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Insect herbivory on high altitude *Eucalyptus pauciflora* Sieb ex. Spreng saplings: the influence of local- and landscape-scale processes

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Declaration

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Abstract

The distribution and abundance of plant species in high mountain ecosystems is thought to depend largely on abiotic factors that play out at both landscape-scales (e.g. steep environmental gradients affected by increasing elevation) and local-scales (e.g. changes in topography, aspect and canopy cover). Relatively little attention, however, has been paid to biotic interactions and how these might also change with landscape- and local-scale factors. Ecological interactions between plants and insect herbivores are likely to alter species performance and affect local abundance but a comprehensive understanding of their role in the Australian alps remains largely ignored. In this study, I examined the extent and distribution of herbivory on saplings of the dominant high altitude tree, Snow Gum *Eucalyptus pauciflora* Sieb. ex Spreng (Myrtaceae). In addition, the potential for biotic interactions, seed origin and environmentally driven changes in plant morphology to influence local scale herbivory patterns was explored.

Using an existing reciprocal transplant experiment, field observations of saplings across altitudes and mountains were used to assess the distribution of herbivory. Across all mountains, herbivory was best explained by the combined influence of plant height, canopy openness, leaf thickness and altitude, while no influence of location or seed origin was found. The potential for ants to act as a biotic defence and influence local scale patterns of herbivory was assessed through a comparison of saplings open to ants to those where ants were excluded. However, no influence of ant presence was found. In addition, the potential for seed predation by ants to influence the dispersal and persistence of saplings was assessed using cafeteria trials. Ant harvesting of seed was observed but removal rates were low compared to that of other systems.

This study highlights the individuality of each mountain environment, at landscape and local-scales, as well as the complexity of relationships between environmental change, plants and insects. The factors that best explain herbivory across mountains suggests that herbivory may increase with increasing productivity associated with altitude, a trend in broad agreement with hypotheses associating leaf area loss to the availability of resources and plant vigour.

Chapter 1

Introduction

Plants dominate our view of the world. However, where there are plants there are almost certainly insects. These two highly diverse groups form relationships both intimate and complex, that have influenced not only their own diversity and evolution, but that of the communities in which they exist (Ehrlich & Raven 1964; Futuyma & Agrawal 2009).

For an insect, a host plant is more than simply a source of food (Schoonhoven *et al.* 1998). However, a major force in the evolution of both these groups stems from interactions whereby plants strive to avoid consumption while insects aim to optimise their utilisation of plants as food (Schoonhoven *et al.* 1998). Thus, there exists an 'evolutionary arms race', a reciprocal interplay with plants developing novel defences and insects' evolving strategies to overcome them (Futuyma & Agrawal 2009). In turn, insect herbivores have become highly specialised feeders (Schoonhoven *et al.* 1998; Strong *et al.* 1984) and plants have evolved complexes of physical and chemical defences (Price 2011; Strong *et al.* 1984), traits that have led herbivory to be highly variable in both space and time (Crawley 1983).

Through the expression of gene and environment interactions, individual plants are morphologically, chemically and nutritionally distinct and thus, vary in their palatability to insects (Strong *et al.* 1984). Such heterogeneity exists not only between species but also within species, populations and stands and, consequently, is the greatest defence a plant has against herbivores (Whitman 1983; Bernays *et al.* 1994). Inter-specific variation is beneficial to plants in two ways: (i) by concealing high quality hosts among others of varying quality, diluting the effect on any one plant, and (ii) through exposing insects to a spectrum of chemical and physical defences, limiting their ability to develop resistance (Rausher 1983; Whitman, 1983).

In the search for a suitable host, the abundance and distribution of plants, spatial and temporal variation in plant quality, and the nature of the surrounding vegetation all influence insect success (Schowalter 1986). Between and within species, plants differ in their suitability to insects. Similarly, different stages of insect development carry different nutritional requirements (Price *et al.* 2011; Schoonhoven *et al.* 1998). Thus, when searching for a suitable host, insects are confronted with a series of decisions and are often highly selective in the host they chose for both oviposition and consumption (Rausher 1983). Such behavioural responses to varying plant quality is thought, in part, an evolutionary response to plant heterogeneity as selecting a plant of low quality carries consequences for development, reproduction and fitness (Rausher 1983; Whitman 1983; Bernays & Chapman 1994).

An important form of variation between plants is in anti-herbivore defences (Crawley 1983), which for plants exist in three main forms - (i) *physical* protection by hairs, spines, waxes or strengthening with lignification; (ii) *chemical* protection with toxic compounds inherent within their tissues; and (iii) *biotic*, where animals such as ants are recruited to protect foliage (Crawley 1983; Rico-Gray & Oliveira 2007). The most common physical defence is that of the leaf surface, whereby waxes, lignin or silica permeate the epidermis forming tissue resistant to puncturing and chewing (Strong *et al.* 1984). In addition, trichomes (hairs) may form impenetrable mats or beds of short spikes that inhibit insects gaining a hold of foliage or biting into tissue (Bernays & Chapman 1994). Chemical defence is in the form of plant secondary metabolites (PSMs) that are suffused within tissues or induced, and act through deterrence of insect feeding, disruption of digestion or as a toxin, with poisoning following ingestion (Crawley 1983; Lawler & Foley 2002). Biotic defences are often in the form of mutualisms with ants whereby plants offer rewards in the form of nectar in extra floral nectaries or support populations of honeydew secreting hemiptera, prompting ants to act territorially and protect their foliage (Buckley 1987; Crawley 1983).

When searching for a host, insects are faced with a mosaic of resistance within which they must distinguish the suitable from unsuitable (Rausher 1983; Bernays *et al.* 1994). Owing to the consequences associated with selecting a host of poor quality, insects are often extremely selective in terms of the plants they chose to consume, resulting in the uneven distribution of damage among plant hosts within a single community (Crawley 1989; Rausher 1983; Bernays 1994). Resource allocation, plant vigour and plant stress are the three most widely acknowledged hypotheses used to describe this variation in plant host selection (Cornelissen *et al.* 2008; Hunter & Price 1992; Landsberg & Gilleson 1995; Maschinski & Whitman 1989; Williams & Cronin 2004). The 'resource allocation hypothesis' predicts the regulation of resources allocated to either plant growth or defence is determined by their availability. Hence, as the production of secondary metabolites and physical defences can be expensive for a plant to produce, their production is thought beneficial only when the benefits gained in protection are greater than their cost (Coley 1985). Consequently, plants of low nutrient environments are likely to allocate more to defence than growth, as the development of new tissue production becomes costly, whereas plants of high resource environments are likely to favour growth due to the availability of resources allowing the replacement of lost tissue (Coley 1985). The 'plant vigour hypothesis' suggests plants that grow vigorously within a population, or modules that grow vigorously within a plant, will be preferred by herbivores, and consequently lose a greater portion of tissue (Price 1991). Vigour in this sense is defined as any plant, or module within a plant, that grows quickly and reaches a size greater than the population average (Price 1991). Such plants are thought favourable due to higher quality tissues, less chemical and physical defences and an increased availability of resources in flush growth (Cornelissen 2008; Price 1991). Conversely, the 'plant stress hypothesis' suggests stressed plants are more attractive to herbivores as the accessibility of nutrients within a plant increases with the withdrawal of resources from senescing tissue (White 1984).

Thus, the abiotic environment, through its influence on the resources available to plants and insect demography, is largely responsible for relationships between herbivores and hosts, and consequently, the patterns of herbivory observed within a population (Crawley 1983;1989; Bernays *et al.* 1994). Interactions at both landscape (e.g. soil, temperature, aspect, rainfall) and local scales (e.g. topographically induced microclimates, soil moisture and nutrient availability, canopy cover and light availability), suggests abiotic factors will influence not only populations but also individuals in ways that alter plant palatability and herbivore abundance. This, in turn, will define the distribution of herbivory among hosts and populations and, subsequently, community dynamics (Crawley 1983; Price 2011).

The potential for biotic interactions to influence insect herbivory directly through the aggressive deterrence of herbivores, or indirectly by altering local abiotic conditions, is also important when discussing plant defence (Crawley 1983; Rico-Gray & Olievier 2007). Mutualisms between ants and plants that result in plant protection against herbivores are widespread, and acknowledged as having significant influence over insect herbivore-plant dynamics (Buckley 1987; Rico-Gray & Olievier 2007). Such relationships exist with different levels of investment by plants. While some can be direct, whereby the plant offers rewards to ants in the form of extra-floral nectaries and nesting sites, others are mediated through hemiptera that harvest plant resources and recruit ants with the presence of honeydew (Rosumek *et al.* 2009). While the outcomes of such interactions are highly variable, they have the potential to significantly alter the fitness of a plant and its persistence within a landscape (Messina 1981; Rico-Gray & Olievier 2007).

Another ant-plant mutualism, of interest when discussing plant fitness and herbivory, is that of seed dispersal. Here, the benefit to the plant lies in the relocation of propagules away from the parent tree; with increased distance, seeds are less likely to be affected by competition with the parent, infected by host specific pathogens or predated (Janzen 1970). Additionally, seeds may benefit through the relocation to ant nests, thought to be nutritionally superior micro-sites and thus, a high-quality site for a seed to germinate and grow (Andersen 1988b; Rico-Gray & Olievier 2007; Whitney 2002). In order for seeds to benefit, however, they must be discarded. Thus, many plants have developed the eliasome and arils; external 'ant-attractants' affixed to seeds that prompt ants to harvest diaspores whole, only later discard the seed unharmed, once the attractive portion is removed (Rico-Gray & Oliveira 2007). Seed predation by ants, where seeds are harvested for compounds inherent in their seed coat or for the nutrient-rich endosperm, is also widespread. Seeds may, however, benefit from such antagonistic interactions if the seed is discarded or forgotten (Beattie 1985; Rico-Gray & Oliveira 2007; Howe & Smallwood 1982).

The effect that variation in chemical, physical and biotic defences has on insect and plant fitness, distribution and persistence within a landscape has been studied in many systems around the world (Suzuki 1998; Coley 1983;1980; Spiller & Agrawal 2003; Fritz *et al.* 2003; Auslander *et al.* 2003; Larsson *et al.* 1986; Alonso & Herrera 1996; Alonso 1999;

Cornelissen *et al.* 2008; Sagers 1992; Crawley & Akhteruzzaman 1988). In Australia, a continent peculiar in its dominance by a single angiosperm genus, the *Eucalyptus,* studies of leaf chemistry and its effects on herbivore host preference and performance have dominated those looking to understand the relationship between insects and this dominant genus. This is understandably so, given that chemical defences have been found to exert an inconsistent, yet considerable influence over insect choice in this genus (Lawler & Foley 2002; Ohmart & Edwards 1991). However, foliage is defended not only chemically but also physically and at times biotically (Nahrung *et al.* 2001), and yet various defences and their relationship with herbivores are often analysed in isolation.

Predominantly an arid continent, Australia has nutrient-poor soils and landscapes prone to frequent fires (Bjorkman *et al.* 1991). Thus, the evolution of eucalypts in Australia was under stressful conditions, the evidence of which is found in the distinct morphology of the genus (Wardell-Johnson *et al.* 1997) and the co-evolutionary adaptations of its herbivores (Carne 1966; Matsuki *et al.* 2011; Morrow *et al.* 1976; Cooper 2001). Soils of Australia are nutrient-poor due to the quantities of nutrients rather than the qualities. The result of long-term weathering, soils are deficient in nutrients essential to the growth of many organisms, including phosphorous, zinc, iodine, cobalt and selenium (Orians & Milewski 2007). However, regardless of such a scarcity in indispensible nutrients, eucalypts are able to photosynthesise at relatively high rates providing sunlight and soil moisture are sufficient, in turn producing sufficient energy for fast growth. Hence, with the pendulous nature of eucalypt foliage that maximises light capture and high levels of insolation across much of Australia, conditions that support photosynthesis are rarely limiting (Orians & Milewski 2007; Williams & Brooker 1997). Through the use of stored nutrients in lignotubers and long-lived leaves or those acquired through symbioses with bacteria, many eucalypts photosynthesise at high rates, producing large quantities of excess carbon-rich energy which they allocate to the synthesis of nutrient-poor wood, or secondary compounds that defend against herbivores (Bell & Williams 1997; Orians & Milewski 2007; Williams & Brooker 1997).

The nutrient-poor soils and aridity of Australia influence not only the chemistry of eucalypt foliage but also its morphology (Williams & Brooker 1997). Sclerophyllous due to high levels of lignification, eucalypt herbivores are presented with foliage that is not only low in nutrients and protected chemically but at times physically unpalatable (Wardell-Johnson 1997; Williams & Brooker 1997). Both adult and juvenile leaves are coated in a thick cuticle overlaid with protective waxes, with countless oil glands throughout the parenchyma, and a suffusion of secondary metabolites within the mesophyll tissue (Boland *et al.* 1991; Brooker 2002). The form of adult leaves varies little between species, while juvenile forms vary considerably in colour, shape, size, orientation and chemistry (Raymond 1998; Williams & Brooker 1997). In turn, such variation affects the ability of insects to exploit different species and various stages of development (Ohmart & Edwards 1991). Additionally, it is not only foliage but also seed capsules and the bark of eucalypts that are suffused in carbon-rich secondary compounds - alkaloids, phenols, tannins and terpenes (Ohmart & Edwards 1991) that vary significantly within and between species (Boland *et al.* 1991). While variation between species is qualitative, within a species variation is usually, but not solely, quantitative in nature (Williams & Brooker 1997). Furthermore, eucalypts are primarily evergreen; however, leaf longevity and timing of new growth vary between species and environments. The shedding of leaves and sprouting of new growth can be constant throughout the year or seasonal, with notable consequences for insects that feed on new growth (Ohmart & Edwards 1991).

Regardless of such defences, insect herbivory on eucalypts is significant, with leaf area losses in the order of 20-50% annually (Ohmart & Edwards 1991). The near absence of herbivory in eucalypts grown outside Australia suggests that within Australia, herbivores are highly specialised, having co-evolved to counter eucalypt defences (Paine *et al.* 2011) However, herbivory varies significantly between species and environments, as well within a single species and stand (Lawler & Foley 2002; Steinbauer *et al.* 2010; Edwards *et al.* 1993). While *Eucalyptus* is a specious genus, comprising more than 700 species that occupy most Australian environments capable of supporting trees (Williams & Brooker 1997), mortality as a result of herbivory is rare (Ohmart & Edwards 1991). When high levels of defoliation do occur, they are linked to changes in plant fitness, morphology, phenology, chemistry, competitive ability (Bryant 1971; Ohgushi 2005; Lowman 1995), and increased susceptibility to pathogen and secondary attack (Kulman 1971; Nahrung *et al.* 2001), and thus have consequences for community dynamics (Ohmart & Edwards 1991).

As is the case of many plant-insect interactions, the hypotheses most commonly used to explain such intra-specific variation in eucalypts are those of plant vigour (Price 1991), plant stress (Fox & Macauley 1977; Mattson 1980; White 1984) and concentrations of chemical defences, specifically PSMs (Fraenkel 1959; Cooper 2001; Lawler & Foley 2002). In all cases, there is particular emphasis of bottom-up influences, namely the effect of the abiotic environment and subsequent availability of nutrients on plant resource allocation (Cornelissen *et al.* 2008; Lawler *et al.* 1997). In addition, eucalypt morphology and defences have a genetic element contributing to their composition and expression (Boland *et al.* 1991; Pryor & Bryant 1958; Lawler & Foley 2002).

Clarifying the role of environment in resource allocation, morphological and chemical trait development and insect-herbivore behaviour has received significant attention within plantation-grown eucalypts, cultivated in monocultures and periodically harvested for timber (for example: Braganca *et al.* 1998; Close *et al.* 2004; Cordero Rivera & Santolamazza Carbone 2000; de Freitas *et al.* 2005; Guedes 2000; Jordan 2002; Judd *et al.* 1996; Paine *et al.* 2011; Rapley *et al.* 2004; Stone 2001). However, relatively little attention has been paid to understanding natural patterns, that may be important in understanding what drives species distributions (Ohmart & Edwards 1991; Davidson & Morton 1985; Burdon & Chilvers 1974a; Steinbauer 2010; Close *et al.* 2005; Lawler *et al.* 1997). This is particularly pertinent for species at the edge of their range or distributed across sharp environmental gradients, like those of sub-alpine and alpine systems whose morphology is often heavily influenced by altitude (Korner 1999; Pryor 1956). Consequently, the potential for sub-alpine environments to change considerably over short distances deems them ideal for investigating the role of environment in eucalypt-insect interaction (Korner 2007).

In the Australian Alps, *Eucalyptus pauciflora* Sieb ex. Spreng (Snow Gum) is the dominant tree of the subalpine vegetation zone, ranging in altitude from 1100 m to the upper treeline at >1900 m (Burdon and Chilvers 1974b). The response of Snow Gum morphology to increasing altitude was first documented by Pryor (1956), who, through a comparison plant traits between altitudes in the Brindabella Ranges, ACT, found distinct trends with elevation that were under both genetic and environment control. Burdon and

Chilvers (1974) examined the effect this variation may have on phytophagous insect herbivores and parasites associated with Snow Gum saplings. They found a negative relationship with altitude, density of herbivores and herbivory. Interestingly, herbivory was found to vary significantly within altitudes, a finding later confirmed by Williams (1990). Both studies failed, however, to demonstrate what underpins insect choice and behaviour that leads to such trends.

Despite recognition of the potential effects of herbivory for plants, many studies of *E. pauciflora* seedling recruitment, distribution and persistence in sub-alpine regions of Australia scarcely acknowledge it (Ball *et al.* 1997; Davidson & Reid 1985; Egerton *et al.* 2000; Ferrar *et al.* 1988; Noble 1980). A closer look at within- and between-altitude variation in young developmental stages of *E. pauciflora* may allow a better understanding of what drives patterns in herbivory, the effect of herbivory on persistence and the potential effects of selection for more palatable plants.

This thesis contains two broad components. First, a study of the extent, type and distribution of herbivory on saplings of *E. pauciflora* in response to seed origin, as well as landscape (e.g. altitude, location) and local (e.g. canopy cover, aspect) scale processes (Chapter 2). Secondly, the potential of biotic interactions between ants and plants to influence herbivory is explored (Chapters 3 & 4). Chapter 5 summarises the major findings of the study and applying these observations, provides some suggestions for future research.

Specifically this study addresses the following questions:

i). Is herbivory of *E. pauciflora* saplings a function of processes acting at the landscape and local scales, and how do such processes and seed origin influence plant traits and palatability to herbivores (Chapter 2)?

ii). Do ants that form tri-trophic relationships involving hemiptera and *E. pauciflora,* influence local herbivory patterns by acting as a biotic defence (Chapter 3)?

iii) Do ants influence the distribution and fitness of *E. pauciflora* saplings through seed dispersal (Chapter 4).

Study Area

The study was conducted in the Victorian alpine region, approximately 350 km north-east from Melbourne (-36° 57' S, +147° 11' E) (Figure 1). Soils are the alpine humus type; highly organic, acidic and nutrient poor (Costin 1955). They are uniform in texture and can reach depths of up to 1 m. Gneiss and granite are the principle rock types throughout the region, with basalt and slate occurring less frequently (Costin 1955).

Average annual precipitation is high (1200-2500 mm), with most (60%) falling as snow (Ashton and Williams 1989). Frosts are frequent, and can occur at any time of the year (Williams 1987). Average monthly temperatures range from approximately 6 – 19 °C in summer and -0.1- 11 °C in winter (Bureau of Meteorology, *unpublished data*) (Figure 2).

Vegetation shifts from forests (more than 20 m high) to woodlands (up to 5 m), dominated by *E. pauciflora*, with an understorey of grasses or shrubs, often determined by both elevation and the parent material of the soil (Ashton & Williams 1989). Above the treeline, grass tussocks and forbs are common (Kirkpatrick & Bridle 1998).

This study focused on five mountains: two in the Mt Hotham resort region (Mt Hotham and Mt Blowhard), and three further south within the Alpine National Park (Mt Howitt, Mt Magdala and King Billy 1) (Figure 1). Both elevation and geology differed between mountains (Table 1). Throughout the study period (August 2011- February 2012), mean monthly minimum and maximum temperatures, and mean monthly rainfall varied little between Mt Hotham and Falls Creek, while Mt Buller, the closest weather station to Mt Howitt, Mt Magdala and King Billy, experienced less rainfall and marginally higher temperatures (Figure 2).

