysophtharta agricola

Analyses of the distribution of feeding damage by *C. agricola* beetles and larvae and the distribution of eggscars of this beetle are summarised in Table 3.1 and the least squares means are plotted in Fig. 3.1, 3.2 and 3.3 respectively. Feeding damage by the adult beetle is found to be significantly greater ($p<0.001$) on the adult foliage than on the juvenile foliage from the same height (Fig. 3.1). No significant response was noted for the foliage from either parental eucalypt species (Table 3.1; Fig. 3.1b). A contrasting pattern was seen by the larval stage. Significantly more feeding damage by the larvae was found on the juvenile foliage ($p<0.001$). The larval feeding damage was significantly more abundant on the *E. nitens* ($p<0.01$; Table 3.1) and similar abundances were found on the F_1 hybrids suggesting a dominance response. The distributions of the *C. agricola* eggscars mirrored that of the larval feeding damage on the different ontogenetic foliage types (Fig. 3.3a), however there was no significant difference ($p>0.05$) in the distributions of the eggscars between genetic classes.

Discussion

Of the twenty taxa examined 80% showed a significant degree of ontogenetic specialisation whereas only 35 % showed a significant response to one or the other pure species host. None of the taxa which showed significant preferences to one of the parental species did so in the absence of a significant response to ontogeny. Furthermore the degree of ontogenetic specialisation was far greater than the specialisation exhibited for one of the parental eucalypt species. A significant response by 40% of the taxa to changing height in the canopy was found demonstrating the necessity of examining ontogenetic responses independently of changing height.

Similar responses by members of the same guilds (species with similar feeding habits) were noted in this study. All of the lepidopteran larvae examined here (six different species) and the majority of the Psyllidae species (83%) showed a significant degree of ontogenetic specialisation for the juvenile foliage. Furthermore, these species did not

differentiate between either *E. globulus* or *E. nitens* and they appeared to be confined to the juvenile foliage regardless of position. The same pattern was shown by the majority of Psyllids. Many lepidopterous larvae and leafhoppers are considered to be monophagous (occurring on only one or a few closely related plant species) (Crawley 1985; Schoonhaven 1998). The results here suggest that the monophagous habit by lepidopteran larvae and Psyllids is occurring between ontogenetic types. It is possible though that with further time for colonisation of the adult foliage, different lepidopteran species (larvae) will be found to colonise the adult foliage.

An interesting response was shown by the chrysomelid leaf eating beetle, *C. agricola.* The adults of this beetle were found to feed on the adult foliage while larval damage and eggscars of this species were found predominantly on the juvenile foliage. It is a possibility that the feeding patterns for this beetle (and potentially other taxa) observed in the field are being altered by inter-specific interactions with competitors and predators of the species, and/or by changes in micro-climate between the juvenile and the adult canopies, this is examined in the Chapter 4.

Significant responses by dependent taxa to the two parental species (*E. nitens* and *E. globulus*) were found in 35% (seven taxa - six insect species and one spider morph) of the taxa examined. Thus, despite the close morphological and taxonomic relationship of these *Eucalyptus* species these seven taxa were able to discriminate between host eucalypt species. *Eucalyptus nitens* was found to be favoured by almost as many species as *E. globulus.*

Six taxa showed a differential response to the hybrid phenotypes. Three of these taxa exhibited a dominance response, two exhibited an intermediate response, and one taxon exhibited a susceptibility response. Four of these six taxa were also species specialists. Whitham *et al.* (1994) noted that generalist species were more commonly found to utilise the hybrid classes while specialist species were more commonly found restricted to parental types (and the closest backcross). In this study 13 taxa were classified as species generalists, eight (62%) of these exhibited no response to the hybrids, whereas the other seven (38%) all appeared to exhibit a susceptibility response. No generalist taxa showed a resistance response to the F_1 hybrids. Thus the findings

from this study support the claims by Whitham *et al.* (1994) that hybrids are more susceptible to colonisation by dependent taxa on an overall community level.

In conclusion , the present analysis of individual responses of dependent taxa to the ontogenetic and genetic gradient present in the field trial is consistent with the results of the overall community analysis. Further, it identified key ontogenetic and genetic (species) specialists responsible for those broader community responses. This idea of differential responses to variation within a tree, specifically ontogenetic variation, has not previously been experimentally verified. For the first time this study shows highly significant preferences by a number of dependent species for adult or juvenile foliage independent of confounding positional, environmental and genetic factors. Moreover the responses by these dependent taxa to the different ontogenetic foliage types are shown to be far greater than the responses to the genetic differences between two eucalypt species.

Chapter 4

Feeding trials with *Chrysophtharta agricola* beetles

Introduction

Chrysomelids (Family Chrysomelidae) are one of the most widespread families of Coleoptera (Schoonhaven 1998). These leaf eating beetles are considered to be major pests of eucalypt plantations in Tasmania (Kile 1974; Elliot *et al.* 1993; Raymond 1995) and New Zealand (Edwards 1982) and can cause as much as 40% leaf area loss in plantations (Kile 1974). The effects of leaf area loss caused by these beetles on the production of commercial forests is of considerable economic importance (Leon 1989; Elliot *et al.* 1993). Of these beetles, *Chrysophtharta bimaculata* is considered to be a serious pest (Greaves 1966; de Little and Madden 1975; de Little 1989; Greener and Candy 1994). However, recently the pest status of *C. agricola* has also been recognised (Ramsden and Elek in press), probably in part due to the increasing use of *E. nitens*, a preferred host of *C. agricola*, as a plantation species in Tasmania (J. Elek pers. comm). Greaves (1966) considered that *C. agricola* is a pest, particularly of the juvenile foliage of *E. nitens*, with the third and fourth larval instars causing the most serious defoliation. The claim by Greaves (1966) and also by de Little (1989) that *C. agricola* is preferentially found on the juvenile foliage are based on observational evidence that does not remove confounding effects of variable genotypes, changing growth conditions and other environmental effects such as inter-specific competition and predation by other insect species. By looking at preferences in an isolated system this chapter aims to elucidate the true feeding preference of the *C. agricola* beetle.

In the previous chapter it was shown that the adult beetle of *C. agricola* was preferentially feeding on the adult foliage. In contrast, the larval stage (and eggscars) were found primarily on the juvenile foliage regardless of canopy position. This difference in the distribution of the different life-stages of the beetle may be caused by interactions with inter-specific competitors and predators. Additionally, environmental factors related to different micro-climates and the dispersal patterns of the beetle may

also influence field distributions. The preference of the adult *C. agricola* beetle for foliage of different ontogenetic and genetic types was therefore examined in the laboratory in order to remove these confounding effects and determine the true feeding preference of the beetle.

The availability of the *E. globulus* x *nitens* trial in the process of phase change enables a detailed comparison of the feeding preferences of *C. agricola* in the absence of the confounding factors mentioned above. Comparisons are made between the juvenile and adult ontogenetic types taken from the same height, upper and lower canopy positions on homoblastic trees, as well as between the different genetic types provided by the *E. globulus* x *E. nitens* hybrid system. This enabled four questions to be addressed:

1) Does the adult *C. agricola* exhibit a direct preference for adult or juvenile foliage?
2) Does the adult *C. agricola* exhibit a preference for particular genetic classes in the *E. globulus* x *nitens* hybrid system?
3) Is there a greater response of *C. agricola* beetles to variation in ontogenetic or genetic foliage type?
4) How does the physiological age of the foliage affect the feeding preferences of *C. agricola*?

Methods

The collection of beetles

Chrysophtharta agricola beetles were collected by hand from the *E. globulus*, *E. nitens* and hybrid trees in the trial at Tyenna for use in the feeding experiments. Collection of these beetles was carried out on the 3rd and 4th of March, 1998. The beetles were maintained in the laboratory in cages in which suitable foliage was placed in water, this foliage was replaced every 3-4 days (depending on quality and the amount eaten). The

cages were kept in an environment of constant temperature (21°C), humidity (55%) and day-length (16 hours daylight; 8 hours darkness).

The collection of foliage

Foliage was collected from the upper and lower canopy of each of the selected trees censused in Chapter 2 for use in the laboratory feeding trial. Thus, for each heteroblastic/ homoblastic pair there was juvenile and adult foliage from the heteroblastic tree, and low juvenile and high juvenile foliage from the homoblastic tree. A single, healthy shoot of approximately 40 cm, with leaves of a range of different physiological ages, was sampled. Shoots were taken which had the least amount of damage possible. The foliage was collected by replicate from the field trial and the same replication was maintained in the laboratory experiments. Shoots of healthy foliage were cut, labelled and placed into cold water. The foliage was taken to the laboratory and stored in a cool room at 6° C until it was required. All of the foliage was used within 48 hours from the time it was gathered from the field. This time differed slightly between replicates, however, within a replicate all of the foliage received the same treatment. Collection of foliage was undertaken between the 3rd March and 17th March, 1998.

Cage feeding

Insect cages (150 x 35 x 35 cm) (Fig 4.1) were used to contain the foliage and the insects for the duration of each experiment. Each cage contained all the foliage from a single replicate in the field trial resulting in 24 shoots in each cage. Thus for each of the six genetic classes (two parental species and four hybrid classes) there were two shoots from the heteroblastic trees (mature and juvenile) and two shoots from the homoblastic trees (low juvenile and high juvenile). On a single shoot there was very young (newly expanded) foliage at the tips, intermediate age (fully expanded, still succulent) foliage, and old (sclerophyllous) foliage of the current seasons growth. The presence of these three different physiological ages of foliage provided the potential to examine beetle preferences for foliage of different ages and to ensure that there was no

bias in the results caused by feeding greater amounts of one type of foliage of a different age. Thus in each replicate (cage) the beetles were exposed to high and low foliage of three different physiological ages from each of the twelve trees.

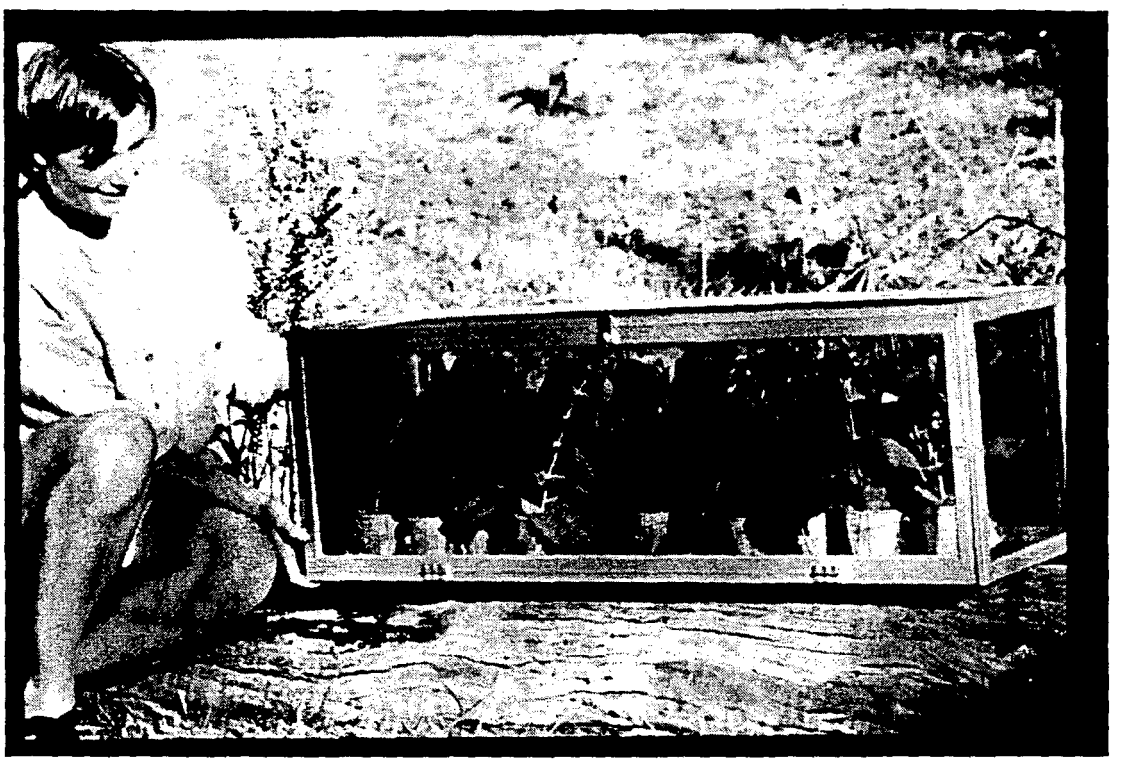


Fig 4.1. The feeding trial was undertaken using insect cages (150 x 35 x 35 cm) in which foliage from a single replicate was placed. Thirty-six beetles were allowed to feed in these cages under controlled environmental conditions for 72 hours.

