The effects of honeybees (*Apis mellifera*) on the biodiversity of manuka (*Leptospermum scoparium*) patches

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ABSTRACT

Honeybees (*Apis mellifera*) are important pollinators of many plant species and are employed globally for crop and honey production. However, little is known about the effects of this species on native pollinator and plant species in areas to which they have been introduced; and previous research has not been able to reach a general consensus as to the type of impact honeybees have on pollination systems. In addition to the effects of exotic pollinators, the loss and fragmentation of natural habitats is also of major concern to the continuing diversity of pollinators and plant populations.

Here, the impact of honeybee density on other pollinator guilds, and levels of remaining pollen and nectar standing crop among 18 patches of the New Zealand native shrub – manuka (*Leptospermum scoparium*) is examined at three different regions within the North Island. The same sites were also used to test the reproductive capabilities of manuka and subsequent pollen limitation among patches. A further 11 sites were utilised to examine biodiversity via intercept and pitfall traps within manuka patches, and the patch variables driving taxa composition.

Large fly (Diptera ≥ 5 mm) abundance was negatively correlated with honeybee abundance and instances of physical disturbance of large flies by honeybees were observed. There was no significant correlation between honeybee abundance and other pollinator guilds. Nectar was a limiting resource for both honeybees and large flies, whereas, pollen was not a limiting resource among any of the major pollinating insect guilds. Pollination treatments revealed that manuka is partially self-compatible, but relies more heavily on cross pollination for higher yields of capsule and seed set. Pollen limitation did not occur significantly at any of the sites. A total of 159 Coleoptera, 125 Diptera, 131 Hymenoptera morphospecies, and 50 other groups of taxa from various orders were collected among sites. Invertebrate richness was higher at lower altitudes and litter invertebrate richness was significantly higher with an increase in the proportion of manuka cover. There were distinct differences in taxa composition between the three regions, with plant community composition and altitude the most significant factors. Patch size also played a part, but a lack of overall variation in patch sizes may understate the effect this has on insect composition.

Overall, honeybees are competing for nectar resources and displacing large flies as a consequence; however, capsule and seed set among manuka patches did not significantly suffer as a consequence. Regional variation in patch characteristics such as altitude, plant community composition, patch size, proportion manuka cover, and plant evenness appear to be influencing insect composition found within manuka patches to varying degrees. Further investigation into the impact of patch size and patch connectivity is also warranted.

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INTRODUCTION

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INTRODUCTION

1.1 The introduction of exotic insects, honeybees, and New Zealand pollinators

1.1.1 Introduction of exotic insect species

Introduced insects can have numerous affects on native biodiversity. They can directly impact via predation, parasitism, or through genetic hybridisation. They can also impact indirectly by transmitting diseases, competing for resources or nesting sites, by having mutual natural enemies, which can create cascading effects throughout the ecosystem (Kenis et al. 2009). Introduced insect pollinators, in particular, could have dynamic effects on ecosystems by driving changes in native and exotic plant fecundity (Goulson 2003), causing changes in plant community composition. Displacement or competition for habitat resources with resident native pollinators may also alter pollinator guild composition and abundance, and such changes could even place pressure on the reproductive strategies of native plant species (Ashman et al. 2004). Therefore, introduced species can have effects at the individual, community and ecosystem level.

1.1.2 Origin and global spread of Apis Mellifera

The honeybee (*Apis mellifera*) has had a long history as an important mediator in plant pollination and honey production. This species is thought to have originated in African, west Asian and southern European regions as early as the Eocene era (Michener 1979). Since then, they have been introduced to other areas and spread their range to nearly every country in the world (Goulson 2003). The European strain of honeybee was first introduced to New Zealand in 1839, when hives were brought over from the UK (Hopkins 1911).

1.1.3 General foraging ecology of honeybees

Like all species of bee, the primary diet of honeybees consists of nectar and pollen collected from various flowers; with the occasional supplementation of other plant products (Michener 1974). As a species, honeybees have been very successful in utilising new habitats as they are generalist foragers, easily adapting to variation in flower sources depending on availability (Goulson 2003). They have been observed to visit more than a hundred different species of plant in a single geographic region (Butz Huryn 1997). Research of honeybee visitation among New Zealand plants revealed they have been observed collecting nectar and/or pollen from 224 plant species from 12 different families (Butz Huryn 1995). As they display such generalised visitation, honeybees have the potential to interrupt unique specialised plant-pollinator syndromes. However, such specialised relationships are fairly rare in New Zealand pollination systems, with most native plants relying on a number of unspecialised indiscriminate native pollinators (Lloyd 1985).

1.1.4 New Zealand Pollinator fauna

Pollinators of New Zealand plants include many taxa: birds, bats, other mammals, and insects, with the latter, entomophily, being the most predominant pollinators (Newstrom & Robertson 2005). The main groups of insect pollinators within New Zealand include many species from the major orders; Hymenoptera (ants, bees, and wasps), Diptera (flies), Lepidoptera (butterflies and moths), Coleoptera (beetles), and the occasional insect from other orders; the biggest disparities in the New Zealand pollinator assemblages being the lack of endemic large, social, long tongued bees and a poor butterfly fauna compared to other countries.

Although, one of the more important insect guilds in terms of pollination, previous research has listed only 40 different species of bees within New Zealand (Donovan

2007), including the introduced honeybee. Among the other more advanced social bees that were introduced for agricultural purposes, are two species from the family Megachilidae, as well as further representatives from the family Apidae, which include four species of bumblebees, *Bombus* (three of which are long-tongued) (Macfarlane & Gurr 1995). However, the most well represented family that includes the majority of New Zealand's native species is the more primitive Colletidae. Within this family there are 18 endemic species of *Leioproctus* and 8 species of *Hylaeus*: 6 endemic, 2 introduced; and two other introduced species from the genus *Euryglossina* and *Hylaeoides*. From the more advanced semi-social family Halictidae, there are four endemic species of Lasioglossum, and an imported species from the genus *Nomia.* Compared to honeybees and the other large social introduced bees, our native bees are primarily solitary and short-tongued (Newstrom & Robertson 2005).

In contrast, New Zealand's Dipteran fauna is very abundant and diverse with just over 2000 species currently recorded (Crowe 2002; Macfarlane et al. 2009). Some are opportunistic nectar and pollen gatherers, but there are also some long-tongued flies which are more specialised for nectar feeding (Newstrom & Robertson 2005). Among the more well-known families within New Zealand are many species of nectar-feeding Tachinidae (c. 100 species) (Newstrom & Robertson 2005) and Tabanidae (c. 20 species) (Crowe, 2002); and pollen-collecting flies from the family Syrphidae (c. 40 species) (Holloway 1976; Hickman et al. 1995; Lehnebach & Robertson 2004). Representatives from other fly families, such as the Bibionidae and Calliphoridae have also been observed frequenting flowers within New Zealand (Primack 1983; Lehnebach & Robertson 2004; Newstrom-Lloyd et al. Unpublished data: See Newstrom & Robertson 2005).

New Zealand also possesses a rich moth fauna, approximately 1800 species, but has one of the poorest diversity of butterflies, with as few as 30 species (Dugdale 1988; Parkinson & Patrick 2000). Although little research has been conducted specifically on butterfly and moth pollination in New Zealand, many researchers have observed species of both butterflies and moths visiting the flowers of many plants within New Zealand (Primack 1983).

Instances of beetle pollination have been demonstrated in previous studies (Godley 1979; Primack 1983; Webb 1994), and some species within the family Mordellidae and Oedemeridae are known to frequently occur on the flowers of some New Zealand plants (Crowe 2002). However, much like the Lepidoptera, little research has been conducted on beetle pollination and their efficiency as pollinators within New Zealand.

A previous study on the pollinator assemblages within various montane and alpine regions in the South Island of New Zealand found that insect visitors to manuka (*Leptospermum scoparium*) flowers were highly variable, and included many native insects (Primack 1978).

1.1.5 The current status of honeybee abundance

In comparison to our native bees, which are primarily solitary, honeybees are social insects; colonies consist of up to 50,000 individuals (Goulson 2003). In regions such as South Australia, wild populations of introduced honeybees have estimated to reach densities of 50-150 nests/km^2 (Oldroyd et al. 1997). These feral population densities are heavily influenced by differences in habitat quality and the availability of adequate sites in which to establish nests (Goulson 2003). Previous estimates from the late seventies have approximated that 8000 tons of honey is collected from upwards of 227,000 hives commercially utilized annually in New Zealand (Donovan 1980). This showcases the amount of available nectar resources that *Apis mellifera* employ.

In recent years, wild populations of honeybees have drastically declined all over New Zealand. Little is known as to why this has occurred, but the varroa mite may have played a part by transmitting deleterious diseases (Shen et al. 2005). However, honey production remains a prominent industry across the globe, especially in New Zealand, with recent research praising the anti-bacterial effects of honey produced from the nectar of manuka (*Leptospermum scoparium*). Subsequently, in New Zealand, many commercially bred hives have been employed and beekeepers may place many hives (in one case 24 hives were observed in one 2km area) in close proximity to patches of manuka. As a result captive bred honeybees may reach in the number of millions in areas of peak flowering periods. Consequently, this could potentially be compromising the amount of available resources for native pollinators.

1.2 Literature Review

1.2.1 The effects of honeybees and other exotics on other pollinators/insects

If honeybees deplete a substantial amount of floral resources within a patch, the resources become limited to other pollinators. Resource limitation can lead to interference competition, in which an organism attempts to physically exclude others from the resources within that area. It can also result in exploitative competition, in which the depletion of available resources by one group causes the displacement or exclusion of others (Schowalter 2006). Therefore, there is a possibility that honeybees may compete with native pollinators for available resources. Honeybees are proposed to displace other bee species due to their large body size, but competition may not be exclusively size related, as honeybees have been found to disrupt bird pollinators foraging within the same patches (Paton 1993).

The majority of papers published on the effect of introduced honeybees on other insect pollinators focus on their impact on native bee species. Many studies have found that native bees appear to decrease in abundance with an increase in the density of honeybees among patches (Roubik 1978; Aizen & Feinsinger 1994; Gross 2001; Roubik & Wolda 2001; Kato & Kawakita 2004). Instances of interference competition have been recorded as observations of direct physical antagonism of native bees by honeybees (Cairns et al. 2005; Gross & Mackay 1998; Roubik 1980). Another study found that honeybees depleted significantly more nectar and pollen within patches than visitor assemblages comprised mainly of native insects (Mallick &

Driessen 2009). Conversely, Horskins and Turner (1999) found that honeybees rarely depleted resources to the detriment of other pollinators, suggesting other factors such as differences in primary resource requirements may influence the level of resource competition that occurs.

A previous review of studies on the direct and indirect effects of honeybees on native bees showed that many studies found conflicting impacts on native bee species (Paini 2004). However, the type of impact honeybees have on other pollinators may vary by competing species life history or the plant species and resources under scrutiny.

More recent publications have also been divided and hesitant to draw any strong conclusions of negative indirect impacts of honeybees on native insects (Kato & Kawakita 2004; Paini 2004; Mallick & Driessen 2009), as mere overlap of niche resources does not infer that honeybees may be having an effect on species population abundance. Nevertheless, many authors have described competition from exotic pollinators, whether direct or indirect, to be a driving force in the possible decline of native pollinators (Kearns et al. 1998; Goulson 2003; Kenis et al. 2009), perhaps second only to habitat disturbance (Goulson 2003).

1.2.2 The effects of honeybees and other exotics on plant reproduction

Attention has also turned to the impact of introduced bees on the pollination success of native plants. Research suggests that exotic bees could possibly reduce pollination or population structure of native plants by employing different methods and efficiency of pollen transfer compared to native pollinators (Butz Huryn 1997; Gross & Mackay 1998).

These concerns are reinforced by studies that have found honeybees to be less efficient pollinators of some species, as they display reduced stigmatic contact compared to other native pollinators (Paton 1993) and have been observed actively removing previously deposited pollen from flowers (Gross & Mackay 1998). They have also been recorded visiting fewer flowers on the same plant for longer periods than native pollinator’s thus promoting self-fertilisation (Gross & Mackay 1998; Klein et al. 2003; Chamberlain & Schlising 2008). Another study found honeybees had a preference for the male flowers of a dioecious plant, *Clusia arrudae,* and hence contributed very little transfer of pollen to stigmas (do Carmo et al. 2004). On the other hand, in generalised pollination systems, honeybees may be beneficial by adding to the biodiversity of pollinator assemblages and increase seed set, as honeybees are capable of carrying larger pollen loads than most other native pollinators (Newstrom & Robertson 2005).

In concordance, other research has found possible positive impacts of honeybees on plant pollination, as honeybees were much more frequent flower visitors compared to native pollinators (Suzuki 2003; Chamberlain & Schlising 2008) with less pollen limitation and higher fruit set occurring in patches of several plant species with higher honeybee density (Suzuki 2003; Gonzalez-Varo et al. 2009). Further publications concluded that there was no difference in plant fruit and seed set between patches high in honeybee density compared with those comprised of mainly native pollinator assemblage (Gross 2001; Dupont et al. 2004; Mallick & Driessen 2009).

These conflicting results have consequently produced strong debate as to whether honeybees are detrimental, beneficial, or merely an addition to pollinator assemblages. Generalisations of the type of effect on native plant populations may be futile however, as plant breeding system, flower size and/or shape, and existing biodiversity may provide a better predictor of the impact honeybees may have (Newstrom & Robertson 2005).

1.2.3 Effects of land use and habitat disturbance on pollinator biodiversity

Another factor at the forefront of ecological concern, are the effects of human disturbance on pollinator guilds. Destruction of natural and semi-natural habitats has been regarded as the primary cause of pollinator decline (Kearns et al. 1998, Goulson et al. 2008). Fragmentation or more generally, habitat loss of natural landscapes causes resident species to confront dynamic modification of their environment such as reduced area, increasing isolation, and novel ecological margins (Fahrig 2003).

A previous review of the effects of habitat fragmentation on invertebrate pollinators proposed the effects on biodiversity to be consistently deleterious (Rathcke & Jules 1993). However, more recent views have outlined the ambiguity in the proposition of such conclusions, as some studies have yielded positive effects among certain species but not others, and the extent of effects may vary depending on what feature of habitat changes are being measured (Debinski & Holt 2000; Fahrig 2003). Early research examining the effects of habitat fragmentation on pollinators focused on butterflies, partially due to the ease in which they are spotted and identified, among tropical forest systems (Dover & Settele 2009). Butterflies are highly influential pollinators within the tropics; however, outside of equatorial regions bees and flies begin to play a more important role as pollinators.

To date, research into the effects of habitat fragmentation on insect pollinators has risen vastly since original publications, especially in regards to other insect pollinators such as the Apoidea – bees. A meta-analysis was recently conducted on literature involving research on the effects of habitat destruction on many different species of bees. Results showed that habitat loss was the strongest factor influencing bee richness and abundance in the 54 studies analysed (Winfree et al. 2009). Many studies have also focused on the effects of fragmentation on certain species of Coleoptera (beetles) (Ewers et al. 2007; Ewers & Didham 2008; Niemela 2001; Nichols et al. 2007). Other research has suggested using specific taxa as indicators of sustainable landscape management (Pearce & Venier 2006; Michaels 2007; Maleque et al. 2009), but there is still a paucity of research delving into the influence habitat modification may have on other insect pollinators such as long-tongued fly species. Some recent articles have begun to investigate the effect of habitat fragmentation on specific fly species belonging to families Glossinidae, and Syrphidae (Ducheyne et al. 2009; Meyer et al. 2009). However, literature on the response of flies to habitat fragmentation is still lacking, the former fly family examined as a focus of pest control of this species and the latter fly family playing a role in broader pollinator ecosystems.

In addition, the particular aspects of habitat loss and the varying degrees of effects they have on different species are not yet clear.

As previously mentioned New Zealand has one of the lowest richness of butterfly species, and as a consequence, bees and flies, especially within alpine areas (Kearns 2001) are more important pollinators of the New Zealand flora (Primack 1983; Newstrom & Robertson 2005). Therefore, in order to examine the effects of habitat fragmentation on pollinator assemblages among more temperate regions, such as New Zealand, further research should include the influence which this phenomenon may have on other keystone guilds or species within more relevant pollinator orders like the Diptera. However, to understand the possible effects of fragmentation, and dynamic changes to surrounding landscape, the diversity which currently occurs among different habitats and the main factors that are driving differences in species diversity must first be established.

1.3 Manuka as a suitable system to study

Manuka (*Leptoseprmum scoparium*) is a native New Zealand plant that is still found in abundance across most of the country. Human disturbance, primarily in the form of habitat loss and culling of the species to make way for agricultural practices has resulted in the fragmentation of once continuous landscapes surrounded by other native forest. On the flip side, such human activity, especially through the agricultural clearing of climax vegetation, may provide ideal conditions for the reestablishment of this seral species. Manuka has also become a valuable species for commercial honey production. Recent studies have found that a key ingredient found in manuka honey – the enzyme methylglyoxal – has anti-bacterial effects (Mavric et al. 2008). This has made this plant species even more highly sought after for its honey products. Consequently, many hives are placed around the Tongariro National Park every year to utilise the numerous patches of manuka within the area. Manuka is known to attract many native pollinators and introduced bees (Butz Huryn 1995; Donovan 1980; Primack 1983), yet little research to date has examined the impact introduced bees may be having on manuka plant populations and the native pollinators that frequent patches of this species for resources.

A preliminary study of the effects of honey bees (*Apis mellifera*) on manuka and *Hebe stricta* around Tongariro National Park found that fly abundance and diversity was affected by the presence of honey bees (Murphy & Robertson 2000). Introduced bees may be antagonising other native bees and pollinators or exploiting resources to the point where it is no longer rewarding for them to visit the flowers.

However, little is known about the pollination requirements of manuka, and due to such findings further research into the influence of introduced honeybees on other insect pollinators and consequent manuka plant fitness is warranted. There are also aspects of the general biology of manuka that need further elucidation. Recent publications delve into the possible effects that introduced bees may have on native pollinator ecosystems in many countries across the globe (Paini 2004), but, little research has focused on the effects of introduced honeybees on exclusive native New Zealand ecosystems (Newstrom & Robertson 2005).

1.4 Research sites and objectives

The main aim of this study is to investigate the impact introduced honeybees have on other pollinator guilds and manuka plant reproduction, as well as examining the biodiversity present within manuka patches and the patch characteristics driving invertebrate assemblages.

Twenty of the sites chosen for this study were within the Tongariro National Park area. This region is rich in several basic vegetation types. Podocarp broadleaf forest is most predominant in Southern areas and at lower altitudes, whereas beech (*Nothofagus* spp.) forest and alpine vegetation become more common at higher altitudes. Western edges are dominated by red tussock grassland and beech forest. The eastern boundaries of the Desert Road are comprised mainly of kanuka (*Kunzea ericoides*) and manuka (*Leptospermum scoparium*) shrubs, mountain beech, and tussock grassland, whilst the northern margins are monopolised by large patches of manuka shrubland and mountain beech forest (Murphy & Robertson 2000). Other study sites were located within the Wairarapa region: three within the Alfredton area approximately 25 km east of Eketahuna which were primarily surrounded by pastureland, and two further South-West on the Northern outskirts of Masterton. These consisted primarily of pastureland, but retained a lot of remnant native forest vegetation. The final four sites were chosen from the Manawatu region. All but one site, which was quite isolated and surrounded by pastoral grass, had a surrounding vegetation mosaic similar to that found at the sites close to Masterton.

Chapter two examines which pollinator guilds are visiting manuka patches and the relative abundance of these flower visitors, as well as the difference in nectar and pollen standing crops in patches with a high or low presence of honeybees.

Chapter three evaluates the pollination requirements and dynamics of manuka; whether manuka is pollinator dependent or has the potential to self pollinate, and the level of pollen limitation between patches with a high or low density of visiting honeybees.

The overall biodiversity within and between manuka patches is covered in Chapter four, as well as the patch characteristics that may be influencing taxa diversity and composition.

2.

The impact of honeybee density on pollinator guild abundance and available resources

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2.1 Introduction

Concerns have been raised over the effect introduced insects have on existing pollinator communities (Donovan 1980; Butz Huryn 1997; Kearns et al. 1998; Goulson 2003). Introduced honeybees specifically have been highlighted, as these social pollinators have been introduced to almost every continent in the world (Goulson 2003) and are extremely important and widely employed for the production of honey products. Indeed, Eickwort & Ginsberg (1980) noted that there was strong evidence that foraging habits of native bees are influenced by the presence of honeybees. However, there is still much debate over the effect honeybees may have on other native pollinators. A study in French Guiana found that the addition of honeybee hives to flower patches visited by native meliponine bees resulted in the reduction of abundance of these native bees. When honeybees were removed from the same area, the native bees increased in abundance (Roubik 1978). Kato & Kawakita (2004) also raised concerns of the impact of introduced honeybees when examining plant-pollinator interactions in New Caledonia. Although bees were the most dominant pollinators among plant communities within this study, 91.5% of bee visitations recorded were by introduced honeybees. The authors therefore suggest that original plant-pollinator relationships have been altered to the point that native bees in New Caledonia may have become endangered and imply that introduced honeybees may play a role in this decline. However, the mechanisms behind such a decline are not clear.

Other studies have focused on more direct measurements of the impacts of introduced honeybees. In Australia, Gross & Mackay (1998) found that honeybees were, in fact, disrupting foraging native bees in 91% of their interactions among patches of Australian blue tongue (*Melastoma affine*). Other studies have also directly observed physical attacks by honeybees on stingless (Meliponinae) bees (Roubik 1980; Carins et al. 2005). In Central Europe, Steffan-Dewenter & Tscharntke (2000) examined bee populations among 15 grassland sites. They found no impact of honeybees on wild and native bees. The number of bee species and brood cells per grassland were not significantly correlated with density of honeybees. Interspecific competition for resources among honeybees also did not have a significant effect on the species richness or abundance of wild bees. In Panama, Roubik & Wolda (2001) collected bee species in two light traps 7 years prior to and 10 years after the introduction of honeybees. Despite instances of resource competition at flower patches and among colonies, there was no evidence of any substantial decrease in population numbers of 15 dominant native bee species. There was little change in native bee abundance among years, even with fluctuations in honeybee abundance. A recent study by Mallick & Driessen (2009) looked at the effect of hive honeybees among patches of Tasmanian leatherwood (*Eucryphia lucida*). They found that honeybees at apiary sites significantly depleted more nectar and pollen compared to control sites. They do note, however, that the variation in abundance of native insects among sites may mask any competitive effects.

In contrast, an Australian study conducted by Horskins & Turner (1999) showed that honeybees foraging among patches of *Eucalyptus costata* seldomly completely depleted available nectar resources. They found that honeybees began foraging earlier in the morning when temperatures were lower, with the majority of these early morning visits primarily involving pollen collection; they did not forage in large numbers until later in the day when native bee presence was higher. These foraging patterns allowed other pollinators access to the high nectar stores built-up overnight. Some studies have also been limited by a number of confounding factors obscuring their results. For example, Aizen and Feinsinger (1994) found that honeybees were more common in smaller patches of subtropical forest in Argentina, whereas native bees were more common in larger patches. Although these results could be construed as implying competitive influences, the authors did not draw any firm conclusions, as they found that patch size was a confounding factor. Gross (2001) found that visitation rates of honey bees and native bees on *Dillwynia juniperina* were negatively correlated, suggesting a negative impact of honeybees on native bees. The author noted, however, that no sound conclusion should be made without examining brood levels of native bees in response to honeybee competition. Examining the effects on subsequent brood sizes would provide stronger evidence of a negative impact of resource limitation as a consequence of competition.

Many studies have touched on the impact honeybees have on native bees and native insects as a collective, but little research has focused on the impact on other specific pollinator guilds. A previous survey of mountain flora pollination found that many species from all the main pollinator orders were found visiting manuka flowers. Among these were a large majority of native bee species, flies, many families of Lepidoptera, and mordellid beetles (Primack 1983). Conservation of manuka and the native pollinator assemblages that co-exist within these communities is of concern. Many hives, and consequently large numbers of introduced honeybees, are being employed for manuka honey production. However, the effects that honeybees may have on the original existing biota are not yet known. Murphy & Robertson (2000) examined the effect of the presence of honeybees on manuka (*Leptospermum scoparium*) and *Hebe stricta* within the Tongariro National Park. Their findings indicated that diptera abundance and diversity was strongly negatively correlated with the presence of honeybees.

The objectives of this chapter were to first record pollinator guilds visiting manuka flowers, and the direct effect of the density of introduced honeybees on these insect pollinators through behavioural observations; and second, to determine the indirect effects of honeybee density through resource competition of available nectar and pollen standing crops within manuka patches.

2.2 **Methods**

2.2.1 Sites and habitat variables

Eighteen study sites were chosen from three main regions; four from the Manawatu (A. Shilton, D. McIntyre, I. Satherley, J. Love), four from the Wairarapa (Campbell, Campbell 2, McKenzie North, McKenzie South), and ten from Tongariro (Bishop, Desert Road, Horopito/Railway, Kapoors Road, Ohakune, Ohakune 2, Papakai, Railway/Erua, Silica Rapids, and Sir Edmund Hilary Outdoor Pursuits Centre) (Appendix 1). These sites were also used to examine pollen requirements and limitation, and nectar and pollen utilisation. Patches at sites varied in elevation, size, vegetation (Appendix 2), and honeybee hive number and proximity. Patch size was recorded by walking the perimeter of each patch and using GPS to calculate the included area. A cover percentage was estimated for manuka as well as the other vascular plants within the patches. From this, ordinations were performed to investigate the association between plant composition within patches with those from the behavioural observation pollinator data, as well as plant richness and evenness. Honeybee number and density was not recorded, but the number of hives within a 2 km radius were noted.

2.2.2 Behavioural Observations

Early attempts were made to experimentally add a number of hives to isolated patches and compare these with control patches. However, this proved impracticable due to issues with sourcing, placing, and moving hives to other patches for comparison. As a result of time constraints, the study was limited to correlational examination.

Within each site, twenty plants along a “transect” (usually the edge of a manuka patch) were chosen and tagged with an individually-numbered plant label. Each transect within a site was sampled twice; once in the morning and once in the afternoon, with the exception of Campbell 2, which was only sampled in the morning.

Four sites (A. Shilton, I. Satherley, McKenzie North, and McKenzie South) were also surveyed on more than one day. Days that were mostly overcast or rainy were avoided. The morning observations began at approximately 10.00 am. Wind speed (m/s), temperature (°C), humidity (%), barometric pressure, and subjective measures of cloud cover (%) and rain intensity were recorded prior to and immediately after observations.

Each of the twenty tagged manuka plants was then visited sequentially and the type and number of insects present on flowers within a one-metre square area were recorded on observation sheets, as well as any obvious signs of agonistic behaviour between pollinator guilds. Insects were scored into the following categories: Honeybees (Hbees), bumblebees, native bees (Nbees), ichneumonids, wasps, tachinids, syrphids, bibionids, tabanids, large flies (large Diptera ≥ 5 mm which may include individuals from the other family categories), small flies (small Diptera ≤ 5 mm which may include individuals from the other family categories), manuka beetles, other Coleoptera, moths, butterflies, and Hemiptera (See Appendix 3). Where identifiable, other insects, especially wasps and large Diptera were identified and recorded to specific families. Any new or unidentifiable insects were collected with a net from plants where they occurred. These insects were placed into plastic containers and labelled by the site, date, time, and plant number.

Nectar and pollen samples were then collected immediately after each observation period. The same process was repeated in the afternoon, with observations beginning at approximately 1.30 pm. The entire area of each site was recorded using a GPS to get the total area of each patch and altitude. Other vegetation within and surrounding the manuka patches were also identified, recorded, and assigned a subjective percentage cover rating.

2.2.3 Insect Vouchers

Insect specimens that were collected in the field were placed into a freezer after every field-day session. They were then pinned, dried out, and stored, or placed in ethanol for later identification. Each specimen was at first sorted to family, then to morphospecies. Expert entomologists Ian Andrews and Barry Donovan, were then able to distinguish to genus, or sometimes species level for the dipterans and hymenopterans which made up the majority of insects caught.

2.2.4 Nectar Extraction

To store the nectar samples collected in the field, polystyrene blocks were placed into cardboard storage folders. The polystyrene blocks were divided and labelled into forty positions, twenty for morning and afternoon samples taken from each site. Chromatography paper was used to absorb the nectar. This was cut into wicks 10 mm x 20 mm, impaled on pins and placed in each labelled cell of the polystyrene in preparation for field collection. In the field one hermaphrodite flower, at half life, was picked from each of the twenty marked plants at each site. Absorption of the nectar was accomplished by using a technique published in Practical Pollination Biology (Dafni et al. 2005), where nectar is absorbed and dried onto filter paper wicks. The wicks are then re-dissolved and quantified using a sulphuric acid anthrone colormetric assay (McKenna & Thomson 1988). Preliminary trials were run in which wicks were rinsed three times. This showed that the majority of sugars present (~85%) were extracted from the first rinse. Therefore, subsequent samples were re-dissolved only once.

2.2.5 Pollen collection, extraction, and dilution

At each site, an open hermaphrodite flower at half life was randomly selected from each of twenty plants within each transect in the morning and again in the afternoon, and placed into plastic vials containing 2 ml saline solution.

In preliminary counts, the first rinse to remove pollen grains from anthers was shown to yield an average of 79.08% of pollen grains from each flower (Figure 2.1). Subsequent rinses only yielded a marginal amount of further pollen grains. Therefore, each pollen sample was vortexed only once for one minute to assist in the removal of pollen grains from the anthers. Once vortexed, the flower was removed from each sample vial and 8 ml of saline solution added to bring the volume to 10 mls.

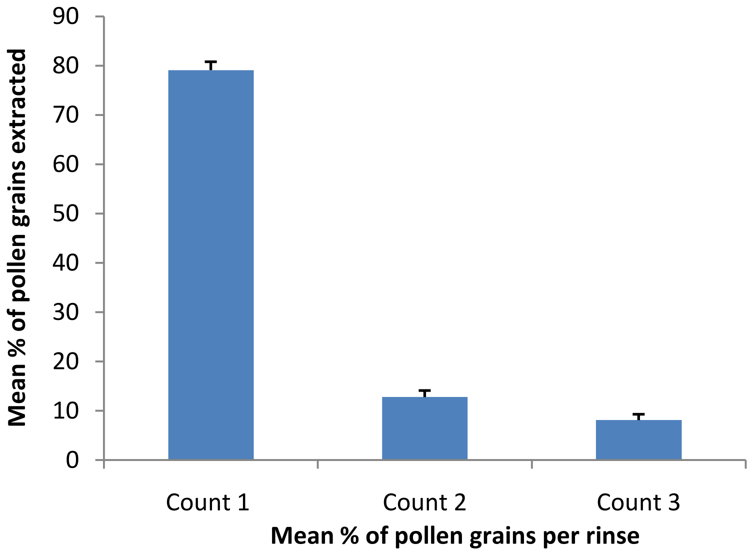

Figure 2.1: Mean and standard error of first, second, and third counts for trial pollen samples.