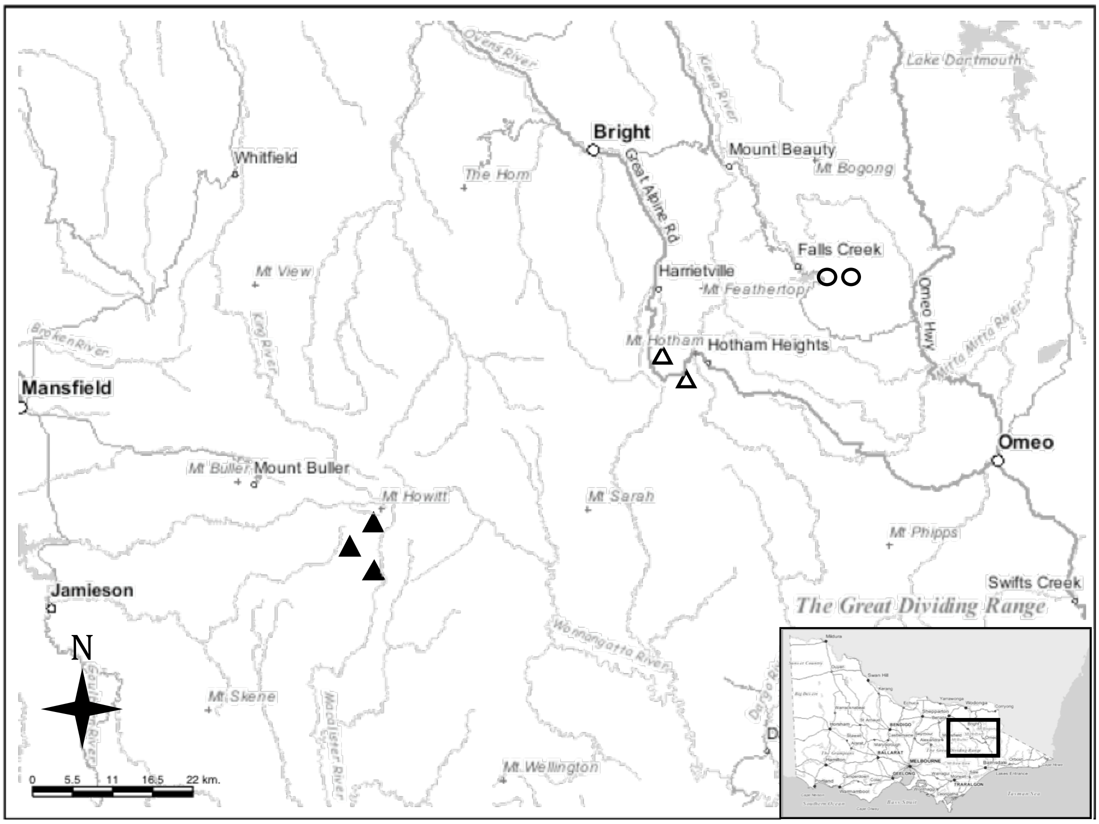


Figure 1. Location of the study sites: Mt Howitt, Mt Magdala and King Billy 1 (▲) Mt Hotham and Mt Blowhard (△), and Falls Creek (○).

Table 1. Description of the mountain study site locations, elevation and geology.

Study site	Location	Elevation (m.a.s.l)	Geology/ Parent material
Mt Hotham	36°58’35’’S 147°07’30’’E	1860	Ordovician sedimentary (marine) siltstones, sandstones and shales
Mt Blowhard	36°59’60’’S 147°06’30’’E	1690	
Mt Howitt	37°10’55’’S 146°38’31’’E	1742	Carboniferous sandstones, mudstones and conglomerates
Mt Magdala	37°15’30’’S 146°37’15’’E	1725	
King Billy	37°12’21’’S 146°36’31’’E	1693	Extrusive Tertiary basalts

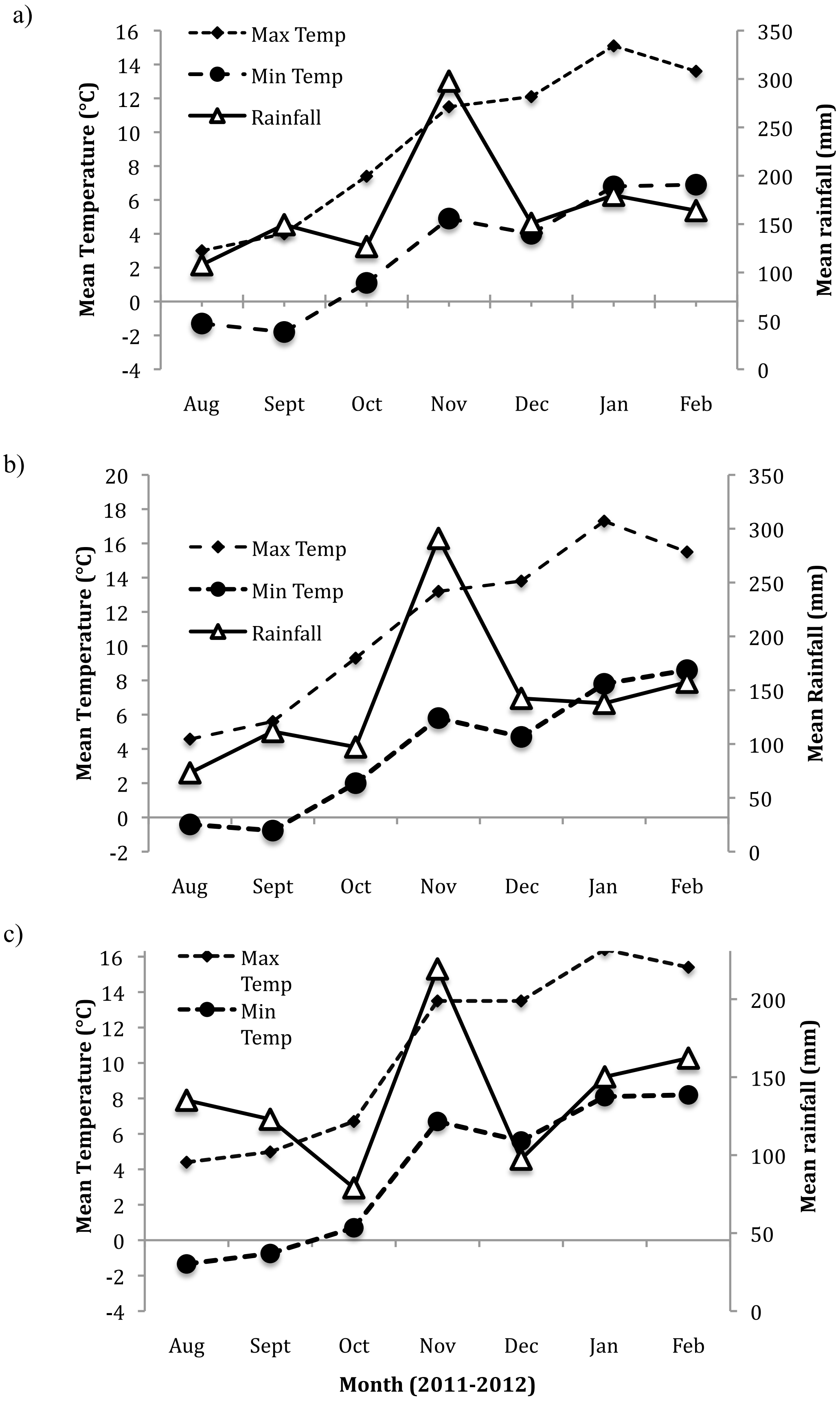


Figure 2. Monthly maximum temperature, minimum temperature, and rainfall for (a) Mt Hotham (data also relevant for Mt Blowhard), (b) Falls Creek and (c) Mt Buller (the closet weather station to Mt Magdala, Mt Howitt and King Billy) over the experimental period.

Chapter 2

Insects and the environmental gradients of mountains

Introduction

For plants, the earliest stages of establishment are considered the most vulnerable (Bell and Williams 1997) with both abiotic (e.g. temperature, frost, drought) and biotic (e.g. competition, interactions with herbivores and pathogens) factors key in determining their success (Ferrar *et al.* 1988; Hodkinson 2005; Korner 1999). Through the use of environmental gradients, the effects of changing abiotic factors on plant survival and physiognomy have been well studied (see Korner, 1999). However, how insect herbivory changes along such gradients has received relatively little attention (Garibaldi *et al.* 2011). The potential for herbivores to significantly alter plant fitness suggests that, in order to understand distributional patterns of plants, knowledge of the interaction between plant and herbivore, and how this co-varies with environmental change, is vital.

The severity of herbivore damage experienced by a plant is a function of three interacting factors: (i) the palatability of the plant, (ii) plant community composition and complexity and, (iii) the dynamics of herbivore populations (Crawley 1983). Plant susceptibility depends primarily on morphological and chemical defences that are determined by genotype, access to resources and climate (Boland *et al.* 1991). Within a plant, the allocation of resources varies with their availability (Coley *et al.* 1985; Massey *et al.* 2007; Wilkens *et al.* 1996). Thus, the concentration of nutrient and non-nutritional compounds that are of importance to herbivores or, alternatively, provide protection to plants, vary in response to the environment (Coley *et al.* 1985).

In high mountain ecosystems, this variation can occur at both local- and landscape-scales (Korner 1999). At the landscape-scale, increasing altitude leads to increased environmental stress which manifests as reduced availability of resources (Korner 2007).

That, in turn, can lead to an increased allocation of resources to tissue defence and less to growth, with distinct changes in plant morphology (Garibaldi *et al.* 2011; Korner 1989; 1999). The local-scale effects of topography on microclimate may, however, override the general effect of altitude to alter site quality over relatively short distances (Hodkinson 2005).

Concentrations of foliar nutrient (K, P and N) and non-nutrient compounds important to herbivores (secondary metabolites: tannins, phenols) are primarily edaphic; however, tree age, leaf age and season contribute substantially to between- and within-plant variation (Bell & Ward 1984; Loney *et al.* 2006; Moore *et al.* 2004). Of all foliar nutrients, leaf N has been shown to significantly influence herbivore preference and performance (Fox & Macauley 1977; Morrow & Fox 1980; Ohmart *et al.* 1985; Bernays & Chapman 1994). Foliar nitrogen concentrations are primarily determined by the availability of nitrogen within the soil (Orians & Milewski 2007), a function of landscape position and the associated microclimate (Bowman *et al.* 2003). However, concentrations available to herbivores are known to increase in senescing tissues through the translocation of nutrients prompted by plant stress (plant stress hypothesis) (White 1984). By contrast, leaf nutrient availability (and hence, susceptibility to herbivores) varies due to the influx of nutrients to new growth in aid of leaf development (plant vigour hypothesis) (Price 1991). Both hypotheses have been proposed as explanations for herbivore attraction to certain tissues within plants and may also help explain insect outbreaks (White 1984; 2009). Owing to the nutrient-poor foliage of eucalypts, these hypotheses may be important in understanding the distribution of insect damage within this genus. While low leaf nitrogen concentrations may be compensated for through increased leaf consumption, such behaviour may be hindered by plant defences, which, in turn, may lead to the preference of tissue with elevated nitrogen concentrations (Ohmart & Edwards 1991).

Leaf consumption by herbivores is often regulated by the presence of PSMs and the varying tolerance of insects to the compounds ingested (Crawley 1983; Bernays & Chapman 1994). Within the foliage of eucalypts, concentrations are particularly high in the form of essential oils and phenolic compounds (Boland *et al.* 1991). However, considerable variation, both qualitative and quantitative, exists between species, across populations and within stands (Andrew *et al.* 2007; Boland *et al.* 1991; Steinbauer 2010). Attempts to understand this variation commonly invoke hypotheses derived from the resource allocation framework, namely the ratio of available carbon to nitrogen (Bryant *et al.* 1983), growth-differentiation (Gershenzon *et al.*), and resource availability (Coley *et al.* 1985), all based on the notion of a physiological trade off between processes of growth and differentiation (Herms & Mattson 1992). Thus, for species occupying alpine ecosystems, stress associated with different altitudes, or variation in resource availability as defined by changes in microclimate, may help define the concentrations of PSMs within foliage and, consequently, palatability to herbivores.

Within eucalypt foliage, both physical and chemical defences (PSMs) are thought to significantly influence the palatability and quality of foliage to insects (Lawler & Foley 2002). Secondary metabolites have been found to act as both deterrents and attractants. However, insect response to these compounds are highly inconsistent suggesting species-specific responses (Edwards *et al.* 1993; Morrow & Fox 1980; Stone & Bacon 1994) or reactions with compounds not yet isolated (Lawler & Foley 2002). Physical defences, by contrast, are in the form of leaf waxes (glaucousness), trichomes or leaf strengthening (sclerophylly), that vary within and between species and individuals (Gutschick 1999). While such traits are determined initially by genotype and leaf age, resource allocation can also significantly influence their expression (Coley *et al.* 1985). Such traits function as defences primarily through restricting the biting and chewing of tissue or in preventing insects grasping the leaf long enough to feed (Cooper 2001). For example, for leaf chewers, edible leaves may be defined by their mandible size and leaf or leaf margin thickness, while leaf waxes may prevent insects gaining a hold of the leaf in the first instance (Sanson *et al.* 2001; Steinbauer *et al.* 1998). Thus, sclerophylly, laminar width and leaf waxes of foliage have the potential to influence the severity of herbivore damage experienced by a plant (Nahrung *et al.* 2001; Steinbauer *et al.* 1998). The influence of resource allocation over the expression of such traits suggests their importance to insect-eucalypt dynamics of alpine systems. As stated above, stress associated with changes of altitude and local variation due to topographically induced microclimates may induce changes at both the landscape and local scales that carry repercussions for palatability.

Within high-mountain ecosystems, insect presence and abundance is defined not only by the availability and palatability of their host plants, but the environmental tolerances of individual species (Bale *et al.* 2002). Thus, key factors involved in determining abundances are those that influence successful development through life history stages, specifically changes within predator populations, temperature required for development and host plant phenology, along with the immediate effects of the local climate on mortality (e.g. wind, snow, rain) (Crawley 1983). Consequently, in montane and sub-alpine woodlands, declining temperature with increasing elevation (i.e. the 'lapse-rate; 0.77 °C in Australia) will likely impose important restrictions on insect community composition, as it does for plants (Korner 1999). Sub-optimal temperatures may affect insect growth and development, while resource availability and stress influence plant growth and fitness (Price *et al.* 2011). Clarifying these relationships may be important in the understanding of recruitment patterns in *E. pauciflora* and the potential changes to both insects and plants that may occur as a result of global warming (Bale *et al.* 2002).

The sub-alpine to alpine woodlands in the Victorian alpine region offer a system well suited to the study of environmental determinants of herbivory in eucalypts, specifically as *E. pauciflora* forms monospecific stands from sub-alpine regions to the treeline, and an elevational gradient offers substantial environmental changes within short distances. Additionally, the use of an existing experiment whereby seedlings have been reciprocally transplanted between altitudes allows the assessment of the potential influence of seed origin on traits important to herbivory.

The relationship between *E. pauciflora* seedling/saplings, herbivory and altitude has been studied previously (Burdon and Chilvers 1974a,b; Williams 1990). Briefly, they found trends of decreased herbivory with increases in elevation and changes to insect species composition, although trends were diluted due to high levels of variation within each altitude, deeming them statistically insignificant. Importantly, however, these studies all centre on a single area within the Brindabella Range of the Australian Capital Territory, with altitude represented by a single site. Hence, it is unclear whether these observations have more general relevance to higher altitude populations of *E. pauciflora* over a larger area of its range. Thus, an investigation of Victorian populations, focusing on variation in plant traits, the influence of environment and their relationship with herbivores across mountains, seems a worthy study.

The aims of this study were:

1) To quantify herbivory on *E. pauciflora* saplings across an altitudinal gradient within and across mountains;
2) To document the relative importance of seed origin and local environment as regulators of herbivory on *E. pauciflora* at different altitudes;
3) To determine how *E. pauciflora* plant and leaf traits vary both within- and between-mountains and how this might effect the level of insect herbivory that occurs;
4) Document the distribution of herbivore types on *E. pauciflora* within- and across-mountains.

Experimental procedure

Experimental design

This study builds on the experimental design of S. Le Bel (LTU Botany PhD student, *unpublished data*) that investigates the effect of altitude on *E. pauciflora* sapling establishment and growth. I utilise the same experimental design to ask: how does the rate and type of herbivory vary with altitude, and does this depend on the effect of seed origin?

The field experiment was established between November and March 2009. At each of five mountains, 3-5 month old seedlings of *E. pauciflora* seedlings were planted. On each mountain, a single transect spanning just over 200 altitudinal metres was established. Along each transect, four experimental planting locations were established: 'alpine' (10-20 m above treeline),'treeline' (positioned at the treeline), 'mid' (100 altitudinal metres below the treeline), and 'low' (200 altitudinal metres below the treeline). These transects represent an average reduction of 13.4% in number of growing degree days between the treeline and the low site (Tinytag Ultra temperature data loggers in Stevensons screens from mid- October 2011 to early February 2012. Appendix 1). As the location of sites along each transect is determined by the position of the treeline, and the location of the treeline differs on each mountain, the absolute altitude of each site therefore differ between mountains (Table 2).

The original planting design tested the effects of seed origin on seedling establishment. Seed was sourced from each of the 'low', 'mid' and 'treeline' sites and grown into seedlings that were reciprocally transplanted back along each transect into each of the four sites. All seedlings were planted back into the mountain from which their seed was sourced.

At each site, seedlings were organised into 15 mini-plots. Each mini-plot contained three seedlings, one from each of the three seed origin sites (low, mid and treeline) (Figure 3). Forty-five seedlings were planted within each site, 15 from each seed source.

Table 2. Elevation, aspect and slope for each site within each mountain.

LOCATION	SITE	ALTITUDE (m)	ASPECT	SLOPE (°)
Mt Hotham	Low	1570	SSW	41
	Mid	1670		
	Tree-line	1780		
	Alpine	1785		
Mt Blowhard	Low	1455	ENE	40
	Mid	1555		
	Tree-line	1665		
	Alpine	1670		
Mt Howitt	Low	1497	S	49
	Mid	1597		
	Tree-line	1697		
	Alpine	1707		
Mt Magdala	Low	1493	S	48
	Mid	1593		
	Tree-line	1693		
	Alpine	1703		
King Billy	Low	1473	E	37
	Mid	1573		
	Tree-line	1692		
	Alpine	1702		

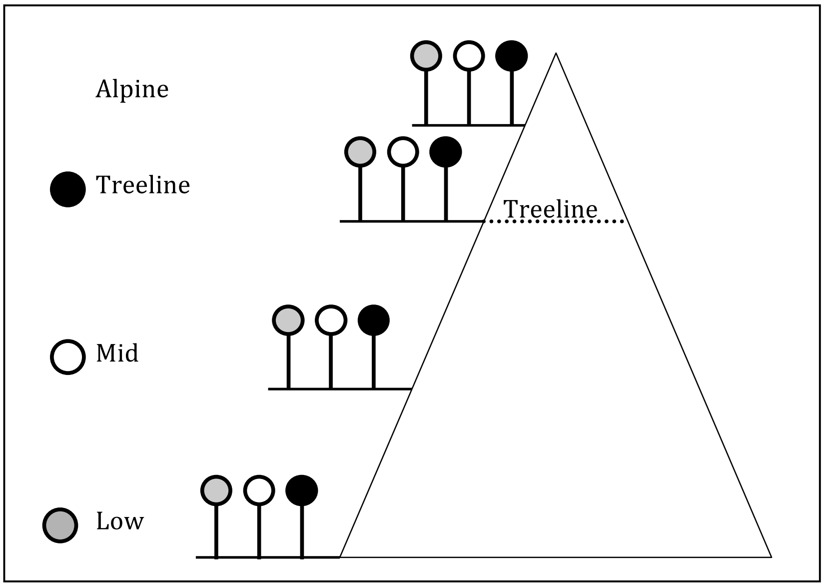


Figure 3. Design of reciprocal seedling transplant experiment, showing a single mini-plot (one of 15) comprising one seedling from each seed origin (low, mid and treeline) planted within all four sites.

Herbivory on established saplings

In November 2011, approximately 18 months after planting, all saplings on all mountains were visited and a visual assessment of their condition was made. For each live sapling, the total amount of herbivory (photosynthetic area lost) of all leaves was estimated by eye (to the nearest 5%). Leaves that were dead, substantially burnt by frost, or reduced to no more than a petiole were not included in herbivory estimates. The focus of this study is solely leaf herbivory and associated folivorous herbivores. While herbivory affects every part of a eucalypt from roots to stems to flowers to seeds (Landsberg & Cork 1997), defoliation is often significant with many flow-on effects for both plant and community (Hanley 1998).

Additionally, the types of herbivore damage on each sapling were categorised *in situ* based on the method of tissue removal (leaf chewers, miners/skeletonises and gall makers). The extent of epiphyll cover and frost damage on all foliage was allocated one of three damage categories: low (0-30% of photosynthetic area affected), moderate (40-70%) and significant (80-100%). Predominantly, observed damage was distinctly insect-induced. However, as wombats, deer, rabbits and hares inhabit many of the sites, saplings suspected of vertebrate damage (top of sapling bitten off, sapling pushed over or branches broken) were not included in analysis.

Herbivory Rate

To determine how the rate of herbivory across one season varied with planting location, photographs were taken of leaves at the start of the growing season (November 2011) and compared to those taken at the end of summer (February 2012).

Individual branches were selected from a subset of saplings at each site. Each leaf, providing it was fully expanded and alive, was placed on a piece of white card with a scale (Figure 4b) and photographed using a digital camera. The base of each branch was affixed with coloured wire, used as a reference in leaf identification. The first leaf above the wire was leaf one, the second leaf two and so forth up the branch (Figure 4a). Alternate leaf orientation of saplings allowed for little confusion in leaf identification with this system.

As leaves were both lost and gained on many branches studied, leaf identity was confirmed by comparing first and second photos before final leaf area measurements were calculated using ImageJ imaging software. Final leaf area was subtracted from initial area to calculate leaf area lost (mm^2 per day).

Initial and final leaf photographs were analysed for herbivory type based on the shape, edge texture and extent of leaf damage (Table 3).