For the feeding trial, the shoots collected from the field trial were cut to approximately 25-30cm lengths. Leaves which had any scalloping damage were avoided or where this was not possible the scalloping damage was cut off to present a straight edge. Efforts were made to put equal proportions of each type of foliage and age category of each foliage into the cages to ensure that no bias in feeding preferences was introduced through different availabilities of different foliage types. The base of each shoot was placed in water in an individual plastic cup. The top of the cup was covered with an aerated plastic bag, which was pierced by the shoot, thereby preventing the beetles from falling in the water. The shoot was secured and held upright using rubber bands. The shoots from a single field replicate were distributed randomly within each cage.

Thirty six even aged beetles were placed in the cage and allowed to feed for approximately 72 hours. The cages were kept in an environment of constant temperature (22°C) and constant humidity (55%) with a photo-period of 16 hours daylight and 8 hours of darkness. After the 72 hours had elapsed the beetles were removed to allow the amount of foliage eaten to be scored.

In order to determine the amount of foliage which had been ingested by the beetles the leaves were removed from the cage, stripped from the stem and placed between two perspex plates. The leaves were classified into the three different physiological ages. The leaf area eaten was measured using a leaf area meter. To determine the area eaten a (black) whiteboard marker pen was used to extrapolate the area to that which would have been present before beetle feeding. The difference between extrapolated area and the remaining area was recorded as the leaf area eaten which could then be converted into the percentage of total leaf area eaten.

Analysis of data

A three-way analysis of variation of individual adult *C. agricola* damage (Appendix 1, damage type 1) was performed on the data. The data best fitted the assumptions of normality using a square root transformation. The analysis was undertaken on the transformed data using the MIXED procedure in SAS (SAS 6.12, 1996). The repeated measures mixed model used for this analysis was as follows:

$$\%\ \text{leaf area eaten} = \textit{replicate} + \text{ctype} + \text{age} + \text{foliage} + \text{foliage*age} + \text{ctype*age} + \text{foliage*ctype} + \text{foliage*age*ctype} + \textit{error} \qquad \textbf{Equation 4.1}$$

where 'ctype' refers to cross type, 'age' refers to the young, intermediate or old physiological age of the foliage and 'foliage' refers to the four ontogenetic types (homoblastic low juvenile, homoblastic high juvenile, heteroblastic low juvenile and heteroblastic high adult). The variables ctype, foliage and age were treated as fixed effects, *replicate* and *error* were treated as random effects and the shoot was treated as the subject. Pairwise comparisons between cross types, ontogenetic types and

positional types were undertaken using the contrast statement in the MIXED procedure as detailed in the previous chapter.

Results

The results from the three-way analysis of variance are presented in Table 4.1. Significant differences in feeding preferences were found between leaves of different ontogenetic type (Fig. 4.2), different canopy position (Fig. 4.2) different physiological age (Fig. 4.3) and changing genetic class (Fig. 4.4). There were no significant interactions between any of the factors (Table 4.1) indicating that effects were consistent for all foliage types.

The specific contrast of adult versus high juvenile foliage in the laboratory trials showed that the beetle had a highly significant preference for the adult foliage (Table 4.1a; Fig. 4.2). The contrast between high juvenile foliage and low juvenile foliage showed a highly significant preference ($p>0.001$) by the beetle for foliage from the high canopy (Table 4.1a; Fig. 4.2). This preference however was not as strong as the preference for adult foliage over juvenile foliage.

Dramatic differences in feeding preferences by the beetles were evident between the young, intermediate and old aged foliage. The beetles ate significantly more of the young foliage than of either the intermediate aged foliage or of the older foliage, the latter of which was left virtually untouched (Fig. 4.3). This preference for different physiological ages did not change between adult and juvenile foliage or between the different genetic classes (Table 4.1).

An examination of the differential responses of *C. agricola* for the different genetic classes showed that the beetles had a significant preference for the introduced *E. nitens* over the native *E. globulus* ($p<0.001$; Fig. 4.4). Additionally, responses to the foliage from the four hybrid classes differed from the responses to the parental types. The beetles showed a preference for the F_1 hybrid which was intermediate to the parental types and not significantly different from the mid-parental value. There was no

significant preference for the F_2 over the F_1. Feeding on the backcross *globulus* was intermediate between the F_1 and *E. globulus* foliage. Feeding on the backcross *nitens* was greater than on *E. nitens* foliage but not significantly so.

Table 4.1. The three-way mixed model analysis of variance of feeding preferences by *C. agricola* in the feeding trial. Effects are: 'foliage' (homoblastic high juvenile, homoblastic low juvenile, heteroblastic low juvenile, heteroblastic high adult); 'age' (young foliage, intermediate aged foliage, old foliage); and 'genetic' (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). The main effects and their interactions are shown in bold. Specific contrasts between foliage types and genetic classes are shown in regular font. Error degrees of freedom = 206.

Effect	**df**	**F**	**Pr > F**
foliage	3	70.5	0.000
age	2	22.8	0.000
genetic	5	3.0	0.012
foliage*age	6	0.9	0.534
genetic*foliage	15	1.5	0.100
genetic*age	10	0.3	0.969
genetic*foliage*age	30	0.7	0.883
Contrasts			
ontogenetic			
homo high (juvenile) vs hetero high (adult)	1	23.2	0.000
position			
homo high (juvenile) vs homo low (juvenile)	1	7.3	0.007
ontomorph			
homo low (juvenile) vs hetero low (juvenile)	1	1.0	0.326
E. globulus vs *E. nitens*	1	4.9	0.027
pure vs hybrids	1	3.0	0.087
F1 vs F2	1	1.6	0.207
F1 vs mid-parent	1	0.1	0.739
F2 vs mid-parent	1	3.1	0.081
E. globulus vs backcross *globulus*	1	1.4	0.241
E. nitens vs backcross *nitens*	1	1.6	0.211

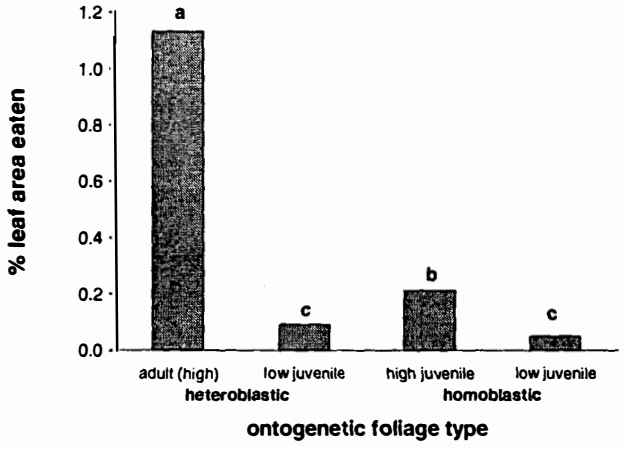


Fig. 4.2. The least squares mean percentage of leaf area eaten by *Chrysophtharta agricola* beetles in the laboratory feeding trial for the four different foliage types. Foliage types correspond to adult foliage and juvenile foliage from high on the homoblastic tree and juvenile foliage from the low canopy of the heteroblastic and homoblastic ontomorphs. Significant differences ($p<0.05$) in feeding preferences are indicated by different letters above the foliage classes.

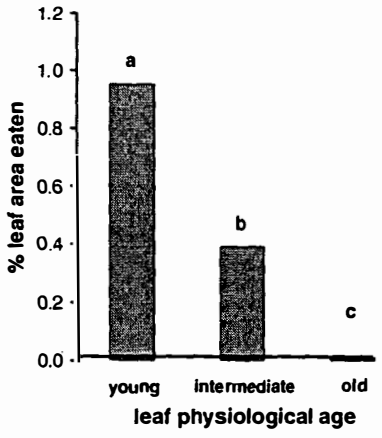


Fig. 4.3. The least squares mean percentage of leaf area eaten by *Chrysophtharta agricola* beetles in the laboratory feeding trial for foliage of different physiological age (young, intermediate, old) from the current seasons growth. Significant ($p<0.05$) differences in feeding preferences are indicated by different letters above the foliage classes.

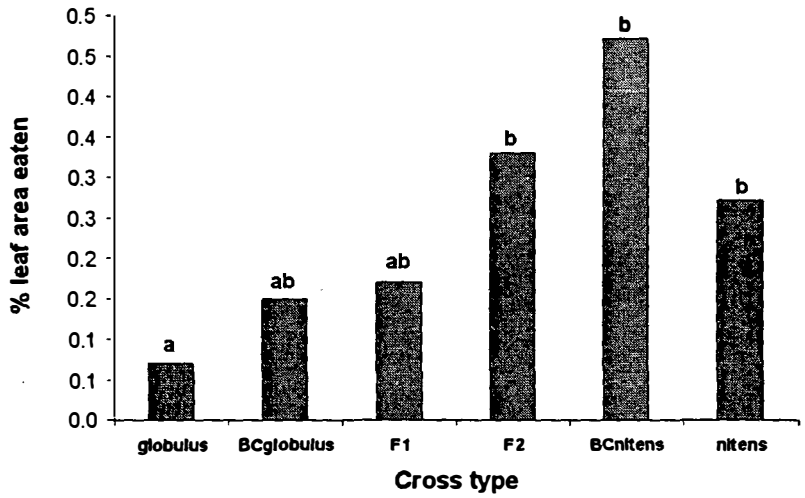


Fig. 4.4. The least squares mean percentage of leaf area eaten by *Chrysophtharta agricola* beetles in the laboratory feeding trial for foliage of the different genetic classes (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). Significant differences ($p<0.05$) in feeding preferences are indicated by different letters above the foliage classes.

Discussion

Responses to changing ontogeny

Under laboratory conditions the adult Chrysomelid beetle, *Chrysophtharta agricola*, exhibits a significant ($p<0.001$) preference for adult foliage. This response mirrors the response shown by the same beetle under field conditions as examined in chapter 3. A key issue is whether the laboratory preferences are a direct response to leaf quality or due to a more general characteristic of the foliage such as habit or leaf orientation. Evidence for the role of leaf quality was provided by an additional feeding trial which was undertaken in conjunction with the cage experiment (the results are not presented here). In this early trial, 52 adult beetles were fed on cut pieces of foliage placed on filter paper in petri dishes. Of the 52 beetles 73% (38 beetles) took their first bite from the adult foliage while only 23% (12 beetles) ate the juvenile foliage first (2 beetles were indecisive). The results of these feeding trials suggest that the response seen in the field, by *C. agricola* beetles, to ontogenetic variation was directly mediated by

features of the individual leaves rather than foliage habit/orientation or inter-specific interactions.

It was found that the larval damage and eggscars of the same species were predominantly on the juvenile foliage (Fig. 3.3a). In the feeding trials it was noted that the beetles were laying their eggs more often on the juvenile foliage. This suggests a partitioning for the different ontogenetic foliage by the different ontogenetic stages of the beetle. The adult beetle feeds principally on the adult foliage, but lays it's eggs and the larvae feed primarily on the juvenile foliage. *C. agricola* has an unusual tarsal structure which enables it to move about on juvenile foliage (Li 1993). Other chrysomelids appear to be excluded from the glaucous juvenile foliage due to an inability to grip the waxy surface (Edwards 1982; Li 1993). Inter-specific displacement may have forced the *C. agricola* beetle to lay it's eggs on the juvenile foliage, even though the adult beetle preferentially feeds on the adult foliage. However, the suitability of juvenile and adult foliage for the growth and development time of the *C. agricola* larvae requires detailed study. *Chrysophtharta agricola* is reported by Greaves (1966) to be a pest of juvenile foliage of eucalypts. This author states that the greatest amount of defoliation is caused by the third and fourth instar larvae. The present results, in combination with observations by Greaves (1966), suggest that after the transition to adult foliage has been made in plantations this beetle should not be considered a significant pest.

Responses to physiological aging

The response of the beetle under laboratory conditions to foliage of different ages was highly significant. The younger foliage was chosen over the intermediate foliage and the oldest foliage remaining virtually untouched. This response to foliage of different ages did not change with varying ontogenetic or genetic type. It is commonly found that herbivores choose the most succulent leaves (i.e. youngest leaves) on which to forage (Scriber and Slansky 1985). Potential reasons for these preferences may be that younger foliage commonly has greater amounts of nitrogen, and a higher water content than older foliage (Scriber and Slansky 1985). However, in many instances it is found that foliage of intermediate age is chosen in preference to younger foliage due to the

fact that the younger, and more precious leaves (Harper 1989) often contain greater amounts of toxic chemicals than older leaves (Cates 1980). The consistent response seen here of *C. agricola* beetles to leaves of different physiological ages regardless of parent species (*E. globulus* and *nitens*) suggests that there is no difference between the two plant species in partitioning of defensive chemicals into foliage of different ages.