2.2.6 Pollen Counts

Each sample was then run through a CASY$^{(R)}$excell Version 2.2 particle counter machine, and 1.2 ml was extracted from each sample in three 400 µl increments. From this, the three rinses of pollen samples were averaged per ml and a graph of the range of pollen grains was produced. Preliminary trials and previous research (Moar 1993) placed the range of pollen grains of manuka between 8 and 23 µm. Any particles extracted below 8 µm were omitted as small dust particles. Any particles above 23 µm were omitted as other foreign contaminants.

2.2.7 Data Analysis

The average number of each insect guild per transect was calculated and $\log_{10}(x+1)$ transformed for each study site for comparison of relative abundance. Non-metric Multidimensional Scaling (NMS) ordinations were run to examine the relationship between site and honeybee density and the abundance of other insect guilds, morning and afternoon variance and the effect of patch variables on insect guilds across different sites, as well as to establish axis 1 and 2 values of the composition of plant taxa between sites. NMS ordinations were run using the “autopilot (slow and thorough)” mode in PC-ORD Version 5.0 with Sorenson distance as the dissimilarity measure. The Insect guild and plant taxa ordinations were finally configured with a 2-dimensional solution, according to stress values from the real and randomised (Monte Carlo test) original iterations. Honeybees were withheld from the ordination and included in the second matrix along with patch variables to examine community structure irrespective of honeybee density. The second matrix therefore included quantitative data of honeybee density, plant ordination axis 1 & 2 scores, manuka coverage (%), altitude, plant species richness and Simpson’s Index (1-$\mathbf{D} = \Sigma\, \mathbf{(n / N)^2}$ on plant cover values to assess evenness; categorical data were included for region, surrounding land use, and frequency of surrounding manuka. Surrounding land use was categorised into three groups: pastoral, native bush or tussock; dependent on the highest proportion of surrounding vegetation types directly around the patch. The proportion of surrounding manuka frequency was also categorised into three categories: sparse, moderate, and abundant. Insect guilds which yielded less than five visits over all sites and plant taxa that occurred only once were omitted from the ordination analyses. Critical values for the guild data were 0.549, based on 19 df and $p = 0.01$. Critical values for patch characteristics (second matrix) were 0.433, based on 19 df and $p = 0.05$. Mutlti-Reponse Permutation Procedures (MRPP’s) were employed to examine the significance of the categorical data: region, land use, and surrounding manuka frequency, on insect guild composition.

For the nectar data, regressions were fitted to the standard solution values for each run of the assays. This was to determine the slope required to calculate the level of sugar in each flower sample. Sugar content for each flower was then calculated. The effects of honeybees and large flies on nectar standing crop were analysed using the statistical programme R (version 2.9.0). $\mathrm{Log}_{10}(x+1)$ transformations were conducted on the mean numbers of these two guilds in each transect and used to run a multiple regression test in this programme. Excel was used to interpret correlation and regression scores between the abundance of honeybees and large flies as a function of the average nectar standing crop recorded at each site.

Pollen density values were calculated by multiplying the overall counts per ml of each sample by the original dilution (which in almost all cases was 10 ml). These were then averaged for each site. The effects of honeybees, native bees and syrphid flies (three primary pollen collectors) on pollen levels were analysed using R (version 2.9.0). $\text{Log}_{10}(x+1)$ transformations were conducted on the average numbers of these three insect guilds and used to run a multiple regression GLM with Gaussian error terms. Excel was used to interpret correlation and regression scores between the abundance of honeybees, native bees, and syrphid flies as a function of the average pollen standing crop recorded at each site.

2.3 **Results**

2.3.1 Behavioural Observations

All three regions varied in insect guild abundance, richness, diversity, and consequently remaining standing nectar crops. The Campbell site located in the Wairarapa had the highest number of honeybee visits. Ian Satherley site from the Manawatu and sites within the Tongariro National Park at the highest altitudes had the lowest number of honeybee visits. All Campbell and McKenzie sites from the Wairarapa, Bishops from Tongariro, and John Love from the Manawatu had the richest insect fauna, whereas Ian Satherley site had the lowest. Patches highest in insect diversity spanned across all three regions, however, again Ian Satherley site had the lowest insect Simpson’s Index. Patch size did not appear to have as much influence on these factors. Region and altitude had the largest impact on insect abundance, richness, and evenness (Table 2.1).

Table 2.1: Basic patch characteristics, total insect values, and average nectar and pollen levels at each site. *CampII site was only sampled once in the morning.

Site	Region	Altitude	Patch size (ha)	Honeybees	Bumblebees	Native bees	Tachinidae	Calliphoridae	Tabanidae	Syrphidae	Bibionidae	Large flies	Small flies	Insect Richness	Insect Simpsons Index	Ave nectar per flower	Ave pollen per flower
AShil_I	Manawatu	180	0.3	5	0	23	37	8	1	19	45	8	0	10	0.710	232±22	47022±2683
AShil_II	Manawatu	180	0.3	3	0	1	66	5	4	6	29	2	1	12	0.455	114±7	40924±2751
Bis	Tongariro	860	1	3	3	48	34	28	72	45	0	15	25	13	0.743	129±6	23239±1871
Camp	Wairarapa	312	0.3	56	7	38	2	6	0	33	3	3	3	13	0.659	311±21	48846±2920
CampII*	Wairarapa	310	0.4	20	1	17	1	5	4	9	0	1	0	12	0.683	293±28	53478±3253
DesRd	Tongariro	870	0.5	35	1	11	1	3	1	8	0	0	0	11	0.470	310±25	20349±1516
DMc	Manawatu	63	7.1	8	6	57	3	1	1	10	1	1	0	10	0.367	366±26	63106±3908
Ho_Ra	Tongariro	760	0.7	22	1	44	4	6	3	8	0	0	0	11	0.522	605±47	28293±1879
ISath_I	Manawatu	160	0.3	0	0	4	197	2	0	20	13	0	0	7	0.176	84±8	48793±3662
ISath_II	Manawatu	160	0.3	0	0	2	233	5	0	6	4	0	0	7	0.097	104±17	41935±2232
JLo	Manawatu	165	0.7	3	3	6	23	11	1	22	36	7	0	13	0.702	288±22	58191±2957
Kapo	Tongariro	780	0.2	2	2	66	6	17	51	34	0	6	0	9	0.643	163±10	24209±1172
McK_N	Wairarapa	195	0.9	27	1	41	2	2	1	8	0	3	0	13	0.578	332±28	65931±2705
McK_S	Wairarapa	180	1.2	27	0	32	5	0	1	11	0	4	1	12	0.509	434±36	48237±4209
Oha	Tongariro	1210	0.3	0	0	34	97	18	27	15	0	7	12	8	0.542	103±7	24612±1252
OhaII	Tongariro	1200	0.3	0	1	9	77	30	33	26	0	34	7	11	0.650	112±24	22482±1132
Papa	Tongariro	645	0.1	4	3	26	4	3	4	9	1	4	3	12	0.600	749±42	49943±3181
Ra_Er	Tongariro	820	0.5	24	1	29	4	14	18	21	0	1	0	10	0.746	473±39	29975±1872
SilRa	Tongariro	1270	0.2	0	0	7	30	15	2	10	1	4	17	11	0.691	226±16	23593±1095
SrEd	Tongariro	636	0.1	20	0	7	4	36	11	15	0	5	1	10	0.647	194±29	21748±1784

Overall, there was a strong negative correlation (r = -0.793, p = <0.001) between the number of honeybees and large flies occurring at each of the study sites (Figure 2.2). Sites with a higher density of honeybees tended to have lower large fly visitation.

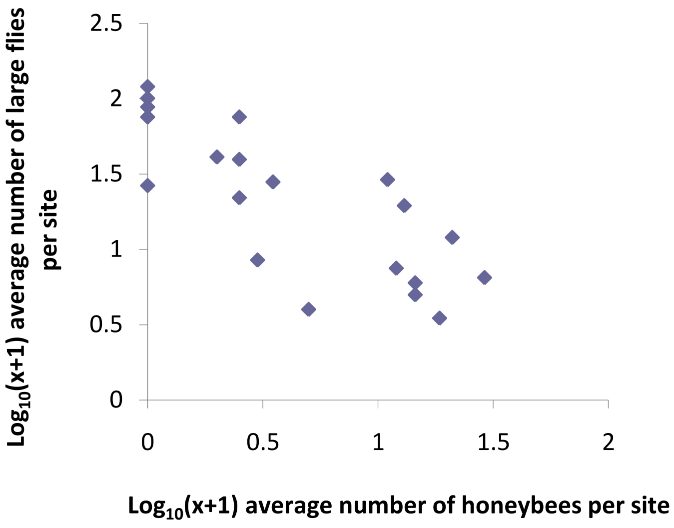

Figure 2.2: $Log_{10}(x+1)$ transformation of the average number of honeybees at each site plotted against the $log_{10}(x+1)$ transformed average number of large flies at each site.

For the categorical variables measured, insect guild abundance varied most strongly among different regions; however land use was also significant (Table 2.2). Therefore, region was used in the ordination graphs to display differences in guild composition. However, land use appears to also have an influence on guild composition.

Table 2.2: MRPP significance of categorical data for behavioural observation data. Significant values shown in bold.

	A	P
Region	**0.130**	**<0.001**
Land Use	**0.070**	**0.022**
Manuka freq.	0.024	0.107

Among the quantitative data, honeybees were the strongest factor influencing insect guild abundance (Table 2.3) and they were highly positively correlated with Axis 1 and highly negatively correlated with Axis 2 (Figure 2.3). Plant ordination axis 1 scores were the second strongest factor and were negatively correlated with Axis 2. Altitude was almost as significant as scores from plant ordination axis 1, but was positively correlated with Axis 2 (Table 2.3). Patch size was also a significant factor influencing insect guild composition (Table 2.3), positively correlated with Axis 1 and negatively correlated with Axis 2, similar to the honeybees.

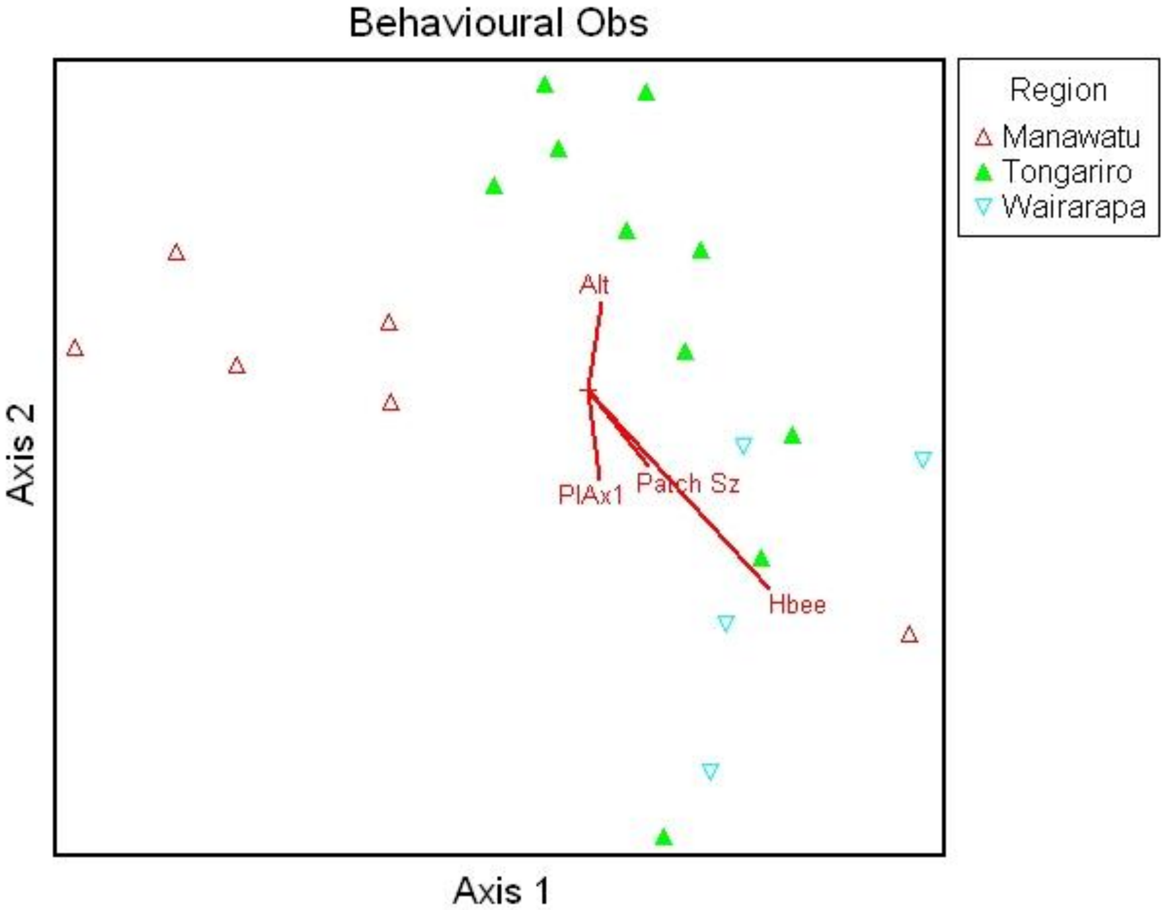

Figure 2.3: Two-dimensional ordination for insect guilds from behavioural observation data. Patch variables that correlated with either axes were overlaid using the Pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations but simply indicates the strongest associations. Hbee = Honeybees, PlAx1 = Plant ordination axis 1 scores, Alt = Altitude, and Patch Sz = Log_{10} patch size (ha).

Table 2.3: Patch variables influencing insect guild composition with the three regions. The strongest correlations are shown in bold.

Variable	Axis 1	Axis 2
Honeybee	**0.654**	**-0.687**
Altitude	0.173	**0.457**
Plant Ordination Axis 1	0.172	**-0.459**
Plant Ordination Axis 2	-0.183	-0.264
Patch size	**0.377**	**-0.426**
Prop.Manuka cover	-0.207	-0.166
Plant Richness	0.200	0.082
Plant Simpsons Index	0.132	0.234

Results from the insect guild ordination were significant (Monte Carlo p-value = 0.019) and the final stress value was 10.64. Bumblebee and native bee scores were significantly positively correlated with Axis 1 (Figure 2.3, Table 2.4), suggesting that these guilds were more commonly observed within patches that had a higher density of honeybees. The combined values of all the large flies (including the tachinids) and the tachinids alone were significantly negatively correlated with Axis 1 and positively correlated with Axis 2 (Figure 2.3, Table 2.4), showing that these guilds were most common at sites that were higher in altitude and where there were lower densities of honeybees. The bibionids were also negatively correlated with Axis 1 (Figure 2.3, Table 2.4), so were found at sites with lower visitation rates of honeybees. Individually, calliphorids, syrphids, tabanids, small flies, and the combined large flies exclusive of tachinids, were significantly positively correlated with Axis 2 (Figure 2.2, Table 2.4). Therefore, these guilds occurred more commonly at higher altitudes.

Table 2.4: Insect guild correlations on the 2 ordination axes for the behavioural observation data. Strongest correlations shown in bold. *Large flies refers to other large Diptera (>5mm) not identified to family. *All large flies refers to all Diptera over 5mm observed.

Insect Guild	Axis One	Axis Two
Bumble bees	**0.647**	-0.223
Native bees	**0.810**	-0.169
Ichneumonidae	0.379	-0.437
Wasp	-0.223	0.325
Tachinidae	**-0.855**	**0.647**
Calliphoridae	-0.070	**0.765**
Tabanidae	0.191	**0.604**
Syrphidae	0.162	**0.568**
Bibionidae	**-0.636**	0.107
Large flies*	0.083	**0.579**
All Large flies*	**-0.721**	**0.869**
Small flies	0.066	**0.598**
Manuka beetle	-0.410	-0.358
Beetles	-0.020	0.041
Butterflies	-0.022	-0.416
Hemiptera	0.244	0.240

2.3.2 Nectar and pollen standing crops

P values from the nectar data show that there was a significant difference between the remaining level of nectar and large fly abundance. However, there appeared to be no significant difference between nectar levels and honeybee abundance, or between honeybees and large flies combined and nectar standing crop (Table 2.5).

Table 2.5: Multiple regressions of nectar levels compared to honeybees ($\log_{10}(x+1)$) and large flies ($\log_{10}(x+1)$). Significant values shown in bold.

	df	Deviance	F	P
Large flies	**1**	**310950**	**20.119**	**<0.001**
Honeybees	1	4440	0.287	0.599
Large flies*Honeybees	1	41196	2.665	0.122
Residual	16	247295		

Accordingly, there was a high negative correlation (r = -0.718, p = <0.001) between large fly abundance and nectar levels at each study site (Figure 2.4). An increase in the number of large flies resulted in a significant decrease in the level of remaining nectar standing crop.

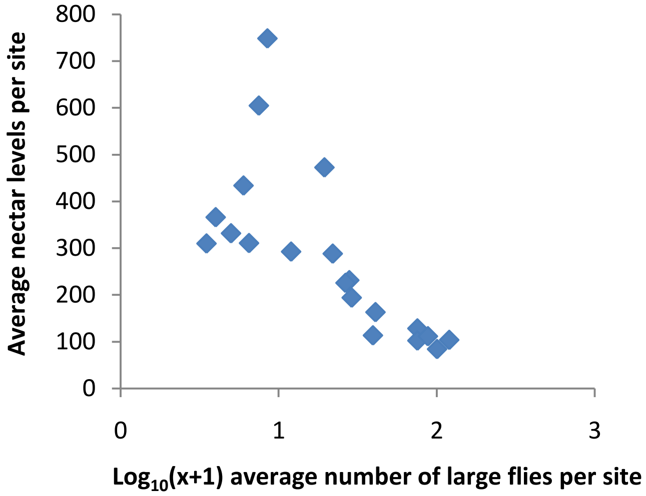

Figure 2.4: $Log_{10}(x+1)$ transformation of the average number of large flies at each site as a function of the average nectar standing crop at each site.

In contrast, there was a moderate positive correlation (r = 0.517, p = 0.020) between honeybee abundance and nectar standing crop at each study site (Figure 2.5). An increase in the number of honeybees results in an increase in the remaining level of nectar standing crop.

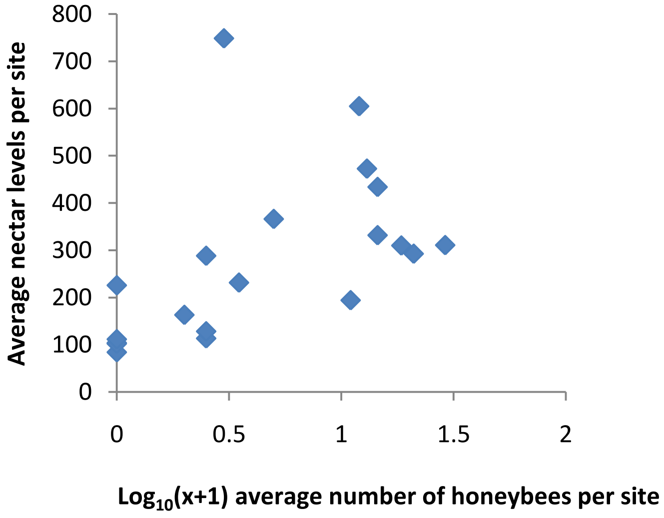

Figure 2.5: $\text{Log}_{10}(x+1)$ transformation of the average number of honeybees at each site as a function of the average nectar standing crop at each site.

However, when combining honeybee and large fly abundance in comparison with nectar levels at each study site there was a strong negative correlation ($r = -0.784$, $p = <0.001$) (Figure 2.6). Therefore, if honeybees and large flies were to commonly occur within the same patches, nectar standing crop would be even lower.

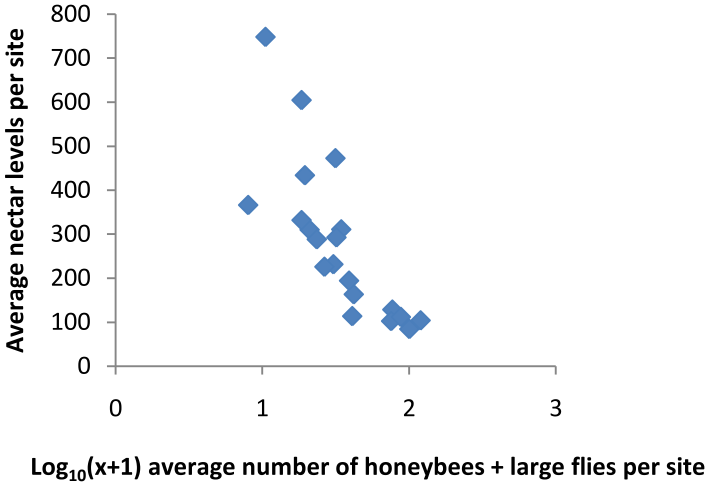

Figure 2.6: Transposition of average honeybees and large flies according to remaining nectar standing crop.

P values from the pollen data show that there was no significant interaction between pollen standing crop and the abundance of any of the pollen-collecting insect guilds (Table 2.6). As predicted in Table 2.6, there is a relatively low correlation (r = 0.224, p = 0.342) between honeybee abundance and site pollen standing crop (Figure 2.7).

Table 2.6: Multiple regression of pollen levels comparative to honeybees ($\log_{10}(x+1)$), native bees ($\log_{10}(x+1)$), and syrphid flies ($\log_{10}(x+1)$) at each site, as well as morning and afternoon sampling variance.

	df	Deviance	F	P
Honeybees	1	379987156	1.364	0.255
Nativebees	1	10876147	0.039	0.845
Syrphidae	1	271251232	0.974	0.334
Am & Pm	1	45878734	0.165	0.689
Honeybees*Nativebees	1	572255772	2.054	0.165
Honeybees*Syrphidae	1	52378590	0.188	0.669
Nativebees*Syrphidae	1	120896793	0.434	0.517
Honeybees*Am_Pm	1	108262	<0.001	0.984
Nativebees*Am_Pm	1	1390334188	0.499	0.487
Syrphidae*Am_Pm	1	254848092	0.915	0.349
Honeybees*Native bees*Syrphidae	1	319850960	1.148	0.295
Honeybees*Nativebees*Am_Pm	1	421228467	1.512	0.231
Honeybees*Syrphidae*Am_Pm	1	80665152	0.290	0.596
Nativebees*Syrphidae*Am_Pm	1	1894381	0.007	0.935
Honeybees*Nativebees*Syrphidae*Am_Pm	1	488046063	1.752	0.199
Residual	23	6406708379		

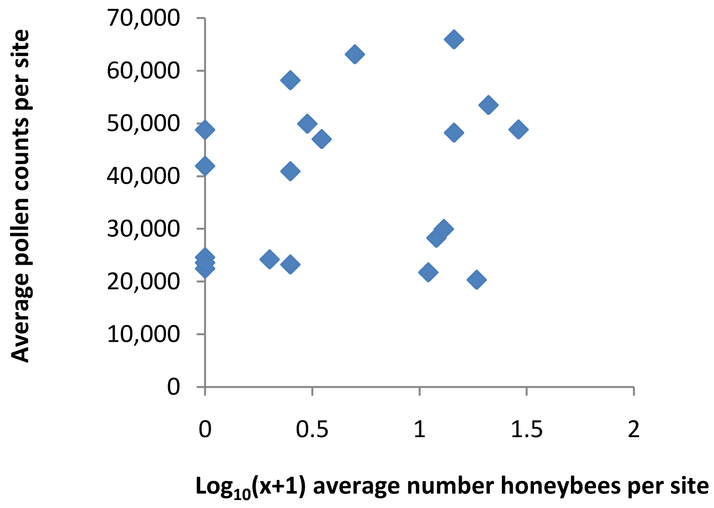

Figure 2. 7: $Log_{10}(x+1)$ transformation of the average number of honeybees as a function of the average pollen standing crop at each site.

2.4 Discussion

In concordance with Primack's (1983) mountain flora survey, many of the same insect species, especially the native bees, were observed visiting manuka flowers in the current study, plus some additional species were observed (Appendix 3). The main discrepancies from the previous survey were fewer observations of Lepidoptera taxa and the addition of catalogued fly species visitors. However, considering Primack's survey sampled sites among the South Island montane ranges, differences among species and taxa composition may be expected in comparison to montane and lower altitude sites within the North Island that were sampled in the present study.

Contrary to previous findings, no physical interference of native bees by honeybees was observed (Gross & Mackay 1998; Cairns et al. 2005); however, a couple of instances of aggressive encounters between honeybees and large flies were witnessed. Density of honeybees did not appear to affect the abundance of native bees within patches. In fact, native bees and bumble bees were more commonly found among patches in which higher densities of honeybees were present. On the other hand, there was a relatively strong negative correlation between the abundance of honeybees and large flies occurring within sites. No other significant relationships between the other insect guilds and honeybee density were apparent. Therefore, the presence of honeybees appeared to have a negative effect on the presence of large flies within patches, but appeared to have less impact on the abundance of other insect guilds. However, as the data was correlational, this negative relationship between honeybees and large flies could be due to a combination of factors as displayed in Figures 2.8 & 2.9.

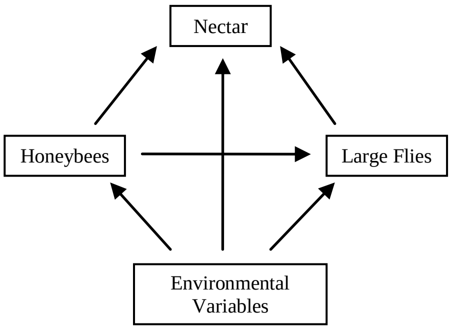

Figure 2.8: Diagram of the relationships that may influence nectar levels.

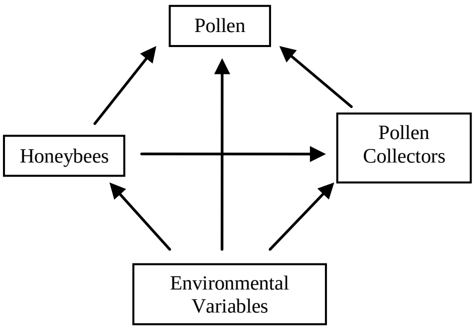

Figure 2.9: Diagram of the relationships that may influence pollen levels.

An early attempt was made to experimentally manipulate the density of honeybees within the manuka patches. Patches of manuka which were isolated and at least two kilometres from any commercial hives or other large patches of manuka were initially sought out. A number of hives were to be placed in half the patches whilst the other half remained a control. The idea was to then move the hives after a week to the control patches. These early experiments did not work out for a number of reasons. Firstly, it was difficult to source the number of hives required for the chosen patch sizes, so patches were not adequately saturated with honeybees for comparison. Secondly, when hives were moved to a different patch (at least 2 km away), the honeybees were still visiting the previous and close-by smaller patches. As the field work season for this study was limited, the decision was made to compare sites with differing proximity to commercial hives, number of hives, and relative abundance of honeybees rather than moving hives. Therefore, further investigation was warranted to try to elucidate which particular patch variables had the strongest effect on large fly presence as this may have had a stronger influence on fly abundance than honeybee density.

Other factors at each site were examined against all insect guilds including the large flies. These included quantitative measures of axis 1 & 2 of the ordination of plant species composition, altitude, patch size, plant species richness and evenness (Simpson's Index); as well as categorical variables such as region, surrounding land use, and frequency of surrounding manuka. The results of the ordination showed that honeybees were indeed the strongest indicator of variation among insect guild composition, followed by plant ordination axis 1 scores; altitude and log patch size respectively. All other factors provided only a negligible contribution. More specifically, honeybees had little effect on the abundance of native bees and other insect guilds, but did appear to be affecting the abundance and distribution of large flies, especially the tachinids. This was consistent with previous findings by Murphy & Robertson (2000) of variance in the abundance of large flies with the presence of honeybees.

Plant composition associated with plant ordination axis 1 scores was the second strongest indicator of the difference in taxa assemblage, followed closely by altitude, although these vectors were strongly contrasted. Plant compositions associated with plant ordination axis 1 scores were found more commonly at lower altitudes. The guilds that occurred most frequently within these patches include the ichneumonid wasps, butterflies, and manuka beetles. These may have been more greatly influenced by plant composition within these patches or lower altitudes. Pollinators more likely to be found at higher altitudes included many of the large flies. It must be noted, however, that the effects of altitude on the variation of insect guilds is hard to disentangle from region, as sites within Tongariro were all higher in elevation than sites from the Wairarapa and Manawatu.

Patch size also appears to have had an influence and was highly correlated with honeybee density. Higher density of honeybees appeared to occur among larger patches, which was very different from previous study findings where honeybee visits were more frequent in smaller compared to large patches (Aizen & Feinsinger 1994; Gonzalez-Varo et al. 2009). However, compared to the previous studies there is less average range size differences between the current study sites. Therefore, it would be unreliable to draw any strong inferences from this trend without further replication with more patch size variation. In addition, the presence of nearby manuka patches may also confound this result, although surrounding manuka frequency was accounted for.

When examining the nectar data, there were no significant differences in the abundance of honeybees within a patch and the amount of remaining nectar. Therefore, consistent with Horskins & Turners (1999) findings, honeybees did not completely deplete available nectar resources. With the exception of large flies, the abundance of other insect guilds did not show a strong relationship with remaining nectar levels. There was a significant negative correlation between large fly abundance and remaining nectar levels, suggesting that large flies, where they occur in high densities, are efficient consumers of nectar resources. When comparing both honeybees and large flies, the results were not significant, as these two guilds rarely occurred together in large numbers. However, when plotted together , there appears to be a distinct trend. If honeybees and large flies were to co-occur within the same patches, we get the best prediction of levels of nectar standing crop. Therefore, nectar appears to be a limiting resource for both of these guilds. During the behavioural observations there were never very high numbers of honeybees recorded within a patch. Where hives were placed, beekeepers appeared to be rather conservative in the number of hives put within an area. This would be an advantage in trying to maximize the yield of honey produced. Both too many and too few hives produces an inefficient use of the hive, which results in uneconomic nectar utilisation. This would be an advantage in respect to other nectar gatherers, as modest levels of nectar are left behind to collect. However, patches where a high abundance of large flies were recorded, the nectar resources were significantly depleted.

When examining pollen levels between sites, there were distinct differences, but they were not predictable for any of the variables that were recorded. There was a very low correlation between the average number of honeybees present per site and pollen levels. When comparing the major pollen collector guilds, which included honeybees, native bees and syrphids, there was also no significant difference. Honeybees and other pollen gatherers rarely appeared to be depleting pollen resources to insufficient levels within patches, which suggest little competition for pollen as a resource. However, as fieldwork for this study was conducted over a limited summer period, changes in food resource requirements may vary for each of these guilds as temperatures or life cycle stages differ.

