Understanding the role and impact of introduced honey bees in a submontane indigenous forest ecosystem

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“Earth's crammed with heaven,

And every common bush afire with God,

But only he who sees takes off his shoes;

The rest sit round and pluck blackberries.”

-Elizabeth Barrett Browning-

Abstract

New Zealand's apiculture industry is the fastest growing in the world, expanding in agricultural landscapes, as well as in native ecosystems. While this has obvious benefits for economy and industry, the impacts on sustainability of native ecosystems are less easy to discern. Honey bees (*Apis mellifera*) have a suite of potential impacts, both positive and negative, on native plants and flower visitors in native ecosystems. This research aimed to investigate the impact of managed introductions of *Apis mellifera* in native forest dominated by *Weinmannia racemosa* and *Ixerba brexioides*, two native forest trees used extensively for monofloral honey production in New Zealand. Research focused on three key areas: 1) timing and availability of floral nectar resources; 2) impacts on plants, particularly *I. brexioides* and *W. racemosa*; and 3) impacts on invertebrate flower visitor communities.

Availability of floral nectar resources from *I. brexioides* and *W. racemosa* was assessed using a combination of nectar collection and phenology data. Pollination potential of honey bees was assessed using video surveillance and effects on seed set of *I. brexioides* and *W. racemosa* were observed using exclusion experiments. Community-level effects on invertebrate flower visitors were assessed using collection of flower visitors and assessment of community data using multivariate and other statistical approaches.

Timing and availability of floral nectar resource showed extreme variation between annual cycles. Nectar sugar production was lowest during a hot, dry summer compared with a cooler, wetter summer, in terms of both sugar production per flower and flower production per tree. At a landscape scale, this can have serious flow-on effects for foraging nectar-feeders, and hence for seed set of flowering plants.

Video surveillance showed that suitability of different flower visitors for pollination of *I. brexioides* and *W. racemosa* differed. For *W. racemosa* all groups of flower visitors contacted reproductive structures, allowing for successful pollination. However for *I. brexioides*, pollination potential was greatest for birds, beetles and native bees and least for spiders, wasps, ants and honey bees. Seed set for *I. brexioides* was highest at pest-proof fenced sites and lowest at high hive density sites, whereas *W. racemosa* seed set was highest at high hive density sites, and lowest at low density sites. *Weinmannia racemosa* had lowest levels of pollen

limitation at sites of high hive density. The combination of *W. racemosa* responses at the high hive density site suggest that small-flowered species, such as *W. racemosa,* have the potential to benefit from increased pollination success in areas where honey bees are frequent visitors.

Community analysis showed differences in flower visitor communities between sites with high and low honey bee hive density. Honey bees were the key species contributing to differences between high and low hive density sites. Diversity of insect flower visitors was higher at low hive density sites. Network analysis highlighted structural differences in networks between high and low hive density sites in terms of connectance, nestedness, and species-level indices. High and low hive density sites had a similar number of species that were native and non-native, but high hive density sites had more frequent interactions with non-natives, and 45 % of those interactions were from honey bees.

Pilot studies investigating methods for studying plant-pollinator interactions highlighted a need to tailor methods of pollen isolation to fit the research question, i.e. whether research is focused on pollination interactions or diet-related questions. Comparison of methods for understanding plant-pollinator interactions concluded that identification of pollen by microscopic means identified a greater breadth of plant-pollinator interactions than that identified by field observations alone. However, DNA-based methods of pollen identification have the potential for even greater specificity, cost-effectiveness, and answering a range of questions not possible with traditional methods.

Analysis and findings from this research support a case indicating that honey bees can affect seed set of native plants, and communities of invertebrate flower visitors in a number of ways. Prevention of permanent changes to flower visitor communities should be prioritised by preserving large areas of intact native forest where low levels of fragmentation create refuges for native flower visitors. Estimates of annual sugar production for *I. brexioides* and *W. racemosa* call attention to the need to build greater flexibility into legislated stocking rates in native forest, to minimise competitive effects on native flower visitors during low production years. Developing these measures will build sustainability into New Zealand models of apicultural practise, ensuring longevity of honey operations and protection of native ecosystems.

Keywords pollination; honey bees; *Ixerba brexioides; Weinmannia racemosa;* exclusion experiments; nectar; phenology; mutualistic networks; network analysis; DNA barcoding; pollen identification; New Zealand; hive management

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Chapter 1

Introduction

1.1 Research Topic

This thesis presents the findings of a three-year study of honey bees and their interactions in a New Zealand submontane forest ecosystem. The research is focused on the Kaimai-Mamaku Range, North Island, New Zealand, in forest which is dominated by *Ixerba brexioides* (tāwari) and *Weinmannia racemosa* (kāmahi). The investigation is centred on understanding the pollination ecology of two canopy flowering trees, *I. brexioides* and *W. racemosa*, with the purpose of identifying the actual and potential impacts of honey bees interacting with native species in this ecosystem. Dynamics of nectar production were estimated by a series of phenological measurements paired with regular nectar collection and analysis, to determine periods of low resource availability and the potential for resource limitation to act as a driver for negative species interactions between flower visitors. Impacts of honey bee introduction on plant reproductive output, were determined by video surveillance of plant-pollinator interactions and experimental pollination treatments. And impacts of honey bee density on invertebrate flower visitor community composition and structure were observed by collecting invertebrate flower visitors and analysing their communities using multivariate and network techniques to identify changes in community composition and structure at sites of varying honey bee hive density. Overall, these investigations act as the basis for recommendations concerning future management of apiaries on conservation land.

1.2 Background

The European-derived honey bee (*Apis mellifera* sspp), the most commonly known honey bee, is one of the world's most widespread bee species. Its native range includes Europe, Africa and the Middle East (Han et al. 2012) but it has since expanded into nearly every continent. This global expansion comes as a result of its usefulness to society and active spread by humans undertaking apiculture. Global pollination services alone are worth at least €153 billion (NZ$255 billion) (Gallai et al. 2009). This estimate includes other insect pollinators, but honey bees make a significant contribution. In addition to pollination services, live bee exports, honey

and other bee-related products, are becoming increasingly profitable (Ministry for Primary Industries 2019).

In New Zealand the economic value of pure honey exports was NZ$348 million in 2018, not including other benefits calculated for pollination, local honey sales, and seed exports resulting from bee pollination (Ministry for Primary Industries 2019), with honey bees also contributing about NZ$5 billion to the New Zealand GDP through pollination services for horticultural and agricultural crops (Newstrom-Lloyd 2013). The first honey bees to be imported to New Zealand in 1839 were European or black honey bees. A number of other subspecies eventually followed including Syrian, Carniolan, Cyprian, Holy Land and Swiss Alpine bees. The most common type in New Zealand today – Italian stock - was first imported in 1880 (Matheson & Reid 2011). This research focuses on honey bees without distinction of the individual sub-species. The service they provide and the potential impacts of such services are generally the same, though there has been discussion around behavioural differences between types that could increase the likelihood of negative impacts, for example by a greater inclination for swarming or greater foraging distances introducing additional pressure on native ecosystems.

The purpose of the following review is to discuss current literature regarding floral nectar dynamics and impacts of increasing honey bee presence in native ecosystems. This background information will provide context for understanding the underlying concerns associated with increasing honey bee presence in New Zealand forest, and justification for the relevance of the research presented in this thesis.

1.2.1 Understanding resource availability in dynamic flowering landscapes

With recent global concern about sustaining pollinator populations, resource availability has become an increasingly important topic. Quantifying resource availability in different ecosystems is important for restoration and management of conservation lands because of implications for sustaining populations of pollinators (Dennis 2010), but also has applications in managing forage for apiarists (Enkegaard et al. 2016; Ausseil et al. 2018). Resource availability is difficult to measure and predict because of confounding sources of variation in nectar and pollen quality, quantity and production rate between plant species, time of day, age of flowers, and consumption rates (Nicolson et al. 2007). Because of these

difficulties, few studies agree on standard methodologies, as they must be adapted to different landscapes, climates, and research aims (Szigeti et al. 2016).

Most often, resource availability is calculated using simple measures, such as flower number or area (Tepedino & Stanton 1981). Few studies delve further into resource value (nectar and pollen volumes) (Zimmerman & Pleasants 1982; Potts et al. 2004), or into quantifying the nutritional constituents of those resources, such as amino acids and sugars, at a landscape level. Zimmerman & Pleasants (1982) demonstrated that results from measuring the actual resource (nectar and pollen) are more conclusive than those relying on proxies such as flower number or area, however in some systems, with sophisticated modelling, reliable estimates of floral resource potentials can be made (Frankl et al. 2005).

Length of study is another important factor of experimental design relating to resource availability. Because of seasonal and inter-annual variation in resource availability and flower-visitor network structure, one season of data is insufficient to draw meaningful conclusions (Alarcón et al. 2008). An additional consideration is the spatial scope of the study and the degree of extrapolation. Because of the time-intensive methods for developing fine resolution data on resource availability, in general, a finer resolution is only possible with a smaller scale of research.

Phenological studies map the timing of availability of floral resources beyond the flower level by recording the timing and abundance of key reproductive events such as flowering. An extensive phenological database has been developed in the United States of America (USA) using citizen science. The USA National Phenology Network (NPN) encourages people to observe plant species and wildlife around them and record different phases using an electronic app. Data in this network covers over 800 species of plant and over 300 species of animal in various clearly defined phenophases. This data can be mapped and downloaded for general use and has been developed as an indicator for climate change (USA-NPN National Coordinating Office 2012; USA-NPN National Coordinating Office 2016). In New Zealand, phenological studies are few. Leathwick (1984) presents a phenology of trees, shrubs and lianes in four central North Island forests that demonstrates timing and abundance of vegetative growth, flowering, and fruiting. Castro (1995) focuses on phenology of food sources for hihi on Kāpiti Island, including timing and abundance of flowering and fruit set. Her study used this data to infer the levels of hihi populations that could be supported by the available resource throughout

different seasons and years. New Zealand also has an online platform, iNaturalist NZ, that is using citizen science to develop a database of species occurrence data and phenological monitoring that will continue to become more useful as data volume increases (iNaturalist 2019).

When done with care, measures of resource availability can be extremely useful. For example, recent identification of pollinator loss in England led to the establishment the Countryside Stewardship scheme with a specific agri-environment package for the care of wild pollinators. Estimates of critical levels of resource availability became a key part of securing the development of the package and advising policy on supporting native pollinators in agricultural systems (Dicks et al. 2015). In North Western European countries, similar problems with loss of natural pollinators in agricultural ecosystems has prompted research into the effects of increasing floral resource availability and diversity in agricultural systems. Recent research has demonstrated that increasing diversity of available resource through wildflower belts and new pasture seed mixes can have positive effects on native pollinator abundance and diversity (Korpela et al. 2013; Scheper et al. 2013; Woodcock et al. 2014; Scheper et al. 2015). In New Zealand, information on resource availability represents a significant knowledge gap that makes estimation of the impacts of honey bee introductions difficult (Beard 2015). This thesis makes a contribution to nectar production methods and data for *I. brexioides* and *W. racemosa* in an effort to begin to understand the potential impacts of a managed invasive flower visitor in New Zealand submontane forest.

1.2.2 Potential impacts of honey bee introduction on native ecosystems

Early introductions of alien honey bees were generally assumed to be beneficial to native ecosystems due to the pollination services they could provide (Paton 1993). However, since that time, research has acknowledged the actual and potential impacts of honey bee on ecosystems, plants and flower visitors that can be both positive and negative (Butz Huryn 1997; Paini 2004; Howlett & Donovan 2010; Beard 2015).

Broader ecosystem effects

Looking at broader effects of invasion on plant-pollinator communities requires analysis at the ecosystem level. Recent literature uses plant-pollinator networks to decipher the role of different species within the ecosystem. Mutualistic networks, like plant-pollinator networks, are generally nested (consisting of a core group of

generalists with increasing layers of specialists interacting with those generalists) and demonstrate heterogeneity in the strength distribution of links (i.e. some links are stronger than expected by chance) (Bascompte 2009). These characteristics give robustness to the network, protecting it from environmental change and random species loss. However, it can also leave networks vulnerable to the loss of key individuals. Within plant-pollinator networks some species act as hubs – generalist species with many interaction links that rely on them – or as connectors – linking together functional groups of pollinators (Tylianakis et al. 2010). The loss of either of these key roles can lead to rapid collapse of the network. Measures of these features of mutualistic networks have been developed and are useful in several applications, particularly conservation of mutualistic networks. For example, Fortuna & Bascompte (2006) demonstrated the effect of habitat loss on structure of plant-pollinator networks, while Memmott et al. (2007) used mutualistic networks to demonstrate the projected impacts of climate change. Network-level analysis of pollination systems is lacking for natural systems in New Zealand.

In addition, there are other broader potential impacts of honey bee introduction into native ecosystems, such as transfer of diseases and pathogens. Flower visitation can facilitate pathogen transmission between plants and other flower visitors (Durrer & Schmid-Hempel 1994; Singh et al. 2010), and honey bee behaviours make them particularly suited to this role, with generalist foraging strategies, high densities in densely stocked areas, and often hives are moved across great distances in large apiculture operations. Myrtle rust (*Austropuccinia psidii*), for example, is a plant pathogen that has been recently introduced to New Zealand, posing significant threats for the longevity of Myrtaceae. Honey bees have been observed actively collecting myrtle rust spores which are taken back to hives and can germinate viably for 9 days (experimental limit) after this (Pattemore et al. 2018). This behaviour could result in further spread of the pathogen through hive movements.

The generalist foraging strategy of honey bees, and a propensity for foraging on introduced species makes honey bees a risk for exacerbation of weed issues (Goulson 2005). Though research in New Zealand has not suggested a strong contribution of honey bees to weed issues (Butz Huryn & Moller 1995), international evidence suggests a link between honey bee foraging and increased weed fecundity for a number of important plant pests, such as purple loosestrife (*Lythrum salicaria*) (Mai et al. 1992), yellow star-thistle (*Centaurea solstitialis*)

(Barthell et al. 2001), Scotch broom (*Cytisus scoparius*) (Simpson et al. 2005) and lupin (*Lupinus arboreus*) (Stout et al. 2002). Though there are other New Zealand pollinators, both introduced and native, that also visit these plants, relative contributions of these pollinators has yet to be discerned (Beard 2015).

Impacts on plants

Considering the potential impacts to plants of increasing honey bee presence in native ecosystems, this thesis looks at the effects of honey bee hive density on the pollination and seed set of two dominant New Zealand forest trees. Visitation of native flowers in New Zealand by honey bees is common; Kelly et al. (2006) cited up to 56.8 % of insect visits to 12 native plant species were made by honey bees. However, one of the concerns associated with increasing honey bee presence in native ecosystems is that flower visitation does not necessarily result in pollination. Butz Huryn (1995) presented an extensive list of the 188 New Zealand native plant species that are used by honey bees for nectar and pollen sources, and Newstrom-Lloyd (2013) identified 97 species (71 native and 26 introduced) recommended by apiarists as good honey bee forage, however the efficacy of honey bees as pollinators of these species has only rarely been investigated (Robertson et al. 2005). Plant reproductive strategy, flower visitor behaviour, and flower architecture are some potential barriers for successful pollination. Different reproductive strategies of plant species may require outcrossing, rejecting pollen received from flowers of the same tree, or even closely-related neighbours (Ferrer & Good 2012). In addition, stigmatic receptivity is often restricted to a short window of time, or a small surface (Souza et al. 2016). Honey bee foraging on New Zealand plants is not limited to entomophilous flowers and is often observed on larger ornithophilous flowers that may not be structured to facilitate pollination by small invertebrates (Anderson 1997; Kelly et al. 2006). However, Schmidt-Adam et al. (2000) demonstrated that pollination is often a function of both pollen transfer and visitation frequency, as insect visitors to *Metrosideros excelsa* (pōhutukawa) can be as effective as birds if they are present in high enough numbers. Because of these factors, visitation rate is only part of a robust measure of pollination efficacy.

Pollination compensation has been identified as a potentially beneficial role of honey bees for New Zealand plant species whose natural pollinators are extinct or in decline (Butz Huryn 1997). Several introduced species in New Zealand and around the world have been linked with this service: ship rats in New Zealand

pollinating rewarewa (Pattemore & Wilcove 2012), silvereyes having an increasing role in pollination in Hawaii (Cox & Elmqvist 2000), and the red-whiskered bulbul (*Pycnonotus jocosus*) visiting flowers of a rare species in Mauritius (Olesen et al. 1998). Dick (2001) demonstrated that pollination compensation by honey bees can be particularly important in fragmented vegetation presumably because of the ability of bees to travel long distances and coordinate foraging. The role of honey bees in pollination compensation in New Zealand is yet unknown. Of the pollinator groups we know about, birds (Kelly et al. 2010), bats (O'Donnell et al. 2018), skinks and geckos (Hitchmough et al. 2013) have shown substantial declines, from loss of species to shrinking population sizes and distributions. It is likely that honey bees may not be effective at pollinating flowers adapted for pollination by larger pollinators, such as birds, bats, skinks and geckos (Kelly et al. 2006). In addition, there is little data on populations of native invertebrates, making declines and extinctions difficult to discern. However, Butz Huryn (1995) identifies several threatened New Zealand plant species used by honey bees which should be investigated for the potential for honey bees to increase seed set if pollination is currently limiting.

In contrast, honey bee visitation of native plants can have negative implications for plant reproduction. Excessive pollen deposition can result in decreased seed set due to pollen tube competition (Young & Young 1992), and high visitation rates can cause mechanical damage of the flower itself, preventing effective pollination and fruit development (Aizen et al. 2014). In addition, honey bees have been seen on occasion to remove the pollen deposited on stigmas by previous flower visitors, decreasing potential for fruit set (Gross 1993; Gross & Mackay 1998). Fruit development can also be affected by the quality and quantity of pollen deposited by flower visitors. Several studies have shown the impact of poor pollination services on the reproductive output of plants. Celebrezze & Paton (2004) demonstrated the comparatively superior pollination service provided by birds over honey bees through a series of exclusion experiments on the Australian small shrub *Brachyloma ericoides*. Despite honey bees contacting reproductive parts of the flowers, and having a higher visitation rate overall, fruit set from shrubs where the birds were excluded was significantly lower. Vaughton (1996) had similar results, but this time bird-excluded treatments for *Grevillea barklyana* showed lower fruit set than treatments where all pollinators were excluded.

Part of the highly efficient foraging behaviour of honey bees may involve nearest neighbour movements, minimising travel distances between flowers. This is not a behaviour peculiar to honey bees. While this is beneficial for the pollinator, it can have detrimental effects on the plant because of lower rates of outcrossing and the potential for inbreeding depression (Vaughton 1996; England et al. 2001). Some New Zealand plants have mechanisms to prevent long-term negative effects, such as self-incompatibility (Godley 1966; Godley & Smith 1976), or high mortality of seedlings produced by selfing (Schmidt-Adam et al. 2000). These honey bee behaviours that can impact native plant species are affected to a large extent by honey bee population density, plant reproductive strategy, the presence and dominance of other pollinators, and environmental effects.

Because of complex biological interactions, long term plant reproductive effects are difficult to confirm based on available data. For example, Taylor & Whelan (1988) inferred a negative effect on plant reproductive success based on observations of flower visitation by honey bees on *Grevillea* resulting in little or no measurable transport of pollen, and no successful deposition of pollen on the flower stigmatic surface; and Gross (1993) observed honey bees removing pollen from plant stigmas that had been deposited by other pollinators (see also Gross & Mackay (1998)). Other studies use comparative visitation rates of honey bees compared with other native flower-visitors to infer a negative impact (Kato & Kawakita 2004). However, these observations are not linked to measures of population-level effects that could confirm long-term impacts.

Impacts on flower visitors

Competition with native flower visitors for resources is frequently cited as a primary concern arising from the introduction of honey bees into native ecosystems. Studies have shown that the removal rate of floral resources by honey bees can be between 80-100% (Paton 1990; Celebrezze & Paton 2004). This represents a significant loss of resources available for forage by native flower visitors. When honey bee density is high, corresponding high levels of resource depletion can cause displacement of native pollinators or expansion of foraging ranges (Paton 1995; Hansen et al. 2002). This results in higher energy expenditure while foraging and will result long-term in adaptation or reduced fitness, affecting composition of flower-visitor communities. This thesis uses community ecology to investigate the

effect of honey bee hive density in native forest on the composition and structure of invertebrate flower-visitor communities.

Literature investigating honey bee impacts on native invertebrates has focused on the interaction between honey bees and native bees, presumably because this is the area of greatest niche overlap. However, many of these studies use observational techniques, and measures such as visitation rate to infer competitive interaction between honey bees and native flower-visiting invertebrates (Kato & Kawakita 2004). Few studies delve into the foundational population level effects that will have an impact in the long term (Sugden & Pyke 1991; Roubik & Wolda 2001; Thomson 2004). Thomson (2004) is one of the few studies which show the effects of honey bee competition on the reproductive fitness and fecundity of a competing invertebrate, in this case a bumble bee (*Bombus occidentalis*) native to North America. Over a three-year period, nest boxes of the native pollinator were set up in an area of vegetation at increasing distances from established apiaries. After each summer season, the nest boxes made it possible to investigate the reproductive success of the native pollinators in terms of cocoons produced, gyne number, and gyne ratio. Results demonstrated that hives in areas of high honey bee density responded to competitive exploitation by reallocating pollen foragers to nectar collection, resulting in lower larval production. In contrast, Paini et al. (2005) found no short-term impact of honey bees on the reproductive success of an Australian native bee. Possible variation in adaptation to high temperature was cited as a potential reason for this, giving the native bee a competitive advantage over honey bees in their natural range. Other studies produced results that were less clear (Roubik 1983; Sugden & Pyke 1991).

There are 27 species of endemic bees distributed throughout New Zealand (Donovan 2007). For the most part they are solitary bees, with the exception of the Halictidae (4 species) which are primitively eusocial. Most species are ground nesting, digging tunnels in bare soil, sand or clay, but some nest in holes in wood (Hylaeinae) (Donovan 1980). In this sense, competition with honey bees for nest sites is not an issue. Concern about competition between native bees and honey bees is instead related to niche overlap in terms of forage preferences and activity periods. Current apiary management practises often see honey bees active throughout the year, with a peak in spring and summer. Native bees are active during late spring to early summer, with some Halictinae active till May (Donovan

1980). New Zealand's native bees are for the most part generalist foragers, with some groups focusing on specific plant families. For example, Colletinae are comprised of three groups which forage either on Myrtaceae, Leguminoseae, or Compositae (Donovan & Macfarlane 1984). Of the native genera used by native bees as forage (Donovan 1980; Donovan 2007), 70 % are recorded as used by honey bees in accounts by Walsh (1978) and Butz Huryn (1995). Experimental studies on the effects of honey bees on native bee abundance and diversity are few in New Zealand. Iwasaki et al. (2018) demonstrated that competition between native bees and honey bees was potentially minimised by resource partitioning and differential access to flowers (i.e. long tongue bees versus short tongue bees).

In many cases the pollinator niche of birds does not overlap as strongly as that of insects and honey bees. Birds are often active earlier in the day, before it is warm enough for honey bees to begin foraging. They are also more capable of foraging during weather conditions that would prevent honey bee foraging, such as cold and wet conditions (Vaughton 1996; Hansen et al. 2002; Celebrezze & Paton 2004). New Zealand's nectar-feeding fauna are also represented by a number of species of endemic bat, skink and gecko, which share flower visitation preferences with honey bees (Whitaker 1987; Pattemore 2011). Temporal separation of flower visitation due to the nocturnal nature of these vertebrate fauna (Eifler 1995; Newstrom & Robertson 2005) may minimise competitive displacement of these species by foraging honey bees, depending on the extent of and time frames for nectar replenishment in forage species. However, there is still potential for competitive interaction and negative impacts between vertebrate flower visitors and honey bees such as pollinator displacement, competition for nest sites, and resource exploitation. In New Zealand, the potential for honey bees to exacerbate the decline of vertebrate flower visitor populations appears small in proportion to the impact of introduced predators and habitat loss (Diamond 1984; O'Donnell 1996; Kelly et al. 2010). However, Castro (1995) demonstrated that resource limitation, a factor with the potential to be exacerbated by honey bees, limited the fecundity of native hihi (*Notiomystis cincta*) on Kāpiti Island.

Though managed honey bees nest in hive boxes, colonies can spread from there to form feral populations, raising concern for competition between native birds and honey bees for nest sites (Butz Huryn 1997; Beard 2015). Competition for nest sites between birds and honey bees has not been investigated in New Zealand, however,

anecdotal evidence suggests that since the arrival of *Varroa*, wild populations of honey bees are few, indicating that the likelihood of competition for nest sites is low (Beard 2015). In Australia, Oldroyd et al. (1994) showed an overlap between nest choices of Cockatoo and honey bees, but demonstrated that 52% of nest sites selected by honey bees were unsuitable for cockatoo, and only 0.7% of available nest hollows were occupied by honey bees. Saunders (1979) identified a season of cockatoo breeding failure that appeared to be connected with honey bee swarming, however in this case, as with many others, the most widespread cause of competition for nest sites is reduced habitat availability via deforestation and degradation of natural vegetation.

1.2.3 Management of honey bees in native ecosystems

Because of the pervasive nature of honey bees and the economic benefits available from apiculture, there are few examples of conservation management of honey bees on conservation land. An example of a complete honey bee elimination project was carried out on Santa Cruz Island, off the coast of California - an island slightly smaller than New Zealand's Great Barrier Island (Wenner & Thorp 1993; Wenner & Thorp 1994; Wenner et al. 2009). Colonies were first identified, then a parasitic mite, *Varroa destructor*, was administered to the colonies as a biocontrol agent. Monitoring was maintained until the last colony had been eliminated in 2003. The elimination of honey bee colonies was conducted in stages so as to study the impacts of honey bee removal along the way. A significant increase was observed in the number of native bees encountered along a transect covering eastern island sites where honey bees had been removed and western sites where they were still present. Honey bees had been established for 110 years prior to removal. This example demonstrates that it is possible to remove honey bees, both wild and managed, in a closed system with clearly defined borders and no route for re-invasion. In most situations, however, this is not the case, and complete eradication would be neither possible nor necessary and would condemn apiculture.

Paton (1990) suggested a resource allocation system for management of honey bee numbers. His work on resource (nectar and pollen) removal from flowers demonstrated that up to 100% of the resource can be removed in honey bee visits, leaving little for native fauna to utilise (Paton 1990; Celebrezze & Paton 2004). By monitoring visitation rate, and understanding likely patterns of resource removal, Paton suggested that apiarists can manage the amount of resource exploited by

honey bees and move hives to a new location once a specified threshold has been reached.

In New Zealand, hives must be registered and maintained within regulation to prevent the spread of honey bee diseases and parasites such as American foulbrood (*Paenibacillus larvae larvae*) and *Varroa destructor* mites. Permission to place honey bee hives on Public Conservation Land (PCL) requires the owner to lodge an application, and the Department of Conservation (DOC) to grant a concession. The process for granting these concessions has changed in recent years in response to growing concern about the unknown impacts of apiculture on native ecosystems (Department of Conservation 2015a). Applications for hive concessions generally require consultation with local iwi to assess cultural impacts, and assessments of environmental impacts, and attract fees for the concession and ongoing monitoring of the apiary (Department of Conservation 2019). This process is designed to minimise ecological impact by restricting beehives in high risk areas and focusing on low risk areas. Attributes classifying areas as high risk include: where the currently approved stocking rate (three hectares per hive) has already been reached or exceeded, where problem weeds that are pollinated by honey bees are being actively managed, large tracts of PCL with a low ratio of edge to interior, EMU (Ecosystem Management Units), rare ecosystems, and areas where vulnerable threatened or at risk fauna and flora are present and have the potential to be impacted by honey bees (Department of Conservation 2015). These processes are intended to improve the management of apiaries on conservation land, but non-compliance still occurs through unregistered and illegally placed hives that cannot be traced back to an owner.

1.2.4 Summary

In summary, this literature review has highlighted a growing global body of research on the potential impacts (positive or negative) of honey bees in native ecosystems. It has become evident that the dynamic nature of the systems is such that a single model or solution is only ever narrowly applicable. It is therefore crucial for research to be undertaken within the New Zealand context to highlight the implications of a widespread, managed invertebrate introduction into New Zealand native ecosystems. This will also enable development of evidence-based management prescriptions.

1.3 Research Objectives and Questions

Three research aims were developed to address the lack of New Zealand focused research on the actual and potential impacts of large-scale introduction of honey bees into native forest ecosystems.

Research Aim One: Understand nectar dynamics for Weinmannia racemosa *(kāmahi) and* Ixerba brexioides *(tāwari)to assess resource availability*

Research Aim One was designed to quantify nectar availability for *I. brexioides* and *W. racemosa* submontane forest in the Kaimai-Mamaku range. Competition for floral resources becomes more likely if resources are limited. Because of this, understanding the dynamics of floral resource production will enable more appropriate management of those resources to minimise competitive interactions between honey bees and native flower visitors.

The critical steps associated with this aim include:

1. Record year-round phenological data
2. Quantify nectar production for *W. racemosa* and *I. brexioides*
3. Track environmental variables

Research Aim Two: Assess the impact of honey bees on two endemic trees: Weinmannia racemosa *(kāmahi) and* Ixerba brexioides *(tāwari)*

Research Aim Two aimed to observe the potential of honey bees to act as pollinators for *I. brexioides* and *W. racemosa*, two native trees with contrasting flower size and structure.

The following critical steps contribute to this aim:

1. Observe flower visitor behaviour
2. Assess pollen limitation of *W. racemosa* and *I. brexioides*
3. Assess seed set success at sites of varying hive density

Research Aim Three: Investigate the effects of honey bee introduction in native forest on flower-visitor communities

Research Aim Three was purposed to analyse the composition and structure of invertebrate flower visitor communities in response to introductions of commercially farmed honey bees. This is an important step towards preserving native interactions between plants and pollinators, native flower visitor survival and successful plant reproduction.

Three critical steps contribute to Research Aim Three:

1. Collect representative communities of flower visitors and identify key participants at sites of low and high honey bee hive density
2. Analyse patterns of community composition at sites of low and high honey bee hive density
3. Analyse plant-pollinator network structure at sites of low and high honey bee hive density

1.4 Thesis Outline

Research that addresses the objectives described above is presented in the following five chapters:

Chapter Two: Nectar dynamics of Weinmannia racemosa *and* Ixerba brexioides *in submontane forest in the Kaimai-Mamaku Range, New Zealand*

Chapter Two uses measurements of nectar volume and sugar content, paired with observations of flowering phenology to describe the dynamics of nectar production for *W. racemosa* and *I. brexioides* in the Kaimai-Mamaku Range, New Zealand. Data from National Vegetation Survey (NVS) plots is used to scale measurements per hectare, highlighting seasonal changes in nectar sugar crops, and implications for apiary management.

*Chapter Three: Introducing honey bees (*Apis mellifera*) into native New Zealand submontane forest: impacts on* Weinmannia racemosa *(kāmahi) and* Ixerba brexioides *(tāwari)*

Chapter Three uses video surveillance and experimental pollination treatments at sites of varying honey bee hive density to explore the pollination potential of honey bees for *W. racemosa* and *I. brexioides* and the effects of honey bee visitation and hive density on seed set.

*Chapter Four: Influence of honey bee (*Apis mellifera*) invasion on invertebrate flower visitors in the Kaimai-Mamaku Range, New Zealand*

Chapter Four details the implications of honey bee invasion on plant-pollinator community composition and structure in native New Zealand forest, focusing on flower visitors of *W. racemosa* and *I. brexioides*. Invertebrate flower visitor communities collected via sweep netting are analysed using community ecology methods and network mapping and compared between sites of varying honey bee

hive density. The implications of hive density on flower visitor communities is discussed.

Chapter Five: Method comparison for identifying and understanding plant-pollinator interactions

Chapter Five uses literature review and pilot studies of pollen identification to review traditional morphological approaches and novel molecular approaches to identifying plant-pollinator species interactions. Contrasting methods were compared to inform recommendations made for future research.

Chapter Six: Synthesis and Recommendations

Chapter Six summarises the findings of all the research presented, including implications for conservation management in New Zealand and directions for further research.

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Chapter 2

Nectar dynamics of *Weinmannia racemosa* and *Ixerba brexioides* in submontane forest in the Kaimai-Mamaku Range, New Zealand

Abstract

Floral resource availability has ecological and environmental implications for conservation management and economic implications in apiculture and agriculture. However, methods of floral resource quantification rarely agree, and are seldom paired with phenological data to inform timing and extent of resource availability beyond the flower level. This chapter uses both phenology and floral resource quantification to investigate the dynamics of nectar availability and sugar content for two dominant native forest trees, *Ixerba brexioides* and *Weinmannia racemosa,* in the Kaimai-Mamaku Range, North Island, New Zealand. Nectar sugar content per floret during the summer of 2017 was less than half the sugar content per floret during 2016 for both *I. brexioides* and *W. racemosa*. In addition, the mean number of inflorescences per tree during 2017 was only one fifth of the number of inflorescences per tree in 2016. These findings highlight large-scale dynamic changes in floral resource availability and hence ecosystem carrying capacity for nectar-feeding fauna from year to year. Understanding this natural variation in nectar production can assist informed resource allocation for sustainability in apiculture and in native forest management.

2.1 Introduction

Quantifying floral resource availability in different ecosystems is important for restoration and management of natural ecosystems because of implications for sustaining wildlife, and the ecosystem services they provide (Dennis 2010). It also has economic implications for agriculture and apiculture that rely on pollination services and floral resources (Enkegaard et al. 2016). However, quantifying large-scale floral resource availability is not a simple task. Floral resource availability is difficult to measure and predict because of the confounding sources of variation in nectar and pollen quality, quantity and production rate between plant species, time of day, age of flowers, and also variable levels of consumption by consumers

(Nicolson et al. 2007). For these reasons, few studies agree on standard methodologies for measuring floral resource availability as they must be adapted to different species, landscapes, climates, and research aims (Szigeti et al. 2016).