Responses to genetic variation

A significant preference by the *C. agricola* beetle for the introduced *E. nitens* over the native *E. globulus* was demonstrated in this feeding trial. The same trend (although not significant) was demonstrated in the field (Fig. 3.1b). Thus, despite the fact that *E. nitens* is an introduced species, it is the preferred host *of C. agricola.* This preference by the adult *C. agricola* for feeding on *E. nitens* was shown by Li (1993) and is noted by de Little (1989) based on field observations. Li (1993) noted that there was a strong correlation of beta-diketones in the foliage with the feeding preferences of *C. agricola.* It has been found that there are major differences in putative defensive chemicals (phenolic glycosides and condensed tannins) between juvenile and mature foliage of *Populus* (Whitham pers. comm). Similarly, differences in the components of oils and waxes between the juvenile and adult foliage of *Eucalyptus* have been demonstrated by Li *et al.* (1997). In eucalypts these chemical differences between juvenile and adult foliage types have not been examined simultaneously with feeding preferences by any insect species and therefore chemical mechanisms behind the preference shown here are little understood.

There were differential responses to the hybrids and parental types shown by the beetle. The susceptibility of the F_1 hybrid was intermediate between the two parental types. These responses may indicate that a putative resistance trait in the native *E. globulus* is of an additive nature, rather than epistatic nature given that the response in the F_2 hybrids is not significantly different from that seen in the F_1's.

Preferences in the field do not exclude the possibility of interactions with other species such as interspecific competition and predation, the existence of differential dispersal patterns, or the potential for responses to differing habits between foliage types.

Moreover, by looking at feeding preferences at multiple levels, it is possible to gain a greater insight into the forces which are shaping the spatial distribution of dependent species. Through the use in this study of controlled laboratory feeding trials it was conclusively shown that the adult foliage of both *E. nitens* and *E. globulus* is preferred by *C. agricola* beetles. This response was in agreement with the results from the field census. There was a significant preference exhibited by *C. agricola* for *E. nitens* over *E. globulus* however, this preference was secondary to the preference shown for the adult foliage.

Chapter 5

Species richness

Introduction

Genetic variation as a result of hybridisation can affect the distributions of dependent species (Strauss 1994; Whitham *et al.* 1997). Hybrid zones and individual hybrids often support greater abundance and numbers of dependent taxa than either of the parental species involved in the hybridisation (Strauss 1994; Whitham *et al.* 1997; c.f. see Boecklen and Spellenberg 1990). Hence, it has been argued that hybrid zones are centres of biodiversity and are important for the conservation of biodiversity (Whitham *et al.* 1991; Morrow *et al.* 1994; Whitham *et al.* 1997). Species richness (termed alpha diversity) is a key component of biodiversity and is simply the number taxa which occur on a given sampling unit (Magurran 1988). Within hybrid zones of *Eucalyptus*, an increase in species richness has been shown on natural hybrids of *E. baxteri* x *E. obliqua* (Morrow *et al.* 1994) and *E. risdonii* x *E. amygdalina* (Whitham *et al.* 1994). A genetic component to this increase in richness has been demonstrated in the *E. risdonii* x *amygdalina* system by Dungey (1996) using a common environment field trial with hybrids of known pedigree. The possibility that hybrids are genetically more susceptible to colonisation by dependent species than their parental types has important implications for pest control in agro-forestry (Whitham 1989; Strauss 1994).

In contrast to the attention which has been given to variation between trees due to hybridisation, the impact of within-tree variation due to ontogenetic change on the richness of dependent species has received little attention (but see Waltz and Whitham 1997). Ontogenetic variation significantly affects the distributions of dependent species (Chapter 2-4) and results in a change in the composition of dependent communities on different

foliage types. Heteroblasty will result in an increase in the heterogeneity of habitats on the one ecological unit (tree). It is predicted that due to this increase in available habitats, the heteroblastic trees will exhibit a greater species richness than the homoblastic trees and a population of heteroblastic trees will have greater species richness than in population of homoblastic trees. Within the *E. risdonii* x *amygdalina* hybrid system an increase in the species richness on the heteroblastic hybrids was found in comparison to the homoblastic parental types (Dungey 1996). However, it is unclear whether the increase in dependent taxa on the hybrid genotypes was due to hybridisation *per se* or simply due to the fact that the hybrids were heteroblastic. The examination of the richness of dependent taxa in a contrasting hybrid system where the same degree of heteroblasty is exhibited by both parental types and hybrids will indicate whether the changing species richness observed on eucalypt hybrids is a response to heteroblasty or a response to hybridisation *per se.*

The present chapter examines the pattern of species richness across the ontomorphic types and across the hybrid types by studying species richness at three different levels (on a given foliage type, on a single tree, within a population). It specifically addresses three questions:

1) Is there a greater species richness on the heteroblastic trees than the homoblastic trees?
2) Is the species richness on the hybrid genotypes greater than the species richness on the pure species?
3) Does the trend in species richness seen at the population level follow the trend in species richness seen at the whole tree level or is there an additional influence due to genetic differences between individual trees?

Methods

The community data, the collection of which is described in Chapter 2, was used for the analysis of species richness (alpha diversity). Species richness was calculated, using the DECODA program, by summing the number of dependent taxa which were present on each ontogenetic foliage type (homoblastic low - juvenile, heteroblastic low - juvenile, homoblastic high - juvenile, heteroblastic high - adult). Further, two aggregated files were created which combined data from: 1) the high and low canopies of each tree; and 2) all the trees of the one genetic class. In this way species richness was calculated at three levels: 1) the individual ontogenetic foliage type level; 2) the whole tree level; and 3) at the population level for each of the genetic classes. This data was exported into SAS for analysis.

The data for individual foliage types and for the individual trees was analysed using the MIXED procedure of SAS (SAS 6.12, 1996). Analysis of species richness on each foliage type was performed using the same repeated measures mixed model as in the analysis of individual species responses in the field (Chapter 3):

richness = mean + *replicate* + pctype + ctype + position + pctype*ctype +ctype*position + pctype*position + pctype*position*ctype + *error* **Equation 5.1**

For the analysis of the whole tree species richness (summed over upper and lower canopy samples), the following model was used:

richness = mean + *replicate* + pctype + ctype +pctype*ctype + *error* **Equation 5.2**

In both models, 'replicate' was the replicate number, 'ctype' is the cross type (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*), 'pctype' is the phase change type (heteroblastic or homoblastic), 'position' is the height in the canopy (upper or lower; equation 5.2 only). *'Replicate'* and *'error'* were treated as random effects and 'ctype',

'pctype' and 'position' were treated as fixed effects. At both levels, species richness followed a normal distribution and the analysis was performed using untransformed data. Pairwise comparisons were undertaken at the individual foliage type and tree level using the CONTRAST statement in PROC MIXED as detailed in Chapter 3. The significance of the differences between cross types in species richness calculated at the population level was tested using a contingency X^2 (chi-squared) test.

Results

The results of the mixed model analysis of the richness of dependent taxa at the foliage and tree level are summarised in Table 5.1 and Table 5.2 respectively. Species richness on 1) the different foliage types (Fig. 5.1), 2) at the individual tree level for the heteroblastic versus the homoblastic ontomorphs (Fig. 5.2) and 3) for the different genetic classes at the individual tree and population level (Fig. 5.3, 5.4).

At the whole tree level there was no significant increase in the richness of dependent taxa on the heteroblastic and homoblastic ontomorphs (Table 5.2). This result was consistent across genetic classes as the interaction between genetic class and ontomorph was not significant (Table 5.1). The species richness on the individual foliage samples varied with both ontogeny and canopy position (Table 5.2), with the interaction being highly significant. The adult foliage had significantly ($p<0.001$) fewer taxa (8) than the juvenile foliage at the same height (10 taxa) (Table 5.1; Fig. 5.1). Additionally, richness was significantly greater ($p<0.001$) on the low (12 taxa) than the high foliage (10 taxa) of the homoblastic trees indicating a significant positional effect (Table 5.1; Fig 5.1).

Table 5.1. Three way analysis of variance for differences in the abundance of dependent taxa (species richness) on the different foliage types (homoblastic low juvenile, homoblastic high juvenile; heteroblastic low juvenile, heteroblastic high juvenile) from each of the six genetic classes (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). The main effects and their interactions are shown in bold. Pairwise contrasts between the ontogenetic, ontomorphic, positional and genetic classes are shown in regular font. Error d.f = 105. The random replicate effect was insignificant.

Effect	d.f.	F	Pr>F
genetic	**5**	**4.5**	**0.001 ****
phase change type (ontomorph)	**1**	**9.1**	**0.003 ****
position	**1**	**81.9**	**0.000 *****
genetic*phase change type	**5**	**0.9**	**0.494 ns**
phase change type*position	**1**	**10.4**	**0.002 ****
genetic*position	**5**	**0.5**	**0.782 ns**
genetic*position*ontogenetic	**5**	**0.3**	**0.908 ns**
ontogenetic			
high juvenile vs high adult	1	26.9	0.000 ***
ontomorphic			
low juvenile (hetero) vs low juvenile (homo)	1	0.1	0.831 ns
position			
high juvenile vs low juvenile (homo)	1	17.2	0.000 ***
E. globulus vs *E. nitens*	1	0.2	0.633 ns
pure vs hybrid	1	19.4	0.000***
F1 vs F2	1	0.4	0.516 ns
F1 vs mid-parental value	1	11.1	0.001 **
F2 vs mid-parental value	1	17.4	0.000 ***
E. globulus vs backcross *globulus*	1	6.8	0.010 *
E. nitens vs backcross *nitens*	1	3.0	0.085 ns

The richness of dependent taxa varied significantly ($p<0.01$) between the genetic classes (Table 5.1). However, the number of dependent taxa on the two parental species *E globulus* and *E. nitens* was effectively the same at the foliage type (Table 5.1), individual tree (Table 5.2) and population levels. At the individual tree level 13 taxa were found on both parental species (Fig. 5.3), while at the population level there were 25 taxa on *E. globulus* and 29 taxa on *E. nitens* (this difference was not significant at the 0.05 level with the contingency $X^2_1 = 0.06$; Fig 5.4).

Table 5.2. Two way analysis of the number of dependent taxa per tree. The analysis examines the effects of ontomorph (heteroblastic versus homoblastic), genetic classes (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*) and their interaction. The main effects and their interaction are shown in bold. Pairwise contrasts between the genetic classes classes are shown in regular font. The random replicate effect was insignificant. Error d.f. are 45.

Effect	d.f	F	Pr>F
genetic	**5**	**4.3**	**0.003 ****
phase change type (ontomorph; heteroblastic vs homoblastic)	**1**	**0.2**	**0.657 ns**
genetic*phase change type	**5**	**1.8**	**0.136 ns**
E. globulus vs *E. nitens*	1	0	0.858 ns
pure vs hybrid	1	20	0.000 ***
F1 vs F2	1	0.2	0.625 ns
F1 vs mid-parental value	1	10.0	0.003 **
F2 vs mid-parental value	1	14.9	0.000 ***
E. globulus vs backcross *globulus*	1	6.45	0.015 *
E. nitens vs backcross *nitens*	1	6.33	0.016 *

A highly significant difference in the richness of dependent taxa was found between the hybrid classes combined and between the two parental types combined (Fig. 5.3; Table 5.1). This trend was seen at the population level (Fig. 5.4; contingency $X^2_5 = 7.55$; $p<0.001$), and at the individual tree level (Table 5.2). Significantly different responses among the different hybrid classes were found, both at the individual tree level and at the population level. At the individual tree or at the population level the species richness on the F_2 hybrid (16 and 39 respectively) was not significantly greater than on the F_1 hybrid (16 and 34 respectively) (Figs. 5.3 and 5.4). The backcross hybrids supported numbers of dependent taxa which were intermediate between the F_1 hybrid and the parental type, again this trend was seen at both the individual tree (Fig. 5.3) and population level (Fig. 5.4).