Overall, the results showed that there appears to be a strong relationship between the abundance of honeybees and large flies. These two pollinator guilds are primarily interested in the collection of nectar, so it is not surprising that there was also a strong correlation between large flies and nectar consumption. As honeybees did not reach as high a density as those recorded for the large flies, the lack of a strong relationship between honeybee density and nectar levels was not unexpected. Results from the pollen data did not reveal any significant trends, suggesting pollen provides at best only a weak competitive drive between guilds.

3.

Pollination capabilities and pollen limitation among patches of manuka (*Leptospermum scoparium*)

3. Pollination capabilities and pollen limitation among patches of manuka (*Leptospermum scoparium*)

3.1 Introduction

Previous research on the effects of introduced insects on pollination processes has primarily focused on the effect of exotic insects such as honeybees - both directly or indirectly through resource competition (Paini 2004). The effects of honeybees on plant pollination and reproduction are just as important and have also been strongly debated (Kenis et al. 2009). Honeybees may potentially displace other pollinators, forcing them to go to other patches, but what may be less obvious are the direct and indirect effects that they have on the pollination process, seed set, and consequently plant fitness.

Long-tongued bees such as honeybees tend to carry very large pollen loads (Newstrom & Robertson 2005), which could supplement the amount of pollen available to receptive stigmas, thus aiding fertilization and leading to an increase in seed set production among native plants. In contrast, honeybees have been found at times to be inefficient in regards to pollen deposition. When pollen is the primary target resource, honeybees are capable of collecting and carrying large loads, but very little may be transferred to receptive stigmas (Wilson & Thomson 1991). Several publications have examined the level of pollen limitation and/or seed production within plant populations in which exotic honeybees have been introduced. However, there is little agreement as to the nature of the effect these introduced bees have on plant fitness.

Some studies have found negative effects on pollination service and consequent seed set. Paton (1993) found honeybees to be less efficient pollinators of *Callistemom* *rugulosus* (a relatively self-incompatible shrub native to Australia) than the nectar-feeding honeyeaters. Honeybees that were nectar-harvesting only made contact with the stigma of flowers of this species in 4.4% of floral visits. Pollen-harvesting honeybees made stigmal contact more frequently, 16.7% of the time, whereas larger honeyeater birds made contact 50% of the time. In this case honeybees were providing a lower quality of pollination service than the honeyeaters that they displace. Another study observed honeybees actively removing previously deposited pollen from stigmas of another Australian plant *Melastoma affine* (Gross & Mackay 1998). The results from that study showed that overall seed set was significantly lower in flowers visited by honeybees than those to which native bees were the last to visit. Similarly, Klein et al. (2003) found that social bees visited fewer flowers per plant and remained on each flower for a longer period than solitary bees, thus promoting self pollination which may result in lower seed/and or fruit production. do Carmo et al. (2004) found that pollen- harvesting honeybees affected the fitness of a dioecious plant, *Clusia arrudae*, by removing almost all of the pollen from male flowers, very rarely visting the female flowers, thus having a possible detrimental effect on fruit and seed set within this species.

In contrast, other research has highlighted the positive impacts honeybees have on plant reproduction. Suzuki (2003) investigated the effect of pollinators on the reproductive success of pollinator-dependent Scotch broom, *Cytisus scoparius*. Here, honeybees appear to be important pollinators of this species. They were the most frequent visitors, and fruit set from a single visit from a honeybee was similar to fruit set under natural conditions. Chamberlain & Schlising (2008) examined the effectiveness of various pollinators on patches of the native Californian plant, *Triteleia laxa*. They suggest honeybees are less efficient pollinators qualitatively, as they visit fewer flowers per minute. However, they argue that honeybees are still important pollinators as they visit flowers far more frequently than other pollinators. Gonzalez-Varo et al. (2009) hold a similar view. Examining varying patches of *Myrtus communis*, they found honeybees to be important contributors to fruit set. Within their study, small populations of this plant species were less diverse in pollinator assemblage and there was a higher density of introduced honeybees in small patches compared to larger patches. They found that the smaller patches which were dominated by honeybees were less pollen-limited than the larger patches.

Conversely, other studies have found little evidence of any impact of honeybees on plant reproduction and/or fitness. Gross (2001) studied the effects of introduced honeybees on populations of *Dillwynia juniperina.* This species, like the plant species discussed in the previous paragraph, are reliant on pollination by bees. Results showed no significant occurrence of pollen limitation among populations in which honeybees were present in high densities compared with those in which low numbers were present. Therefore, Gross (2001) concluded that there was no measureable effect on fruit set. Dupont et al. (2004) also found no significant difference on fruit set between populations of *Echium wildpretti* comprised predominantely of native pollinators and those dominated by honeybees. A recent publication also found little impact of the presence of honeybee apiaries on pollination of patches of *Eucryphia lucida* (Tasmanian leatherwood) in Tasmania (Mallick & Driessen 2009). Several sites were sampled, some in close proximity to commercial apiaries and control sites a minimum of 2 km from the nearest apiary. No difference was found in the total number of pollen grains deposited on stigmas or percentage seed set between the apiary and control sites. These conflicting opinions show that whether the effect of introduced honeybees is beneficial or detrimental to plant fitness is still in debate, and may vary across plant species and habitats. Such contrasting results could be due to variation in pollen deposition. Nectar-collecting honeybees are more likely to deposit a higher amount of pollen than pollen-collecting honeybees (Wilson & Thomson 1991). Time of life cycle or other environmental cues impacting honeybees' primary resource collecting focus, may influence the level of pollination success these bees deliver. Hence, previous research also shows that impact on seed set may rely on the aspect of the pollination process being measured and the reproductive biology and/or morphology of the plant species being examined. Honeybees may have a very different effect on plants that produce a large majority of hermaphroditic flowers than those in which both sexes occur and male flowers are being targeted for pollen. The level of pollen deposition may be dependent on the morphology of flowers of a species in comparison to the morphology of the pollinator of interest.

Each year dozens of hives are placed within areas containing large patches of manuka (*Leptospermum scoparium*). There is, however, little assessment as to the kind of effects the introduction of these exotic insects may have specifically on the reproductive success of manuka. Primack & Lloyd (1980) studied the floral morphology of manuka. They found that manuka is andromonoecious, in which hermaphrodite and male flowers occur on the same plant (Primack & Lloyd 1980), but little is known about its pollination requirements. Burrell (1965) noted that bagged manuka flowers excluding insect visitors still managed to produce capsules. Primack & Lloyd (1980) also postulate that manuka is most likely to be self-fertile and that further research to establish this needed to be undertaken. More recent studies have found that many Australian species of *Leptospermum* have the ability to self-pollinate (O'Brien & Calder 1993; Harris 2000), but little research has focused solely on the pollination capabilities of *Leptospermum scoparium.* Therefore, it has not been established whether manuka is completely pollinator dependent, self-compatible or capable of autonomous self fertilization. Newstrom & Robertson (2005) emphasise the difference between these systems. Most plants rely on pollinators for cross-pollination (fertilization of flowers with pollen from different individuals). Species like these are said to be pollinator dependent. However, plants that are pollinator dependent may or may not be self-compatible and/or capable of autonomous selfing. Plants that are self compatible are able to be fertilized by pollen from flowers of the same individual, whereas some plants that are monoecious, in which male and female reproductive parts are present on the same flower, are capable of being able to self-fertilise with pollen to stigma contact from the same flower.

The objectives of this chapter were to examine the pollination requirements of manuka and assess the levels of pollen limitation among patches close to commercial hives with those of manuka patches isolated from commercial hives in which there is relatively little presence of honeybees.

3.2 Methods

3.2.1 *Pollination requirements*

Six of the observation study sites were originally chosen to receive pollination treatments to determine pollination requirements and breeding system of manuka (Don McIntyre, Ian Satherley, John Love, Ohakune, Sir Edmund Hilary, and Campbells). At each of these sites, six plants were randomly selected and tagged with a plant identification tag to examine the pollination potential of manuka. On each of the six plants I assigned a randomly selected flower-rich branch for the treatments. White mesh bags made of curtain netting were then placed over treatment branches to exlude pollinators. The bags were removed and the branches were checked periodically to determine when the flowers began to open. This was to ensure that the flowers that had been tagged were hermaphroditic and not male flowers. As a consequence, twelve flowers, four of each of three treatments were ultimately used on each plant. The three treatments were as follows: to examine the capacity of manuka for autonomous selfing, four unopened flowers were tagged with bright green wire which received no pollination treatment and pollinators were excluded by the presence of the mesh bags (hence referred to as “Bagged”). To determine self compatibility, another four flowers were tagged with blue wire which received pollen from the same plant (“Selfed”). This was achieved by randomly selecting flowers from the same plant, then choosing and removing a pollen rich anther from one of the flowers using forceps and rubbing the tip of the anther on the stigma of the recipient flowers. The last four flowers were tagged with red wire that received a third treatment, to get an idea of potential levels of seed following outcrossing. These flowers were hand pollinated as above, but using an anther from another plant within the same patch (“Crossed”). The branches were then re-covered with the white mesh bags to exclude pollinators from visiting the flowers as they opened. Following treatment, the bags were left covering each of the treatments on the selected branches. The branches were then periodically checked for capsule formation and then collected. Unfortunately the bagged treatments from two sites were so badly damaged by cattle and bad weather that only data from four sites were used for analysis.

3.2.2 Pollen Limitation

At all 17 observation sites, six plants were randomly selected and tagged to receive two additional pollination treatments. On these plants, eight hermaphroditic flowers at half life were selected and assigned to be either left unbagged for pollinators to be able to visit - “Natural” control, or were also left unbagged but given supplemental pollen from another plant – “Supplemental” treatment. The difference in seed set by these two treatments is a measure of pollen limitation – the loss of seed production due to limited pollen supply.

3.2.3 Capsule and Seed set

Five manuka plants on the Massey University Turitea, Palmerston North campus, were tagged as an indicator as to when seeds may begin to shed. Unfortunately, these plants were not good indicators and had not begun to shed seed round the time that plants from experimental sites had. Capsules were collected mid-winter and as a consequence of these location differences in seed shedding, some of the seeds had begun to shed prior to collection.

Some capsules, especially for the bagged treatments had been aborted. Therefore, only data from four of the pollination treatment sites could be analysed. Back in the lab, each capsule was opened to reveal two of the five locules present in a manuka capsule. The numbers of viable and inviable seeds were then counted in these two locules. The viability of seeds was determined by the shape and texture of the seeds using a dissecting microscope. Seeds that appeared to be full or somewhat expanded (thick) and were soft to the touch were deemed viable. Seeds deflated/empty (thin) and hard were deemed inviable. As some of the capsules had already begun to shed seeds, capsules in which the total number of seeds from two locules was less than 50 were omitted from the analyses. As a consequence, the total numbers of seeds were not known for some capsules. However, the proportion of seeds was the point of interest for the analyses, so to check whether there was a bias in the proportion of viable seeds caused by different degrees of shedding in the capsules, total seed counts were plotted against viable seeds for both the “Natural” and “Supplemental” treatments (Figure 3.1). As this graph shows, there appears to be no significant differences in the proportion of viable seeds over the range of total number of seeds recorded from each capsule. If anything, the proportion of viable seeds is slightly lower for both treatments with an increase in the total number of seeds counted.

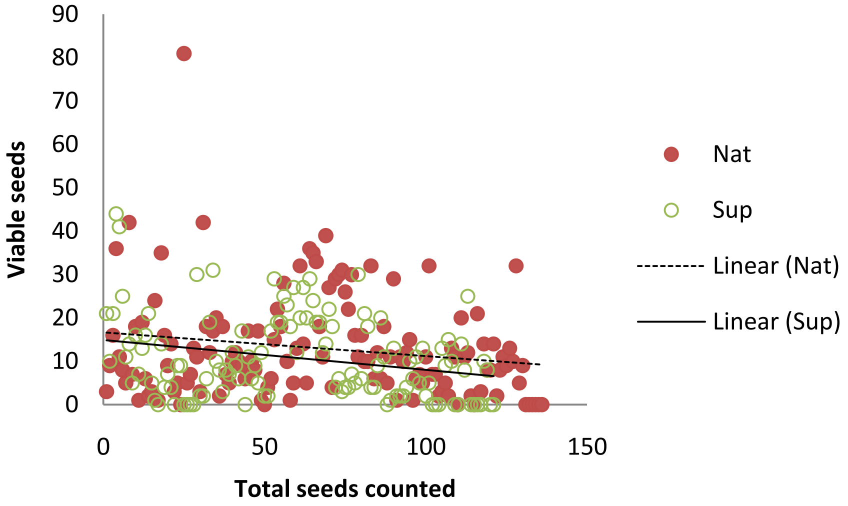

Figure 3.1: Percentage of viable seeds as a function of the total number of seeds counted for both the “Natural” and “Supplemental” treatments.

3.2.4 Data Analysis

For both tests of pollination treatments and pollen limitation, site and treatment effects were considered using a balanced 2-way factorial design in the R version 2.9.0 statistical programme. Differences among sites and treatments for both capsule set and seed set were tested using Binomial Generalised Linear Models and logit link functions in this programme. Seeds per flower were calculated (consistently underestimated as only two locules were counted and some capsules had begun to shed) as well as Autonomous Selfing Index (A.S.I.), Self Compatibility Index (S.C.I.), and Pollen Limitation Index (P.L.I.) values based on site averages. A.S.I. = Bagged % seed set/Selfed % seed set. S.C.I. = Selfed % seed set/Crossed % seed set. P.L.I. = 1 -

Natural % seed set/Supplemental % seed set. Newstrom & Robertson (2005) found these indices very useful in obtaining levels of self compatibility and pollen limitation in a review of pollination requirements of New Zealand flora.

3.3 Results

3.3.1 Bagged Flower Treatments

Overall, capsule set and the proportion of seeds viable within a capsule was highest for the crossed hand pollination treatments. Capsule set was also relatively high for the self pollinated treatments, although the proportion of viable seeds within the capsules produced was much lower. Capsule set and the proportion of seeds viable for the ”bagged” unpollinated flowers yielded the lowest values.

Self Compatibility Index (S.C.I.) values were variable across sites (Table 3.1). A value of 0 represents complete self incompatibility. Values closer to 1 represent a higher level of self compatibility. All the sites except for Campbell show little ability to self pollinate. The Campbell site showed a moderate to high level of self compatibility.

The Autonomous Selfing Index (A.S.I.) for manuka revealed relatively low values, apart from the John Love site with an A.S.I. of 0.986 (Table 3.1). Values closer to 0 represent an inability of flowers to autonomously self. Values closer to 1 represent the ability for a flower to autonomously self or pollinate in the absence of pollinators to the level allowed by the self compatibility of the population.

Table 1: Average capsule set, proportion of viable seeds, Self Compatibility Index (S.C.I.) and Autonomous Selfing Index (A.S.I.) at each of the bagged treatment sites.

		Capsule set			**Proportion viable**				
Site	**Region**	**Bag**	**Self**	**Cross**	**Bag**	**Self**	**Cross**	**S.C.I**	**A.S.I**
D. McIntyre	Manawatu	0.167	0.75	0.917	0.316	0.040	0.227	0.144	0.001
I. Satherley	Manawatu	0.000	1.000	1.000	0.000	0.015	0.059	0.254	0.000
J. Love	Manawatu	0.273	0.273	1.000	0.069	0.070	0.236	0.081	0.986
Campbells	Wairarapa	0.167	0.500	0.833	0.000	0.054	0.041	0.791	0.000

Analysis of the capsule set data for the bagged pollination treatments did not reveal a significant effect of site, but did show a significant difference at the treatment level (Table 3.2). However, there was a weak significant interaction between site and treatment (Table 3.2). This is due to variation in the self compatibility scores shown in Table 3.1.

Table 3.2: Chi square values for capsule set for the bagged treatments. Significant values shown in bold.

	df	**Deviance**	**P**
Site	3	1.470	0.689
Treatment	**2**	**45.111**	**<0.001**
Site*Treatment	6	12.843	0.046
Residual	0	<0.001	

Analysis of the proportion of viable seed data for the bagged pollination treatments revealed a significant effect of both site and treatment, as well as a significant interaction between site and treatment (Table 3.3), which is also highlighted by the self-compatibilty and autonomous selfing indices in Table 3.1.

Table 3.3: Chi square values of the proportion of seeds viable for the bagged treatments. Significant values shown in bold.

	df	**Deviance**	**P**
Site	**3**	**179.22**	**<0.001**
Treatment	**2**	**299.78**	**<0.001**
Site*Treatment	**4**	**40.42**	**<0.001**
Residual	44	595.76	

Based on capsule set and the proportion of seeds that were viable, figure 3.2 shows the average percent seed set that was produced at each bagged treatment site. The “Bagged” treatment in which no pollination was applied shows very low levels of seed production. Capsules from the hand self-pollinated (“Selfed”) treatments produced a higher percentage of seeds per capsule at most sites, whereas the "Crossed" hand pollinated treatment revealed the highest proportion of seeds produced per capsule.

Although the Campbell site appears to be self-compatible, flowers at this site did not autonomously self pollinate. Therefore, all populations are pollinator-dependent, and at D. McIntyre, I. Satherley, and J. Love sites, cross pollination is needed to produce maximum seed set.

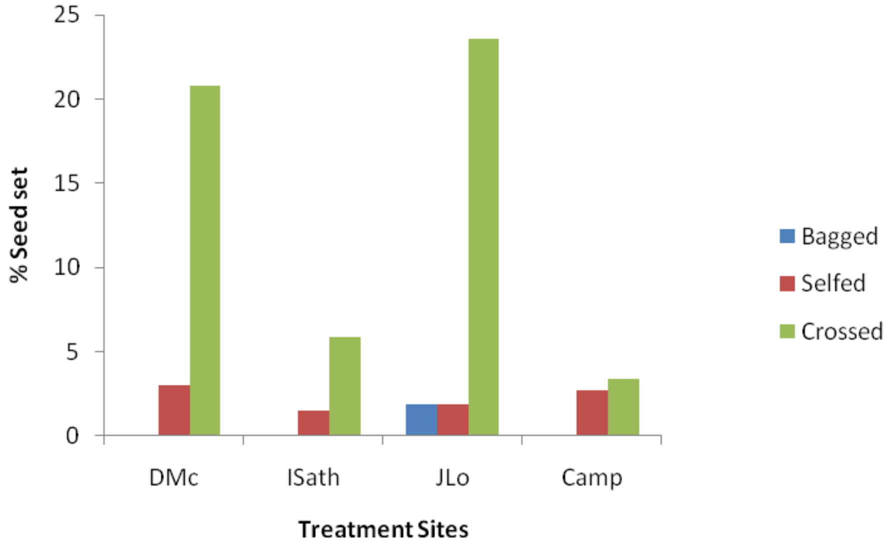

Figure 3.2: Percentage seed set at each of the bagged treatment sites.

3.3.2 Pollen Limitation

Table 4 shows the mean capsule set and proportion of viable seeds for both the "Natural" and "Supplemental" treatments at all sites. The last column lists the Pollination Limitation Index (P.L.I.) per study site. A P.L.I. value less than zero indicates that flowers that are supplementarily pollinated set seed less than natural treatments. The McKenzie (Nth) site from the Wairarapa region had the lowest P.L.I. value of -1.6279, representing little pollen limitation was occurring at this site, whereas A. Shilton site from the Manawatu region had the highest P.L.I. value of 0.4945, suggesting that this site was pollen limited.

Overall there appears to be a lot of variation in the P.L.I. values across study sites and regions, but there are only six sites (A. Shilton, D. McIntyre, Horopito/Railway, Papakai, Railway/Erua, and Silica Rapids) in which pollen appears to be limited, and the degree of pollen limitation is still quite low at three of these sites (Table 3.4).

Table 3.4: Average capsule set, proportion of viable seeds, and Pollen Limitation Index (P.L.I.) for the pollen limitation flower treatment sites. Nat = Natural, Sup = Supplemental.

		Capsule set		Proportion viable seeds		
Site	**Region**	**Nat**	**Sup**	**Nat**	**Sup**	**P.L.I**
A. Shilton	Manawatu	1	1	0.092	0.182	0.4945
I. Satherley	Manawatu	1	1	0.133	0.067	-0.9851
D. McIntyre	Manawatu	0.583	0.75	0.069	0.078	0.3124
J. Love	Manawatu	1	0.667	0.146	0.146	-0.4993
Campbells	Wairarapa	1	1	0.078	0.055	-0.4182
McKenzie (Nth)	Wairarapa	1	1	0.113	0.043	-1.6279
McKenzie (Sth)	Wairarapa	1	0.75	0.109	0.083	-0.7510
Bishops	Tongariro	1	0.833	0.123	0.08	-0.8457
Desert Rd	Tongariro	1	0.7	0.165	0.141	-0.6717
Horopito/Railway	Tongariro	0.9	0.8	0.096	0.129	0.1628
Kapoors Rd	Tongariro	1	1	0.191	0.162	-0.1790
Ohakune	Tongariro	0.9	0.9	0.082	0.077	-0.0649
Ohakune_2	Tongariro	0.9	0.9	0.079	0.067	-0.1791
Papakai	Tongariro	0.75	0.75	0.12	0.147	0.1837
Railway/Erua	Tongariro	1	1	0.089	0.114	0.2193
Silica Rapids	Tongariro	0.923	1	0.155	0.175	0.1825
Sir Ed Outdoor	Tongariro	1	0.833	0.167	0.082	-1.4449

Results from capsule set data for pollination limitation treatments reveal a significant effect of site, but no significant effect of treatment or interaction between site and treatment (Table 3.5).

Table 3.5: Chi square values of capsule set for the pollen limitation treatments. Significant values shown in bold.

	df	Deviance	P
Site	**16**	**36.269**	**0.003**
Treatment	1	2.570	0.109
Site*Treatment	16	15.205	0.510
Residual	0	<0.001	

The proportion of viable seeds produced from the pollination limitation treatments reveals a significant effect of site but no significant effect of treatment. There was, however, a significant interaction between site and treatment, showing that the effect of the supplemental pollination is strongly site dependent (Table 3.6).

Table 3.6: Chi square values of the proportion of seeds viable for the pollen limitation treatments. Significant values shown in bold.

	df	Deviance	P
Site	**16**	**304.96**	**<0.001**
Treatment	1	2.70	0.100
Site*Treatment	**16**	**109.74**	**<0.001**
Residual	223	1553.43	

The correlation between seed set and honeybee density for natural treatment showed a non significant relationship (r = -0.063, p = 0.810) (Figure 3.3). The correlation between seed set and honeybee density for the supplemental treatment was also not significant (r = -0.342, p = 0.179).

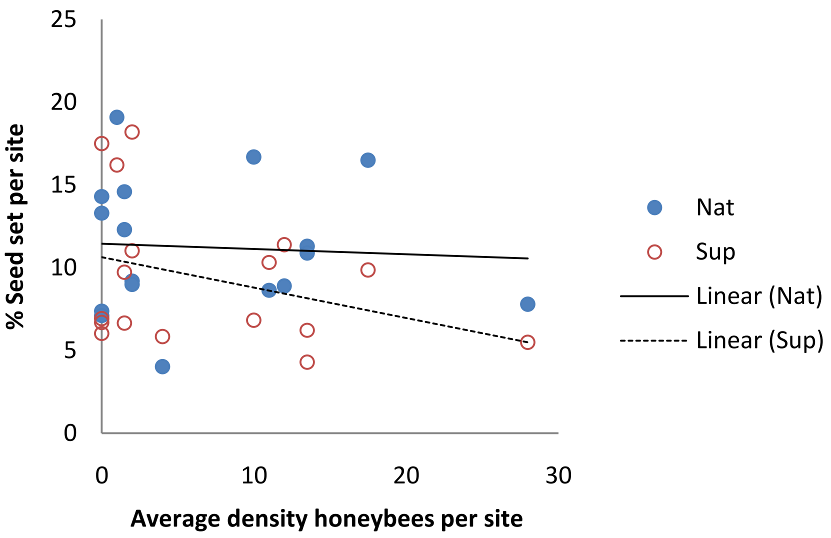

Figure 3.3: Percentage seed set produced for "Supplemental" treatment and "Natural" control as a function of the average density of honeybees per site.

The correlation between seed set and large fly abundance for the natural treatment was not significant (r = 0.046, p = 0.860). The correlation between seed set and large fly density for the supplemental treatment was also not significant (r = -0.115, p = 0.659) (Figure 3.4).

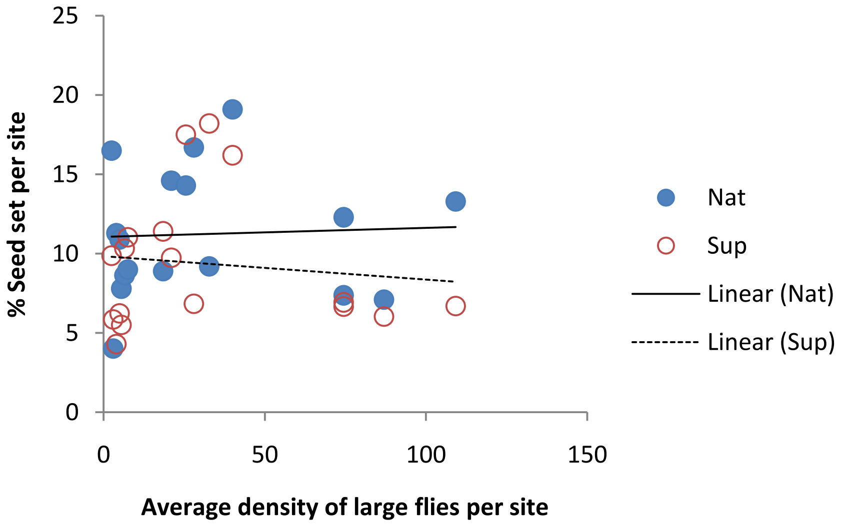

Figure 3.4: Percentage seed set produced for "Supplemental" treatment and "Natural" control as a function of the average density of large flies per site.

The correlation between the P.L.I. and average honeybee density at each site was not significant (r = -0.286, p = 0.266). Therefore, honeybee density did not appear to affect the level of pollen limitation among study sites (Figure 3.5).

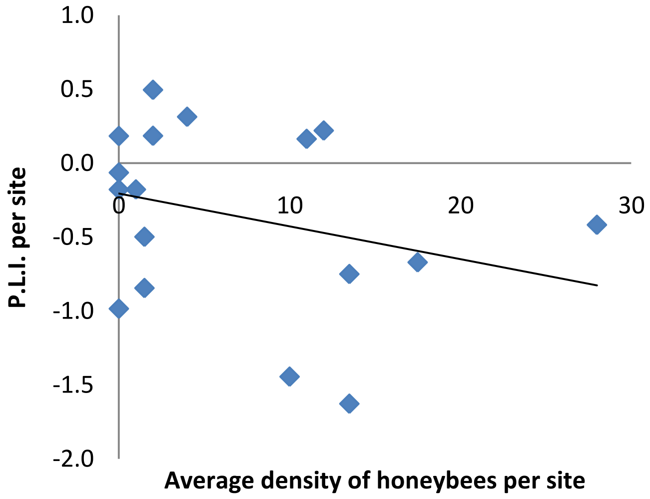

Figure 3.5: Pollen Limitation Index (P.L.I.) as a function of density of honeybees per site.

The correlation between P.L.I. and average large fly density at each site was not significant (r = -0.116, p = 0.656), hence large flies also did not significantly affect the level of pollen limitation among the study sites (Figure 3.6).

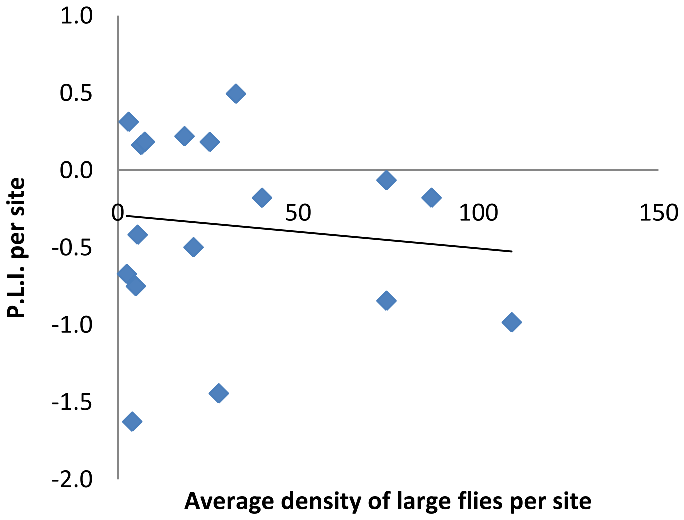

Figure 3.6: Pollen Limitation Index (P.L.I.) as a function of density of large flies per site.

The correlation between the P.L.I. and $\log_{10}$ patch size (ha) at each site displayed a weak positive relationship and was not significant (r = 0.10863, p = 0.67812). Patch size, therefore, did not appear to affect the level of pollination limitation at the sites (Figure 3.7).

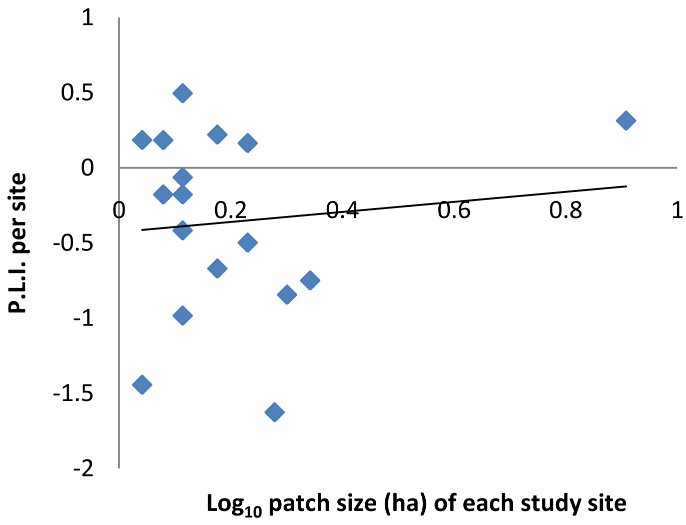

Figure 3.7: Pollen Limitation Index (P.L.I.) as a function of $\log_{10}$ patch size (ha) per site.