Mapping fluctuations in flower numbers per plant is generally undertaken using phenological studies which measure the timing and magnitude of flowering events. Phenological studies are useful for resource estimation and, when carried out over extended time scales, can provide a useful indicator of climate change (Chmielewski & Rötzer 2001; Visser Marcel & Both 2005; Peñuelas & Filella 2009; Brown et al. 2016) and population dynamics (Bewick et al. 2016; McLean et al. 2016). Despite their usefulness, long running phenological studies are few. An exception is the USA National Phenology Network (NPN), which uses citizen-science to record flora and fauna in various clearly defined phenophases using an electronic app. Data in this network covers over 800 species of plant and over 300 of these can be mapped and downloaded for general use (USA-NPN National Coordinating Office 2012; USA-NPN National Coordinating Office 2016). In contrast, phenological data from New Zealand ecosystems is sparse: Leathwick (1984) presented a phenology of trees, shrubs and lianes in four central North Island forests that demonstrates timing of vegetative growth, flowering, and fruiting; and Castro (1995) focused on phenology of food sources for hihi on Kāpiti Island, including timing and abundance of flowering and fruit set. New Zealand also has an online platform, iNaturalist NZ, that is using citizen science to develop a database of species occurrence data and phenological monitoring that will continue to become more useful as data volume increases (iNaturalist 2019). Rarely do phenological studies translate into measures of floral resource availability, particularly in New Zealand where data on per-flower floral resource production is extremely limited.

Most often, floral resource availability is calculated using simple measures, such as flower number or flowers per unit area (Tepedino & Stanton 1981). Few studies have delved further into resource value (nectar and pollen volumes) (Zimmerman & Pleasants 1982; Potts et al. 2004), or into quantifying the nutritional constituents of those resources, such as amino acids and sugars, at a landscape scale. Zimmerman & Pleasants (1982) demonstrated that results from measuring the actual resource (nectar and pollen) are more conclusive than those relying on proxies such as flower number or flowers per unit area, however in some systems,

with sophisticated modelling, reliable estimates of floral resource potentials can be made (Frankl et al. 2005). Length of study is another important factor of experimental design relating to resource availability. Because of seasonal and inter-annual variation in resource availability and flower-visitor network structure, one season of data is insufficient to draw meaningful conclusions (Alarcón et al. 2008). An additional consideration is the spatial scope of the study and the degree of extrapolation. In general, because of the time-intensive methods needed to develop fine resolution data on resource availability, a finer resolution is only possible with a smaller scale of research.

Floral nectar is an important part of the diet of nectar-feeding New Zealand native birds, insects, geckos, skinks and bats (Newstrom-Lloyd 2013), but is also important for managed populations of introduced bees that are vital for agriculture, such as honey bees (Donovan 2007). Not only is nectar a main energy source for honey bees (Nicolson 2011), but it also forms the basis of their usefulness in apiculture as the pre-cursor to honey. As land-use changes and habitat modification reduce the availability of nectar worldwide (Kremen et al. 2007; Giannini et al. 2012; Otto et al. 2016), inability to sufficiently provide for nectar requirements of managed honey bees can result in lower industry returns (Al-Ghamdi et al. 2016), and increased competition with native nectar-feeders for resources (Butz Huryn 1997). In an assessment of the potential impacts of increasing presence of managed honey bees on conservation land, Beard (2015) identified limited information on availability and limitation of floral resources as a significant knowledge gap in New Zealand.

This chapter combines methods of floral resource quantification with phenological research to explore the dynamics of nectar availability for two abundant endemic New Zealand trees – *Ixerba brexioides* and *Weinmannia racemosa.* These trees are native to New Zealand, and commonly targeted by apiarists for the production of monofloral honey. Both species are similar in habit and co-occurring in habitat. Both are primarily insect-visited but differ in floral structure and floral reward display. This study demonstrates the ecological, environmental, and economic value of quantifying floral resource availability beyond simply phenological or per flower resource measures for improved application in conservation management and sustainable apiculture. It was expected that reliable inter-annual estimates of

floral resource availability would require a combination of both types of resource measurement.

2.2 Methods

2.2.1 Study Sites

Research for this study occurred in the submontane belt (400 to 800 metres above sea level) of the Kaimai-Mamaku range over two peak flowering periods: November 2016 to January 2017 and November 2017 to January 2018. Sites were selected based on accessibility, proximity to honey bee hives, and presence or absence of target species. Sampling was carried out at four sites each year, with sites on average 40 km apart. The initial sampling season focused on the Kaimai-Mamaku Range, New Zealand, with field sites accessed from Department of Conservation (DOC) tracks Tuahu, North-South (at the State Highway 29 lookout), Woods Mill, and Mokaihaha. In season two (2017-2018) the North-South track at SH29 was abandoned due to accessibility issues, low flowering, and low pollinator activity. Maungatautari Sanctuary Mountain was included in the second season of sampling to replace the North-South site. Figure 2.1 shows the location of study sites.

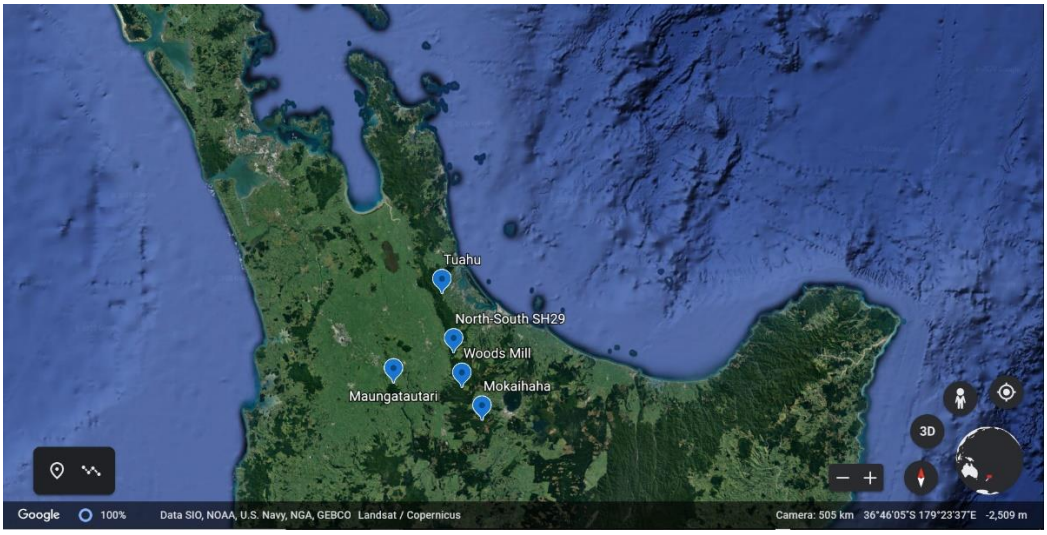

Figure 2.1:Location of field sites, North Island, New Zealand.

Field sites were categorised based on known hive density within 5 km and pest management status (Table 2.1). Hive density categories were assigned based on 2016 hive numbers and categories were maintained for 2017. Hive density categorisation presented challenges due to the difficulty of obtaining data on the density of existing hives. Data on registered hive numbers and locations are kept by AsureQuality and though it can be made available for research purposes, the process

took considerable time between request and receipt of data (over 12 months) and the level of detail available was low. The number of unregistered hives and feral colonies was unknown, but both are likely to be present at study sites. The pest management category separated the Maungatautari site from the others on the strength of it being a pest-proof sanctuary.

The Kaimai-Mamaku range is a large tract of native forest in the central North Island of New Zealand with a long history of disturbance due to demand for timber, gold mining, farming and stock droving. Today it is a protected natural area administered by the Department of Conservation (Department of Conservation 2006). The Forest Park stretches from State Highway 2 through the Karangahake Gorge southwards to State Highway 5 near Rotorua. The sites selected for this research represent the latitudinal span of the Kaimai-Mamaku range and are located in the submontane belt (400 to 800 metres above sea level).

Vegetation at each of the study sites has a canopy dominated by *I. brexioides* and *W. racemosa*, with *Beilschmiedia tawa* (tawa) and *Knightia excelsa* (rewarewa) also common. The understory is comprised mostly of *I. brexioides*, *Coprosma grandifolia* (kanono), *Hedycarya arborea* (pigeon wood), *Melicytus ramiflorus* (māhoe) and tree fern species *Cyathea dealbata* (silver fern) and *Dicksonia squarrosa* (wheki). In addition to previously mentioned species, the shrub layer is often dominated by *Freycinetia banksii* (kiekie), *Alseuosmia macrophylla* (toropapa), *Parablechnum novae-zelandiae* (kiokio), *Geniostoma ligustrifolium* var. *ligustrifolium* (hangehange) and *Leucopogon fasciculatus* (mingimingi). Ground cover includes several species of native ferns such as *Lomaria discolor* (crown fern) and *Asplenium bulbiferum* as well as native sedges and grasses *Carex uncinata* (hook sedge) and *Microlaena avenacea* (bush rice grass).

Maungatautari Sanctuary Mountain comprises 3,400 hectares of mainly native forest in a matrix of farmland that has been surrounded by 47 km of pest-proof fencing since 2006. Non-native pest species eradicated from the mountain include hedgehogs (*Erinaceus europaeus occidentalis*), cats (*Felis cattus*), Norway rats (*Rattus norvegicus*), ship rats (*Rattus rattus*), stoats (*Mustela erminea*), ferrets (*Mustela furo*), weasels (*Mustela nivalis vulgar*), rabbits (*Oryctolagus cuniculus cuniculus*), hares (*Lepus europaeus occidentalis*), possums (*Trichosurus vulpecula*), deer (*Cervus spp.*), pigs (*Sus scrofa*) and goats (*Capra hircus*).

Surveillance and volunteer maintenance are designed to prevent reinvasion of pest species (Sanctuary Mountain 2018).

Table 2.1: Study site location (latitude and longitude), altitude (m), registered hive numbers within a 5 km radius of the sites in 2016 and 2017, hive density category (L=low, H=high), and pest management status whether unmanaged (U) or within a pest-proof fenced sanctuary (F).

Site	Lat.	Lon.	Alt.	2016	2017	Hives	Pests
Woods Mill	-38.03	175.98	500	140	150	L	U
North-South	-37.87	175.93	500	374	-	H	U
Mokaihaha	-38.18	176.10	600	97	395	L	U
Tuahu	-37.60	175.86	400	822	1444	H	U
Maungatautari	-38.01	175.58	500	-	431	H	F

2.2.2 Study Species

Ixerba brexioides (tāwari) is an endemic tree that grows in submontane forest north of 38 degrees latitude. Its most conspicuous feature is its large white inflorescences that open *en masse* each year in December. *Ixerba brexioides* grows up to 20 metres in height. The pollination system of *I. brexioides* has been elucidated by Thomson (2013) and was found to be primarily entomophilous, though bird visitation has been reported by other authors (Schneider 2007; Dawson et al. 2011). Nectar collected from *I. brexioides* by European-derived *Apis mellifera* (honey bee) is important for the production of tāwari honey. Honey bees represented up to 10% of flower visitors to *I. brexioides* in a 2012 study on the Mamaku plateau (R Thomson unpublished data).

Weinmannia racemosa (kāmahi) is also one of New Zealand's native trees from which honey is produced. It is ecologically significant as our most abundant forest tree and an important food source for native fauna. It is evergreen and grows up to 25 metres in height and, like *I. brexioides*, produces abundant white inflorescences during November and December. Florets are arranged in brush-like racemes that develop a red tinge with age (Wardle & MacRae 1966). While it is generally agreed that *W. racemosa* is insect pollinated, bird visitation has also been recorded (Castro & Robertson 1997; Newstrom & Robertson 2005). Whitaker (1987) also suggested investigating the possibility of lizard pollination.

Figure 2.2: A) Inflorescences of W. racemosa *and* I. brexioides *growing on adjacent trees; B)* W. racemosa *florets; C)* I. brexioides *florets. B and C reproduced with permission from Larry Jensen (University of Auckland).*

2.2.3 Environmental monitoring

Measurements of temperature and humidity were logged year round using DS1923 iButton Hygrochrons (Maxim Integrated). At each site two iButtons were suspended in the canopy within an aluminium shield. Information on rainfall and sunlight hours was obtained from weather station data nearest each site, accessed from the NIWA online database (NIWA 2018) (See Appendix for detailed information on weather stations used). Of the searched stations, sunlight data during the observation period was only available from station 26645 in Hamilton and

rainfall was available from stations 1587 near Matamata and 1617 at Whakamarama.

2.2.4 Nectar Production

Nectar and pollen collection and measurement techniques were, of necessity, adapted to the structure of the florets of each species and the pollinator reward system they employ (see Figure 2.2 for contrast in floret size and nectar presentation). During November to January nectar was collected from *I. brexioides* and *W. racemosa* at each study site. Samples were collected both from un-bagged open florets and florets that were bagged as buds and remained bagged over the flowering season to prevent flower visits. For both species, nectar samples were routinely taken at 8 am, 12 pm, 4 pm and 8 pm on sampling days. Sampling was not conducted during rainy conditions. In total there were 16 sampling days each season. Collection of *I. brexioides* nectar occurred on 24 days total, and *W. racemosa* occurred on 17 days total. Sampling days for each site were dependent on weather conditions and availability of flowers: 14 days at Tuahu, 12 days at Woods Mill, 9 days at Mokaihaha and 3 days at Maungatautari (where sampling only occurred in one season). In total, 12 *I. brexioides* trees were sampled and 17 *W. racemosa* trees.

Ixerba brexioides nectar was collected using 100 µl microcapillary tubes (Sigma Aldritch). The volume was assessed by measuring the length of the nectar column using digital callipers. Sugar concentration was then measured using a digital refractometer (PAL1, Atago, Japan).

Nectar collection from *W. racemosa* was challenging because of the small size of the florets. Samples were taken as a pooled sample across five florets. Each floret was washed with 5 µl of distilled water using an auto pipette. The wash was collected in a micro-centrifuge tube, and then transferred to a microcapillary tube to assess volume. Sugar concentration was then measured using a handheld refractometer. This was converted to sugar content in milligrams by multiplying the percentage sugar reading by the volume and density of sucrose at the observed concentration (Bolten et al. 1979) using the equation and conversion table supplied by Dafni (1992). Washes were repeated until the sugar concentration was zero, and pooled to represent the total sugar content for the five sampled florets.

Nectar replenishment was measured for *W. racemosa* by repeated nectar sampling. Five inflorescences were selected and bagged as buds. After bloom, five florets were identified from each inflorescence for sampling. From those florets nectar was collected and sugar content measured every four hours at 8am, 12 pm, 4 pm and 8 pm in one 24-hour period. Inflorescences were bagged before and after each sampling. Replenishment was not measured for *I. brexioides* in this study, but was estimated from previous research by Thomson (2013).

2.2.5 Phenological monitoring

Phenology of the submontane forest in the Kaimai-Mamaku Range was assessed by carrying out monthly surveys at each study site throughout the year over two flowering seasons. At each site, the most abundant flowering trees and shrubs were identified and five individuals of that species selected and tagged. Tree selection was based on estimated age range (ensuring all trees were old enough for flowering and fruiting to occur), and whether the species was a documented nectar or pollen resource for honey bees. Each month, tagged trees were visited and an estimate of number of flowers and fruit at each phenological stage was recorded (Table 2.2). The full species selection for each site is given in Table 2.3.

Table 2.2: Phenological event categories and abundances (number per tree)

	Phenological stages	*Abundances*
Flowers	Flower buds	1-10
	Expanding flower buds	10-50
	Flowers	50-100
	Petal fall	100-500
Fruit	Unripe fruit	500-1000
	Ripe fruit	1000-5000
	Fruit dehisced or dispersed	5000-10000
		10000 +

Table 2.3: Species for phenological studies carried out at four sites in the Kaimai-Mamaku Range between November 2016 and November 2018.

Site	Species	Trees
Kaimai Summit	*Beilschmiedia tawa*	5
	Coprosma grandifolia	5
	Hedycarya arborea	3
	Ixerba brexioides	5
	Melicytus ramiflorus	5
	Raukaua edgerleyi	5
	Schefflera digitata	5
	Weinmannia racemosa	1
Mokaihaha	*Alseuosmia macrophylla*	5
	Aristotelia serrata	1
	Coprosma grandifolia	5
	Ixerba brexioides	4
	Melicytus ramiflorus	5
	Pseudopanax arboreus	5
	Schefflera digitata	5
	Weinmannia racemosa	5
Tuahu	*Coprosma grandifolia*	5
	Geniostoma ligustrifolium	5
	Hedycarya arborea	6
	Ixerba brexioides	5
	Leucopogon fasciculatus	5
	Melicytus ramiflorus	6
	Myrsine australis	3
	Olearia rani	5
	Pseudowintera axillaris	4
	Weinmannia racemosa	4
Woods Mill	*Alseuosmia macrophylla*	4
	Aristotelia serrata	1
	Beilschmiedia tawa	1
	Brachyglottis repanda	3
	Coprosma grandifolia	3
	Coprosma robusta	3
	Geniostoma ligustrifolium	4
	Ixerba brexioides	5
	Knightia excelsa	1
	Leucopogon fasciculatus	3
	Melicytus ramiflorus	2
	Myrsine salicina	1
	Pseudopanax arboreus	3
	Schefflera digitata	1
	Weinmannia racemosa	5
Maungatautari	*Alseuosmia macrophylla*	6
	Coprosma lucida	5
	Geniostoma ligustrifolium	5
	Ixerba brexioides	5
	Knightia excelsa	5
	Kunzea robusta	5
	Olearia rani	5
	Weinmannia racemosa	5
	Total Trees	198

2.2.6 Landscape scaling of resource production

Nectar measurements from *I. brexioides* and *W. racemosa* were used in concert with phenology data and NVS data to estimate the production of nectar per hectare for those focal species. This was done using the maximum number of flowers observed for each tree each week over the flowering period. The peak weekly flower numbers were adjusted based on a 2-week flower lifespan (based on observations from (Thomson 2013)), and multiplied by the average number of florets per inflorescence (92 for *W. racemosa*; *I. brexioides* florets were counted as flowers during phenology monitoring). This total floret number per tree was then multiplied by the average nectar sugar per floret for each species in each respective year to give an estimate of sugar production per tree. Because of limited data on nectar replenishment for these species, two scenarios were considered: where no replenishment of nectar sugar occurs after resource removal; and where replenishment occurs after 24 hours for *I. brexioides* (Thomson, 2013 unpublished data), and 48 hours for *W. racemosa*.

Data from 106 National Vegetation Survey (NVS) plots were used to determine the average number of trees per hectare for *I. brexioides* and *W. racemosa* (Hurst & Allen 2007). Only data from plots located in the Kaimai-Mamaku Range were used. Any repeat sampling of plots was ignored, and only the most recent data were used. Stem diameter information was used to determine the number of trees >5 cm DBH per plot (40 m x 40 m) for each species, and this was scaled up to trees per hectare. This identified an average of 341 *I. brexioides* and 528 *W. racemosa* trees per hectare.

Values of nectar sugar production per hectare for *I. brexioides* and *W. racemosa* were compared to hive sugar requirements based on hive stocking rates at each site. Hive sugar requirements were calculated using the average New Zealand, North Island hive honey yields from 2010 to 2019 (Ministry for Primary Industries 2019), assuming that honey surplus represents 27 % of total honey required for the hive (Southwick & Pimentel 1981) and an average water content of 17.5 % for *W. racemosa* and *I. brexioides* honey (Vanhanen et al. 2011).

2.2.7 Statistical analysis

Statistical differences in nectar production between sites, sampling times and years were assessed using R (RStudio Team 2018). Only measures from bagged florets were used. Normality of the data was assessed using the Anderson-Darling

normality test from the R 'nortest' package (Gross & Ligges 2015). ANOVAs were applied to normally distributed data using the 'aov' function. Where data did not fit a normal distribution, non-parametric Kruskal Wallis rank sum tests were applied using the 'kruskal.test' function. Environmental variables were treated similarly to discern significance of differences in temperature and humidity between sampling years and study sites.

Summary statistics for data were generated using the R 'psych' package and the functions 'describe' and 'describeBy' for variables and by groups respectively (Revelle 2019). Throughout the text averages are given as mean value ± standard error of the mean. Graphs were produced using the R 'ggplot2' package (Wickham et al. 2019). Box plots representing median values with interquartile ranges were produced where the upper whiskers show the 75th percentile to the largest value no further than 1.5 times the interquartile range. Data outside this range were plotted individually as outliers, and asterisks represent the mean. Bar graphs throughout the text show averages with error bars representing 95 % confidence intervals.

2.3 Results

2.3.1 Environmental monitoring

Overall, sampling season one was warmer, sunnier, drier and less windy than season two. Temperature (p<0.01) and humidity (p<0.01) were both significantly different between sampling years. During November 2016 to January 2017 the average temperature was 17.33 °C with 76.12 % relative humidity and average wind speed of 0.51 ms^{-1}. Average daily rainfall and radiation were 3.18 mm and 21.66 MJ/m^2 respectively. During November 2017 to January 2018 the average temperature was 21.11 °C with 56.01 % relative humidity and average wind speed of 0.18 ms^{-1}. Average daily rainfall and radiation were 2.65 mm and 24.38 MJ/m^2 respectively.

2.3.2 Nectar production

Yearly patterns of nectar production

Average nectar sugar production per floret in 2016 from bagged florets of *I. brexioides* and *W. racemosa* was two-fold higher than in 2017. *Weinmannia racemosa* florets produced on average 0.13 ± 0.07 mg of sugar per floret in 2016 and 0.06 ± 0.04 mg in 2017. *W. racemosa* inflorescences had an average of 92 florets per raceme, indicating an average sugar production per raceme of 11.96 mg of sugar in 2016 and 5.52 mg of sugar in 2017. Nectar sugar production from bagged *I. brexioides* florets was similarly more than two-fold higher in 2016 than

in 2017. Nectar sugar averaged 1.63 ± 0.44 mg of sugar per floret in 2016 compared with 0.55 ± 0.11 mg of sugar per floret in 2017. Nectar volume per floret was an average of 20.25 ± 1.36 µl in 2016 and 10.30 ± 0.77 µl in 2017. Nectar concentration for *I. brexioides* was 21.39 ± 1.57 % in 2016 and 23.13 ± 1.46 % in 2017. Sugar data did not follow a normal distribution for either *W. racemosa* (p<0.01) or *I. brexioides* (p<0.01). Non-parametric analyses demonstrated significant differences in patterns of nectar production between years for *I. brexioides* (p=0.002) but not for *W. racemosa* (p=0.12) (Figure 2.3).

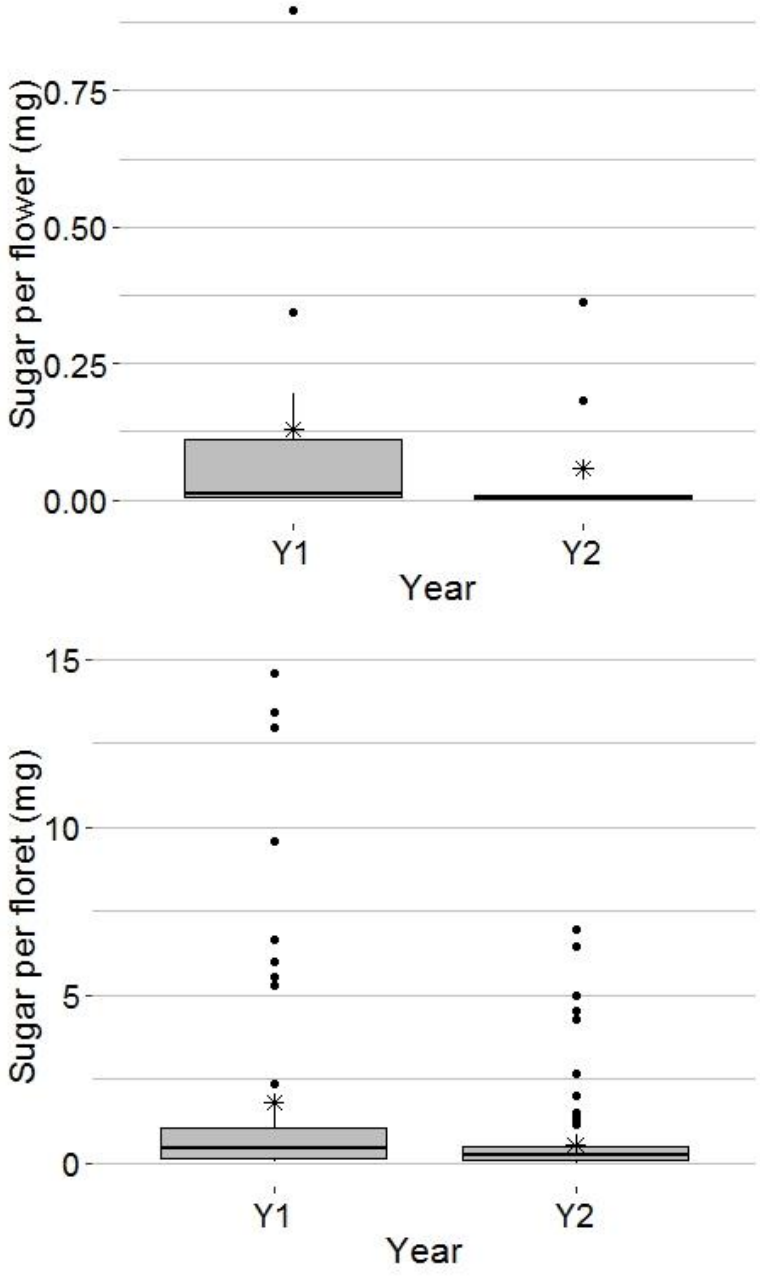

Figure 2.3: Boxplot of nectar sugar content (mg) for bagged florets of W. racemosa (top) *and* I. brexioides (bottom) *from 2016 (Y1) and 2017 (Y2) samples. See section 2.2.7 for a description of boxplot parameters.*

Weekly patterns of nectar production

Weekly patterns of nectar sugar production across the sampling years were not consistent across species or years (Figure 2.4). Nectar sugar production for bagged *W. racemosa* florets trended upwards over four sampling weeks in 2016 and 2017, but differences in sugar production were not significant (p=0.644). Nectar sugar production for bagged *I. brexioides* florets was significantly different across sampling weeks (p=0.002), increasing over 2016 and decreasing over 2017. Variability was high in Week 1 of 2016 due to low sample numbers.

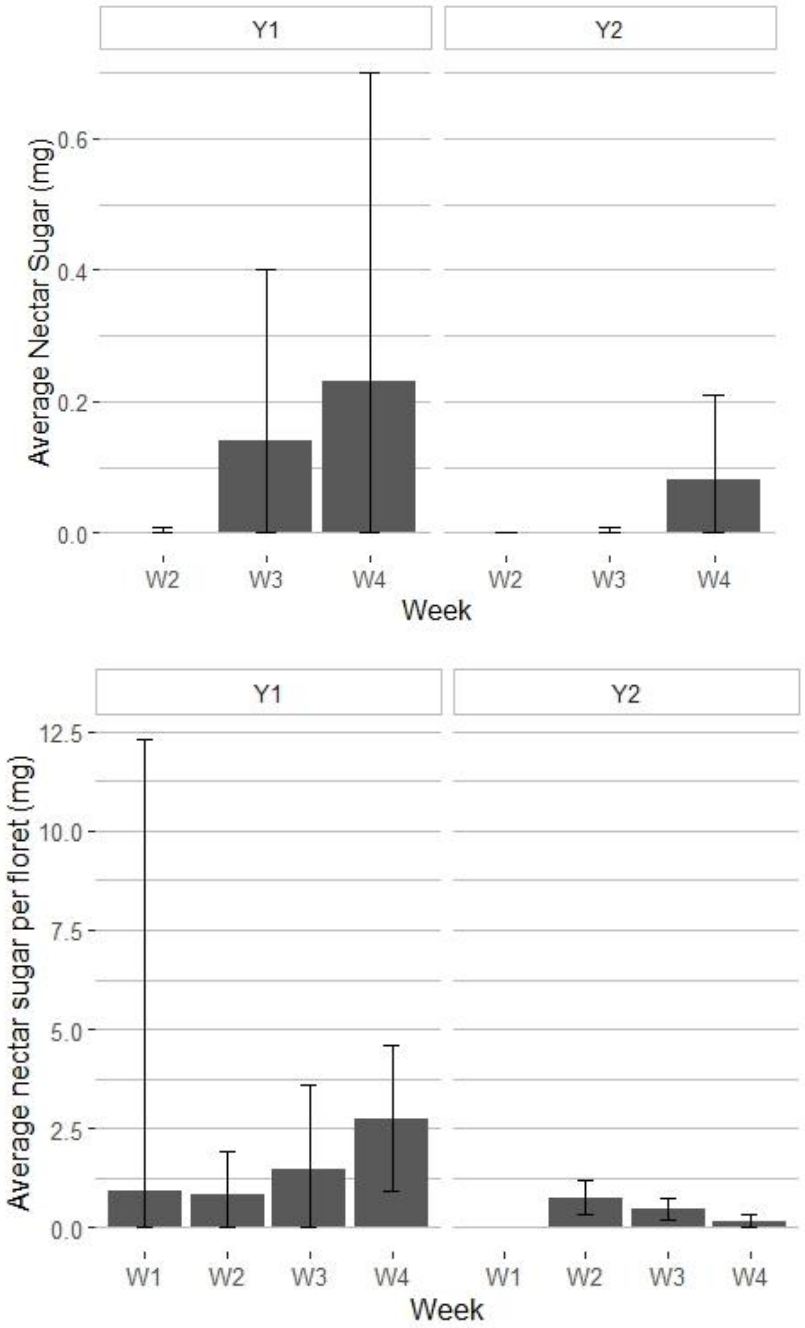

Figure 2.4: Average nectar sugar per floret (mg) for W. racemosa *(top) and* I. brexioides *(bottom) over four sampling weeks and two sampling years. Error bars represent 95 % confidence intervals.*

Daily patterns of nectar production

Nectar sugar per floret was not significantly different among times of the day for *I. brexioides* (p=0.44) or *W. racemosa* (p=0.08) (Figure 2.5). However, nectar volume and concentration for *I. brexioides* florets were significantly different among times of the day (p<0.01 for both variables) (Figure 2.6).

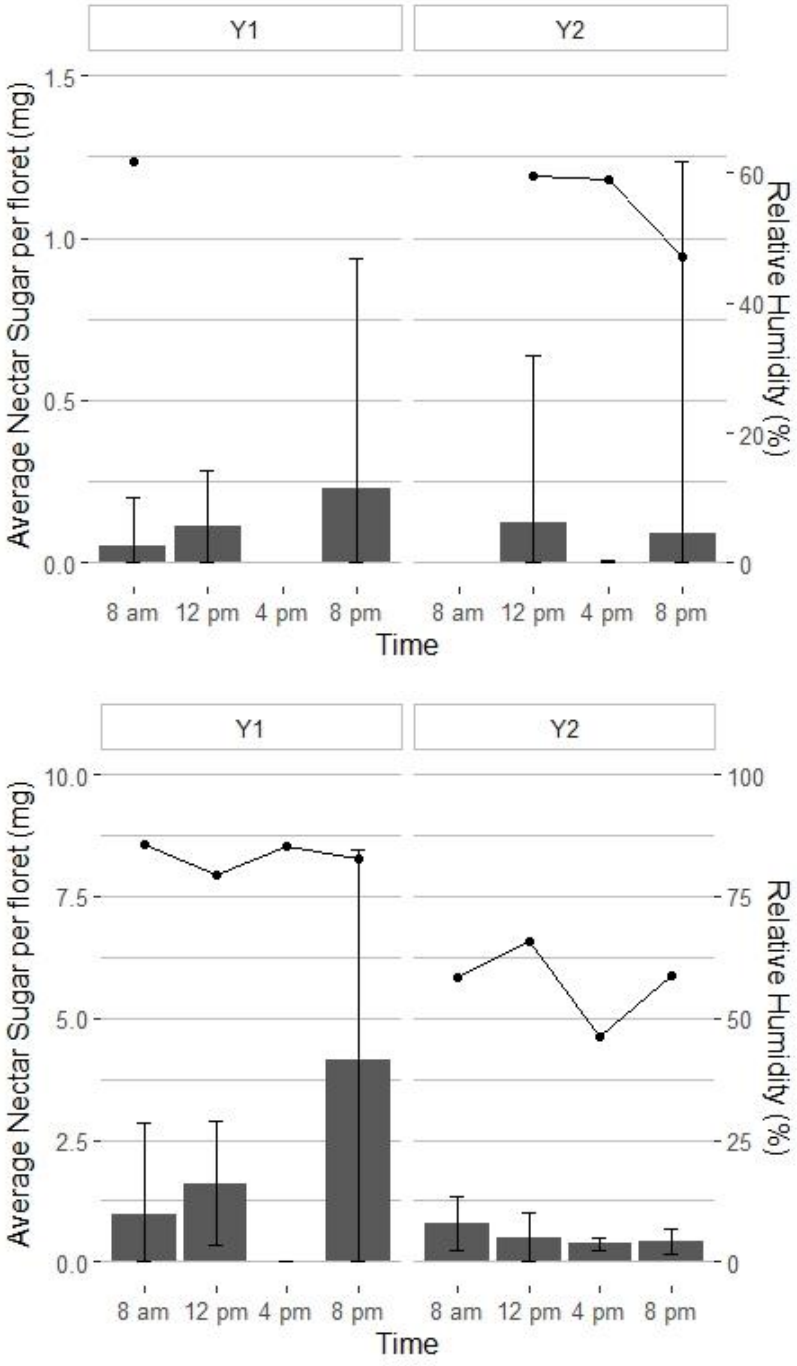

Figure 2.5: Bar chart of W. racemosa *(top) and* I. brexioides *(bottom) average nectar sugar per floret (mg) from bagged florets across daily sampling times in Y1 (2016) and Y2 (2017). The line graph represents relative humidity (%). Error bars represent 95 % confidence intervals.*

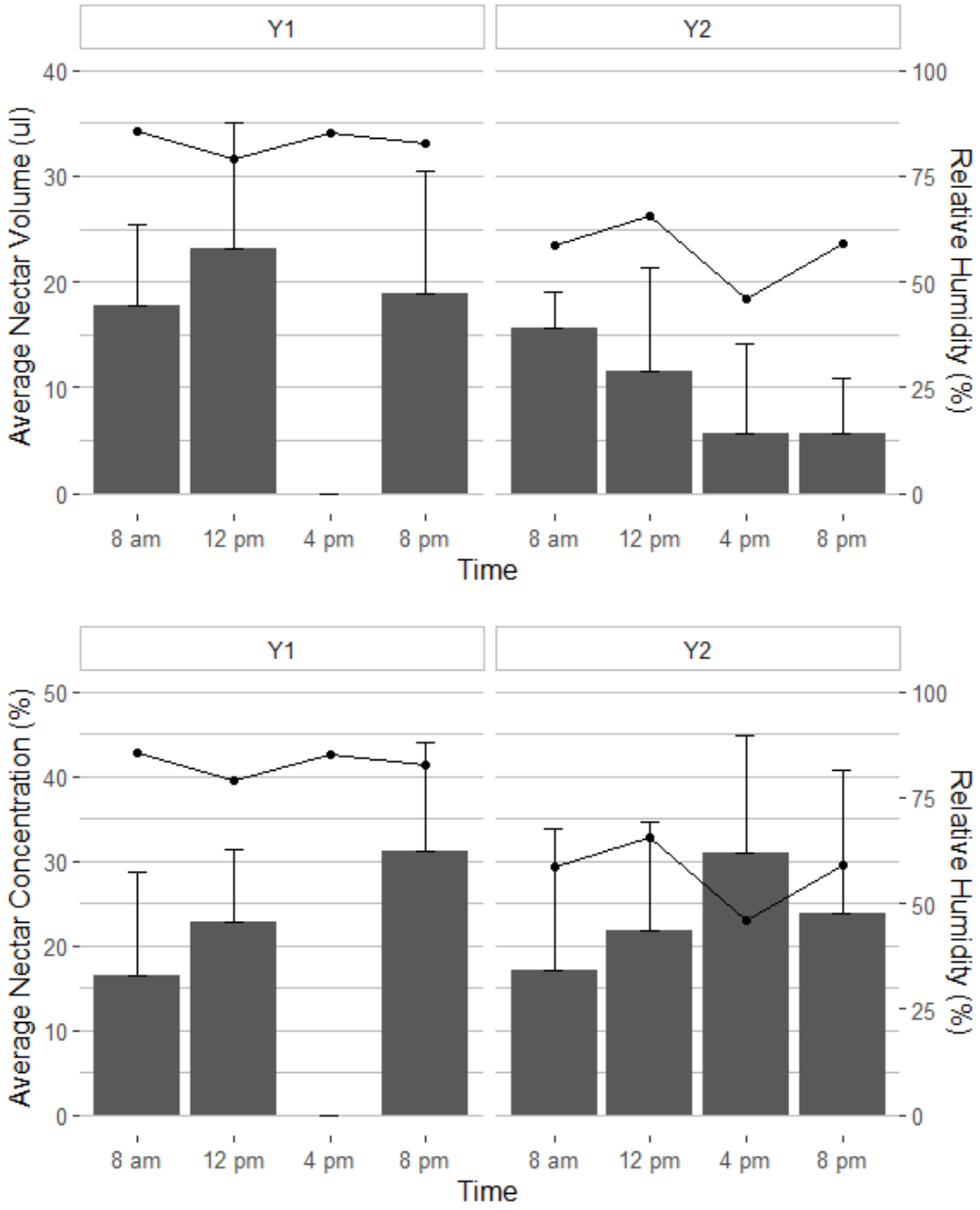

Figure 2.6: Bar chart of I. brexioides *average nectar volume per floret (ul) (top) and nectar concentration (%) (bottom) from bagged florets across daily sampling times in Y1 (2016) and Y2 (2017). The line graph represents relative humidity (%). Error bars represent standard deviation.*

Bagging treatments

Bagged samples had significantly higher nectar sugar content than un-bagged samples, indicating that significant nectar harvest was occurring on un-bagged florets (Figure 2.7). For both *I. brexioides* and *W. racemosa* this difference was significant overall (p<0.001) and for 2016 (p<0.001) and 2017 (p<0.001) sampling years.