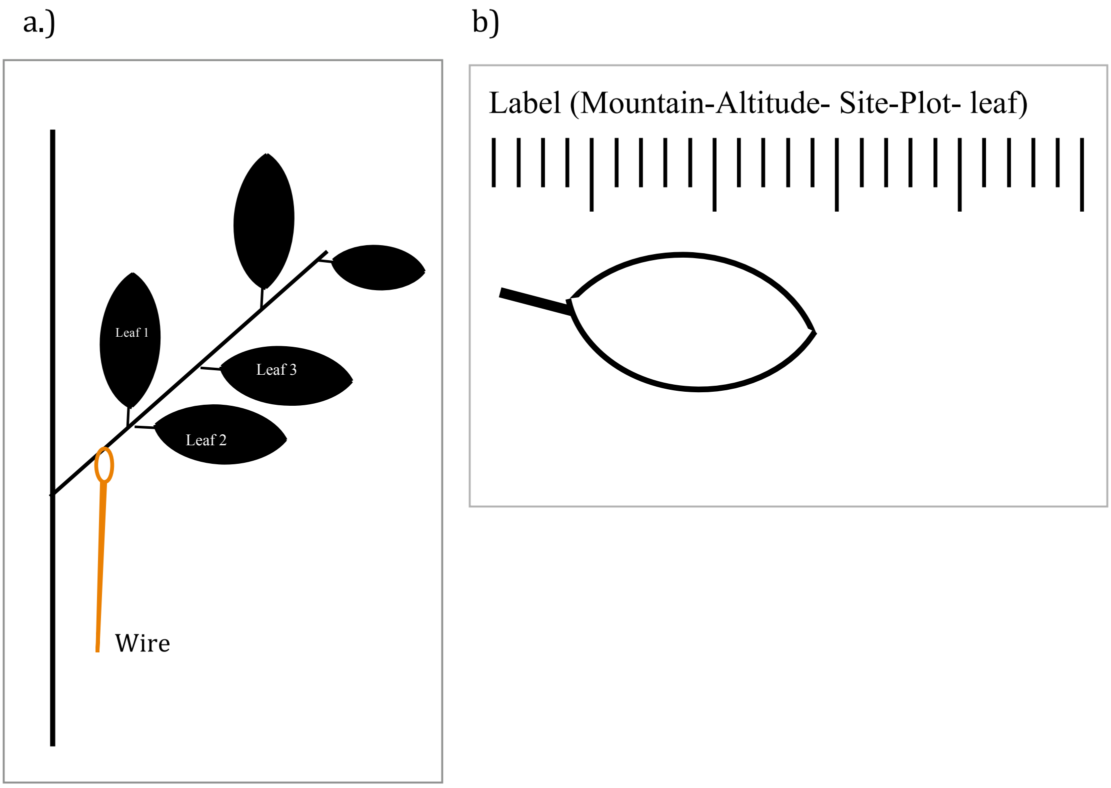


Figure 4. (a) Labelling of leaves on a sapling branch with reference to wire tag, (b) graphic example of white board, with scale used as a backdrop for leaf photographs

Table 3. Types of herbivory observed on saplings categorised based on chew mark characteristics and the suspected associated herbivores

Leaf damage	**Example**	**Herbivores**
Chewing- disc shaped (neat)		Leaf beetles
Chewing- Disc shaped, curved (irregular)		Leaf beetles Other beetles
Chewing-Rough edge left		Beetle larvae
Chewing- smooth edge, large blocks of leaf removed		Larvae
Leaf mining- (serpentine or blotch)		Larvae
Leaf skeletonising		Larvae

Drivers of herbivory

To assess the spatial variation in herbivore damage, data was collected from live saplings on (a) plant traits, (b) local environment, (c) altitude and (d) seed origin. Sapling height (in metres) was measured as the distance from the soil surface to the tallest meristem and leaf number excluded dead and developing leaves, those not yet fully expanded. Local environment observations included (a) the type of understorey species (percent cover of grass, shrub, herb) in a 1 m radius surrounding each sapling and (b) canopy openness (%) above each mini-plot.

Understorey species were surveyed *in situ;* however, percent canopy openness was calculated for each mini-plot using hemispherical fisheye photographs taken in the previous year (provided by S. Le Bel). Hemispherical photographs were analysed using imaging software, GAP Light Analyser version 2.0 (Frazer *et al.* 1999), which allows the calculation of canopy structure and light penetration measurements from colour fisheye photographs.

Leaf traits

Leaves were collected during the 4-week period from mid January to mid-February, from all live saplings supporting leaves suitable for analysis. Guidelines for leaf selection, collection and storage followed Cornelissen *et al.* (2003). Selected leaves were fully-expanded, current season’s growth, without substantial herbivore or pathogen damage or coating by epiphylls. Each leaf was carefully removed from the stem by cutting the base of the petiole and immediately wrapped in moist paper towel, sealed in a plastic bag and stored in an esky for transportation to the lab whereupon they were stored between 0-4 °C.

In preparation for leaf trait analysis, leaves (laminar and petiole) were carefully dried to remove excess water, weighed, photographed (using same methods described above), and oven dried at 80 °C for 48 hrs. Dry weight (mg) was recorded immediately following the 48 hr oven-drying period. These leaf attributes provided the information required to calculate leaf traits.

Formula used for the calculation of leaf traits are as follows:

Specific leaf area (mm^2 mg^{-1}) (SLA): one sided leaf area (mm^2) divided by dry mass (mg).

Leaf dry matter content (mg): dry weight (mg)/fresh weight (mg)

Leaf thickness (mm) estimation as described by Vile *et al* (2005): (Specific leaf area x leaf dry matter content)$^{-1}$

Leaf water content: Fresh weight (mg) - dry weight (mg).

For the analysis of leaf nitrogen concentrations, dry leaves were individually placed in a Retch MM 400 ball mill, at a frequency of 200 1/s for 23 seconds and ground into a fine powder. Careful to sterilise between samples, powdered leaves were stored in individual zip lock bags. In preparation for analysis, 20-30mg of dried leaf material was weighed, sealed in a small foil cup, re-weighed and stored until analysis. A Vario EL III element analyser (Manufacturer: Elementar Analysensysteme GmbH, Donaustraße 7, D-63452 Hanau-Germany) was used to analyse nitrogen concentrations within samples.

Differences in the level of herbivory, rate of herbivory (mm^2/day), plant traits (height, leaf number), leaf traits (SLA, LWC, leaf thickness) and canopy openness between each mountain and within altitudes of a single mountain were tested using one-way ANOVA with Bonferroni post-hoc comparisons. Levenes and Kolmogorov-Smirnov test statistics were used to test for normality and homogeneity of variances. When significant, data was transformed (log, square root, arcsine); if transformation failed to improve normality, the non-parametric Kruskall-Wallis test was used with Mann-Whitney U tests for post-hoc comparisons.

All subset regression modelling

All subset linear regression modelling is a multiple linear regression technique whereby the relationship between the dependent variable and a set of explanatory variables is estimated (Quinn and Keough 2002). Explanatory variables are added to the model in succession, allowing the comparison of all possible variable combinations and their explanatory power.

Distinguishing the 'best' models able to explain observed data is conducted through hierarchical partitioning, whereby the improvement of a model with the contribution of each new variable is compared to all equivalent models from where that variable is absent and averaged across all models where present. Hence, hierarchical partitioning allows those variables whose correlation with the dependant variable may be of importance to be distinguished from those that are not (Mac Nally 1996).

Model selection is done with the aid of Akaike and Schwarz Bayesian information criterion (AIC and BIC, respectively) which incorporate sample size of variables and the number of model predictors to provide a summary of model information (Mac Nally 1996). . For both these criterion, lower values are indicative of more parsimonious models (Quinn and Keough 2002).

$$\mathrm{BIC} = n\,(\ln(\mathrm{SS}_{\mathrm{Residual}})) + (p + 1)\ln(n) - n\ln(n)$$

$$\mathrm{AIC} = n\,(\ln(\mathrm{SS}_{\mathrm{Residual}})) + 2(p + 1) - n\ln(n)$$

where n is the number of observations and p the number of predictor variables

Average herbivory (dependent variable) was modelled against the explanatory variables location (mountain), altitude (site), plant height, leaf number, canopy openness, leaf thickness, leaf water content (LWC), specific leaf area (SLA) and understorey vegetation type using all subset regression modelling. Before model selection, collinearity of predictor variables was tested through graphical exploration of data using scatterplot matrices and post regression analyses using tolerance values. Predictors found to co-vary were not used in model analysis. All statistical analyses were conducted using SPSS 18.0.

Insects

When observed, insects found upon foliage of *E. pauciflora* of all ages were collected for identification. Insects were placed directly from trees into vials of 70% ethanol, and stored until identification.

Results

Herbivory as defined by mountains, sites and seed origin

Mean herbivore damage, measured as total photosynthetic area lost, differed significantly between mountains (Kruskall-Wallis = 74.84, df = 4, p = <0.001). Mean herbivory was lowest on Mt Hotham and highest for King Billy (Figure 5).

Within mountains, mean herbivory differed between sites. In general, the biggest difference was between 'alpine' (lowest herbivory) and 'low' sites (highest herbivory). For individual saplings, damage ranged from zero to 90% leaf area loss, and was highly variable within sites and the mini-plots (Figure 6).

Seed origin had no significant effect on leaf area lost by saplings for all mountains (Kruskall-Wallis = 0.206, df = 2, p = 0.902).

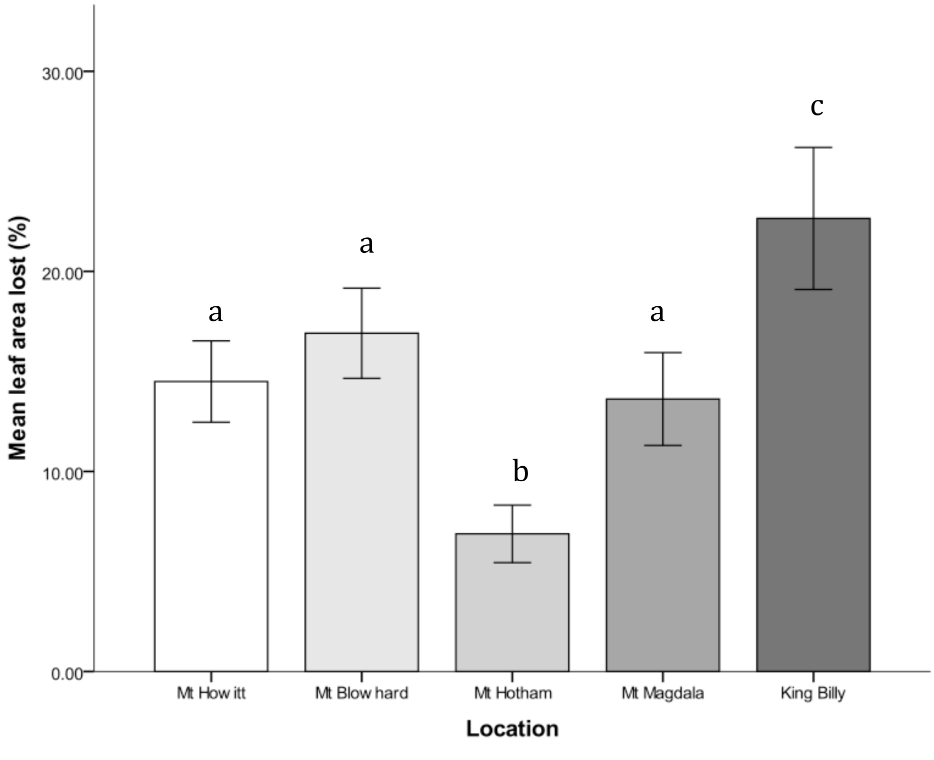


Figure 5. Mean leaf area lost (± S.E) for each mountain. Columns with different letters are significantly different at the p = 0.05 level of significance level.

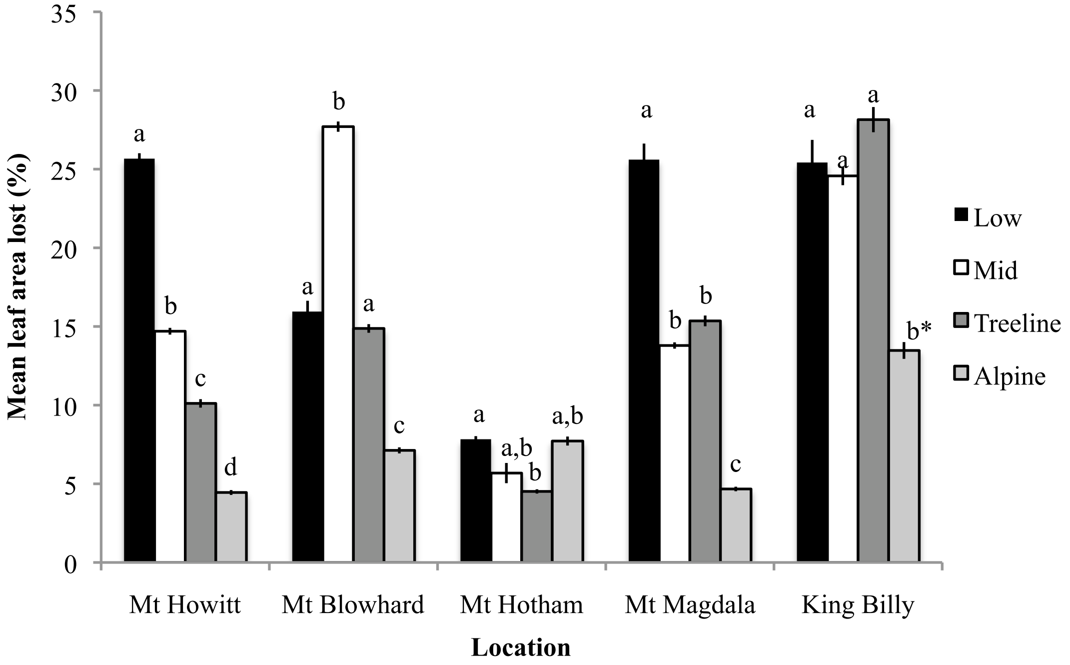


Figure 6. Mean leaf area lost (± S.E) for each site within each mountain. Columns with different letters are different at the $p = 0.05$ level significance. Comparisons are between sites within each mountain, not between mountains.

* Significantly different at the $p = 0.1$ level.

Herbivory rate

Herbivory rate was calculated for all mountains. However, due to unforseen levels of leaf loss and sapling growth, the collated data did not accurately represent the levels of herbivory observed. Leaf loss significantly reduced sample numbers from all mountains, but most significantly within Mt Howitt. New leaf production, which was earlier and faster than expected, impacted results by re-directing herbivory away from tagged leaves to those developing. In consequence, collated data has not been presented (Appendix 4).

Damage by frost and fungi

In all saplings frost and fungal damage was reduced between first and second observations. This is likely due to the production of new leaves, resulting in the loss of leaves most significantly affected (older leaves). Frost damage was rare by the second survey, however, fungal infection, while substantially reduced, was observed on all saplings.

All subsets linear regression model

Eleven models (of 128 total models) were significant (*p* <0.05) when explaining herbivory (Table 4). Mean leaf area loss was best explained by the combination of altitude, canopy openness, leaf thickness and plant height. Most leaf traits (SLA, LWC) and local vegetation patterns (% herb, shrub, grass) were never significant in models, nor was location. While the model excluding altitude is the more parsimonious, and equally supported by AIC and Schwarz BIC, altitude explained a further 3% of observed leaf loss, which is considerable in light of the low explanatory power of the model as a whole.

Table 4. Significant models built using all subset linear regression modelling that explain observed patterns of herbivory, 'best' models in bold text.

No. Predictors	Model	r^2	Adj r^2	*p*	AIC	Schwarz (BIC)
1	Altitude	0.202	0.201	<0.001	2664.82	2668.12
1	Canopy openness	0.173	0.171	<0.001	2840.52	2843.89
1	Plant height	0.089	0.088	<0.001	2718.49	2721.77
1	Leaf thickness	0.011	0.008	0.045	1987.86	1990.80
2	Altitude + Canopy openness	0.228	0.225	<0.001	2649.40	2651.68
2	Altitude + Plant height	0.232	0.23	<0.001	2628.39	2630.67
2	Altitude + leaf thickness	0.217	0.212	<0.001	1900.18	1902.13
2	Canopy openness + Plant height	0.222	0.219	<0.001	2635.99	2638.28
2	Canopy openness + Leaf thickness	0.211	0.207	<0.001	1903.03	1904.97
2	Plant height + Leaf thickness	0.119	0.115	<0.001	1941.05	1943.00
3	Altitude + Canopy openness + Plant height	0.233	0.229	<0.001	2629.97	2631.26
3	Altitude + Canopy openness + Leaf thickness	0.226	0.22	<0.001	1897.61	1898.56
3	Altitude + Plant height + Leaf thickness	0.239	0.233	<0.001	1886.96	1887.90
4	**Canopy openness + leaf thickness + Plant height**	**0.243**	**0.237**	**<0.001**	**1871.44**	**1871.38**
4	**Canopy openness + Leaf thickness + Plant height + Altitude**	**0.274**	**0.267**	**<0.001**	**1870.968**	**1870.91**

Herbivory- combined for all mountains

Combined for all mountains, mean leaf area loss differed significantly between sites (Kruskall-Wallis = 117.49, df = 3, p = < 0.001). As indicated by individual mountain analyses, leaf area loss was lowest at the alpine site and highest at the low and mid sites (Figure 7).

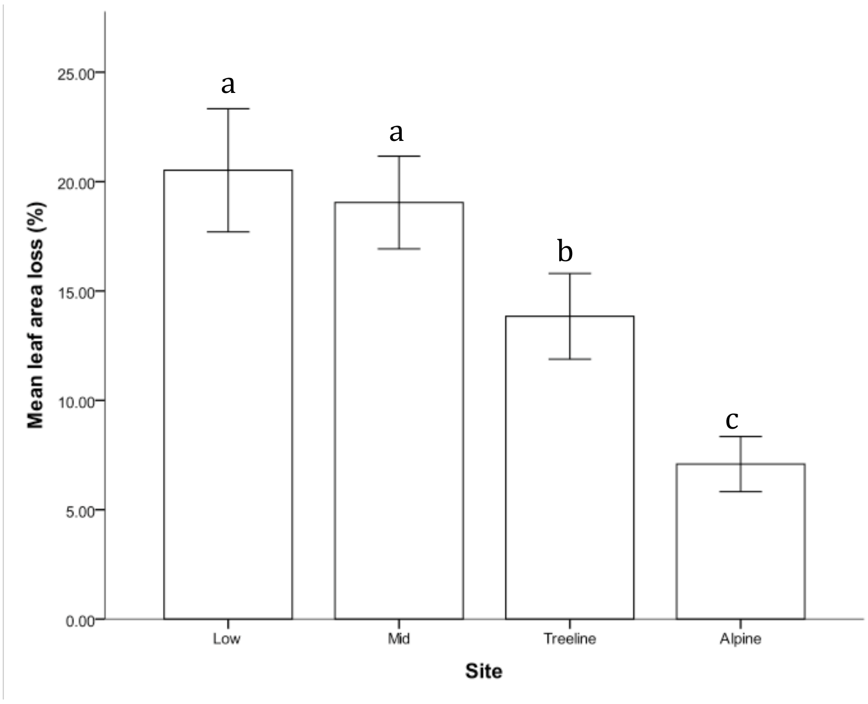


Figure 7. Mean leaf area lost (%) combined for sites of all mountains. Bars represent 95% confidence intervals. Columns with different letters differ significantly at the p = 0.05 level.

Leaf traits

Leaf traits varied both within and between mountains. Between mountains, significant differences were found within mean SLA (Kruskall-Wallis =15.31, df = 4, *p* = 0.004), mean leaf thickness (Kruskall-Wallis = 28.85, df = 4, *p* = <0.001) and mean LWC (F= 158.43, *p* = <0.001). SLA decreased with altitude within Mt Blowhard, Mt Hotham and King Billy, while Mt Howitt and Mt Magdala showed no linear response to altitude. Mt Blowhard had both the lowest and highest mean SLA values while Mt Howitt showed the lowest variability between all sites (Appendix 2).

Leaf thickness showed a linear response with altitude on all mountains. Mt Blowhard had both the highest and lowest values, ranging from approximately 0.30 mm to 0.55 mm thick. Between sites at Mt Howitt, leaf thickness varied least (Figure 8). Mean leaf area loss showed a marginally positive relationship with decreases in leaf thickness, however this relationship was not strong. Additionally, mean leaf area lost was highly variable for each class of leaf thickness (Figure 9).

LWC was the least variable leaf trait between sites within mountains; however, mountains differed significantly (ANOVA F = 158.43, *p* = <0.001). No significant relationship with altitude was found within mountains. Mt Howitt had the highest LWC and Mt Blowhard and Mt Hotham the lowest. Mt Hotham and Mt Blowhard were significantly different from Mt Howitt, Mt Magdala and King Billy, suggesting the potential influence of mountain location on this trait (Appendix 2).

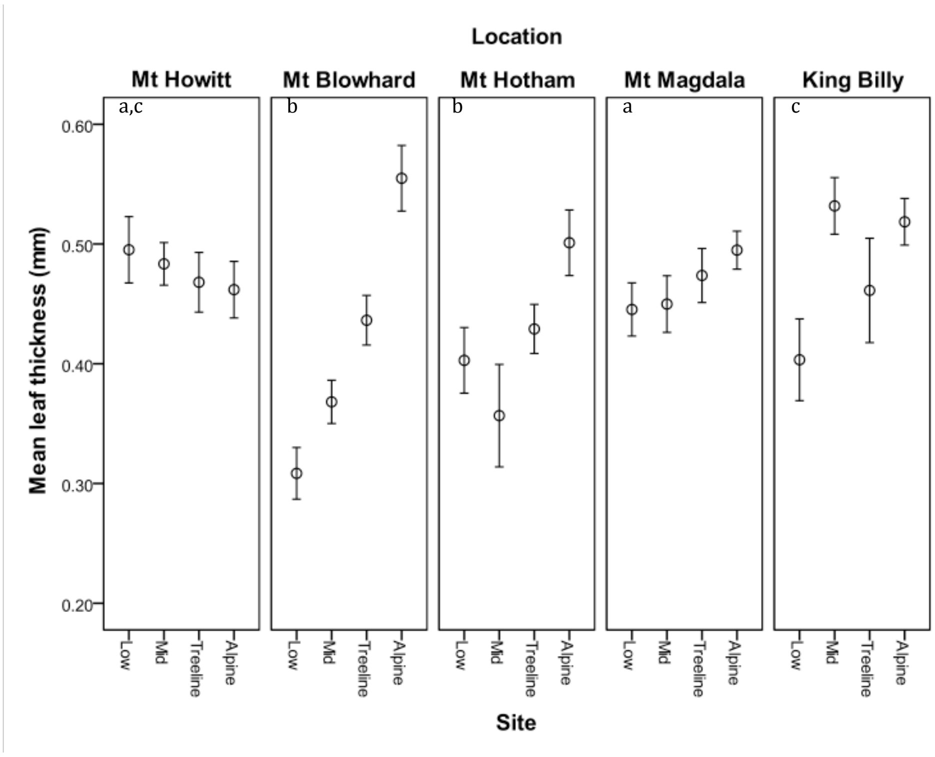


Figure 8. Comparison of leaf thickness between sites within mountains Bars represent 95% confidence intervals. Columns with different letters are different at the p= 0.05 level.