3.4 Discussion

Results from the bagged treatments show that manuka has little capability for autonomous self-fertilisation. A.S.I. values for all but one site were zero; however the John Love site yielded an A.S.I. value of 0.986. This would suggest a high level of ability to autonomously self fertilise, although an A.S.I. value is only reliable if a reasonable level of self-compatibility is established. John Love’s site, however, had a lower score of self-compatibility, so confounding variables may have yielded the high A.S.I. values found at this particular site. When the capsules were collected, a number of the no pollination “Bagged” treatment flowers had been aborted, presumably due to a low or complete lack of viable seeds being produced. This is reinforced by the significant difference in capsule set for each treatment. At John Love’s site in particular, only two capsules for the “Bagged” treatment were left on the plants. Therefore, there was little baseline data to compare with other treatments for this site. One of the capsules had produced no viable seeds, whereas the other produced a ratio comparable to the “Selfed” treatments. In this particular case, either the anther may have somehow been contaminated with pollen from another flower within the bag, or smaller pollinators may have been able to gain entrance to the bag. For example, thysanopterans (thrips) were frequently observed on manuka flowers. Thrips are very small and little is known about their pollination capabilities, but it has been suggested that they may play a part. Thrips have been observed on many species of New Zealand plants in which pollen has been found adhering to their thorax, legs, and abdomen (Norton 1984) and some individuals may have been able to enter through the minute holes in the bags.

Results from the “Selfed” treatments reveal some level of self-compatibility in this species. All but one site, Campbell, show a low to moderate level of self compatibility. This indicates that manuka appears to be at least partially self compatible. The Campbell site revealed a S.C.I. value of 0.791, indicating that flowers at this site appear to be much more self compatible than at the other sites. Site differences like this, and the higher A.S.I. score at the John Love site in the previous paragraph, are reinforced by the presence of a strongly significant interaction between sites for the bagged treatments and the type of treatment. Interestingly, Campbell had a higher density of honeybees than observed at the other experimental sites.

Manuka is heavily reliant on pollinators for seed production. These results provide interesting foresight into the pollination requirements of manuka. Many other species from Australia within the same genus, *Leptospermum*, are andromonoecious (Andersen 1990). Two species in particular, *L. myrsinoides* and *L. continentale*, have been found to be self-compatible (O’Brien & Calder 1993). Furthermore, both these species displayed self-compatibility levels consistent with those found in this study - partial self-compatibility and limited autonomous selfing capabilities (O’Brien 1994). However, the author also states that the level of self-compatibility may be linked to genotype. Therefore, additional experiments spanning a much larger number of plants across more geographic regions could further validate the pollination capabilities of manuka and the possibility of genotype variation in pollination capabilities.

Results from the pollen limitation data show that all study sites do not appear to be pollen limited. Overall, there was no significant difference between capsule set and proportion of seeds viable in supplemental and natural flowers. This shows that natural pollination conditions did not significantly differ from supplemental cross pollination treatments. Therefore, pollinators present within patches of each site are adequately pollinating the flowers. This did not significantly differ with an increase in density of honeybees within site patches for either the natural or supplemental treatments.

However, there was a significant difference between capsule set and the proportion of viable seeds produced and site. As explained in the methods, manuka plants around the Massey Palmerston North campus were tagged as an indicator of capsule set. Unfortunately, this population was not a good indication of patches at other locations, as capsules at some sites had begun to split earlier than others. A study by Harris (2002) showed extensive variation of capsule splitting between populations, even amongst those located within the same localized areas. He found much variation in capsule splitting and seed shedding among 61 populations of manuka across New Zealand. Populations showed strong latitudinal differences in timing, with northern patches displaying more serotinous patterns. Factors shown to affect the onset of capsule splitting and the shedding of seeds within manuka include fire, drought, transplantation, and cold snaps (Burrell 1965). Thus, suggesting the timing of these processes is under pressure from environmental variation. This may help explain why sites closer to Tongariro National Park began splitting capsules earlier than sites from other regions.

There was also considerable variation between sites with regard to which treatment was more efficient, as is shown by the P.L.I. values in Table 3.4. However, there was no significant correlation between percentage seed for either the natural control or supplemental treatment in relation to the density of honeybees or large flies.

Conversely, there was no significant difference between P.L.I. and honeybee or large fly density for each study site suggesting that pollination requirements tend to be met in both the presence and absence of honeybees or large flies.

Dupont et al. (2004) found that honeybees spent more time foraging at flowers of the same plant than native pollinators, therefore possibly contributing to a higher level of self pollination. Other publications have found similar patterns (Paton 1993; Chamberlain & Schlising 2008). During behavioural observations I noticed that honeybees appeared to forage on the same plant for longer than other pollinators, however no accurate measurements were recorded. If this pattern is confirmed, rather than solely focusing on the quantity of pollen transferred by honeybees, perhaps the quality of pollen should be more closely examined also. There was no evidence of pollen limitation occurring between patches, but this does not provide insight into the possible quality of pollen being transferred by honeybees. If honeybees primarily visit flowers on the same individual plant and spend more time at each plant than other pollinators during foraging, they are more likely to be promoting a higher level of self fertilization. This could have long term effects on plant fitness and genetic variation.

In conclusion, manuka clearly relies largely on pollinators for capsule set and seed production, although it does appear to display a moderate level of self compatibility. Despite being andromonoecious, results do not show much support for the favouribility of the monoecious manuka flowers to autonomously self pollinate. In turn, self fertilization treatments developed fewer capsules and substantially less viable seeds than flowers that were cross-pollinated. There was also no evidence of pollen limitation occurring within the study sites. Therefore, honeybees do not directly appear to have any impact on the quantity of pollen transferred or consequent seed set. However, more complex interactions, such as the quality of pollination service provided by honeybees, should also be reviewed before we can make a general assumption that honeybees may not have any negative impacts on the reproductive fitness of *Leptospermum scoparium*.

4.

The effects of manuka patch variables on taxa diversity

4. The effect of manuka patch characteristics on invertebrate taxa diversity

4.1 Introduction

The loss of large areas of native bush and original grasslands worldwide has generated interest about the management of remaining natural habitats. Human disturbance and consequent destruction or fragmentation of natural and semi-natural habitats has been proposed as one of the major threats to biodiversity (Kearns et al. 1998; Fahrig 2003; Goulson et al. 2008). Such habitat loss can cause a reduction in species richness and abundance, as well as impact on invertebrate diversity and plant reproduction, by altering species composition and the behaviour of pollinators within patches (Aizen & Feinsinger 1994; Steffan-Dewenter & Tscharntke 1999; Steffan-Dewenter & Westphal 2008). Pollinator limitation, due to changes in pollinator composition, may also lower the fecundity of host plants, resulting in a lower quantity and/or quality of pollen transfer (Robertson et al. 1999; Ashman et al. 2004). Pollinator limitation may even drive changes in reproductive strategies to favour autogamous plant species (Ashman et al. 2004). Habitat fragmentation has also been linked to shifts in pollinator behaviour toward more generalised flower visitation (Taki & Kevan 2007). Dick et al., (2003) propose that in these cases generalist pollinators, such as honeybees, may help link fragmented landscapes by expanding neighbouring patches, as they move to nearby patches regardless of plant species composition.

Nevertheless, other species may be more sensitive to habitat fragmentation and a recent meta-analysis conducted on literature involving research of the effects of habitat destruction on various bee species indicated that habitat loss was the strongest influencing factor on bee richness and abundance in the 54 studies analysed (Winfree et al. 2009). Many characteristics of habitat fragments have been shown to influence population abundance, diversity, and community composition.

These include patch size, isolation, quality, proportion of edges, as well as surrounding landscape (Tscharntke et al. 2002). However, which particular aspects of habitat loss and/or fragmentation, and the varying degrees of effects that they have on different species, are not as clear, although a disproportionate amount of research has focused on patch size, surrounding landscape, and habitat quality in regards to invertebrate and insect pollinator groups.

Previous research tends to suggest that fragment size has an effect on biodiversity. Aizen & Feinsinger (1994) investigated pollinator assemblage in response to the size of forest fragments in Chaco Serrano, Argentina. They found that the frequency of visitation and species richness of native pollinators declined with a decrease in patch size; smaller patches were increasingly monopolized by feral honeybees. A recent study by Gonzalez-Varo et al. (2009) examined the effects of fragmentation on pollen limitation and seed production as well as pollinator assemblage of populations of *Myrtus communis.* Larger patches had higher taxonomic diversity, whilst small patches were dominated by honeybees. This shows that either native pollinator's were more susceptible to the presence of honeybees at smaller landscape scales, or that smaller patches do not provide the resources needed to support a diverse community of flower visitors. Another study by Martinko et al. (2006) also found that insect richness was higher among larger patches (5000 m^2) compared to medium-sized (1728 m^2) or small patches (480 m^2). Conversely, other studies have found that fragment size has no effect on species diversity. Donaldson et al. (2002) examined the effects of habitat fragmentation on pollinator diversity on several plant species in South African shrub-lands. Their results showed that species abundance of three major pollinators: bees, butterflies and flies decreased with increasing distance from large fragments, although patch size alone did not significantly affect species richness.

Many recent studies have also examined the effects of agricultural land use, and subsequent habitat loss on invertebrate diversity. Intensity of agricultural land use is characterized by farming practices, such as machinery, and the use of fertilizers, that may be detrimental to the health and survival of resident pollinators (Steffan-Dewenter & Westphal 2008). Hendrickx et al. (2007) examined the effect of the intensity of agricultural land use, landscape structure, and habitat diversity on species richness within 24 European agricultural landscapes. The taxa of interest included wild bees (Apoidea), carabid beetles (Carabidae), true bugs (Heteroptera), hoverflies (Syrphidae), and spiders (Araneae). Results showed that species richness among all of these invertebrate groups was negatively affected by decreasing plant species diversity and increasing intensity of surrounding land use. Spiders in particular also responded negatively to a decrease in proximity to semi-natural habitat patches. A similar study examined four groups of pollinators: bees, beetles, butterflies, and hoverflies within semi-natural grasslands in Sweden (Sjodin et al. 2008). Beetle and hoverfly species richness and abundance declined with an increase in grazing intensity, whereas there were no significant differences among the bees and butterflies. The groups also varied in species richness, abundance, and composition, in response to local habitat and landscape diversity, suggesting that different taxa may, in fact, vary in their responses to different habitat disturbances. Steffan-Dewenter et al. (2002) found that landscape context significantly affected pollinator abundance and diversity. Honeybee richness and abundance decreased in response to a lower proportion of semi-natural habitat at larger spatial scales (up to 3km), in comparison to solitary bees which responded more strongly to lower proportions of semi-natural habitat at smaller scales (up to 750m). Therefore, they conclude that solitary bees are affected more than social bees by local landscape destruction.

Other recent studies have highlighted the influence of plant species composition on invertebrates among patches. In Switzerland, Diekotter et al. (2008) examined the difference in landscape and habitat diversity within two differing agricultural landscapes on the partitioning of wild bees, carabid beetles, and true bugs. Their results indicate that landscape structure has an effect on species richness, with each insect guild preferring different plant compositions in varying landscapes. Indeed, Franzen & Nilsson (2008) found a significant positive correlation between plant species composition and species richness among patches. They found that species richness of solitary bees and butterflies was higher at increased densities of the plant *Knautia arvensis* within the agricultural patches examined, and decreased in areas with a higher proportion of grazed grassland. Crist et al. (2006) used an experimental approach to examine species responses to habitat variables. They found that plant richness and composition were the strongest predictors of insect composition and abundance. From such studies, it is apparent that grazing intensity and plant species composition appears to heavily influence the species composition of invertebrates in agricultural landscapes. However, the influence of plant composition is not limited to agricultural settings. Variation in species diversity may also occur within the context of the landscape of focus.

Research among regenerating fire-stricken Mediterranean landscapes on the Greek Islands of Lesvos found that bee richness was correlated with floral and nectar resource diversity (Potts et al. 2006). Brosi et al. (2008) examined the effects of fragment size, shape, isolation and landscape context (patch variables) on bee community composition within a tropical countryside in Southern Costa Rica. Their results showed that different guilds of bees responded differently to fragment and landscape characteristics, highlighting the complexity of the variability of effects habitat changes among different taxa. Another recent study found that bee abundance and species richness was lower in forest habitats, and was higher in agricultural, suburban, and urban landscapes in Sourthern New Jersey in the USA (Winfree et al. 2007). However, this may be as a result of particular species' preferences, as 18 of the 130 bee species examined were more strongly associated with forest habitats. This highlights the complexity of the effects on species rather than focusing on groups of taxa (as different species may be affected by fragmentation differently).

It is vital to have a baseline understanding of the type of biodiversity present within certain landscapes in order to examine the effects of habitat modification and put them into context. In a survey of montane and subalpine pollinators, Primack (1983) found many insect visitors from all the main orders visiting manuka plants, which provides a valuable baseline to stem from. Therefore, the biodiversity among manuka patches would be expected to increase in the presence of a higher density of flowering plants. The degree of specialisation of particular species is important when examining the effects of habitat loss, as specialist species rely on specific hosts, prey, habitat, and therefore, have little flexibility to adapt to environmental changes (Didham et al. 1996). As New Zealand pollinators, regardless of the introduction of honeybees are generalist pollinators, they may be less susceptible to detrimental effects of habitat loss and fragmentation. However, the effects of patch size and local habitat characteristics on manuka fitness and invertebrate communities are unknown. As manuka is a highly valuable native plant and many endemic insects frequent its flowers, conservation of this plant-pollinator system is of importance.

The aim of this chapter is to examine the biodiversity of invertebrates occurring among patches of manuka and which patch characteristics help explain some of the difference in invertebrate taxa abundance, diversity and insect family composition.

4.2 Methods

4.2.1 Study sites

Intercept and pitfall traps were set up at each of the eighteen study sites referred to in chapter two, as well as eleven additional sites (Appendix 1 & 2) including one from the Wairarapa and ten from the Tongariro region. The patch characteristics described in chapter 2 were also recorded for the additional eleven sites. For the purposes of this chapter, surrounding land use and the frequency of surrounding manuka cover was also recorded. Surrounding land use was classified into one of three categories: Pastoral, native bush, or tussock. The frequency of surrounding manuka cover was recordedin one of three categories: Sparse, moderate, or abundant.

4.2.2 Intercept Traps

One intercept trap was set up at each of the twenty nine study sites. The set up of the intercept trap is shown in figure 4.1. The trap had both a top and bottom chamber leading to plastic containers filled to a one third measure of propylene glycol. The traps were left out for a period of at least one week at a time, more often two weeks. At the eighteen main study sites the traps were sampled at least three times. The other eleven sites were only sampled once, but the traps were left out for a period of three weeks. The traps were placed towards the centre of each patch and in sites sampled more than once the trap was moved into a different position each time they were emptied, but always within the middle of the patch.

Figure 4.1: Set up of intercept traps, displaying bottom and top chambers without propylene glycol added. Clear Perspex baffles are inserted to intercept invertebrates which can migrate upwards into the top chamber or fall down into the bottom chamber.

4.2.3 Pitfall Traps

A pitfall trap- was also set up at each of the 29 sites. The traps were constructed by digging holes within the patches and positioning a plastic container half-filled with propylene glycol in the hole flush with the ground. The lids were then removed and a metal plate positioned over the top leaving at least a 2 cm gap to allow invertebrates access. All sites were only sampled once. The traps were left for a period of three weeks before being collected.

4.2.4 Sample sorting

Each invertebrate within a sample was initially identified to order. Acarina, Collembola, and Thysanoptera were excluded due to time constraints and logistics of small size and overabundance within the traps. Once divided into order, six major insect orders were identified to family level. These included the Coleoptera, Diptera, Hymenoptera, Lepidoptera, Orthoptera, and Hemiptera. To examine trends more closely, the main pollinators: Coleoptera, Diptera, Hymenoptera (with the exception of lepidopterans) were identified to morphospecies level. The Lepidoptera were not identified to morphospecies level as they were the least abundant insect visitors during the daytime observations and in trap collections and many were too damaged or unable to be identified to morphospecies.

4.2.5 Data Analysis

For the intercept trap data, all sites sampled more than once were averaged. Preliminary results of the ordinations revealed distinct separation between morphospecies found in the top or bottom chambers of the intercept traps (Figure 4.2). As a consequence, data from the top and bottom trap chambers were analysed separately, and together. The most commonly occurring morphospecies and orders (ie. those that occurred in the traps on more than one occasion and had an average higher than 0.3 when transformed to a logarithmic scale) were plotted on a bar graph to identify which invertebrates favoured one or the other chamber.

The effects of patch characteristics on invertebrate richness and evenness (Simpson's Index:

$$1 - \sum \left(\frac{n}{N}\right)^2$$

for both the intercept (combined) and pitfall trap data were considered using a generalised linear model multiple regression in R (version 2.9.0). Insect richness and evenness were calculated for each site for both the intercept combined and pitfall traps for all sites across regions, and for Tongariro separately to examine differences exclusive of region as altitude was a confounding factor among regions. I ran multiple regressions of invertebrate richness and evenness against the patch characteristics at each site. The patch characteristics included quantitative measures of altitude, proportion maunka cover (%), plant richness, plant evenness (Simpson's Index), $\log_{10}$ patch size (ha), and plant ordination axis 1 & 2 scores, as well as categorical variables of region, surrounding land use, and frequency of surrounding manuka.

For ordination analysis, non-metric multidimensional scaling (NMS) ordinations were originally performed at the invertebrate morphospecies level within the main matrix, however, many morphospecies only occurred at one or two sites and overall the data was quite sparse, resulting in little overlap to compare between sites. So in the final results, ordinations were run at the insect family level. Compositional gradients of insect families from each site were investigated using non-metric multidimensional scaling (NMS) ordinations. NMS ordinations were performed for the combined intercept data, as well as the top and bottom chambers separately, and the pitfall traps. As there was large variation among the Tongariro sites, NMS ordinations were also calculated exclusively for this region. For the intercept traps, ordinations were initially run with data averaged for each site, whereas later iterations were run with a $\log_{10}(x+1)$ transformation of the data to account for the sparseness of the raw data and many 0 values. The pitfall trap data were also $\log_{10}(x+1)$ transformed. All NMS ordinations were run using the "autopilot (slow and thorough)" mode in PC-ORD Version 5.0 with Sorensen distance as the dissimilarity measure. All but one data set, the intercept top chambers, were finally configured in a 3-dimensional solution as the recommended best fit; the top intercept chamber was fitted with a 2-dimensional solution, according to stress values from the real and randomised (Monte Carlo test) original iterations. Multi-Response Permutation Procedures (MRPP's) were also run to examine the significance of the categorical variables: region, surrounding land use, and surrounding manuka frequency on community assembly. Ordination results were displayed using the axes that best described the variation in invertebrate assemblage; some were best explained by axis 1 and 2, others axis 1 and 3 or axis 2 and 3.

4.3 Results

A total of 159 Coleoptera (beetles), 125 Diptera (flies), 131 Hymenoptera (ants, bees, and wasps) morphospecies, and 50 other groups of taxa from various orders were collected among the intercept and pitfall traps (Appendix 4).

Larger Coleoptera and Diptera made up a large quantity of the invertebrates caught in the bottom intercept chambers (Table 4.1). Smaller Diptera morphospecies and other orders comprised a larger majority of invertebrates collected in the top chambers of the intercept traps. Coleoptera occurred less frequently in the top intercept chambers, and where they were collected, they were mostly smaller morphospecies. The distribution of orders was comparatively more uniform in the pitfall traps. However, the morphospecies of insects, as expected, were quite distinct from those collected in the intercept traps. The majority of the hymenopterans collected in the pitfalls comprised of ant species, and the Coleoptera of ground beetle families such as the Carabidae and Staphylinidae.

Table 4.1: Average totals and morphospecies richness per trap with standard errors for different taxa groups collected within intercept top and bottom, and pitfall traps at all sites.

	INTERCEPT BOTTOM		**INTERCEPT TOP**		**PITFALL**	
Taxa	**Individuals caught**	**Richness**	**Individuals caught**	**Richness**	**Individuals caught**	**Richness**
Coleoptera	12.53 ± 1.77	3.40 ± 0.60	0.50 ± 0.14	0.30 ± 0.07	7.44 ± 2.03	2.17 ± 0.31
Diptera	13.06 ± 1.92	4.82 ± 0.42	2.60 ± 0.43	1.80 ± 0.28	8.79 ± 1.72	2.52 ± 0.20
Hymenoptera	2.89 ± 0.72	1.62 ± 0.22	1.50 ± 0.28	0.60 ± 0.17	14.79 ± 3.92	2.38 ± 0.29
Other orders	6.51 ± 0.61	3.95 ± 0.28	3.10 ± 0.37	1.80 ± 0.17	12.17 ± 1.64	4.76 ± 0.25

4.3.1 Intercept Traps

Preliminary ordination results showed a distinct separation in morphospecies collected in the top and bottom chambers of the intercept traps (Figure 4.2). Therefore, ordinations were run separately for the insects caught in the top and bottom traps as well as the combined catch.

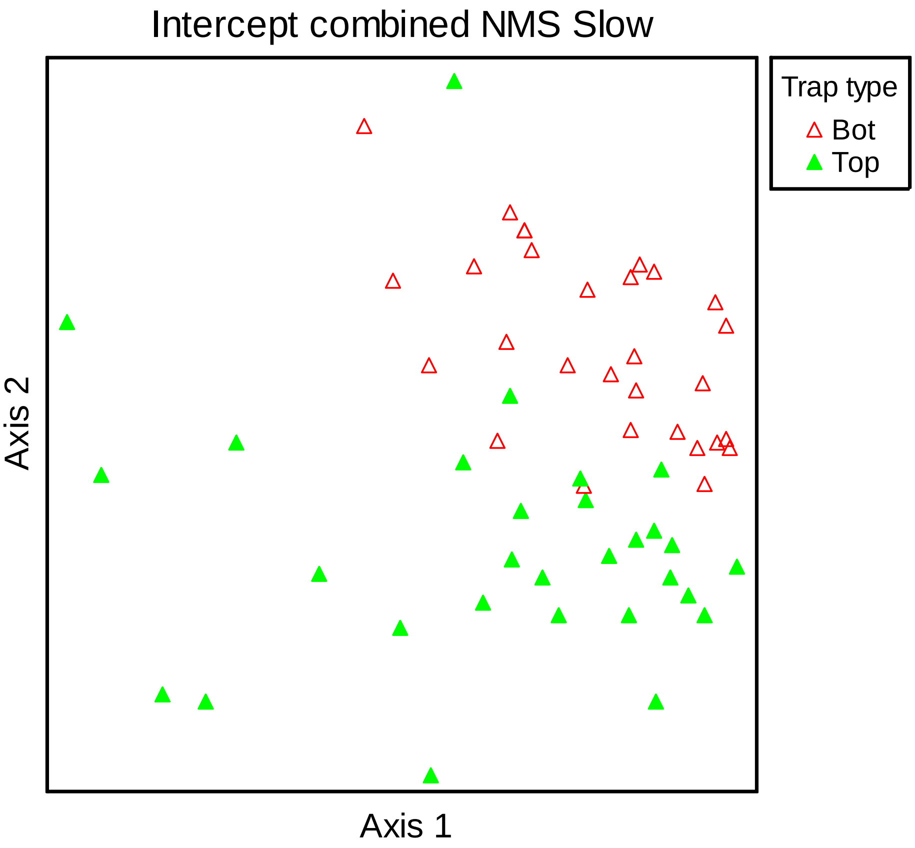

Figure 4.2: Composition of invertebrate taxa collected in the top and bottom chambers of the intercept traps.

As shown in figure 4.2, the two types of chambers were quite distinct in the type of taxa caught. Figure 4.3 displays the most common coleopteran, dipteran, and hymenopteran morphospecies present in the intercept traps and which chambers of the traps they more frequently occurred. All the common Coleoptera were caught more often within the bottom chamber of the intercept traps.

Both Diptera and Hymenoptera were also found more frequently in the bottom chambers than in the top. However, most notably, dolichopodid (D2) flies appeared more often in the top chamber and phorid (D9) flies were caught fairly evenly between the top and bottom chambers. The other discrepancies were wasps from the family Eulophidae which occurred more frequently in the top chamber of the traps.

Among the other orders, most were found in the bottom chamber of the intercept traps, with the exception of Araneida (spiders) and Lepidoptera (moths & butterflies). Spiders, much like the phorid flies, were found more uniformly in the top and bottom chambers, whereas lepidopterans were more likely to be caught in the top chamber.

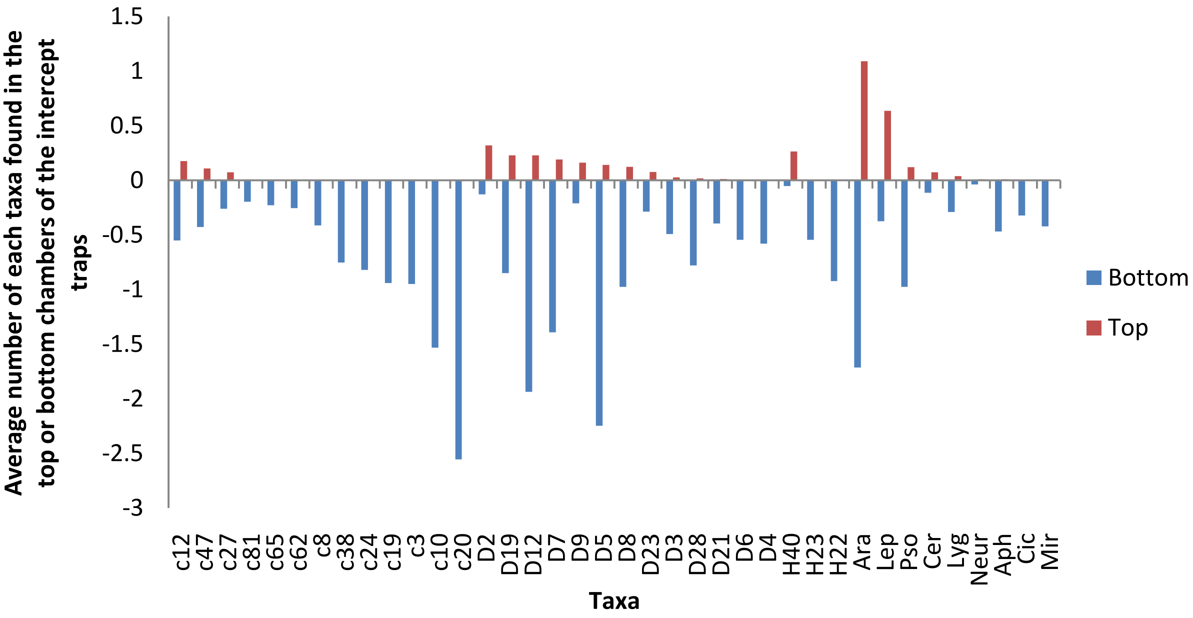

Figure 4.3: The most frequently occurring invertebrate taxa within Intercept traps and their distribution within the top and bottom chambers. Taxa classified with a "C" and subsequent numbers are morphospecies within the Order Coleoptera, those with a "D" are Diptera, and "H" denotes Hymenoptera. The remaining invertebrates are from other diverse orders. Ara = Araneida, Lep = Lepidoptera, Pso = Psocoptera, Cer = Cercopidae, Lyg = Lygaeidae, Neur = Neuroptera, Aph = Aphidae, Cic = Cicadidae, Mir = Miridae.

4.3.2 Intercept trap diversity

Among the combined intercept trap taxa between sites, invertebrate richness was significantly influenced by region and altitude (Table 4.2). Invertebrate richness was negatively correlated with altitude (r = -0.800; p = <0.001). It was highest within the Manawatu, followed by the Wairarapa, and was lowest within Tongariro; and lower at sites at higher altitudes (Figure 4.4).

Table 4.2: Multiple regression of invertebrate taxa richness against environmental factors for intercept traps from all regions. Significant values are shown in bold.

	df	Deviance	F	P
Region	**3**	**6351.2**	**16.30**	**<0.001**
Land Use	2	387.4	1.50	0.259
Manuka freq.	2	269.4	1.04	0.380
Altitude	**1**	**1436.5**	**11.05**	**0.005**
Prop. Manuka cover	1	113.2	1.03	0.328
Plant Richness	1	32.9	0.25	0.623
Plant Evenness	1	5.1	0.04	0.845
Patch size	1	65.8	0.51	0.489
Plant Ordination Axis 1	1	3.9	0.03	0.865
Plant Ordination Axis 2	1	2.2	0.02	0.900
Residual	14	1819.3		

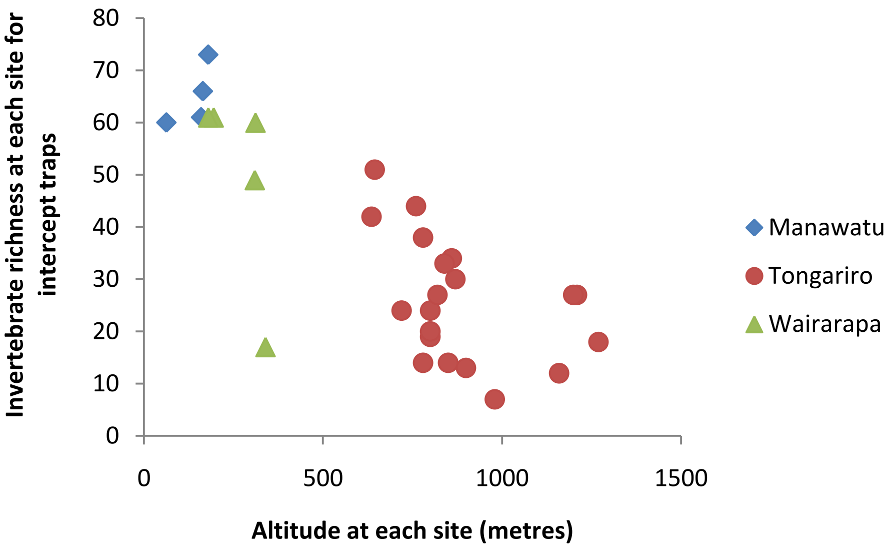

Figure 4.4: The relationship between invertebrate richness and altitude for intercept trap data with regions shown by colour symbols.

However, region and altitude are confounded, as the sites within the Tongariro National Park were higher in altitude compared to the Manawatu and Wairarapa regions. Therefore, in order to examine whether altitude was actually having a strong influence independent of region, another multiple regression was run analysing only the Tongariro sites against environmental variables (Table 4.3; Figure 4.5).