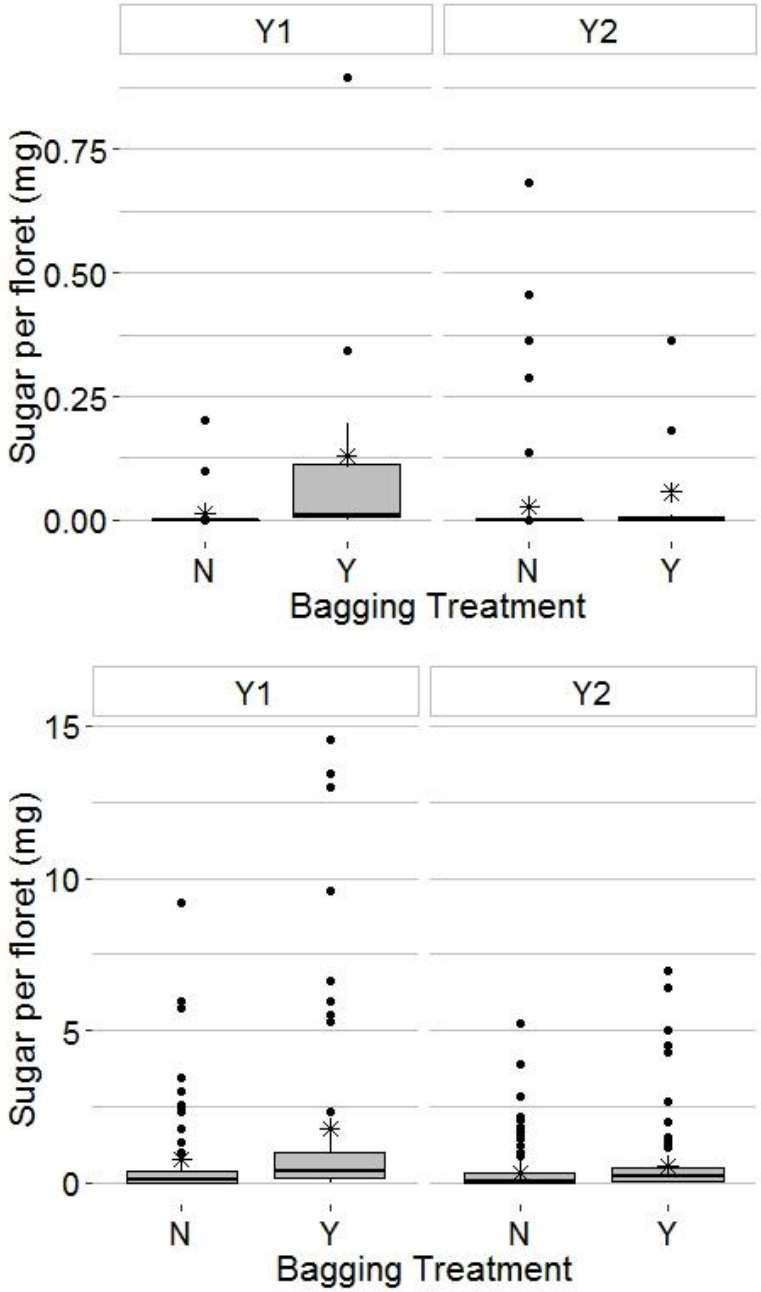

Figure 2.7: Sugar per floret (mg) for W. racemosa *(top) and* I. brexioides *(bottom) between un-bagged (N) and bagged treatments (Y). See section 2.2.7 for a description of boxplot parameters.*

Sampling sites

There was no significant difference in nectar sugar per floret between sampling sites for *W. racemosa* (p=0.35) or *I. brexioides* (p=0.29).

2.3.3 Nectar replenishment

Nectar of *W. racemosa* started with an average of 2.20 ± 1.4 µg sugar per floret. At every sampling period following initial sampling 0.00 µg of sugar was measured. This indicates either that each floret of *W. racemosa* takes more than 24 hours (limit

of study) to replenish nectar sugar or does not replenish at all. Replenishment times were not investigated for *I. brexioides.*

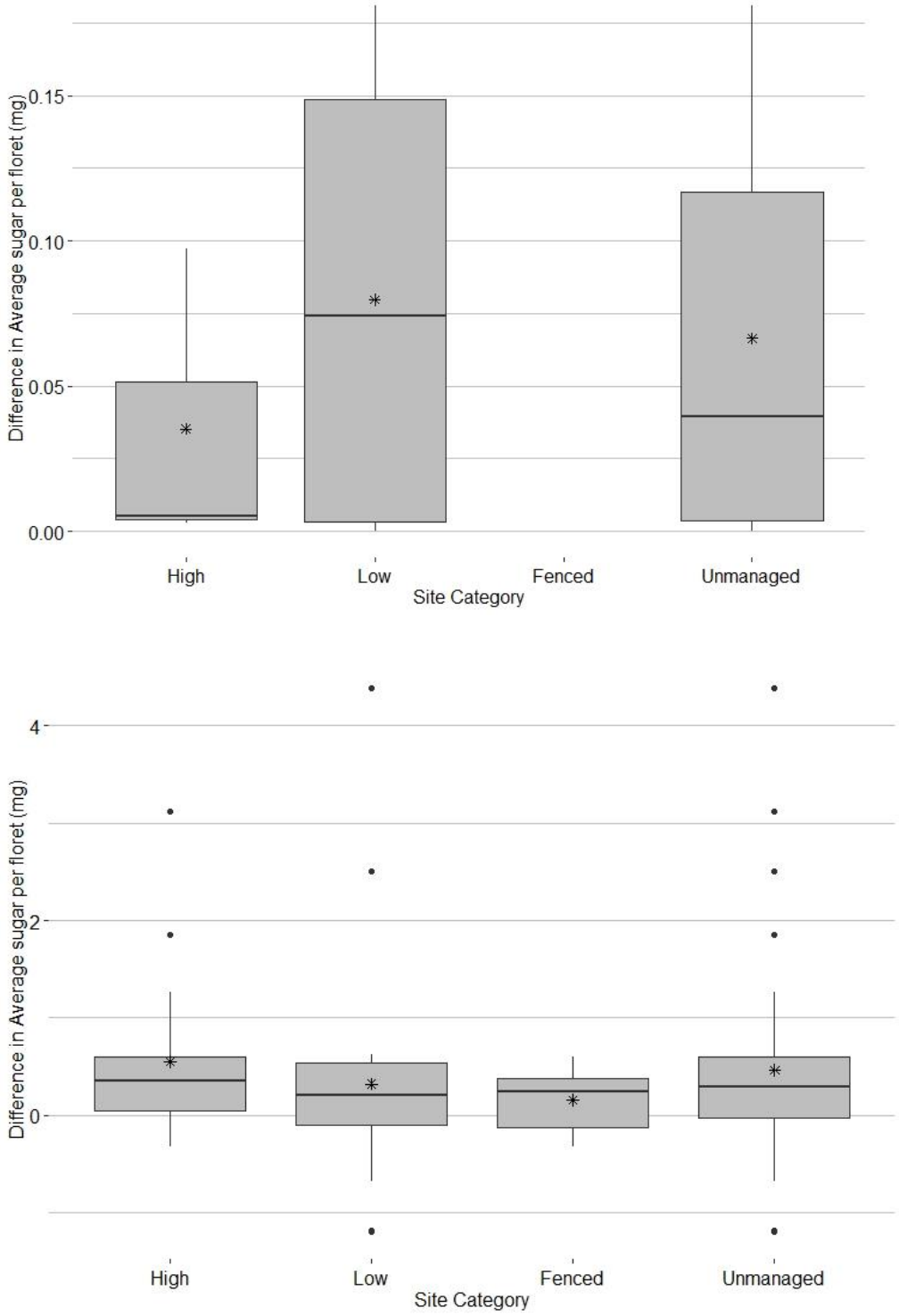

Figure 2.8: Difference in average sugar production per floret between bagged and un-bagged florets for W. racemosa *(top) and* I. brexioides *(bottom) at sites with high and low hive densities and unmanaged sites and a fenced sanctuary free of mammalian pests. Asterisks indicate mean values. Positive values indicate higher sugar production in bagged florets compared to un-bagged florets. See section 2.2.7 for a description of boxplot parameters.*

2.3.4 Hive density effects

Figure 2.8 illustrates the effect of site management on differences in average sugar observed per floret (mg) between bagged and un-bagged florets for *I. brexioides* and *W. racemosa* to identify the extent of nectar use by flower visitors. A larger difference indicates where more of the available nectar crop is being utilised. Kruskal-Wallis tests identified no significant differences in average sugar between bagged and un-bagged florets for *W. racemosa* at high and low hive density sites (p=0.732), or for *I. brexioides* at sites of differing hive density (p=0.234) or pest management (p=0.596). Differences between sampling years were not significant for *I. brexioides* (p=0.114) or *W. racemosa* (p=0.602).

2.3.5 Phenological monitoring

Figure 2.9 demonstrates how per-tree flower production fluctuated between high and low annual production. *Ixerba brexioides* showed high per-tree flower production in 2016, and low production in 2017. *Weinmannia racemosa* also displayed high flower production in 2016 and low flower production in 2017.

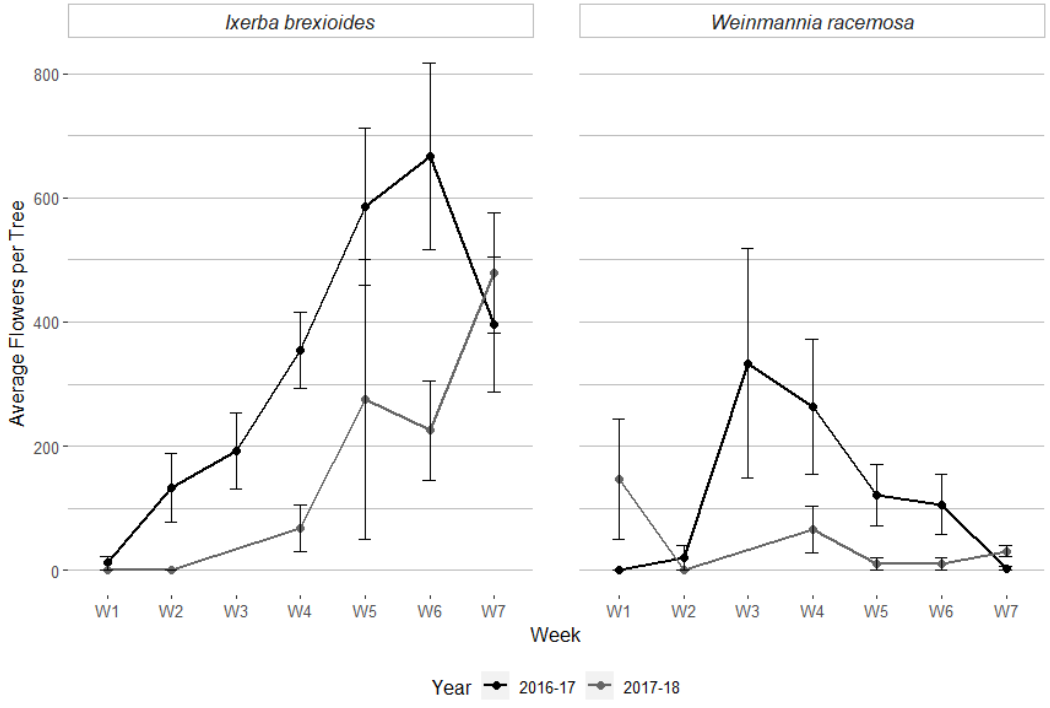

Figure 2.9: Average numbers of inflorescences per tree for I. brexioides *and* W. racemosa *over 7 weeks from 28 November 2016 to 14 January 2017 and 13 November 2017 to 1 January 2018. Error bars show standard error.*

Figure 2.10 shows numbers of flowers for all species sampled between November 2016 and August 2018. Peak floral resource availability occurred during summer months, but was overall higher during the 2016-2017 summer than the 2017-2018 summer.

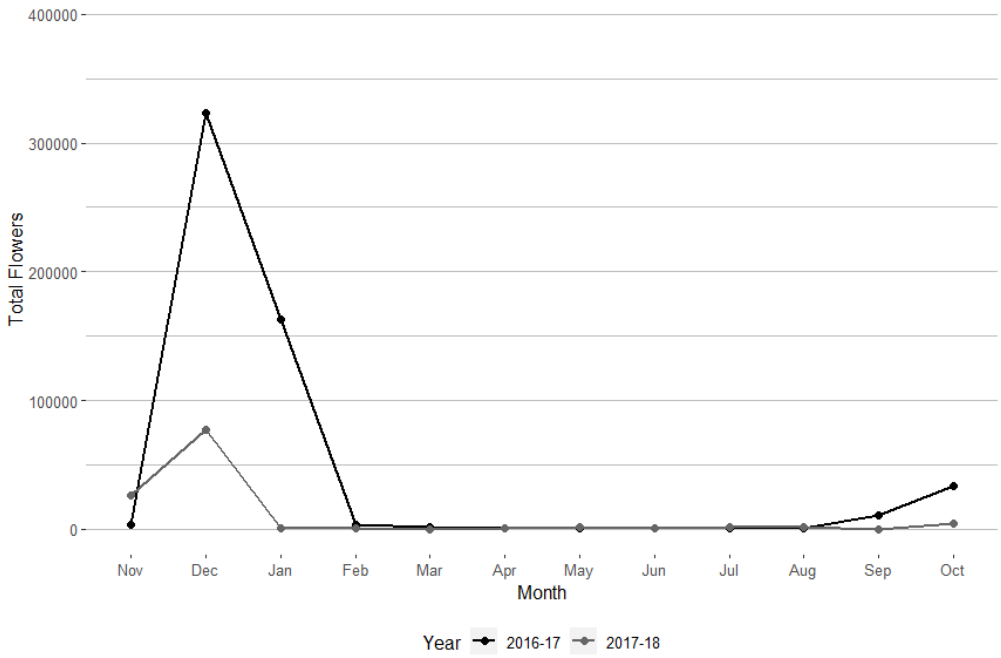

Figure 2.10: Total maximum flower numbers across 152 trees of 20 species observed monthly between November 2016 and October 2018

Flowering onset was consistent across both sampling years, generally starting in the first week of December for *I. brexioides* and ranging between mid-November and early December for *W. racemosa* (Figure 2.11). Latitudinal and altitudinal gradients did not appear to affect flowering onset at this scale.

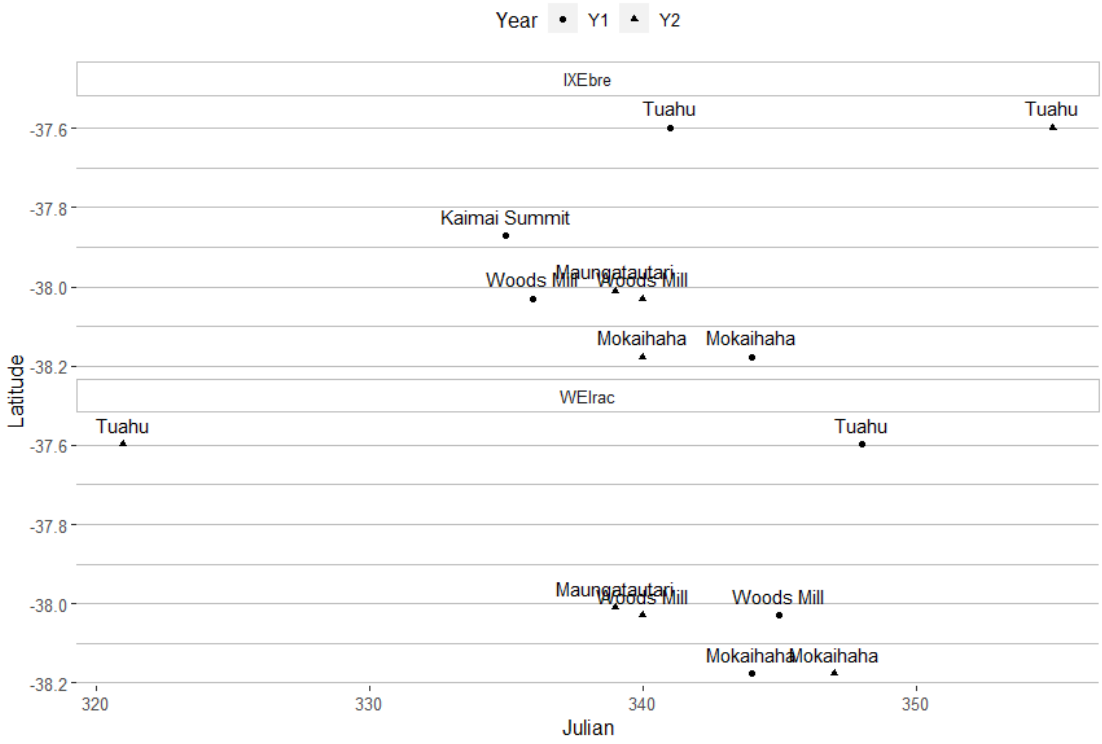

Figure 2.11: Flowering onset for I. brexioides *(top) and* W. racemosa *(bottom). Y axis shows site latitude X axis shows the date as Julian calendar day across two sampling years.*

2.3.6 Landscape scaling of nectar production

Nectar sugar production per hectare for *I. brexioides* was a maximum of 2.6 kg per hectare in 2016 under a 24-hour replenishment scenario. *Weinmannia racemosa* nectar sugar production in 2016 was a maximum of 5.7 kg per hectare under a 48-hour replenishment scenario. Nectar sugar production was in 2017 was 19% of 2016 production for *I. brexioides* and 12 % of 2016 production for *W. racemosa.* Details are given in Table 2.4.

Table 2.4: Nectar sugar production (kg per hectare) for I. brexioides *and* W. racemosa *during November 2016 to January 2017 (Y1) and November 2017 to January 2018 (Y2). Scenarios of nectar replenishment (24 hours for* I. brexioides *and 48 hours for* W. racemosa*) and no nectar replenishment are given due to limited data on nectar replenishment for these species.*

	No nectar replenishment		Nectar replenishment	
	Y1	Y2	Y1	Y2
Weinmannia racemosa	1.6	0.2	5.7	0.7
Ixerba brexioides	0.4	0.1	2.6	0.5
Total	2.0	0.3	8.3	1.2

Table 2.5: Average hive sugar requirement as a % of total nectar sugar production of I. brexioides *and* W. racemosa *at 5 km radius study sites in the Kaimai-Mamaku range during November 2016 to January 2017 (Y1) and November 2017 to January 2018 (Y2). Hectares per hive indicates density of hives at each site based on data from AssureQuality. Max site represents a fictitious site where Department of Conservation maximum recommended stocking rates are met. Scenarios of nectar replenishment (24 hours for* I. brexioides *and 48 hours for* W. racemosa*) and no nectar replenishment are given due to limited data on nectar replenishment for these species. Highlighted cells indicate where hive sugar requirements exceed nectar sugar production.*

	Hectares per hive		No Replenishment		Replenishment	
Site	Y1	Y2	Y1	Y2	Y1	Y2
Woods Mill	56	52	74%	592%	18%	134%
Kaimai Summit	21		198%		48%	
Mokaihaha	81	20	51%	1560%	12%	353%
Tuahu	10	5	436%	5702%	105%	1289%
Maungatautari		18		505%		114%
Max Site	3	3	1388%	10331%	333%	2336%

Table 2.5 compares the average hive sugar requirements for each site with the total nectar sugar production from *I. brexioides* and *W. racemosa.* Max site represents the New Zealand Department of Conservation maximum stocking rate of 3 hectares per hive (Department of Conservation 2015). Under maximum stocking conditions hive nectar sugar requirements exceed total sugar production in year one and year two if there is no nectar replenishment occurring. However, assuming nectar replenishment, total nectar sugar production from *I. brexioides* and *W. racemosa* is sufficient to meet the demands of hives in year one, at all but the highest hive density site. Nectar sugar production cannot meet hive demands at any site in year two, regardless of whether nectar replenishment is occurring.

2.4 Discussion and Conclusions

2.4.1 Variability in floral nectar availability

Findings from this research highlight the dynamic nature of nectar production in annual cycles. *Ixerba brexioides* and *W. racemosa* both demonstrated large-scale changes in inflorescence numbers per tree between flowering seasons across the latitudinal range of the Kaimai-Mamaku forest. Large-scale fluctuations in flowering intensity are not uncommon and have been linked to a range of climatic cues, such as air temperature prior to budding, and rainfall, rather than other environmental stressors such as disturbance (Law et al. 2000). In addition to variation in inflorescence number, the average sugar production per floret varied by

as much as 100% between years for both tree species. Enkegaard et al. (2016) showed similar patterns between sampling years for *Calluna vulgaris* (heather), with fewer flowers and lower sugar production in an overall dryer season. Lack (1982) also demonstrated significant variability in nectar secretion between years, attributed to unusually warm and dry weather in the previous summer, with some plants differing by a magnitude of five between years. Nectar concentration of *Corymbia maculata* flowers was also observed to vary by a factor of two or three between sampling years (Law & Chidel 2008). In contrast, other studies show less variation between sampling years than between months within flowering seasons (Hernández-Conrique et al. 2007).

Patterns of nectar sugar production across the weeks of the flowering season were significant across sampling weeks for *I. brexioides,* but not *W. racemosa.* Both species demonstrated increasing trends in sugar production over the 2016 flowering season, and in the 2017 flowering season *I. brexioides* showed a decreasing trend while *W. racemosa* showed an increasing trend. Increasing sugar production toward late season was documented by Bernhardt & Calder (1981) who related it to the availability of different flower foragers later in the season. In contrast, a higher quantity or quality of nectar resource at the beginning of the season, as seen with *W. racemosa,* is a trend observed by a number of studies (Pleasants 1983; Zimmerman & Pyke 1986; Torres & Galetto 1998; McDade & Weeks 2004), where it is suggested as a strategy that entrains flower visitors as the flowering season begins (McDade & Weeks 2004). Peak production in the middle of the season is another observed trend (Lack 1982). However, as was the case in this study, it is not uncommon for nectar secretion to vary in seasonal pattern from year to year, increasing throughout the season one year, and decreasing or demonstrating a mid-season peak in the following year (Hernández-Conrique et al. 2007). This pattern suggests that environmental variables have as much effect as the intrinsic phenology of flowering of each species.

Significant variation in diurnal cycles of nectar volume and concentration was observed for *I. brexioides.* Sugar concentration was lowest in the morning, increasing toward afternoon and evening. This is an expected trend, often related to diurnal changes in relative humidity (Pleasants 1983) and consequent evaporation of the liquid constituents of nectar. However, in this case, particularly in year one of sampling, nectar sugar concentration peaked with volume, and occurred at the

warmest and driest time of the day, indicating that changes in concentration are not likely to be linked to evaporation. In year two samples, concentration increased throughout the day as volume decreased. This pattern is more typical of humidity and evaporation effects and is likely more apparent in year two due to the higher temperatures and lower relative humidity overall.

At the landscape level, there was significant disparity between sugar production in year one and year two. For *I. brexioides* a combination of lower sugar per floret, and lower inflorescence numbers per tree overall reduced the sugar crop from 2.6 kg per hectare per year, to 0.5 kg per hectare per year. Similarly, for *W. racemosa*, production decreased from 5.7 kg per hectare per year to 0.7 kg per hectare per year. These floral nectar production estimates are low compared to New Zealand and global estimates, likely as a result of limited evidence of nectar replenishment over the lifetime of the floret, and because estimates were based on single species measurements of nectar production, rather than all nectar-producers in the submontane forest. Floral resource modelling by Ausseil et al. (2018) estimated nectar production generally between 4 and 60 kg per hectare per year for a range of mixed-species ecosystems. An exception was mānuka-kānuka vegetation which produces well outside that range at over 100 kg per hectare per year. Studies of United Kingdom ecosystems also had comparable results, estimating 6-40 kg per hectare per year for annual/perennial meadows, and 70 kg per hectare per year for broad-leaved vegetation, and 14.4 kg per hectare per year for conifer forest types (Hicks et al. 2016). Law & Chidel (2008) quantified nectar standing crop produced nocturnally by *Corymbia maculata*, with production supplying 2.2 kg per hectare over one night. Generally, models of landscape scale resource availability do not account for large-scale seasonal fluctuations in resource availability as this is difficult to demonstrate without long-term data on species specific production of floral resources. Other methods, however, have improved usefulness by providing estimates of pollen resources, and by combining floral resource estimates from mixtures of plant species, rather than individual species. In the system presented in this research *I. brexioides* and *W. racemosa* are the main nectar producers. Other species observed flowering at the same time included *Alseuosmia macrophylla*, *Hedycarya arborea, Melicytus ramiflorus, Pseudopanax arboreus,* and *Pseudowintera axillaris.* Future research on nectar production of other natives, such as these, as well as pollen resource production, will enable greater accuracy of landscape scale resource estimates for New Zealand submontane forest.

2.4.2 Factors affecting variation in nectar production

Temperature is known to affect nectar secretion and composition. For example, some studies have shown increased nectar secretion and concentration with temperature (Petanidou & Smets 1996), while others have shown decreased nectar secretion during hotter, drier climate events (Mu et al. 2015; Takkis et al. 2015; Phillips et al. 2018; Takkis et al. 2018) and even negative effects on nectar glucose:fructose ratios (Hoover et al. 2012). Nectar secretion over 2016 and 2017 for *I. brexioides* and *W. racemosa* indicate similar trends of decreased nectar secretion and concentration in hotter temperatures, giving cause for concern under future climate change scenarios. On a larger scale, temperature has been shown to act as a cue for flowering, for example, for several species of Myrtaceae cool temperatures prior to budding acting as a strong predictor of flowering (Law et al. 2000).

Temperature is often related to water availability, which similarly affects nectar secretion. Increased moisture via high relative humidity generally results in larger volumes of more dilute nectar (Wyatt et al. 1992). In addition, rainfall or watering can significantly increase both nectar volume and sugar production within the season of measurement (Wyatt et al. 1992). Conversely, extended periods of drought can lead to poor flowering and lower nectar production per flower (Law et al. 2000). In this study, care was taken to avoid bagging inflorescences in fabric that would not breathe, to prevent higher humidity conditions inside bagged treatments, however, the outdoor study set up meant that rainfall could not be controlled for. Nectar volume appeared unrelated to relative humidity in 2016, with lower nectar volumes for *I. brexioides* being recorded at periods of highest relative humidity.

The ability for flowers to replenish nectar after removal is another factor that can contribute particularly to diurnal patterns of nectar availability. *Weinmannia racemosa* has a comparatively long replenishment period, taking more than 24 hours (experimental limit). In contrast some species of *Aralia* can replenish floral nectar within 15 minutes to increase attractiveness to flower visitors (Thomson et al. 1989). Other species, such as *Tillandsia* respond to nectar removal by producing over three times more nectar volume than when no nectar removal is conducted, but not necessarily increasing the secretion of additional sugar (Ordano & Ornelas 2004).

Total tree carbon budgets have been shown to affect the quality of reproductive units produced by each tree. For example, if a tree produces fewer fruit, generally the fruit are bigger and/or have higher sugar content (Guardiola & García-Luis 2000). If nectar production is a significant component of the total tree carbon budget, generally we would predict that if there are fewer flowers then there would be more nectar per flower. In our study we observed that in 2017 when flower numbers were significantly lower than in 2016, nectar sugar content was also low compared to 2016 levels. This suggests that nectar flow is not related to tree resources, and it is likely that environmental constraints have a more important role, or that both flowering and nectar flow per flower are constrained by the same resource limitation.

Individual variation in nectar production was not investigated in this project, however, previous research on *I. brexioides* showed no significant difference in nectar production from different trees (Thomson 2013). Studies on other taxa observe significant individual variation (Hodges 1993; McDade & Weeks 2004; Żywiec et al. 2012; Parachnowitsch et al. 2018), as well as marked differences in visitor preference between high and low producing plant individuals (Enkegaard et al. 2016). Klinkhamer et al. (2001) suggest that these differences in production rate may persist because of spatial arrangement of plants, with low producing plants receiving comparable rates of visitation if found in close proximity to high producing plants. Other sources of variation in nectar secretion include heritable traits (McDade & Weeks 2004), as well as flower size (Herrera 1985), environmental conditions (Pacini et al. 2003), structural characteristics of flowers such as position in the inflorescence, number of flowers on each plant, flower age status, plant size, and gender phases (Devlin et al. 1987).

2.4.3 Implications of nectar variability for flower visitor support

Marked differences in both flower number and sugar mass per flower between flowering seasons represents a yearly fluctuation in forest carrying capacity for nectar feeding fauna that often goes unnoticed because of the lack of data on phenology and flower resource quantification. Table 2.5 indicates that at maximum recommended hive stocking rates (Department of Conservation 2015) hive sugar requirements exceed total nectar sugar production from *I. brexiodies* and *W. racemosa* when there is no nectar replenishment, or low flowering. When both those condition were combined, hive sugar requirements exceeded nectar

production by 10,331 %. This highlights the need for active management of apiary numbers in areas of native vegetation that support a complement of native nectar-feeders. Estimates of total nectar sugar production for year one and year two of sampling highlight how variable carrying capacity of an apiary site can be from year to year. This affects both the productivity of commercial hives, as well as the viability of supporting native populations of nectar feeding flower visitors.

Comparing nectar available from bagged and un-bagged florets was intended to highlight differences between the *W. racemosa* and *I. brexioides* in terms of levels of competition for nectar at different sites (Figure 2.8). A higher average difference in sugar between bagged and un-bagged florets suggests a greater level of nectar removal by flower visitors and hence greater competition. Though the average difference in sugar per floret between bagged and un-bagged florets was greater at low hive density sites for *W. racemosa*, and greater at high hive density sites for *I. brexioides*, this was not statistically significant. This suggests that competition for nectar resource was not significantly different between sites of different categories. Measurements were not taken for *W. racemosa* at the fenced site due to lack of flowering, but *I. brexioides* showed lower average difference at fenced sites, again, not statistically significant. This is contrary to what was expected, as observations at the fenced sites indicated that frequent bird visitation might result in greater nectar competition than at sites where no bird visitation was observed. In addition, differences between bagged and un-bagged florets were not statistically different between sampling years. This suggests, that despite lower availability of floral resources, competition was not different between sampling years for either *I. brexioides* or *W. racemosa.*

Generalised foraging strategies utilised by native bee species have been observed in several studies, which indicate that flower preference differs between locations, depending on what is available for forage (Donovan 1980; Donovan 2007; Hart 2007). These differences in foraging preferences can reduce competition between honey bees and native solitary bees (Iwasaki et al. 2018). In addition, key reproductive stages for most native bees fall during the period of peak nectar flow (November to February) (Donovan 2007), when resource availability is at its highest (Figure 2.9). Though this is generally the time when competing honey bee hives would be present, greater availability of resources may reduce the potential for negative competitive interactions (Beard 2015). In addition, other research in

Northland, New Zealand demonstrated that when native bees visit flowers, they targeted pollen-producing floral parts, rather than the nectar-producing parts most often targeted by honey bees (Hart 2007). Resource partitioning of this nature could also protect native bees from the negative effects of honey bee competition. To date, there are no studies in New Zealand which investigate direct effects of honey bee competition on native bee reproduction, but work by Hart (2007), examining the nest architecture, dynamics and foraging of native bees provides essential groundwork for designing future studies.