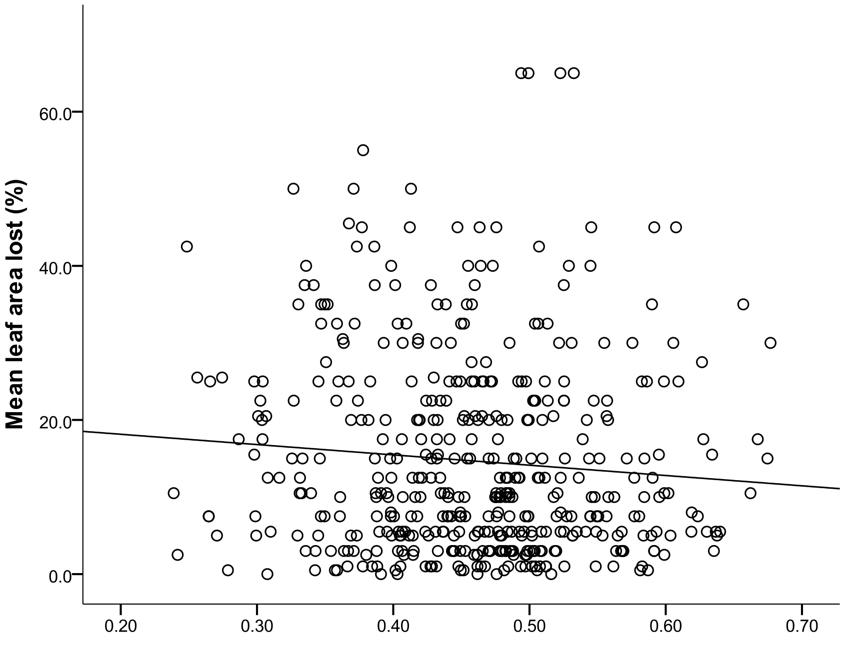


Figure 9. Linear relationship between mean leaf area loss (%) and leaf thickness (mm) for all mountains

Plant traits

Mountains were found to differ significantly in both the mean height of saplings (ANOVA F = 93.89, *p* = <0.001) and mean leaf number per sapling (ANOVA F= 76.42, *p* = <0.001). Mean height of saplings on Mt Howitt, Mt Blowhard and Mt Hotham decreased linearly with increasing altitude, while King Billy showed the reverse trend and Mt Magdala no linear trend with altitude (Figure 10). Mean plant height ranged from 0.15 m to ~0.6 m. The highest saplings on average were within Mt Howitt and lowest Mt Hotham.

Mean leaf area loss showed a positive linear response to increased plant height; however, the extent of area lost within each plant height was highly variable (Figure 11).

Mean leaf number was variable between sites for all mountains and ranged from ten to over eighty per sapling. Of all mountains, only King Billy displayed a relationship with altitude, as leaf number linearly increased with altitude. Mt Howitt saplings displayed consistently high mean leaf number at all sites, while Mt Hotham and Mt Blowhard were consistently low (Appendix 2).

Canopy openness

Mean canopy openness ranged from 15% to over 80%, with all alpine sites a minimum of 60% open. Average canopy openness varied significantly between mountains (Kruskall-Wallis = 52.55, df = 4, *p* = <0.001). Canopy openness was highest at alpine sites and lowest at low sites for all mountains bar King Billy. A linear trend of decreasing openness with decreasing altitude was observed for all sites other than King Billy where the treeline site had less openness of canopy than the low. Mt Howitt was the most open for all sites, while Mt Blowhard was consistently lower excluding the alpine site (Figure 12).

Mean leaf area loss showed a negative linear relationship with canopy openness, however mean area loss was variable within each level of openness (Figure 13).

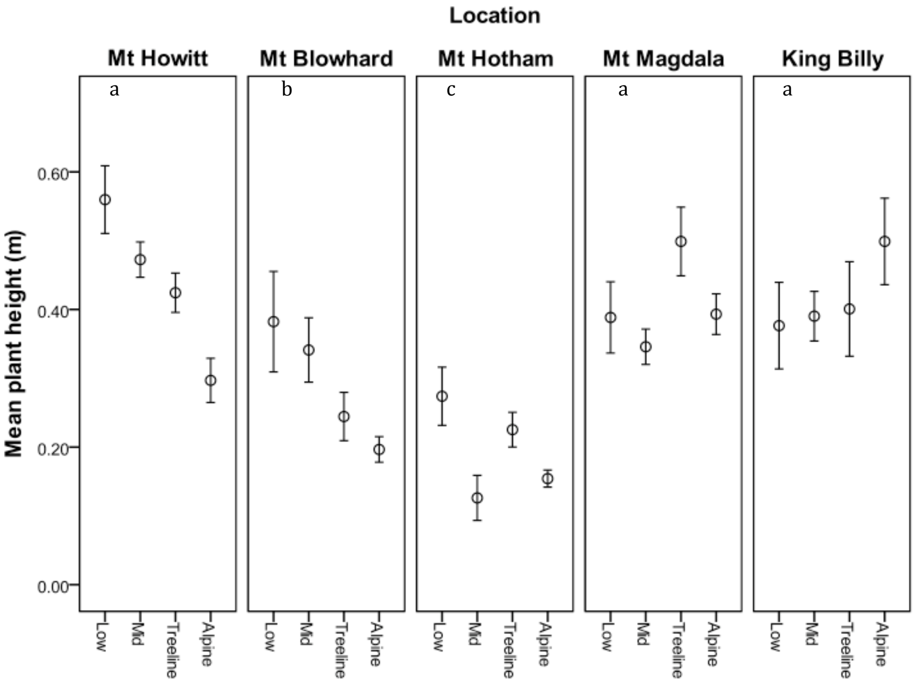


Figure 10. Mean plant height for sites within each of the five mountains. Bars around means represent 95% confidence intervals. Columns with different letters differ at the *p* = 0.05 level of significance.

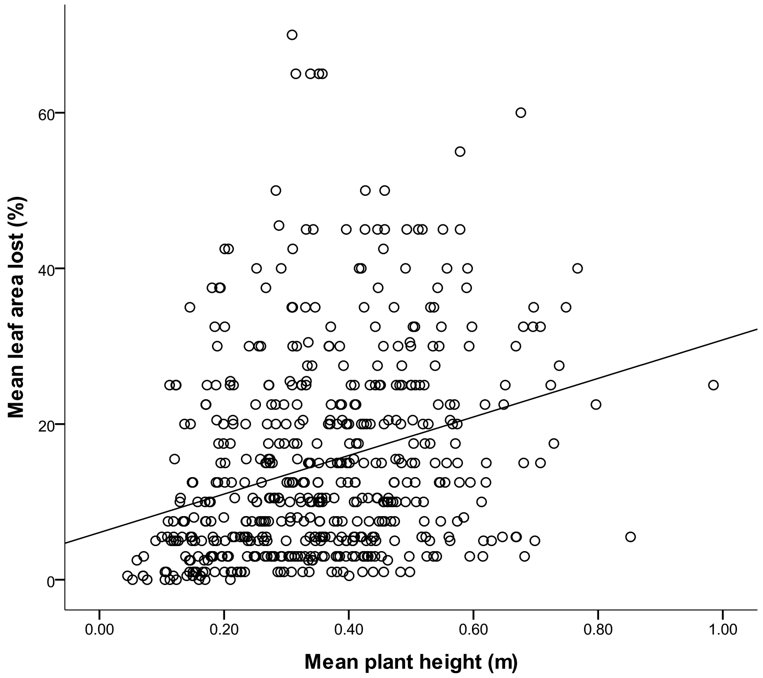


Figure 11. Linear relationship between mean leaf area loss (%) and mean plant height (m) combined for all mountains.

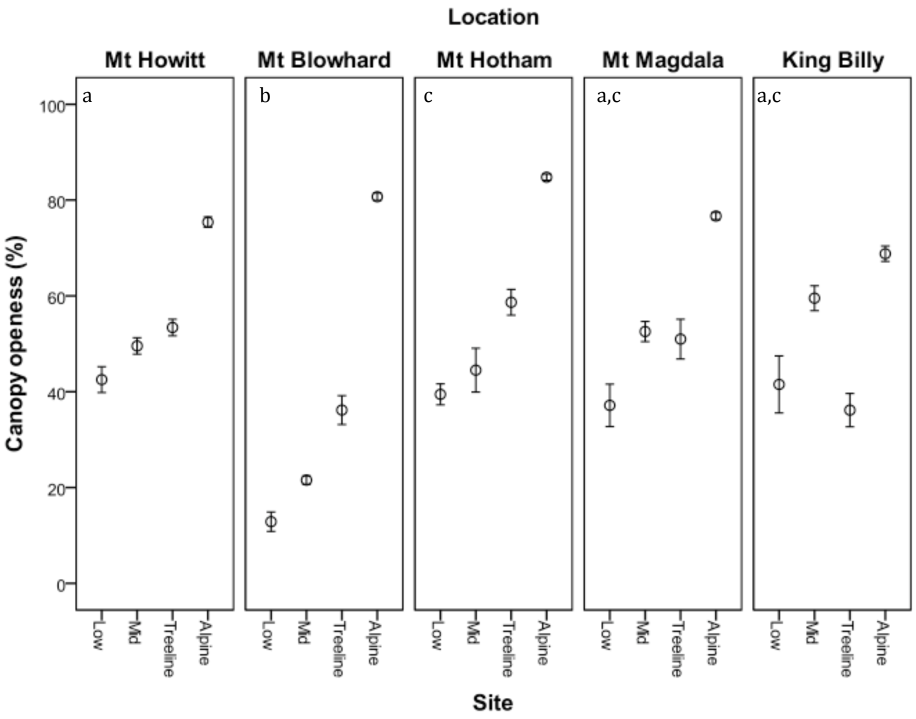


Figure 12. Mean canopy openness of sites within each mountain. Bars represent 95% confidence intervals. Columns with different letters differ at the p = 0.05 level of significance.

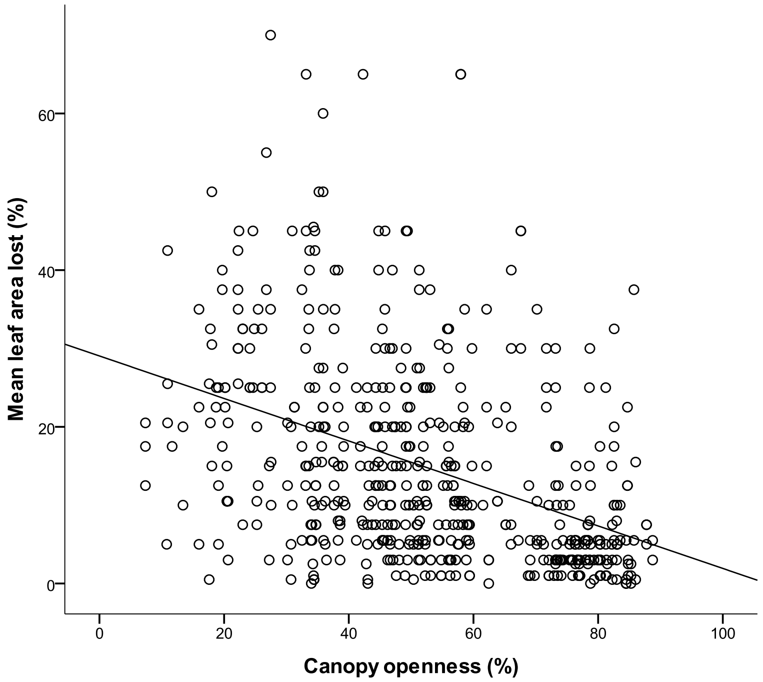
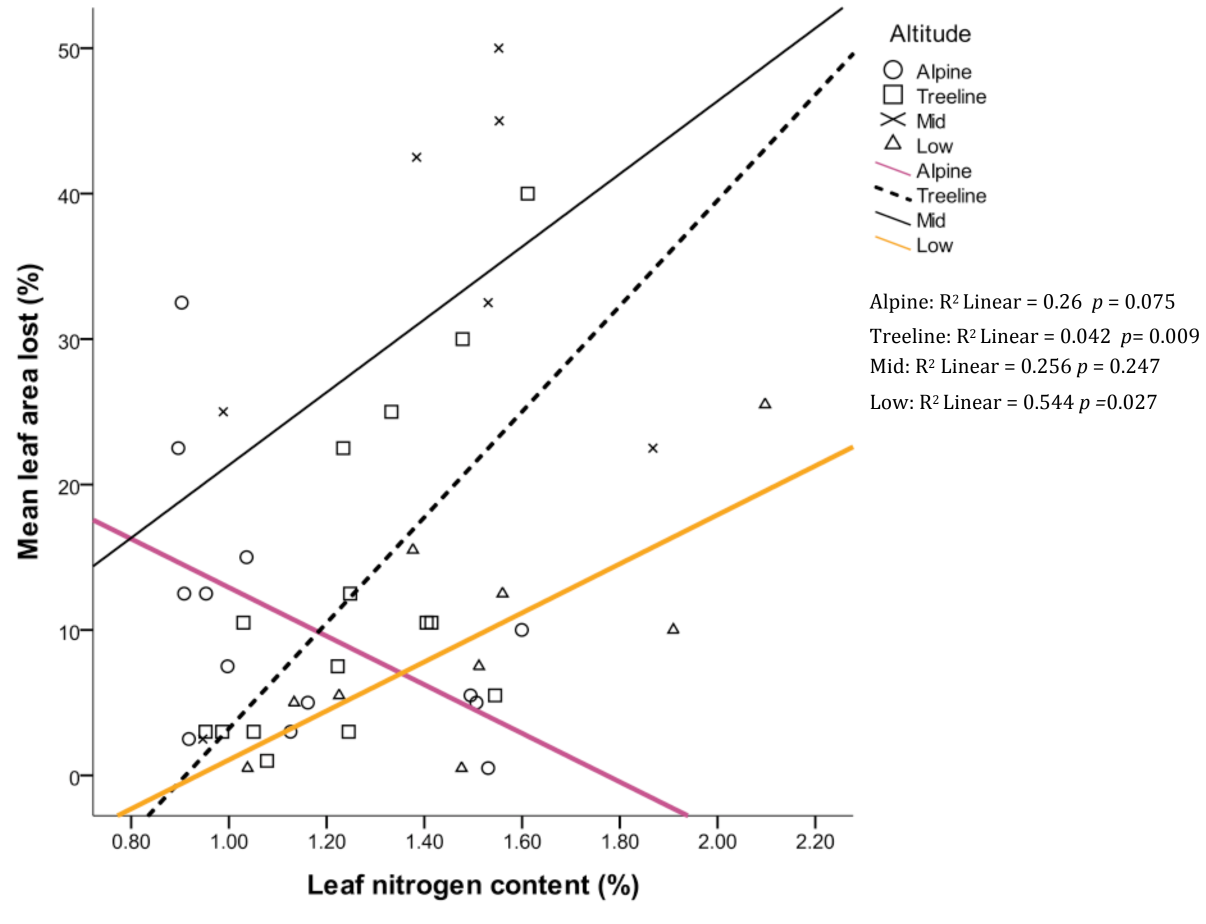


Figure 13. Linear relationship between mean leaf area lost (%) and canopy openness (%) for all mountains combined.

Leaf nitrogen content

Leaf nitrogen content ranged from 0.9% to 2.1% with significant differences observed between sites within mountains (Kruskall-Wallis = 9.17, df = 3, $p = 0.027$). Mean leaf loss was found to increase with higher concentrations of leaf nitrogen for all sites other than the ‘alpine’ which showed a negative relationship, however this was found to be insignificant. The relationship between the amount of leaf lost and concentrations of leaf nitrogen varied between sites, suggesting site effects (Figure 14). Correlations between leaf area loss and leaf nitrogen concentrations were strongest at the low site and weakest for the treeline. Saplings within the mid site showed the highest level of leaf loss for the lowest nitrogen concentrations.



Alpine: R^2 Linear = 0.26 $p = 0.075$

Treeline: R^2 Linear = 0.042 $p = 0.009$

Mid: R^2 Linear = 0.256 $p = 0.247$

Low: R^2 Linear = 0.544 $p = 0.027$

Figure 14. Comparison of linear relationships between leaf nitrogen concentrations (%) and mean leaf area lost (%) in saplings between sites of Mt Blowhard and Mt Hotham.

Insects

Few insects were observed on the planted saplings, although many were seen on the foliage of juvenile and mature trees in the surrounding landscape. Most insects observed were from the order Coleoptera, the beetles. Where possible, they were identified to genus. Across all sites, 12 species in the family *Chrysomelidae* (leaf beetles) were found, comprising seven within the genus *Paropsis*, three morpho-species within the subfamily *Cryptocephalinea,* a single species within the genus *Ethomela* (common at all sites) and one within the genus *Macrones* (long horn beetle). Beetles within the families *Curculionidae* (Weevils), Family *Belidae* (Belid weevils) and *Scarabaeidae* (Scarab beetles) were also found. Within *Scarabaeidae* were the genera *Antitrogus* (three species) and *Heterony* (one species). Nine morpho-species were identified within the *Curculionidae*. Within the family Belidae, one species, *Rhadinosoma lacordairei,* was found. Lone individuals, one within the family *Elatridae* and one within the family *Cantharidae*, of the genus *Chauliognathus* were also observed (Figure 15).

Below treeline, larvae - predominantly sawfly (Family *Pergidae*) and *Paropsis sp.* - were abundant and conspicuous (Figure 15). Adults of all groups were rarely seen on foliage below the treeline.

Sap-sucking herbivores from the order *Hemiptera* were also common, predominantly leaf hoppers (family Cicadellidae), sap feeders and juveniles and adults of *Amorbus rubiginosus* (Coreidae) (Figure 15).

Distribution of herbivore damage types

Distinct Chrysomelid beetle damage (neat discs removed) was highest at the alpine sites, decreasing with altitude. Larval damage was highest at the low and mid sites. Other beetle damage was consistent throughout, with a slight reduction above treeline. Leaf mining and skeletonising was consistently low, however, frequency was highest at low sites and steadily decreased with increasing altitude (Figure 16).

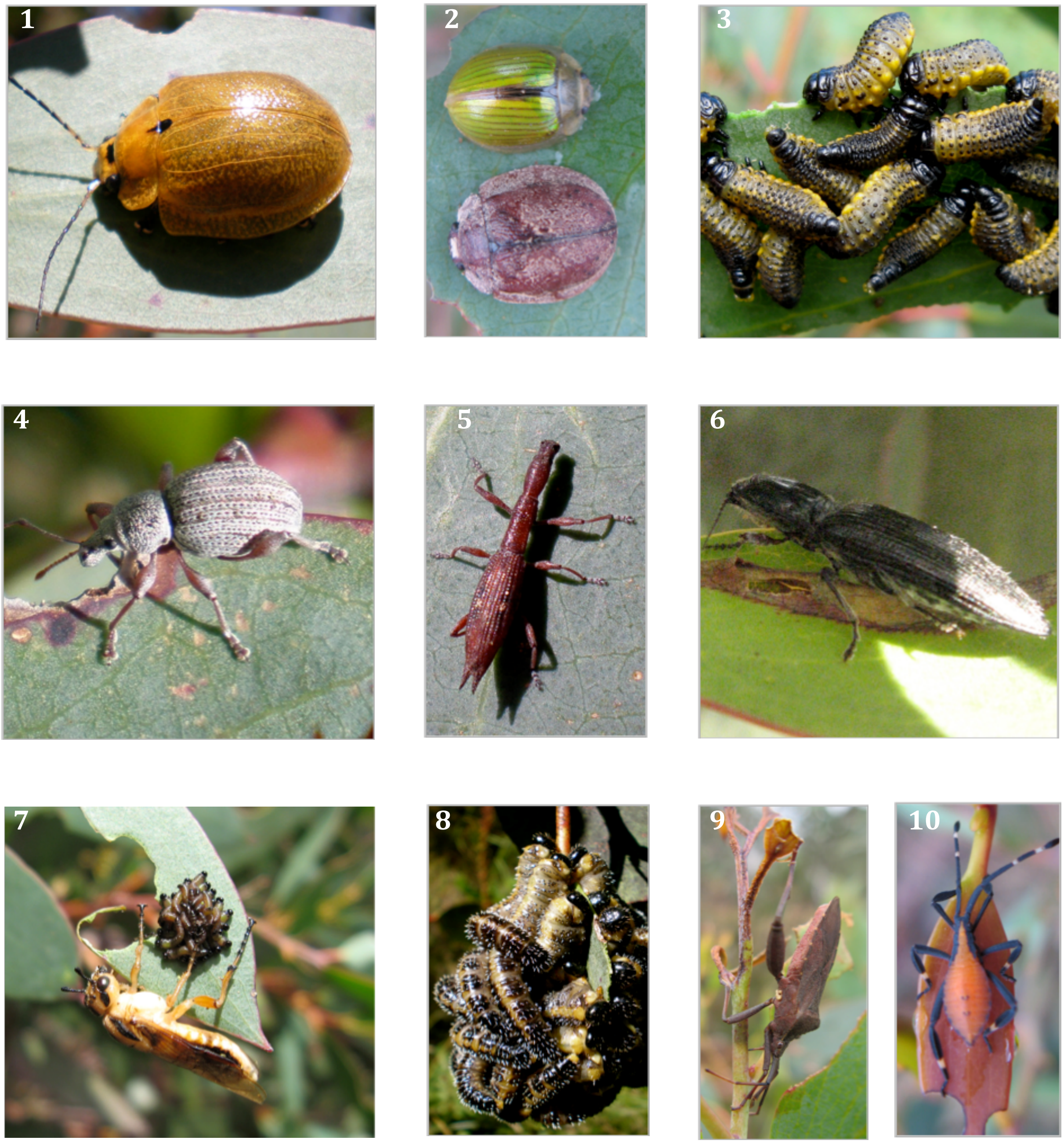


Figure 15. A subset of the dominant families of insect fauna collected from foliage of *E. pauciflora*. Family Chrysomelidae: 1). *Paropsis augusta* . 2). *Paropsis sp.* 3). *Paropsis* larvae. Family Curclionidae: 4). Morphpospecies # 2 Family Belidae: 5). *Rhadinosoma lacordairei.* Family Elatrididae: 6). Unknown sp. Family Pergidae: 7). Female Sawfly with larvae, 8). Sawfly larvae (older instars). Family Coreidae: 9). *Amorbus rubiginosus* 10). Juvenile *Amorbus rubiginosus.*