Discussion

It was expected that the more heterogeneous environment on the heteroblastic trees, provided by two different foliage types would, on average, have resulted in a greater number of taxa on the heteroblastic ontomorphs. However, it was seen that although there was a slight tendency for an increase in the richness of dependent taxa on the heteroblastic trees the difference was not significant. Therefore the hypothesis that there would be a greater species richness on the heteroblastic ontomorphs than on the homoblastic partner was proven to be incorrect. It is thought that a major reason that the expected increase on the heteroblastic ontomorphs was not observed was that the adult foliage supported far fewer taxa than the high juvenile foliage. Therefore any increase in species richness due to the presence of adult foliage may have been masked by the much higher richness on the high juvenile foliage. A potential reason for the paucity of species observed on the adult foliage compared with the juvenile foliage at the same height is the lack of a source of colonisation. It is evident that there is a considerable degree of specialisation for adult or juvenile foliage by dependent species (as has been shown in the earlier chapters of this study) and therefore when the trees undergo phase change to the adult foliage type there are few dependent species in the vicinity which are specialised for colonising adult foliage. Observations in natural stands of *E. globulus* through 1998 indicated that the same guilds of species are present in the natural situation as were found in this plantation. As there are no colonising sources of *E. globulus* in the immediate vicinity (the nearest *E. globulus* population is 10km away just outside the boundaries of Mt Field National Park) there is not an available source for the rapid colonisation of the adult foliage. In contrast there were dependent species which were well adapted to juvenile foliage already present in the trial

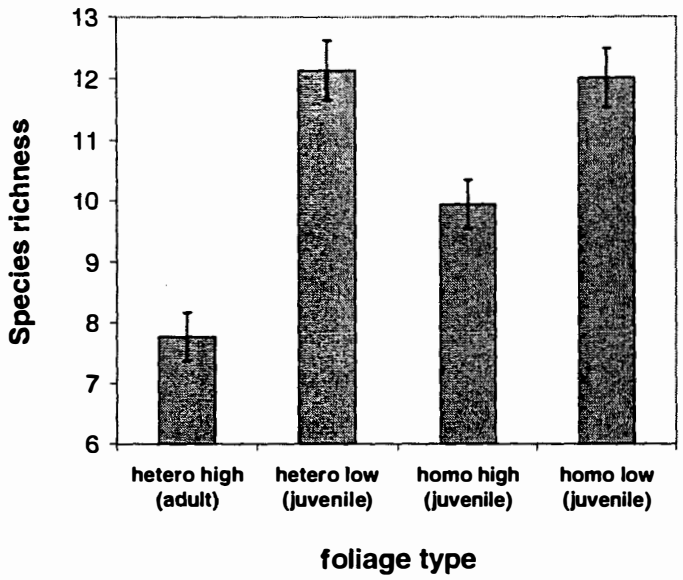


Fig. 5.1. The richness of dependent taxa on the four ontogenetic foliage types (homoblastic low juvenile, homoblastic high juvenile; heteroblastic low juvenile, heteroblastic high juvenile). There are significant differences in richness between; the (high) adult foliage (8 taxa) and the high juvenile foliage (10 taxa) ($p<0.001$) and between the juvenile foliage from the upper and lower canopies of the homoblastic trees (12 taxa on both ontomorphs) ($p<0.001$).

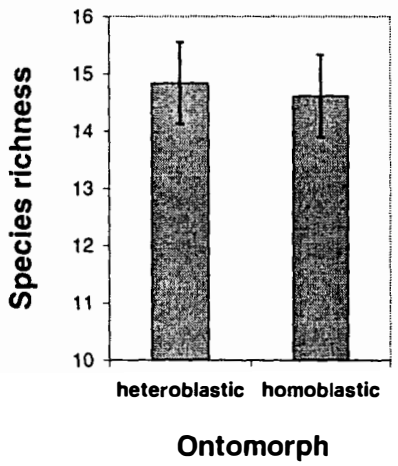


Fig. 5.2. The mean richness of dependent taxa at the individual tree level (pooled over samples from the upper and lower canopy) on the heteroblastic and homoblastic ontomorphs. On average, 14.8 taxa were found on the heteroblastic trees compared with 14.6 taxa on the homoblastic trees. The difference between these two types was not significant ($p>0.05$).

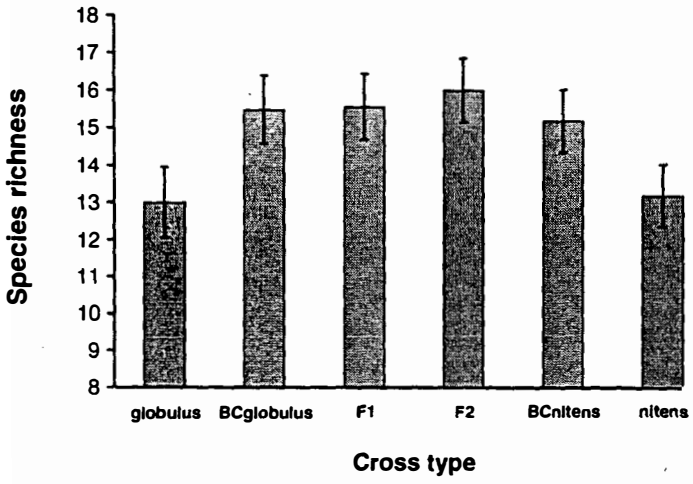


Fig. 5.3. The richness of dependent taxa at the individual tree level averaged across genetic classes (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). There is no significant difference in species richness between *E. globulus* (13 taxa) and *E. nitens* (13 taxa) trees. Significant differences ($p<0.001$) are seen between the pure species and the hybrids; on average the hybrids supported 15.5 taxa while the parentals supported only 13 taxa. The F_1 and F_2 (16 taxa on both) hybrids have a significantly greater number of dependent taxa on them than both parental types (13 taxa each).

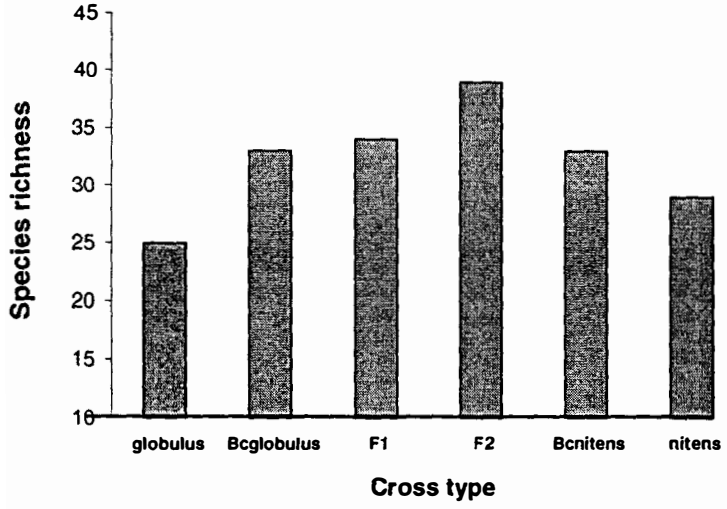


Fig. 5.4. The richness of dependent taxa (alpha diversity) in the population of each genetic class (*E. globulus*, backcross *globulus*, F_1, F_2, backcross *nitens*, *E. nitens*). The difference between the two parental types is not significant (contingency X^2_5 ns), although there is a slightly greater number of taxa on *E. nitens* (29 taxa) compared to the *E. globulus* (25 taxa) population. There is a significant difference in dependent taxa richness on the pure species (average = 27 taxa) versus all of the hybrids (average = 34.75) (contingency $X^2_5 = 7.55$; $p<0.001$).

from the previous three years of colonisation which could act as a source of colonisation for the new juvenile foliage.

It is possible that with more time for growth and subsequent colonisation, a greater number of dependent species would occur on the adult foliage and thereby cause an increase the overall richness of the heteroblastic trees. However it is of interest to note that the nature of eucalypt establishment (i.e. growth in a competitive, disturbed environment) is such that in a natural population, where trees are situated in close proximity to one another, the juvenile leaves will inevitably be lost as the transition to adult foliage accompanying canopy closure occurs. Therefore, as proposed here, an increase in species richness due to heteroblasty will effectively only occur for a brief period of time. Thus in reality there is likely to be a small amount of time, just prior to the complete conversion to adult foliage, when these heteroblastic trees will exhibit a greater species richness than their homoblastic counterparts. So perhaps the greatest impact of phase change in eucalypts on the richness of dependent communities in the species examined here will be that a different suite of species will be associated with stands of different ages. It is predicted that the greatest biodiversity will occur where both juvenile and adult trees are found together in one area.

Significant differences in numbers of colonising dependent taxa were found on the different genetic classes. However, the two parental species, the native *E. globulus* and the exotic *E. nitens*, supported effectively the same numbers of dependent taxa at the individual tree level. It is commonly found that introduced plants are relatively free from pest attack compared to native plants (New 1988), a phenomenon which is not supported here. However there may be two reasons which explain this: 1) the two species are similar morphologically and are taxonomically close and therefore traits which attract or deter pests will be similar, and 2) there was coupe of juvenile *E. nitens* trees growing adjacent to the trial which could have acted as a source of colonisation for the *E. nitens*.

The first generation and the advanced generation hybrids of *E. globulus* x *nitens* examined in this project all exhibited a significantly greater richness of dependent taxa than both

parental types. This is in agreement with a large proportion of studies which have been undertaken in the natural situation where the hybrid phenotypes are found to support a greater number of dependent dependent species than the parental types (Strauss 1994; Whitham *et al.* 1994; Christensen *et al.* 1995). However, there is only a single study known where the dependent species richness has been shown to have a genetic basis using pedigreed hybrids and parental genotypes grown in a common environment (Dungey 1996). The use of such common environment trials with progeny of known genotype removes environmental factors and therefore responses can be attributed to genetic causes. This study by Dungey (1996) of *E. amygdalina x risdonii* mirrored what was seen by Whitham *et al.* (1994) in the natural hybrid zone, but the responses observed by Dungey (1996) may have confounded potential effects of an increase in heteroblasty on the hybrid phenotypes. In the present study the increase in species richness on the hybrid genotypes demonstrates a response to hybridisation *per se* as it is not confounded by changing deegrees of heteroblasty between the hybrid and parental types.

It has previously been suggested that the increasing susceptibility of hybrids may be due to the breakdown of co-adapted gene complexes in advanced generation hybrids (Fritz *et al.* 1994). In this study it was found that the advanced generation hybrids were no more susceptible to dependent species than the F_1 hybrid arguing against the breakdown of co-adapted gene complexes. Alternatively it suggests that the trait is under additive control and i.e. that traits which impart resistance to dependent species are under the control of a large number of independent genes which become diluted in the hybrid genotypes. This is further supported by the intermediate susceptibility of the backcross hybrids.

The *E .globulus* x *E. nitens* hybrid system is not naturally occurring, however the results of this study showing the responses of dependent communities to the artificially induced hybrid serves to increase our understanding of the genetic basis of community structure and therefore the relative effects of 'bottom-up' influences, due to plant quality, compared with 'top-down' influences as a result of competition and predation (Dickson and Whitham 1996). *E. globulus* and *E. nitens* are taxonomically and morphologically closely related

and it might consequently be expected that the hybrid genotypes would exhibit little difference in dependent species colonisation than the parental types. This study clearly demonstrates that, according to the dependent species, there are in fact major differences between the hybrids versus the parental types. Additionally as the hybrids exhibit the same degree of heteroblasty as the parental types the increase in richness of dependent taxa on the hybrids is a response to hybridisation *per se*.

Chapter 6

General conclusions

The present study attempted to quantify and compare the responses of dependent species, at the individual and community level, to ontogenetic and genetic variation in eucalypts. Responses of dependent communities to changing plant quality are widely reported in the literature. However, these responses mostly refer to between-plant variation as a result of inter- and intra-specific differences (Maddox and Root 1987; Fritz and Price 1988; Moran and Whitham 1990; Paige *et al.* 1990; Boecklen and Price 1991). The concept that dependent communities also respond to within-plant variation through the different ontogenetic stages has only recently been addressed (Kearsley and Whitham 1989, 1998; Waltz and Whitham 1997). Within *Eucalyptus*, there are thought to be a number of species which respond to ontogenetic variation (Farrow *et al.* 1994; de Little 1989; Dungey *et al.* 1997), however these claims are based mainly on anecdotal evidence or on experimental work which confounds the factor of changing height by taking juvenile foliage from low in the canopy and adult foliage from high in the canopy.

Due to the striking nature of heteroblasty in *Eucalyptus* (Pryor 1976), the genus is an ideal system in which to study the effects of ontogenetic variation on dependent community structure. A further factor which makes eucalypts suitable for such studies is the strong genetic control of height and timing to phase change and the large amount of variation in the trait found within and between populations (Dutkowski and Potts in press; Jordan *et al.* submitted). This allows juvenile and adult foliage to be compared independent of confounding positional effects. In the *E. globulus* x *nitens* hybrid system it was clearly demonstrated that the response to ontogenetic variation by dependent communities is far greater than the response to genetic variation between the pure species and their hybrids.