Sugar quantity is a nectar characteristic that has been linked to visitation frequency and preference by flower visitors, including honey bees (Real & Rathcke 1991; Rabinowitch et al. 1993), though there are various other factors involved, such as non-sugar nectar constituents (Afik et al. 2006), sugar composition (Dötterl et al. 2014), flower fertility (Rabinowitch et al. 1993) and flower gender-specific traits (Gonzalez et al. 1995; Aizen & Basilio 1998; Ashworth & Galetto 2002). Differential attractiveness to flower visitors can affect not only composition of flower visitor fauna, but also reproductive fitness of plants by reducing potential seed set (Real & Rathcke 1991). Honey bee visitation to *I. brexioides* and *W. racemosa* (recorded in Chapter 4 of this Thesis) fell between the first two weeks of flowering for *W. racemosa* and the second two weeks of flowering for *I. brexioides.* In addition, visitation by honey bees occurred only at 12 pm sampling times for *I. brexioides* but occurred throughout the day for *W. racemosa*. These visitation periods correspond with generally lower nectar volume and low nectar sugar concentration for both *I. brexioides* and *W. racemosa,* indicating that sugar quantity is unlikely to be a significant driver of attractiveness for honey bees.

2.4.4 Applications of resource inventory

When undertaken with care, measures of resource availability can be extremely useful in supporting conservation of native fauna in degraded or invaded environments. For example, recent identification of pollinator loss in England led to the establishment of the Countryside Stewardship scheme with a specific agri-environment package for the care of wild pollinators. Estimates of critical levels of resource availability became a key part of securing the development of the package and advising on policy for supporting native pollinators in agricultural systems (Dicks et al. 2015). In North Western European countries, similar problems with loss of natural pollinators in agricultural ecosystems has prompted research into the

effects of increasing floral resource availability and diversity in agricultural systems. Research has demonstrated that increasing diversity of available resource through wildflower belts and new pasture seed mixes can have positive effects on native pollinator abundance and diversity (Korpela et al. 2013; Scheper et al. 2013; Woodcock et al. 2014; Scheper et al. 2015). Furthermore, resource inventory can have economic benefits for improving management of apiculture. For example, Enkegaard et al. (2016) tailored measures of *Calluna vulgaris* nectar production toward a tool for supporting apiarist decisions around spatial and temporal hive movements. This approach to improving stocking rate management by budgeting resource production and allocation across landscape levels is not new but is gaining momentum as sustainability becomes an increasingly recognised part of business management (Paton 1990; Dicks et al. 2015; Arundel et al. 2016; Ausseil et al. 2018).

Despite the benefits of a detailed inventory of floral resources, availability of accurate data is a major limitation to application, particularly in New Zealand. As part of this study, a list of major flowering plants in Kaimai-Mamaku forests was the basis of a literature search for data on nectar or pollen production. Of 260 species researched, nectar data were available for only nine, and pollen data were available for only seven; in addition, there was little agreement on measurement techniques, or reporting form. This highlights the paucity of information that is currently available for determining resource availability in New Zealand native forest. Agreement on sampling techniques, and further data are required for floral resources to be accurately measured for wider ecosystems and species across New Zealand. One way that this could be achieved is by utilising the collective phenological knowledge and regular observations of apiarists, NZPCN observations, (New Zealand Plant Conservation Network 2013) or online platforms such as iNaturalist (iNaturalist 2019) to begin generating long term and country-wide data sets. As part of good practice and profit maximisation, beekeepers generally take note of phenological patterns, and many already utilise hive management software and applications that could be modified to allow recording of data for wider use.

2.4.5 Summary

In summary, this research highlights the dynamic nature of nectar production for two endemic New Zealand trees in annual cycles. The flow-on effects of this

variability extend to foraging nectar-feeders and effects on plant reproduction. Floral resource availability data, compiled from both phenological recording and flower resource quantification, are needed to complete the picture of resource availability for forest ecosystems and to more accurately inform conservation efforts and management decisions for apiaries on conservation land. Developing global information bases on floral phenology and floral resource quantification, and effects of projected climate change on floral physiology will go a long way toward deepening our understanding of natural ecosystems from a resource dynamics point of view, enabling us to manage resource demands more appropriately.

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Appendix: NIWA Climate Station Details

Name	*Agent Number*	*Network Number*	*Latitude (dec.deg)*	*Longitude (dec.deg)*	*Height (m)*
Mamaku 2	11755	B86102	-38.1	176.086	570
Waihi	1550	B75381	-37.394	175.841	91
Waihi C.B.	1557	B75481	-37.469	175.86	213
Te Aroha	1565	B75571	-37.547	175.716	18
Mt Te Aroha Tv Stn	1566	B75572	-37.537	175.741	951
Wharawhara Water Stn	1567	B75581	-37.5723	175.8621	132
Shaftesbury	1579	B75681	-37.6172	175.7982	55
Katikati Lockington	1580	B75691	-37.625	175.91	107
Te Ariki Falls, Matamata Water Treatment Plant	1587	B75782	-37.751	175.881	253
Kaimai Tunnel	1588	B75791	-37.7	175.933	305
Whakamarama 1	1589	B75792	-37.7519	175.9696	392
Whakamarama 2	1590	B75793	-37.72	175.991	260
Kaimai School	1597	B75891	-37.838	175.96	376
Kaimai Summit	1599	B75893	-37.874	175.928	488
Old Kaimai Rd	1600	B75894	-37.863	175.944	457
Valley View	1601	B75895	-37.836	175.982	280
Kakahu Downs	1604	B75991	-37.938	175.892	122
Varteg Hill	1605	B75992	-37.932	175.892	137
Whakamarama Edr	1609	B76603	-37.693	176	20
Whakamarama	1617	B76701	-37.7321	176.0022	255
Omanawa	1634	B76803	-37.865	176.069	274
Ngawharo, Mamaku	1655	B76913	-37.958	176.172	466
Lloyd Mandeno	17080	B76804	-37.855	176.029	275
Mamaku School	1732	B86001	-38.102	176.079	570
Mamaku Aero	1733	B86002	-38.034	175.999	518
Mamaku, Dansey Road	1737	B86014	-38.095	176.136	457
Mamaku	1759	B86101	-38.1	176.067	576
Paradise Valley	1761	B86112	-38.13	176.143	366
Whakarewarewa	1765	B86124	-38.164	176.263	307
Mamaku South Forest	1780	B86212	-38.2	176.1	640
Te Awamutu, Paepaerahi	18040	C85042	-38.058	175.472	140
Cambridge 2	2114	C75842	-37.883	175.467	70
Roto O Rangi 2	2122	C75941	-37.981	175.47	61
Maungatautari	2129	C75954	-37.9627	175.5469	183
Maungatautari 1	2189	C85051	-38.05	175.583	183
Arapuni Power Stn	2190	C85061	-38.073	175.642	123
Waihi, Woodlands Road	25877	B75482	-37.4804	175.8657	290
Holland Road, Hamilton	26645	B75735	-37.748	175.367	45
Mamaku Radar Wxt Aws	38671	B86003	-38.066	176.062	617

Chapter 3

Introducing honey bees (*Apis mellifera)* into native New Zealand submontane forest: impacts on *Weinmannia racemosa* (kāmahi) and *Ixerba brexioides* (tāwari)

Abstract

Honey bees are a relatively recent addition to New Zealand ecosystems. Increasing value of native honey products has resulted in a 70% increase in hive numbers over the last five years on conservation land dominated by native vegetation. This chapter presents research identifying impacts of honey bees on two native trees valued for honey production: *Weinmannia racemosa* (kāmahi), and *Ixerba brexioides* (tāwari). Video surveillance identified honey bees as unsuitable pollinators for *I. brexioides*, a large-flowered native tree, whereas small-flowered *W. racemosa* trees are likely to be pollinated by a range of visitors, regardless of visitor size. Pollination experiments suggested *W. racemosa* had low pollen limitation at a high hive density site, and medium pollen limitation at low hive density sites. Highest seed set for *W. racemosa* was also observed at high hive density sites. Seed set of *I. brexioides* was highest where total removal of major mammalian pests resulted in higher rates of bird visitation. Honey bee impacts can be positive or negative depending on the density of hive stocking, the behaviour of honey bees at the flower level, and the flower structure of the plants they interact with.

3.1 Introduction

Honey bees were first introduced to New Zealand in 1839 (Hopkins 1911). A boom in profitability of apicultural enterprise has seen the number of registered hives in New Zealand double over the last 10 years (Airborne Honey 2018). On conservation land, that increase has exceeded the national trend, increasing by 70% in the last five years (C. Beard pers. comm.). Several reports since the nineteen eighties have reviewed the impact of honey bees in New Zealand native ecosystems since the eighties (Donovan 1980; Butz Huryn 1995, 1997; Howlett & Donovan 2010; Beard 2015) each with a similar conclusion – honey bees have a suite of potential impacts, both positive and negative, but conclusive research to confirm

those impacts is lacking. Despite this repeated message, studies investigating potential impacts of honey bees in native New Zealand ecosystems are few. This research aimed to contribute to appropriately identifying actual impacts to native plants of large-scale introduction of honey bees in native forest, by observing pollinator efficacy, pollen limitation and seed set of two dominant New Zealand tree species, *Weinmannia racemosa,* and *Ixerba brexioides,* at sites of varying honey bee hive density.

The degree to which honey bee introduction can affect native plant species is influenced by factors such as density of the invading species (Aizen et al. 2014), reproductive strategy of native plant species, and presence of other pollinators (Butz Huryn 1997; Goulson 2003). Considering these factors, impacts of honey bees on flora will vary between locations, seasons, and vegetation types. Because of the difficulty of studying directly the impacts of honey bees on pollination systems, most studies rely on indirect measures of honey bee impacts by isolating metrics that have the potential to affect native plants or animals. For example, Taylor and Whelan (1988) used observations of pollen collection by honey bees from *Grevillea* to infer effects on plant reproductive success; and Gross (1993) observed honey bee behaviour at flower level, identifying honey bees removing pollen from plant stigmas that had been deposited by other pollinators. Many other studies use comparative visitation rates of honey bees compared with other native flower-visitors to infer a negative impact (Kato & Kawakita 2004).

In addition to research difficulties, public opinion can also be a barrier to research on the impacts of widespread, managed introduction of honey bees in native ecosystems. Public discussion around the world in recent years has focused on saving the honey bee, planting gardens to provide them with forage and avoiding using pesticides (Greenpeace 2014; Honeybee Conservancy 2018; Save the Bees 2018), and New Zealand is no exception. Few voices have expressed the idea that saving honey bees should not be applicable in areas where they are not native (Geldmann & González-Varo 2018), because the general public are not aware that honey bees are introduced, and that in most cases there is an array of native species that are important for pollination.

The aim of this study was to quantify the role honey bees played in the pollination of *W. racemosa* and *I. brexioides and* assess whether increasing densities of bees led to negative effects on plant reproduction. We utilised a combination of video

surveillance and plant reproductive experiments to understand how effective honey bees are as pollinators of native plants, and how plant reproductive success is impacted by honey bee visitation. We predicted that higher bee densities would result in reduced reproductive output from *I. brexioides* and increased or no effects on *W. racemosa* as a result of contrasting flower architecture and visitor behaviour.

3.2 Methods

3.2.1 Study Sites and species

Research for this study occurred in the submontane belt (400 to 800 metres above sea level) of the Kaimai-Mamaku range between November 2017 and January 2018. Sites were selected based on accessibility, proximity to honey bee hives, and presence or absence of target species. Sampling was carried out at four sites on average 40 km apart. Field sites were based on the Department of Conservation (DOC) tracks Tuahu, Woods Mill, and Mokaihaha. Maungatautari Sanctuary Mountain was also included, and is located just west of the Kaimai-Mamaku range. Figure 2.1 (pg 37) shows the location of field sites.

Table 3.1: Details of study sites including latitude and longitude, altitude (m), hive numbers found within a 5 km radius of the sites in 2016 and 2017, hive density category (L=low, H=high), pest management status whether unmanaged (U) or with intensive management (IM)

Site	Lat.	Lon.	Alt.	2016	2017	Hives	Pests
Woods Mill	-38.03	175.98	500	140	150	L	U
Mokaihaha	-38.18	176.10	600	97	395	L	U
Tuahu	-37.60	175.86	400	822	1444	H	U
Maungatautari	-38.01	175.58	500	-	431	H	IM

Table 3.1 shows categorisation of sites based on hive density within 5 km and pest management status. Hive density categories were assigned based on 2016 hive numbers and categories were maintained for 2017. Hive density categorisation presented challenges due to the difficulty of obtaining data on the density of existing hives. The data is kept by AsureQuality and though it can be made available for research purposes, the process took considerable time between request and receipt of data (over 12 months) and the level of detail available was low. Due to privacy issues the exact location of hives was not available. Hive numbers were given for between November 2016 and January 2017 and November 2017 and January 2018.

Pest management categories separated the Maungatautari site from other sites because of its unique situation as a pest-proof sanctuary.

Fragmentation of surrounding land matrix was determined using ArcGIS 10.5.1 2017 to analyse land use in the land matrix within a buffer radius of 5 km. Land cover classes were categorised using LCDB v4.1 - Land Cover Database version 4.1, Mainland New Zealand data (Landcare Research 2015). Fragmentation was based on the percentage of native forest cover within the 5 km radius. Overall, within a 5 km radius, native forest had the largest percentage land cover (54%), followed by agricultural pasture (28%) and forestry (16%) (Table 3.2). Woods Mill and Tuahu had the highest percentage of native forest cover (69 % and 66% respectively), but Tuahu also had the highest percentage of horticultural land use (8%, including orchards and perennial cropping). Mokaihaha was located adjacent to a plantation forestry estate and had a high percentage of exotic forestry cover.

Table 3.2: Land cover percentage within a 5 km radius of field study sites

Land use	Maungatautari	Mokaihaha	Tuahu	Woods Mill	Total
Native forest	42.4	51.3	66.1	69.1	53.6
Pasture	56.6	11.4	19.1	5.8	28.1
Forestry	0.7	37.1	6.0	24.3	15.7
Horticulture	0.1	0.0	8.0	0.0	2.1
Other	0.3	0.2	0.8	0.7	0.6

Tuahu, the most northern site, was located near Katikati in an area of the Bay of Plenty that is important for kiwifruit and avocado production. Woods Mill is part of a large area of conservation estate that runs alongside State Highway 5 and is a popular place for hunting. Mokaihaha is an ecological reserve that is near Mamaku, in an area surrounded by exotic pine forestry. Finally, Maungatautari is a unique site, as a reserve that is free of vertebrate pests (except mice), and which is surrounded by a pest-proof fence (Sanctuary Mountain 2018).

The Kaimai-Mamaku range has a long history of disturbance due to demand for timber, gold mining, farming and stock droving but today is a Department of Conservation administered forest park (Department of Conservation 2006). The canopy vegetation at the study sites is dominated by *I. brexioides, W. racemosa*,

with *Beilschmiedia tawa* (tawa) and *Knightia excelsa* (rewarewa) also common. The understory was dominated by *Coprosma grandifolia* (kanono), *Hedycarya arborea* (pigeonwood), *Melicytus ramiflorus* (māhoe) and tree fern species *Cyathea dealbata* (silver fern) and *Dicksonia squarrosa* (wheki).

Figure 3.1: Views of forest at study sites, from North-South site (top) and Tuahu (bottom)

The focal study species located at these sites are *Ixerba brexioides* and *Weinmannia racemosa.* Both species are endemic to New Zealand and are nectar producing trees that are frequently used in honey production but represent a contrast in flower structure and arrangement. *Ixerba brexioides* flowers average 2.5 cm across (Nepia 2018) and are structured in a pentagonal arrangement, with a lobed, nectar-secreting disk at the base of the flower, a twisted stigma in the centre of the disk, and anthers

at the end of long filaments inserted between the disk lobes (Figure 2.2, pg. 39). These flowers are clustered into inflorescences of up to 20 flowers that are generally displayed over the month of December each year. In contrast, *Weinmannia racemosa* inflorescences are racemes which can be made up of over 100 smal1 (~2 mm across), densely packed florets. In each floret anthers and stigma are arranged over a cup-like nectary. Flowering occurs from mid-November to December each year, and flowers are generally white, changing to a red colour with age.

3.2.2 Video surveillance

Flower visitation of *W. racemosa* and *I. brexioides* was filmed using video cameras during December 2017 during dry daylight hours. The cameras that were used were Veho Muvi K-Series K2 NPNG. Two were deployed at a time and swapped out every two hours for cameras with fresh batteries. A total of 61 hours of footage was collected over 11 days across all sites for *I. brexioides* and at Mokaihaha for *W. racemosa* (Table 3.3). Customs issues prevented deployment of video equipment in time for *W. racemosa* flowering at all sites but Mokaihaha. Observations were categorised according to the floral parts contacted at each visit, duration of visit, and identification of each visitor. Flower visitors were categorised by morphogroups as the video quality did not allow identification to species level (Manaaki Whenua Landcare Research 2016). All flowers under observation remained bagged before and after filming. Seed capsules were collected the following March, after which the seed heads were dissected, and the proportion of viable and non-viable seeds noted. Viability of *I. brexioides* seeds can easily be assessed based on the size of the seed (Thomson 2013), so there is no need for dissection or staining. *Weinmannia racemosa* florets that do not progress to seed set naturally dehisce, leaving only seeds to populate the raceme stalk. Withered buds were not counted.

Table 3.3: Duration of video footage collected at the flower level for W. racemosa *and* I. brexioides *in submontane forest at four study sites during December 2017*

Species	Location	Days	Duration (hr)	Trees
Weinmannia racemosa	Mokaihaha	3	14.0	2
Ixerba brexioides	Maungatautari	2	16.5	4
	Mokaihaha	2	7.1	1
	Tuahu	2	10.0	1
	Woods Mill	2	14.2	2
Total	All Sites	11	61.8	10

3.2.3 Exclusion experiments

Exclusion experiments were set up to examine the degree of pollen limitation in *I. brexioides* and *W. racemosa* (Table 3.4). Exclusion experiment methods were based largely on the work of Boulter et al. (2006), where experimental trees are selected based on reproductive stage and accessibility, i.e. in bud, and with flowers within reach of a ladder. The selected flowers were tagged and randomly assigned to a treatment (Table 3.4). Table 3.5 shows sample sizes for each treatment.

Table 3.4: Exclusion experiments

No.	Pollination Treatment	Bagged	Emasculated	Pollen source
1	Control	no	no	open
2	Caged	no	no	open/no birds
3	Cross-pollen	no	no	different tree
4	Autonomous	yes	no	None/self

Control treatments were tagged for identification, but were not manipulated, to provide a baseline for seed set under natural conditions. The caged treatment was designed to identify the extent to which bird visitation affects seed set, by excluding birds but not insects. These inflorescences were enclosed in a wire mesh cage fastened with wire. Supplemental cross-pollination treatments were set up to demonstrate the extent to which seed set is limited by the availability of cross pollen. Flowers were left open for pollination and also received additional pollen from flowers of the same species nearby via paintbrush. Autonomous treatments were designed to assess the degree to which seed set can occur without pollinating agents. These were enclosed in mesh bags with adjustable openings at either end to allow the bag to enclose the inflorescence and be fastened around the branch at one end, and closed off to pollinators at the other end. The results of the pollination treatments were assessed after fruit maturation by assessing seed viability.

Table 3.5:Sample sizes for exclusion experiments for I. brexioides and W. racemosa *at sites in the Kaimai-Mamaku Range, New Zealand.*

Species	Site	Treatment	Flowers	Trees
Ixerba brexioides	Maungatautari	Autonomous	5	4
		Cage	4	4
		Camera	7	4
		Control	15	4
		Hand-Cross	6	4
	Tuahu	Camera	2	1
		Control	4	1
		Hand-Cross	6	1
	Woods Mill	Autonomous	9	4
		Cage	3	3
		Camera	4	4
		Control	17	4
		Hand-Cross	6	4
			88	9
Weinmannia racemosa	Mokaihaha	Autonomous	4	4
		Cage	6	5
		Camera	4	4
		Control	10	5
		Hand-Cross	5	5
	Tuahu	Autonomous	11	6
		Caged	11	6
		Camera	5	5
		Control	15	6
		Hand	12	6
	Woods Mill	Autonomous	5	2
		Cage	17	2
		Control	8	2
		Hand-Cross	8	2
			121	12
Grand Total			209	21

Pollen limitation index (PLI) was used to determine the extent of pollen limitation for *I. brexioides* and *W. racemosa*. PLI is calculated by comparing seed set from supplementary cross-pollinated flowers (P_s) and from naturally pollinated flowers (P_o) using the following formula (Larson and Barrett (2000)):

$$PLI = 1 - (P_o/P_s)$$

The formula assumes that all the pollen necessary for maximum seed set will be supplied by cross-pollination. Any negative difference between hand cross and naturally pollinated seed set can therefore be attributed to insufficient pollen

deposition. A value of zero means there is no pollen limitation as both natural and hand cross pollinated flowers have the same proportion of seed set. Newstrom and Robertson (2005) indicated that PLI values are classified as low pollen limitation if PLI <0.2, medium pollen limitation if 0.2<PLI<0.75, and high pollen limitation if PLI>0.75. In this study PLI was pooled by site rather than by tree as tree origin was not recorded at the time of seed collection. This is not recommended for future studies as it loses variation in PLI between individual trees at each site.

Self-compatibility was assessed using the Autonomous Selfing Index (ASI). Autonomous selfing refers to a species ability to reproduce without pollen vectors, relying on its own pollen. Autonomous selfing species must be self-compatible, but self-compatible species are not necessarily autonomously selfing. ASI is calculated as the seed set of pollinator-excluded treatments relative to cross-pollinated treatments. This index gives an indication of possible back-up reproduction mechanisms if pollen limitation is an issue. Plants with an ASI of over 0.5 are classified as 'autonomously selfing'. In this study ASI was pooled by site rather than by tree as individual tree source was not recorded at the time of seed collection. This is not recommended for future studies as it loses variation in ASI between individual trees at each site.

3.2.4 Statistical Analysis

Visit duration was tested for normal distribution using the Anderson-Darling normality test from the R 'nortest' package (Gross & Ligges 2015) and was not normally distributed ($p<0.01$). Non-parametric Kruskal Wallis rank sum tests were applied to visit duration data in R (RStudio Team 2018) to determine significance of differences between flower visitors. A Friedman Test was applied in R to identify significant differences between visit duration at different sites, taking into account differences between visitor groups.

Flower number (determined by number of florets) was compared to seed set by using the 'lm' function in R (RStudio Team 2018) to produce a linear regression model. The default settings were used, indicating QR decomposition method of analysis with unweighted factors. The relationship was considered significant if $p<0.05$.

Throughout the text averages are given as mean value ± standard error of the mean. Graphs were produced using the 'ggplot2' package in R (Wickham et al. 2019).

Box plots represent median values with interquartile ranges. Upper whiskers show 75th percentile to the largest value no further than 1.5 times the interquartile range. Data beyond these are plotted individually as outliers, and asterisks represent the mean. Bar graphs throughout the text show averages with error bars representing 95 % confidence intervals.

3.3 Results

3.3.1 Physical capacity for pollination

Physical capacity for potential pollination differed between groups of flower visitors as a result of differing rates of contact with floral reproductive parts (Figure 3.2). The anther and stigma *of W. racemosa* flowers were contacted in 100% of visits by all groups except the small flies. Lower rates of anther and stigma contact were observed in *I. brexioides*, with the highest contact rates exhibited by birds (100%) and beetles (100%), followed by native bees (94%). Groups exhibiting the lowest rates of contact with the reproductive organs of *I. brexioides* included spiders (0%), wasps (0%), ants (6%), honey bees (11%) and flies (12%). Overall, 100% of the observed visits to *W. racemosa* flowers could potentially result in successful pollination based on contact with floral reproductive parts. In contrast, only 44% of visits to *I. brexioides* were likely to result in successful pollination.

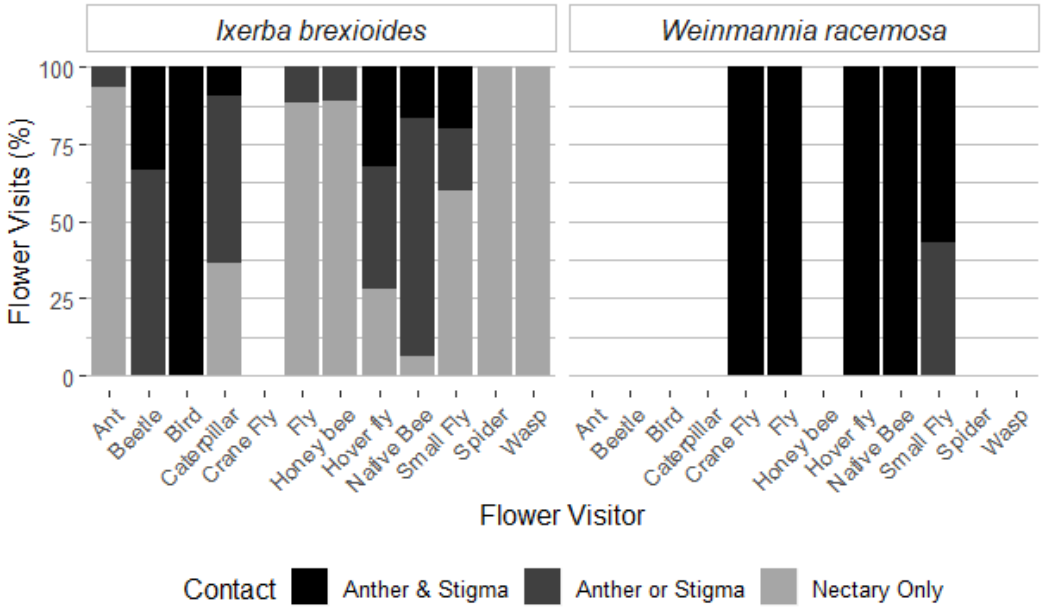

Figure 3.2: Percentage of visits to W. racemosa *and* I. brexioides *flowers where both anther and stigma were contacted (black), anther or stigma were contacted (medium grey), or only the nectary was contacted (light grey)*

Table 3.6: Summary table showing visit frequency per minute for visitors of I. brexioides *and* W. racemosa *at high and low hive density sites. Average of flower visits (± standard deviation) is also given for visits contacting flower anther and stigma (AS), anther or stigma (A/S) or nectary only (N).*

Hives	Visitor	Visits per minute	AS	A/S	N
Ixerba brexioides					
High	Ant	0.101		0.05 ± 0.23	0.84 ± 0.44
	Bird	0.001	1.00 ± 0.00		
	Fly	0.018		0.03 ± 0.18	0.92 ± 0.37
	Honey bee	0.023		0.11 ± 0.31	0.88 ± 0.31
	Hover fly	0.012	0.26 ± 0.45	0.31 ± 0.47	0.36 ± 0.49
	Native Bee	0.008	0.16 ± 0.38	0.5 ± 0.52	0.33 ± 0.49
	Wasp	0.003			1.00 ± 0.00
Low	Beetle	0.005	0.33 ± 0.51	0.66 ± 0.51	
	Caterpillar	0.009	0.08 ± 0.28	0.5 ± 0.52	0.33 ± 0.49
	Fly	0.021		0.18 ± 0.39	0.74 ± 0.44
	Hover fly	0.006	0.37 ± 0.51	0.5 ± 0.53	
	Native Bee	0.081	0.16 ± 0.37	0.80 ± 0.39	0.02 ± 0.16
	Small fly	0.004	0.2 ± 0.44	0.2 ± 0.44	0.6 ± 0.54
	Wasp	0.002			1.00 ± 0.00
Weinmannia racemosa					
Low	Crane Fly	0.001	1.00 ± 0.00		
	Fly	0.004	1.00 ± 0.00		
	Hover fly	0.010	1.00 ± 0.00		
	Native Bee	0.007	1.00 ± 0.00		
	Small fly	0.010	0.5 ± 0.53	0.37 ± 0.51	

Visitation duration (Table 3.6) differed significantly between flower visitors when looking at combined visitation durations for both species ($p<0.01$), and for *I. brexioides* inflorescences ($p<0.01$), but not *W. racemosa* inflorescences ($p=0.19$). Visitors with significantly longer visitation times on *I. brexioides* inflorescences included ants (average = 5.4 min), beetles (average = 4.4 min), and caterpillars (average = 5.7 min), although native bees also had visits lasting up to 7.5 minutes. Wasp visits to *I. brexioides* inflorescences were on average the shortest, at 0.25 minutes (Figure 3.3). Native bees demonstrated the longest visits to *W. racemosa* inflorescences, averaging 3.9 minutes per visit, with visits lasting up to 12.6 minutes (Figure 3.4). Visit duration between visitors and sites of differing hive density was not significantly different ($p=0.37$), or sites of differing pest management level ($p=0.13$).

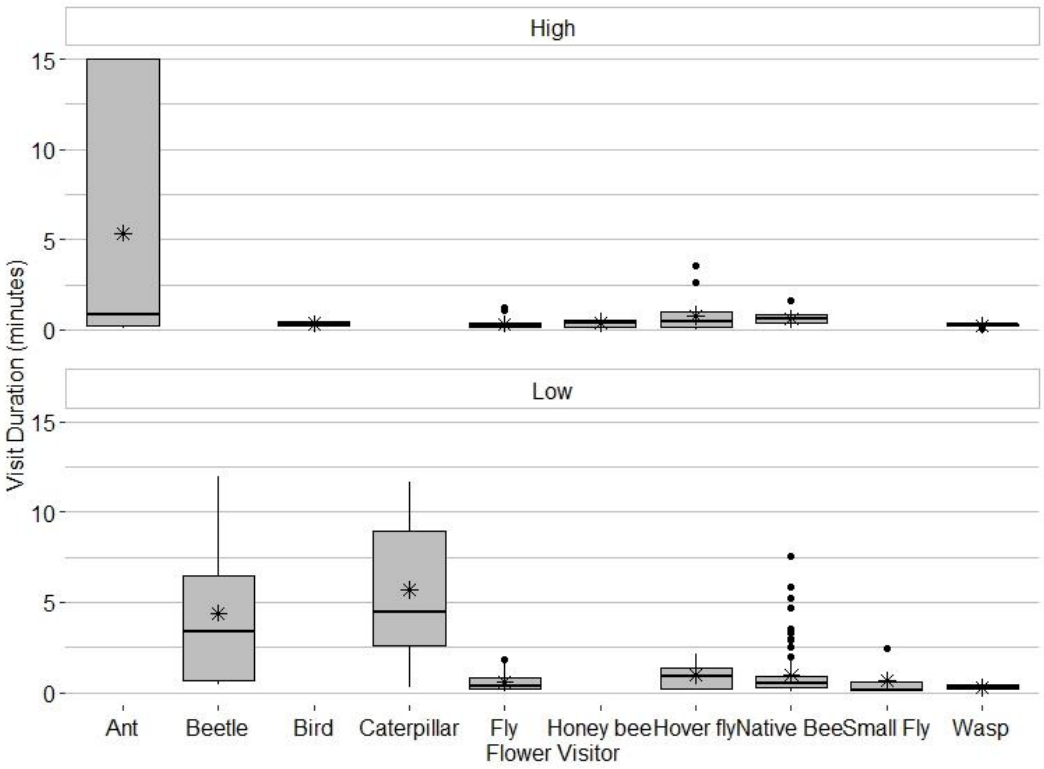

Figure 3.3: Visit duration of flower visitors observed on I. brexioides *flowers during video surveillance at sites of high (top) and low honey bee hive density (bottom)*

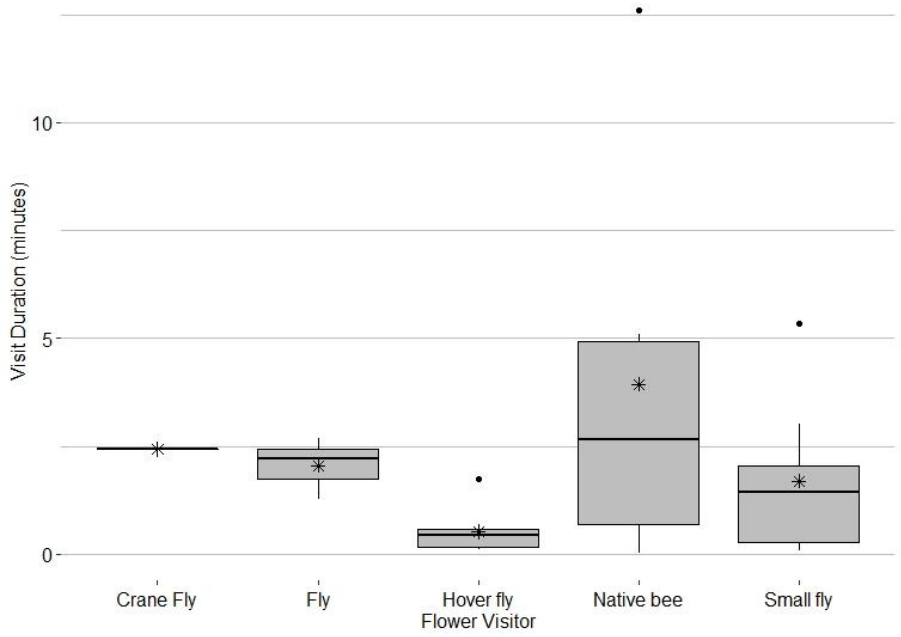

Figure 3.4: Visit duration of flower visitors observed on W. racemosa *flowers during video surveillance at Mokaihaha, December 2017*

3.3.2 Pollen limitation and seed set

Differences in seed set for *W. racemosa* were not significant (p=0.16) across sites, though average seed set appeared highest at Maungatautari for *I. brexioides* and at

Tuahu for *W. racemosa* (see Figure 3.5).*Weinmannia racemosa* seed capsules under open pollination had an average percentage seed set of 32 ± 6 %. Percentage seed set per inflorescence was highest at Tuahu but showed a high level of variation between inflorescences at each of the sites. On average 19 ± 11 % of all capsules failed to reach seed formation. Caged treatments averaged 36 ± 9 % seed set, with the highest caged seed set occurring at Woods Mill (50%). *Weinmannia racemosa* pollen limitation indices ranged from 0.03 at the Tuahu site to 0.43 at Woods Mill and Mokaihaha. This indicates that seed set is not limited by available pollen at the Tuahu site but has moderate pollen limitation at Woods Mill and Mokaihaha. Autonomous selfing index (ASI) was 0.4 on average across all sites, indicating that *W. racemosa* is not classified as autonomously selfing. A non-parametric Kruskal-Wallis rank sum test identified significant differences in seed set between treatments (p = 0.01). Linear model regression showed a significant correlation between flower number and seed set (p<0.01) and seed set percentage. Larger inflorescences with more florets produced more seeds (R^2 =0.6025) and had higher percent seed set (R^2=0.0982).