Table 4.3: Multiple regression of invertebrate taxa richness against environmental factors for intercept traps from Tongariro only. Significant values are shown in bold.

	df	**Deviance**	**F**	**P**
Land Use	1	0.730	0.015	0.907
Manuka freq.	2	149.04	1.486	0.277
Altitude	**1**	**1269.50**	**25.307**	**<0.001**
Prop. Manuka cover	1	101.25	2.018	0.190
Plant Richness	1	175.37	3.496	0.094
Plant Evenness	1	50.27	1.002	0.343
Patch size	1	188.31	3.754	0.085
Plant Ordination Axis 1	1	16.77	0.334	0.577
Plant Ordination Axis 2	1	249.07	4.965	0.053
Residual	9	451.48		

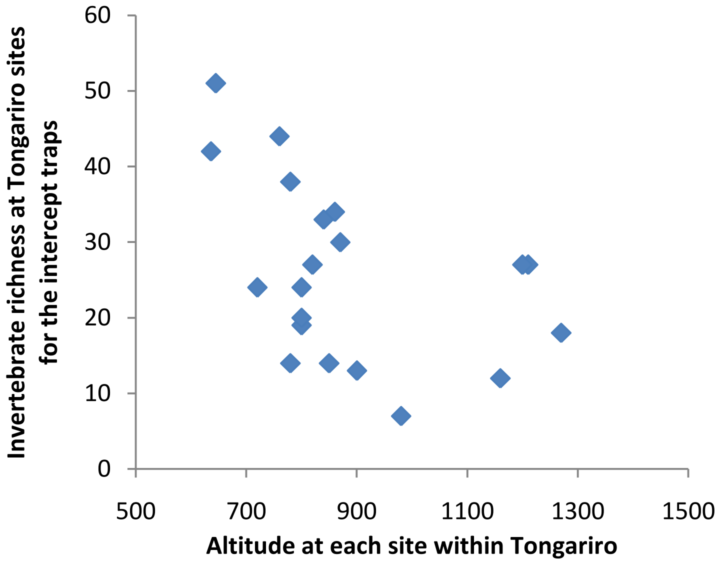

Figure 4.5: Taxa richness as a function of altitude for the intercept traps at the Tongariro sites.

There is a distinct variation in altitude among sites within Tongariro (Figure 4.5). When the environmental variables were analysed by sites only within this region, altitude was still a significant factor influencing taxa richness (Table 4.3), showing a negative correlation with altitude (r = -0.460; p = 0.047), consistent with figure 4.4, in which taxa richness was lower at higher altitudes (Figure 4.5). Therefore, altitude, independent of region, appears to have an effect on taxa richness. None of the other patch variables were significantly associated with invertebrate richness.

Invertebrate evenness (Simpsons Index) for the intercept traps was significantly different among the regions (Table 4.4; Figure 4.6). Sites within Tongariro had the most even assemblages, followed by sites within the Wairarapa, with the Manawatu sites having the lowest evenness (Figure 4.6). Again, no other patch characteristics significantly affected invertebrate evenness.

Table 4.4: Multiple regression of invertebrate taxa evenness against environmental factors for intercept traps from all regions. Significant values are shown in bold.

	df	Deviance	F	P
Region	**3**	**0.259**	**9.0**	**0.001**
Land Use	2	0.017	0.9	0.437
Manuka freq.	2	0.009	0.5	0.629
Altitude	1	0.003	0.3	0.601
Prop. Manuka cover	1	0.025	2.6	0.129
Plant Richness	1	0.003	0.3	0.568
Plant Evenness	1	0.002	0.2	0.648
Patch size	1	0.005	0.5	0.478
Plant Ordination Axis 1	1	0.007	0.7	0.405
Plant Ordination Axis 2	1	0.017	1.8	0.203
Residual	14	0.135		

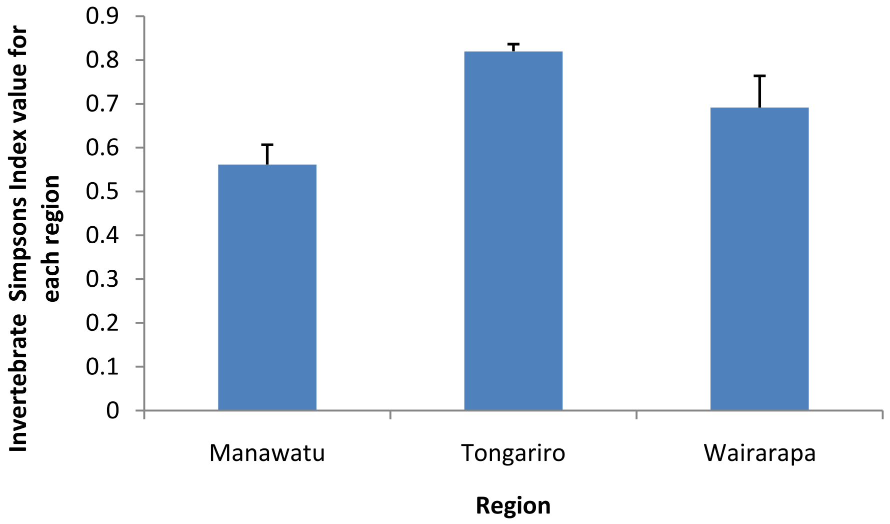

Figure 4.6: Average invertebrate evenness value and standard error for each region of the intercept trap data.

When excluding the other regions and comparing Simpson’s Index for Tongariro only, no patch variables were significant (Table 4.5).

Table 4.5: Multiple regression of invertebrate taxa evenness against environmental factors for intercept traps from Tongariro only.

	df	Deviance	F	P
Land Use	1	0.012	2.180	0.171
Manuka freq.	1	0.002	0.396	0.543
Altitude	1	0.009	1.609	0.233
Prop. Manuka cover	1	0.015	2.805	0.125
Plant Richness	1	0.002	0.414	0.535
Plant Evenness	1	0.006	1.106	0.318
Patch size	1	<0.001	<0.001	0.985
Plant Ordination Axis 1	1	0.003	0.556	0.473
Plant Ordination Axis 2	1	<0.001	0.123	0.733
Residual	10	0.053		

4.3.3 Intercept combined trap ordination

Multi-Response Permutation Procedure (MRPP) tests were run to examine which categorical factors were significantly affecting invertebrate assemblage. For the intercept combined data, intercept bottom chamber, and intercept top chamber, region was the only significant variable determining differences in taxa composition (Table 4.6). For the pitfall trap data both region and land use were significant, although region was a much stronger predictor. Therefore, for the purposes of the ordination graphs, region was used to group the composition of the insect family data for all trap types.

Table 4.6: Multi-Response Permutation Procedure (MRPP) tests of categorical data as predictors of intercept and pitfall trap assemblages at all sites. "A" represents the critical value. Significant values are shown in bold.

	Intercept combined		**Intercept bottom chamber**		**Intercept top chamber**		**Pitfall**	
	A	**P**	**A**	**P**	**A**	**P**	**A**	**P**
Region	**0.024**	**0.050**	**0.078**	**<0.001**	**0.044**	**<0.001**	**0.061**	**<0.001**
Land Use	0.008	0.267	0.019	0.059	-0.003	0.613	**0.031**	**0.009**
Manuka freq.	0.010	0.096	0.008	0.142	<-0.001	0.472	0.005	0.255

The relationship between insect family composition and other patch characteristics, such as plant composition, plant diversity, patch size, altitude, and categorical factors including land use and surrounding manuka cover frequency arranged sites from the three regions into groups occupying different sectors of ordination space and are presented in figures 4.7 & 4.8, using a 3-dimensional NMS ordination. Results from the combined intercept ordination were significant (Monte Carlo p-value = 0.004) and the final stress value was 10.98 (A stress value range of 10 – 20 represents a fair representation of the data. Stress values above 20 represent a poor representation of data). The Manawatu, Wairarapa, and Tongariro regions occupied different sectors of the ordination space. Therefore, there were distinct differences in insect family composition among the three regions. Sites from the Manawatu and Wairarapa were quite tightly clustered, suggesting less variation in insect family composition within these regions, whereas sites within the Tongariro were much more variable.

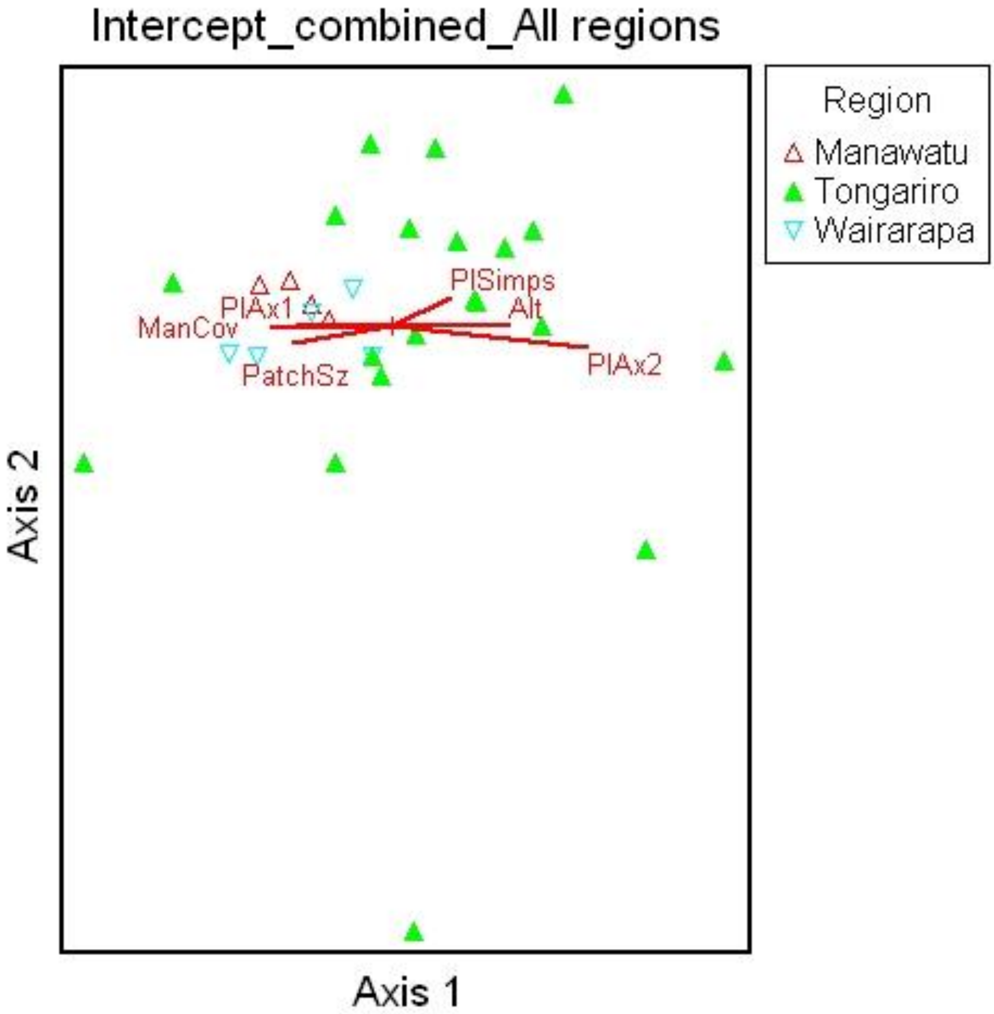

Figure 4.7: Three-Dimensional NMS ordination for insect families from combined intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = Altitude, PlAx1 = Plant ordination axis 1 scores, Plax2 = Plant ordination axis 2 scores, ManCov = Proportion manuka cover (%), PatchSz = Log$_{10}$ patch size (ha), and PlSimps = Plant Simpsons Index.

There also appears to be a lot of overlap in assemblages between the Manawatu and Wairarapa (Figure 4.7 & 4.8). The outlying site at the bottom of figure 4.7 had a higher composition of Lepidoptera from the family Noctuidae.

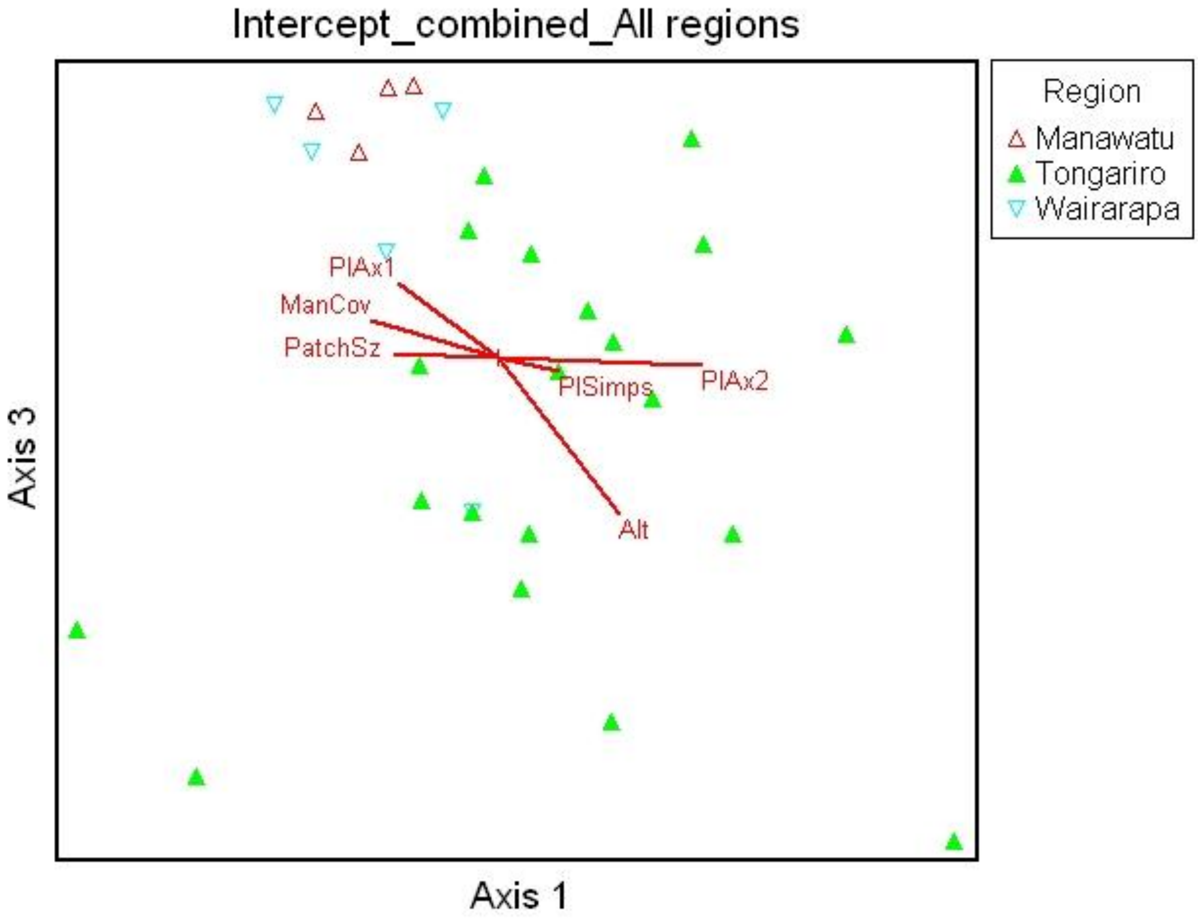

Figure 4.8: Three-Dimensional NMS ordination for insect families from combined intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = Altitude, PlAx1 = Plant ordination axis 1 scores, Plax2 = Plant ordination axis 2 scores, ManCov = Proportion manuka cover (%), PatchSz = Log$_{10}$ patch size (ha), and PlSimps = Plant Simpsons Index.

Correlations between insect family composition and other patch characteristics are shown as vectors overlaid on the ordination graphs (Figure 4.7 & 4.8). The ordination axes were correlated with altitude, plant ordination axis 1 and 2 scores, proportion manuka cover, patch size, and plant evenness. Altitude, plant ordination axis 2 scores, and plant evenness were positively correlated with axis 1 and were higher at sites within Tongariro. Proportion manuka cover (%), log$_{10}$ patch size (ha), and scores from plant ordination axis 1 were negatively correlated with axis 1 and therefore higher at sites within the Manawatu, Wairarapa, and a few sites within Tongariro. Altitude was also negatively correlated with axis 3, and plant ordination axis 1 scores positively correlated with axis 3 (Table 4.7; Figure 4.7 & 4.8).

Table 4.7: Patch variables influencing insect family composition for the combined intercept traps within all three regions. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2	Axis 3
Altitude	**0.527**	0.046	**-0.596**
Prop. Manuka cover	**-0.539**	-0.072	0.286
Plant Richness	0.191	0.299	-0.077
Plant Simpsons Index	**0.371**	0.262	-0.182
Patch Size	**-0.489**	-0.207	0.098
Plant Ordination Axis 1	**-0.476**	0.019	**0.410**
Plant Ordination Axis 2	**0.682**	-0.232	-0.130

Taxonomic differences tended to be separated by axis 3, which also separated the regions and was negatively correlated with altitude (Table 4.7 & 4.8; Figure 4.8). All insect families with a critical r value of 0.470 or greater were considered. All the significant Coleoptera families were positively correlated with axis 3, indicating they were more common at the low altitude sites in the Manawatu and Wairarapa. Chrysomelid and staphylinid beetles were also negatively correlated with axis 1, so were also more common at some sites within Tongariro with high manuka cover and larger patch size. Dipteran families showed similar trends with all but one, the Mycetophilidae, positively correlated with axis 3. The mycetophilids, fungus gnats, were negatively correlated with axis 1, so were more common not only in the Manawatu and Wairarapa, but among sites within Tongariro with higher manuka cover (Table 4.8; Figure 4.7).

All four significant Hymenoptera families were correlated with axis 3, as were the Lepidoptera from the families Crambidae and Geometridae. However, one Lepidopteran family, the Noctuidae, were negatively correlated with axis 2 (an axis that didn't correlate significantly with any patch variable), and found at only two sites within Tongariro. Four families of Hemiptera were positively correlated with axis 3, whereas family Cicadellidae were negatively correlated with axis 1 and hence more common at outlying Tongariro and high manuka cover sites (Table 4.8; Figure 4.7). Overall, the Manawatu and Wairarapa regions tend to have quite distinct uniform assemblages, at the order level, of invertebrates compared to Tongariro, in which a lot more variation in composition occurs.

Table 4.8: Insect families significantly correlated with at least one of the 3 ordination axes for the intercept combined data from all three regions. Families that were not significantly correlated with one of the three axes are not shown. The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2	Axis 3
Coleoptera	Corticariidae	Scavenger beetles	-0.330	0.105	**0.745**
	Corylophidae	Minute fungus beetles	-0.453	-0.020	**0.522**
	Cryptophagidae	Silken fungus beetles	-0.327	0.022	**0.734**
	Chrysomelidae	Leaf beetles	**-0.502**	-0.032	**0.588**
	Curculionidae	Weevils	-0.112	0.423	**0.500**
	Elateridae	Click beetles	-0.404	0.139	**0.573**
	Melyridae	Soft-winged flower beetles	0.147	0.260	**0.541**
	Mordellidae	Tumbling flower beetles	-0.180	-0.292	**0.496**
	Salpingidae	Narrow-waisted bark beetles	-0.376	0.038	**0.514**
	Scarabaeidae	Scarabs	0.043	0.140	**0.567**
	Staphylinidae	Rove beetles	**-0.545**	0.110	**0.611**
Diptera	Cecidomyiidae	Gall midges	**-0.471**	0.270	**0.475**
	Chironomidae	Midges	-0.291	0.419	**0.666**
	Dolichopodidae	Long-legged flies	-0.215	-0.022	**0.562**
	Mycetophilidae	Fungus gnats	**-0.623**	0.101	0.246
	Phoridae	Hump-backed flies	-0.172	0.221	**0.615**
	Psychodidae	Moth flies	-0.389	0.012	**0.504**
	Sciaridae	Dark-winged fungus gnats	-0.097	0.096	**0.596**
Hymenoptera	Aphelinidae	-	-0.421	0.024	**0.508**
	Bethylidae	-	-0.186	0.059	**0.581**
	Pompilidae	Spider wasps	-0.384	0.058	**0.579**
	Scelionidae	Scelionid wasps	-0.414	0.298	**0.571**
Hemiptera	Aphidae	Aphids	-0.302	0.137	**0.587**
	Cercopidae	Spittle bugs	-0.148	0.195	**0.571**
	Cicadellidae	Leafhoppers	**-0.586**	0.005	-0.295
	Cicadidae	Cicadas	-0.208	0.014	**0.531**
	Miridae	Plant bugs	-0.213	0.148	**0.590**
Lepidoptera	Crambidae	Snout moths	-0.260	0.160	**0.511**
	Geometridae	Geometrid moths	-0.349	-0.004	**0.529**
	Noctuidae	Noctuid moths	0.137	**-0.486**	-0.137

4.3.4 Intercept combined trap ordination for Tongariro only

MRPP tests were also run to evaluate which categorical factors were significantly affecting insect family assemblages in Tongariro (Table 4.9). Of the two categorical variables, neither surrounding land use nor surrounding manuka frequency was significant. However, surrounding land use was a better predictor; therefore land use was used to group the composition of insect families for intercept and pitfall trap data from Tongariro.

Table 4.9: Multi-Response Permutation Procedure (MRPP) tests of categorical data as predictors of intercept and pitfall trap assemblages within Tongariro only. "A" represents the critical value.

	Intercept combined		**Pitfall**	
	A	**P**	**A**	**P**
Land Use	0.011	0.110	0.007	0.258
Manuka freq.	0.012	0.189	-0.005	0.572

As there was a lot of variation between sites within Tongariro, a 3-dimensional NMS ordination was run to examine site differences within this region, arranged by land use. Results from the Tongariro combined intercept ordination were significant (Monte Carlo p-value = 0.004), final stress value was 10.96. There were no apparent clusters between different land use types, but there were some distinct outliers. The two sites at the bottom left hand corner of figure 9 had a higher composition of Hemiptera from the family Cicadellidae, and were associated with larger patch size (ha). The site in the top left hand corner and midway down Axis 2 on the left hand side also had a higher composition of cicadellids (Figure 4.9). The three outliers shown in the right hand side of figure 4.10 had a higher composition of mordellid beetles and were associated with higher plant ordination axis 2 scores.

The ordination axes for the Tongariro intercept traps were correlated with plant ordination axis 2 scores and $\log_{10}$ patch size (ha). Plant ordination axis 2 scores were positively associated with axis 2 and $\log_{10}$ patch size (ha) negatively associated with axis 1 (Table 4.10; Figure 4.9 & 4.10).

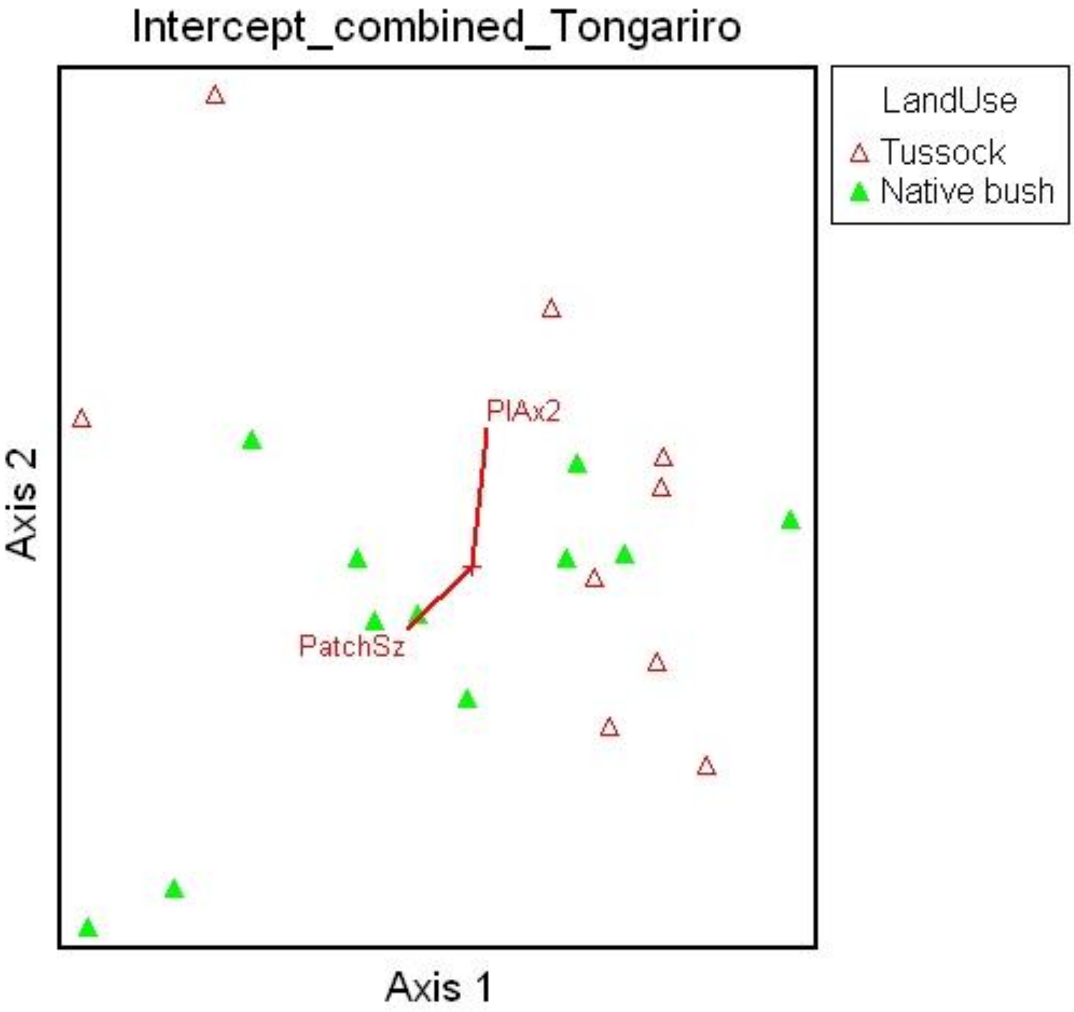

Figure 4.9: Three-Dimensional NMS ordination for insect families from combined intercept traps within Tongariro only. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. PlAx2 = Plant ordination axis 2 scores and PatchSz = Log_{10} patch size (ha).

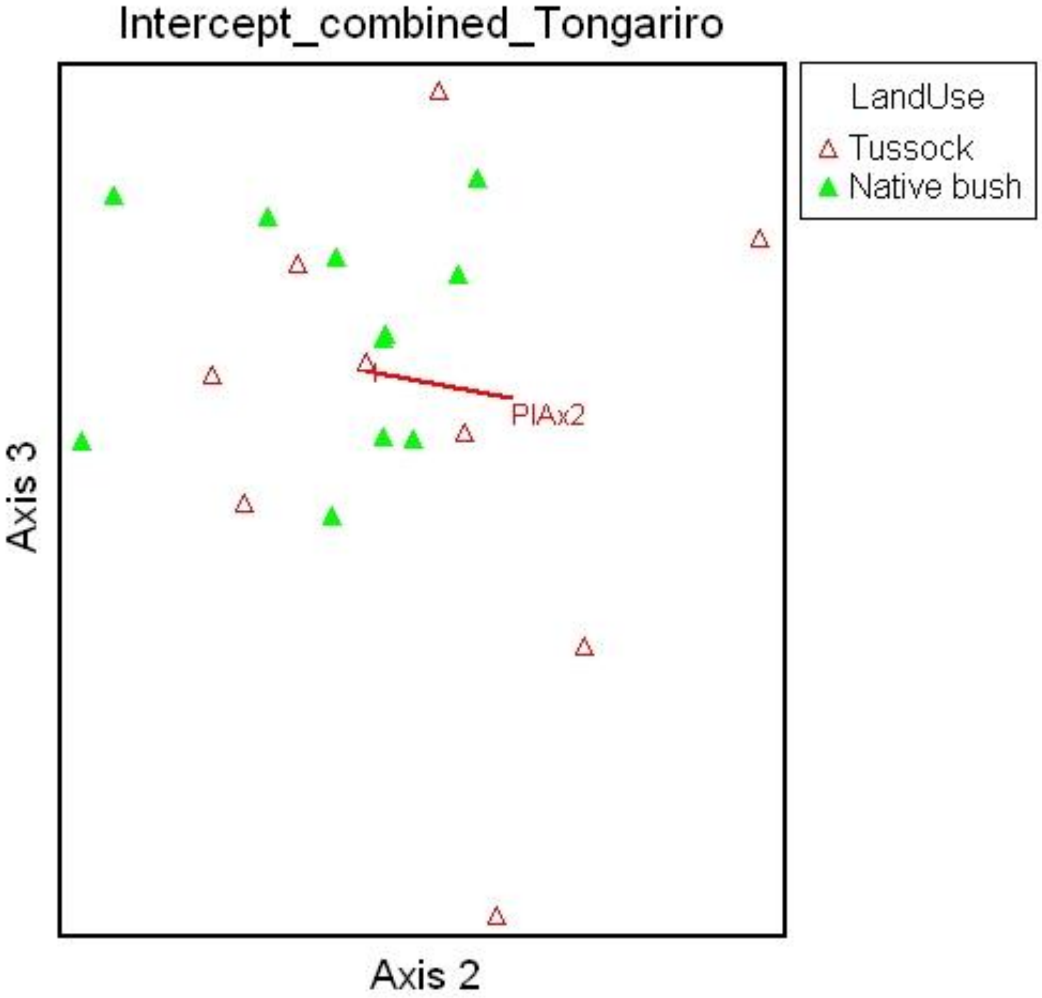

Figure 4.10: Three-Dimensional NMS ordination for insect families from combined intercept traps within Tongariro only. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Vectors are scaled in proportion to the correlation coefficient, e.g. the correlation between PlAx2 and axis 2 is 0.651 in the negative direction. PlAx2 = Plant ordination axis 2 scores and PatchSz = Log_{10} patch size (ha).

Table 4.10: Patch variables influencing insect family composition for the combined intercept traps at Tongariro. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2	Axis 3
Altitude	0.093	0.208	0.179
Prop. Manuka cover	-0.220	-0.341	-0.153
Plant Richness	0.268	-0.030	0.337
Plant Simpsons Index	0.378	0.006	0.261
Patch Size	**-0.396**	-0.390	-0.282
Plant Ordination Axis 1	-0.056	-0.200	-0.031
Plant Ordination Axis 2	0.189	**0.578**	-0.251

Taxonomic differences among the Tongariro sites tended to be separated by axis 1 (Table 4.11). All insect families with a critical r value of 0.561 or greater are shown. Coleoptera from the family Melyridae, Diptera from families Ceratopogonidae, Chironomidae, and Empididae, as well as the Apidae were positively associated with axis 1. Hemiptera from the family Cicadellidae were negatively associated with axis 2, and Coleoptera from the family Mordellidae were negatively associated with axis 3 (Table 4.11). Both these groups were higher in composition at the outlying sites described from figure 4.9 & 4.10.