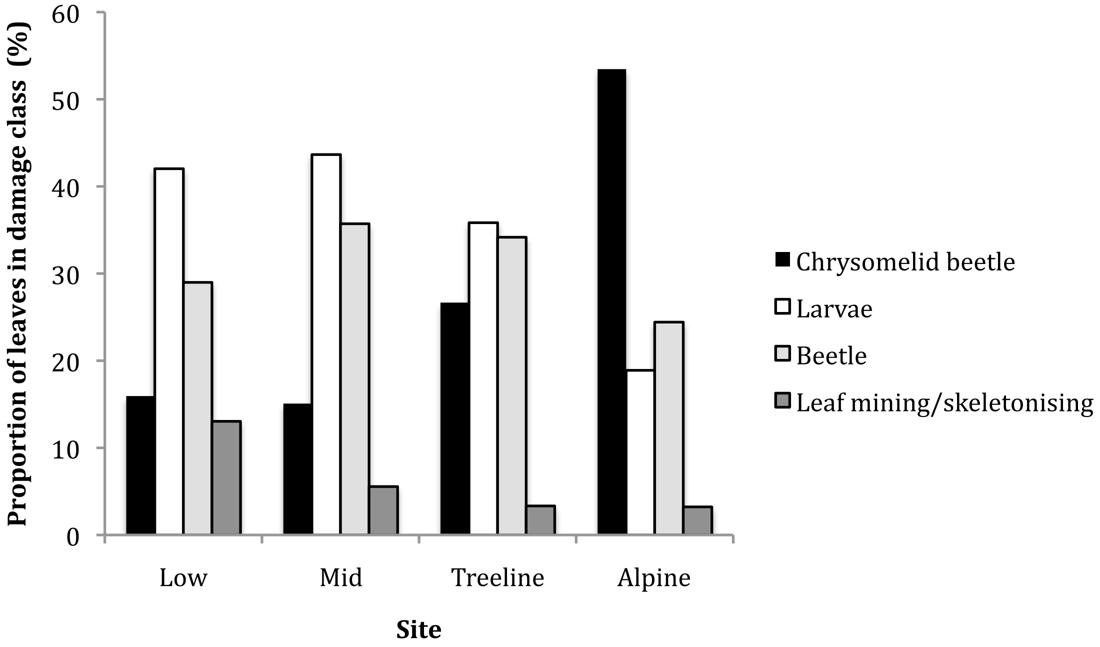


Figure 16. Frequency of herbivory damage, as defined by suspected herbivore/herbivory class, for each altitude. Data combined for all mountains.

Discussion

The aim of this study was to document the extent, type and distribution of herbivory on saplings of *E. pauciflora* (Snow Gum) along an altitudinal gradient established at each of five mountains in the Victorian Alps. Additionally, the influence of location (mountain), altitude (position relative to treeline) and seed origin on leaf traits, plant traits and characteristics of the environment were assessed, as was their potential to influence herbivory. The results of this study suggest that the extent of leaf area lost to herbivores is influenced consistently by altitude, while plant traits, leaf traits, and the local environment also contribute, their relationship with leaf area loss is highly variable both within and between altitudes and mountains. Overall, the amount of herbivory observed was best explained by the combined influence of canopy 'openness', plant height, leaf thickness and altitude. This suggests that herbivory may increase with increasing productivity associated with altitude, a trend in broad agreement with hypotheses associating leaf area loss to the availability of resources and plant vigour.

Mean leaf area loss, combined for all mountains, was 7% for alpine sites, 14% at the treeline, 19% at the mid- and 21% at the low-site. The extent of herbivory observed was consistent with studies of herbivory on *E. pauciflora* saplings elsewhere in Australian alpine regions. Burdon and Chilvers (1974), for instance, found ~8% leaf are loss at 1750 m, 19% at 1480 m and 21% at 1220 m. This suggests, altitude is important for herbivory across both these sites.

Seed origin had no effect on sapling morphology or the proportion of leaf area lost at both the local and landscape scales. It is highly likely that the maternal plants that were initially sourced for the experiment were very similar, with low- to high-altitude populations only 200-400 m distant from one another. These findings differ from those of Pryor (1956) who documented inherent differences in *E. pauciflora* seedlings within their first year of growth as determined by their altitude of origin. The five sites used by Pryor (1956) were separated by 150 altitudinal metres, suggesting the 100 altitudinal metre gradient used in the present study is sufficient to demonstrate differences in sapling genotype. These traits were, however, detected upon seedlings grown in the glasshouse, not in their natural environment. Thus, it is possible that while inherent differences may exist between seedlings of different sites in the present study, the effects of altitude, local site variation and climate may influence seedlings in such as way as to conceal their expression.

Mountains differed in both the extent of herbivory and its distribution between sites. The inherent differences between mountains and transects may partially explain this variation. Transects differed in their aspect and true altitudes, while mountains differed in their geology and location. Aspect and altitude influence local temperatures and, in doing so, may alter the environment experienced by a seedling directly, through daily temperature fluctuations, or indirectly through long-term effects on surrounding plant growth and the formation of canopy cover (Korner 1999). While all soils in these regions are of the 'alpine humus' type (Costin 1955), the underlying parent material will define the nutrients available to seedlings and the composition of species with which they co-exist (Kirkpatrick and Bridle 1998). Within this study, the expression of plant traits in eucalypts differed. Mt Hotham and Mt Blowhard, comprised of shales, showed distinct differences to Mt Magdala and King Billy (formed of mudstones/sandstones and basalt, respectively), in the average height of plants, sapling leaf number and leaf water content. These differences may be induced through variation in the availability of soil nutrient and water resources (Landsberg & Gillieson 1995). Mt Howitt differed from all mountains for most traits.

The effect of altitude on herbivory may be explained through increases in the level of stress associated with increases in elevation, known to influence plant traits and insect abundance. The effects of altitude on both insect abundance and herbivory have been well documented for a wide range of species. For example, Scheidel *et al.* (2003) found a reduction of slug herbivory of a montane Asteraceae at high altitudes with evidence for adaptations to plant traits related to altitude. Additionally Suzuki (1998) found beetle activity decreased at higher altitudes, resulting in less leaf damage of its herbaceous host despite the foliage being of superior nutritional quality. Garibaldi *et al.* (2011), in a study of herbivory on *Nothofagus pumilio* (Nothofagaceae) found herbivory decreased with increasing elevation due to changes in leaf traits and insect abundance.

In this study, I did not document herbivore identity extensively. However, preliminary assessment of leaf damage within sites suggests that different altitudes support different herbivores, with beetles dominating above treeline and larvae of various orders below. Such findings are consistent with those of Burdon and Chilvers (1974) in that herbivore density and diversity associated with *E. pauciflora* decreased at higher altitudes. However, they also describe high levels of damage by Chrysomelid and Scarab beetles at low sites, a finding that contrasts to that found in the present study. Importantly, techniques for assessing this damage differed between studies; the present study assessed individual leaf damage while Burdon and Chilvers (1974) made collections of herbivores from foliage. Hence, I offer these results with caution, as identifying herbivores through damage is difficult considering similar damage can result from very different insects. Furthermore, a weakness within this type of assessment is in accumulative damage, whereby herbivores feeding on a pre-damaged leaf may erase traces of those who have eaten before.

Plant and leaf traits varied in their responses to altitude across mountains. However, consistent with other studies (Burdon and Chilvers 1974b; Garibaldi *et al.* 2011; Landsberg & Gillieson 1995; Suzuki 1998), seedlings at higher elevations, where higher levels of abiotic stress is encountered, were shorter, had tougher (SLA) and thicker leaves, and had more leaves per sapling than those at lower more favourable altitudes. Such changes to plant morphology are consistent with theories of plant responses to physical stress, whereby resource-limitation restricts growth rates and alters resource allocation (Coley *et al.* 1985). Additionally, resource allocation theory states that such plants will have higher concentrations of chemical defences; however, these were not measured in the present study. Physical traits alone, however, may limit the type of insect able to exploit plant resources and the amount they are able to digest through physical restrictions defined by their mouthparts. Nahrung *et al.* (2001) showed leaf margin thickness altered the rate and success of feeding in Chrysomelid beetle larvae feeding on eucalypt foliage, due to variation in mandible size.

Plants often occur in patches or clumps, the size of which can influence insect abundance and herbivore damage upon individual plants (Strong *et al.* 1984). In large patches dominated by a single species, insect abundance is often greater and herbivory higher than small patches (Bernays & Chapman 1994). It is suggested such patches are both easier to find amongst a mosaic of unsuitable hosts (Steinbauer *et al.* 2004), and provide a stable resource with insects able to easily feed among numerous plants (Strong *et al.* 1984). Hence, reduced structural complexity within alpine sites, due to the complete lack of trees or shrubs, isolates transplanted saplings so they may be likened to islands surrounded by vegetation of little interest to eucalypt herbivores, which, in turn, may reduce their conspicuousness (Strong *et al.* 1984). In addition, the shorter growing season for treeline and alpine seedlings (due to longer snow cover duration) may reduce the time available for detection by insects and subsequent defoliation to occur. Thus, sapling conspicuousness above the treeline may be less than for those below.

For all plant traits measured, there was considerable variation within sites at each mountain. Determining the causes of such variation, however, was beyond the scope of this study. It is possible, however, to speculate that such variation may result from differences in microclimate, where plant position may substantially alter fitness. Variation in slope (Davidson and Reid 1985), proximity to other plants (Ball *et al.* 1997; Venn *et al.* 2009), soil depth and water content (Costin 1981), canopy openness (Close *et al.* 2003) and soil water availability (Ferrar *et al.* 1988) can all vary within short distances and influence the type and availability of resources available to individual plants (Costin 1981). In addition, microclimate variation may alter the likelihood or intensity of insect herbivory. For example, as the environment directly influences insect behaviour and abundance, environmentally driven differences in herbivore distributions may result in an uneven distribution of herbivory. Burger and Louda (1994) showed variation in canopy cover significantly altered local scale herbivory of the grassland cactus *Opuntia fragilis* (Cactaceae).

The inclusion of plant height, leaf thickness and canopy openness as factors best able to explain herbivory across these mountains suggests that such inter-site variation may important to the understanding drivers of herbivory. Preference for taller plants lends support to the plant vigour hypothesis, where insects select the most vigorous plant within a population (Price 1991), while preference of thinner leaves supports the resource allocation hypothesis where thinner leaves are indicative of less defences (Coley *et al.* 1985).

Variation in the availability of sunlight between plants may explain the relationship of decreasing herbivory with greater canopy openness. While the resource allocation hypothesis suggests that plants with greater availability of resources, in this case sunlight, may allocate less to defence and more to growth, and thus become more attractive to herbivores. A major threat to sapling persistence in high mountain environments is that of cold-induced photoinhibition (Ball *et al.* 1991), whereby plant exposure to low temperatures and high levels of irradiance decreases the efficiency on photosystem II, resulting in a reduction of photosynthetic capacity and subsequent plant fitness (Close *et* *al.* 2000). Additionally, open sites may provide less protection from both predators and the environment for herbivores, resulting in less insect activity away from vegetation cover (Burger and Louda 1994).

The last trait, leaf thickness, was highly variable within and between sites. While there is a positive relationship of thinner leaves and herbivory, it was not strongly supported. Such variation may reflect different feeding capacities of herbivore guilds, developmental stages of herbivores or facilitation by gregarious feeding or previous damage to leaf margins altering leaf thickness. For example, Nahrung *et al.* (2001) found larval feeding on pre-damaged leaves of eucalypts significantly increased leaf area ingested and survival rate of herbivores.

Importantly, these traits combined explained 27% of observed herbivory. While variation between mountains may have diluted effects through contrasting results, it is likely other factors not included in the study are also important. For example, leaf chemical defences are plant attributes known to affect herbivore choice (Fox & Macauley 1977; Jordan 2002; Journet 1980; Lawler 2002; Loney *et al.* 2006; Morrow & Fox 1980; Nahrung *et al.* 2001; Steinbauer *et al.* 2004; Steinbauer 2010; Steinbauer *et al.* 1998; Stone & Bacon 1994) and likely important in high mountain eucalypts.

Of all leaf chemicals thought to be attractive to insects, leaf nitrogen is the most consistent across guilds and plant communities (Mattson 1980). Analysis of concentrations of leaf nitrogen and its relationship with herbivory within Mt Blowhard and Mt Hotham showed no strong relationship with altitude, unlike Suzuki *et al.* (1998), who found leaf nitrogen content increased with altitude. However, within low, mid and treeline sites, higher levels of herbivory were associated with increases in concentrations of leaf nitrogen. Although not all relationships were significant, these findings are consistent with studies that indicate nitrogen levels influence host selection by insects (Fox & Macauley 1977; Landsberg & Gillieson 1995; Mattson 1980; Morrow & Fox 1980; Ohmart *et al.* 1985; Stone & Bacon 1994).

Conclusion

This study has shown that mean leaf area loss for seedlings of *E. pauciflora* is extremely variable. Indeed, it varies between mountains, between altitudes on a single mountain, and within sites where altitude is constant. Additionally, plant and leaf traits varied between mountains and within sites (i.e. inconsistently) meaning that the relationship between plants as food sources and herbivores remains unclear. This is unlikely an issue of sample size but rather, reflects the complex and patch nature of plant-animal interactions in this environment. Plant vigour and resource allocation hypotheses best explain the positive relationships observed between sites, plant traits and herbivory, while no evidence of plant stress influencing herbivory was found. Leaf nitrogen showed potential to be an important leaf trait to herbivores within this system, however further analysis is required. These results highlight the individuality of each mountain environment, at landscape and local-scales, as well as the complexity of relationships between environmental change, plants and insects.

Chapter 3

Biotic interactions and their influence on herbivory

Introduction

Relationships between insect fauna and plants are far more complex than simply that of herbivore and host. Having an intimate history of co-evolution, these two major groups have formed both mutualistic and antagonistic relationships that are geographically widespread (Crawley 1983; Bernays & Chapman 1994). Of all insects, the ants (Hymenoptera: Formicidae) are likely the most dominant, both in numbers and ecological influence (Rico-Gray & Oliveira 2007).

The success of the ants is likely, in part, due to the eusociality of all species, that form highly complicated societies, organised through caste systems and the division of labour (Holldobler & Wilson 1994). Through specialisation of anatomy and behaviour, ants form colonies both large and small. They fill a vast range of niches which they dominate through numbers and the evolution of complex communication, allowing recruits to be called to aid food collection or protection of colony and resources (Holldobler and Wilson 1990; Sudd 1967). Such complex social behaviour is positively associated with the evolution of mutualisms (Beattie 1985). Of the relationships between ants and plants, mutualisms involving seed dispersal, pollination, plant feeding and plant protection are widespread and can substantially affect plant fitness and the structure and organisation of the greater community (Andersen 1988b; Beattie 1985; Rico-Gray & Oliveira 2007; Rouz & Ilková 2008).

Many ant-plant associations involve ants acting as a biotic defence, whereby they defend plants against herbivores, pathogens or other plant competitors and, in return, are provided with food or shelter (Rosumek *et al.* 2009). Ants are predominantly omnivorous and feed solely on liquids, which on plants is primarily nectar (Beattie 1985). Due to restrictions applied by their lack of masticatory mouthparts and enzymes required for cellulose digestion, ants are unable to directly exploit plant resources within foliage or phloem (Rico-Gray & Oliveira 2007). Such resources are made available only through the hemolymph of plant eaters or the honeydew secretions of sap sucking herbivores, the homoptera (Hemiptera) (Beattie 1985; Rico-Gray & Oliveira 2007). Hence, ant-plant mutualisms can be direct, whereby the plant offers rewards to ants in the form of extra-floral nectaries and nesting sites, or indirect, as mediated through hemiptera that harvest plant resources and recruit ants with the presence of honeydew (Rosumek *et al.* 2009).

Comprising four superfamilies, the Aphidoidea, Aleurodoidea, Coccoidea and Psylloidea, the hemiptera are sap feeders that secrete excess compounds as honeydew, a sugary exudate commonly harvested by ants (Morgan 1984). Comprised not only of sugars, but minerals, amino acids and vitamins, honeydew secretions are a valuable resource that is both predictable and renewable (Rico-Gray & Oliveira 2007). Hence, ant harvesting of honeydew has seen the presence of hemiptera increase the foraging intensity of ants upon plants and, in many cases, develop mutualisms whereby ants tend and farm hemiptera, cleaning them of excess sugars and defending against predators and pathogens in exchange for honeydew and occasional protein (note: ants may occasionally also eat hemiptera) (Ando & Ohgushi 2008; Buckley 1987; Fritz 1983). However, the outcome of such mutualisms for the plants upon which they occur is variable (Beattie 1985). While ants are known to become increasingly aggressive to non-hemiptera herbivores, in some cases reducing herbivory, their presence may also increase naturally low populations of hemiptera that directly harvest plant resources and act as vectors for disease (Buckley 1987; Del-Claro & Oliveira 2000).

In consequence, the outcome of such mutualisms for plants is conditional (Billick & Tonkel 2003; Cushman 1991; Rico-Gray & Oliveira 2007). Indeed, in order for plants to benefit from such associations through the reduction of herbivory, certain criteria need be met. First, the hemiptera tended by ants should not be the principle herbivore. Second, populations of hemiptera should not be allowed, by ants, to reach numbers detrimental to plants and finally, ants should be successful in deterring non-hemipteran herbivores (Del-

Claro & Oliveira 2000; Eastwood 2004; Messina 1981; Rico-Gray & Oliveira 2007). Hence, the outcome for plants is dependant first on the threat of herbivory and its extent and then on the outcome of the ant-hemiptera interactions (Rico-Gray & Oliveira 2007).

However, for both ant and hemiptera, the outcomes of their mutualisms are not static but spatially and temporally variable (Cushman 1991). The strength of their association may be altered by climate, plant phenology and seasonal host plant use (Del-Claro & Oliveira 1999), fluctuating population densities (Breton & Addicott 1992), life history stages (Cushman & Whitham 1989) and species-specific behaviours (Eastwood 2004; Stadler & Dixon 1998). Thus, although described as a mutualism, a relationship beneficial to the two groups involved, the final results of such associations are clearly dependent on many factors intrinsically linked with such systems and must be considered as such (Rico-Gray & Oliveira 2007). Hence, ant-plant relationships as mediated by hemiptera are one of the most facultative, inconsistent and opportunistic, with highly unpredictable outcomes for plants (Rico-Gray & Oliveira 2007; Rosumek *et al.* 2009).

The outcomes of these tri-trophic relationships incur changes that are felt at the level of community. For example, plant selection may occur when ant-tended individuals are protected and incur greater fitness than their non-tended neighbours (Messina 1981). Similarly, the selective exclusion of plant herbivores, their eggs and hemipteran predators by ants can influence the type and local abundance of insect fauna (Rico-Gray & Oliveira 2007). Thus, the role of ants as a biotic defence, along with the availability of resources, plant defences and seasonal influences, may help explain the variable nature of herbivory in many of the species they affect (Mackay 1991).

In *Eucalyptus*, a genus extensively studied for its intra-specific variation in palatability to herbivores and resulting damage (Abbott *et al.* 1993; Auslander *et al.* 2003; Braganca *et al.* 1998; Burdon & Chilvers 1974a; Floyd *et al.* 2002; Fox & Macauley 1977; Fox & Morrow 1983; Jordan 2002; Journet 1981; Landsberg & Cork 1997; Lowman & Heatwole 1987; Majer *et al.* 1997; Matsuki *et al.* 2011; Morrow & Fox 1980; Morrow 1977; Nahrung *et al.* 2001; Ohmart *et al.* 1985; Raymond 1995; Stone 2001), the potential for ant associations to influence herbivory has been largely unstudied (Mackay 1991). In many eucalypts, despite the absence of specific ant attractants, ants are commonly seen ascending trunks and patrolling foliage (Mackay 1991), often in aid of tending hemiptera and harvesting honeydew (Vranjic & Gullan 1990).

Of the hemiptera associated with eucalypts, the leafhoppers (Eurymelidae), scale insects (Eriococcidae), psyllids (Psyllidae) and coreid bugs (Coreidae), the psyllids pose the greatest threat to plant fitness; causing high levels of defoliation that is, at times, lethal (Landsberg & Cork 1997). Psyllid damage is caused by sedentary nymphs which, under the cover of protective starch shells, use their stylet to penetrate leaf tissue and feed directly from the phloem (Landsberg & Cork 1997). Ants often harvest honeydew secretions that result from nymph feeding, with strong associations observed with psyllids from ants of the genus *Iridomyrmex* (Eastwood 2004; van Wilgenburg & Elgar 2007).

Iridomyrmex, members of the dominant family Dolichoderinae (Shattuck 1999), are common throughout Australia and the alpine regions (M. Nash *pers. comm.*; Green 2002 as cited in Green 2009; Shattuck 1999). Nests of *Iridomyrmex* are typically built within the soil, erupting into distinct mounds on the soil surface. Additionally, many colonies display polydomy, the building of multiple nests. Thought to be constructed in the aid of reducing foraging time, such colonies often position nests close to stable food sources such as honeydew producing hemiptera (van Wilgenburg & Elgar 2007).