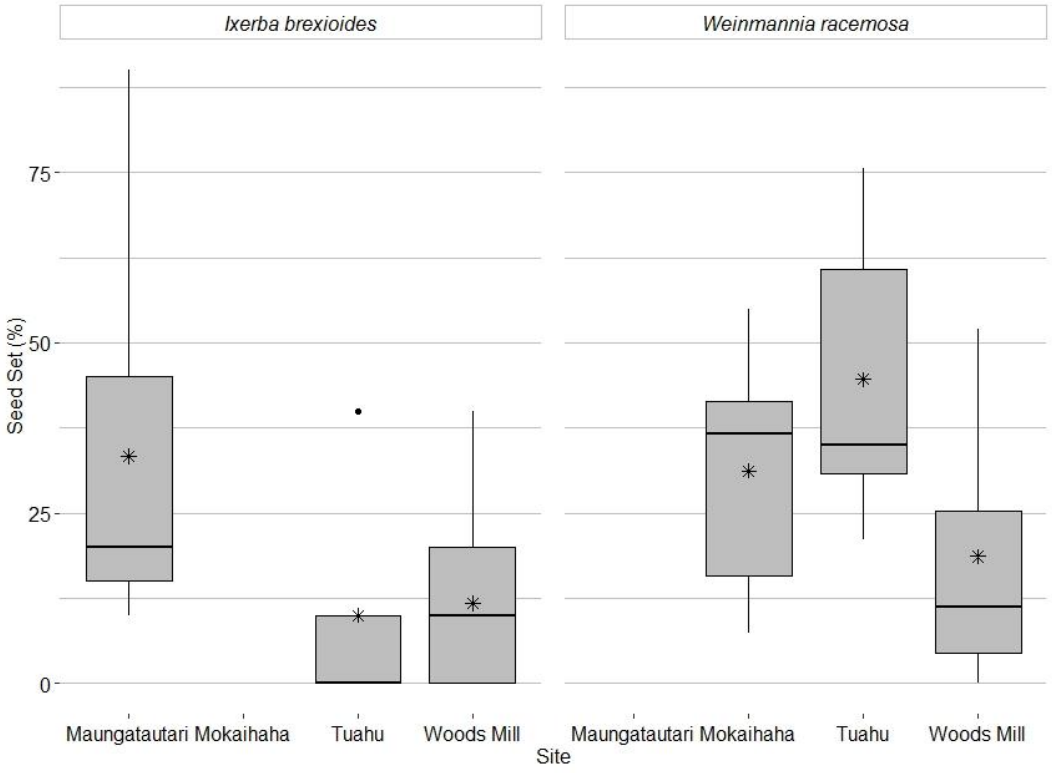

Figure 3.5: Seed set percentage for open pollinated I. brexioides *and* W. racemosa *flowers at sites in the Kaimai-Mamaku range. Asterisks indicate mean values.*

Open pollinated flowers of *I. brexioides* showed significant differences in seed set between sites of differing hive density (p=0.04) and pest management (p=0.003), mostly attributable to the Maungatautari site. Average seed set for *I. brexioides* was highest at the fenced site, and at the high honey bee hive density sites. *Ixerba brexioides* seed capsules under control conditions had an average percentage seed set of 21 ± 4 %. Percentage seed set per inflorescence was highest at Maungatautari (33 ±7 %). On average 29 ± 3 % of capsules were aborted or destroyed (e.g. by fungal rot or predation) before seed formation. Caged treatments had the lowest average seed set for *I. brexioides,* averaging 7 ± 4 %. At Woods Mill capsules reached maturity, and averaged 17 ± 7 % seed set, whereas none of the caged capsules at Maungatautari made it to seed production stage.

Ixerba brexioides exhibited a PLI of 0.60 at low honey bee hive density sites, 0.00 at high honey bee hive density sites (Figure 3.6). Exclusion experiments at Maungatautari, the pest-proof fenced site, also had a PLI of 0.00. PLI values of zero or less indicate a failure of the hand-cross treatment, indicating either the pollen used was no longer viable, the stigma was not receptive, or the brush damaged the stigma. Autonomous selfing index (ASI) was 2.52 on average across all sites, indicating that *I. brexioides* is classified as autonomously selfing. Seed set data did not fit a normal distribution (Anderson-Darling p = <0.01). A non-parametric Kruskal-Wallis rank sum test identified no difference between treatments (p = 0.12). Inflorescence size had no relation to percentage of viable seed set (R^2 = 0.05).

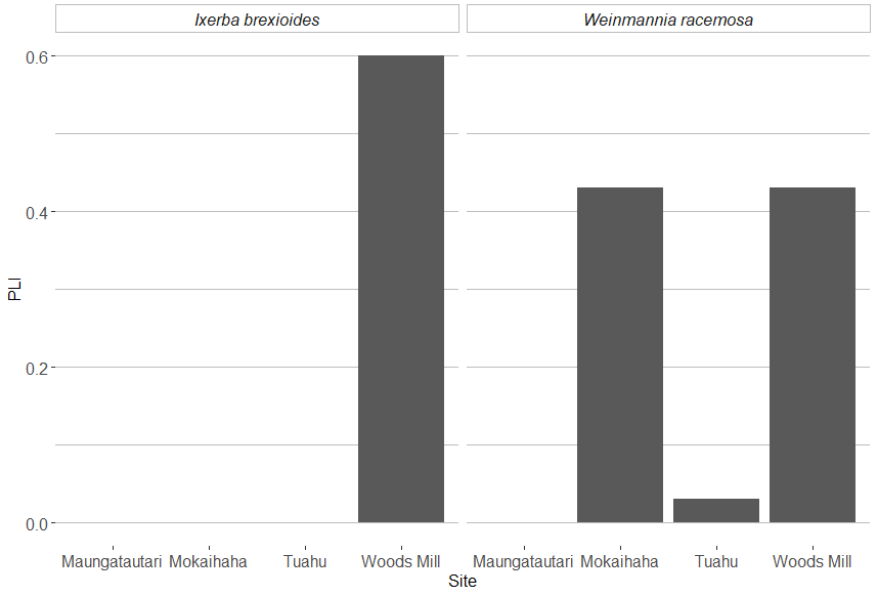

Figure 3.6: Pollen limitation indices for I. brexioides *(left panel) and* W. racemosa *(right panel) at sites in the Kaimai-Mamaku Range, New Zealand*

3.4 Discussion and Conclusions

Results of this study highlight the impacts of increasing honey bee numbers on *I. brexioides* and *W. racemosa* in terms of the pollination potential of various flower visitors and implications of hive density conditions on seed set and pollen limitation. This has implications for considering management of apiaries in native forest and long-term effects on plant reproductive success. Key findings and their implications will be discussed in the paragraphs that follow.

3.4.1 Pollination compensation by introduced species

This study determined differences in the potential for pollination compensation by honey bees for the small-flowered *W. racemosa* and large flowered *I. brexioides.* Pollination compensation refers to the ability of an introduced species to replace, completely or in part, the pollination services previously provided by natural pollinators which are extinct or in decline (Pattemore & Wilcove 2012). Global research suggests this as one of the potentially beneficial roles of introduced honey bees in native ecosystems (Butz Huryn 1997), particularly in fragmented vegetation presumably because of the ability of honey bees to affect gene transfer over long distances between forest patches (Dick 2001). Butz Huryn (1995) presents a list of 188 species where honey bee visitation has been observed in New Zealand and suggests a particular benefit to some threatened plants. However, pollination compensation by introduced species is only successful if flower visitation results in pollination (Butz Huryn 1997).

Observations from this current study suggest that flower architecture and visitor behaviour present barriers to pollination compensation by honey bees for *I. brexioides.* Flower structure of *I. brexioides* favours pollination by large beetles (most commonly large long-horn beetles) and birds. Native bees (commonly *Hylaeus*), by virtue of their behaviour on and around the flowers, were also probable pollinators. Native bees had among the longest average duration of flower visits. Their visitation behaviour included foraging at the anther and moving between anthers by crawling on filaments and the central style, thus 94% of their visits involved contact with either stigma or anther. In contrast, honey bees spent less than half as much time foraging at each flower, and predominantly foraged at the nectary, not contacting either anthers or stigmas. Hence, the potential for honey bees to supplement pollination of *I. brexioides* is low. In contrast, the structure of *W. racemosa* florets readily allows contact between flower visitor and floral

reproductive parts while foraging. Because of their small size, even the smallest flower visitors (midges) contact anther or stigma at 100% of visits. Honey bee visitation to *W. racemosa* flowers was not observed by video surveillance in this study, however personal observations of visitation and floral structure suggest that pollination compensation potential by honey bees is high.

Pollen limitation indices for *W. racemosa* agreed with measures of pollination potential, demonstrating the lowest pollen limitation at the Tuahu site, where honey bees represented 10% of the visitor fauna overall. These results suggest that honey bees do not impede pollination and may even improve the pollination of small-flowered natives, such as *W. racemosa,* though there are likely other factors also in play (such as latitude, diversity of visitor fauna, or climatic conditions). In contrast, evidence of pollen limitation for *I. brexioides* was not obvious due to failed hand-cross treatments. However, previous research reported moderate pollen limitation of seed set in mainland, modified forest environments (Thomson 2013). It is not clear whether this indicates inadequate pollination because baseline PLI is not currently available. Unmodified *I. brexioides* forest is rare and hard to access (e.g. on Little Barrier Island), and most areas are affected by agricultural land-use in surrounding areas, and the invasion of mammalian pests which have significant effects on both bird and invertebrate fauna. Maungatautari, surrounded by a pest-proof fence, supports a bird fauna that is better representative of pre-human settlement conditions. However, given that the sanctuary sits within an extensive agricultural matrix, honey bees were still common at this site.

Examples of successful pollination compensation in New Zealand and around the world are not uncommon. Some examples include ship rats in New Zealand pollinating rewarewa (Pattemore & Wilcove 2012), silvereyes having an increasing role in plant pollination in Hawaii (Cox & Elmqvist 2000), exotic flies maintaining pollination services in agricultural settings where native species are sparse (Stavert et al. 2018), and the red-whiskered bulbul (*Pycnonotus jocosus*) visiting flowers of a rare species in Mauritius (Olesen et al. 1998). However, there are also examples of interactions representing a potential for compensation, which result in negative impacts on plant reproduction due to incompatible floral architecture or visitor behaviour. Flower structure and visitor behaviour prevent pollen supplementation of *Rhabdothamnus solandri* by introduced bird visitors because a long corolla tube prevents short-tongued visitors from reaching the nectar reward. This results in

introduced bird visitors ripping the corolla tube and damaging the flower (Anderson et al. 2011). And for *Dactylanthus taylorii* visitation by introduced possums (*Trichosurus vulpecula*), ship rats (*Rattus rattus*), and kiore (*Rattus exulans*) reduces successful pollination because of the significant inflorescence damage that occurs while nectar foraging, often before even fully opening (Ecroyd 1996).

While honey bees can supply pollination compensation services in some instances, research suggests that honey bee visitation can also have high costs for flower health and seed production. Young and Young (1992), demonstrated that excessive pollen deposition by honey bees resulted in decreased seed set due to pollen tube competition. Aizen et al. (2014) showed that high visitation rates by honey bees caused mechanical damage of the flower itself, preventing effective pollination and fruit development. In addition, honey bees were observed removing pollen deposited on stigmas of *Melastoma affine* (Melastomataceae) by previous flower visitors, decreasing potential for fruit set (Gross & Mackay 1998). Honey bees exhibit extremely efficient foraging behaviour, often involving nearest neighbour movements to minimise travel distances between flowers. This is not a behaviour peculiar to honey bees. While this is beneficial for the pollinator, it can have detrimental effects on the plant because of lower rates of outcrossing and the potential for inbreeding depression (Vaughton 1996; England et al. 2001). Some plants have mechanisms such as self-incompatibility (Godley 1966; Godley & Smith 1976), or high mortality of seedlings produced by selfing (Schmidt-Adam et al. 2000) to prevent any long-term effects, making this negative impact difficult to detect.

Despite low recovery of capsules from caged treatments, seed set percentages indicated that the contribution of birds to successful pollination is greater for *I. brexioides* than for *W. racemosa.* Different groups of flower visitors can also have different levels of pollination potential based on the quality and quantity of pollen that they deposit. Celebrezze and Paton (2004) demonstrated through a series of exclusion experiments that bird visitation resulted in a superior pollination service for *Brachyloma ericoides* (Epacridaceae) when compared to honey bee visitation. Though honey bees contacted floral reproductive structures, and had a higher visitation rate overall, fruit set from shrubs where birds were excluded was significantly lower. Vaughton (1996) had similar results with *Grevillea barklyana*

(Proteaceae) but demonstrated that bird-excluded treatments showed lower fruit set than treatments where all pollinators were excluded.

When pollination compensation does not occur, or when visitation by a widespread, introduced, exotic species has negative impacts on successful pollination, this can have long-term effects on vegetation composition, biodiversity or frequency of visitation by native pollinators. In New Zealand, honey bees have a great potential to impact native pollination interactions because of the absence of some regulators from their native range (Olesen et al. 2002), their increasing abundance in native ecosystems (Vázquez et al. 2007; Giannini et al. 2015), and the generalist strategy of both honey bees (Norfolk et al. 2018) and many native plants that they visit (Olesen et al. 2002). However, care should be taken in promoting pollination services of introduced pollinators to native plant species because the quality of fruit output may not be comparable. As this study demonstrates, because of floral architecture and flower visitor behaviour, native pollinators, in some instances, can provide higher quality pollination services that improve seed set at an extent that is not replaceable, even by managed colonies of honey bees (MacInnis & Forrest ; Garibaldi et al. 2013).

3.4.2 Impact of introduced pollinators on seed set of native plants

Exclusion experiments identified that for *I. brexioides* seed set was highest at Maungatautari, a pest-proof fenced site where bird visitation was common. *Weinmannia racemosa* showed the highest percentage seed set, and lowest level of pollen limitation at sites of high honey bee hive density. These findings are consistent with our expectations based on the floral architecture of these species and the known behaviour of floral visitors and demonstrates the flow-on effects that differing pollination potentials of flower visitors can affect. The following paragraphs go beyond flower-level architecture and visitor behaviour to explore other potential causes of reduced seed set, such as resource partitioning, plant traits, and selective abortion, and the long-term impacts of these.

Resource partitioning, selective abortion, flower structure and ovule number are important ways that plants manage reproductive output in response to resource availability and resource limitation, independent of pollen deposition. Resource availability is variable at the landscape scale due to seasonal change and topographical effects, and at the plant scale due to inflorescence ontogeny, flower position, and proximity to sources of photosynthates (Diggle 1995; Wesselingh

2007). Because of this variability, plants must be able to respond to differing reproductive conditions to maximise seed set. Ida et al. (2013) demonstrated flexibility in reproductive investment, through increased resource allocation to fully cross-pollinated plants, and the ability of completely defoliated plants to produce viable seed. This research considered results from supplemental pollination experiments to infer to what extent seed set is limited by available pollen. The methodology used prevents inference of seed limitation attributable to resource limitation.

Seed abortion is a strategy that allows plants to manipulate seed production, in response to resource availability (Meyer et al. 2014), insect herbivory (Stephenson 1981; Fernandes & Whitham 1989; Phillips & Walker 1997), damage, or disease (Meyer et al. 2014), regardless of adequate pollen supply (Pearse et al. 2015). *Weinmannia racemosa* and *I. brexioides* both displayed fairly low rates of abortion compared with some species of soy bean (Chanprasert 1988), but higher than some native species (Haase 1986; Allen & Wilson 1992). In most cases the cause of abortion was not apparent, but was likely due to disease, damage, or herbivory.

Ovule number certainly affects the number of seeds that may be produced by a single flower, but it can also affect the allocation of resources towards individual flowers. Strelin and Aizen (2018) demonstrated this effect with raspberry (*Rubus idaeus*) which showed differential resource allocation to fruits from flowers with many ovules. Even flower structure plays a role in the potential seed set of a plant. Dai et al. (2018) demonstrate that pollen supplementation increased fruiting of single flowers, but not complex inflorescences. Though *W. racemosa* did demonstrate evidence of greater seed set on racemes with greater floret numbers, this is insufficient to surmise structural differences affecting seed production.

Ixerba brexioides demonstrated higher seed set in open pollinated (control) conditions over hand supplemented cross-pollination. This leads to the general assumption that seed set must be limited by resource availability. Because of limitations in the methodology, it is difficult to distinguish the effect of pollen limitation from that of resource limitation. Other possible reasons for poor seed set of hand pollinated flowers could be that the crossed pollen was from too near a relative, the timing of the treatment was outside of the window of stigmatic receptivity, or if sufficient pollination had already been achieved by other means, the treatment could have removed pollen already deposited or damaged the flowers.

Because of the breadth of this study, depth was sacrificed in some instances, limiting the application of some data. This research is further limited because it shows only one season and because poor flower numbers during the growing season prevented a greater scale of replication. For future research, it is recommended that the comprehensive methodology suggested by Wesselingh (2007) be followed as closely as possible. This method includes describing breeding system and pollinators, as well as spatial and temporal arrangement of plant architecture and phenology, before observing natural patterns of fruit and seed set, and pollen deposition, before carrying out experimental manipulation of resource availability and pollen limitation. For many species in New Zealand where information on plant breeding systems and reproductive biology are lacking, the methodology of Wesselingh (2007) is often not feasible under research funding and time constraints but should be the ultimate goal.

Large-scale limitation or improvement of seed set has the potential to alter population demographics (Turnbull et al. 2000), and ultimately long-term vegetation composition and structure if the extent of the effect is marked enough. In this case, improving *W. racemosa* seed set in areas of high hive density, and limiting *I. brexioides* seed set could lead to a shift in vegetation dominance toward *W. racemosa* and other small-flowered tree species, over *I. brexioides* and other large-flowered tree species.

3.4.3 Landscape matrix

There is strong evidence in the literature to suggest that the matrix of land use in a particular area will have significant impacts on biodiversity of species, conservation values, and other ecosystem properties (Jules & Shahani 2003). For most sites in this research native forest was the main land cover type within a 5 km radius. Other land cover classes that were common were agricultural pasture, which was the dominant surrounding for Maungatautari and a main constituent at Tuahu, along with horticultural production, which was also prevalent at Tuahu.

At Tuahu, the main horticultural products are kiwifruit, and avocado, though berries, hydrangeas, and olive crops are also found nearby. Kiwifruit and avocado flowering and pollination generally occurs earlier in spring than flowering of *W. racemosa* and *I. brexioides*, but in the case of early flowering coinciding with late crop pollination, this could be a source of honey bee drift into native forest, which may account for the higher rates of honey bee visitation at this site. Growers can

mitigate the risk of drift by swapping out hives regularly, supplementary feeding of honey bees (especially for kiwifruit pollination as the flowers do not produce nectar) or enclosing orchards in nets (New Zealand Kiwifruit Growers 2018), or they may compensate for drift by having high colony stocking rates. However, research suggests that honey bee foraging declines with distance from colonies (Cunningham et al. 2016), indicating that though the maximal range of honey bees is large, the energetic cost of foraging long distances means that it is not likely to be a common foraging strategy (Pyke et al. 1977).

For this research, surrounding land use has implications, not just for the abundance and diversity of native pollinators, but also for the density of honey bees. Feral populations are not a significant consideration because, while they were once well established, arrival and spread of parasites and diseases such as *Varroa destructor* (parasitic mites), *Nosema spp*. and American foulbrood (*Paenibacillus larvae*) in New Zealand has resulted in wild populations no longer surviving in significant numbers (Beard 2015). However, hives on private land bordering conservation areas are becoming an increasing concern as 'border stacking' often means there are many more bees operating in an area than hive consents will allow (Newstrom-Lloyd 2016; Burns-Francis 2017).

Research has demonstrated that decline of natural and semi-natural landscapes and increasing isolation within agricultural matrices can reduce abundance of some species of wild bees (Jauker et al. 2009; Stavert et al. 2018). Landscape pattern can also greatly influence dispersal ability and survival of pollinators constrained by resource availability and habitat (Viana et al. 2012). Agricultural practises within those landscapes can also affect the diversity of pollinators, particularly with regard to cropping regimes and use of pesticides (Holzschuh et al. 2008; Macdonald et al. 2018). Despite being set in a matrix dominated by agricultural pasture, Maungatautari showed the highest percentage of seed set for open pollinated *I. brexioides* flowers. One of the key characteristics of this site that likely contributed to this result is the higher incidence of bird visitation as a result of the pest-proof fence that surrounds the 2500 hectare reserve. Maungatautari was the only site where bird visitation of *I. brexioides* flowers was observed, and video footage demonstrated a high potential for pollination with each visit.

3.4.4 Summary and recommendations

In summary, this research illustrates that the potential impacts of introduced honey bees on native plants are not straight forward and depend in a large part upon the floral architecture of the plant, and the behaviour of the visitor. For *I. brexioides* flower visitation by birds, beetles and native bees is more effective overall for pollination than visitation by honey bees, though results may indicate a positive outcome for *W. racemosa* reproductive output in areas of high honey bee hive density. Further research is required to assess whether any long-term effects come from increasing honey bee presence in native forest, such as alteration of forest composition and structure or impacts on plant genetic diversity.

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Chapter 4

The effects of the introduction of managed honey bees (*Apis mellifera*) on invertebrate flower visitors in the Kaimai-Mamaku Range, New Zealand

Abstract

Increasing presence of managed honey bee (*Apis mellifera*) colonies in areas of native vegetation is an issue of growing concern. Introduction of honey bees into native plant-pollinator networks has been shown to decrease links between native pollinators and plants and affect plant reproductive success. In New Zealand, rapid increases in hive numbers, attributable to success in the apiculture industry, are particularly notable in areas of native vegetation under conservation protection. We investigate the influence of managed honey bee introduction on plant-pollinator communities and network structure in the Kaimai-Mamaku Range, New Zealand, and the implications for sustainable management of honey enterprises and native vegetation. Flower visitors were collected from five sites of varying beehive density by sweep netting between November and December of 2016 and 2017 and identified anatomically. Community composition, species co-occurrence and network analysis were carried out in R, and compared across sites with high and low honey bee hive density. Data demonstrated that high density of managed honey bee hives in native forest was correlated with differences in communities of invertebrate flower visitors in terms of patterns of diversity, network structure and interactions, species co-occurrence, and community composition. We conclude that this provides evidence of honey bee impacts on plant-pollinator networks and that these networks are affected by invader density. Regulation of beehive presence in areas of native vegetation is advised to prevent long term and potentially irreversible network-level change that will disconnect native pollinators from networks and negatively affect diversity of native flower visitor communities.

4.1 Introduction

Around the world, there is growing public concern for the decline of honey bees (*Apis mellifera*) (Goulson et al. 2015). However, public concern does not distinguish between risks to honey bees that are wild and operating in their native

range, and honey bee colonies being managed for apiculture outside their native range. In fact, as honey products rise in popularity, honey bees are becoming a significant presence in ecosystems outside of their native home range, particularly in New Zealand. Honey bees were introduced to New Zealand in 1839 (Matheson & Reid 2011), where there is now one of the fastest growing apiculture industries in the world, with a 95 % increase in hive numbers over the last five years (Airborne Honey 2018). In addition, use of native forest reserves for apiculture has substantially increased in New Zealand over the last five years, showing a 70 % increase in hive numbers on land administered by the Department of Conservation (C. Beard pers. comm.). This growth has prompted questions around the sustainability of continued increases in hive numbers on conservation land, and the potential impacts of such on native ecosystems (Beard 2015). This chapter reviews factors influencing the likelihood of honey bee establishment in plant pollinator communities following introduction and the potential impacts of such. In addition, multivariate and network theory approaches are applied to data on communities of invertebrate flower visitors from the Kaimai-Mamaku Range, New Zealand, to assess the effects of honey bee hive density on these communities.

4.1.1 Factors affecting success of introduced pollinators invading pollination networks

Because of pests and diseases, such as parasitic *Varroa destructor* mites, honey bee colonies are unlikely to survive in the wild without active management. Hence, invasion, as used in this text, refers to these alien species being introduced and incorporated into otherwise largely 'native' pollination networks, rather than their ability to autonomously invade and persist in native ecosystems. The impact that increasing hive numbers have on flower visitor communities in native forest ecosystems will depend largely upon factors that affect invasion success. Flower visitation strategies of invading pollinators, for example, play a role. Generalist flower visitors, with low dependency on specific limited plant species, are involved in a significantly higher number of interactions than other pollinators, making it easier for them to become established in native plant-pollinator networks. In the pollination networks of five of the Galápagos Islands, five super-generalist pollinators were identified, three of which were aliens (Traveset et al. 2013). Santos et al. (2012); Giannini et al. (2015); Norfolk et al. (2018) all found honey bees to be super-generalists in networks they had invaded. Norfolk et al. (2018) compared a non-invaded high mountain network to a low mountain network in Egypt that was

invaded by the honey bee as a result of managed hives in the area. Honey bees were found to be involved in <5% of all interactions in the high mountain network but accounted for 27% of all interactions in the low mountain network. The honey bee was only a super-generalist in the low mountain network where it was highly abundant and was not a super-generalist in the high mountain network where managed hives were absent.

Literature suggests integration of invasive species into pollination networks is also assisted by native generalist plants. Olesen et al. (2002) demonstrated that on two oceanic islands where super-generalists were common, invasive pollinators could quickly establish in the pollination network. This occurs because generalised strategies on the part of the native plants or the introduced pollinator increases the likelihood of developing strong plant-pollinator relationships in the new environment. Islands can be ideal sanctuaries for native species, as isolation deters invasion of non-native species. However, because island ecosystems generally have high abundance of generalist species, pollination networks on islands may be more susceptible to invasion in these conditions (Olesen et al. 2002).

Abundance strongly affects degree metrics of an introduced pollinator (Vázquez et al. 2007; Giannini et al. 2015), or the number of plant species it will interact with (Bascompte 2009). Aizen et al. (2008) demonstrated, from research in the Southern Andes, that invading pollinators that were initially rare in plant-pollinator networks persisted only by interacting with native generalist plants. However, disturbance and/or a lack of regulatory processes (e.g. predation or parasitism), allowed an increase in abundance that facilitated expansion of invaders pollination niche.

Habitat modification can exacerbate an invasive species' impact on a network. The strength of the interaction, measured by the dependencies of the plant species it interacts with (Giannini et al. 2015), can increase to a greater extent than would be expected by the effect of abundance alone, when habitat modification favours an invading pollinator (Didham et al. 2007). Habitat modification can be particularly damaging on isolated islands, where generalist strategies make plant-pollinator networks more susceptible to invasion (Didham et al. 2007). Prevention of initial invasion is important to protect native pollination networks on islands.

Though honey bees might not meet the definition of invasive species in New Zealand due to their inability to survive on their own without the use of synthetic

miticides, they do have the ability to invade native plant-pollinator networks based on the factors discussed above. They demonstrate a generalist visitation strategy, having been seen visiting 188 species in New Zealand across a wide variety of plant life forms and habitats (Butz Huryn 1995). In addition, the New Zealand flora has a lack of specialisation (Heine 1937; Lloyd 1985), with predominantly simple, radial flower structures, with exposed pollen structures and simple dish or tube blossom classes. These characteristics of flower simplicity also tend toward pollination in non-specific ways, indicating a generalised strategy toward pollination. In terms of regulatory processes specific to honey bees, though there are some diseases and competitors that are present in New Zealand ecosystems (Howlett & Donovan 2010), such as American Foulbrood (Palmer-Jones 1964), *Varroa destructor* mite (Zhang 2000), and wasps (Donovan 1984), there are other threats that are not yet here, such as small hive beetle and European Foulbrood (Goodwin 2004). In addition, honey bees are managed by their owners to minimise the effects of these regulatory processes, as well as normal environmental regulators, through pest management and supplementary nutrition (Matheson & Reid 2011). Many New Zealand habitats have also been highly modified in ways which are likely to improve conditions for honey bee invasion, particularly with introduction of exotic flowering species that are common on farmland and forest edges, and the conversion of large areas of native forest to exotic pasture.

4.1.2 Potential impacts of invasion

Invasion of an alien pollinator can have wide-reaching effects, which can only be observed at an appropriate depth by looking at the community or network as a whole. Plant-pollinator networks are described as bipartite, and mutualistic because they generally involve only two trophic levels (plants, and pollinators) with a bi-directional flow of benefits. Several measures of mutualistic network structure have been developed to identify features of and track changes in the architecture of interactions between plants and their pollinators. These measures are useful in a number of applications, particularly conservation of mutualistic networks. For example, Fortuna & Bascompte (2006) demonstrated that the effects of habitat loss were modulated by structure of plant-pollinator networks, and Memmott et al. (2007) used mutualistic networks to demonstrate the projected impacts of climate change. Invasion of mutualistic networks by an alien species can affect networks in several key ways: network interactions, connectance and nestedness. In addition,

these changes can have flow-on effects to plant reproduction that can lead to further ecosystem degradation.

Network interactions

Mutualistic networks, like plant-pollinator networks, are generally nested (consisting of a core group of generalists with increasing layers of specialists interacting with those generalists). This arrangement gives robustness to the network, protecting it from environmental change and random species loss by creating functional redundancy and providing pathways for the persistence of specialists (Bascompte et al. 2003; Bascompte & Jordano 2007). However mutualistic networks also generally demonstrate heterogeneity in the strength distribution of links (i.e. some links are stronger than expected by chance) (Bascompte 2009). This can give the network strength as key individuals hold the network together, but it can also cause rapid network collapse if those key species are lost. These key species may act as hubs – generalist species with many interaction links that rely on them – or as connectors – linking together functional groups of pollinators (Tylianakis et al. 2010).

The extent of invasive pollinator effects on network interactions becomes more pronounced as the extent of invasion increases. Traveset et al. (2013) identified an increasing dependence on alien species in pollination networks with increasing establishment of the alien species. Although only 21% of the network were aliens, they were disproportionately engaged in 38% of all interactions in networks studied on five Galápagos Islands. And on the oldest and most disturbed island, San Cristóbal, the most important pollinators in terms of linkage level were alien species. In highly invaded webs, more species interact with and become dependent on generalist alien pollinators for pollination services (Aizen et al. 2008). This has implications for conservation and restoration of pollination networks. If native plants have become highly dependent on invasive pollinators, removal of those pollinators may lead to species co-extinction.

Invasive pollinators can also affect the strength of existing mutualisms. Mutualistic interactions are typically asymmetric, demonstrating unequal dependence on the part of the plant and pollinator (Bascompte, Jordano, & Olesen, 2006). This is the result of selection against pollinators that specialise on rare plants due to population fluctuations of the host, favouring pollinators that specialise on common plants. At

the same time, rare plants benefit from having a range of different pollinators, favouring specialist plants that are pollinated by generalist pollinators. In a network of ants and extrafloral nectary-bearing plants on the oceanic Ogasawara (Bonin) Islands in Japan, invasion of exotic ants led to the loss of native ant-plant links (Sugiura 2010). Aizen et al. (2008) demonstrated that in highly invaded webs interactions involving at least one alien were more asymmetric than those between natives. Generalist alien species in highly invaded networks are generally involved in a large proportion of the most asymmetric interactions in highly invaded webs and become central nodes within the network (Aizen et al. 2008). This can have implications for management of invasive pollinators because of over dependence of native plants on introduced pollinators.

Connectance

Network connectance, referring to the proportion of realised versus potential interactions, affects robustness of networks to co-extinction (Okuyama & Holland 2008; Campbell et al. 2012; James et al. 2012; Vieira & Almeida-Neto 2015). However, connectance is often low in real networks and, indeed, networks function better in these conditions as high connectivity can indicate high rates of competition (Santos et al. 2012; Traveset et al. 2013; Valdovinos et al. 2016; Norfolk et al. 2018). Research has shown that network connectance is generally not affected by invasive pollinators (Santos et al. 2012; Traveset et al. 2013; Norfolk et al. 2018), however, invasion can alter the distribution of interaction links without changing the overall connectance (Aizen et al. 2008). Aizen et al. (2008) demonstrated that native mutualists were normally more connected among themselves in the lightly invaded networks than in highly invaded networks. Connectance did not vary overall in highly invaded networks, as a loss of connectance among natives was compensated for by increased connectance with alien species.