Table 4.11: Insect families significantly correlated with at least one of the 3 ordination axes for both chambers of intercept traps at Tongariro. Families that were not significantly correlated with one of the three axes are not shown.The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2	Axis 3
Coleoptera	Melyridae	Soft-winged flower beetles	**0.621**	-0.029	-0.098
	Mordellidae	Tumbling flower beetles	0.089	0.144	**-0.653**
Diptera	Ceratopogonidae	Biting midges	**0.723**	-0.055	0.241
	Chironomidae	Midges	**0.607**	-0.346	0.299
	Empididae	Dance flies	**0.590**	-0.056	0.032
	Mycetophilidae	Fungus gnats	-0.066	-0.691	0.085
Hymenoptera	Apidae	Bees	**0.634**	0.169	-0.165
Hemiptera	Cicadellidae	Leafhoppers	-0.542	**-0.651**	0.172

4.3.5 Intercept bottom chamber ordination

Results from the intercept bottom chambers ordination were significant (Monte Carlo p-value = 0.004). Final stress was 10.98. Similar to the combined intercept traps, the bottom chambers showed that Manawatu, Wairarapa, and Tongariro regions occupied different sectors of the ordination space. This reinforces distinct differences in insect composition among the three regions as the majority of invertebrates were caught in the bottom chamber. Again, sites from the Manawatu and Wairarapa were quite clustered, suggesting little variation in insect family composition, whereas sites within the Tongariro were much more variable (Figure 4.11 & 4.12). There were no distinct family composition accounting for variation of three outlying sites in the right hand side of figure 4.11, however they are associated with higher plant ordination axis 2 scores (Table 4.12; Figure 4.11).

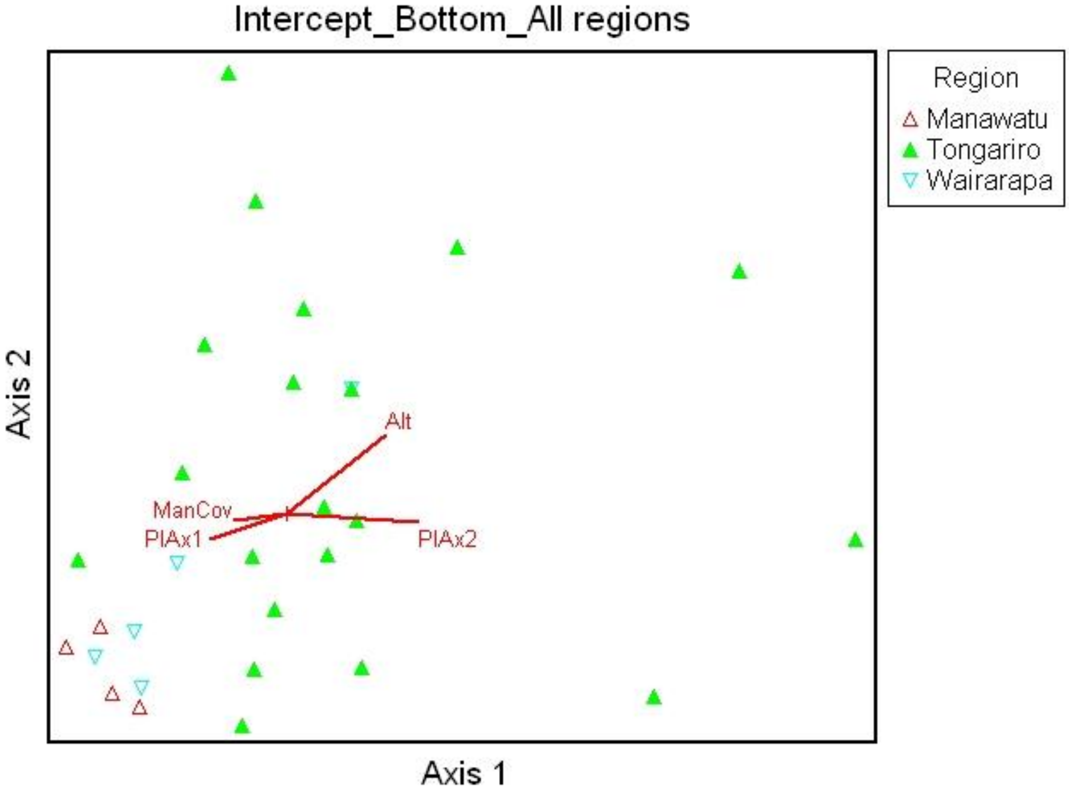

Figure 4.11: Three-Dimensional NMS ordination for insect families from the bottom chamber of the intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = Altitude, Plax1 = Plant ordination axis 1 scores, PlAx2 = Plant ordination axis 2 scores, and ManCov = Proportion manuka cover (%).

There were also three outlying sites present in figure 4.12 when examining axis 3. The bottom two sites are associated with higher altitudes and higher plant ordination axis 2 scores, whereas the site at the top right hand corner of the graph is not strongly associated with any of the patch variables (Table 4.12; Figure 4.12). This site is, however, associated with a higher composition of Hymenoptera from the family Formicidae (Table 4.13; Figure 4.12).

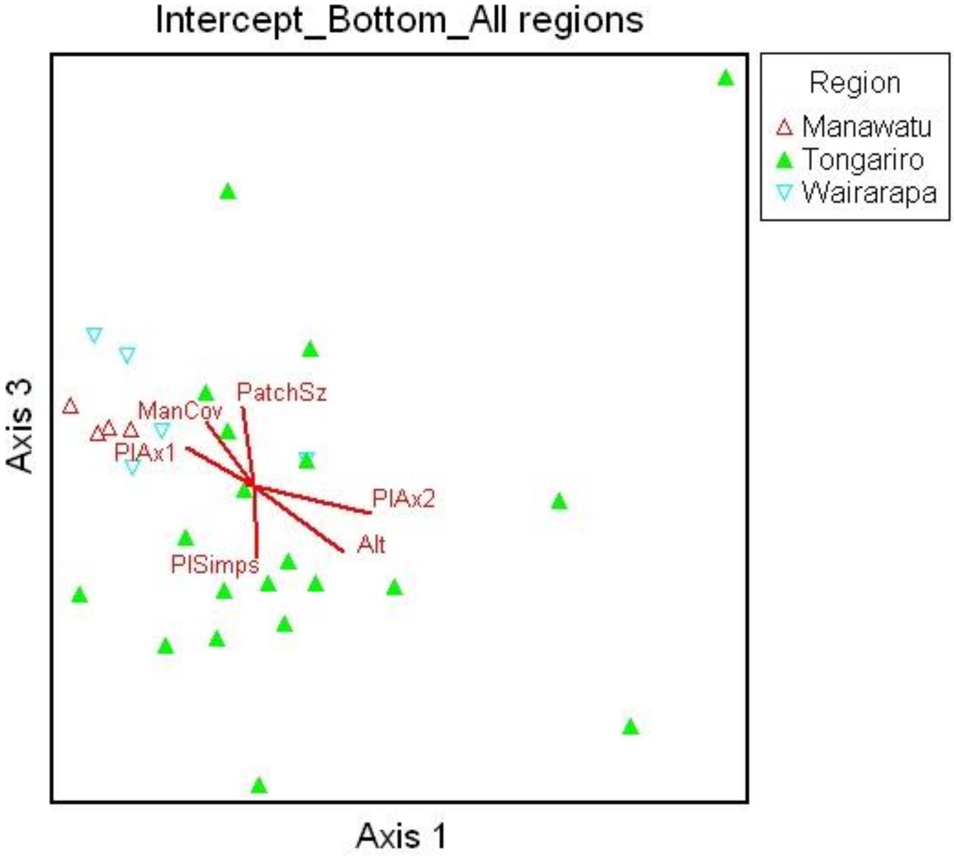

Figure 4.12: Three-Dimensional NMS ordination for insect families from the bottom chamber of the intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations.Alt = Altitude, Plax1 = Plant ordination axis 1 scores, PlAx2 = Plant ordination axis 2 scores, ManCov = Proportion manuka cover (%), PatchSz = Log$_{10}$ patch size (ha), and PlSimps = Plant Simpson's Index.

The ordination axes for the bottom chambers of the intercept traps were correlated with altitude, plant ordination axis 1 and 2 scores, proportion manuka cover, patch size, and plant evenness (Figure 4.11 & 4.12) similar to the combined intercept results. Again, altitude and plant ordination axis 2 scores were positively associated with axis 1 and were higher at sites within Tongariro. Proportion manuka cover (%) and scores from plant ordination axis 1 were negatively associated with axis 1 and therefore higher at sites within the Manawatu, Wairarapa, and a few sites within Tongariro (Table 4.12; Figure 4.11 & 4.12). Altitude was also positively associated with axis 2 (Table 4.12; Figure 11) and together with plant evenness was negatively associated with axis 3, whereas proportion manuka cover and $\log_{10}$ patch size were positively associated with axis 3 (Table 4.12; Figure 4.12).

Table 4.12: Patch variables influencing insect family composition for the intercept bottom chambers for all three regions. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2	Axis 3
Altitude	**0.503**	**0.446**	**-0.432**
Prop. Manuka cover	**-0.368**	-0.134	**0.427**
Plant Richness	-0.054	0.084	-0.348
Plant Simpsons Index	0.111	0.124	**-0.447**
Patch Size	-0.177	0.013	**0.474**
Plant Ordination Axis 1	**-0.440**	-0.259	0.333
Plant Ordination Axis 2	**0.577**	-0.142	-0.283

Most of the taxonomic variation for the intercept bottom chambers were separated by axes 1 and 2 (Table 4.13). From the Coleoptera, families Coricariidae, Cryptophagidae, Chrysomelidae, Curculionidae, Elateridae, Mycetophagidae, and Staphylinidae were significantly negatively correlated with axis 1. Diptera from the families Cecidomyiidae, Chironomidae, Phoridae, and Tachinidae, along with Hymenoptera from the families Pompilidae and Scelionidae, and Hemiptera from the family Aphidae were also negatively correlated with axis 1 (Table 4.13; Figure 14.1).

Coleoptera from the families Corticariidae, Cryptophagidae were also negatively associated with axis 2 as well as families Melyridae, Mordellidae, and Scarabaeidae; Diptera families Chironomidae, Dolichopodidae, Phoridae, and Sciaridae; Hymneoptera families Apidae and Bethylidae; and Hemiptera families Cercopidae, Cicadellidae, Cicadidae, and Miridae and consequently higher in composition among sites from the Manawatu and Wairarapa and certain sites within Tongariro (Table 4.13; Figure 4.11).

Finally, Coleoptera from the family Scirtidae and Diptera family Tipulidae were negatively associated with axis 3 so higher in composition at sites within Tongariro, whereas family Formicidae from the Hymenoptera were positively associated with axis 3 (Table 4.13; Figure 4.12).

Table 4.13: Insect families significantly correlated with at least one of the 3 ordination axes for the intercept bottom chamber data from all three regions. Families that were not significantly correlated with one of the three axes are not shown. The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2	Axis 3
Coleoptera	Corticariidae	Scavenger beetles	**-0.551**	**-0.628**	0.210
	Cryptophagidae	Silken fungus beetles	**-0.491**	**-0.636**	0.267
	Chrysomelidae	Leaf beetles	**-0.494**	-0.443	0.405
	Curculionidae	Weevils	**-0.534**	-0.378	-0.186
	Elateridae	Click beetles	**-0.544**	-0.421	0.216
	Melyridae	Soft-winged flower beetles	-0.304	**-0.540**	-0.230
	Mordellidae	Tumbling flower beetles	-0.114	**-0.513**	0.378
	Mycetophagidae	Hairy fungus beetles	**-0.508**	-0.024	0.351
	Scarabaeidae	Scarabs	-0.295	**-0.561**	-0.073
	Scirtidae	Marsh beetles	0.159	0.147	**-0.524**
	Staphylinidae	Rove beetles	**-0.618**	-0.420	0.331
Diptera	Cecidomyiidae	Gall midges	**-0.624**	-0.269	0.154
	Chironomidae	Midges	**-0.697**	**-0.489**	-0.052
	Dolichopodidae	Long-legged flies	-0.333	**-0.508**	0.211
	Phoridae	Hump-backed flies	**-0.485**	**-0.521**	0.012
	Sciaridae	Dark-winged fungus gnats	-0.356	**-0.554**	0.052
	Tachinidae	Bristle flies	**-0.518**	-0.330	-0.060
	Tipulidae	Crane flies	0.066	0.177	**-0.561**
Hymenoptera	Apidae	Bees	-0.039	**-0.503**	-0.280
	Bethylidae	-	-0.376	**-0.518**	0.135
	Formicidae	Ants	-0.014	-0.159	**0.537**
	Pompilidae	Spider wasps	**-0.483**	-0.452	0.263
	Scelionidae	Scelionid wasps	**-0.650**	-0.380	0.106
Hemiptera	Aphidae	Aphids	**-0.491**	-0.469	0.153
	Cercopidae	Spittle bugs	-0.438	**-0.490**	0.010
	Cicadellidae	Leafhoppers	-0.206	**0.493**	0.343
	Cicadidae	Cicadas	-0.339	**-0.470**	0.177
	Miridae	Plant bugs	-0.451	**-0.499**	0.088

4.3.6 *Intercept top chamber ordination*

Results from the top chamber of the intercept traps were significant (Monte Carlo p-value = 0.028). The final stress value was 17.86. The regions were less clustered than those for the previous intercept trap ordinations, but the Manawatu sites were again clustered close together and there was still some overlap among the Manawatu and Wairarapa regions (Figure 4.13 & 4.14). One particular site from the Wairarapa is separated from the other sites within this region. No patch characteristics could explain the variation, but this site appears to have a higher composition of families Psyllidae and Cicadellidae (Table 4.15; Figure 4.14).

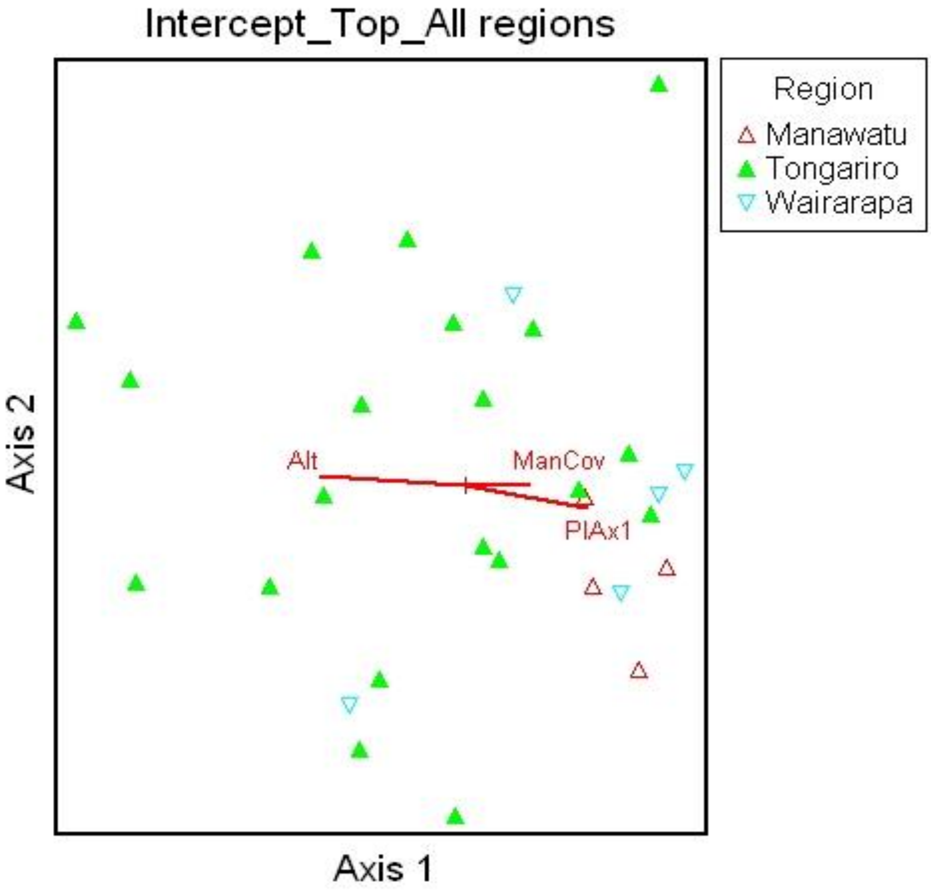

Figure 4.13: Three-Dimensional NMS ordination for insect families from the top chamber of the intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = altitude, ManCov = Proportion manuka cover (%), and PlAx1 = Plant ordination axis 1 scores.

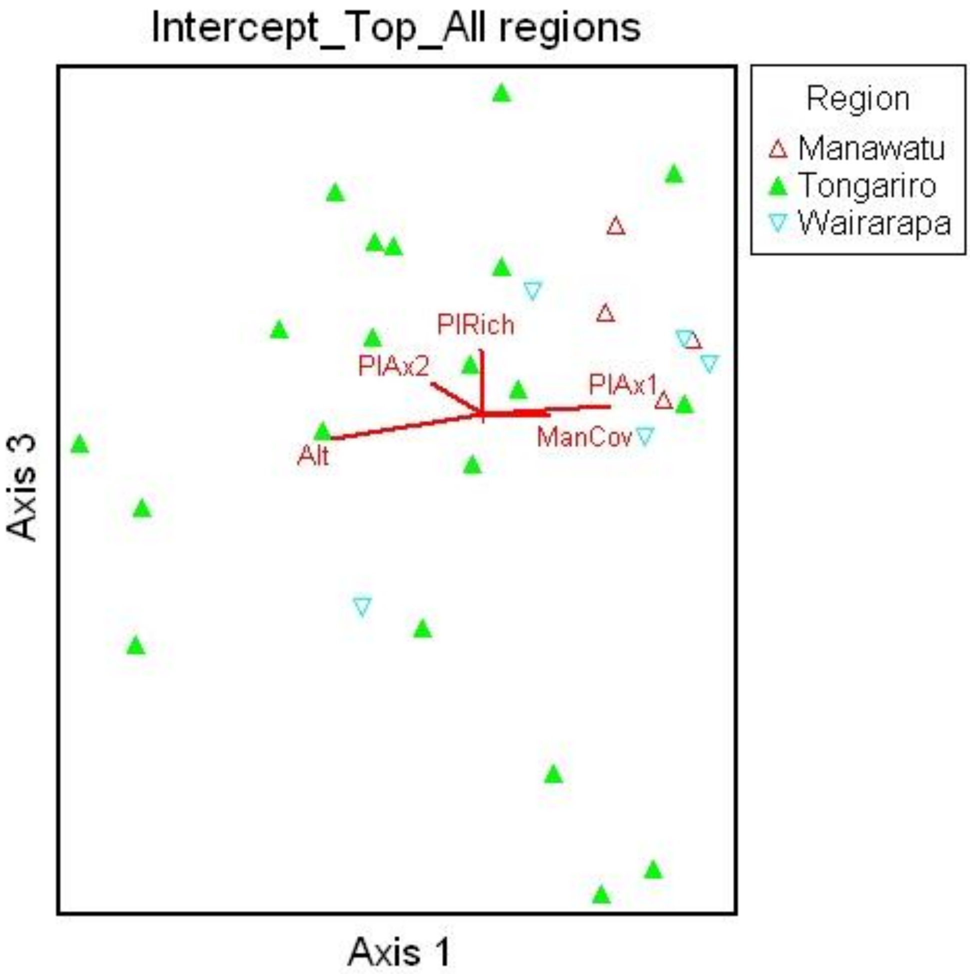

Figure 4.14: Three-Dimensional NMS ordination for insect families from the top chamber of the intercept traps. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = altitude, PlAx1 = Plant ordination axis 1 scores PlAx2 = Plant ordination axis 2 scores, PlRich = Plant richness, and ManCov = Proportion manuka cover (%).

Correlations between insect family composition and patch variables could be seen in the vectors overlaid in the top chamber ordinations. The ordination axes were correlated with altitude, plant ordination axis 1 & 2 scores, proportion manuka cover (%), and plant richness (Figure 13 & 14). Altitude was negatively associated with axis 1. The proportion manuka cover (%) and plant ordination axis 1 scores were positively associated with axis 1, so were higher at sites within the Manawatu and Wairarapa (Table 4.14; Figure 4.13 & 4.14). Plant ordination axis 2 scores and plant richness were positively associated with axis 3 (Table 4.14; Figure 4.14).

Table 4.14: Patch variables influencing insect family composition for intercept top chambers for all three regions. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2	Axis 3
Altitude	**-0.614**	0.158	-0.247
Prop. Manuka cover	**0.407**	0.017	-0.068
Plant Richness	-0.067	0.028	**0.404**
Plant Simpsons Index	-0.320	-0.103	0.119
Patch Size	0.261	-0.084	-0.156
Plant Ordination Axis 1	**0.562**	-0.246	0.140
Plant Ordination Axis 2	-0.359	0.120	**0.379**

Much of the taxonomic variation among the top chambers of the intercept traps were separated by axis 1 & 3 (Table 4.15). There were no significant Coleoptera families occurring in the top chambers, but families from the orders Diptera, Hymenoptera, and Hemiptera were represented. Diptera from the family Psychodidae and Hymenoptera from the families Aphelinidae and Scelionidae were significantly positively correlated with axis 1, indicating they were comprised a higher composition of assemblages of sites within the Manawatu, Wairarapa, and some sites within Tongariro and therefore associated with sites with a higher proportion of manuka cover and plant ordination axis 1 scores.

In contrast, Hemiptera from the family Psyllidae were negatively correlated with axis 1, so were higher in composition at sites within Tongariro and that were higher in altitude (Table 4.15; Figure 4.13 & 4.14). Family Scelionidae were also negatively correlated with axis 2 so were higher in composition at sites within the Manawatu (Table 4.15; Figure 4.13).

Diptera from the families Phoridae and Sciaridae were positively correlated with axis 3, suggesting they were higher in composition at sites among all three regions and that were associated with a higher value of plant richness, whereas Hemiptera from the family Cicadellidae were negatively associated with axis 3, and therefore higher in composition at sites within Tongariro and one particular site within the Wairarapa (Table 4.15; Figure 4.14).

Table 4.15: Insect families significantly correlated with one of the 3 ordination axes for the intercept top trap data from all three regions. Families that were not significantly correlated with one of the three axes are not shown. The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2	Axis 3
Diptera	Phoridae	Hump-backed flies	0.098	0.013	**0.681**
	Psychodidae	Moth flies	**0.496**	0.005	0.236
	Sciaridae	Dark-winged fungus gnats	0.187	-0.087	**0.487**
Hymenoptera	Aphelinidae	-	**0.542**	-0.159	0.171
	Scelionidae	-	**0.471**	**-0.566**	0.421
Hemiptera	Cicadellidae	Leafhoppers	0.335	-0.022	**-0.559**
	Psyllidae	Jumping plant lice	**-0.690**	0.307	-0.028

4.3.7 Pitfall trap diversity

Invertebrate richness for the pitfall traps among sites was significantly influenced by region (p = 0.010) and increased with the proportion of manuka cover within the patch (p = 0.018) (Table 4.16; Figure 4.15).

Table 4.16: Multiple regression of invertebrate taxa richness against environmental factors for pitfall traps from all regions. Significant values are shown in bold.

	df	Deviance	F	P
Region	**3**	**147.89**	**5.57**	**0.010**
Land Use	2	37.90	2.14	0.154
Manuka freq.	2	11.03	0.62	0.551
Altitude	1	8.34	0.94	0.348
Prop. Manuka cover	**1**	**63.76**	**7.20**	**0.018**
Plant Richness	1	26.76	3.02	0.104
Plant Evenness	1	2.92	0.33	0.575
Patch size	1	0.80	0.09	0.768
Plant Ordination Axis 1	1	3.95	0.45	0.515
Plant Ordination Axis 2	1	5.40	0.61	0.448
Residual	14	123.93		

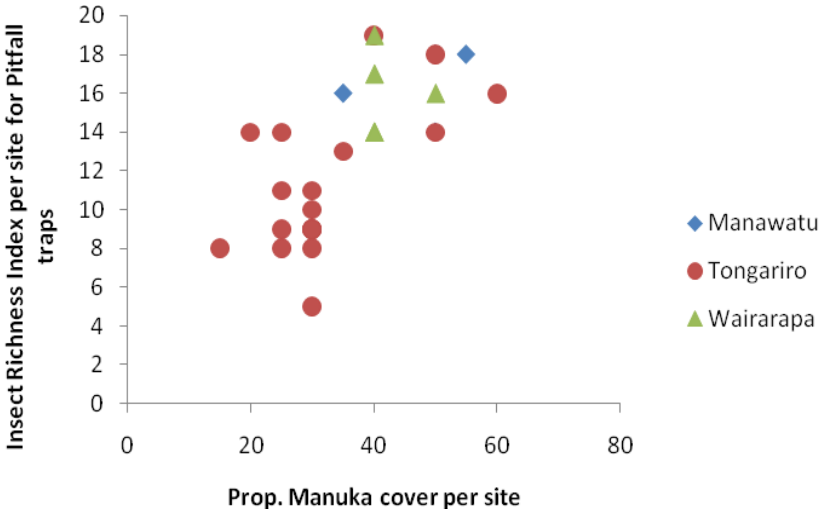

Figure 4.15: The relationship between insect richness and proportion manuka cover (r = 0.688; p = <0.001) for pitfall traps with regions shown by colour symbols.

Invertebrate richness among pitfall traps within Tongariro were also significantly related to proportion manuka cover, but not significantly related to altitude like the intercept traps (Table 4.17). Much like the results for all regions, insect richness within Tongariro was positively correlated with proportion manuka cover (r = 0.627; p = 0.003) (Figure 4.16).

Table 4.17: Multiple regression of invertebrate taxa richness against environmental factors for pitfall traps from Tongariro only. Significant values are shown in bold.

	df	**Deviance**	**F**	**P**
Land Use	1	18.618	1.659	0.230
Manuka freq.	2	24.821	1.106	0.372
Altitude	1	9.742	0.868	0.376
Prop. Manuka cover	**1**	**67.874**	**6.049**	**0.036**
Plant Richness	1	14.653	1.306	0.283
Plant Evennes	1	5.304	0.473	0.509
Patch size	1	4.828	0.430	0.528
Plant Ordination Axis 1	1	11.279	1.005	0.342
Plant Ordination Axis 2	1	0.690	0.062	0.810
Residual	9	100.992		

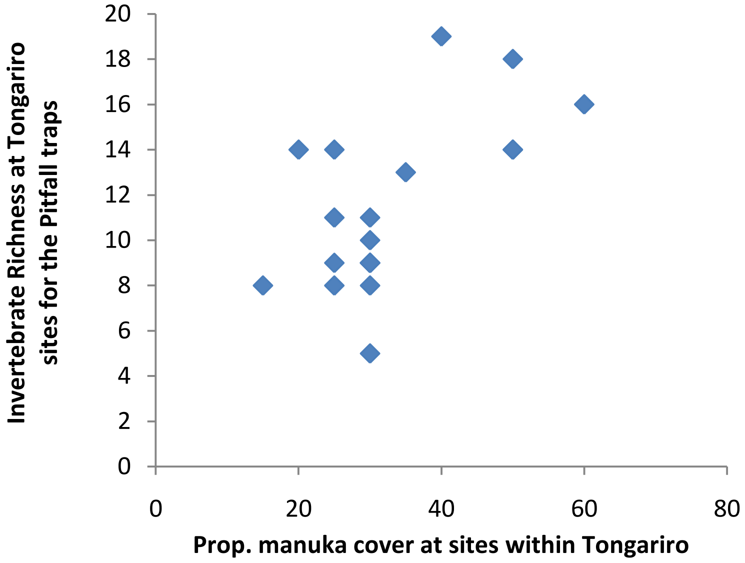

Figure 4.16: The relationship between invertebrate richness and proportion manuka cover for pitfall traps at the Tongariro sites.

Insect evenness (Simpson's Index) on the other hand, was most significantly related to plant evenness among patches within all the regions in the multivariate analysis (Table 4.18).

Table 4.18: Multiple regression of invertebrate taxa evenness against environmental factors for pitfall traps from all regions. Significant values are shown in bold.

	df	Deviance	F	P
Region	3	0.108	1.54	0.248
Land Use	2	0.061	1.31	0.302
Manuka freq.	2	0.012	0.25	0.779
Altitude	1	0.037	1.57	0.231
Prop. Manuka cover	1	0.052	2.23	0.158
Plant Richness	1	0.001	0.05	0.834
Plant Evenness	**1**	**0.190**	**8.11**	**0.013**
Patch size	1	0.054	2.28	0.153
Plant Ordination Axis 1	1	0.056	2.40	0.143
Plant Ordination Axis 2	1	0.091	3.86	0.070
Residual	14	0.328		

However, when examining the univariate correlation between insect evenness and plant evenness there was only a weak negative correlation (r = -0.183; p = 0.332) (Figure 4.17).

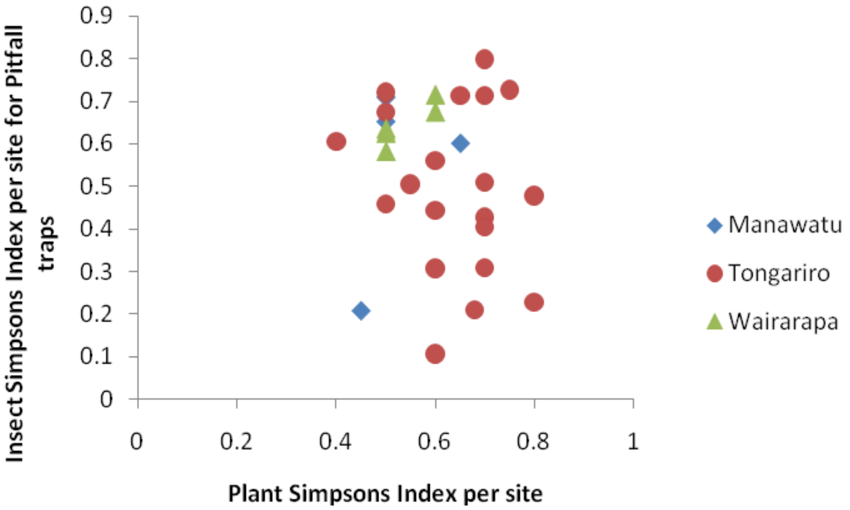

Figure 4.17: The relationship between invertebrate evenness and plant evenness (using Simpsons Index) for pitfall traps with regions shown by colour symbols.

When looking solely at taxa evenness within Tongariro, plant evenness and proportion manuka cover are significant drivers in the multiple regressions (Table 4.19). However, much like the results for all regions, the univariate relationship between invertebrate evenness and plant evenness was a weak negative correlation (r = -0.186; p = 0.432) (Figure 4.18).