The strong response to ontogenetic variation has profound ecological implications. Different communities of dependent species will be associated with different aged stands of *Eucalypts.* In eucalypt forests sudden conversions to juvenile foliage occur

following major disturbance events such as wildfire and these are likely to lead to a dramatic change in the communities of associated dependent species. These findings also have important implications for pest management in eucalypts as the suite of associated pests will change dramatically as plantation trees undergo phase change.

In the present study one of the insects involved in the community change was the *Chrysophtharta agricola* leaf eating beetle. The feeding preferences exhibited by this beetle in the field were maintained in controlled feeding trials. Such feeding trials are important to eliminate factors such as inter-specific competition, predation, changing dispersal patterns and environmental effects which may cause differential preferences in the field. Therefore, in the case of the *C. agricola* beetle the feeding preferences in the field were a direct response to foliage quality. However, the traits which are changing to cause this response to ontogenetic variation are not well understood. It is likely that various dependent taxa will respond to different traits associated with ontogenetic change. There are clear morphological differences between the two foliage types (Johnson 1926; Pryor 1979). The juvenile leaves are far more glaucous than the adult leaves, a trait which is known to affect the foraging traits of Chrysomelid leaf eating beetles (Edwards 1982; Li 1993). The orientation of the two foliage types differs, a characteristic which in itself may have an influence on the foraging habits of dependent species. Finally, it has been found that there are differences in the compositions of oils and waxes between the adult and juvenile leaves in *E. globulus* and other Tasmanian species (Li *et al.* 1997a,b). However, these chemical differences need to be studied in conjunction with studies of feeding preferences such as the one undertaken for *C. agricola* in this study.

The adaptive significance of phase change in plants is not understood, the strong genetic control of the trait suggests that it is under selective pressure (Bell and Williams 1997). It is possible that the differing susceptibilities of the ontogenetic foliage types to pest species may drive the selection for retention of juvenile foliage for a relatively longer period or alternatively a switch to adult foliage earlier than is the norm. The differential response to *Mycosphaerella* leaf disease (Dungey *et al.* 1997) and Autumn Gum Moth (Floyd and Farrow 1994) are possible examples. The present study has shown a strong preference of some dependent species for adult foliage and of

others for juvenile foliage, indicating that one pest species will rarely act in isolation and the biotic selection forces on this trait will be complex.

Eucalyptus globulus and *E. nitens* are taxonomically very close and morphologically similar. The leaves of a single ontogenetic type between the two species are quite indistinct (Brooker and Kleinig 1990). In contrast, there is a large difference between the juvenile and adult foliage types within a tree in these two species (Brooker and Kleinig 1990). Therefore the strong response in this particular system by dependent communities to ontogenetic compared to genetic change is an extreme case. However, these results do suggest there are likely to be considerable responses in other heteroblastic species (e.g. *Salix* - Bryant *et al.* 1985 and *Populus* - Kearsley and Whitham 1989,1998), particularly amongst other eucalypts which are characterised by pronounced ontogenetic change (Brooker and Kleinig 1990).

Hybridisation is a significant source of genetic variation in natural populations (Potts and Reid 1985). The dynamics of herbivore, pathogen and higher trophic level interactions in these hybrid systems have received considerable attention in the past decade (Whitham *et al.* 1997). Variable responses are reported. These range from: 1) 'resistance', which involves a decrease in the number of dependent taxa found on the hybrid phenotypes (Aguilar and Boecklen 1992; Boecklen and Spellenberg 1990); 2) 'intermediate' responses (Gange 1995); and 3) hybrid 'susceptibility' (Whitham 1989; Whitham *et al.* 1994) where hybrids host a greater diversity of dependent taxa than the parental types. Susceptibility is the most widely reported response from other studies (reviewed by Strauss 1994; Whitham *et al.* 1997). Most previous studies have been undertaken in natural systems whereas this study of the *E. globulus* x *nitens* system shows a susceptibility response in a common environment trial. The widespread occurrence of this susceptibility response has led Whitham *et al.* (1994) to implicate hybrids and hybrid zones as being important in ecological space and evolutionary time as centres of biodiversity and as an important foci for evolution. Consequently, the conservation of hybrid zones is considered important in the long term for the conservation of biodiversity (*Whitham et al.* 1992).

A true genetic basis was demonstrated for this susceptibility response in *E. globulus* x *E. nitens* hybrids through the use of a common environment trial, allowing an insight

into the genetic mechanisms responsible for this hybrid susceptibility. The genetic variation on the F_2 hybrids was not significantly greater than that found on the F_1 hybrids suggesting an additive rather than epistatic basis for resistance traits. Ontogenetic variation in *E. globulus* and *E. nitens* has been shown to be an extremely important factor in determining the composition of dependent communities. There is a true genetic basis for variation in dependent community structure, in the traditional sense of variation in plant genotypes between plants and in the additional sense of ontogenetic variation within a plant. Furthermore, this study is one of the first to provide evidence a genetic basis for the increases in biodiversity observed in hybrid zones.

Bibliography

Abbott, I. (1993). Insect pest problems of eucalypt plantations in Australia. Western Australia. *Australian Forestry* **56 (4)** 381-384.

Abrahamson, W.G. (Ed.) (1989). Plant-Animal interactions. McGraw-Hill Book Company.

Aguilar, J.M. and Boecklen, W.J. (1992). Patterns of herbivory in the *Quercus grisea* x *Quercus gambelii* species complex. *Oikos* **64,** 498-504.

Ashton, D.H. and Turner, J.S. (1979). Studies on the light compensation point of *Eucalyptus regnans* F. Muell. *Australian Journal of Botany* **27**, 439-458.

Beadle, C.L., McLeod, D.E., Turnbull, C.R.A., Ratkowsky, D.A. (1989). Juvenile/total foliage ratios in *E. nitens* and the growth of stands and individual trees. *Trees* **3**, 117-124.

Beckmann, R. (1991). Trees that resist insect attack. *Ecos* **70**, 23-25.

Bell, D.T. and Williams, J.E. (1997). Eucalypt ecophysiology. Chapter 8 in: *Eucalypt Ecology; Individuals to Ecosystems.* Woinarski, J. and Williams, J. Editors. Cambridge University Press. UK. 168-196.

Boecklen, W.J. and Price, P.W. (1991). Non-eqilibrium community structure of sawflies on Arrayo willow. *Oecologia* **85**, 483-491.

Boecklen, W.J. and Spellenberg, R. (1990) Structure of herbivore communities in two oak (*Quercus spp*) hybrid zones. *Oecologia* **85**, 92 –100.

Bowman, D.J.M.S and Minchin, P.R. (1987). Environmental relationships of woody vegetation patterns in the Australian monsoon tropics. *Australian Journal of Botany* **35,** 151-169.

Brink, R.A. (1962). Phase change in higher plants and somatic cell heredity. *Quarterly Review of Biology* **37,** 1-22

Brooker, M.I.H. and Kleinig, D.A. (1990). *A Field Guide to Eucalypts*, Volume 1. Inkata Press, Australia.

Bryant, J.P., Weiland, G.D., Clausen, T. and Kuropat, P. (1985). Interactions of Snowshoe Hare and Feltleaf Willow in Alaska. *Ecology* **66 (5)**, 1564-1573.

C.S.I.R.O. (1979). The Insects of Australia. Melbourne University Press.

Cameron, R.J. (1970). Light intensity and the growth of *Eucalyptus* seedlings. 1. Ontogenetic variation in *E. fastigata. Australian Journal of Botany* **18**, 29-43

Carne, P.B., Greaves, R.G.W. and McInnes, R.S. (1974). Insect damage to plantation-grown eucalypts in north coastal N.S.W., with particular reference to Christmas beetles (Coleoptera: Scarabaoidae). *Journal of Australian Entomological Society* **13**, 189-206.

Cates R.G. (1980). Feeding patterns of monophagous, oligophagous and polyphagous insect herbivores, the effect of resource abundance and plant chemistry. *Oecologia* **46**, 22-31.

Cauvin, B. (1981). Rejuvenilisation multiplication d'ortets seniles - *Eucalyptus. Extrait des Annales Afocel.* 74-105.

Christensen, K.M., Whitham, T.G. and Keim, P. (1995). Herbivory and tree mortality across a pnyon pine hybrid zone. *Oecologia* **101,** 29-36.

Crawley, M.J. (1985). Reduction of oak fecundity by low-density herbivore populations. *Nature* **314,** 163-164.

Curtis, H. and Barnes, N.S. (1989). *Invitation to Biology.* Worth Publishers, New York.

Dargie, T.C.D. (1984). On the integrated interpretation of indirect site ordinations: a case study using semi-arid vegetation in southeastern Spain. *Vegetatio* **55,** 37-55.

de Little, D.W. (1989). Paropsine chrysomelid attack on plantations of *Eucalyptus nitens* in Tasmania. *New Zealand Journal of Forest Science* **19 (2)**, 223-227.

de Little, D.W. and Madden, J.L. (1975). Host preference in the Tasmanian eucalypt defoliating Paropsini (Coleoptera: Chrysomelidae) with particular reference to *Chrysophtharta bimaculata* (Olivier) and *C. agricola* (Chapuis). *J. Aust. Ent. Soc.* **14**, 387-394.

Dickson, L.L. and Whitham, T.G. (1996). Genetically based resistance traits affect arthropods, fungi, and birds. *Oecologia* **106**, 400-406.

Donovan, L.A. and Ehrlinger, J.R. (1991). Ecophysiological differences among juvenile and reproductive plants of several woody species. *Oecologia* **86,** 594-597.

Duncan, F. (1989). Systematic affinities, hybridisation and clinal variation within Tasmanian eucalypts. *Tasforests* **1**, 13-25.

Dungey, H.S. (1996) *The Susceptibility of hybrids to pests.* Unpublished PhD Thesis, The Univesity of Tasmania, Hobart, Australia.

Dungey, H.S., Potts, B.M., Carnegie, A.J., and Ades, P.K. (1997). *Mycosphaerella* leaf disease: genetic variation in damage to *Eucalyptus nitens, Eucalptus globulus*, and their F_1 hybrid. *Canadian Journal of Forest Research* **27,** 750-759.

Dutkowski, G.W.and Potts, B.M. (in press). Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. *Australian Journal of Botany* **46 (5).**

Edwards, P.B. (1982). Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? *Australian Journal of Ecology* **7**, 347-352.

Edwards, P.B., Wanjura, W.J., Brown, W.V. (1990). Mosaic resistance in plants. *Nature* **347**, 434.

Eldridge, K., Davidson, J., Hardwood, C. and Van Wyk, G. (1994). *Eucalypt domestication and breeding*. Clarendon Press, Oxford.

Elliot, H.J. and Bashford, R. (1978). The life history of *Mnesampela privata* (Guen.) (Lepidoptera: Geometridae), a defoliator of young eucalypts. *Journal of the Australian Entomological society* **17,** 201-204.

Ericson, L., Burdon, J.J. and Wennstrom, A. (1993). Inter-specific host hybrids and phalacrid beetles implicated in the local survival of smut pathogens. *Oikos* 68, 393-400.

Farrell , G.S., and New, T.R. (1980). Some aspects of the biology of the eucalypt mining sawfly *Phylacteophaga froggati* Reik (Hymenoptera: Pergidae.) *Australian Journal of Zooology* **28**, 83-90.

Farrow, R.A., Floyd, R.B., and Neumann, F.G. (1994). Inter-provenance variation in resistance of *Eucalyptus globulus* juvenile foliage to insect feeding. *Australian Forestry* **57**, 65-68.

Floate, K.D. and Whitham, T.G. (1995). Insects as traits in plant systematics. *Canadian Journal of Botany* **73,** 1-13.

Floate, K.D., Martinsen, G.D. and Whitham, T.G. (1993). Elevated herbivory in plant hybrid zones: *Chrysomela confluens*, *Populus* and phenological sinks. *Ecology* 74, 2056-2065.

Fortanier, E.J. and Jonkers, H. (1976). Juvenility and maturity of plants as influenced by their ontogenetical and physiological aging. *Acta Horticulturae* **56**, 37-43.

Fritz, R.S. and Price, P.W. (1988). Genetic variation among plants and insect community structure: willows and sawflies. *Ecology* **69**, 845-856.

Fritz, R.S., Nichols-Orians, C.M. and Brunsfield, S.J. (1994). Inter-specific hybridisation of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia* **97**, 106-117.

Fritz, R.S, McDonough, S.E. Rhoads, A.G. (1997) Effects of plant hybridisation on herbivore/parasitoid interaction. *Oecologia **110 (3),*** 360-367.