Nestedness

Nestedness is a structural property of mutualistic networks where specialists interact with a subset of generalists, and generalists interact amongst themselves (Bascompte 2009). This limited reciprocal dependency provides structural stability, robustness to extinction and minimises competition (Bastolla et al. 2009). Invaders can increase nestedness by interacting with generalists in plant-pollinator networks. In a study of five islands in the Galápagos, Traveset et al. (2013) demonstrated all networks were significantly nested, and the most nested islands were also the most

invaded. Because nestedness is often related to network stability, this suggests a positive relationship between network stability and network degradation (Traveset et al. 2013). Honey bees have been shown to increase nestedness with invasion, and simulated removal of honey bees leads to a decrease in nestedness (Santos et al. 2012; Norfolk et al. 2018). Giannini et al. (2015) showed that while the non-native super-generalist honey bee had a strong effect on nestedness, the native super-generalist *Trigona spinipes,* a stingless native bee, did not. Increased nestedness is typically associated with increased network stability and species diversity (Bastolla et al. 2009; Thébault & Fontaine 2010), as the generalist core prevent extinctions of specialists and may also include other rare specialists in the network. This has negative implications for the restoration of pollination networks, as generalist invasive pollinators monopolise many interactions in the network. Before restoration is attempted by simply removing invasive pollinators, a network should be investigated to determine what species are structurally important (Olesen et al. 2007).

4.1.3 Summary

When novel generalist pollinators such as honey bees invade a new ecosystem, they establish interactions with a broad suite of plant species that are also pollinated by a large host of native pollinators. Thus, impacts of invading generalist pollinators can be highly complex and far-reaching. As such, these impacts cannot be understood by looking at pairwise interactions alone and require understanding of changes to the entire community and interaction network. In particular, structural properties such as network connectance, nestedness, modularity, etc. are all network-level properties that could be affected, yet these impacts could go unseen if only pairwise plant-pollinator interactions are assessed. This chapter assesses changes to flower visitor communities and interaction networks observed in the Kaimai-Mamaku range affected by differing densities of managed honey bee hives. Based on the literature reviewed earlier, we expected that sites with high honey bee hive density would be significantly different to sites with low honey bee hive density in terms of species composition and structure. In particular, we expected that plant-pollinator communities at sites of high hive density would demonstrate increased nestedness, and more asymmetrical interactions, without affecting overall connectance.

4.2 Methods

4.2.1 Study Sites

Research for this study occurred in the submontane belt (400 to 800 metres above sea level) of the Kaimai-Mamaku range between November 2016 and January 2017 and November 2017 and January 2018. Sites were selected based on accessibility, proximity to honey bee hives, and presence or absence of target tree species – *Weinmannia racemosa* and *Ixerba brexioides*. Field sites were based on Department of Conservation (DOC) tracks at Tuahu, North-South (at the State Highway 29 lookout), Woods Mill, and Mokaihaha. The North-South track was abandoned in the second season of sampling due to accessibility issues, low flowering, and low pollinator activity. Maungatautari Sanctuary Mountain was included in the second season of sampling and is located just west of the Kaimai-Mamaku range. Figure 2.1 (pg 37) shows the location of field sites.

Table 4.1: Details of study sites including latitude and longitude, altitude (m), hive numbers found within a 5 km radius of the sites in 2016 and 2017, hive density category (L=low, H=high), pest management status whether unmanaged (U) or with intensive management (IM), and fragmentation level whether fragmented (F) or unfragmented (U).

Site	Lat.	Lon.	Alt.	2016	2017	Hives	Pests	Matrix
Woods Mill	-38.03	175.98	500	140	150	L	U	U
North-South	-37.87	175.93	500	374	-	H	U	D
Mokaihaha	-38.18	176.10	600	97	395	L	U	U
Tuahu	-37.60	175.86	400	822	1444	H	U	U
Maungatautari	-38.01	175.58	500	-	431	H	IM	F

Table 4.1 shows categorisation of sites based on hive density within 5 km, as well as pest management and fragmentation of the surrounding land matrix. Hive density categories were assigned based on 2016 hive numbers and categories were maintained for 2017. Hive density categorisation presented challenges due to the difficulty of obtaining data on the density of existing hives. The data is kept by AsureQuality and though it can be made available for research purposes, the process took considerable time between request and receipt of data (over 12 months) and the level of detail available was low. Pest management categories separated the Maungatautari site from other sites because all mammalian pests except mice have been eradicated from this sanctuary (Sanctuary Mountain 2018). Fragmentation of

surrounding land matrix was determined using ArcGIS 10.5.1 2017 to analyse land use in the land matrix within a buffer radius of 5 km. Land cover classes were categorised using LCDB v4.1 - Land Cover Database version 4.1, Mainland New Zealand data (Landcare Research 2015). Sites were categorised as 'fragmented' if native forest cover was less than 50 % of the surrounding land matrix within a 5 km radius.

4.2.2 Insect collection

Invertebrate visitors to *W. racemosa* and *I. brexioides* flowers were collected by sweep netting flowering trees at each study site for ten-minute intervals at 8 am, 12 pm, 4 pm, and 8 pm daily. Sampling of a broader range of flowering tree species was conducted for network analysis, including *Melicytus ramiflorus, Leucopogon fasciculatus, Kunzea ericoides, Olearia rani, Geniostoma ligustrifolium var ligustrifolium, Pseudopanax arboreus, Streblus heterophyllus, Pseudowintera axillaris,* and *Schefflera digitata.* Collected invertebrates were stored in individual tubes and frozen, and later identified by a contracted taxonomic expert.

Table 4.2: Summary of sampling effort at sites of high and low hive density for sweep netting. 'Trees' outlines the number of separate trees sampled. 'Y1','Y2' and 'Total' indicate the number of sampling periods for each species.

Hives	Site	Species	Trees	Y1	Y2	Total
High	Kaimai Summit	*Ixerba brexioides*	1	1		1
		Schefflera digitata	1	1		1
	Maungatautari	*Ixerba brexioides*	4		10	10
	Tuahu	*Brachyglottis repanda*	2		2	2
		Geniostoma rupestre	3		3	3
		Ixerba brexioides	2	6	7	13
		Leucopogon fasciculatus	3		3	3
		Melicytus ramiflorus	2	1	2	3
		Olearia rani	3		3	3
		Pseudopanax arboreus	1		1	1
		Pseudowintera axillaris	1		1	1
		Streblus heterophyllus	2		2	2
		Weinmannia racemosa	5	12	15	27
Low	Mokaihaha	*Ixerba brexioides*	1	2	1	3
		Kunzea ericoides	1	1		1
		Pseudopanax arboreus	1		1	1
		Schefflera digitata	1	1		1
		Weinmannia racemosa	4	12	11	23
	Woods Mill	*Ixerba brexioides*	3	8	4	12
		Pseudopanax arboreus	1		1	1
		Weinmannia racemosa	3	9	5	14
		Total	*45*	*54*	*72*	*126*

More than 1500 flower visitors were collected from the five sites across 126 sampling periods. Sampling was not undertaken in rainy conditions. Although we recognise that not all flower visitors are effective pollinators, for the purposes of simplicity in the description of the analysis we refer to these collected flower visitors from here on as 'pollinators'.

4.2.3 Community analysis

NMDS ordination was carried out on data collected from sweep netting and summarised into a matrix based on sampling sites and periods, and flower visitor abundance. Flower visitors were grouped by the highest taxonomic identification. Communities were analysed separately for visitors of *I. brexioides* and *W. racemosa.* Community matrices were also separated by sample collection year as initial analysis showed significant differences in flower visitor communities based on collection season for *W. racemosa* (p=0.001). Analysis was done using the 'vegan' package in R with code adapted from Lefcheck (2012). The 'metaMDS' function was used to produce NMDS scores with Wisconsin double standardisation. Parameters were set to two dimensions, maximum of 5000 runs. The parameter 'noshare' set to 0.1 which triggers a step-across function if the site pairs with no shared species exceeds 10 %. The 'vegdist' function (Oksanen et al. 2019) was used to generate a distance matrix from the flower visitor community data. Bray-Curtis was the selected distance measure. The 'envfit' function was used to assess the fit of environmental variables with the data, including hive density, main surrounding land use, season, Shannon diversity, species richness, proportion of native species, and average wind speed (ms^{-1}), relative humidity (%) and temperature (° C).

The 'simper' function in Vegan (Oksanen et al. 2019) was used to identify key species contributing to dissimilarities between communities, in this case, between sites categorised by honey bee hive density. In addition, indicator species analysis was used to assess key groups of species that are indicative of site categories. This was done using the 'indicspecies' package in R (De Caceres 2013; De Caceres & Jansen 2016).

4.2.4 Co-occurrence analysis

Species co-occurrence analysis can identify patterns of competition, mutualism and predation, and is a vital part of community composition (Diamond 1975). Analysis of species co-occurrence is used here, in addition to network analysis, to provide a specific pair-wise analysis of species interactions, and to demonstrate which species

within plant-pollinator communities in the Kaimai-Mamaku range have significant positive or negative relationships with honey bees based on their patterns of occurrence in both time and space. Input matrices treated community data from *I. brexioides* and *W. racemosa* separately, with separate matrices for each sampling year. Flower visitors which were present in only one sampling event were excluded.

Species co-occurrence was analysed in R (Version 3.5.3) using the "co-occur" package with code adapted from Tulloch et al. (2018). This package uses a probabilistic model to compare observed co-occurrence with expected co-occurrence, calculated as the product of the two species probability of occurrence multiplied by the number of sampling sites: $E(N_{1,2}) = P(1) \times P(2) \times N$ (Veech 2013). Output includes *p* values indicating significantly positive or negative interactions between species pairs. Code from Griffith et al. (2016) was used to summarise co-occurrence results into graphs using the "cooccur" package in R.

4.2.5 Network Analysis

Network-level analysis was conducted using the 'bipartite' package in R Studio (Dormann et al. 2009; Dormann et al. 2018). Community data was assembled from sweep netting key flowering species throughout the year, and represents annual pollination networks, rather than point in time assemblages. Flower visitor collection was concentrated on *I. brexioides* and *W. racemosa.* This presents some challenges with representing the network appropriately with regard to the variety of plant species. The original plan for this work relied on analysis of pollen occurring on the bodies of the flower visitors that were collected, allowing broader capture of real network interactions. Trials of the microscopic pollen identification were carried out (see Chapter 5), but after consulting with a pollen expert molecular identification of pollen, using DNA sequencing, was pursued instead. This method, however, took longer to develop than anticipated, and it became necessary to complete analysis without the DNA data, with the intention of completing the DNA sequencing work before publication of the results.

Community data was arranged into a matrices with flower visitor species represented as columns and plant species represented as rows; the values within the table indicated interaction density. Interaction density was corrected by sampling intensity in terms of sampling time at each site. Community matrices were analysed for all samples combined, as well as separately for samples from high hive density sites and low hive density sites. Networks were mapped using the 'plotweb'

function and network indices were calculated for the networks as a whole using the 'networklevel' function and for individual species using the 'specieslevel' function under default conditions.

4.2.6 Statistical analysis

Statistical differences in species diversity among sites were first assessed by calculating the Shannon Diversity index using the 'diversity' function in the Vegan package of R. Rarefaction analysis also used the Vegan package in R, with the function 'rarefy' (Oksanen 2019). Function 'rarecurve' was used to produce a rarefaction curve. The sample was designated as min(rowSums(community_matrix)). Normality of the data was assessed using the Anderson-Darling normality test from the R 'nortest' package (Gross & Ligges 2015) and was not normally distributed (p<0.01). Non-parametric Kruskal Wallis rank sum tests were applied to Shannon diversity data in R using the 'kruskal.test' function (RStudio Team 2018) to determine significance of differences in diversity between sites categorised by hive density.

Statistical analysis of community ordination data was conducted on distance matrices produced using Bray-Curtis as the distance measure. Permutational multivariate analysis of variance (PERMANOVA) was carried out using the 'adonis' function in Vegan to discern differences between community ordinations in R. The Vegan function 'betadisper' was also used on the distance matrices to assess multivariate homogeneity of group variances. Variances were plotted to visualise differences. Site categories were treated individually in this research, but future work will investigate mixed effects models for comparison.

Statistical analysis of network parameters was done using the 'bipartite' package in R (Dormann et al. 2018). Indices were compared with randomly generated networks to determine whether observed values were significantly different to null models using the function 'null.t.test'.

4.3 Results

4.3.1 Invertebrate visitor fauna

Over the five study sites, Tuahu consistently had the highest percentage of honey bees visiting flowers (10 % of all flower visitors) and the lowest percentage of native species (47 %). In comparison, the other sites had between 0 % and 1% honey bee incidence with 67 % to 91 % native species occurrence. Tuahu also had the

greatest richness of flower visitors (65 species in 2016 and 110 species in 2017), followed by Mokaihaha (92 species in 2016 and 52 species in 2017). Table 4.3 summarises these findings.

Table 4.3: Summary of invertebrates collected by sweep netting at five sites in the Kaimai-Mamaku range. Y1 = Year 1, November to December 2016, Y2 = Year 2, November to December 2017. Honey bee % = percentage of all samples collected that were honey bees; Periods = number of total sampling periods; Samples/period = average samples collected per sampling period.

Site	Tuahu		Nth-Sth	Maungatautari	Woods Mill		Mokaihaha	
Year	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
Honeybee %	7	11	0	1	0	0	0	0
Periods	17	39	2	10	16	7	14	12
Samples/period	11	12	17	10	13	20	21	12
Species	65	110	15	33	62	41	92	52
Native (%)	67	39	91	80	85	42	83	52
Introduced (%)	33	61	9	20	15	58	17	48
Tree species	4	10	2	1	2	3	4	3

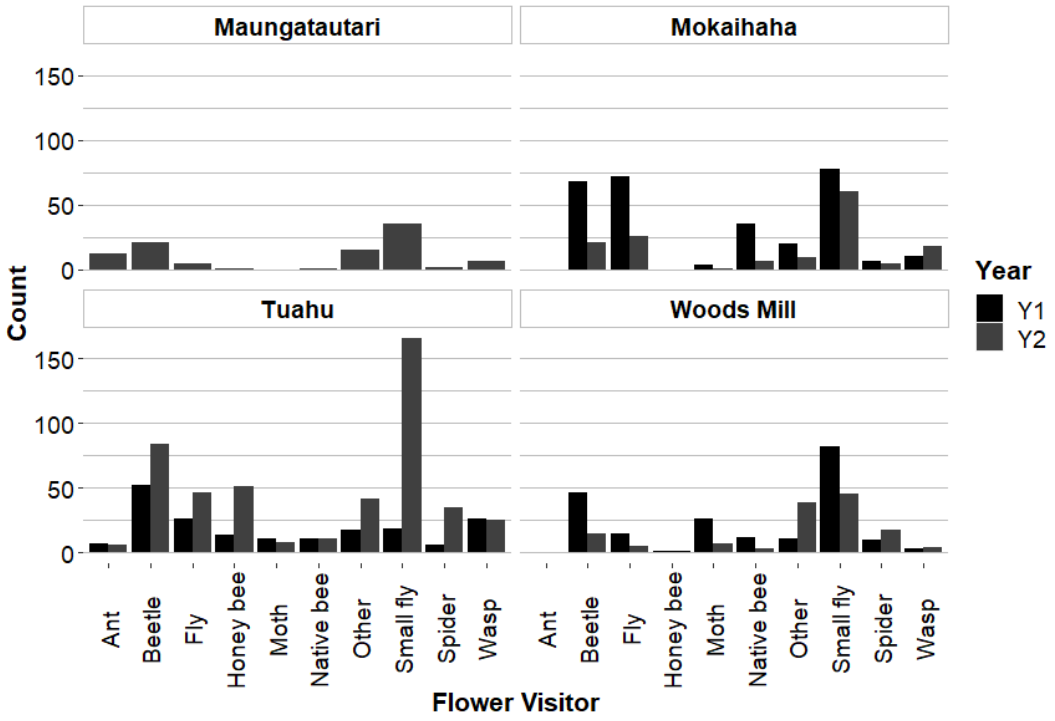

Figure 4.1: Composition of visitor fauna as collected by sweep netting for four sites during 2016 (Y1) and 2017(Y2)

Figure 4.1 shows the composition of flower-visitors collected by sweep netting from November to December 2016 and 2017 at four sites: Maungatautari, Mokaihaha, Tuahu and Woods Mill. Across both years, small flies (<5mm) made up the greatest proportion of visitors (26-41 %), followed by beetles (13-29 %) and

flies (6-15 %). The majority of the invertebrates collected (78 %) which were identifiable to genus or species did not have data on threat status. A further 9 % were data deficient and 11 % were naturally uncommon (Buckley et al. 2012; Leschen et al. 2012; Department of Conservation 2013; Ward et al. 2014; Buckley et al. 2015; Heath et al. 2015; Hoare et al. 2015; Thomas R. Buckley 2016; Trewick et al. 2016). One species, *Orthodera novae-zelandiae* (New Zealand praying mantis), is in decline according to the New Zealand threat classification system (Buckley et al. 2012).

Shannon diversity of samples differed between sites of high (1.64±0.08) and low (1.93±0.08) hive density (p=0.028), with low hive density sites exhibiting higher species diversity than high hive density sites. In addition, diversity was higher at sites where native forest was the dominant surrounding land use (1.8±0.06) (within a 5 km radius), compared to sites surrounded by pasture (1.36±0.22) (p=0.017). Diversity at intensively managed (1.37±0.25) and unmanaged (1.8±0.06) sites was not significantly different (p=0.089).

Rarefaction analysis was done on flower visitor matrices pooled by high and low hive density. Rarefied diversity at sample size 731 was 99.9 for high hive density sites, and 102 for low hive density sites. Slope at sample size 731 was 0.023 for high hive density sites and 0.000 for low hive density sites (Figure 4.2).

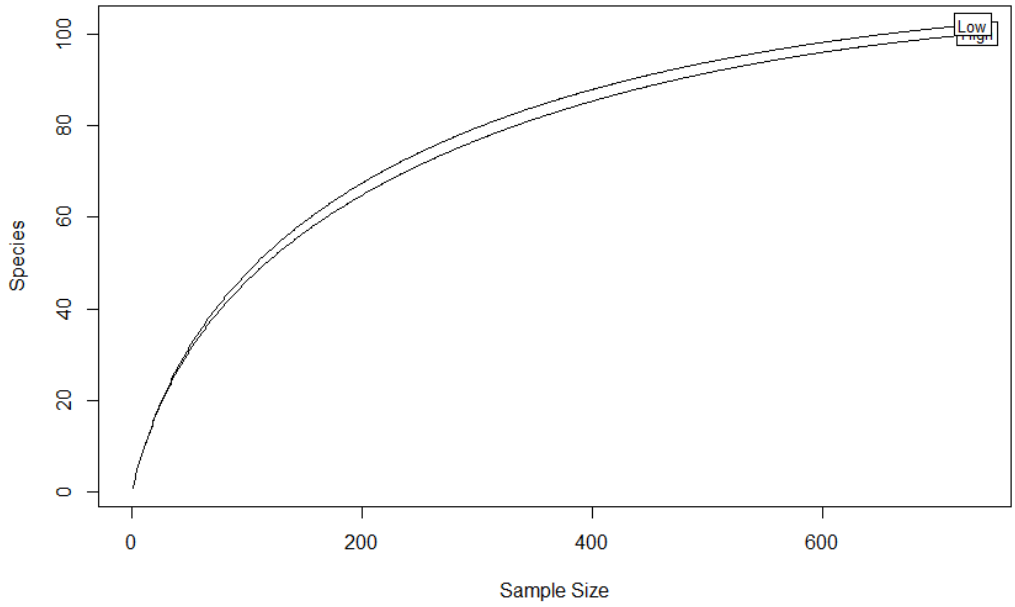

Figure 4.2: Rarefaction curve for flower visitor samples collected at sites of high and low hive density in the Kaimai-Mamaku Range, New Zealand

4.3.2 Community analysis

Ordination visually demonstrated differences between flower visitor communities present at sites of differing hive density. Figure 4.3 and 4.4 show metaNMDS plots for flower visitors collected from *I. brexioides* and *W. racemosa* over two sampling seasons. The coloured polygons show groupings based on levels of hive density. The overlay of species names indicates which species are most indicative of the community.

A range of factors were assessed for significant associations with the community matrix used in the metaNMDS. In *I. brexioides* flower visitor communities the percentage of native species (p=0.036) and species diversity (p=0.006) were most significantly correlated with community composition in year one and year two of sampling respectively. For *W. racemosa* communities there were no significantly correlated parameters in year one, and species richness (p=0.024) was most significant in year two of sampling.

PERMANOVA was used to illustrate statistically significant differences in species composition between high and low hive density sites. Highly significant differences were observed between the flower visitor communities collected from *W. racemosa* at high and low hive density sites (p=0.001 in Year 1 and Year 2). Flower visitor communities from *I. brexioides* had significant differences between high and low hive density sites in Year 1 (p=0.005) but not Year 2 (p=0.064).

Dispersion analysis was used to identify statistically significant differences between communities based on dissimilarity (see Table 4.4). Low and high hive density sites showed communities that were not significantly different with the exception of *W. racemosa* communities in year two of sampling (p=0.001).

Table 4.4: Results from dispersion analysis (999 permutations) comparing sites categorised by hive density for flower visitor community dissimilarity for visitors from W. racemosa *and* I. brexioides.

Species	Variable	Year	Df	Sum Sq	Mean Sq	F	Pr(>F)
I. brexioides	Hive Density	Y1	1	0.01	0.01	0.97	0.356
		Y2	1	0.00	0.00	0.17	0.673
	Site	Y1	3	0.64	0.21	18.75	0.001
		Y2	3	0.30	0.10	5.44	0.008
W. racemosa	Hive Density	Y1	1	0.06	0.06	16.56	0.001
		Y2	1	0.00	0.00	0.61	0.425
	Site	Y1	2	0.03	0.01	3.15	0.068
		Y2	2	0.02	0.01	1.20	0.319

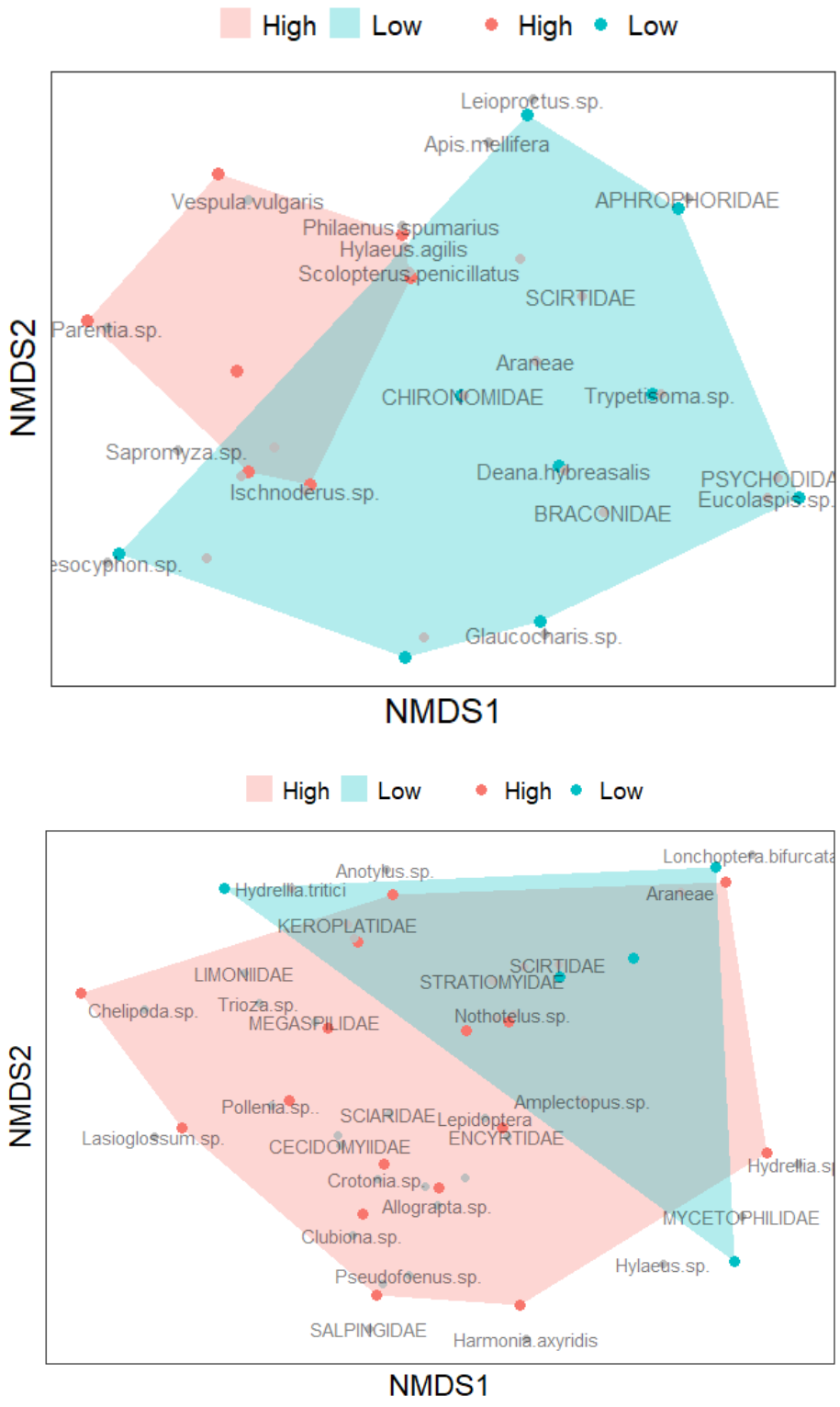

Figure 4.3: NMDS Ordination plots for communities of flower visitors collected from I. brexioides *during December 2016 (top) and December 2017 (bottom). Plots were constructed using the Bray-Curtis distance measure and show axis 1 and 2 from a 2-dimensional solution. Coloured hulls indicate high (red) or low (blue) hive density within a 5 km radius of the site.*

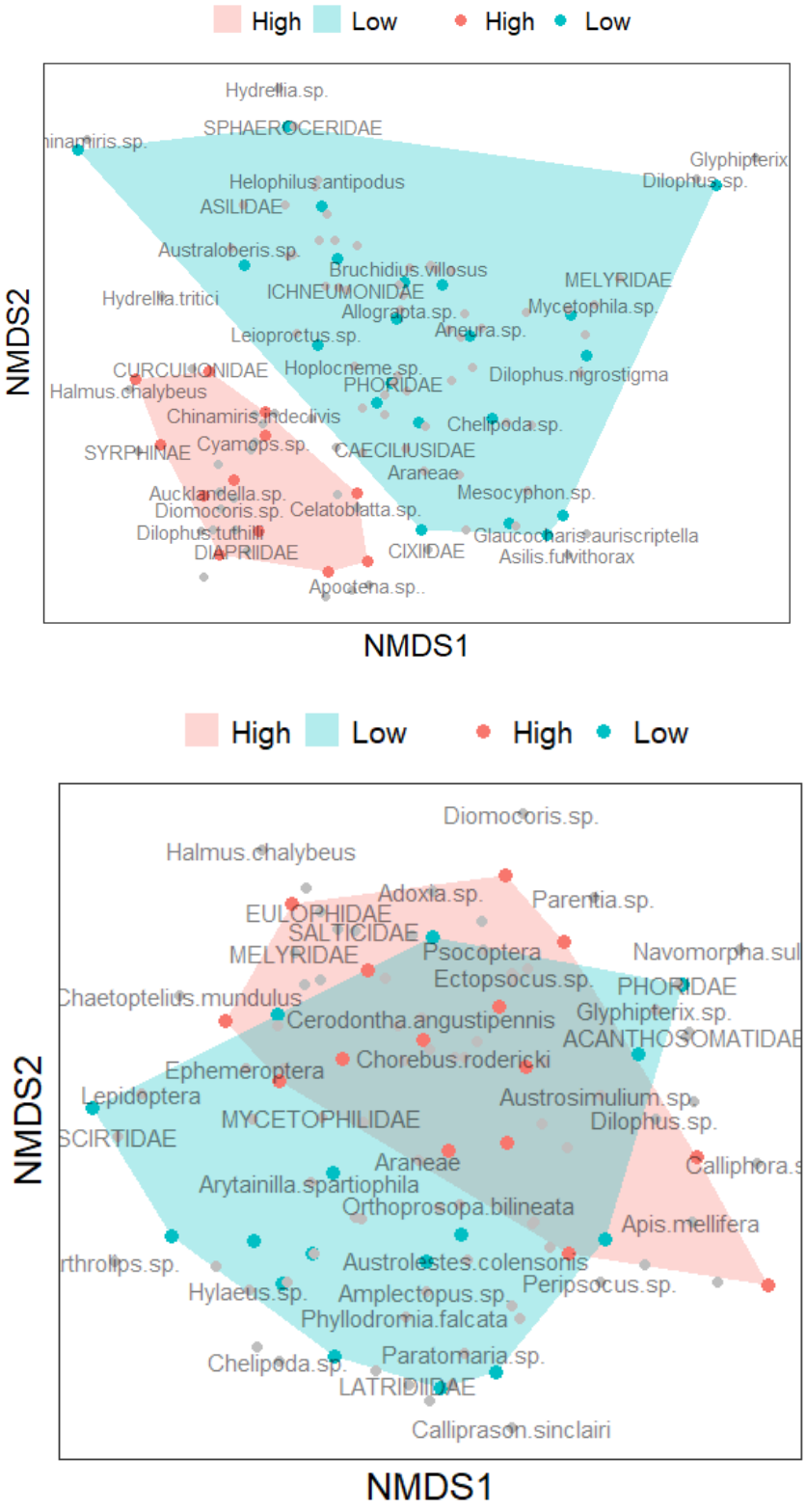

Figure 4.4: NMDS Ordination plots for communities of flower visitors collected from W. racemosa *during November to December 2016 (top) and November to December 2017 (bottom). Plots were constructed using the Bray-Curtis distance measure and show axis 1 and 2 from a 2-dimensional solution. Coloured hulls indicate high (red) or low (blue) hive density within a 5 km radius of the site.*

Influential species groups contributing to dissimilarities between communities were identified using the 'SIMPER' function in the 'Vegan' R package (Table 4.5). In *I. brexioides* flower visitor communities Mycetophilidae, *Tetragoneura sp.* and *Vespula vulgaris* were among the top contributors, with over 15% of differences attributable to those groups. In contrast, *Apis mellifera* contributed only 2%. For communities sampled from *W. racemosa, Apis mellifera* had the highest total contribution (14 %) and average contribution (7 %) over sampling years.

Table 4.5: SIMPER analysis showing influential contributors to dissimilarities between flower visitor communities collected from W. racemosa *and* I. brexioides *at sites sites of high and low hive density. Communities were sampled between Nov-Dec 2016 (Y1) and Nov-Dec 2017 (Y2). The displayed contributors are the most influential, contributing over 4 % to the total community difference.*

Contributor	Y1	Y2	Total	Average
Ixerba brexioides				
MYCETOPHILIDAE	2%	15%	18%	9%
Tetragoneura sp.	15%		15%	8%
Vespula vulgaris	15%		15%	15%
SCIARIDAE	3%	9%	12%	6%
Psocoptera		11%	11%	6%
LIMONIIDAE	8%	2%	10%	5%
Araneae	3%	7%	10%	5%
SCIRTIDAE	3%	4%	7%	4%
Hylaeus sp.	6%		6%	4%
PSYCHODIDAE	3%	3%	5%	3%
Chelipoda sp.		5%	5%	5%
Eucolaspis sp.	2%	2%	5%	2%
Hylaeus agilis	4%		4%	4%
Hydrellia tritici	1%	3%	4%	2%
Weinmannia racemosa				
Apis mellifera	5%	9%	14%	7%
Eucolaspis sp.	8%	3%	11%	6%
Hydrellia tritici	4%	4%	7%	4%
Leioproctus sp.	3%	3%	6%	3%
Araneae	2%	3%	6%	3%
Hoplocneme sp.	4%	2%	5%	3%
LIMONIIDAE	4%	2%	5%	3%
SPHAEROCERIDAE	1%	4%	5%	3%
Tetragoneura sp.	5%		5%	5%
CHIRONOMIDAE		4%	4%	2%
Technomyrmex jocosus	3%	1%	4%	2%

Indicator species analysis identified flower visitor species, shown in Table 4.6, that were significantly indicative of high and low hive density sites. Honey bees were a key indicator for communities sampled from *W. racemosa* at high hive density sites,

during both sampling years. In contrast, *Vespula vulgaris* were most indicative of *I. brexioides* flower visitor communities at high hive density sites during the first year of sampling, and Psocoptera were most indicative during the second year. No significant indicators were detected for low hive density sites for *I. brexioides* communities, and *Mesocyphon sp.* was the only significant indicator detected for low hive density communities sampled from *W. racemosa*.

Table 4.6: Significant ($p<0.05$) indicator species for flower visitors communities collected from I. brexioides *and* W. racemosa *at sites of high and low honey bee hive density between Nov-Dec 2016 (Y1) and Nov-Dec 2017 (Y2). A = specificity or positive predictive value of the functional groups as indicator of the site group. B=fidelity or sensitivity of the functional group as indicator of the target site group. Stat = indicator value. P value = probability of reaching as high indicator value over 999 iterations.*

Year	Hives	Indicator Species	A	B	Stat	*p* value
Ixerba brexioides						
Y1	High	*Vespula vulgaris*	1	0.571	0.756	0.019
Y2	High	Psocoptera	1	0.688	0.829	0.027
Weinmannia racemosa						
Y1	High	*Apis mellifera*	1	0.417	0.645	0.002
		Cerodontha sp.	1	0.250	0.500	0.034
		Dilophus tuthilli	1	0.333	0.577	0.009
		Eucolaspis sp.	0.799	0.917	0.856	0.001
		Halmus chalybeus	1	0.250	0.500	0.034
		SCIARIDAE	1	0.333	0.577	0.010
		Technomyrmex jocosus	1	0.500	0.707	0.001
Y1	Low	*Mesocyphon sp.*	1	0.333	0.577	0.047
Y2	High	*Apis mellifera*	1	0.600	0.775	0.001
		CERATOPOGONIDAE	0.933	0.400	0.611	0.018
		Hydrellia tritici	0.941	0.533	0.708	0.003
		ICHNEUMONIDAE	1	0.267	0.516	0.041
		Phytomyza sp.	1	0.333	0.577	0.015
		PSYCHODIDAE	1	0.267	0.516	0.045

4.3.3 Invertebrate flower visitor co-occurrence

The Veech (2013) method characterises species co-occurrence relationships as positive, negative, or random based on whether the observed frequency of co-occurrence is greater than expected, less than expected, or not significantly different than expected, respectively. Co-occurrence was analysed for flower-visitor communities from *I. brexioides* and *W. racemosa* over flowering periods in two years. On average 2 % of *W. racemosa* community associations were non-random, and all non-random associations were positive interactions. Communities from *I.*

brexioides had on average 1.1 % non-random associations, with 4 positive associations and 1 negative association. Honey bees had two significant associations with *Dilophus tuthilli* (p=0.007) and SCIARIDAE (p=0.007), which occurred more often than expected by chance. The invasive wasp *Vespula vulgaris* also had a positive interaction with the native bee *Hylaeus agilis* (p=0.05). Figure 4.5 shows heat maps of the interactions, with yellow squares representing negative co-occurrences, and blue representing positive co-occurrences.