Table 4.19: Multiple regression of invertebrate taxa evenness against environmental factors for pitfall traps from Tongariro only. Significant values are shown in bold.

	df	Deviance	F	P
Land Use	1	0.025	1.055	0.331
Manuka freq.	2	0.018	0.376	0.697
Altitude	1	0.044	1.868	0.205
Prop. Manuka cover	**1**	**0.124**	**5.235**	**0.048**
Plant Richness	1	0.003	0.107	0.751
Plant Evenness	**1**	**0.197**	**8.344**	**0.018**
Patch size	1	0.050	2.096	0.182
Plant Ordination Axis 1	1	0.021	0.903	0.367
Plant Ordination Axis 2	1	0.039	1.667	0.229
Residual	9	0.212		

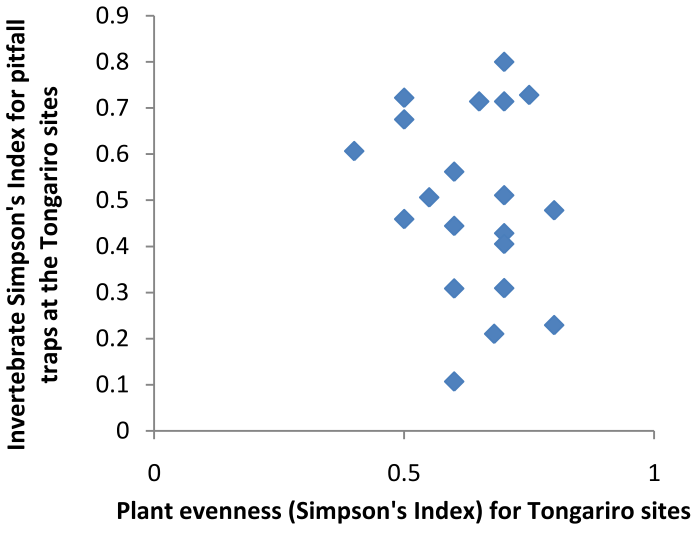

Figure 4.18: The relationship between invertebrate evenness and plant evenness for pitfall traps at the Tongariro sites.

The relationship between invertebrate evenness and proportion manuka cover on the other hand, showed a stronger positive correlation (r = 0.412; p = 0.071) (Figure 4.19). Therefore, within Tongariro, plant evenness did not have a major influence on invertebrate evenness, but proportion manuka cover does appear to affect invertebrate evenness to a degree.

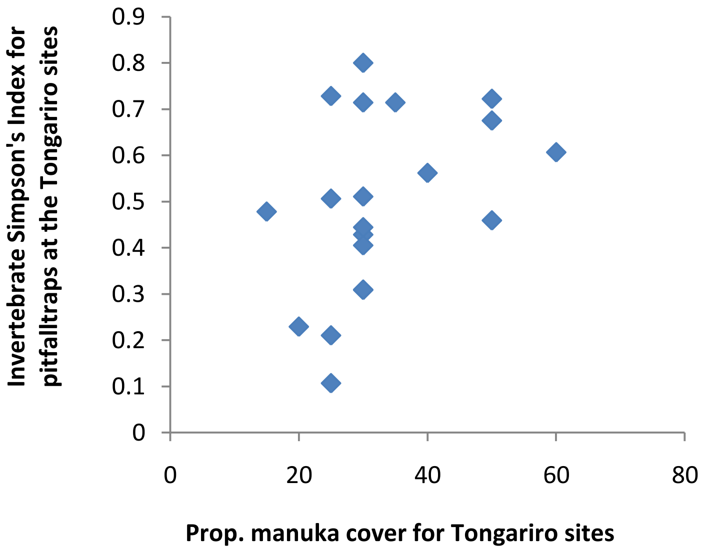

Figure 4.19: The relationship between invertebrate evenness and proportion manuka cover for pitfall traps at the Tongariro sites.

4.3.8 *Pitfall trap ordination*

As shown in Table 4.6, the strongest categorical predictor of insect family composition for the pitfall traps was region; hence it is used to display the ordination results.

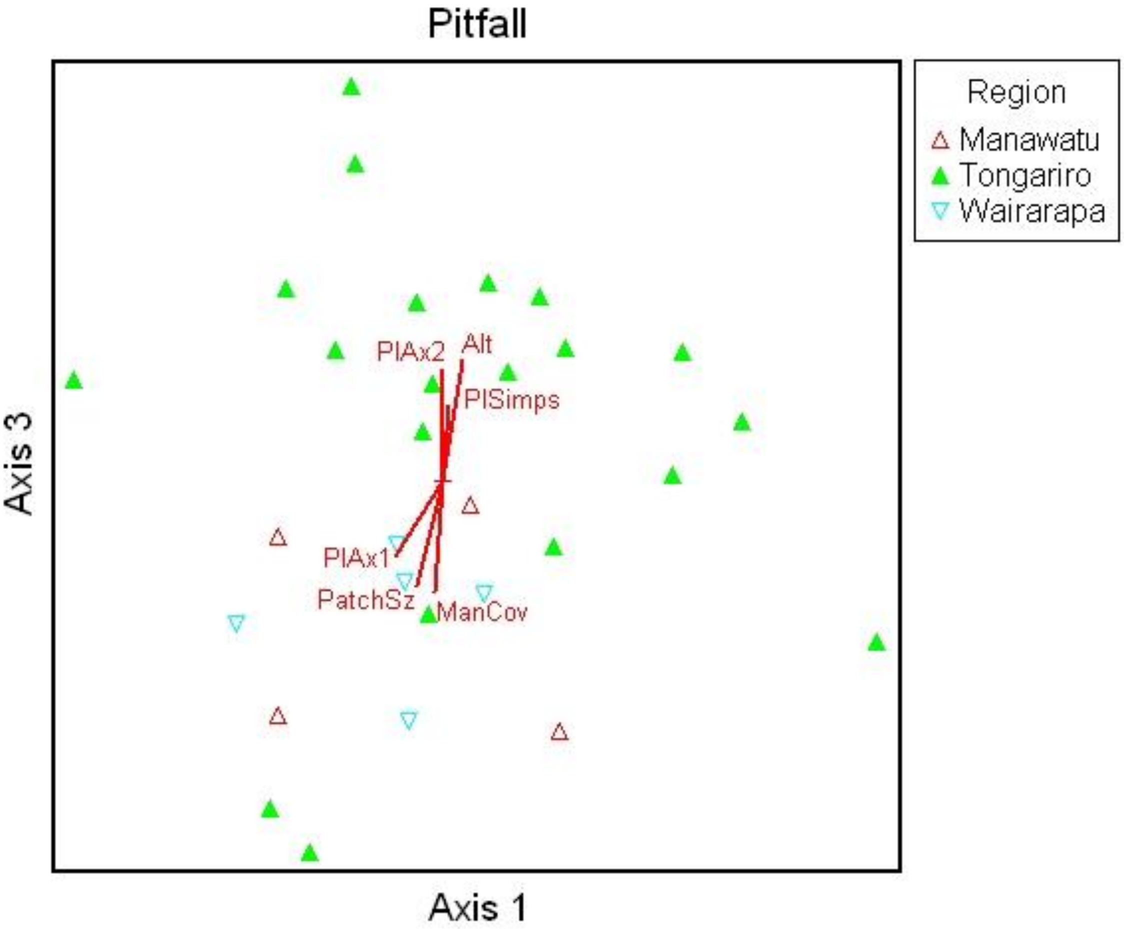

Figure 4.20: Three-Dimensional NMS ordination for insect families from the pitfall traps at all sites. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = altitude, PlAx1 = Plant ordination axis 1 scores PlAx2 = Plant ordination axis 2 scores, PatchSz = Log_{10} patch size (ha), PlSimps = Plant Simpson's index, and ManCov = Proportion manuka cover (%).

Results from the pitfall trap ordination were significant (Monte Carlo p-value = 0.012), with final stress = 15.069. The three regions still occupied different sectors of the ordination space, displaying similar results with the intercept trap ordinations. There are two outlying Tongariro sites in the left bottom corner of Figure 4.20 & 4.21: Bones on Desert Rd and Desert Rd. These sites were associated with larger patches and a higher proportion of manuka cover (%) (Table 4.20; Figure 4.20 & 4.21), and had a higher composition of coleopteran families Corticariidae and Staphylinidae (Table 4.21).

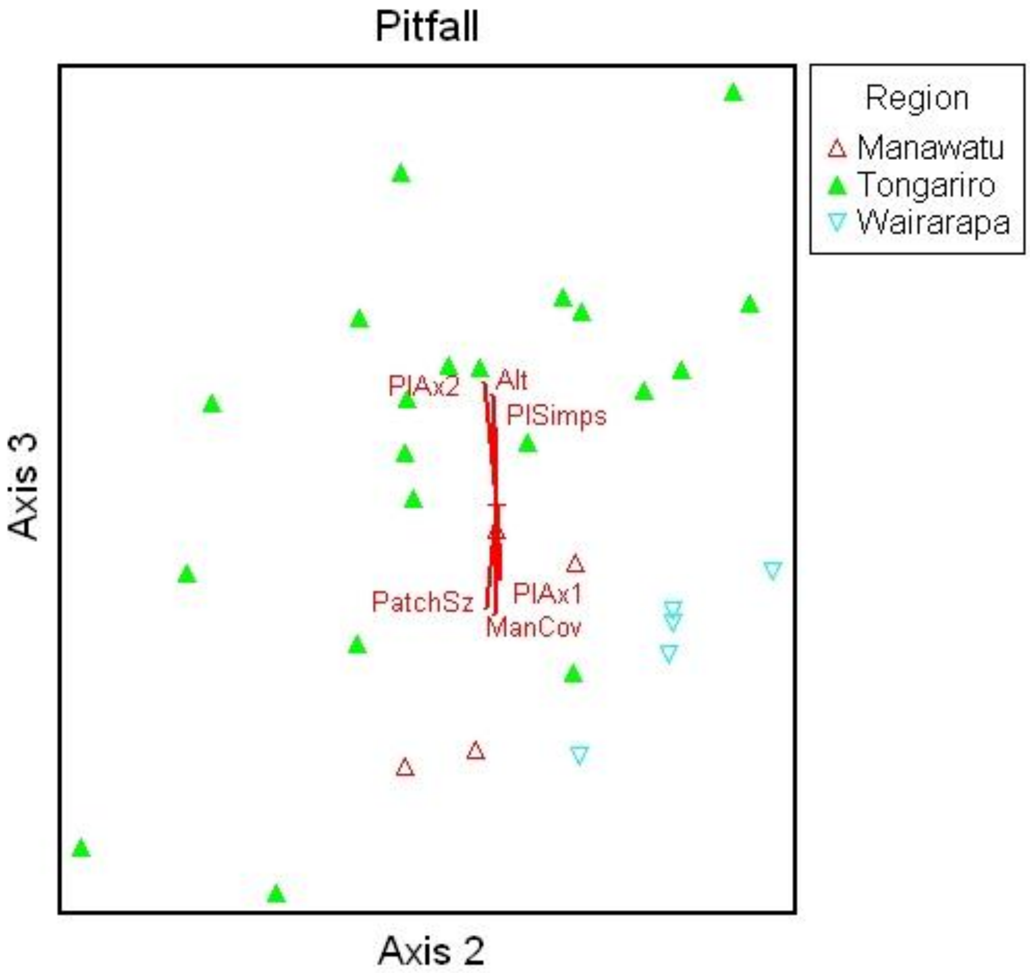

Figure 4.21: Three-Dimensional NMS ordination for insect families from the pitfall traps at all sites. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. Alt = altitude, PlAx1 = Plant ordination axis 1 scores PlAx2 = Plant ordination axis 2 scores, PatchSz = Log$_{10}$ patch size (ha), PlSimps = Plant Simpson's index, and ManCov = Proportion manuka cover (%).

The ordination axes for the pitfall traps were correlated with altitude, scores from plant ordination axis 1 & 2, log$_{10}$ patch size (ha), proportion manuka cover (%), and plant Simpson's index (evenness) (Figure 4.20 & 4.21). Altitude, plant evenness, and plant ordination axis 2 scores were significantly positively correlated with axis 3, whereas proportion manuka cover (%), patch size, and plant ordination axis 1 scores were significantly negatively correlated with axis 3 (Table 4.20; Figure 4.20 & 4.21).

Table 4.20: Patch variables influencing insect family composition for the pitfall traps from all three regions. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2	Axis 3
Altitude	0.217	-0.171	**0.551**
Prop. Manuka cover	-0.142	-0.086	**-0.525**
Plant Richness	0.069	-0.024	0.193
Plant Simpsons Index	0.112	0.049	**0.436**
Patch Size	-0.253	-0.154	**-0.511**
Plant Ordination Axis 1	-0.343	0.097	**-0.434**
Plant Ordination Axis 2	0.027	-0.112	**0.526**

Taxonomic differences for the pitfall trap families were mainly separated by axis 1 & 3 (Table 4.21). All insect families with a critical value of 0.470 or greater are shown. Coleoptera from the families Corticariidae and Staphylinidae were negatively correlated with axis 3, and the staphylinids were also negatively correlated with axis 2. Therefore, they were associated with sites that were higher in patch size, proportion of manuka cover (%), and plant ordination axis 1 scores, and in the case of the staphylinids, more common at some sites within Tongariro (Table 4.21; Figure 4.20 & 4.21). The Diptera family Chironomidae and family Formicidae from the Hymenoptera were positively correlated with axis 1 and Diptera from the family Phoridae were positively correlated with axis 3, so these three families tended to be more common within Tongariro (Table 4.21; Figure 4.20 & 4.21).

Table 4.21: Insect families significantly correlated with one of the 3 ordination axes for the pitfall trap data from all three regions. Families that were not significantly correlated with one of the three axes are not shown. The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2	Axis 3
Coleoptera	Corticariidae	Scavenger beetles	-0.340	0.089	**-0.600**
	Staphylinidae	Rove beetles	-0.235	**-0.633**	**-0.666**
Diptera	Chironomindae	Midges	**0.633**	0.045	0.017
	Phoridae	Hump-backed flies	-0.141	0.060	**0.601**
Hymenoptera	Formicidae	Ants	**0.663**	0.417	0.012

4.3.9 Pitfall trap ordination – Tongariro only

As shown in Table 4.9, neither land use nor surrounding manuka frequency were significant predictors of variation in insect family composition for the pitfall traps at Tongariro. However, land use was stronger than surrounding manuka frequency so is used to graphically display the ordination.

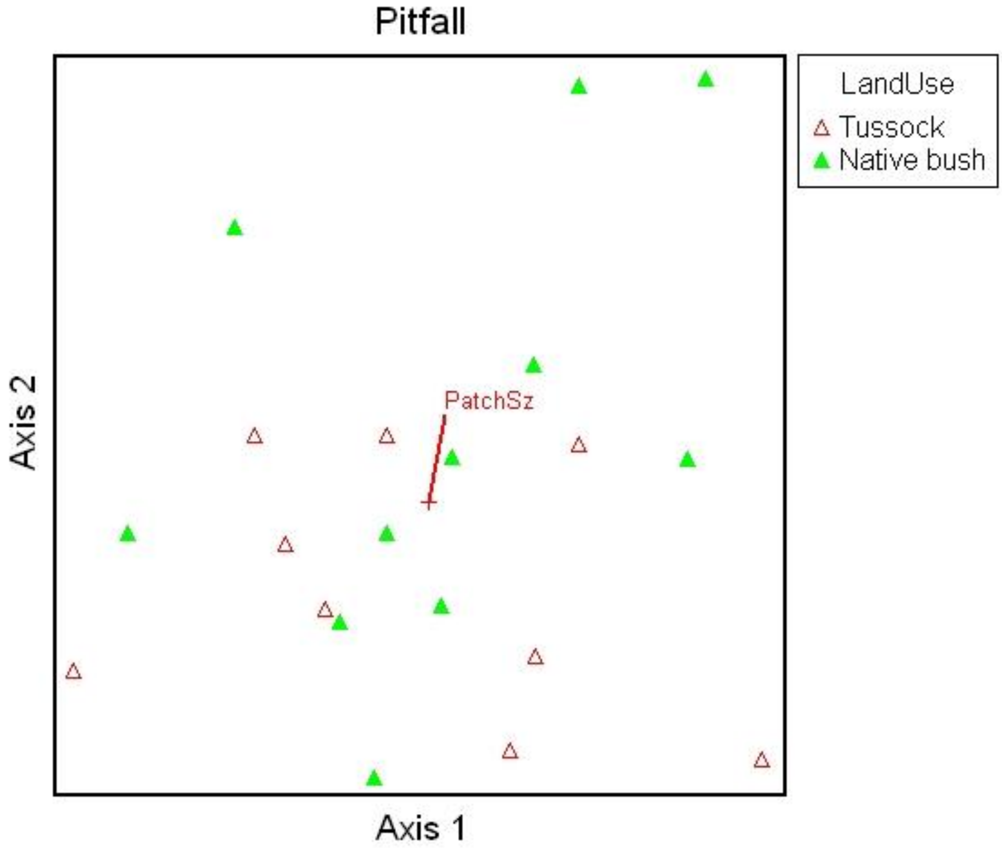

Figure 4.22: Two-Dimensional NMS ordination for insect families from the pitfall traps at Tongariro only. Patch variables that correlated with either axes were overlaid using the pearsons correlation coefficient critical value for the number of sites as a criteria for inclusion. This is not intended as a formal significance test of the associations. PatchSz = Log$_{10}$ patch size (ha).

A 2-dimensional NMS ordination was applied to the pitfall traps from sites within Tongariro for comparison. Results from this ordination were significant (Monte-Carlo p-value = 0.0199), and the final stress was 18.98. There were no obvious clusters between different habitat types; however, similar outliers were occurring as those for the combined region pitfall ordination. The two sites at the top right hand of figure

4.22 were the same two outlying sites in figures 4.20 & 4.21: Bones on Desert Rd and the Desert Rd sites. Again these sites are associated with a higher patch size and families Corticariidae and Staphylinidae (Table 4.22 & 4.23; Figure 4.22). The ordination axes for the Tongariro pitfall traps were correlated with $\log_{10}$ patch size (ha), and is significantly positively correlated with axis 2 (Table 4.22; Figure 4.22).

Table 4.22: Patch variables influencing insect family composition for the pitfall traps within Tongariro. The strongest correlations are shown in bold.

Patch Variables	Axis 1	Axis 2
Altitude	-0.356	-0.257
Prop. Manuka cover	0.295	0.268
Plant Richness	-0.017	-0.050
Plant Simpsons Index	-0.217	-0.225
Patch Size	0.210	**0.496**
Plant Ordination Axis 1	0.098	0.329
Plant Ordination Axis 2	-0.265	-0.066

There were few taxonomic differences at the family level among the pitfall traps within Tongariro, but those that were significant tended to be separated by axis 2 (Table 4.23). All insect families with a critical value of 0.561 or greater are shown. Coleoptera from the families Corticariidae and Staphylinidae were positively correlated with axis 2, with the staphylinids also positively correlated with axis 1, so tended to be more common in larger patches. In contrast, Hymenoptera from the family Formicidae were negatively correlated with axis 2, indicating they were more common in smaller patches (Table 4.23; Figure 4.22).

Table 4.23: Insect families significantly correlated with one of the 2 ordination axes for the pitfall trap data for Tongariro. Families that were not significantly correlated with one of the two axes are not shown. The strongest correlations are shown in bold.

Order	Family	Common name	Axis 1	Axis 2
Coleoptera	Corticariidae	Scavenger beetles	0.079	**0.681**
	Staphylinidae	Rove beetles	**0.564**	**0.740**
Hymenoptera	Formicidae	Ants	0.008	**-0.632**

4.4 Discussion

Taxa from the main pollinating orders were well represented in both the intercept and pitfall traps. Among the study sites 159 Coleoptera, 125 Diptera, and 131 Hymenoptera morphospecies were collected, as well as 50 other groups of taxa from various orders (Appendix 4).

When examining variation of invertebrate taxa caught in the intercept traps, it became apparent that there was a difference between those caught in the top and bottom chambers. This implies variation in behavioural or geotactic responses among invertebrate taxa. Indeed, invertebrates may exhibit a wide variety of tactic responses e.g. moths are positively phototactic, cockroaches negatively phototactic, and flies negatively geotactic; as well as behavioural responses e.g. beetles' drop response to in reaction to disturbance (Carthy 1958).

The majority of invertebrates caught in the bottom chambers of the intercept traps were beetles and larger flies and bees, whereas the majority of insects caught in the top chambers of the intercept traps were smaller non-pollinating taxa and invertebrates from other orders. Larger beetles and flies were more frequently found in the bottom chamber of the intercept traps within the Manawatu and Wairarapa, whereas larger Hymenoptera, especially honeybees, were more commonly found within traps from Tongariro, and for a smaller part, the Wairarapa. This coincided with data from behavioural observations in which a higher number of honeybees were observed visiting the flowers of patches within Tongariro. Many of the insects caught in the top chamber of the intercept traps were smaller beetles, flies, and minute hymenopterans which were not observed visiting manuka flowers. The Araneida (spiders) were one of the more prevalent orders also occurring in both the top and bottom chambers. Lepidopterans (moths and butterflies), were predictably more common in the top chambers of the intercept traps.

The composition of the pitfall traps were more evenly distributed amongst the different orders. The beetles appearing in the pitfall traps were primarily ground beetles from the families Carabidae and Staphylinidae. The diptera were primarily smaller fly species, and the Hymenoptera consisted of a high number of ant species. Pitfalls differed from intercept traps most obviously by the appearance of a wide range of soil invertebrates, namely Amphipoda, Annelida, Chilopoda, Diplopoda, and Platyhelminthes. These results illustrate a large amount of diversity between manuka patches. This can be observed on both a site and regional level, especially within Tongariro, and many patch characteristics appear to be playing a role in the diversity and composition of the taxa.

When examining the intercept traps as a whole, both invertebrate richness and evenness varied significantly between the three regions, with altitude also being an influential factor. Sites within the Manawatu region had the highest level of taxa richness, followed by the Wairarapa, and Tongariro, which had the lowest taxa richness. The problem with inferring correlational effects of altitude on invertebrate richness, however, is that altitude within this study was congruous with region. Sites within the Tongariro were higher in altitude as this area of National Park is a montane region, in contrast to the Wairarapa and Manawatu which are primarily lower altitude farmland. Therefore, analyses of patch characteristics exclusively against the Tongariro sites were conducted. When region was accounted for, altitude still had a significant effect on taxa richness. Hence, it is still apparent that altitude plays a role in invertebrate diversity among the patches, with lower taxa richness with an increase in altitude.

Much like the results for the intercept traps, invertebrate taxa richness within the pitfall traps significantly differed between the regions, and was influenced by the proportion of manuka cover within the patches. This suggests that some invertebrates may have mutualistic relationships with specific plant taxa, as diversity of litter invertebrates was higher within patches with a larger proportion of manuka cover. Invertebrate evenness on the other hand was not significantly different among the regions. Plant evenness was the strongest variable influencing invertebrate evenness; however, the relationship was not significant and negligible at best. Contrary to previous findings, neither patch size (Aizen & Feinsinger 1994; Martinko et al. 2006; Gonzalez-Varo et al. 2009) nor plant species diversity or surrounding habitat type (Hendrickx et al. 2007; Sjodin et al. 2008) had a significant effect on invertebrate taxa diversity. However, in addition to the effects of region, altitude, and proportion of manuka cover on invertebrate diversity, ordinations of insect taxa from both the intercept and pitfall data revealed environmental characteristics that were affecting insect family composition among the regions.

Plant composition, altitude, proportion manuka cover, patch size, and plant evenness were all indicators of the habitat variation influencing insect family composition within insects caught from the intercept traps. Patch size and plant composition were also important drivers of insect family composition among the Tongariro sites. But, as mentioned it is very difficult to distinguish whether differences are due to altitudinal gradients or other environmental factors that are present among the regions, as plant composition differed with altitude among the three regions. Sites within the Manawatu and Wairarapa regions had a higher homogeneity of plant species between them, quite different from the plant composition within Tongariro. The proportion of manuka cover within patches was also typically higher at sites within the Manawatu and Wairarapa which appeared to have a significant effect on invertebrate diversity and insect family composition. This is consistent with previous observations that pollinator richness was higher with an increase in the proportion cover of target plant species within patches (Franzen & Nilsson 2008). This may suggest that plant species composition may be a more important driving factor. However, without further investigation into other altitudinal related affects, such as ambient temperature, no sound generalisations can be made.

The NMS ordinations were originally analysed at the invertebrate morphospecies level, but the data was too sparse and final stress values were comparatively higher than ordinations focusing on insect families (Intercepts - ~15, Pitfall - ~25), so they were a less accurate representation of the data. However, results at the morphospecies level were fairly similar to the insect family analyses. Similar patch variables were significantly influencing taxa at the morphospecies, with the exception of patch size and plant evenness which became important for the intercept combined and bottom chamber at the insect family level. For morphospecies from the top chambers of the intercept traps, only plant composition (ordination axis 2) was significant), but for the insect families, other patch characteristics (altitude, plant ordination axis 1, proportion manuka cover, and plant richness) also had an influence on assemblages. The same patch variables were significant for the pitfall traps at both the morphospecies and family level. In addition, for all trap types, more taxa were significantly represented in the ordinations at the insect family level as opposed to morphospecies, with negligible alterations.

For the pitfall trap ordinations, the same patch variables were playing an integral part in insect family composition. Every factor except plant richness was significant. Unlike results for the intercept traps, categorical data of surrounding land use was significant as well as region; surrounding land use appearing to be more influential for less mobile soil invertebrates. The results also reinforced the variation in habitat composition among the regions. Sites within the Tongariro in addition to being higher in altitude and closely associated with plant composition from plant ordination axis 2 scores, also had a higher level of plant evenness. In comparison, sites within the Manawatu and the Wairarapa had a different overall plant composition, higher proportion of manuka cover, and larger average patch sizes. Corresponding to the intercept data, pitfall litter invertebrates showed preferences for certain sites, probably as a consequence of many of the habitat variables associated within them. Yet, plant composition, proportion manuka cover, and patch size among regions appeared to be the strongest influencing factors.

When examining differences at the site level, sites within regions were fairly similar in terms of diversity. Manawatu and the Wairarapa appeared to have less variation in insect family composition than Tongariro which was more disparate compared to the other two regions. From the Tongariro pitfall trap ordination, part of the variation of insect family composition between sites within Tongariro appears to be attributed to patch size. Surrounding land type was used to display the Tongariro ordinations and some sites within this region were composed mainly of pastoral grass and native bush compared to others with a more montane composition of plants. The Manawatu and

Wairarapa on the other hand, were much more homogenous in plant composition, all sites comprised of a mixture of pastoral land and forest remnants. However, surrounding land type did not appear to account for differences in diversity or insect family composition among the sites.

Overall, altitude was a significant factor for the regional analyses of both invertebrate taxa diversity and ordinations of insect family ordinations, as well as analyses of Tongariro sites exclusive of region. This suggests that altitude may play a part in determining invertebrate diversity, but no solid conclusions on the influence of altitude on insect family composition can be made. Further replication with more variation between regions and analyses at the insect genus or species level may reveal significant results. Inter-patch movements and loss of nesting habitat (connectivity) are also factors that should be taken into consideration when examining pollinator communities, rather than solely focusing on plant species fragments within which species forage (Cane 2001). Results for the pitfalls were similar to the intercept traps, except proportion manuka cover and patch size appear to have a stronger influence on litter insect composition. As litter invertebrates are less mobile than flying insects they may rely more heavily on specific plant species identity and more vulnerable to local habitat disturbance. Therefore, it is difficult to generalise what kind of an impact different habitat dynamics have on ecological communities, as different functional groups or taxa may respond very differently to habitat changes.

5.

DISCUSSION

5. Discussion

5.1 Relationships within pollination systems and the how it applies to the reproductive capabilities of manuka

Pollination systems are prone to a number of disruptions, including changes to pollinator composition and abundance and plant reproduction with the introduction of exotic insects, and due to changes to the local habitat, such as fragmentation (Kearns et al. 1998; Wilcock & Neiland 2002). Plant reproductive success, patch variation and pollinator behaviour are all intrinsically linked components within pollination systems; each affects and is affected by the others (Figure 5.1).

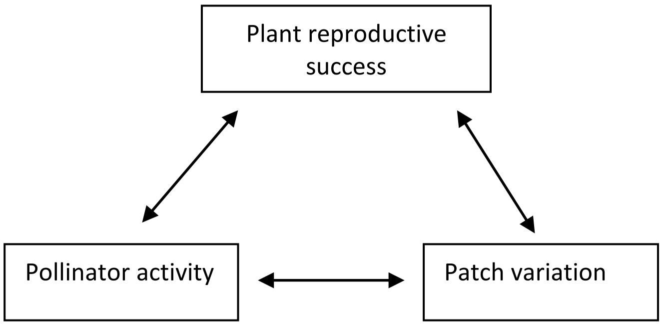

Figure 5.1: Intrinsic link between main variable components of pollination systems.

The introduction of exotic pollinators have the potential to alter the abundance, diversity, and subsequent composition of native pollinators, which in turn may impact on plant reproduction if they are less efficient pollinators than the taxa they displace (Celebrezze & Paton 2004; Dupont et al. 2004). Changes in plant reproduction may also consequently alter habitat dynamics. The type of mating system a plant species displays also has an effect on the severity of impacts on plant reproduction, pollinator activity, and consequent patch dynamics. Plants that rely on outcrossing via animal pollinators are at the highest risk of suffering as a result of pollen limitation; as they are dependent on the successful collection, transportation, and deposition of pollen from such pollinators (Wilcock & Neiland 2002; Ashman et al. 2004).

Manuka is andromonoecious (displays both hermaphrodite and male flowers). Evolutionary speaking, hermaphroditism is generally considered the ancestral mating system in plants; dioecy deriving to help desist the occurrence of self fertilisation, which can lead to inbreeding depression and the discounting of pollen (Barrett 2002). Andromonoecy is a kind of intermedium between these two systems – selfing is still possible, but competing outcrossing is likely on female flowers (Primack & Lloyd 1980). I have shown that in manuka, the hermaphrodite flowers are capable of some self-fertilisation, displaying a low to moderate level of self-compatibility, but the highest yield of capsule and seed set is produced via cross-pollination. In the absence of pollinators manuka had little autonomous selfing capability and under such controlled conditions produced a negligible number of capsules and viable seeds. This suggests that the flower morphology of this plant is not adapted to assist self-fertilisation in the absence of pollinators. So manuka, like closely related Australian andromonoecious *Leptospermum* species is capable of self-fertilisation, but relies heavily on pollinators and more specifically cross-pollination for maximal seed set.

"Pollen discounting" due to geitonogamy (transfer of pollen between flowers on the same plant), refers to the loss of outcrossing opportunities, which is more likely to occur among hermaphrodite species, especially within plants that display mass flowering. The addition of male-only flowers, which has developed in manuka, may help to combat a loss of male flower reproductive fitness due to pollen discounting; making more pollen available for outcrossing. During the summer flowering periods, as well as flowering bursts in mid-autumn, when behavioural observations and capsule collection were taking place, I witnessed hermaphrodite and male flowers on manuka appear to open at different times; anthesis occurring earlier in hermaphrodite flowers (Primack & Lloyd 1980). Hermaphrodite flowers also tend to dominate the top of the plants and terminal tips of branches (Primack & Lloyd 1980). Such temporal and spatial separation may be another functional adaption to promote outcrossing (Barrett 2002).