Gange, A.C. (1995). Aphid performance in an alder (*Alnus*) hybrid zone. *Ecology* **76,** 2074-2083.

Gore, P.L., Potts, B.M., Volker, P.W., and Megalos, J. (1990). Unilateral cross-incompatibility in *Eucalyptus:* the case of hybridisation between *E. globulus* and *E. nitens. Australian Journal of Botany* **38**, 185-194.

Greaves, R. (1966). Insect defoliation of eucalypt growth in the Florentine valley, Tasmania. *Appita* **19**, 119-126.

Greener, A. and Candy, S.G. (1994) Effect of the biotic insecticide *Bacillus thuringiensis* and a pyrethroid on survival of predators of *Chrysophtharta bimaculata* (Oliver) (Coleoptera:Chrysomelidae). *Journal of the Australian Entomological Society* **33**, 321-324.

Greig-Smith, P. (1983). *Quantitative Plant Ecology* (3rd Edition). Blackwell Scientific, Oxford.

Harper, J.L. (1989). The value of a leaf. *Oecologia* **80 (1),** 53-58.

Haukioja, E. (1980). On the role of plant defenses in the fluctuation of herbivore populations. *Oikos* **35,** 202-213.

Haukioja, E., and Niemela, P. (1976). Birch leaves as a resource for herbivores: seasonal occurrence of increased resistance in foliage after mechanical damage of adjacent leaves. *Oecologia* **39**, 151-159.

Johnson, E.D. (1926). A comparison of the juvenile and adult leaves of *Eucalyptus globulus. New Phytologist,* **26** 202-212.

Jordan, G.J., Potts, B.M., Kirkpatrick, J.B. and Gardiner, C. (1993). Variation in the *Eucalyptus globulus* complex revisited. *Australian Journal of Botany* **41**, 763-785.

Karban R. (1987). Herbivory dependent on plant age: a hypothesis based on acquired resistance. *Oikos* **48 (3)** 336-337.

Keane, P.J., Wall, E., Park, R.F. (1981). Pathology of *Eucalyptus* leaf diseases. In 'Eucalyptus dieback in forests and woodlands. Proceedings of a conference held at the CSIRO Division of Forest Research'. (Eds K. Old, G.A.Kile and C.P. Ohmart.) pp. 151-160. Canberra4-6 August 1980. (CSIRO Australia).

Kile, G.A. (1974). Insect defoliation in the eucalypt regrowth forests of southern Tasmania. *Australian Forest Research* **6,** 9-18.

Kile, G.A. and Ohmart, C.P. *Eucalypt Dieback in Forests and Woodlands.* Proceedings of the CSIRO Division of Forest Research, Canberra, 4-6 August 1980.

Kearsley, M.J. and Whitham, T.G. (1998). The developmental stream of cottonwoods affects ramet growth and resistance to galling aphids. *Ecology*, **79 (1)**, 178-191.

Kearsley, M.J., and Whitham, T.G. (1989). Developmental changes in resistance to herbivory: implications for individuals and populations. *Ecology* **70**, 422-434.

Koslowski, T.T. (1971). *Growth and Development of Trees.* Volume 1. Academic Press, New York, New York, U.S.A.

Landsberg, J.J. and Cork, S.J. (1997). Chapter 14 in: *Eucalypt Ecology; individuals to ecosystems.* Woinarski, J. and Williams, J. Editors. Cambridge University Press. UK.

Larson, K.C. and Whither, T.G. (1998) The sink compensation hypothesis: plant architecture affects resistance to gall aphids. *Oecologia* (in press)

Li, H. (1993). 'Phytochemistry of *Eucalyptus* spp. and its role in insect-host-tree selection'. Unpublished PhD Thesis, Tasmania, Australia.

Li, H., Madden, J.L. and Potts, B.M. (1995). Variation in volatile leaf oils of the Tasmanian *Eucalyptus* species – 1. Subgenus *Monocalyptus. Biochemical Systematics and Ecology* **23, 3** 229-318.

Li, H., Madden, J.L. and Potts, B.M. (1997). Variation in leaf waxes of the Tasmanian Eucalyptus species - 1. Subgenus *Symphyomyrtus. Biochemical Systematics and Ecology* **25, 7**, 631-657.

Li, H., Madden, J.L. and Potts, B.M. (1994) Variation in leaf oils of Tasmanian eucalypt species. 1. Subgenus *Monocalyptus. Biochemical Systematics and Ecology* **23**, 199-318.

Linhart, Y.B. (1989). Interactions between genetic and ecological patchiness in forest trees and their dependent species. In: *Evolutionary Ecology of Plants.* Editors, Bock, J.H. and Linhart, Y.B. Warview Press, Boulder, Colorado.

MaCauley, B.J. and Fox, L.R. (1980). Variation in total phenols and condensed tannins in *Eucalyptus*: leaf phenology and insect grazing. *Australian Journal of Ecology* **5,** 31-35.

Maddox, G.D. and Root, R.B. (1987). Resistance to sixteen diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia* **72,** 8-14.

Magurran, A.E. (1988). *Ecological Diversity and it's Measurement.* Croom Helm Limited, London.

Majer, J.D., Recher, H.F. and Ganeshanandam, S. (1992). Variation in foliar nutrients in *Eucalyptus* trees in eastern and Western Australia. *Australian Journal of Ecology* **13,** 269-278.

Mattson, J.W. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11,** 119-161.

May, T.W. and Simpson, J.A. (1997). Fungal diversity and ecology in eucalypt ecosystems. Chapter 11 in: *Eucalypt Ecology; Individuals to Ecosystems.* Woinarski, J. and Williams, J. Editors. Cambridge University Press. UK. 168-196.

Messina, F.J., Richards, J.H. and Durant McArthur, E. (1996). Variable responses of insects to hybrid versus parental sagebrush in common gardens. *Oecologia* **107,** 513-521.

Minchin P.R. (1987). An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* 71, 145-156.

Moran, N.A. and Whitham, T.G. (1990). Evolutionary reduction of complex lifecycles: loss of host alternation in *Pemphigus* (Homoptera: Aphidadae). *Evolution* **42,** 717-728.

Morrow, P.A. (1977). Host specificity of insects in a community of three co-dominant eucalypt species. *Australian Journal of Ecology* **2,** 89-106.

Morrow, P.A., Whitham, T.G., Potts, B.M., Ladiges, P., Ashton, D.H., and Williams, J.B. (1994). Gall forming insects consentrate on hybrid phenotypes of Eucalyptus. Pages 121-134 in The Ecology and Evolution of Gall-forming Insects. P.W. Price, W.J. Mattson and Y.N. Baranchikov, eds.).

Neumann, F.G. and Collett, N. G. (1997). Insecticide trials for control of Autumn Gum Moth (*Mnesampela privata*), a primary defoliator in commercial eucalypt plantations prior to canopy closure. *Australian Forestry* **60, 2,** 130-137.

New, T.R. (1988). *Associations between Insects and Plants.* Australian Institute of Biology.

Ohmart, C.P. and Edwards, P.B. (1991). Insect herbivory in *Eucalypts. Annual Review of Entomology* **36,** 637-657.

Paige, K.N., Keim, P., Whitham, T.G., and Lark, K.G. (1990). The use of restriction length polymorphisms to study the ecology and evolutionary biology of aphid-plant interactions. In Mechanisms of aphid-plant genotype interactions. Edited by R.D. Eikenbary and R.K. Campbell. Elsevier Science Publishers B.V., Amsterdam, The Netherlands. pp. 69-87.

Park, R.F. and Keane, P.J. (1982). Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Trans. Br. Mycological Society* **79 (1)**, 101-151

Paton, D.M. (1991). Eucalypt juvenility. *Woody Plant Biotechnology*, Ed. Ahuja, M.R. Plenum Press, New York.

Patton, R.F. (1961). The effect of age upon susceptibility of Eastern White Pine to infection by *Cronartium ribicola. Phytopathology* **51,** 429-434.

Pederick, L.A. (1979). Natural variation in the Shining Gum (*Eucalyptus nitens*). *Australian Forest Research* **9,** 41-63.

Poethig, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. *Science* **250,** 923-930.

Potts, B.M. and Reid, J.B. (1985) Hybridisation as a dispersal machanism. *Evolution* **42**, 1245-1255.

Potts, B.M. (1989). Population variation and conservation status of a rare Tasmanian endemic, *Eucalyptus cordata.* Research Report no. 4, Tasmanian Forest Research Council Inc.

Potts, B.M. and Wiltshire, R.J.E. (1997). Genetics and Genecology. Chapter 4 in: *Eucalypt Ecology; individuals to ecosystems.* Woinarski, J. and Williams, J. Editors. Cambridge University Press. UK. 51-91.

Potts, B.M., and Reid, J.B. (1985). Variation in the *Eucalyptus gunnii - archeri* complex. II. The origin of variation. *Australian Journal of Botany* **33**, 519-541.

Potts, B.M., Volker, P.W. Dungey, H.S. (1992). Barriers to the production of interspecific hybrids in *Eucalyptus.* Mass production technology for genetically improved fast growing forest tree species. Actes Proceedings, Bordeaux, 14-18 September, 1992) 193-204.

Pryor, L.D. (1976). *The Biology of Eucalypts.* (Edward Arnold: London.)

Raymond, C.A. (1995). Genetic variation in *Eucalyptus regnans* and *Eucalyptus nitens* for levels of observed defoliation caused by the Eucalyptus leaf beetle, *Chrysoptharta bimaculata* Olivier, in Tasmania. *Forest Ecology and Management* **72**, 21-29.

Recher, H.F., Majer, J.D. and Ganesh, S. (1996). Eucalypts, arthropods and birds – on the relation between foliar nutrients and species richness. *Forest Ecology and Management* **85(1-3)**, 177-195.

Reid, J.B., Hill, R.S., Brown, M.J. and Hovenden, M. (1998). *Vegetation of Tasmania.* University of Tasmania, Forestry Tasmania.

Rhoades, D.F. (1979). Evolution of chemical defense against herbivores. – In: Rosenthal, G.A. and Janzen, D.H. (Ed.), *Herbivores: their interrrelationships with plant secondary constituents*, New York, pp3-54.

Schaffalitzky de Muckadell, M. (1954) Juvinile changes in woody plants. *Physiologia Plantarum* **7**:782-796.

Schoonhaven, L.M., Jermy, T. and van Loon, J.J.A. (1998). Insect-Plant Biology: From physiology to evolution. Chapman and Hall, London.

Scriber, J.M. and Slansky, F. (1985). Food consumption and utillisation, pp 87-163 in Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 4. (eds. G.A. Kerkut and L.I. Gilbert). Pergamon Press, Oxford.

Strauss, S.Y. (1994). Levels of herbivory and parasitism in host hybrid zones. *Trends in Ecology and Evolution* **9,** 209-214.

Suomela, J., and Ayres, P. (1994). Within-tree and among-tree variation in leaf characteristics of Mountain Birch and it's implications for herbivory *Oikos* **70,** 212-222.

Tibbits, W.N. (1986). Eucalypt plantations in Tasmania. *Australian Forestry*

Volker, P.W., Owen, J.W. and Borralho, N.M.G. (1994). Genetic variances and co-variances for frost tolerance in *Eucalyptus globulus* and *E. nitens*. *Silvae Genetica* **43,** 366-372.

Waltz, A.M. and Whitham, T.G. (1997). Plant development affects arthropod communities: opposing impacts of species removal. *Ecology* **78 (7),** 2133-2144.

Warwick, R.M., Clarke, K.R. and Suharnso. (1990). A statistical analysis of coral community responses to the 1982-1983 El Nino in the Thousand Islands, Indonesia. *Coral Reefs* **8,** 171-179.

Whitham, T.G. (1989). Plant hybrid zones as sinks for pests. *Science* **244**, 1490-1493.

Whitham, T.G., Morrow, P.A. and Potts, B.M. (1994). Conservation of Hybrid Plants. *Science* **254,** 779-780.

Whitham, T.G., Morrow, P.A. and Potts, B.M. (1994). Plant hybrid zones as centres for biodiversity: the herbivore community of two endemic Tasmanian eucalypts. *Oecologia* **97**, 481-490

Whitham, T.G., Martinsen, G.D., Floate, K.D., Dungey, H.S., Potts, B.M. and Keim, P. (1997). Plant hybrid zones affect biodiversity: Tools for a genetic based understanding of community structure. *Ecology*

William, A.G. and Whitham, T.G. (1986). Premature leaf abscission: an induced defense against gall aphids. *Ecology* 67, 1619-1627.