In the Victorian alpine region, amongst the grass and shrub understorey of *E. pauciflora* sub-alpine woodlands, ant densities can be extremely high, with multiple nests within a single square metre (C. Jewson-Brown *pers. obs.*). Additionally, it is not uncommon for seedlings, saplings and juveniles of *E. pauciflora* to have the base of their main stem encircled by a nest, with individuals ascending, patrolling foliage, and at times tending hemiptera. Considering the frequency with which ants are associated with seedlings and saplings of *E. pauciflora,* the tendency for such trees to support populations of psyllid and other hemiptera within particular areas of the alpine region and the variable nature of herbivory within this genus, investigating the potential for ants to act as biotic defences within the system seems worthy of further study.

Hence, the aims of this study were to investigate:

i). If ants positively affect populations of hemiptera on saplings, and;

ii). If ants and the tending of hemiptera on saplings reduces foliovore herbivory.

Experimental procedure

Site selection and description

A single study area was selected at 'The Lanes' (-36° 53' 6.10"S, +147° 21' 29.98"E; 1600 m) within the Alpine National Park. An unburnt *E. pauciflora* woodland with predominantly grass (*Poa phillipsiana*) understorey was chosen as it did not vary in topography or vegetative structure. Ant activity and nest abundance appeared consistently high across the site. However, nest density was not quantified due to the sheer abundance of nests and the inability to differentiate true nests from satellites. However, all saplings observed within the site (approximating 50 m x 200 m), supported a nest around their base and a stream of ants patrolling between nests and foliage.

Seventeen saplings, all less than 40 cm high, each with an ant nest (*Iridomyrmex mjobergi)* at the base of the main stem and ant-tended psyllids (*Glycaspis* sp.) present on leaves, were randomly selected for study. To control for variation in sapling morphology and chemistry that may alter a plants susceptibility to herbivore attack, all treatments were conducted within a sapling (see Figure 17).

On each sapling, three to four treatments were applied on separate branches. These consisted of two main treatments: 'exclusion' (where ant access was prevented by way of a barrier) and 'non-exclusion' (where no barrier was applied). Barriers consisted of masking tape, approximately 3 cm wide, tightly wrapped around the branch and coated with Tree

Guard®, a sticky natural paste designed as an insect barrier. The exclusion treatments differed in that one was supporting a psyllid population tended by ants, referred to as ‘no ant + psyllids’ (-A+P) exclusion while the other ‘no-ant + no-psyllids’ (-A-P) was not. Non-exclusion treatments consisted of only masking tape, to control for any tape effect on ants; no Tree Guard was applied. Again the treatments differed in that one supported a population of psyllids, ‘ants +psyllids’ (+A+P) and the other, ‘ants + no-psyllids’ (+A-P) did not (Figure 17). As the saplings were short (less than 40 cm), not all were supporting psyllids on more than one branch, on these saplings the +A+P treatment was absent.

Ant access to exclusion treatments via blades of surrounding grasses or leaves on non-exclusion branches was mitigated through cutting grass surrounding each sapling, and trimming/removing linking leaves, provided they were not part of a study. Tree Guard re-application, grass cutting and leaf removal/trimming was monitored and maintained at 3-4 week intervals between December 2011 and February 2012.

Sapling herbivory was measured through the analysis of leaf photographs with leaves labelled, photographed and analysed as described previously. Additionally, leaf photographs were used to count the number of psyllids on leaves.

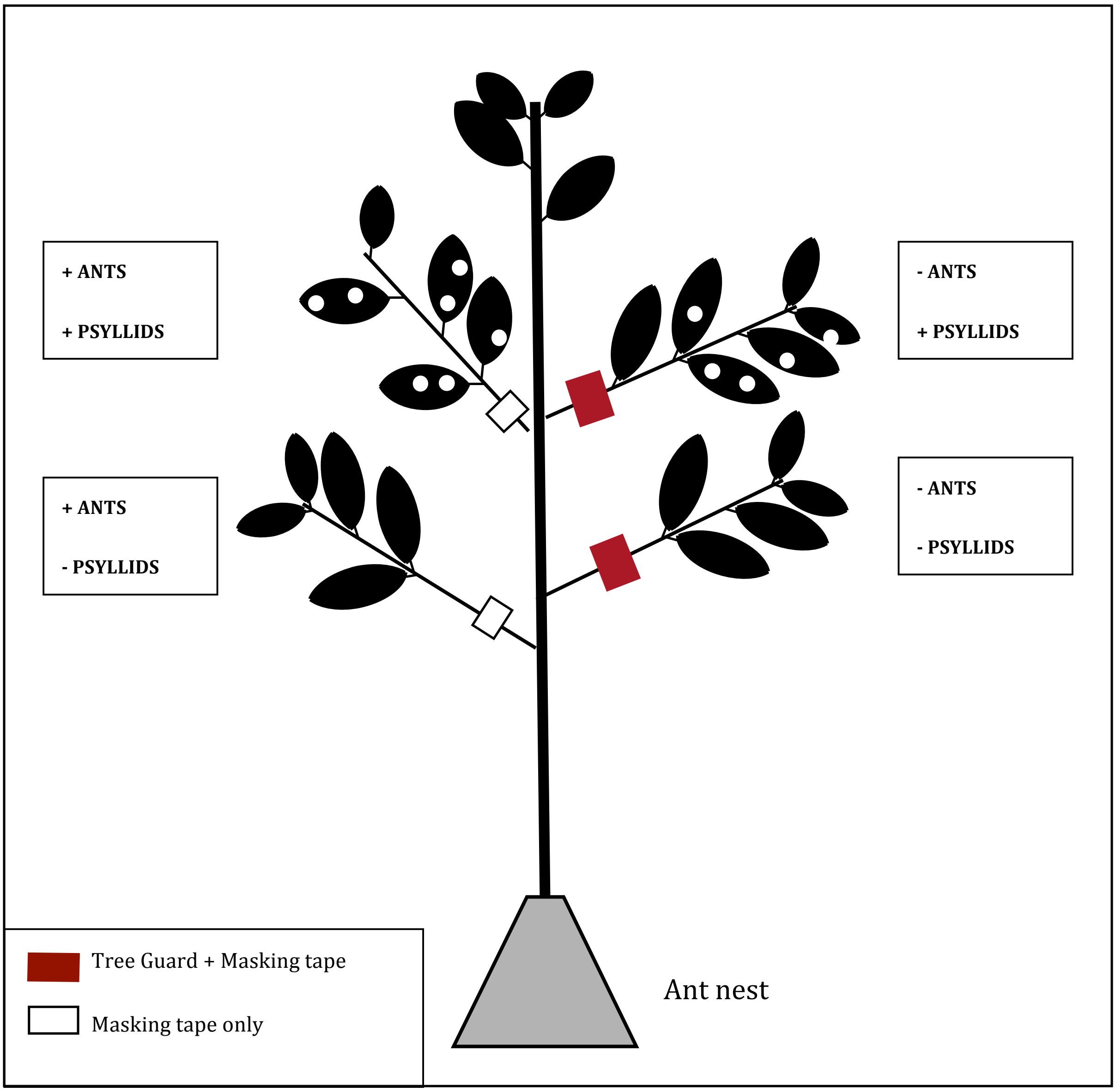


Figure 17. An example of exclusion treatments (red squares), and non-exclusion treatments (white squares) within a single sapling, complete with an ant nest at the base.

Data analysis

Chi-square goodness of fit tests were used to examine whether observed frequencies of leaf area loss (<25%, >25%) within treatments differed from those expected at random.

Measurements of area lost for each leaf within a single treatment (and sapling) were combined to give an average total for each treatment branch. Differences in psyllid numbers pre- and post-treatment were calculated for each treatment within each sapling. Comparisons of average leaf area loss between treatments and changes to psyllid numbers within each sapling were tested using ANOVA. For leaf area loss comparisons, nested ANOVA was used with saplings nested within treatments. Where significant, Bonferroni post-hoc comparisons were used. Homogeneity of variances was tested using the Levenes, Shapiro-Wilk and Kolmogorov-Smirnov test statistics. If significant, data was transformed (square root, log). If transformations failed to improve data distribution, non-parametric Kruskall-Wallis test was performed with Mann-Whitney U tests for post-hoc comparisons.

To test whether there was a relationship between the proportion of leaf area lost and new leaf production on branches between treatments, a Pearson correlation was used.

Results

Mean leaf area loss differed significantly from random under all treatments ($X^2 = 110.46$, df = 6, $p = <0.001$). There were significant differences between leaf area lost between treatments within each sapling (ANOVA F= 3.36, $p = <0.001$) but no significant differences between saplings (ANOVA F = 1.49, $p = 0.171$). Mean damage ranged from 5.4% to 9.5% of leaf area lost. On average, the highest proportion of leaf loss was on branches with ants present but no psyllid populations. The least damage was found on branches with psyllids present but ants excluded (Figure 18).

New leaf production mirrored mean leaf loss in all treatments (Figure 19); however, no significant relationship between leaf loss and leaf production was found (Pearson-correlation $p = 0.451$).

Change to psyllid numbers differed significantly between treatments (Kruskall-Wallis = 21.47, df = 3, $p = <0.001$). Of the non-exclusion treatments harbouring psyllids (Ants + Psyllids), psyllid numbers were reduced in all saplings by the end of the treatment period

(Figure 20). Exclusion treatments with psyllids (No ants + Psyllids) showed a reduction of psyllid numbers in all saplings bar two. Of the exclusion treatments initiated without psyllids (No ants No Psyllids) two saplings were supporting psyllid populations by the end of treatment. Finally, of the non-exclusion treatments initiated without psyllids (Ants –No Psyllids), one sapling of the seventeen gained psyllids by the end of treatment.

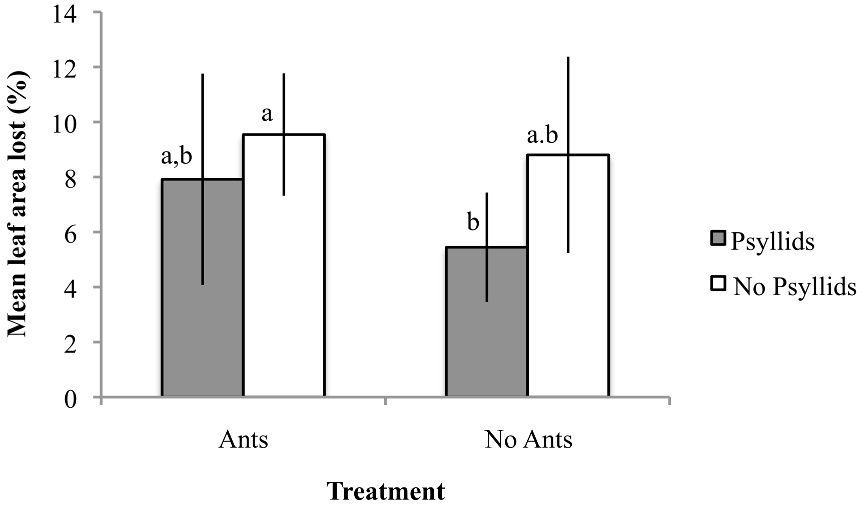


Figure 18. Mean leaf area lost (± S.E) for each of the exclusion (no ants) and non-exclusion (ants present) treatments. Columns with different letters differ at the *p* = 0.05 level of significance.

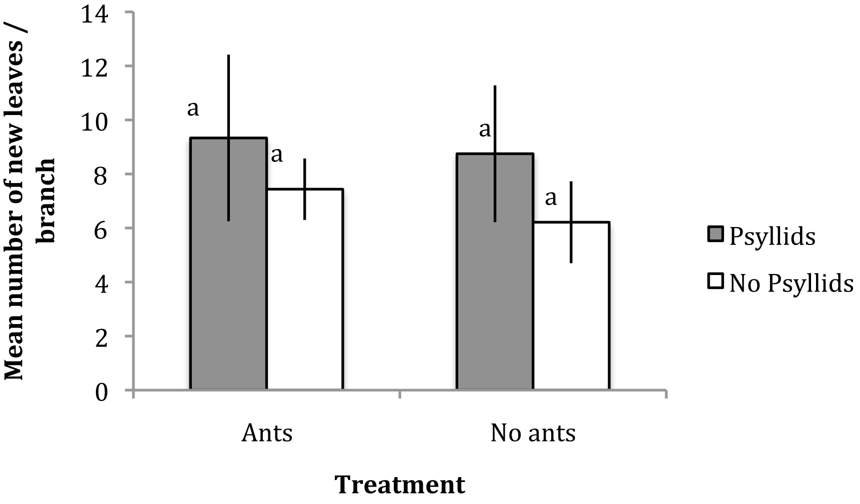


Figure 19. Mean number of new leaves (± S.E) at the end of experimentation for each of the exclusion (no ants) and non-exclusion (ants) treatments. Columns with different letters differ at the *p* = 0.05 level of significance.

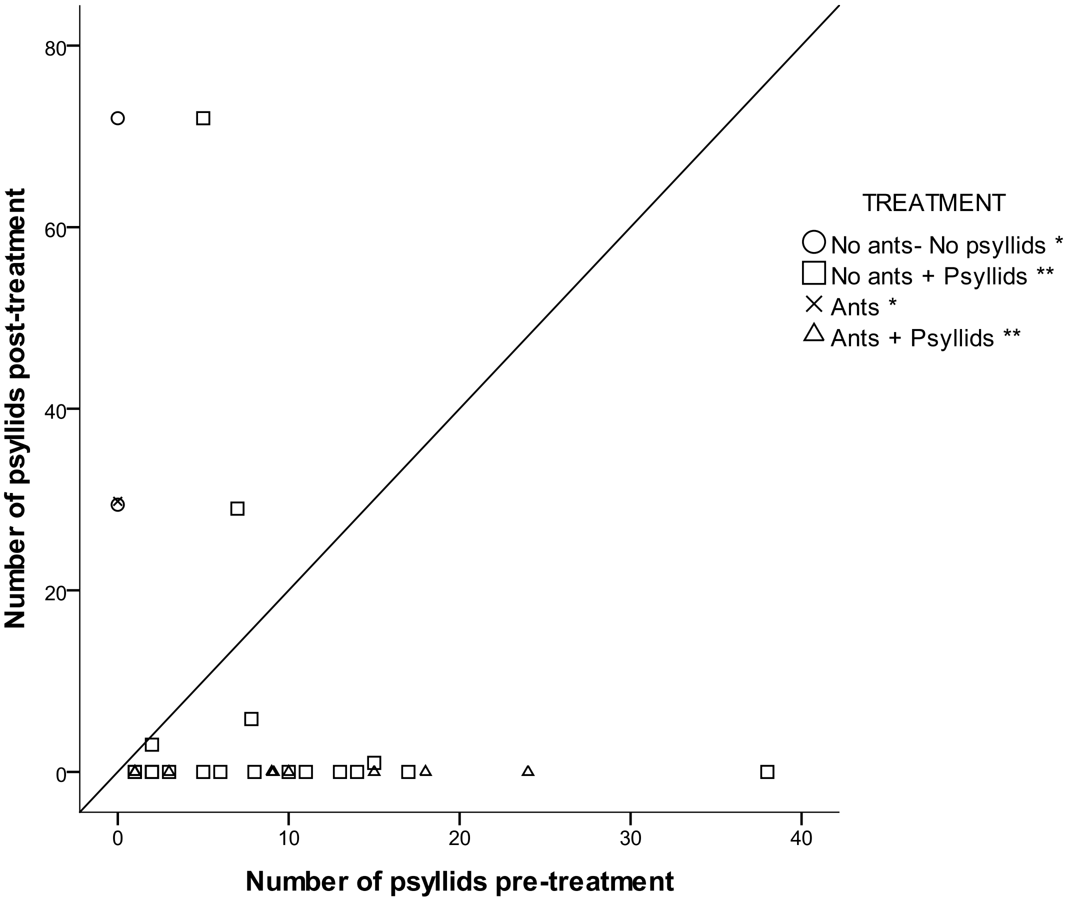


Figure 20. Number of psyllids pre-treatment compared to post-treatment for exclusion and non-exclusion treatments on saplings. Saplings below the line show a reduction in psyllid numbers, while those above have increased throughout the treatment period. Differences in the number of asterisks along side treatments within the graph key represent treatments that differ significantly at the *p* = 0.05 level.

Discussion

The aim of this study was to assess the influence of tending ants (*I. mjobergi*) on populations of the psyllid *Glycaspis sp.* (Hemiptera) associated with saplings of *E. pauciflora* (Snow Gum), while investigating the potential for ants to act as a biotic defence, reducing leaf herbivory. The results suggest that the amount of leaf area lost to herbivores was not significantly influenced by the presence of ants on foliage, but that psyllids potentially have negative effects on herbivory. Leaf area lost, while typically low for all saplings (< 10 %), was not significantly influenced by ant presence in treatments of psyllids nor without, suggesting that ants did not actively defend saplings against herbivores. These results differ from many studies that show ants can significantly alter plant fitness by mitigating herbivory (Floate & Whitham 1994; Lach 2003; Lach & Hoffmann 2011; Skinner & Whittaker 1981; Way & Khoo 1992; Whittaker & Warrington 1985). Grinath *et al* (2012) showed ants tending honeydew producing membracids (Hemiptera) significantly reduced herbivory by leaf beetles on *Chrysothamnus viscidiflorus* (Asteraceae). Similarly, Messina (1981) showed hemiptera-inspired ant protection of *Solidago* sp. (Asteraceae) resulted in increased plant growth and seed set.

In addition, populations of psyllids were significantly reduced by the end of experimentation (71 days) on twelve of seventeen saplings, regardless of treatment; this suggests factors other than ant presence may have influenced psyllid populations. Moreover, as most populations were substantially reduced or disappeared independent of ant presence, the potential for ant tending to intensify psyllid effects on plants remains unclear.

The absence of a significant negative effect of ants on herbivores was unexpected given the literature on ant-psyllid interactions. There are several potential explanations for this observation. *I. mjobergi*, the ants found to be tending psyllids in the present study, are typically small (< 4 mm) and not aggressive (Heterick & Shattuck 2011) and thus, may be limited in their ability to deter non-hemipteran herbivores. Messina (1981) also found that small ants (< 3.5 mm) were unsuccessful in reducing beetle density and subsequent plant damage. Additionally, Cushman and Whitman (1989) found the strength of an ant-hemiptera mutualism, as measured by the benefits to hemiptera, was dependent on the abundance of a hemiptera-specific predator.

As ant presence is often strongly linked to that of hemiptera, the loss of psyllids from saplings throughout this experiment may have resulted in a reduction of ant tending or aggression towards non-hemipteran herbivores, with consequences for plant protection.

Wood (1982) and Cushman and Whitman (1989) describe positive outcomes for nymphs of ant-tended membracids (Hemiptera: Membracidae) as density-dependent due to few ants tending, and benefiting small aggregations. Similarly, Grinath *et al.* (2012) showed a ten-fold increase in ant abundance on individual *C. viscidiflorus* (Asteraceae) hosting honeydew producing membracids (Hemiptera), compared to their neighbours that were not.

While the cause of declining psyllid populations is not known, mortality in *Glycaspis* sp. typically results from poor host quality (Clarke & Dallwitz 1974). More specifically, White (1970) has shown success of psyllid nymphs may depend on the availability of nitrogen within foliage, with increased survival and significant population expansion associated with stressed plants known to have higher concentrations of nitrogen within their phloem. While leaf chemistry was not assessed in this study, others have shown ant nests to be superior sites, in terms of soil moisture and nutrients, for plant growth (Beattie & Culver 1983; Holldobler & Wilson 1994). Thus, seedlings growing within ant nests, as in the present study, may be less likely to experience nutritional or moisture stress, proving a poor host for hemipteran nymphs. This is, however, highly speculative and in need of further research. Briefly, temperature fluctuations and water stress are also known to influence psyllid mortality. However, as it is unknown at what stage of experimentation psyllid numbers declined, nor if reduction was simultaneous for all saplings or staggered, it is difficult to associate variation in weather to mortality. Furthermore, analysis of monthly temperatures (minimum and maximum) and rainfall for the Falls Creek region during the study period reveals few major fluctuations from long-term records, other than higher than average rainfall for November 2011 (Bureau of Meteorology *unpublished data*). Wet conditions are, however, considered favourable for psyllid nymphs (Hodkinson 1974) and are unlikely to have been detrimental here.

New leaf production varied between treatments. In both ant and non-ant treatments, branches containing psyllids grew, on average, two leaves less than those without psyllids. These branches also received the least herbivory, suggesting psyllids may have influenced leaf area loss with consequences for new leaf production. As psyllid feeding directly removes resources from leaves, the nutritional value for herbivores such as leaf chewers may be reduced, in turn altering their palatability. Brown and Weis (1995) found prior damage to leaves of *Solidago missouriensis* (Asteraceae) led to reduced consumption by Chrysomelid beetles and consequences for larval growth and development. Denno *et al.* (2000) also found phloem feeding by the membracid *Prokelisia dolus* (Hemiptera) significantly reduced the quality of its plant host, effecting the development of a second membracid species occurring later in the season.

Conclusion

This study has shown the presence of *I. mjobergi* on the foliage of *E. pauciflora* saplings has little effect on the amount of leaf area lost to herbivores. One explanation for an absence of ant protection is the loss of psyllids causing a reduction in both ant aggression and presence on saplings. Furthermore, populations of *Glycaspis* sp. were reduced significantly regardless of ant presence, suggesting a force other than ant tending influenced populations. Importantly, the presence of *Glycaspis* sp. feeding may also have altered plant quality, effectively modifying branch palatability and reducing herbivory, regardless of ant presence. Studies have shown relationships between ants and hemiptera to be highly variable through space and time (Billick & Tonkel 2003; Breton & Addicott 1992; Cushman & Whitham 1989; Eastwood 2004; Messina 1981) and hence, a longer exclusion period that allows multiple generations of *Glycaspis* sp. to be observed may prove beneficial to understanding the nature of the interaction between ants-psyllids and herbivores more clearly. However, on the whole, the outcome of the present study suggests that at this local scale there is little evidence that herbivore damage and subsequent fitness of *E. pauciflora* saplings is altered by biotic interactions.