4.3.4 Network analysis

Network indices at high and low hive density sites (Table 4.7) demonstrate that sites with low hive density have higher connectance and are on average more nested than sites with high hive density. Indices for sites of different pest management cannot be meaningfully interpreted because the fenced category includes only one site, where insects were collected from only *I. brexioides* flowers. T-tests indicated that both the high and low hive density networks were significantly more nested and less connected than expected by chance. Interaction strength asymmetry was also higher than expected by chance for both networks.

Table 4.7: Network indices for bipartite networks at sites categorised by hive density (L=low, H=high)

	Hives	
Network Indices	L	H
Connectance	0.28	0.14
Weighted connectance	0.07	0.05
Web asymmetry	0.94	0.86
Links per species	1.35	1.40
Compartments	1.00	1.00
Nestedness	25.66	11.11
Weighted nestedness	0.62	0.62
NODF	39.05	31.64
Weighted NODF	24.92	14.06
Interaction strength asymmetry	0.68	0.61
Linkage density	0.62	0.62

High and low hive density communities had similar numbers of total species, and non-native species (Table 4.8), but high-density communities showed a greater proportion of interactions by non-native species. Interactions with honey bees were rarely observed outside of the high-density sites.

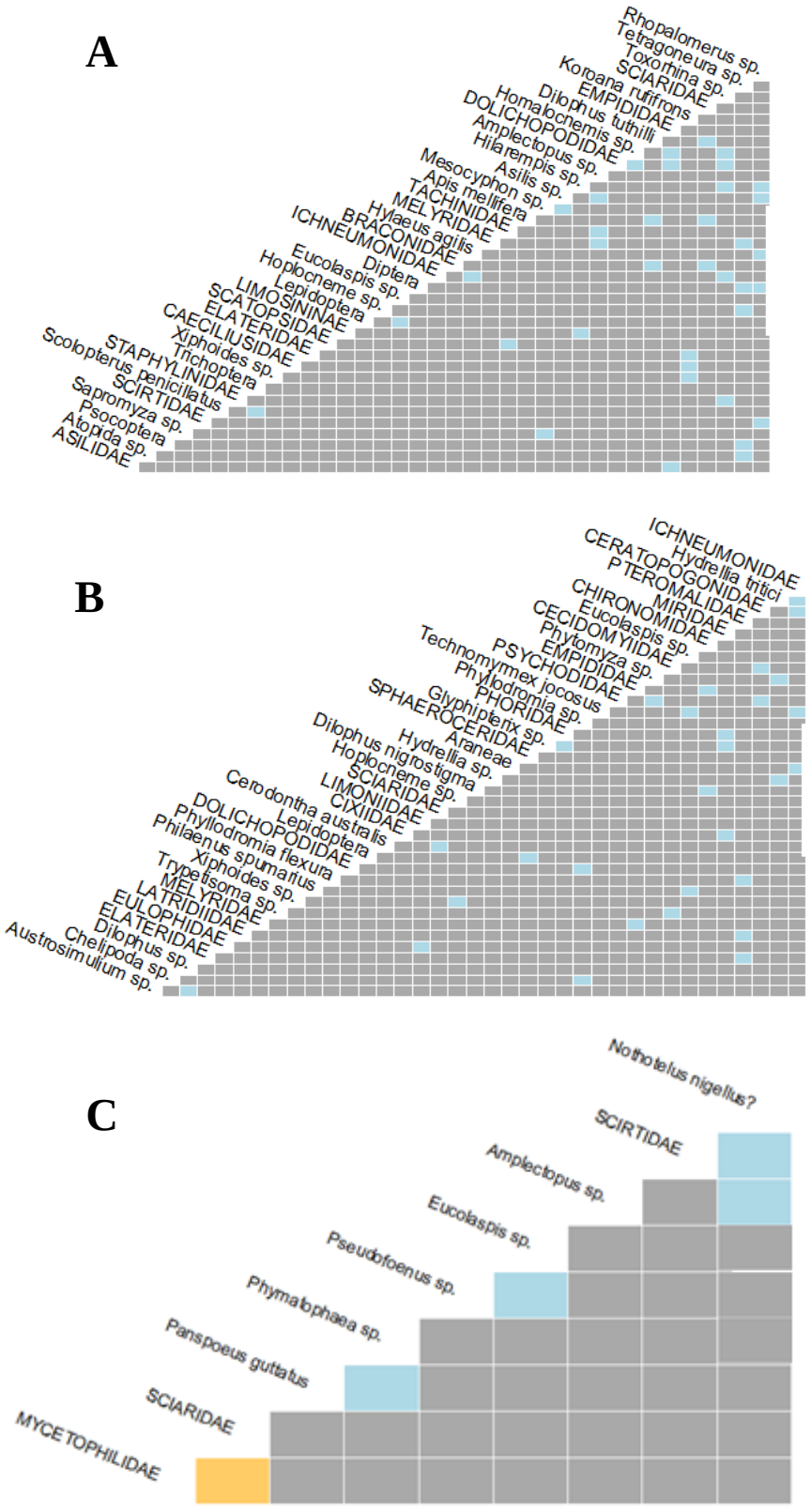

Figure 4.5: Heat map of invertebrate flower visitor co-occurrence generated using function 'cooccur' in the 'vegan' package in R studio. Interactions are classed as positive (blue) if occurring more frequently than expected by chance, negative (yellow) if occurring less frequently than expected by chance, or random (grey). Graphs represent flower visitor communities collected from A) W. racemosa *over November to December 2016; B)* W. racemosa *over November to December 2016; and C)* I. brexioides *over November to December 2017. There were no significant interactions for* I. brexioides *for November to December 2016.*

Table 4.8: Invertebrate flower visitor community species and interactions by native and non-native species for sites categorised by hive density (L=low, H=high)

	L	H
Total Species	153	160
Non-Native species	16	17
% Non-Native	10	11
Total interactions	753	793
Non-Native interactions	54	145
% Non-Native	7	18
Honey bee interactions	1	65
% Honey bee interactions	0	8

Species level indices (Table 4.9) identify honey bees as high positive contributors to nestedness in the network at the high hive density site, and negative contributors at the low hive density site. Pollination support index (PSI) (Dormann 2011) is a measure of how important a pollinator is to the plants in the network based on whether it is common and specialised and is a product of the dependences of the pollinator and the plant. It is calculated by the following equation where p_{ji} is the dependence of pollinator j on visits to plant species i. The exponent β adjusts for the number of visits required for pollination but is usually set to β=1 as this data is difficult to source.

$$PSI_j = \sum_i (p_{ij} \cdot p_{ji}^{\beta})$$

Values range from 0 to 1, with 1 indicating a high level of usefulness. PSI and species strength both indicate a greater importance of honey bees at high hive density sites than at low hive density sites. Specialisation measures showed a higher degree of specialisation for honey bees than expected, demonstrated by low normalised degree, and low betweenness and closeness centrality.

Table 4.9: Species level indices for honey bees in communities categorised by hive density within 5 km

	Low	High
Nested contribution	-0.17	1.32
PSI	0.01	0.72
Normalised degree	0.20	0.45
Betweenness	0.00	0.03
Closeness	0.01	0.01
Species strength	0.01	1.33
Interaction push pull	-0.99	0.07
Species specificity index	0.01	0.72

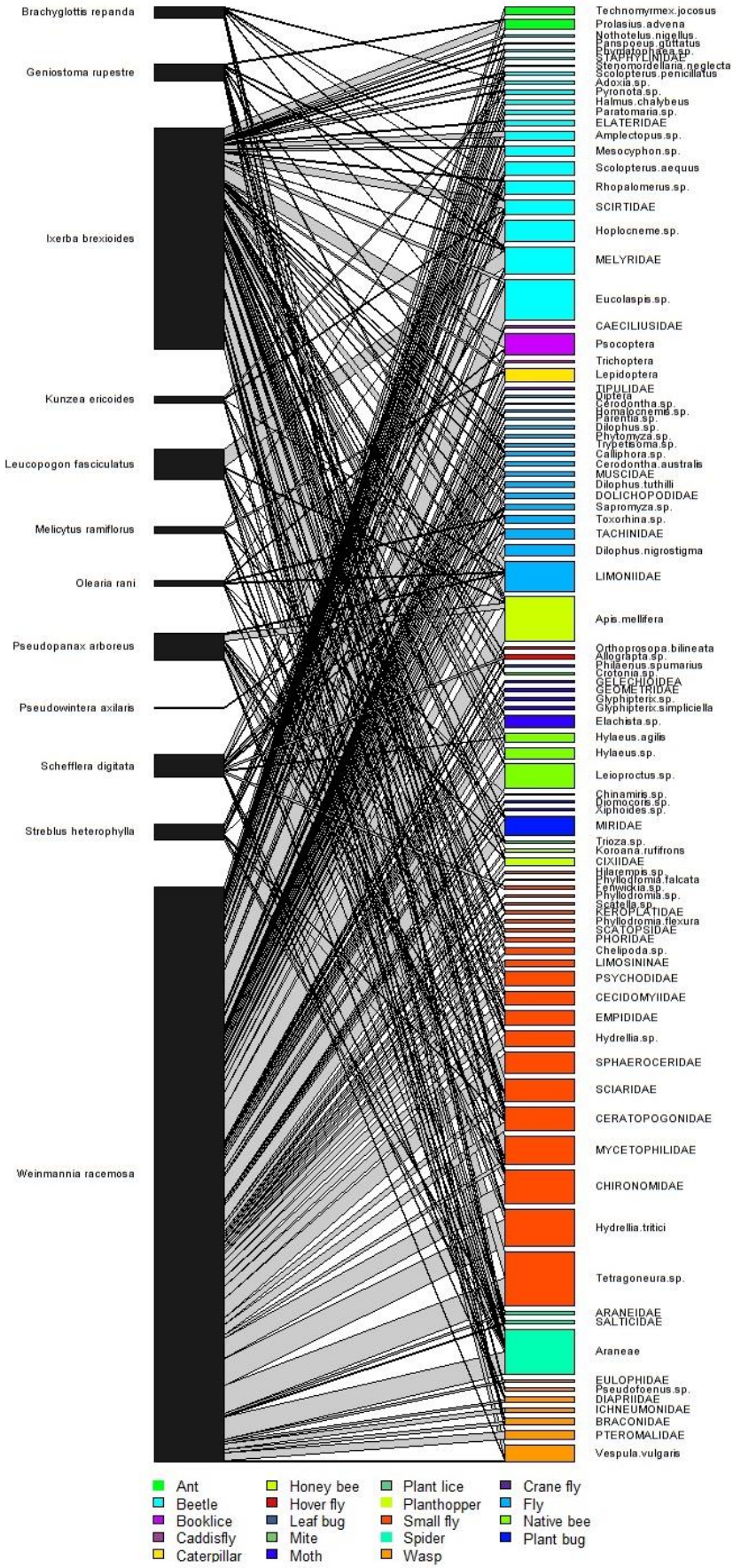

Figure 4.6: Interaction web for insect flower visitors and plants collected from a range of tree species in the Kaimai-Mamaku Range (interactions occurring more than twice are depicted for simplicity and colour-coded by flower visitor functional group)

4.4 Discussion and Conclusions

Results from the range of analyses conducted highlight four key ways in which high densities of managed honey bee hives can affect communities of invertebrate flower visitors: patterns of diversity, species co-occurrence, community composition, and network structure and interactions. We found lower diversity of flower visiting species at sites that had a high density of honey bee hives and statistically significant differences in community composition and relative abundance between sites with high and low honey bee hive density. In addition, we identified that the main species contributing to the differences between the high- and low-density sites was the honey bee, which also acted as a key indicator species for the high hive density community. Sites with a high density of honey bee hives demonstrated effects on network structure in terms of several key network properties: connectance, nestedness, and species-level indices. These findings are discussed in relation to the relevant literature in the following paragraphs.

4.4.1 Impacts of introduced honey bees on diversity of invertebrate flower visitors

This study identified lower diversity of flower visiting species at sites that had a high density of honey bee hives. This finding is consistent with several earlier studies. Badano & Vergara (2011) highlighted declines in native pollinator diversity with increasing honey bee numbers, which had downstream effects on productivity of coffee plantations, and several other studies have reported declines in flower visitation by native species as honey bee hive numbers increase (Wenner & Thorp 1994; Kato et al. 1999; Roubik & Wolda 2001; Hansen et al. 2002; Kato & Kawakita 2004). Indeed, biotic interactions, such as between competing pollinators, have been shown in some cases to be more important drivers of ecological community structure than environmental conditions (Ohlmann et al. 2018).

These observations are by no means conclusive. Though they indicate a negative relationship between honey bee hive density and flower visitor diversity, longer term studies and monitoring of invertebrate populations are required to elucidate whether changes in diversity are the result of population level decline, seasonal trends, or other community responses to change, such as resource partitioning. Clear experimental evidence of honey bees affecting fecundity and population dynamics of native pollinators is rare (Thomson 2004), and studies often cannot demonstrate long-term population pressure (Goulson 2003), with the exception of effects within the *Apis* genus due to pathogen sharing (Moritz & Hartel 2005).

However, this research offers evidence of ideas that have been raised and reviewed in New Zealand for decades (Donovan 1980; Butz Huryn 1997; Howlett & Donovan 2010); that honey bees are a potential threat to biodiversity of native pollinators and should be managed appropriately.

Landscape mapping also allowed a further comparison on matrix land use, identifying lower diversity in sites where native forest comprised less than 50 % of the surrounding land within a 5 km radius. This finding has been echoed in other regions of New Zealand (Macdonald et al. 2018; Stavert et al. 2018) as well as other regions around the world as the ecological impacts of agricultural intensification are becoming better understood (Steffan-Dewenter & Tscharntke 1999; Gallai et al. 2009; Jauker et al. 2009; Decourtye et al. 2010; Woodcock et al. 2014). In many places this agricultural intensification and its downstream effects on biodiversity are linked to the on-going issue of habitat fragmentation. Fragmentation has been shown to improve accessibility of forage by honey bees, and to reduce flower visitation and native bee foraging (Aizen & Feinsinger 1994; Dick 2001). In addition, one of the sites that was included in the study had pasture as the main surrounding land use but was also part of a fenced and fully predator-proof ecological sanctuary, a site of intense restoration and conservation activities. Though the diversity of nectar-feeding bird visitors was markedly higher at this site, diversity of invertebrate pollinators was still low compared to the low hive density sites that had native forest as their main matrix constituent. This finding could indicate that pest-proof fencing does not necessarily improve diversity of invertebrate flower visitors, particularly if the surrounding land matrix is not intact.

4.4.2 Impacts on species co-occurrence and community composition

Invertebrate communities in this research displayed a higher rate of positive associations than negative. This could indicate that competitive pressure is not a strong factor affecting community assembly of invertebrate flower visitors at our sites. Honey bees demonstrated positive association with *Dilophus tuthilli* and SCIARIDAE, indicating that this co-occurrence happened more often than expected by chance. Negative associations are generally associated with competition (Hausdorf & Hennig 2007), though there is theoretical and experimental research to suggest that this link has been overestimated (Hastings 1987; Brazeau & Schamp 2019). Gross (2001) demonstrated a negative correlation

between native bee presence and honey bee presence at *Dillwynia juniperina*, an Australian native bush.

Multivariate analysis highlighted statistically significant differences in community composition and relative abundance between sites with high and low honey bee hive density, particularly for *W. racemosa* flower visitor communities. In addition, we identified that the main species contributing to the differences between the high- and low-density sites was the honey bee, which also acted as a key indicator species for the high hive density community. There are several ways in which honey bees can either directly or indirectly affect other flower visitors in their communities to affect community composition. The most obvious is competition for floral resources. Honey bees shared interactions with 33% of native plant species visited by native bees, and 80-100% of plant species visited by native wasps and soft-wing flower beetles. This overlap in resource preference can lead to compositional community changes, depending on the level of resource availability, the level of resource depletion following visitation, and the comparative efficiency in foraging by competitors (Stout & Morales 2009).

Other potential direct affects that have been observed for invasive pollinators in the literature include competition for nesting sites (Saunders 1979), pathogen transmission through pollen transfer or shared use of flowers (Durrer & Schmid-Hempel 1994; Singh et al. 2010), and reproductive disruption via mating with con-generics (Kanbe et al. 2008). Nesting considerations are not relevant in New Zealand due to the predominantly solitary and ground nesting strategies of New Zealand bees (Donovan 2007), and relatively little or no persistence of wild honey bee populations in native forest since the introduction of *Varroa destructor* around the year 2000 (Beard 2015). Reproductive disruption is, likewise, unlikely to be an issue in New Zealand as there are no native invertebrates that are closely related enough to honey bees to allow hybridisation. However, pathogen transmission is a real threat, highlighted by recent research demonstrating the transmission of honey bee diseases, black queen cell virus, sacbrood virus and deformed wing virus to hoverflies of the genus *Eristalis* (Bailes et al. 2018). This represents an area requiring future research for New Zealand invertebrates.

4.4.3 Impacts of honey bee introduction on network structure and interactions

Sites with a high density of honey bee hives demonstrated effects on network structure in terms of a few key network properties: connectance, nestedness, and

species-level indices. In contrast to trends reported in recent studies (Santos et al. 2012; Traveset et al. 2013; Norfolk et al. 2018), plant-pollinator networks in the Kaimai-Mamaku range had higher connectance in sites that were uninvaded by honey bees. Invasion of pollinators such as honey bees generally increases connectance because of their generalist strategy, resulting in more links within the network. In our research, lower connectance in high hive density sites could be a result of honey bees working fewer plant species, focusing on the honey producers; or it could indicate displacement other species that have the potential to make more connections. Co-occurrence analysis did not detect any significant negative associations between honey bees and other flower visitors, however. Higher connectance at low hive density sites indicates greater network complexity (Landi et al. 2018). Allesina & Tang (2012) indicate that connectivity in a network has a negative relationship to network stability and decreases resilience to extinction (Vieira & Almeida-Neto 2015). However, other studies demonstrate the opposite effect (Okuyama & Holland 2008; Thébault & Fontaine 2010).

Nestedness and modularity are two key attributes of network architecture that affect the functioning of ecological networks. Though modularity is a more common feature of food webs, larger and more complex mutualistic networks do demonstrate modularity (Olesen et al. 2007). The networks observed in the current research were of a relatively small size, and exhibited no evidence of modularity, which is thought to be a good for network persistence and resilience in mutualistic networks (Thébault & Fontaine 2010). Invasion generally increases nestedness as invasive species tend to interact largely with generalists (Traveset et al. 2013). However, our networks demonstrated higher nestedness at sites with low honey bee hive density. Song et al. (2017) suggested that more nested networks were associated with higher temperature seasonality and that greater nestedness can increase the range of environmental conditions the network can be compatible with. Nestedness can also be more common in environments subject to environmental perturbations. Though extreme nestedness can have negative effects on network stability, facilitating extinction cascades (Campbell et al. 2012), some level of nestedness generally indicates greater stability, and robustness to extinction (Aizen et al. 2008).

Different species contribute to community nestedness to different extents. In the low hive density sites, high contributors to nestedness include the native beetles

Eucolaspis and *Navomorpha sulcata,* as well as a native fungus gnat *Tetragoneura.* These high contributors are important for the persistence of the network, but are also more prone to extinction (Saavedra et al. 2011). Honey bees were positive contributors to nestedness for the high hive density network and were negative contributors in the low hive density network. This indicates that they make an important contribution to network robustness in the high hive density area, but not the low hive density area.

The most important flower visitors in terms of PSI in low density networks were CHIRONOMIDAE (native midge, PSI=0.38) and *Pyronata* sp. (native beetle, PSI=0.29), though PSI values were generally low in this network. In contrast, analysis identified *Sapromyza sp*. (native fly, PSI=0.96), and *Apis mellifera* (honey bee, PSI=0.72) as important pollinators in high density sites. It is not clear at what point these introduced flower visitors, such as honey bees, become important, and future research using a gradient-based approach to sites based on hive density is recommended.

Networks in the Kaimai Mamaku range showed varying degrees of mutualism strength and symmetry that can give us an indication of the extent of invasion of invasive pollinators, such as the honey bee. Successful invasion often results in asymmetric interactions, where one species is more dependent on the other (Aizen et al. 2008). Honey bees had a positive interaction push/pull metric in high density sites (0.07), but a negative metric in low density sites (-0.99). High hive density sites were the only sites where this positive interaction metric was observed. This indicates that at high density sites honey bees exhibit an effect on their interaction partners, without reciprocal effects, while at other sites honey bees are affected by their interactions partners without having a strong effect on them (Vázquez et al. 2007). This suggests that invasion success and impacts on network structure are density dependent (Vázquez et al. 2007; Giannini et al. 2015) and supports efforts to manage rising numbers of managed invertebrate flower visitors on conservation land (Department of Conservation 2015b).

In addition to affecting network structure, honey bee hive density affected the type of interactions observed in flower-visitor networks. Honey bees were disproportionately responsible for a large proportion of non-native interactions at sites with high hive density. Non-native species accounted for 11% and 10% of species in high- and low-density sites respectively but constituted 18 % of

interactions in high density sites and 7 % of interactions in low density sites. Honey bees accounted for 45 % of non-native interactions in the high-density network, and 2 % of non-native interactions in the low-density network. This is consistent with findings from Traveset et al. (2013) and highlights the reality of the threat of invasive pollinators displacing native flower visitors.

Using network metrics gives us a way to look at ecological communities to assess their structure, the roles of different species within the community and the response of the community to perturbation (Bascompte & Jordano 2007; Bascompte 2009). The analyses are relatively easy to perform using platforms such as R, and the literature on their use continues to grow. However, the nature of the models means they may be sensitive to differences in the data used to calculate the metrics, such as the number of species, and interactions in the matrix. Nestedness, in particular, is highly affected by single observations of an interaction, and by the overall balance of species in different trophic levels (Dormann et al. 2009). Interaction strength asymmetry is also affected by whether there are more plants or flower visitors in the network (Dormann et al. 2009). This presents some limitations to conclusions drawn from network metrics. In addition, conclusions from this research are limited by the number of sites in each category and the detail revealed from the insect collection method. Smaller numbers of sites were selected in favour of depth of sampling at each site. In addition, sweep netting at flowering trees provided a limited snap shot in time of interactions between flower visitors and trees. Future research has been designed to improve this method and identify a greater number of interactions. This will be accomplished by identifying plant species using pollen identification from grains adhering to flower visitor bodies. See Chapter 5 for a literature review and discussion concerning this approach.

4.4.4 Management

The aim of this chapter was to investigate how introduction of managed honey bee hives can affect invertebrate flower visitors in submontane forest in the Kaimai-Mamaku range. The analysis and results in this chapter provide evidence that honey bees do affect invertebrate flower visitor communities in several ways, including effects on diversity, network structure, species co-occurrence and community composition. In order to maintain sustainable native ecosystems, we must be aware of the impacts of large-scale, managed introductions of non-native species in order to regulate them appropriately.

Management strategies for apiaries in native ecosystems range from complete eradication and exclusion (Wenner et al. 2009) to, more commonly, systems for limiting hive stocking (Department of Conservation 2015a; Department of Conservation 2015b). Increasingly, research seeks to inform and improve stocking rate management by budgeting resource production and allocation across landscape scales (Paton 1990; Dicks et al. 2015; Arundel et al. 2016; Ausseil et al. 2018). Cane & Tepedino (2017) recently presented a metric for gauging the impact of honey bee stocking rates on communities of native bees. This uses estimates of pollen collection by honey bee hives converted to equivalent numbers of native bee progeny, used in concert with walking bee surveys across apiary sites. However, application in New Zealand would require research into resource collection by native bees and quantification of reproductive output, as well as training and monitoring resources for beekeepers. While this is a step in the right direction toward monitoring actual population level effects on native bees, as this study has shown, the extent of native flower visitor fauna that can be impacted by apiaries extends beyond native bees alone.

4.4.5 Summary and directions for further research

This research provides some evidence that invasive pollinators can affect structure and function of plant-pollinator networks in native environments, and that the magnitude of these effects corresponds to invasive species abundance. Prevention of permanent changes to flower visitor communities should be prioritised by preserving large areas of intact native forest where low levels of fragmentation create refuges for native flower visitors. As it stands, this research represents an observational approach to considering the effects of honey bee introduction on native flower visitor communities. However, further research is required to experimentally test these findings, as well as other potential impacts which have been identified in the literature. For example, experiments should include investigation into pathogen transfer to native bees, quantification of resource depletion by honey bees on New Zealand native plants, and effects of current advised hive stocking rates on population dynamics and fecundity of native flower visitors. Future research should also address the response of plant-pollinator networks to restoration and removal of invasive species to inform management strategies and improve sustainability of native flower visitor communities in native ecosystems.

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Chapter 5

Method comparison for identifying and understanding plant-pollinator interactions

Abstract

Species interactions are critical to ecosystem structure and function, but new developments are evolving rapidly, making choice of methods complex. Traditional methods of studying plant-pollinator interactions, including field observations and more detailed microscopy surveys of pollen adhering to flower visitors, remain as important methods of ecological research, but have recognised constraints. DNA barcoding tools present a potential to improve identification accuracy, standardisation and efficiency. Despite leaps in progress with these methods, applying them to detailed plant-pollinator network analysis is not yet fully operationalised and no such analyses have yet been completed in New Zealand. This chapter presents the results of a pilot study of pollen collection and identification and a literature review on comparative molecular tools for pollen identification. Traditional and DNA-based methods for identifying and understanding plant-pollinator interactions are compared to inform future research in the species interaction space. Literature suggests one barrier to adoption of molecular tools is a general lack of agreement in DNA extraction, amplification and sequencing protocols that prevent research comparison. A cohesive approach to pollination network research in New Zealand is needed to align research and create a space for comparison that will be useful for conservation and restoration management in natural environments and urban spaces.

5.1 Introduction

Understanding species interactions is a key part of managing global change (Tylianakis et al. 2008). In particular, plant-pollinator interactions have been a focus of attention in global research as apparent declines in plant pollinator interactions threaten agriculture and food production (Bartomeus et al. 2018). Network analysis maps species interactions to put them into a broader community context, providing a way to track changes in the structure of communities, the kinds of interactions that are occurring and the frequency of occurrence (Bascompte

2009). In this way, network indices can act as indicators of conservation success and offer a unique way to monitor conservation efforts with a community level approach (Kaiser-Bunbury & Blüthgen 2015; Kaiser-Bunbury et al. 2017). In addition, network analysis can identify key functional network participants, focusing efforts for preservation of key species (Memmott et al. 2004), and preventing erosion of species interactions following invasion (Aizen et al. 2008). Plant-pollinator networks also underpin key ecosystem services with value in agriculture, conservation, and even home gardening, informing policy aimed at valuing ecosystem services for a more comprehensive approach to ecosystem management (Bascompte 2009).

The study of network interactions is not new, with concepts dating as far back as the seventeenth and eighteenth centuries (Egerton 2007). But since that time tools for analysing species interactions have developed considerably (Pavlopoulos et al. 2008; Bell et al. 2017; Pornon et al. 2017). Originally starting as hand drawn maps of interactions and descriptions of who ate whom (Darwin 1859), statistical tools now allow the computation of indices to quantify structure of interaction networks, strength of interactions, and roles of key species within those networks. Analysis of large community interaction matrices can now be done relatively simply in statistical software such as R (RStudio Team 2018), where specific packages have been designed for analysis of bipartite interactions, such as plant-pollinator networks (Dormann et al. 2009; Dormann et al. 2018). Methods of visualising network interactions to analyse species interactions have also developed considerably. Programs such as Medusa (Hooper & Bork 2005), Osprey (Breitkreutz et al. 2003), Cytoscape (Doncheva et al. 2012), and Network3D (Williams 2010) can produce 2D or 3D representations of species interactions for visualising complex communities, allowing changes in network structure over time or disturbance to be more easily discerned.

Though these modern computation tools allow for streamlined analysis of species interactions, they are still limited by input data. Techniques for sampling plant-pollinator interactions can be expensive and time-consuming, requiring multiple repeat samples as observed interactions can change significantly over time, space, and climatic conditions (Hegland et al. 2010). In addition, current methods of studying plant-pollinator networks also rely on morphological identification of pollen and flower visitors (Kraaijeveld et al. 2015), which can also be time

consuming and expensive activities. Accuracy of identifications can also vary widely depending on the skill level of the observer, paired with the available time and resources (Cranston & Hillman 1992; New 1996). In addition, specimen quality can affect identification accuracy, with cryptic species, immature life stages, and specimen damage posing difficulties for taxonomic identifications (Armstrong & Ball 2005). Overall, the lack of standardisation in data collection and identification limits accurate reflections of species interaction networks (Wilkie et al. 2003).

Molecular methods, however, have the potential to further improve research on species interactions through increasing standardisation, accuracy and efficiency. Molecular techniques make it possible to standardise the identification of plant-pollinator interactions by removing human error and standardisation issues inherent in traditional methods due to variance in skill level and experience of identifiers (Macgregor et al. 2018). Pornon et al. (2016) found that applying DNA barcoding methods to pollination research revealed 2.5 times more plant species involved in plant-pollinator interactions than traditional observational approaches. This was linked to difficulties in identifying cryptic species on densely populated slides, potential inflation of interactions from metabarcoding and increased sensitivity of barcoding methods. In addition, specificity of identification for network participants is often limited to genus or family levels in traditional methods, whereas species specific identification can be possible in more than 90% of samples when DNA barcoding techniques are used (Hawkins et al. 2015; Sickel et al. 2015). Because of the level of detail that can be revealed through DNA barcoding techniques it is now possible to see the history of pollinator visitation throughout the day, plant parentage, pollination and dispersal distances, and pollination efficiency by using DNA to highlight genetic differences between pollen samples (Matsuki et al. 2008; Hasegawa et al. 2009; Ashley 2010; Isagi 2011).

One barrier to wide-spread adoption of DNA techniques in the study of plant-pollinator interactions is a general lack of agreement in DNA extraction, amplification and sequencing protocols that prevent research comparison, and make it difficult for community ecologists to adopt new methods. A cohesive and simple approach to applying molecular tools to allow comparisons that will be useful for conservation and restoration management in natural environments and urban spaces. This chapter aims to review molecular methods currently used to study

plant-pollinator interactions and compare these to traditional methods to provide recommendations on future research approaches.

5.2 Methods

5.3.1 Literature review

A literature review was conducted using Web of Science and Google Scholar, with the search terms 'pollen' and 'DNA barcoding'. Papers for review were selected where DNA was extracted from pollen grains for DNA barcoding, regardless of sample type or intended application, and where detailed methods were provided in the text. This uncovered 12 papers, ranging from descriptions of protocols for DNA extraction from single pollen grains, honey samples, pollen-bearing plant structures, and insect collected pollen. Each paper was reviewed to compare the DNA extraction protocols, including use of beads for mechanical pollen disruption, wash buffer constituents, incubation and centrifugation, DNA extraction kits, primers, PCR cycles and resulting specificity.

5.3.2 Pilot study

A pilot study was conducted to test methods and feasibility of identifying plant-pollinator interactions using DNA based methods and to compare these with field observation and microscopy. Insects were collected at five sites in the Kaimai-Mamaku Range, a large tract of native forest in the central North Island of New Zealand (Department of Conservation 2006). Maungatautari is an additional site, close to the Kaimai-Mamaku range, and is a unique site, being free from mammalian predators (except mice), and surrounded by a predator-proof fence (Sanctuary Mountain 2018). Sites were selected based on accessibility, proximity to honey bee hives, and presence or absence of target tree species – *Weinmannia racemosa* and *Ixerba brexioides*.

Table 5.1 shows categorisation of sites based on hive density within 5 km, as well as pest management and disturbance of the surrounding land matrix. Hive density categories were assigned based on 2016 hive numbers and categories were maintained for 2017. Hive density categorisation presented challenges due to the difficulty of obtaining data on the density of existing hives. The data is kept by AsureQuality and though it can be made available for research purposes, the process took considerable time between request and receipt of data (over 12 months) and the level of detail available was low. Pest management categories separated the Maungatautari site from other sites because of its unique situation as a pest-proof

sanctuary. Fragmentation of the surrounding land matrix was determined using ArcGIS 10.5.1 2017 to analyse land use in the land matrix within a buffer radius of 5 km (Landcare Research 2015). Sites were categorised as 'fragmented' if native forest cover was less than 50 % of the surrounding land matrix within a 5 km radius.

Table 5.1: Details of study sites including latitude and longitude, altitude (m), hive numbers found within a 5 km radius of the sites in 2016 and 2017, hive density category (L=low, H=high), pest management status whether unmanaged (U) or within a pest-proof fenced sanctuary (F), and disturbance level whether fragmented (F) or unfragmented (U).

Site	Lat.	Lon.	Alt.	2016	2017	Hives	Pests	Matrix
Woods Mill	-38.03	175.98	500	140	150	L	U	U
North-South	-37.87	175.93	500	374	-	H	U	D
Mokaihaha	-38.18	176.10	600	97	395	L	U	U
Tuahu	-37.60	175.86	400	822	1444	H	U	U
Maungatautari	-38.01	175.58	500	-	431	H	F	F

Invertebrate flower visitors were collected by sweep netting flowering trees for 10-minute intervals at four times each day (8 am, 12 pm, 4 pm, and 8 pm) during November to December in two sampling years. Collected invertebrates were stored in individual tubes and frozen. More than 1600 flower visitors were collected from the five sites across 117 sampling periods. Sampling was not undertaken in rainy conditions.