5.2 Impact of honeybees within manuka patches

Plant species which are reliant on cross-pollination need to preserve adequate abundance and assemblage of pollinators, or at least suitable replacements/additions. The introduction of exotic pollinators, such as honeybees into pollination systems may have either direct effects on plant reproduction or indirect effects if the pollinators they displace are more efficient in terms of the quantity and/or quality of pollen they transfer. Manuka attracts a wide range of insect pollinators during peak flowering periods as shown in this study and elsewhere by Primack (1978, 1983; Murphy & Robertson 2000), including many native species. Native bees frequently visit manuka flowers, and contrary to previous findings in other systems (Roubik 1980; Gross & Mackay 1998; Cairns et al. 2005), honeybees did not appear to be antagonising them during the observed peak flowering period. The presence of honeybees did not deter native bees from foraging, nor did the abundance of native bees significantly decline in patches with high honeybee densities. An insufficient overlap in the primary resource requirements of these two pollinator guilds is a plausible reason for this lack of interference. Both groups of bees collect both pollen and nectar, though honeybees may be primarily interested in collecting nectar, whereas native bees may be more focused on gathering pollen; since they don’t store honey, but do provision their nests with pollen (Donovan 2007). As pollen does not seem to be a limiting resource judging by the high standing crops of pollen even in heavily visited patches, these two guilds appear to gain sufficiently to forage within the same patches.

However, I observed honeybees physically disrupting large flies and it appears that nectar is a limiting resource for both these pollinator guilds. Competition between these two groups was both directly observed and statistically inferred. Aside from the physical antagonism observed, higher densities of honeybees appeared to affect the large fly abundance within the same patch. This is consistent with previous findings by Murphy & Robertson (2000). Moreover, an increase in the abundance of large flies resulted in a significantly lower level of remaining nectar standing crop; honeybees did not have a significant effect on remaining nectar levels. The large flies, where they did occur among patches, reached very high densities, allowing large amounts of nectar to be collected. Honeybees did not reach densities as high as the large flies within patches, so even in targeting nectar; there was more chance of a higher level of remaining nectar standing crop among patches dominated by honeybees. The combined abundance of large flies and honeybees plotted against the remaining standing nectar crops explained more variance in nectar standing crop than either guild alone. Therefore, regardless of abundance, these two guilds gave the best prediction of nectar standing crop. It is possible that in addition to physically disrupting large flies, honeybees were depleting nectar levels to a point where it was no longer economical for the large flies to remain within the same patches. This is substantiated by the ordination results, in which honeybees were the most influential patch characteristic associated with variation in pollinator guild composition among patches. In contradiction, honeybee density and nectar levels displayed a positive association – the higher number of honeybees, the higher standing nectar crop probably because honeybees tend to be common in sites where large flies are not and therefore in sites that are heavily depleted by the flies. However, when examining the combined abundance of large flies and honeybees we get the best predictor of variance in nectar levels which suggests that the combined effect is to deplete nectar levels still further.

Regardless of honeybee density and displacement of large flies, there was no significant occurrence of pollen limitation among the study patches (although honeybees never reached extremely high densities). Adequate capsule and seed set appeared to be produced among all the patches; however the quality of the pollen and the subsequent seeds produced were not examined. As previous research has suggested, honeybees tended to spend more time on a single flower than native bees, as well as longer on a single plant (Klein et al. 2003; Dupont et al. 2004). Prolonged single flower foraging by honeybees was evident in the behavioural observations, but was not measured. Such foraging behaviour may promote the transfer of pollen from the same plant, resulting in self-fertilisation which could lead to inbreeding depression. A recent study on pollinator visitation to New Zealand pohutakawa (*Metrosideros excelsa*) found that native bees and honeybees spent a similar amount of time visiting the flowers (Schmidt-Adam et al. 2009). The stigma from this species, however, is embedded among the multiple brush-like stamens of the inflorescence. Therefore the nature of the blossom, rather than species behaviour, may determine how long a pollinator forages on a single flower. The accessibility of the target resource in regards to floral morphology and the morphological traits of pollinator feeding mouthparts would either impede or enhance the availability of resources and the chance of stigma contact.

Subsequently, the type of floral resource required may have affects on pollination deposition also. Pollen-harvesting honeybees have been shown to make contact with floral stigmas more frequently than nectar-collecting honeybees on several different plant species (Wilson & Thomson 1991; Paton 1993; Schmidt-Adam et al. 2009). In order to more closely examine the long-term effects of honeybees on manuka plant fitness, these aspects should be investigated. The relationship between morphological characteristics and the effectiveness of pollen deposition on manuka flowers should also be investigated.

In their review of New Zealand pollination systems, Newstrom & Robertson (2005) proposed a useful matrix for determining functional groups of pollinators according to flower blossom classes. Manuka flowers are small, white, and can be characterised as “dish/bowl/knob” flowers. According to Newstrom & Robertson’s matrix, long-tongued bees, flies, and moths are associated with ineffective pollination potential of this flower type. If this proposition is correct, short-tongued native bees may be more efficient pollinators of manuka than honeybees. An effective way of investigating this could be to expose virgin stigmas to visits from different types of bees; recording the amount of average pollen deposition and removal of each “single visit” (Wolfe & Barrett 1989). As there was no indication of any competition occurring between these two guilds, the reproductive fitness of manuka may be largely unaffected by honeybees. As many New Zealand plants display this kind of flower morphology, such research could potentially be generalised to other species which display similar floral traits.

5.3 Effects of patch characteristics on invertebrate diversity and insect family composition

Besides plant biology and responses among pollinator interactions, other patch characteristics influence invertebrate diversity and insect composition. The main factor which influenced richness of more mobile invertebrates in this study was altitude. Differences in invertebrate diversity and insect family composition in response to altitude is a complex interaction. Environmental conditions may vary substantially at increasing elevations, including variation in temperature, precipitation, partial pressure of atmospheric gases, turbulence and wind speed, as well as ultra violet radiation wavelengths (Barry, 1992). These environmental variables can affect biological and morphological traits within insect groups and plant species, consequently affecting plant community composition and concomitantly invertebrate assemblages (Hodkinson, 2005). Previous research has found that species richness of Lepidoptera and Hymenoptera - with the exception of bumblebees (Galen 1989); probably due to greater thermoregulatory capabilities (Corbet et al. 1993) - tend to be lower with an increase in altitude (Warren et al. 1988), whereas flies tend to become more prevalent and important for pollination of montane plants at higher altitudes (Warren et al. 1988; Kearns, 1992; Totland, 1993). Large flies were well represented at sites within Tongariro at the highest altitudes and in the absence of honeybees. With this in mind, large flies may be less affected by displacement in being able to readily utilise higher altitude patches in conditions less favourable for other pollinators. For soil invertebrates caught in the pitfall traps, the manuka cover in patches was most influential. It has been well recognised that specific plant species can significantly affect the type of insects that occur within patches, and that the abundance of favourable plant species will influence the abundance of insect visitors (Price, 1997). As manuka attracts a diverse range of pollinators, albeit many generalists, it is not surprising that an increase in the proportion of manuka cover would attract a higher abundance of insect visitors, which could have an effect on invertebrate diversity.

The proportion of manuka cover and plant composition within patches also had a large influence on insect family composition for insects caught in both the intercept and pitfall traps. Plant composition was a significant influence on insect family composition for both intercept and pitfall trap insects, but plant diversity (richness and evenness) in some cases was not, suggesting that plant species identity and plant community composition are more important drivers of insect assemblage than a rich diversity of available floral and/or plant resources. A previous study by Perner et al. (2005) on the affect of various habitat characteristics on arthropod abundance in montane grasslands in central Germany showed similar findings. The authors found that plant community composition significantly affected the abundance of specific invertebrate functional groups, whereas plant diversity alone did not have a strong influence. Altitude had strong effects while patch size, although significant, displayed a weaker influence on insect family composition. The range of patch size within my study was not very large and there were few large patches (Range = 0.1ha – 7.1 ha; median = 0.3), so the fact that patch size appears to be having some influence, even among patch sizes with little variation, is worthy of further investigation.

The majority of insects collected in the intercept traps were not from the families of insects that were visiting the manuka flowers in the behavioural observations. Some of them were parasitoids and insect predators that may have been drawn to patches by an increase in pollinator activity during the peak flowering period. As the main pollinators were not well represented in the intercept traps, we cannot assume that the patch characteristics which had an effect on insect family composition were a good indication of the influence driving pollinator composition specifically. Species experience landscapes differently, and flying insects may not be as affected as strongly as litter invertebrates by changes in habitat. Pollinators that are more readily adaptable to generalised foraging are also at less of a disadvantage. The presence of honeybees and altitude were the most important factors influencing pollinator guilds; also sites with a higher visitation of honeybees tended to have higher overall patch invertebrate diversity. This highlights the complexity of interactions that occur within ecosystems, especially pollination systems, and that different taxa are affected by patch characteristics in different ways. Very different results may also occur depending on the functional groups of focus. There were obvious factors influencing the composition of insect family communities, but many more may become evident if insects were identified to species level. Unfortunately, the data collected at the morphospecies level were too sparse to be able to apply a reliable ordination output. Even so, information about insect composition at the family level provides useful information as insects within family units generally display similar ecological characteristics.

5.4 Further applications

Experimental replications of this research repeated over a number of years would help validate the current findings and more direct measures of competition, such as consequent survival, fecundity, and population density, would provide valuable information on the longer term effects of resource competition. Nevertheless, indirect measurements of competition, such as resource overlap, visitation rates, and resource harvesting patterns are helpful in assessing trends warranting further investigation.

Experimental manipulation of mobile insect pollinators is no easy feat. In order to accomplish this, beekeepers are needed who are willing to supply and transport a large number of hives. Honeybees in search of resources have been reported to travel an average of 1.1 km in suburban landscapes (Waddington et al., 1994), and 2.26 km in temperate forest mosaics (Visscher & Seeley, 1982), and in some instances, may travel up to 10 km to forage (Steffan-Dewenter & Kuhn, 2003). Therefore, sourcing sufficient areas of manuka with few honeybees within at least a 10 km radius is also difficult considering the popularity of honey produced from manuka. One possibility is to utilise isolated and restricted patches of manuka on Department of Conservation land, in which honeybee hives are prohibited, although vigilance must be adhered to when choosing patches as hives may still be placed just outside of the boundaries, allowing honeybees to access plant patches within restricted areas. However, restricted areas such as these could be used as control patches in which to further validate the current findings.

5.5 Conclusion

It appears that honeybees have an effect on the abundance of large flies among manuka patches, but the long-term consequences are not known. Pollen limitation was not occurring among manuka patches regardless of the presence of honeybees, therefore sufficient capsule and seed set was produced at all sites. Environmental effects of altitude appear to play a contributing factor in the taxa richness of flying and more mobile invertebrates, and the proportion of manuka within patches on taxa richness of litter invertebrates. These patch characteristics, including patch size and plant composition also have an influence on insect family composition. The fact that patches with a higher proportion of manuka have a higher diversity of invertebrates is a surprising result given that this would appear to produce a more uniform habitat than more mixed patches. This may be worthy of further investigation, especially in terms of the consideration for biodiversity conservation. Many ecosystem variables and interactions must be taken into account when examining the effect of introduced species into novel habitats. However, without direct experimental manipulation it is difficult to apply generalised causation on the hierarchical contribution of different habitat dynamics on native insect and plant species. It is fair to say that many complex ecological interactions have an influence on taxa abundance, diversity and composition; and such impacts may vary dramatically, depending on the taxonomic level or habitat type of focus.

REFERENCES

REFERENCES

Aizen, MA. & Feinsinger, P. 1994. Habitat fragmentation, native insect pollinators, and feral honey-bees in Argentine Chaco Serrano. *Ecological Applications* 4(2): 378-392.

Andersen, AN. 1990. Andromonoecy in four Australian species of *Leptospermum*. *Australian Journal of Botany* 38(5): 511-515.

Ashman, TL; Knight, TM; Steets, JA; Amarasekare, P; Burd, M; Campbell, DR; Dudash, MR; Johnston, MO; Mazer, SJ; Mitchell, RJ; Morgan, MT; & Wilson, WG. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85(9): 2408-2421.

Barrett, SCH. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics*. 3(4): 274-284.

Barry, RG. 1992. Mountain climatology and past and potential future climatic changes in mountain regions – a review. *Mountain Research and Development* 12(1): 71-86.

Brosi, BJ., Daily, GC., Shih, TM., Oviedo, F., & Duran, G. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology* 45(3): 773-783.

Burrell, J. 1965. Ecology of *Leptospermum* in Otago. *New Zealand Journal of Botany* 3: 3-16.

Butz Huryn, VM. 1995. Use of New Zealand plants by honeybees (*Apis mellifera* L.): a review. *New Zealand Journal of Botany* 33: 497-512.

Butz Huryn, VM. 1997. Ecological impacts of introduced honeybees. *Quarterly Review Of Biology* 72(3): 275-297.

Cairns, CE; Villanueva-Gutierrez, R; Koptur, S; & Bray DB. 2005. Bee populations, Forest disturbance, and africanization in Mexico. *Biotropica* 37(4): 686-692.

Cane, JH. 2001. Habitat fragmentation and native bees: a premature verdict? *Conservation Ecology* 5(1).

Carthy, JD. 1958. An introduction to the behaviour of invertebrates. London: Allen & Unwin. 380 pp.

Celebrezze, T. & Paton, DC. 2004. Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brahcyloma ericoides* (Epacridaceae). *Austral Ecology* 29: 129-136.

Chamberlain, SA. & Schlising, RA. 2008. Role of honey bees (Hymenoptera: Apidae) in the pollination biology of a California native plant, *Triteleia laxa* (Asparagales: Themidaceae). *Environmental Entomology* 37(3): 808-816.

Corbet, SA; Fussell, M; Ake, R; Fraser, A; Gunson, C; Savage, A ; Smith, K. 1993. Temperature and the pollinating activity of social bees. *Ecological Entomology* 18(1): 17-30.

Crist, TO; Pradhan-Devare, SV; & Summerville, KS. 2006. Spatial variation in insect community and species responses to habitat loss and plant community composition. *Oecologia* 147(3): 510-521.

Crowe, A. 2002. Which New Zealand insect? Auckland, Penguin Books. 127 pp.

Dafni, A; Kevan, PG; & Husband, BC *eds*. 2005. Practical pollination biology. Cambridge, Ontario, Canada: Enviroquest Ltd.

Debinski, DM. & Holt, RD. 2000. A survey and overview of habitat fragmentation experiments. *Conservation Biology* 14(2): 342-355.

Dick, CW; Etchelecu, G; & Austerlitz; F. 2003. Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12(3): 735-764.

Didham, RK; Ghazoul, J; Stork, NE; & Davis, AJ. 1996. Insects in fragmented forests: A functional approach. *Trends in Ecology & Evolution* 11(6): 255-260.

Diekotter, T; Billeter, R; & Crist, TO. 2008. Effects of landscape connectivity on the spatial distribution of insect diversity in agricultural mosaic landscapes. *Basic and Applied Ecology* 9(3): 298-307.

do Carmo, RM; Franceschinelli, EV; & da Silveira. 2004. Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. *Biotropica* 36(3): 371-376.

Donaldson, J; Nanni, I; Zachariades, C; Kemper, J; & Thompson, JD. 2002. Effects of Habitat fragmentation on pollinator diversity and plant reproductive success in Renosterveld shrublands of South Africa. *Conservation Biology* 16(5): 1267-1276.

Donovan, BJ. 1980. Interactions between native and introduced bees in New Zealand. *New Zealand Journal of Ecology* 3: 104-116.

Donovan, BJ. 2007. Apoidea (Insecta: Hymenoptera). Fauna of New Zealand no. 57. Lincoln, Canterbury, New Zealand, Manaaki Whenua Press.

Dover, J. & Settele, J. 2009. The influence of landscape structure on butterfly distribution and movement: a review. *Journal of Insect Conservation* 13(1): 3-27.

Ducheyne, E; Mweempwa, C; De Pus, C; Vernieuwe, H; De Deken, R; Hendrickx, G; & Van den Bossche, P. 2009. The impact of habitat fragmentation on tsetse abundance on the plateau of eastern Zambia. *Preventive Veterinary Medicine* 91(1): 11-18.

Dugdale, JS. 1988. Lepidoptera - annotated catalogue, and keys to family-group taxa. Fauna of New Zealand no.14. Wellington, DSIR Science Information Publishing Centre.

Dupont, YL; Hansen, DM; Valido, A; & Olesen, JM. 2004. Impact of honey bees on native pollination interactions of the endemic *Echium wildpretii* (Boraginaceae) on Tenerife, Canary Islands. *Biological Conservation* 118: 301-311.

Eickwort, GC. & Ginsberg, HS. 1980. Foraging and mating-behaviour in Apoidea. *Annual Review of Entomology* 25: 421-446.

Ewers, RM; Thorpe, S; Didham, RK. 2007. Synergistic interactions between edge and area effects in a heavily fragmented landscape. *Ecology* 88(1): 96-106.

Ewers, RM. & Didham, RK. 2008. Pervasive impact of large-scale edge effects on a beetle community. *Proceedings of the National Academy of Sciences of the United States of America* 105(14): 5426-5429.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology Evolution and Systematics* 34: 487-515.

Franzen, M. & Nilsson, SG. 2008. How can we preserve and restore species richness of pollinating insects on agricultural land? *Ecography* 31(6): 698-708.

Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: Bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43(4): 882-890.

Godley, EJ. 1979. Flower biology in New Zealand. *New Zealand Journal of Botany* 17: 441-466.

Gonzalez-Varo, JP; Arroyo, J; & Aparicio, A. 2009. Effects of fragmentation on Pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*). *Biological Conservation* 142(5): 1058-1065.

Goulson, D. 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology Evolution and Systematics* 34: 1-26.

Goulson, D; Lye, GC; & Darvill, B. 2008. Decline and conservation of bumblebees. *Annual Review of Entomology* 53: 191-208.

Gross, CL. & Mackay, D. 1998. Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). *Biological Conservation* 86(2): 169-178.

Gross, CL. 2001. The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. *Biological Conservation* 102(1): 89-95.

Harris, W. 2000. Improvement of the ornamental qualities of *Leptospermum* by inter-specific hybridisation. *Acta Horticulturae* 508: 345-346.

Harris, W. 2002. Variation of inherent seed capsule splitting in populations of *Leptospermum scoparium* (Myrtaceae) in New Zealand. *New Zealand Journal of Botany* 40: 405-417.

Hendrickx, F; Maelfait, JP; Van Wingerden, W; Schweiger, O; Speelmans, M; Aviron, S; Augenstein, I; Billeter, R; Bailey, D; Bukacek, R; Burel, F; Diekotter, T; Dirksen, J; Herzog, F; Liira, J; Roubalova, M; Vandomme, V; & Bugter, R. 2007. How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. *Journal of Applied Ecology* 44(2): 340-351.

Hickman, JM; Lovei, GL; & Wratten, SD. 1995. Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). *New Zealand Journal of Zoology* 22: 387-392.

Hodkinson, ID. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* 80(3): 489-513.

Holloway, BA. 1976. Pollen-feeding hover flies (Diptera: Syrphidae). *New Zealand Journal of Zoology* 3: 339-350.

Hopkins I. 1911. *Australasian Bee Manual*. Wellington, NZ: Gordon & Gotch. 173 pp.

Horskins, K. & Turner VB. 1999. Resource use and foraging patterns of honeybees, *Apis mellifera,* and native insects on flowers of *Eucalyptus costata*. *Australian Journal of Ecology* 24(3): 221-227.

Kato, M. & Kawakita, A. 2004. Plant-pollinator interactions in New Zealand influenced by introduced honey bees. *American Journal of Botany* 91(11): 1814-1827.

Kearns, CA. 1992. Antophilous fly distribution across an elevation gradient. *American Midland Naturalist* 127(1): 172-182.

Kearns, CA., Inouye, DW., & Waser, NM. 1998. Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29: 83-112.

Kearns, CA. 2001. North American Dipteran pollinators: Assessing their value and conservation status. *Conservation Ecology* 5(1): Article 5.

Kenis, M., Auger-Rozenberg, M., Rogues, A., Timms, L., Pere, C., Cock, MJW., Settele, J., Augustin, S., & Lopez-Vaamonde, C. 2009. Ecological effects of invasive alien insects. *Biological Invasions* 11(1): 21-45.

Klein, AM., Steffan-Dewenter, I., & Tscharntke, T. 2003. Fruit set of highland coffee Increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270(1518): 955-961.

Lehnebach, CA & Robertson, AW. 2004. Pollination of four epiphytic orchids of New Zealand. *Annals of Botany* 93(6): 773-781.

Lloyd, DG. 1985. Progress in understanding the natural-history of New-Zealand plants. *New Zealand Journal of Botany* 23(4): 707-722.

Macfarlane, RP. & Gurr, L. 1995. Distribution of bumble bees in New Zealand. *New Zealand Entomologist* 18: 29-36.

Macfarlane, RP; Andrew IG; Sinclair BJ; Harrison RA; Dugdale JS; Boothroyd IKG; Toft R; Marshall SA; Johns PM; Forsyth DJ; Matile L; Holder P; Oliver HA. 2009. Checklist of New Zealand Diptera. Entomological Society of New Zealand http://www.ento.org.nz (Accessed 8 August 2009).

Maleque, MA; Maeto, K; Ishii, HT. 2009. Arthropods as bioindicators of sustainable forest management, with a focus on plantation forests. *Applied Entomology and Zoology* 44(1): 1-11.

Mallick, SA. & Driessen, MM. 2009. Impact of hive honeybees on Tasmanian leatherwood *Eucryphia lucida* Labill. (Eucryphiaceae). *Austral Ecology* 34: 185-195.

Martinko, EA; Hagen, RH; & Griffith, JA. 2006. Successional changes in the insect community of a fragmented landscape. *Landscape Ecology* 21: 711-721.

Mavric, E; Wittmann, S; Barth, G; & Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research* 52(4): 483-489.

McKenna, MA., & Thomson, JD. 1988. A technique for sampling and measuring small amounts of floral nectar. *Ecology* 69(4): 1306-1307.

Meyer, B; Jauker, F; & Steffan-Dewenter, I. 2009. Contrasting resource-dependent responses of hoverfly richness and density to landscape structure. *Basic and Applied Ecology* 10(2): 178-186.

Michaels, KF. 2007. Using staphylinid and tenebrionid beetles as indicators of Sustainable landscape management in Australia: a review. *Australian Journal of Experimental Agriculture* 47(4): 435-449.

Michener CD. 1974. *The Social Behavior of the Bees: A Comparative Study.* Cambridge, MA: Harvard Univ. Press. 404 pp. 2nd ed.

Michener CD. 1979. Biogeography of bees. *Annals of the Missouri Botanical Garden* 66(3): 277–347.

Moar, NT. 1993. *Pollen grains of New Zealand dicotyledonous plants*. Manaaki Whenua Press, Lincoln, New Zealand. 200 pp.

Murphy, C. & Robertson, A. 2000. Preliminary study of the effects of honey bees (*Apis Mellifera*) in Tongriro National Park. *Science for Conservation* 139. Department of Conservation, Wellington, New Zealand.

Newstrom, L., & Robertson, A. 2005. Progress in understanding pollination systems in New Zealand. *New Zealand Journal of Botany* 43: 1-59.

Nichols, E; Larse, T; Spector, S; Davis, AL; Escobar, F; Favila, M; & Vuline, K. 2007. Global dung beetle response to tropical forest modification and fragmentation: A quantitative literature review and meta-analysis. *Biological Conservation* 137(1): 1-19.

Niemela, J. 2001. Carabid beetles (Coleoptera : Carabidae) and habitat fragmentation: a review. *European Journal of Entomology* 98(2): 127-132.

Norton, SA. 1984. Thrips pollination in the lowland forest of New Zealand. *New Zealand Journal of Ecology* 7: 157-164.

O'Brien, SP., & Calder, DM. 1993. Reproductive biology and floral phonologies of the sympatric species *Leptospermum myrsinoides* and *L. continientale* (Myrtaceae). *Australian Journal of Botany* 41(4-5): 527-539.

O'Brien, SP. 1994. Andromonoecy and fruit set in *Leptospermum myrsinoides* and *L. continentale* (Myrtaceae). *Australian Journal of Botany* 42(6): 751-762.

Oldroyd BP, Thexton EG, Lawler SH, Crozier RH. 1997. Population demography of Australian feral bees (*Apis mellifera*). *Oecologia* 111(3): 381–87.

Paini, DR. 2004 Impact of the introduced honey bee (*Apis meliifera*) (Hymenoptera: Apidae) on native bees: A review. *Austral Ecology* 29(4): 399-407.

Parkinson, B. & Patrick, B. 2000. Butterflies and moths of New Zealand. New Zealand Nature Series, Auckland, Reed. 64 p.

Paton, DC. 1993. Honeybees in the Australian environment. Does *Apis mellifera* disrupt or benefit the native biota? *Bioscience* 43(2): 95-103.

Pearce, JL. & Venier, LA. 2006. The use of ground beetles (Coleoptera : Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: A review. *Ecological Indicators* 6(4): 780-793.

Perner, J., Wyrtrykush, C., Kahmen, A., Buchmann, N., Egerer, I., Creutzburg, S., Odat, N., Audroff, V., & Weisser, W.W. 2005. Effects of plant diversity, plant productivity and habitat parameters on arthropod abundance in montane European grasslands. *Ecography* 28(4): 429-442.

Potts, SG., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A., & Willmer, P. 2006. Plant- pollinator biodiversity and pollination services in a complex Mediterranean Mediterranean landscape. *Biological Conservation* 129(4): 519-529.

Price, PW. 1997. *Insect Ecology.* New York: Wiley. 3rd ed.

Primack, RB. 1978. Variability in New Zealand montane and alpine pollinator assemblages. *New Zealand Journal of Ecology* 1: 66-73.

Primack, RB. 1983. Insect pollination in the New Zealand mountain flora. *New Zealand Journal of Botany* 21: 317-333.

Primack, RB. & Lloyd, DG. 1980. Andromonoecy in the New Zealand montane shrub manuka, *Leptospermum scoparium* (Myrtaceae). *American Journal of Botany* 67(3): 361-368.

Rathcke, BJ. & Jules, ES. 1993. Habitat fragmentation and plant pollinator interactions. *Current Science* 65(3): 273-277.

Robertson, AW; Kelly, D; Ladley, JJ; & Sparrow, AD. 1999. Effects of pollinator loss on endemic New Zealand mistletoes (Loranthaceae). *Conservation Biology* 13(3): 499-508.

Roubik, DW. 1978. Competitive interactions between neotropical pollinators and Africanized honey bees. *Science* 201(4360): 1030-1032.

Roubik, DW. 1980. Foraging behaviour of competing Africanized honeybees and stingless bees. *Ecology* 61(4): 836-845.

Roubik, DW. & Wolda H. 2001. Do competing honey bees matter? Dynamics and abundance of native bees before and after honey bee invasion. *Population Ecology* 43(1): 53-62.

Schmidt-Adam, G., Murray, BG., & Young, AG. 2009. The relative importance of birds and bees in the pollination of *Metrosideros excelsa* (Myrtaceae). *Austral Ecology* 34(5): 490-498.

Schowalter, TD. 2006. *Insect Ecology: An ecosystem approach*. San Diego: Elsevier-Burlington, Academic Press, 2nd ed.

Shen, MQ., Yang, XL., Cox-Foster, D., & Cui, LW. 2005. The role of varroa mites in Infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology* 342(1): 141-149.

Sjodin, NE; Bengtsson, J; & Ekbom, B. 2008. The influence of grazing intensity and Landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology* 45(3): 763-772.

Steffan-Dewenter, I. & Tscharntke, T. 1999. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121(3): 432-440.

Steffan-Dewenter, I. & Tscharntke, T. 2000. Resource overlap and possible competition between honey bees and wild bees in central Europe. *Oecologica* 122(2): 288-296.

Steffan-Dewenter, I; Munzenberg, U; Burger, C; Thies, C; & Tscharntke, T. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83(5): 1421-1432.

Steffan-Dewenter, I. & Kuhn, A. 2003. Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society of London, Series B* 270: 569-575.

Steffan-Dewenter, I. & Westphal, C. 2008. The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology* 45(3): 737-741.

Suzuki, N. 2003. Significance of flower exploding pollination on the reproduction of the Scotch broom, *Cytisus scoparius* (Leguminosae). *Ecological research* 18: 523-532.

Taki, H. & Kevan, PG. 2007. Does habitat loss affect the communities of plants and Insects equally in plant-pollinator interactions? Preliminary findings. *Biodiversity and Conservation* 16(11): 3147-3161.

Totland, O. 1993. Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant communities. *Canadian Journal of Botany* 71: 1072-1079.

Tscharntke, T; Steffan-Dewenter, I; Kruess, A; & Thies, C. 2002. Characteristics of insect populations on habitat fragments: A mini review. *Ecological Research* 17: 229-239.

Visscher, PK., & Seeley, TD. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* 63(6): 1790-1801.

Waddington, KD., Visscher, PK., Herbert, TJ., & Richter, MR. 1994. Comparisons of forager distributions from matched honey-bee colonies in suburban environments.*Behavioral Ecology and Sociobiology* 35(6): 423-429.

Warren, SD., Harper, KT., & Booth, GM. 1988. Elevational distribution of insect pollinators. *American Midland Naturalist* 120(2): 325-330.

Webb, CJ. 1994. Pollination, self-incompatibility, and fruit production in *Corokia cotoneaster* (Escalloniaceae). *New Zealand Journal of Botany* 32(3): 385-392.

Wilcock, C. & Neiland, R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant science* 7(6): 270-277.

Wilson, P. & Thomson, JD. 1991. Heterogeneity among floral visitors leads to Discordance between removal and deposition of pollen. *Ecology* 72(4): 1503-1507.

Winfree, R; Griswold, T; & Kremen, C. 2007. Effect of human disturbance on bee communities in a forested system. *Conservation Biology* 21(1): 213-223.

Winfree, R; Aguilar, R; Vazquez, DP; LeBuhn, G; & Aizen, MA. 2009. A meta-analysis of bees’ responses to anthropogenic disturbance. *Ecology* 90(8): 2068-2076.

Wolfe, LM. & Barrett, SCH. 1989. Patterns of pollen removal and deposition in tristylous *Pontederia-cordata l*. (Pontederiaceae). *Biological Journal of the Linnean Society* 36(4): 317-329.