Chapter 4

Ants and seed harvesting: a pilot study

Introduction

A significant proportion of interactions between ants and plants centre on seed predation (Rico-Gray & Oliveira 2007). Such interactions, where ants harvest seeds as food, are often antagonistic with little potential for plants to benefit (Beattie 1985; Howe & Smallwood 1982; Hulme 1998; Risch & Carroll 1986; Zwolak & Crone 2012). However, within every antagonistic interaction is the potential for mutualism (Rico-Gray & Oliveira 2007; Siepielski & Benkman 2008), a quality well supported by plants and ant seed harvesters (Boulay *et al.* 2009; Howe & Smallwood 1982; Levey & Byrne 1993; Ohnishi & Suzuki 2011; Retana *et al.* 2004; Vander Wall *et al.* 2005). Importantly, in order for plants to benefit from seed predation, a seed must be harvested and later discarded. Whether abandonment occurs on the way to a nest, within underground granaries or in the refuse pile of a colony, the relationship has transformed from that of predation, to one of seed dispersal (Levey & Byrne 1993; Rico-Gray & Oliveira 2007).

Myrmecochory, the dispersal of seeds by ants, is a widespread dispersal syndrome, within which plants have invested to varying degrees (Aronne & Wilcock 1994; Davidson & Morton 1981; Giladi 2006; Whitney 2002). Indeed, to promote the harvesting of seeds, some plant species enhance seed attraction through anatomical and chemical adaptations like that of the eliasome or aril, prompting ants to harvest the diaspore as a whole and discard the seed once the attractive portion is removed. Other seeds, however, lack external attractants but are harvested for compounds inherent in their seed coat or for their protein-rich endosperm (Rico-Gray & Oliveira 2007; Howe & Smallwood 1982). For a seed, the outcome of ant dispersal is determined by the end point, namely where the seed is discarded (Bas *et al.* 2009; Hanzawa *et al.* 1988). Although highly variable due to seed traits and the different levels of competency with which different ant species harvest seeds (Andersen 1988a; Bas *et al.* 2009; Howe & Smallwood 1982; Rico-Gray & Oliveira

2007), the potential for seeds to benefit lies in their relocation away from the parent tree and, through the transportation to ant nests, a nutrient-rich site that often supports germination (Augspurger 1984; Levey & Byrne 1993; Retana *et al.* 2004; Rico-Gray & Oliveira 2007).

Soils of ant nests typically differ from their surrounds in their chemical composition, temperature, and moisture (Beattie & Culver 1983; Holldobler & Wilson 1994). Thus, seeds returned to nests and discarded are relocated to nutrient-rich nest middens, regarded as high quality micro-sites within which to germinate and grow (Rico-Gray & Oliveira 2007; Whitney 2002). Anderson (1988b) showed growth of seedlings in soil derived from ant nests increased root and shoot production of tested plants by 50%. Similarly, Passos and Oliveira (2002) found nests of *Pachycondyla striata* (Formicidae: Ponerinae) contained higher concentrations of phosphorus and nitrogen, and significantly improved seedling growth within their first year. Furthermore, within nest deposition carries the added benefit of protection from non-ant predators and fire (Rico-Gray & Oliveira 2007).

As described by the ‘escape hypothesis’ (Janzen 1970), with increased distance from the parent tree, seeds are exposed to less competition from both parent and fellow seeds. Additionally, the threat of predation and infection with pathogens is reduced as predators are likely to target known ‘dense’ resources, like that of a parent, and pathogen attack may occur through the transferral of parental infection. As a consequence, the dispersal of a seed away from a parent may enhance seedling fitness (Howe & Smallwood 1982).

The optimal dispersal distance for a seed will differ between species and environments (Howe & Smallwood 1982). However, it is generally considered longer distances incur greater benefits, with short distances attracting little chance that a seed may benefit (Boulay *et al.* 2009; Gomez & Espadaler 1998). Consequently, for seeds in low nutrient habitats of Australia, where ant dispersal is typically less than 5 m, the ‘benefits’ are considered minimal (Andersen 1988a; Auld 1986; Kjellsson 1991; Whitney 2002), However, as described by Andersen (1988a), short distances may be just as beneficial as long in reducing competition between parent and offspring, particularly in habitats where sites suitable for seed germination and growth are rare (Andersen 1988a). Furthermore, in extreme environments, where short distances can incur significant changes to microclimate (Bowman *et al.* 2003; Korner 1999; Smith *et al.* 2003), the short dispersal of a seed may prove beneficial if exposed to the new microclimate more conducive to growth.

Eucalyptus pauciflora, the dominant tree species in the subalpine regions of south-eastern Australia, is not known to have seed dispersed by ants (Green 2009). However, typical for a species that occupies an environment of extremes, successful germination and establishment of seedlings is low relative to the number of seed produced (Atkin *et al.* 2000; Ball *et al.* 1991; Green 2009; Hattenschwiler & Smith 1999). Snow cover, low temperatures, frost, photoinhibition, intra-specific competition and characteristics of the soil all significantly contribute to seedling mortality (Ball *et al.* 1997; Blennow *et al.* 1998; Davidson & Reid 1985; Ferrar *et al.* 1988). Hence, given the evidence for benefits gained by dispersal by ants, it seems likely that seeds able to exploit the superior quality of ant nests or ant-aided dispersal would benefit significantly in such an environment.

Ants of the genus *Iridomyrmex* (Hymenoptera: Formicidae) are present in great numbers within areas of the Australian alpine region (M. Nash *pers. comm.*; Green 2002 as cited in Green 2009; Shattuck 1999), and often form nests around the base of *E. pauciflora* seedlings, saplings and juveniles (C. Jewson-Brown *pers. obs*). Thus, an investigation into potential interactions between these two groups involving seed dispersal and subsequent facilitation seemed worthy.

The first question to arise was that of seed attraction. *E. pauciflora* seeds are not typical of a myrmecochore in that they lack both eliasome and aril. However, Ashton (1979) in a study of ant harvesting of *E. regnans* seeds, describes seeds similar in their nudity, which are highly attractive to ants and heavily predated. While there are many aspects of this relationship worthy of investigation, this study set out to first determine the attractiveness of *E. pauciflora* seeds to ants in the alpine environment and, second, assess the potential effect of patch type on seed detection rates by ants and subsequent harvesting.

Experimental procedure

Site selection and description

This study was conducted at Marums Point (-36° 52' 43"S, +147° 19' 53"E, 1600 m) in the Alpine National Park. Chosen primarily due to a diversity of understorey structure within a small area, the site consists of an *E. pauciflora* woodland with an understorey comprising herbs (predominantly *Goodenia hederacea*), grass (*Poa hiemata*) and heath (*Prostanthera cuneata, Bossiaea foliosa*). Both topography and understorey density varied within the site; from flat, open herb-fields, to close-set grasses and dense shrubs reaching over 2 m in height. Regardless of understorey density or species composition, foraging ants (*I. mjobergi*) and nest mounds were conspicuous and abundant. Ant nest density appeared consistent throughout the site, other than deep under shrubs where it was reduced. Nest density was not quantified due to the difficulty in determining discrete nests from satellites, a structure commonly seen in the *Iridomyrmex* genus (Heterick & Shattuck 2011).

Seed collection and preparation

Mature capsules of *E. pauciflora* were harvested from trees in the Alpine National Park in January 2011. To extract seeds, capsules were oven dried at 150 °C for 20-30 mins, capsules then opened and seeds and chaff were released. To control for any affect of the oven treatment on seeds, capsules were collected a second time, and while in paper bags placed in the sun to desiccate for 2 hrs, prompting seed release.

Ant seed harvesting

Before beginning these experiments a pilot study testing ant response to (a) empty petri-dish and (b) mixture of honey and canned tuna (plain in distilled water), a positive control, was conducted on a ski slope behind the Falls Creek village (-36° 51' 59"S, +147° 16' 38"E; 1600 m). Honey and tuna were chosen as a positive control as both substances are attractive to ants (Andersen and Patel 1994; Romero and Jaffe 1989). Additionally, by combining both, sugar and protein it is attractive to a greater diversity of ant species. Ants, generally curious of new objects within their territory, explored empty dishes, effectively utilising holes around the base, seemingly undisturbed. When confronted with the mixture of honey and tuna (1:1 ratio), ant behaviour was both positive and negative. While ant numbers increased quickly suggesting recruitment, and confirming a positive response, the number of negative responses was reduced when the proportion of tuna within the mixture was reduced. Thus a 2:1 ratio of honey to tuna was used during experimentation.

Initially, dishes were placed complete with lid to reduce seed disturbance by wind, other invertebrates, and vertebrates. However, when in full sun, ants feeding inside often died, suggesting temperatures may have been lethal. Consequently, lids were removed from dishes during experiments, bar those left overnight. The three days (19th-20th January 2012, and 3rd February 2012) over which the experiment was conducted were consistently sunny, warm and still, suggesting minimal effect of wind. No non-ant insects, birds or other vertebrates were observed within or nearby petri dishes containing seed or tuna/honey during observations. Thus, it is likely ants were responsible for any observed seed removal.

Experiment 1: Overnight

Twenty petri dishes, each with 10 holes (approximately 3 mm in diameter) evenly spaced around the base, were laid in a grid pattern, spanning 2250 m^2. Each dish, a minimum of 15 m away from its neighbours, was laid flat on the soil surface with the lid removed.

Of the 20 dishes, 6 were placed beneath shrubs, 9 beneath grass and 5 on bare ground. To verify ant activity, a positive control of combined tuna and honey was placed in five dishes, of the same design (3 in grass, 1 in shrubs and 1 on bare ground). Fifteen eucalypt seeds (differentiated from chaff based on size and colour) were placed in each of the 15 dishes not holding positive control. The time of placement for both seed and tuna/honey was recorded for each dish.

Each dish was revisited three times during a twenty-hour period: mid-morning, mid-afternoon and mid-morning of the following day. At each revisitation, remaining seeds were carefully counted without disturbing petri dish or seeds. For positive controls, ant presence and number was estimated to the nearest ten ants, where more than one hundred ants were present, numbers were recorded as simply greater than one hundred.

Experiment 2- Increased density

To test for the effect of seed detection contributing to harvesting rate, the experiment was repeated with increased density. Thirty-eight dishes were distributed within the same area, each dish a minimum of 10 m away from its nearest neighbour. Of the 38 dishes, 16 were placed beneath shrubs, 16 beneath grass and 6 on bare ground. Positive controls were placed in five of the 38 dishes (2 in grass, 2 in shrubs and 1 on bare ground) and fifteen eucalypt seeds in each of the 33 dishes remaining.

Each dish was revisited four times from mid morning to early evening at approximately 2 hr intervals, seeds were again counted and ant presence noted.

Experiment 3- sundried seeds

Ant harvesting of *E. pauciflora* seeds seems counterintuitive as they lack the typical ant-attractants of eliasome or aril (Green 2009). However, it is not uncommon for seeds lacking a specific ant attractant to be harvested for compounds inherent in their seed coat (Rico-Gray & Oliveira 2007).

The sensitivity to temperature of many compounds thought to aid in attraction (amino acids, fats, sugars) prompted the test of potential effects of oven-drying at 150 °C on the attractiveness of seeds to ants. A simple experiment was conducted using seed dried in the sun.

The study site, an open woodland with an understorey of grass and heath, was situated near the Langford West aqueduct car park (-36° 53' 4"S, +147° 19' 14"E; 1600 m) in the Alpine National Park. This experiment was conducted on the 3rd of February 2012.

Twenty-eight petri dishes were laid in a grid pattern spanning 2100 m^2. Each dish, a minimum of 7 m away from its neighbours, was laid flat on the soil surface or where grass was too dense, on flat grass, all with their lids removed. Of the 28 dishes, five were placed beneath shrubs, 15 within grass and four on bare ground. The treatments were uneven due to a dense grassy understorey throughout most of the area making bare ground sparse. Positive controls were placed in four dishes (2 in grass, 1 in shrubs and 1 on bare ground). Seeds were placed in dishes as described above.

Each dish was revisited three times during a 3-hour period (at hourly intervals), from mid-morning to noon. Seeds were counted and the positive control monitored as described above.

Data analysis

The number of seeds remaining at the end of the experimental period was combined for each vegetation type to give an average group total for each of the three experiments. Group means (three for each experiment) were used to statistically test for differences in seed removal rates between the three vegetation types within each experiment, and for differences in seed removal rates between experiments one and two.

One-way ANOVA with Bonferroni post hoc comparisons were used to compare seed harvesting between treatments. Normal distribution of data and the homogeneity of variances were tested using the Levenes and Kolmogorov-Smirnov test statistics. When significant data was transformed (log, square root, fourth root), if transformation failed to improve data normality, non-parametric Kruskall- wallis tests with Mann-Whitney U tests were used for post-hoc comparisons.

All statistical analyses were conducted using SPSS 18.0.

Results

Experiment 1: Seed removal overnight

Average number of seeds removed after more than 20 hrs of exposure ranged from less than one in grass treatments to three under shrubs. Seed was removed by the second visit (4.54 hrs), and again by the final visit (20.36 hrs) from shrubs and bare ground. No seed removal occurred within grass treatments until 5 hrs post exposure; this removal occurred while lids were covering samples. No significant difference was found in the number of seeds remaining between vegetation types at the end of twenty and a half hours (Kruskall-Wallis = 1.19, df = 2, $p = 0.552$) (Figure 21).

Experiment 2: Seed removal with increased sample density

Less than 2 seeds were removed, on average, from treatments after more than 10 hrs of exposure. Seeds remained untouched in all treatments until 3.75 hrs post exposure (early afternoon). No significant difference was found in the number of seed removed between vegetation types (Kruskall-Wallis = 0.27, df = 2, $p = 0.873$) or between seed removal in experiment one and that of increased density in experiment two (Mann-Whitney U test $z = -0.61$, $p = 0.951$) (Figure 21).

Experiment 3: Removal of sundried seeds

No seeds were removed from either the shrub or bare ground treatments throughout the three hours of exposure. On average less than one seed was removed from the grass treatments. Seed removal from grass samples occurred within an hour of exposure. Treatments were significantly different at the 0.05 but not at 0.01 level (Kruskall-Wallis test = 7.2, df = 2, $p = 0.027$) (Figure 21).

Positive control experiment 1, 2 and 3- honey and tuna

Time till ant discovery of positive samples varied between treatments and experiments. However, once a sample was discovered, ant numbers increased and remained high until the resource was consumed in full (Figure 22). No treatments were left undiscovered or unharvested at the completion of each of the three experiments.

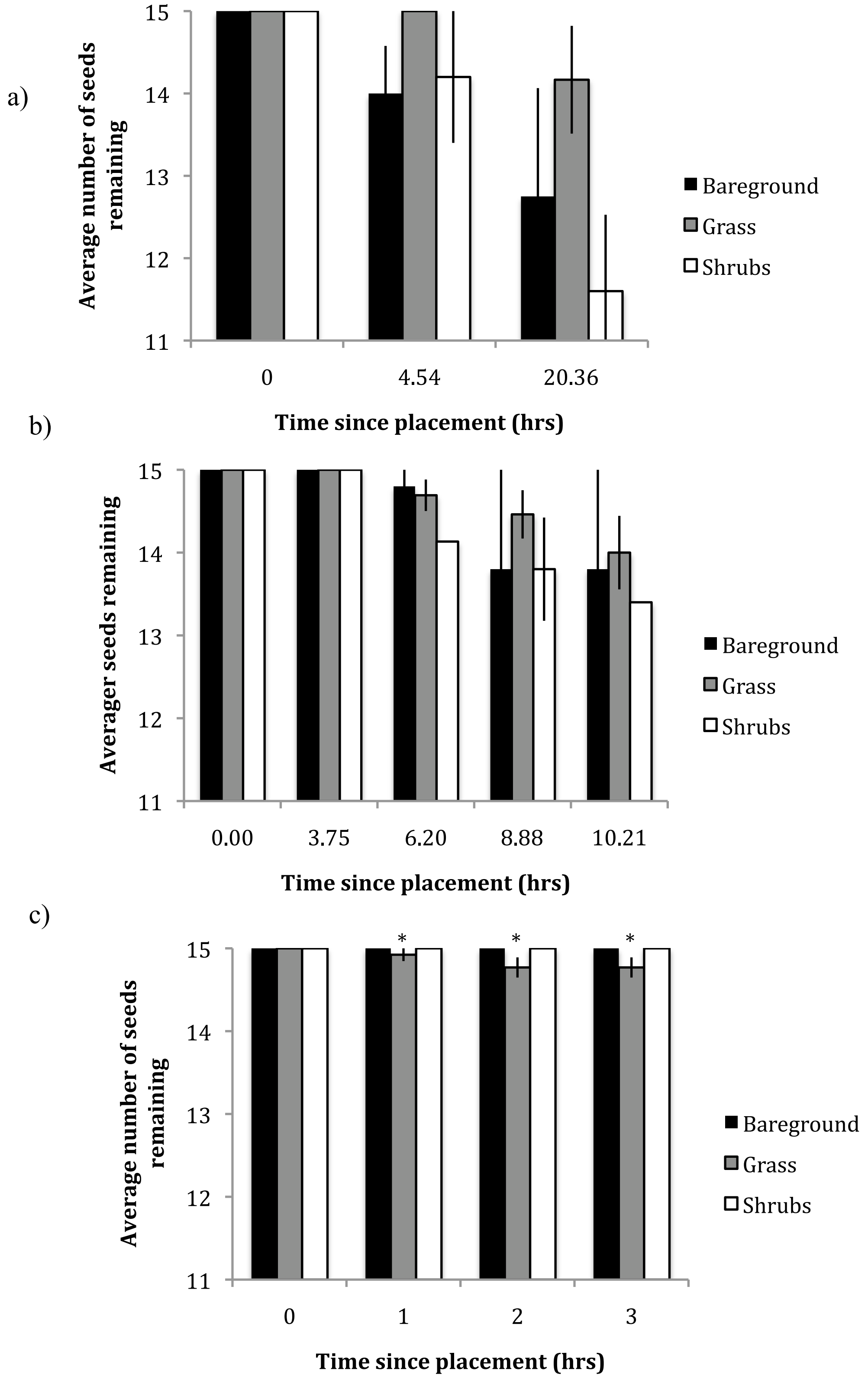


Figure 21. Average number of seeds remaining in the three vegetation treatments at different times since seed placement in (a) the overnight experiment, (b) full day experiment with increased sample density and (c) sundried seed experiment. * Significant at the 0.05 level.

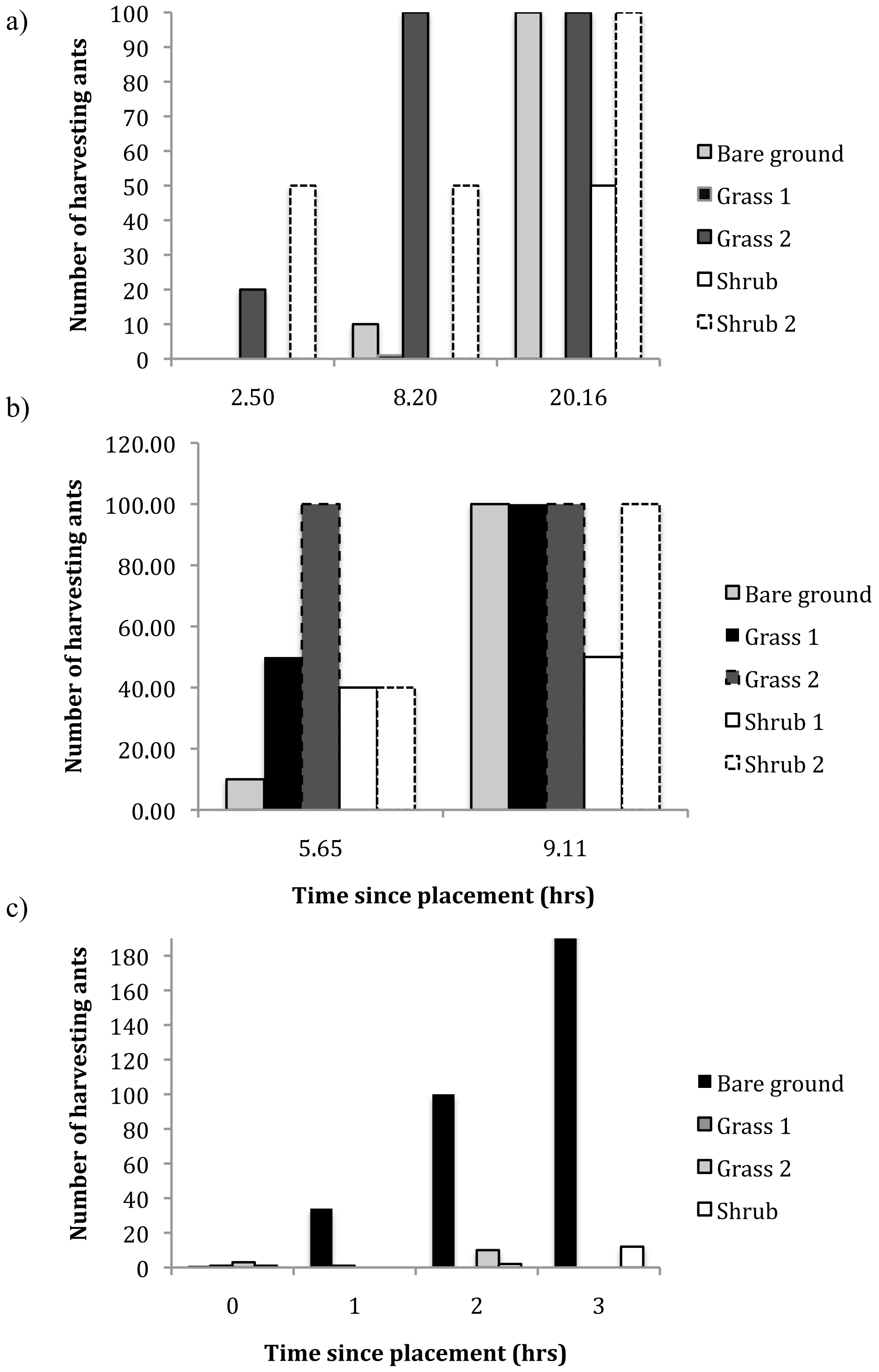


Figure 22. Ants observed harvesting honey/tuna mix within different vegetation treatments at time since exposure in (a) experiment one (b) experiment two and (c) experiment three.