Wiltshire, R.J.E. and Reid, J.B. (1992). The pattern of juvenility within *Eucalyptus tenuiramis* Miq. Saplings. Proceedings: Mass production technology for genetically improved fast growing forest tree species, Bordeaux, France 14-18 September.

Wiltshire, R.J.E., Murfet, I.C., and Reid, J.B. (1994). The genetic control of heterochrony: evidence from developmental mutants of *Pisum sativum* L. *Journal of Evlutionary Biology*, **7 (4)**, 447-465.

Wiltshire, R.J.E., Potts, B.M. and Reid, J.B. (1991). A paedomorphocline in *Eucalyptus*. Variation in seedling morphology in the *Eucalyptus risdonnii/Eucalyptus tenuiramis* complex. *Australian Journal of Botany* **39,** 545-566.

Wiltshire, R.J.E., Potts, B.M. and Reid, J.B. (1992). A paedomorphocline in *Eucalyptus*. II. Variation in seedling morphology in the *Eucalyptus risdonnii/Eucalyptus tenuiramis* complex. *Australian Journal of Botany* **40 (6),** 789-805.

Wiltshire, R.J.E., Potts,B.M., and Reid, J.B. (1998). Genetic control of reproductive and vegetative phase change in the *Eucalyptus risdonnii-E.tenuiramis* complex. *Australian Journal of Botany* **46 (7).**

Zagory, D. and Libby, W.J. (1985). Maturation-related resistance of *Pinus radiata* to Western Gall Rust. *Phytopathology* **75**, 1443-1447.

Appendix 1

Examples of the individual dependent taxa used in the analysis of responses to plant variation. The numbers correspond to the taxa number in Table 2.1.

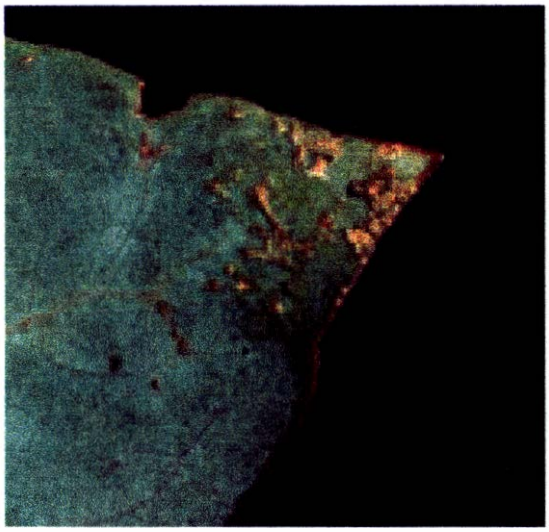
1) *Chrysoptharta agricola* beetle and damage

2) Chrysomelid bettle #2

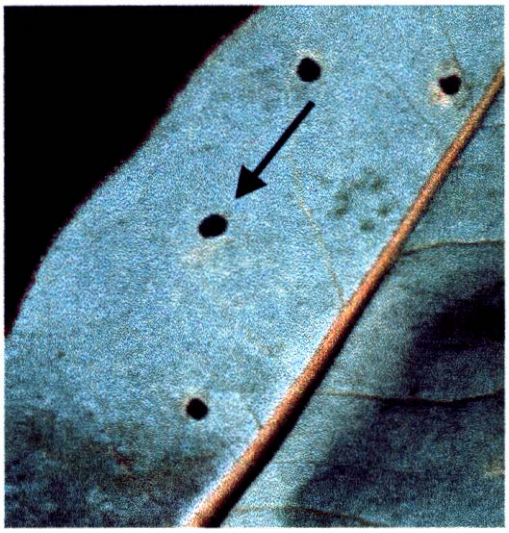
3) *Ctenarytaina eucalypti* (Psyllid #1)

4) *Chrysoptharta agricola* larvae and damage

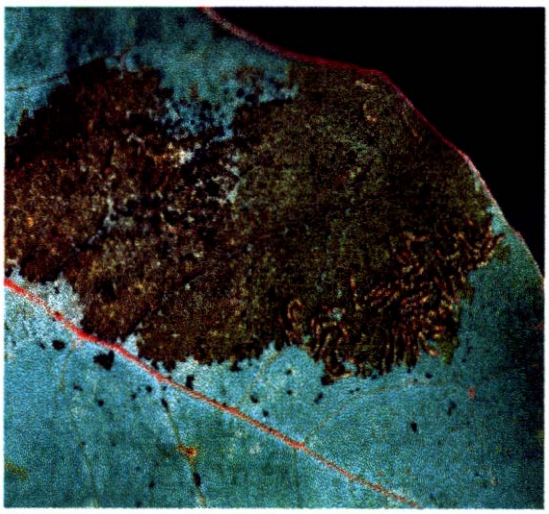
5) *Chrysoptharta agricola* eggscar



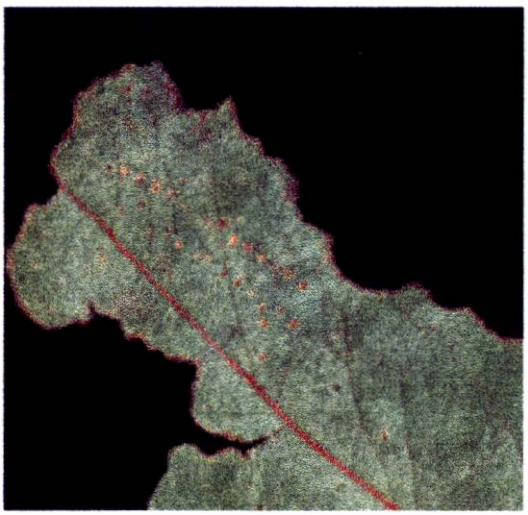
6) Psyllidae #2



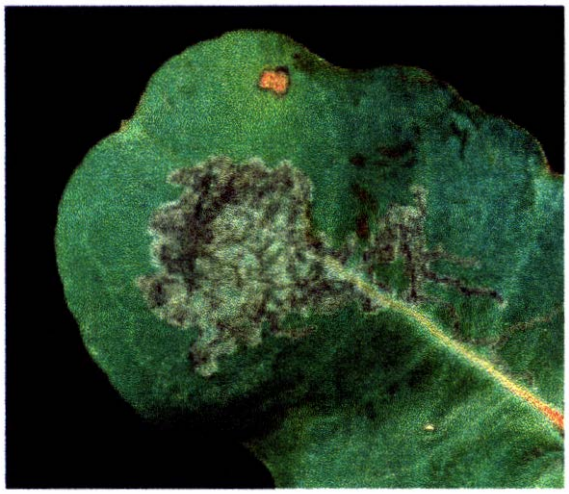
7) *Mnesempala Privata* (Autumn Gum Moth)



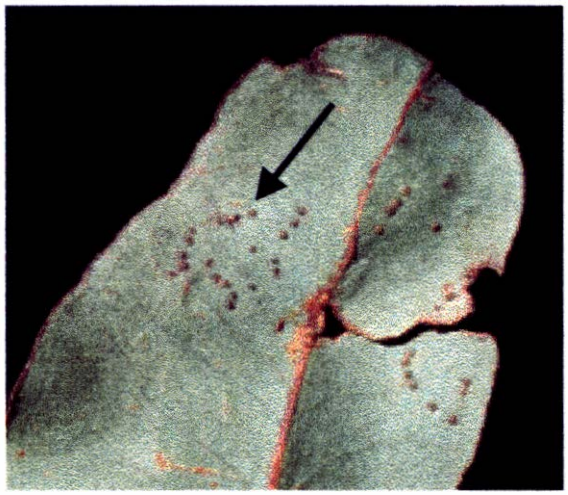
8) *Chrysoptharta bimaculata* eggscar



9) *Microlepidopteran #1* (Genus *Acrocercops)*



10) Hymenopteran #1

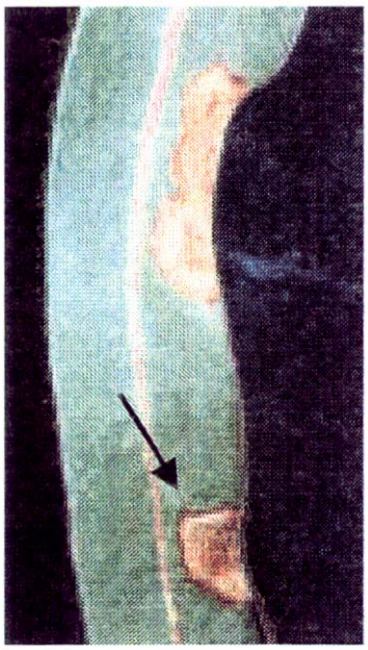


11) Hymenopteran #2

12) Hymenopteran #3

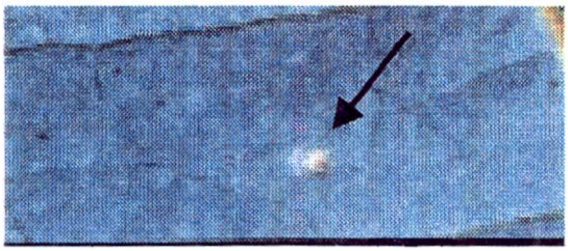
13) Homoptera #1 *Erriococcuss sp.*

14) Hymenopteran #4

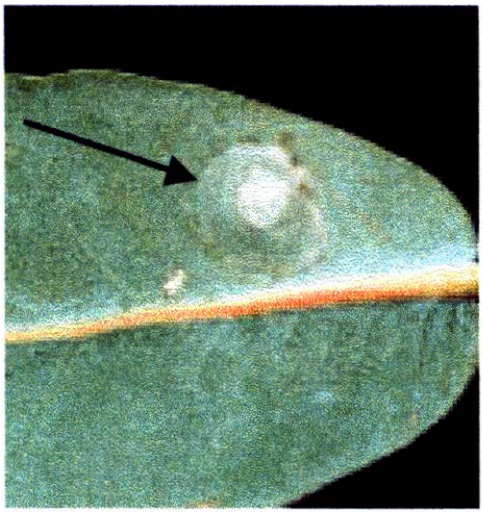


15) *Heteronyx sp.*

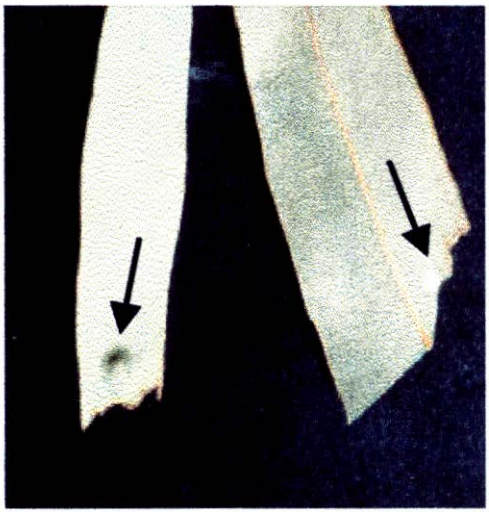
16) Psyllidae #3



17) Psyllidae #4 (Genus *Hyalinaspis*)

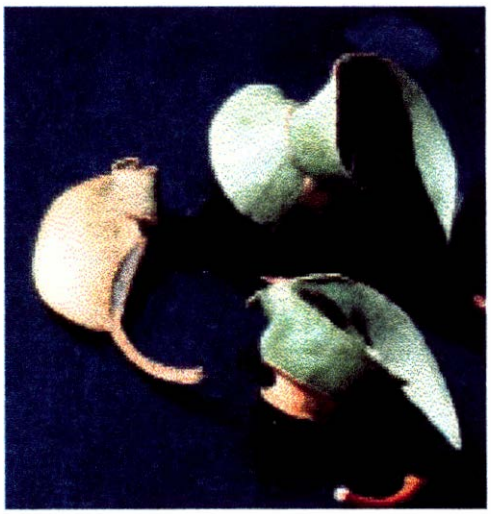


18) Mite gall

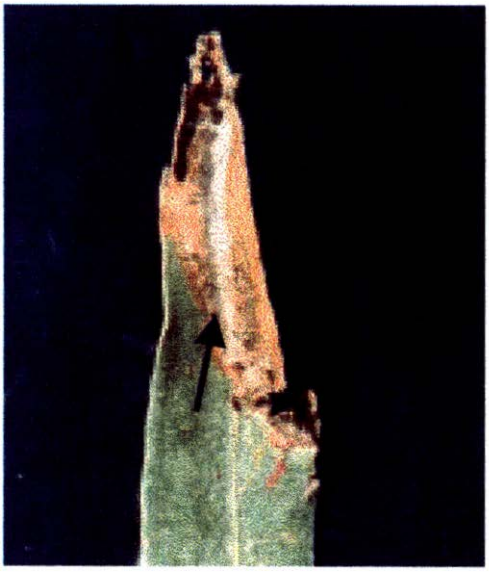


19) Psyllidae #5

20) Psyllidae #6

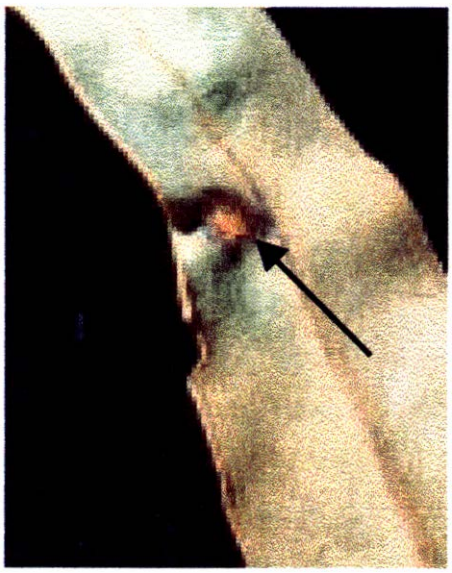


21) Lepidopteran #2

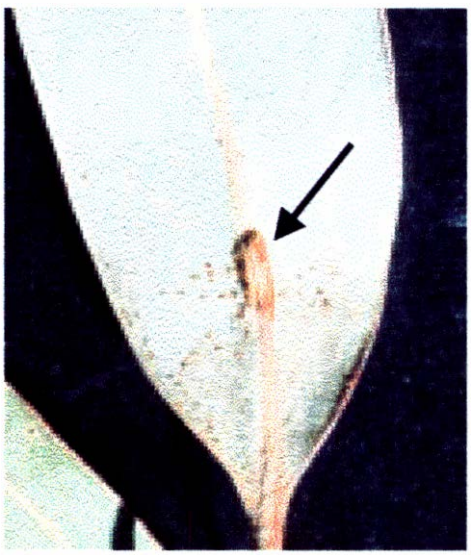


22) Arachnae #1

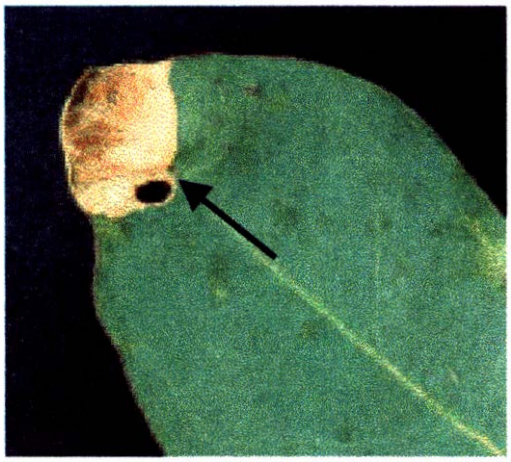
23) Leaf Miner #3



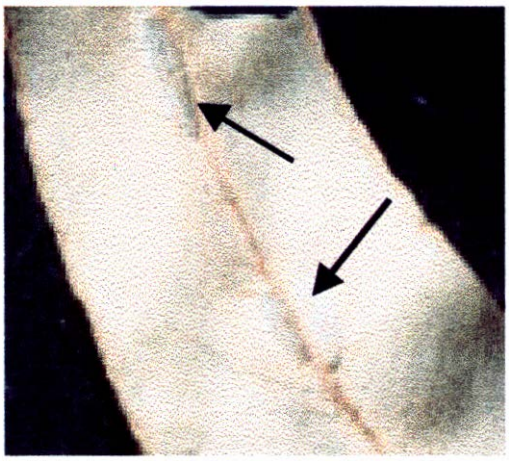
24) Hymenopteran #5



25) Microlepidopteran #2



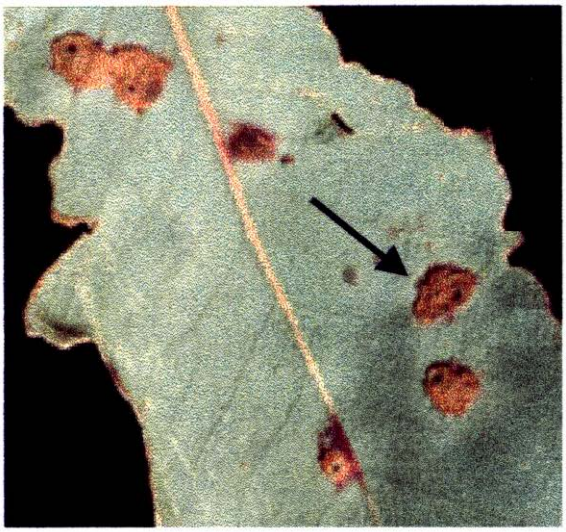
26) Hymenopteran #6



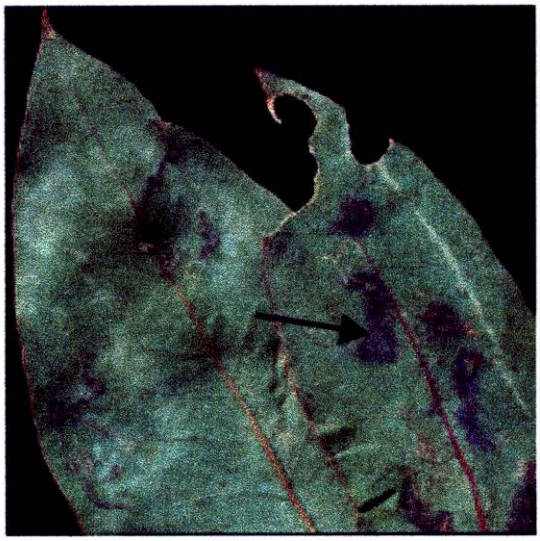
27) Hymenopteran #7

28) Arachnae #2

29) Leaf miner #4



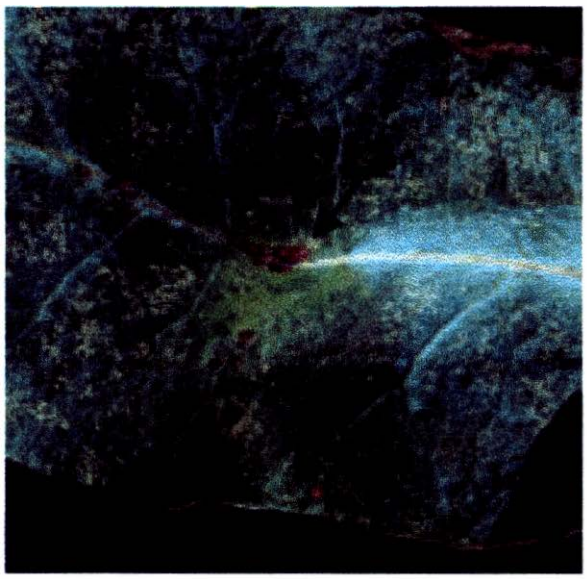
30) Homoptera #2 (Coccoidae)



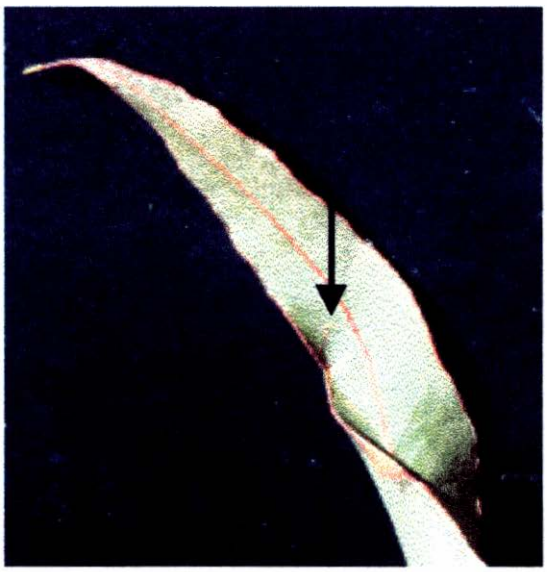
31) Lepidopteran #2

32) Microlepidopteran #3

33) Hymenopteran #3



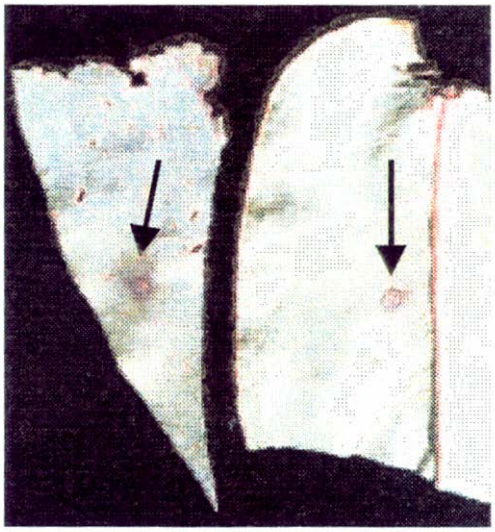
34) Microlepidopteran #4



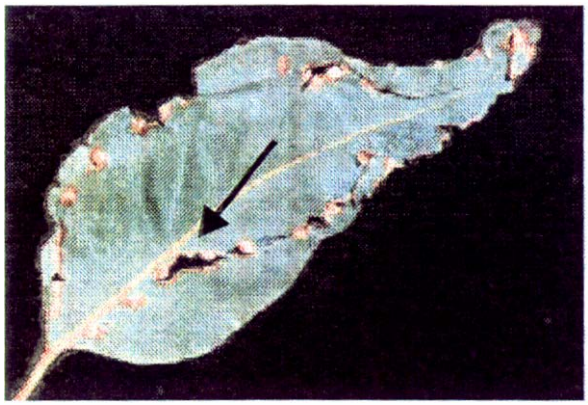
35) Dipteran gall

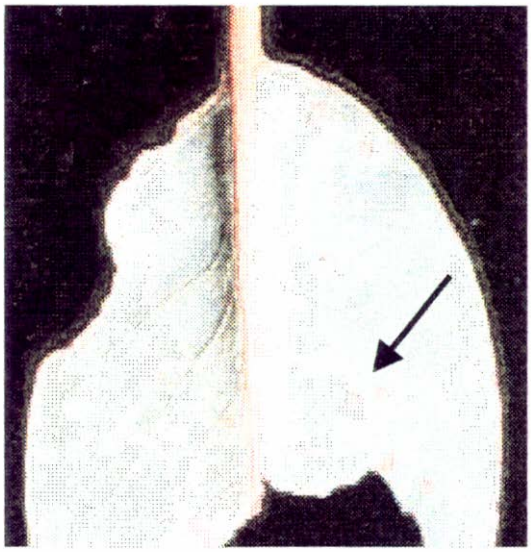
36) Weevil damage (*Gonipterus spp.)*

37) fungal type A



38) fungal type B



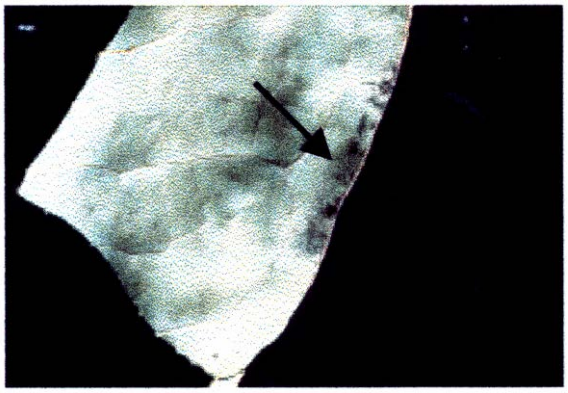


39) fungal type C

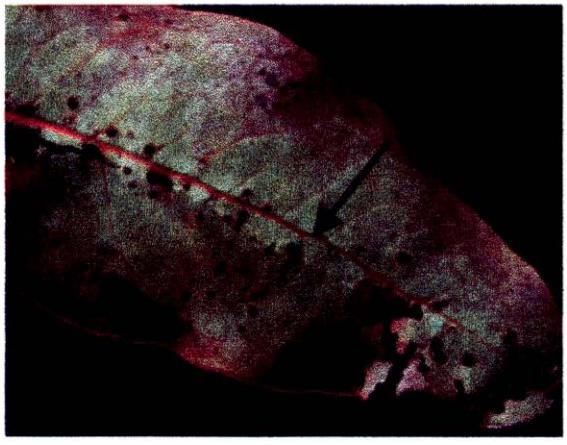
40) *Cylindrosporium samueli*
(fungal type D)

41) fungal type E

42) fungal type G



43) Fungal type H



44) fungal type I