Discussion

The aim of this study was to assess the attractiveness of *E. pauciflora* seeds to ants (*I. mjobergi*), and the potential for ant predation to benefit seeds through dispersal. Ants harvested *E. pauciflora* seeds at low rates independent of ‘patch-type’ i.e. where seeds were positioned relative to vegetation type and cover. Ant presence, as indicated by the harvesting of the positive control, was consistently high in all experiments, with a propensity for recruitment following resource detection. Furthermore, detection of positive controls occurred consistently within the time between initial placement and the first revisit. Thus, it can be assumed that a failure to detect seed is not the reason that so few seeds were harvested. Increasing the density of seeds available within the experimental area had no influence on seed harvesting rates, nor did the desiccation of seeds in the sun rather than the oven. While harvesting of sundried seeds occurred earlier than those in the first two experiments, the rates of removal did not differ after the first seed was removed.

Seed removal rates for all experiments were, on average, less than two for each vegetation type by the end of exposure (20 hrs in experiment one, 10 hrs in experiment two and 3 hrs in experiment three). Comparisons with studies of seed removal in other systems suggest the removal rates of the present study are considerably low. O’Dowd and Gill (1984) describe ant removal of *E. delegatensis* (Alpine ash) seed totalling over 260 per week in an unburnt stand. Similarly, Wellington and Noble (1985), describe seed removal by ants of unburnt stands of the semi arid *E. incrassata* as more than 100 seeds over four days. Andersen (1987) has shown ants harvesting 84% of shed *E. baxteri* seeds in an unburnt woodland, with notable consequences for seedling recruitment. Additionally, most studies of ant seed harvesters (including those discussed above) describe multiple species harvesting a single resource with different levels of competency (Ashton 1979; Briese & Macauley 1981; Drake 1981; O'Dowd & Gill 1984; Passos & Oliveira 2002). In turn, the present study is likely removal rates of a single species, as only *I. mjobergi* was found within the experimental area. This lack of ant diversity may help explain the low removal rates of this system.

While ant response to petri dishes was tested before experimentation and thought to have little influence on ant behaviour, Andersen and Ashton (1985) have shown petri dish seed baits substantially reduced natural seed harvesting for up to 48 hrs post exposure. Moreover, the clumping of seeds within baits, an artificial design compared to that of natural seed rain, may also have altered seed removal. Again Anderson and Ashton (1985) found clumped seeds were harvested significantly less than single seeds, for a variety of seeds and ant species. Observations of *I. mjobergi* behaviour in response to the seeds may shed light on these results.

High densities of ant nests throughout the site, irrespective of vegetation type, may explain the absence of structural influence on harvesting rates. Furthermore, vegetation structure may be more likely to influence seed dispersal distance rather than initial harvesting. Crist and Weins (1994) found the distance and speed of ants travelling between nest sites and known food resources was influenced by vegetation structure, primarily due to differences in the proportion of bare ground and density of predators associated with different structures.

Conclusion

The removal of seeds indicates the ability for *I. mjobergi* to influence *E. pauciflora* through seed predation, either by dispersal of seeds or the harvesting of seeds as food. In turn, the results of this study indicate further research into snow gum seed harvesting by ants, along with ant nest soil chemistry and its effect on seedling growth, has the potential to shed light on seedling germination and recruitment patterns within this ecosystem. However, the low seed removal rates suggests seed predation is not as significant as that of other systems. Moreover, an influence of herbivory (granivory in the present case) on *E. pauciflora* fitness does not appear to be exerted at this small scale.

Chapter 5

Synthesis

The distribution and abundance of plant species in high mountain ecosystems is thought to depend largely on abiotic factors that play out at both landscape-scales (e.g. steep environmental gradients affected by increasing elevation) and local-scales (e.g. changes in topography, aspect and canopy cover). Relatively little attention, however, has been paid to biotic interactions and how these might also change with landscape- and local-scale factors. Ecological interactions such as competition, facilitation and herbivory are all likely to modulate species performance and affect local abundance but a comprehensive understanding of their role in the Australian alps remains largely ignored. In this study, I examined the role of herbivory and its potential to affect aspects of the performance of the dominant high altitude tree *Eucalyptus pauciflora* (Snow Gum).

Through the expression of gene and environment interactions, plants differ both morphologically and chemically. Such inter-plant heterogeneity is well recognised as having significant influence over insect abundance and performance (Bernays & Chapman 1994; Edwards *et al.* 1993; Garibaldi *et al.* 2011; Loney *et al.* 2006; Louda & Sharon 1992; Maschinski & Whitham 1989; Mattson 1980; Ohmart & Edwards 1991; Schowalter *et al.* 1986; Strauss & Agrewal 1999; Suzuki 1998). As described in Chapter 1, resource allocation, plant vigour and plant stress hypotheses are three of the most widely acknowledged theories used to explain the varied distributions of insect herbivores between their host plants at local- and landscape-scales. In high mountain ecosystems, with increasing altitude and hence, declining temperature, plants are likely to allocate more to defence within tissues and this should lead to lower herbivory at high elevation. Microclimate variation at the local scale, however, may promote plants that vary in their degree of stress and vigour that, in turn, makes them vary in palatability to herbivores.

This study aimed to assess the above hypotheses by documenting the extent, type and distribution of herbivory on Snow Gum saplings along altitudinal gradients of five mountains in the Victorian alps. Additionally, the influence of location (mountain), altitude (position relative to treeline) and seed origin on leaf traits, plant traits and characteristics of the environment were assessed, as was their potential to influence herbivory.

Herbivory and plant traits along altitudinal gradients

Consistent with other studies (Burdon & Chilvers 1974a,b; Garibaldi *et al.* 2011; Suzuki 1998; Williams 1990), the results presented in Chapter 2 indicate the extent of leaf area lost to herbivores was influenced by altitude. In addition to altitude, the amount of herbivory observed was best explained by the combined influence of canopy 'openness', plant height, and leaf thickness. This suggests that, at the landscape-scale, herbivory may increase with increasing productivity associated with altitude, a trend in broad agreement with those hypotheses associating leaf area loss to plant vigour and the availability of resources.

Herbivory in response to local scale variation

Environmentally driven trends in plant traits, leaf traits, and the local environment also contributed to observed herbivory patterns; however, the strength of their association varied highly within and between altitudes and mountains. Such variation supports the tendency for different microclimates to alter plant morphology, chemistry and palatability independent of altitude. Additionally, inherent differences between mountains in geology, subsequent soil properties and aspect are likely to substantially affect local interactions.

Ants and local herbivory patterns

Herbivory of seeds (by ant predation) and leaves (in the presence/absence of ants that defend psyllids) are likely to contribute to the local population dynamics of Snow Gum but such interactions have been poorly described. Chapters 3 and 4 describe the potential for biotic interactions to influence herbivory at smaller-scales. I documented the influence of tending ants (*I. mjobergi*) on populations of the psyllid *Glycaspis sp.* (Hemiptera) associated with saplings, along with the consequences of their tending for sapling herbivory. Despite the presence of ants and psyllids on Snow Gum leaves, no influence of ant presence on the rate of herbivory was detected. Additionally, ants, while removing a small fraction of seeds presented in cafeteria trials, appear unlikely to play a significant role in seed dispersal of this species.

Further Research

The present study would have benefited from analysis of leaf chemistry, as the role of plant secondary metabolites as deterrents, and leaf nitrogen concentrations as attractants is acknowledged for many plant genera including *Eucalyptus.* However, due to technical difficulties, only a portion of leaves were analysed for leaf nitrogen content and none for the secondary metabolites thought to influence beetle host selection. The results from leaf N analysis of the present study suggest that concentrations within leaves have the potential to influence the severity of herbivore damage a plant experiences. With increasing leaf N, on average, more herbivory was observed. This suggests that higher concentrations of N increase the quality of tissue to herbivores.

While there is uncertainty within the literature regarding concentrations of plant secondary metabolites and herbivore host selection and consumption rates, the potential for 1, 8 Cineole to influence Chrysomelid beetle behaviour is relatively well supported. Hence, there is some suggestion that it could also influence herbivory in Snow Gums, although the underlying causes for variation in PSMs within and between sapling populations needs to also be quantified. Considering a significant portion of the damage observed in this study resulted from this group of herbivores, analysis of PSM concentrations may prove fruitful and an obvious candidate for better understanding the substantial variation observed here.

A further limitation was the short duration over which observations were made, i.e. a single growing season. The complexity of herbivore-host dynamics, and the potential for previous seasons damage and climate to influence both plant morphology and chemistry, suggest that a study spanning multiple seasons may help better understand the complex variation observed. Additionally, both studies involving ants are likely to benefit from a longer experimentation period. For ant and psyllid dynamics, a relationship well supported in the literature in the benefits to plants and temporal variation, it seems an accurate representation of the relationship and its outcome, requires documentation over numerous seasons. While for ant seed harvesters, longer seed exposure may reveal study design effects, as well as give a more accurate estimation of removal rates.

Additional benefits may lie in the documentation of herbivory by sap-suckering herbivores (Hemiptera) and analysis of fungal pathogens associated with saplings across different mountains and altitudes. As previous studies have suggested (Burdon & Chilvers 1974a,b), pathogen distributions are also influenced by altitude in this species, the distribution of pathogens on saplings as well as the role they may play in altering plant palatability should be considered for future research. Furthermore, hemiptera have the potential to significantly alter a plants fitness, thus while it is difficult to measure, estimates of such damage on snow gum saplings may also be beneficial.

Conclusion

At all scales studied, herbivory was detected. However, defoliation was rarely significant and unlikely to lead to sapling mortality. The variable nature of herbivory in Snow Gum at high altitudes suggests that some plants in the population are able to avoid damage and others not. What governs such differences between saplings is still unclear. While the results of this study suggest herbivores are responding to the environment directly, and indirectly through its influence on plant traits, at both landscape- and local-scales, there is still much that remains to be unravelled. In particular, the high levels of variation in herbivory within and between populations almost suggests that plant-herbivore interactions are random in nature. In order to gain a better understanding of the drivers of insect host selection and feeding, the unique behaviours and requirements of different insect groups needs to be studied, as does the chemical nature of plant tissues and their influence on host suitability.

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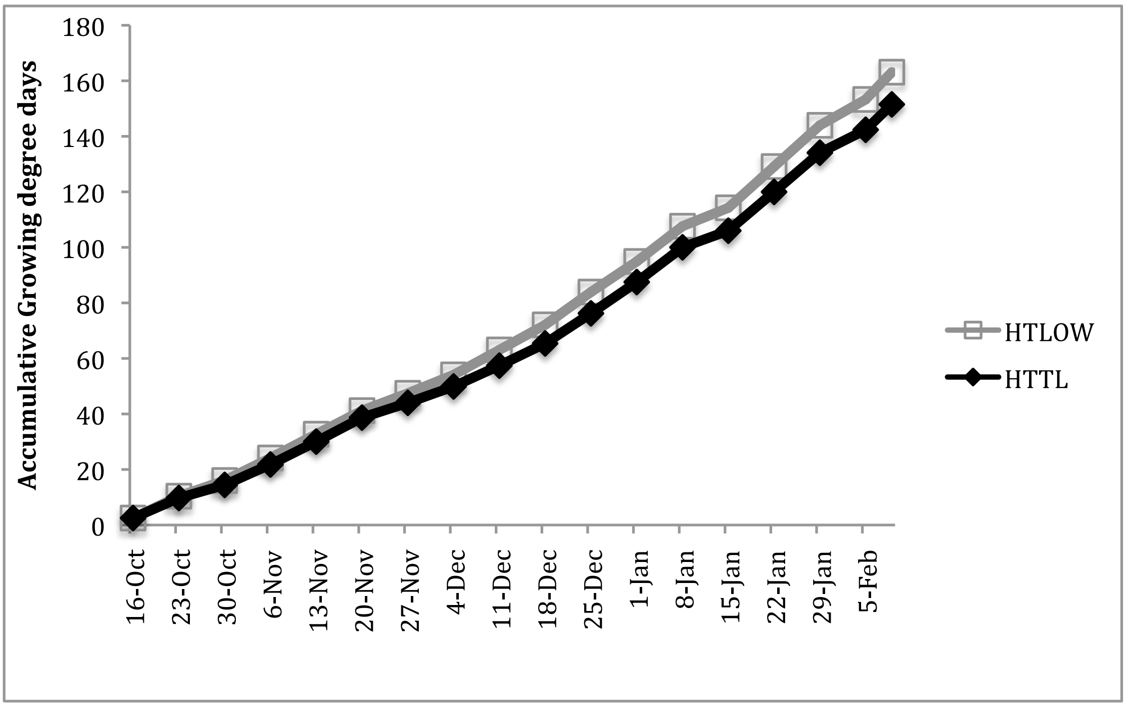
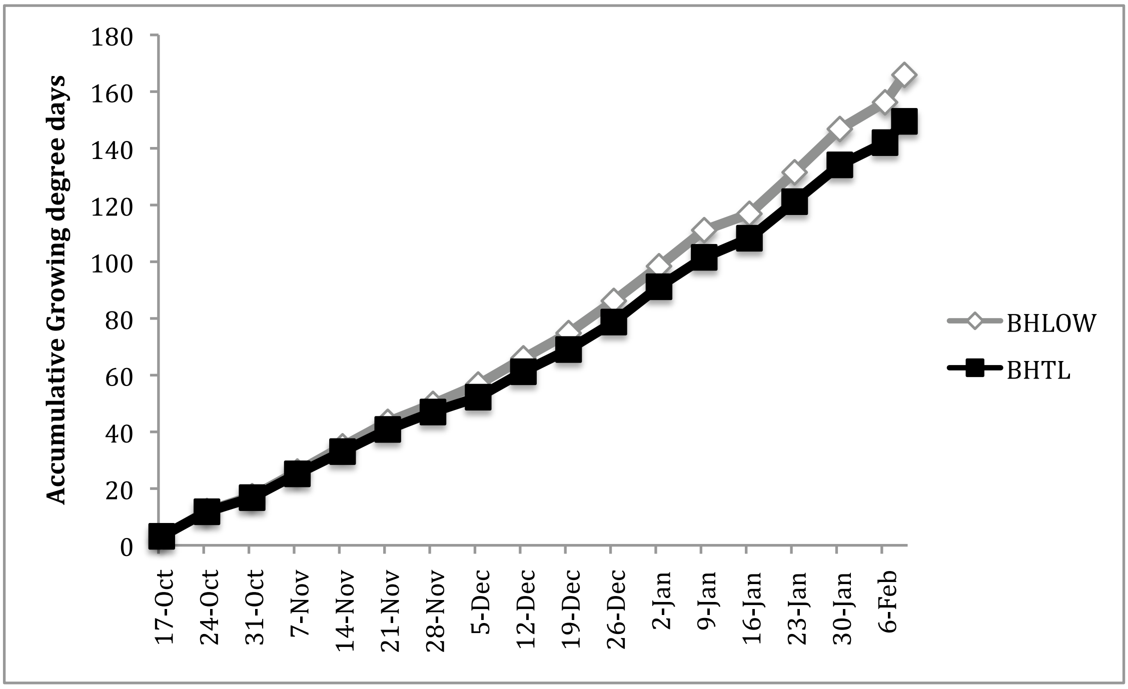
APPENDIX 1

a). Accumulative growing degree days for Mt Hotham and b), accumulative growing degree days for Mt Blowhard.

a)

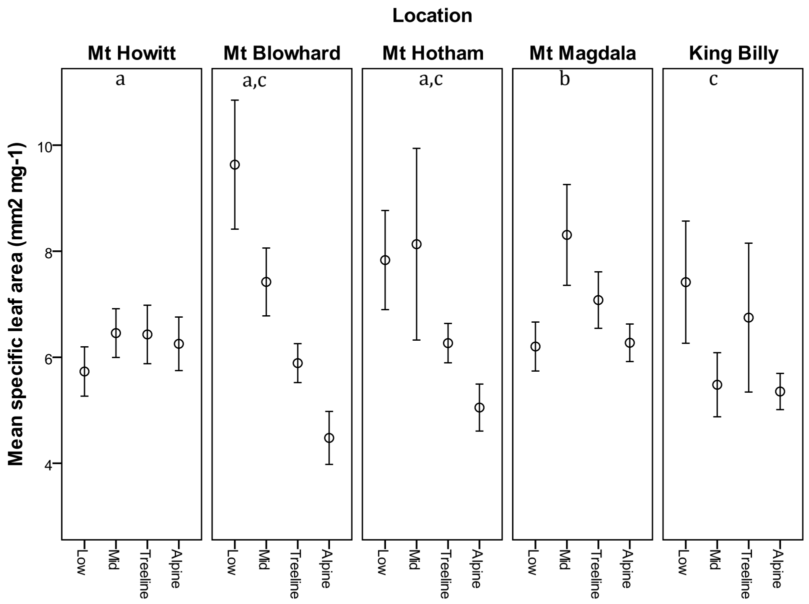


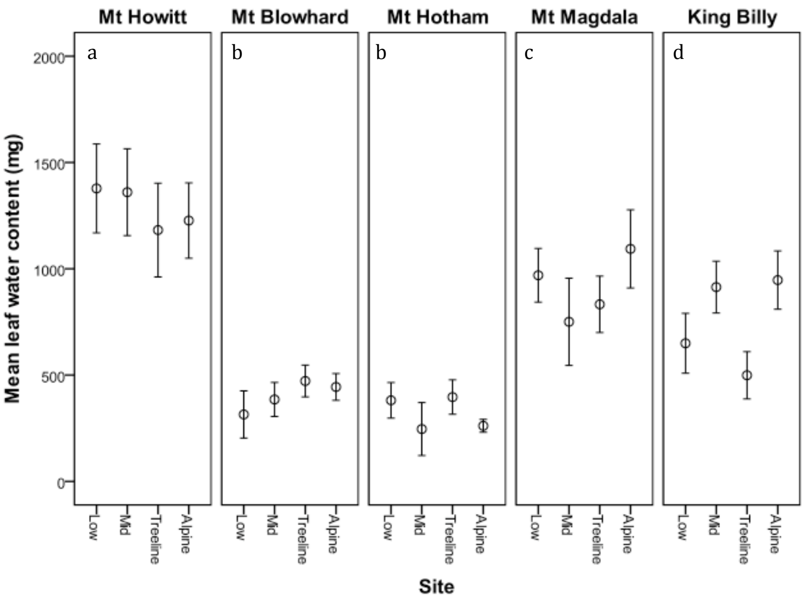
b)



APPENDIX 2

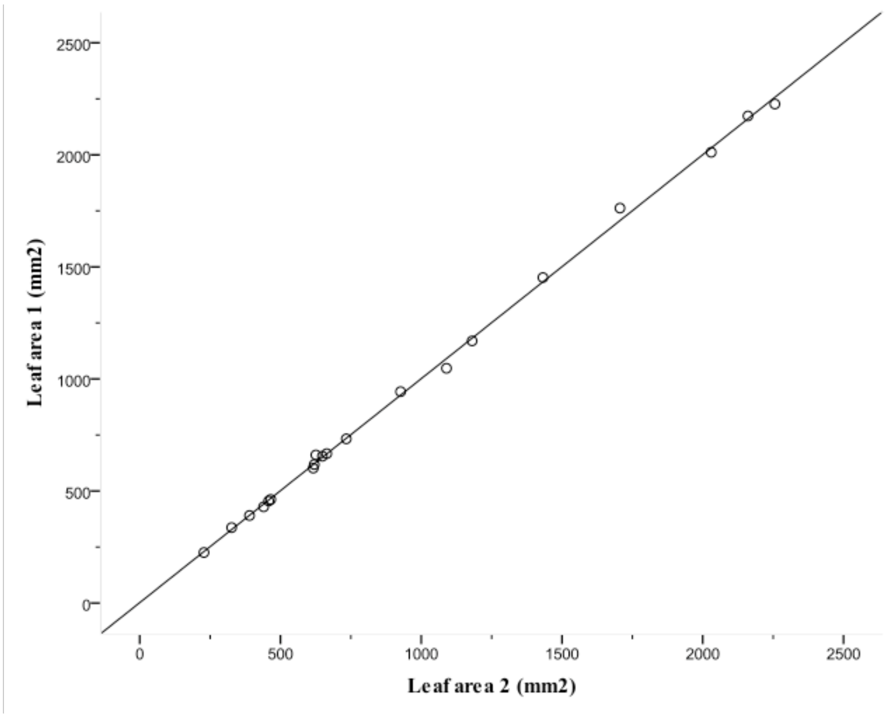
Relationship between leaf traits and altitude for each mountain





APPENDIX 3

To accurately calculate leaf area (mm^2), photographs of leaves were analysed using imaging software program ImageJ. Preparation of each photograph required the establishment of a scale, and at times alteration (erasure of shadow or darkening of over-exposed areas) for the program to measure accurately. Due to the sensitivity of the ImageJ software, slight variations in scale or alterations could lead to variations in area calculations. To investigate the potential error associated with this technique, twenty leaf photographs from the first set of field sampling were randomly selected, twice measured, and the leaf area calculations compared (see Figure 1). Results from a linear correlation show a 0.001% level of error occurred during this process. I can therefore be confident that through this technique leaf area comparisons will be accurate.



Linear correlation illustrating the relationship between pairs of leaf area measurements

APPENDIX 4

Average leaf area lost (%/day) for all mountains