Table 5.2: Specificity of invertebrate identifications

Specificity	Samples	Percent
Order level	1628	100%
Family level	1487	91%
Genus level	542	33%
Species level	364	22%

Identification of insect samples was contracted to an expert invertebrate taxonomist. Identification of approximately 1600 specimens cost $3,000 for labour costs, with hosting, travel costs, and lab space hire adding an additional $2,000 of costs. Care had to be taken to avoid contamination or loss of the pollen on the specimens, so identification had to be non-destructive. For 1628 samples, 91 % were identifiable to family level, 33 % to genus level and 22 % to species level (Table 5.2). Some difficulties were encountered due to the level of care required to preserve pollen

samples, damaged invertebrate specimens, and lack of information on New Zealand invertebrates.

Pollen preparation and identification

Sixty-six invertebrate flower visitors were randomly selected from the pool of 1600 collected insects (see Table 5.4). Pollen was removed from the surface of each insect individually using a distilled water wash. Flower visitors were examined under a dissection microscope before and after washing and pollen was scored to ensure wash efficiency. Pollen scoring identified approximate numbers of pollen grains, as shown in Table 5.3, for mouthparts, wings, legs and dorsal and ventral head, thorax, and abdomen. Water was added to cover the sample, which was then vortexed for one minute. The insect was then removed and (if applicable) forceps were used to remove the elytra (wing coverings) and extend the wings. The wash process was then repeated, and the insect was examined to ensure pollen removal, and then transferred to a new vial and stored at -20 ˚C. The pollen wash liquid was centrifuged, the supernatant was then removed and the pollen pellet was stored at -20 ˚C. Pollen samples were prepared in a room that was clean, regularly decontaminated and physically separated from post-PCR work to prevent contamination of samples from environmental pollen (Pornon et al. 2016).

Table 5.3: Ranges for scoring adherent pollen

Score	Pollen Grains
1	<1
2	1
3	10
4	100
5	10,000
6	1,000,000

Acetolysis was used to prepare pollen samples for identification. This process uses chemical treatments to remove the outer layer of pollen grains, exposing the underlying architecture and making identification easier. Each sample was initially washed with glacial acetic acid, after which a mixture of acetic anhydride and sulfuric acid were added under a fume hood. The sample was then added to a water bath until digested (up to 25 minutes). A further glacial acetic acid wash after acetolysis was done to stop the reaction, and then a series of water rinses were

carried out. The protocol from Jones (2014) was followed (see Appendix for detailed steps). A combination of sample preparation methods was tested, including performing acetolysis on pollen samples washed from insect surfaces, and performing acetolysis on whole flower visitors after removal of external adherent pollen. A digestion method was designed to allow comparison of pollen from internal tissues with those apparent on external surfaces. Digested samples were filtered using 90-micron nylon mesh to remove undigested fragments of insect tissue. Samples of acetolysed pollen were set onto microscope slides in 10 and 20 µl aliquots, stained with Safranin O and fixed with a glycerine jelly preparation. The process of acetolysis and slide preparation took approximately 15 minutes per sample. An empty sample tube was included as a negative control to account for between-sample contamination. A second control came from a short length of cellulose tape left sticky side up on the lab workspace overnight, to account for environmental contamination.

Table 5.4: Sample numbers for pilot study of pollen identification from washed adherent pollen, and digested washed insects

	Samples	Wash	Digest	Control
Ant	1	1		
Beetle	14	11	10	
Cello tape	4			4
Crane Fly	5	4	4	
Fly	13	10	11	
Midge	13	3	13	
Moth	2	2	1	
Native Bee	8	6	6	
Nothing	4			4
Small Fly	4	3	4	
Spider	4	3	2	
Wasp	2	2	2	
	74	45	53	8

Identification of pollen types was carried out to the highest achievable taxonomic resolution using a compound microscope and pollen reference guide from Moar (1993). If no pollen was identified from the 10 µl aliquot, the 20 µl aliquot was also examined. Identification time was dependent on the density of pollen in the sample, and the ease of species identification, but generally decreased with practise. Identification required an average of 25 - 50 minutes per sample, however additional expert advice was required for species which were difficult to identify.

5.3. Pilot study results

The wash protocol was generally efficient at removing adherent pollen, with only four out of fifty specimens requiring a second and third wash. Native bees and large pollinating flies were the only flower visitor groups to require a second wash. This was particularly an issue where pollen packing had occurred on bee leg segments and required the legs to be removed and added to the external pollen sample. Overall, native bees, flies and wasps carried the most pollen. Spiders, small flies, ants and moths carried the least (see Figure 5.1). Pollen was generally most abundant on the abdomen, head and thorax, followed by legs and wings.

Figure 5.1: Average pollen scores across body parts before wash treatments for eight flower visitor groups, and an overall average. Error bars represent 95 % confidence intervals.

Flower visitors were observed in the field interacting on one to two different tree species. In contrast, 57 different types of pollen were identified from insect-carried pollen loads after acetolysis and microscope identification (see Figure 5.2). Flies and native bees carried among the most diverse pollen loads, with 23 and 14 types identified respectively. Ants and moths carried the least diverse pollen loads.

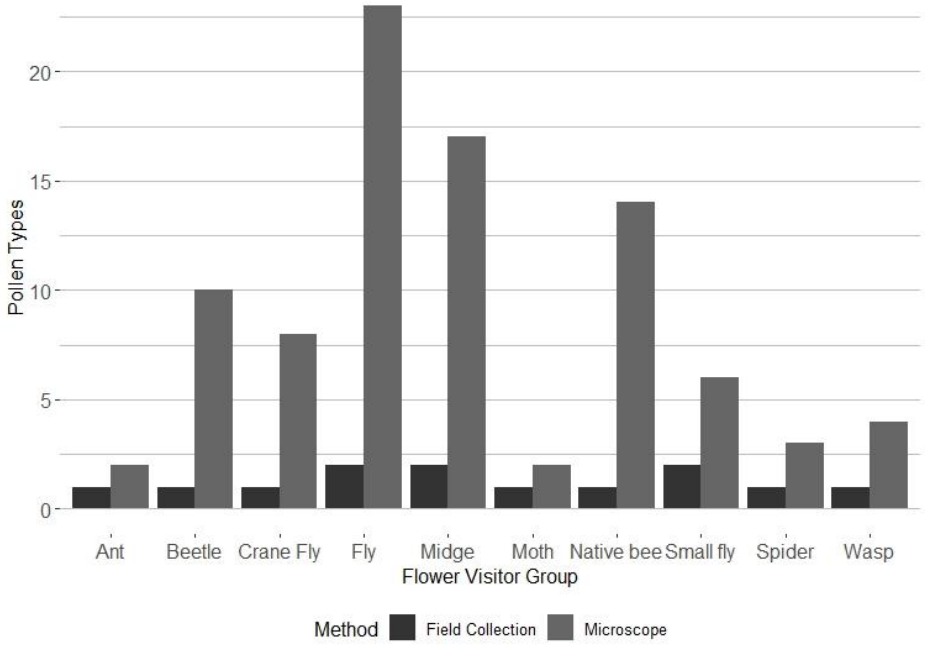

Figure 5.2: Comparison of the number of pollen species identified by field collection and microscopic methods

Differences in the composition of the pollen that was collected from flower visitor exteriors and internal digestions were identified using wash and digest treatments (Figure 5.3). Overall, 31.5 % of pollen species were found on flower visitors both externally and internally, 41.5 % were found only exterior and 27 % were found only interior. Most flower visitor groups had a mixture of pollen species that were exterior only, interior only, and shared between the interior and exterior. However, pollen was only found on the exterior of ants, and wasps had no pollen unique to the exterior surface.

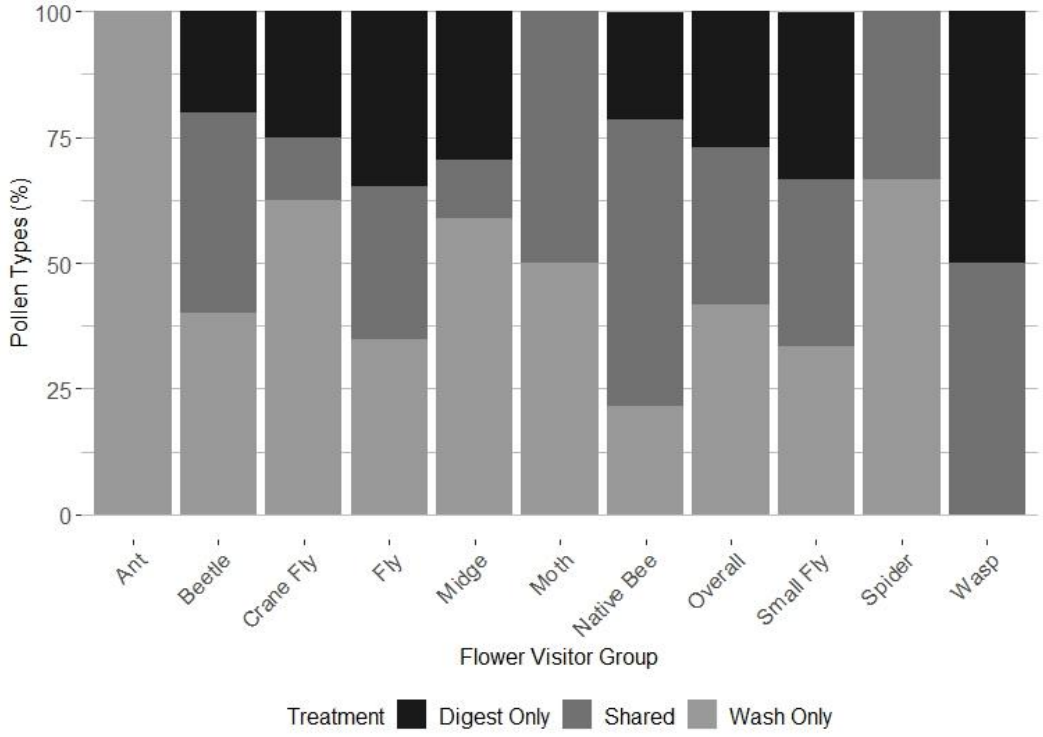

Figure 5.3: Percentage of pollen species removed from invertebrate flower-visitors using wash and digestion techniques

5.4 Literature review results

The following sections compare the methods discussed in the reviewed literature with reference to key methodological parameters. A summary is given in Table 5.5.

5.2.1 Sampling

Analysis of DNA from pollen has been undertaken using a number of sampling approaches and preparations depending on the application of the data. DNA extraction of pollen from honey samples has enabled identification of plant constituents of multi-floral honeys (Lalhmangaihi et al. 2014; Hawkins et al. 2015; Torricelli et al. 2016) but the method requires special attention to avoid problems from the high carbohydrate content of the honey. Other approaches have looked at airborne pollen for applications in allergy studies (Kraaijeveld et al. 2015), fossil pollen from soil cores to assess historical vegetation (Suyama et al. 1996), or behaviour of pollen-collecting insects (Sickel et al. 2015; Park & Nieh 2017). Few studies have used DNA surveys of pollen for analysing plant-pollinator interactions, but those that do also use a range of sampling methods. Bell et al. (2017) used pollen extracted from bees, Widmer et al. (2000) used pollen directly from orchid pollenaria collected off fresh bees or bees from preserved insect collections, and Matsuki et al. (2008) used single pollen grains adherent to insects.

5.2.2 Kits

A range of DNA extraction kits are recommended for extraction of DNA from pollen, most commonly, the DNeasy Plant kit (Qiagen) (Galimberti et al. 2014; Hawkins et al. 2015; Park & Nieh 2017), followed by the Macherey-Nagel Food Kit (Macherey-Nagel) (Sickel et al. 2015; Bell et al. 2017). Both kits make the extraction process straightforward and are designed to produce small amounts of pure DNA for PCR purposes. Users report ease of use for the DNeasy Plant kit, but with often small yields and difficulty in working with tissues high in cellulose and pectin, making sample preparation important (Daudi 2009).

5.2.3 Mechanical pollen disruption

Pollen grains are often surrounded by a thick and hardy coating which can make DNA extraction difficult using traditional methods. Using beads for sample grinding can be an effective way minimise the effect of high concentration polysaccharides and ensure that pollen coats are sufficiently disrupted for effective DNA extraction (Lalhmangaihi et al. 2014; Soares et al. 2015). Other methods suggest mechanical disruption should be avoided to prevent mechanical DNA fragmentation (Torricelli et al. 2016). The majority of studies assessed in this review used standard extraction protocols without bead treatment but were still able to extract DNA from pollen.

5.2.4 Potential barcodes

ITS and rbcL were the most commonly recommended barcodes for identification of pollen using DNA barcoding. ITS2 is recommended by Lear et al. (2018) for green plants because it is universally present with a high copy number, has conserved rRNA flanks, and generally reflects an appropriate length for current Illumina sequencing, a common and cost-effective method of next-generation sequencing (Shokralla et al. 2012). This recommendation is backed by other studies (Widmer et al. 2000; Gu et al. 2013). Chen et al. (2010) compares seven candidate DNA barcodes (psbA-trnH, matK, rbcL, rpoC1, ycf5, ITS2 and ITS) from medicinal plant species. The ranking criteria included PCR amplification efficiency, differential intra-and inter-specific divergences, and the DNA barcoding gap. Data suggests that ITS2 of nuclear ribosomal DNA represents the most suitable region for DNA barcoding applications. The discrimination ability of ITS2 was tested in more than 6600 plant samples belonging to 4800 species from 753 distinct genera and found 92.7% identification rate at the species level.

If DNA analysis is being carried out on pollen samples from soil samples, or other environmental samples, appropriate primer selection can help to avoid poor results through environmentally degraded DNA. Pornon et al. (2016) used trnL and ITS1 with the justification that trnL has the P6 loop that is good for studying DNA that may be degraded, such as in environmental pollen samples; while ITS1 gives the level of specificity that is not possible with trnL alone. Torricelli et al. (2016) also evaluated *actin* and *tRNA-Leu* plant-specific genes on pollen that was extracted from honey. Matsuki et al. (2007b) used *trnL* and *trnF* and Suyama et al. (1996) used *rrn5* and *trnR* to sequence grains of fossil pollen for *Abies* species.

CBOL Plant working group, suggests two plastid (chloroplast) genome sequences, rbcL and matK, for plant identification. Lalhmangaihi et al. (2014) used *mat*K and Galimberti et al. (2014) used *rbcL* and *trnH-psbA.* Results suggested that rbcL alone could not distinguish among congeneric plants; however, *psbA-trnH* identified most of the pollen samples at the species level.

5.2.5 PCR Cycles

The number of PCR cycles used in the DNA amplification process ranged from 25-40 cycles, with an average of 33.5 cycles. The majority of studies used 35 cycles. For those studies that reported it, cycle temperatures differed by one to five degrees Celsius for each step in the PCR reaction between studies. Cycle durations also had only minor differences, 15 seconds to 5.5 minutes for PCR stages. Studies most commonly reported initiation at 95 ˚C for anything between 15 seconds and 15 minutes, denaturation at 95 ˚C for 35 cycles at 30 seconds, annealing at 55 ˚C for 30 seconds, extension at 72 ˚C for 1 minute, and final elongation at 72 ˚C for 5 minutes.

5.2.6 Reported results

Few publications reported data on specificity of pollen identification achieved through DNA barcoding. Sickel et al. (2015) reported the highest levels of specificity for the reviewed studies, identifying 95% of samples to species level using a dual-indexing approach with ITS-S2F and ITS4R. Bell et al. (2017) reported 40% specificity to species level and 38% specificity to genus level when using ITS2, and 55% specificity to genus level or higher using rbcL, and Hawkins et al. (2015) reported 93% specificity to family level or higher using rbcL.

Methods of investigating DNA quality and quantity from pollen samples included recording concentration of DNA per microliter of sample, using photometrically discerned absorbance ratios (at the 260-280 spectrum), as well as electrophoretic analysis and inhibition testing. Torricelli et al. (2016) used the most comprehensive range of techniques to report DNA quality and quantity, demonstrating DNA concentration of 10-160 ng/µl, absorbance ratios of 1.80 to 1.90, inhibition results within accepted parameters, and electrophoretic analysis showed some small fragmentation of DNA extracted from honey and pollen samples. Galimberti et al. (2014) reported high quality and concentration of DNA extracted from pollen samples collected from beehives (>20 ng/µl for each sample). Lalhmangaihi et al. (2014) reported DNA concentrations of 20-45 ng/µl (average 32.25 ng/µl) from pollen isolated from honey samples, and 1.62-1.82 for 260/280 optical density (average 1.74).

5.5 Discussion

Overall, microscope identification was more accurate and identified a greater breadth of interactions between invertebrates and flowers than was observed through field observations alone, but DNA barcoding has the potential for greater accuracy and improved standardisation. Samples of 10-20 microliters were used for microscopic pollen identification and counted once. Illumina sequencing can improve sampling depth because, rather than using a subsample as is done in microscope methods, DNA is extracted, amplified, and sequenced from each of the pollen grains in the sample. In addition, while we identified 57 pollen types using microscopic identification, 95 % of those were only identified to genus level. In contrast, studies using DNA-based methods of pollen identification reported up to 95 % of samples identified to species level (Sickel et al. 2015), indicating a potential for far greater specificity in species identification using DNA-based methods.

Table 5.5: Review of DNA extraction and sequencing protocols from relevant literature

Sample	*Wash buffer*	*Beads*	*Incubation /Centrifuge*	*DNA extraction*	*Primers*	*PCR cycles*	*Reference*
Pollen extracted from bees		Mini beadBeater-96 for 2 minutes		Macherey-Nagel NucleoSpin Food kit	ITS2 and rbcL2 and	35	Bell et al. (2017)
Leaf tissue dried in silica gel				Plant Genomic DNA Kit	psbA-trnH, matK, rbcL, rpoC1, ycf5 and ITS		Chen et al. (2010)
Freeze-dried pollen, ground to powder				Plant DNeasy Isolation and Purification kit. Plant DNeasy.	rbcL and lrnH-psbA	35	Galimberti et al. (2014)
Honey, diluted with ultrapure water	AP1 from DNeasy Plant Mini Kit with Proteinase K added.	TissueLyser II with 3 mm tungsten carbide beads	Incubated 10 minutes at 65 degrees in a water bath	DNeasy Plant Mini Kit (QIAShredder column and second wash stage omitted).	rbcL	30	Hawkins et al. (2015)
Airborne pollen collected on an adhesive tape	DNA isolation buffer (155 mm NaCl, 125 mm Tris-HCl pH 8.0, 15 mm EDTA, 1.25% polyvinylpyrrolidone, 0.6% SDS, 3 mm 1,10-phenanthroline monohydrate, 0.1% betamercaptoethanol	5 Stainless steel 3.2 mm balls.	100 µl of 5% SDS added and then incubated at 65°C for 30 min	QIAamp DNA Mini kit	trnL	25	Kraaijeveld et al. (2015)

Sample	*Wash buffer*	*Beads*	*Incubation*	*DNA extraction*	*Primers*	*PCR cycles*	*Reference*
Pollen extracted from honey	100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5	Sterilized glass beads 0.5 g	56 (1 h) prior to CTAB. 65 (overnight).	Phenol-chloroform-isoamyl alcohol	matK22F and matK22R	35	Lalhmangaihi et al. (2014)
Single pollen grain	0.01% SDS, 0.1 µg/µL proteinase K (TaKaRa) and 1 × PCR (polymerase chain reaction) buffer (containing 1.5 mm MgCl2) of AmpliTaq Gold	Crushed using pipette tip on wall of vial	incubated at 37 °C for 60 min then at 95 °C for 10 min	Pollen grain used directly for PCR after crushing	trnL and trnF	35	Matsuki et al. (2007a)
Pollen				DNeasy plant mini kit.	rbcLa and matK	35	Park & Nieh (2017)
Pollen from solitary bee nest				Macherey-Nagel Food Kit	ITS-S2F and ITS4R.	37	Sickel et al. (2015)
Fossil pollen from soil cores	10 mM Tris-HCl, pH 8.3 at 20 degrees c; 1.5 mM MgCl2, 50 mM KCl, 0.01% Proteinase-K, 0.01% SDS		60 min at 37 degrees C, heated for 10 min at 95 degrees c.		Rrn5 and trnR	25	Suyama et al. (1996)
Pollen extracted from honey	CTAB extraction buffer				Actin and tRNA-Leu	40	Torricelli et al. (2016)
Pollinarium	Standard CTAB		15 minutes, 60 degrees	chloroform-isoamyl alcohol	ITS1	30-35	Widmer et al. (2000)

Sample quality can affect the accuracy and specificity of DNA barcoding. Though pollen grains are by nature resilient to damage, DNA can quickly degrade if samples are not properly stored (Leontidou et al. 2017). Selection of suitable barcodes, in terms of their ability to be accurately and reliably sequenced in diverse sample sets, can also affect the quality of results (Hollingsworth et al. 2011). The range of reference sequences available for the DNA region and species in the geographic sampling location can affect the level of specificity in sample identification that is possible (Keller et al. 2015). In addition, differing amplification efficiencies can bias data, resulting in overrepresentation of species that are easily amplified and under representation of others (Elbrecht & Leese 2015).

Though the major consideration for method selection should be the quality and accuracy of the results, financial constraints must also be considered. The estimated cost for DNA barcoding of samples collected in this study was on average $30-50 per sample, including the initial purchase of DNA barcodes, DNA extraction costs, and Illumina sequencing. This is comparative with costs quoted by Bell et al. (2017) at US$30 per sample (NZD$45). Labour costs are additional. Although this represents a significant cost per sample, contracting taxonomic experts is likely to be higher in some cases. Expert identification may be required for both flower visitors and pollen grains for research on plant-pollinator interactions, whereas, when using DNA metabarcoding approaches often both taxonomic groups can be identified together. This study averaged at $3.00 per sample for insect identification including labour costs and related expenses.

Palynology analysis generally incurs a per-sample rate, and an hourly rate for identification, as well as travel and any other expenses. This differs greatly but can be around NZD$160 to NZD$300 per sample (Universita Degli Studi Firenze 2014). The per sample cost for taxonomic pollen identification will also be influenced by the diversity of pollen in the sample, and the amount of training for local pollen context that is required (Keller et al. 2015). Automatic identification of pollen is another alternative to improve the speed and accuracy of morphological pollen identification, but it is still in development and has barriers to usefulness (Marcos et al. 2015). Pollen grains are 3D structures that can be present on slides in any orientation, making standardised identification difficult, and sample debris can present additional challenges (France et al. 1997). In addition, large volumes of data are initially required for this method to be effective, including high-quality

images of pollen grains with descriptions of features and accurate identifications, which may be limited for some habitats and geographic areas (Stillman & Flenley 1996). Looking at overall costs, DNA analysis can in some cases be a more cost-effective method.

Traditional and molecular methods can provide different kinds of information. For this reason, selecting the appropriate method for identification of species interactions should consider both the accuracy and detail required from results, and the question to be answered. Acetolysis wash and digestion treatments revealed different pollen species found on the interior and exterior of the flower visitors. If acetolysis is being used to prepare pollen samples for identification, this finding highlights a need to tailor the digestion method to fit the purpose. Research questions concerning plant-pollinator interactions should focus on externally washed pollen, because using digestions of insects as a whole helps identify plant species that may be part of the insect diet, but not necessarily those they pollinate due to lack of pollen adherence on external tissues (Jones 2012). In addition, when external pollen is the focus, pollen scoring identified a need to monitor wash protocols to ensure effective pollen removal. Particular attention should be paid to flower visitor groups such as bees and flies to prevent loss of external pollen through inefficient wash protocols. Traditional methods involving microscopic identification of flower visitors and pollen can provide different information about interactions that can be helpful in answering behaviour or physiological questions, for example, identifying patterns of pollen adherence to flower visitor bodies (Figure 5.1), insect diets, or behavioural interactions at the flower level (Jones 2012). Alternatively, DNA barcoding can be manipulated to provide answers to questions about parentage, hybridisation, cross-pollination and dispersal distances (Matsuki et al. 2008; Hasegawa et al. 2009; Ashley 2010; Isagi 2011).

DNA barcoding results can reliably be applied to analysis of plant-pollinator interactions, however, identifying the strength of interactions is limited by a poor correlation between sequence reads and pollen abundance within a sample. Though some studies report a correlation (Pornon et al. 2016), species-specific differences in pollen shelf-life, DNA extraction and PCR efficiency, and copy number of ITS2 and rbcL limit confidence in the applicability of this correlation (Keller et al. 2015; Kraaijeveld et al. 2015; Richardson et al. 2015; Bell et al. 2016). Bell et al. (2017) recommends quantifying plant-pollinator reactions by recording the frequency of

presence-absence interactions, rather than using sequencing read proportions. Field observation on focal plants alone can overestimate specificity in insect interactions (Bosch et al. 2009), but identification of species interaction using pollen identification can be affected by pollinator grooming behaviour, body size, and pollen attributes (Harder 1990). However, these two methods, if combined, can provide meaningful measures of interaction strength, rather than simply presence-absence (Coux et al. 2016).

Available skills and training can also affect method selection decisions. Learning taxonomic pollen identification is a skill that requires considerable time investment, and training from palynological experts as well as access to quality pollen type collections. DNA barcoding, though a more technical approach to pollen identification, is a process that has been simplified through the availability of kits for extraction, and automated technology. Expert training is generally easier to come by for molecular methods and less time intensive than may be the case for taxonomic pollen identification. These can be considered in concert with project funding to determine the most appropriate method.

5.6 Future research directions

Considering accuracy and specificity, time and cost, questions to be answered and available skills and training, we determined that for our research project molecular-based methods will be the most appropriate for answering questions related to interactions between plants and pollinators. Observational approaches have been used in analysis and presented in this thesis (Chapter 3 and Chapter 4) due to constraints related to available training, funding, and time. However, we are currently working to further develop molecular research methods for application to data collected in this research project. The goal of this is to answer questions about how introducing non-native pollinators affects native plant-pollinator interactions in the Kaimai-Mamaku Range, New Zealand. This will allow further comparison of molecular and observational methods. Additional funding has been successfully applied for from New Zealand's Biological Heritage National Science Challenge and work will commence in the near future. This research will be part of a greater effort to address barriers to the wide-spread and cohesive adoption of DNA meta-barcoding in ecology in New Zealand (Holdaway et al. 2017).

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Appendix: Acetolysis Procedure from Jones (2014)

Step 1 – Sample Preparation

1. If the samples are fresh or very wet after being thawed
    a) Crush the sample with a wooden stick, glass stirring rod or motorized pestle.
    b) Go to step 2, Pre-acetolysis, glacial acetic acid.
2. If the samples are relatively dry after thawing
    a) Crush the sample with a wooden stick, glass stirring rod or motorized pestle.
    b) Go to step 3, Acetolysis.
3. Samples that are dry

a) Crush the sample with a wooden stick, glass stirring rod or motorized pestle.
b) Go to step 3, Acetolysis.

Step 2 – Pre-Acetolysis, Glacial Acetic Acid

1. Mix the centrifuge tube for 15 seconds with a stick, vortex stirrer, etc.
2. Add 5 ml of glacial acetic acid (glacial) from the squirt bottle
    a. Stir with a wooden applicator stick as the glacial is being added.
    b. Use a different applicator stick for each sample
    c. While stirring, slowly remove the applicator stick rinsing it with glacial acetic acid as it is lifted from the liquid
    d. Place the applicator stick into the sodium bicarbonate waste beaker
3. Centrifuge samples for 3 minutes at 1060 x g
4. Decant the supernatant into an acid waste collector or follow your safety officer's directions for acid waste disposal

Step 3 – Acetolysis (must be conducted in a fume hood)

1. Put on the face shield
2. Mix the samples well for 15-20 seconds with a stick, vortex stirrer, etc.
3. Make the acetolysis mixture (9:1 ratio of acetic anhydride to sulfuric acid).
    a. From a stock bottle, pour about 38 ml of acetic anhydride into a clean labelled beaker. If this beaker is very clean, the excess can be poured back into the stock bottle.
    b. Add 36 ml of the acetic anhydride from the beaker into a 50 ml clean, dry graduated cylinder.
    c. Add 5 ml of concentrated sulfuric acid into its labelled beaker
    d. Slowly add 4 ml of the sulfuric acid from the beaker into the graduated cylinder that has the acetic anhydride in it.
    e. Let the sulfuric acid run along the inside of the graduated cylinder as it is poured in.
    f. The graduated cylinder will become hot to the touch, and often turns yellow. If the mixture turns very dark, the chemicals could be bad (usually the sulfuric), the amount of sulfuric acid is too high and the acetolysis mixture is considered "hot", or the chemicals are contaminated with another chemical. In those cases, discard and start over with new chemicals.
4. Slowly pour about 1 ml of the acetolysis mixture into the sample
    a. Gently stir the sample with a wooden applicator stick as the acetolysis mixture is added
    b. If there is no reaction, add a little more acetolysis mixture, stirring while adding.
    c. Add a total of 3 ml to the sample, then go to the next sample
    d. Add more acetolysis mixture until all samples have 3 ml of acetolysis mixture
        i. For small pollinators (insects, spiders, etc.) and pollinator tissues do not add any more
        ii. 3 ml will reach the top of the curve of a glass conical bottom centrifuge tube
    e. For large pollinators, whole insects, and multiple samples as one sample, and filter paper, add 2 more ml of acetolysis mixture to each sample
        i. Stirring as it is added

        ii. 5 ml of acetolysis mixture will reach to the middle of a 12-15 ml glass conical bottom centrifuge tube.
    f. Place the wooden applicator sticks into the sodium bicarbonate beaker
5. Place the samples into the pre-heated hot block
    a. If boiling water is used, the water needs to be boiling before making the acetolysis mixture.
    b. The centrifuge tubes should not be able to fall into the water
    c. The level of the boiling water should not bubble up and over into the samples
6. Cook the samples for 5-25 minutes, depending on the sample
    a. For large, whole squashed pollinators, cook for 15-20 minutes
    b. For small, squashed pollinators or its tissue, cook for 10 minutes
    c. For pollen pellets, cook for 5 minutes
    d. For whole, squashed moths or butterflies, cook for 15-25 minutes. The scales that don't dissolve will have to be strained out after acetolysis.
    e. For filter paper, cook for 20 minutes
7. Every three minutes, stir each sample with the clean wooden applicator stick
    a. Use a different applicator stick for each sample
    b. Do not leave the wooden applicator sticks in the centrifuge tubes because the acetolysis mixture will dissolve them.
    c. Continue to stir as the applicator stick is lifted up out of the acetolysis mixture
    d. Place each applicator stick into the beaker that contains the sodium bicarbonate.
8. After the allotted amount of time, remove the samples from the hot block

Step 4 – Post-Acetolysis, Glacial Acetic Acid

1. Add 5 ml of glacial acetic acid to each sample
    a. Stir the samples with an applicator stick as the glacial acetic acid is added
    b. Make sure that the pollen residue plug becomes dislodged and mixed
    c. While stirring, rinse the applicator stick with the glacial acetic acid as it is lifted out of the sample
    d. Place the applicator stick into the waste sodium bicarbonate beaker
2. Centrifuge the samples for 3 minutes
3. Decant the supernatant into a waste beaker
4. Mix the samples very well, at least 15 seconds

Step 5 – Water Rinses

1. The face shield can be removed, but keep the lab coat and gloves on
2. Using a squirt bottle, fill the tube with distilled water to about 2.5 cm (1 inch) from the top
    a. In one motion, aim the tip of the squirt bottle to the centre bottom of the centrifuge tube and squirt water into the sample hard
    b. Once the initial hard pressure is used, back off of the pressure but don't stop squirting
    c. When you start squirting water in to the sample, don't stop until the tube is full

    d. Turn the centrifuge tube as you squirt the water down the inside of the tube so that the sides are rinsed
    e. Make the water level even in the tubes
    f. Don't place the tip of the squirt bottle into the centrifuge tube. This will contaminate the squirt bottle.
3. Centrifuge the samples for 3 minutes at 1060 x g
4. Decant the supernatant into the waste beaker
5. Mix well for 15 seconds
6. Repeat the water rinse steps at least two more times
    a. Continue water rinses until the liquid is clear and no longer smells like glacial acetic acid.
    b. This may take more than three water rinses.

Step 6 – Straining the Samples

1. Skip this step if there are no visible large body parts remaining.
2. Bend the screen slightly so that it has a depression or well in the centre and will rest on the top of a 100 ml plastic or glass beaker (fig. 4).
    a. Use a separate beaker and screen for each sample
    b. Make sure that each beaker is labelled with the sample number
3. Mix the pollen residue for 15 seconds
4. Pour the pollen residue onto the screen, allowing the liquid to go into the beaker
5. Holding the centrifuge tube at an angle, squirt a small amount of water into the centrifuge tube and allow it to run out onto the screen and into the beaker
6. Repeat the above several times, or until all the pollen residue and large body parts are on the screen.
7. Squirt water on the sides of the test tube and allow it to run out onto the screen and into the beaker
8. Squash the body parts with a clean spatula then rinse the screen and body residue several times with distilled water
9. Tap the screen several times on the top of beaker to dislodge any pollen grains stuck to the bottom side of the screen
10. Pour the contents of the beaker back into its original centrifuge tube
11. Centrifuge, decant, and mix
12. If there is more water and pollen residue in the beaker, pour it into the centrifuge tube
13. Add enough water so that the liquid is about 2 cm (1 inch) from the top of the centrifuge tube
14. Centrifuge, decant, and mix
    a. When all the water and residue are back in the centrifuge tube
    b. Rinse the beaker several times with distilled water allowing the water rinse to go into the centrifuge tube
15. Be sure to rinse the sides of the beaker

Step 7 – ETOH Rinse

1. Mix the samples for 15 seconds
2. Add 5 ml of 95% ETOH to each sample
3. Centrifuge for 3 minutes at 1060 x g
4. Decant the supernatant

Chapter 6

Synthesis and Recommendations

6.1 Discussion

This thesis contributes new knowledge on the complex effects of introducing a non-native invertebrate into a native New Zealand forest ecosystem, with wider practical applications for apiculture management and forest conservation. It has quantified floral nectar production for *I. brexioides* and *W. racemosa* over two contrasting sampling years and highlighted the dynamic nature of floral nectar availability and consequences for supporting communities of nectar-feeders (Chapter 2). It has compared the potential effectiveness of honey bee visitation for seed set in a dominant large-flowered tree and a dominant small-flowered tree (Chapter 3), and explored the evidence of community-level disruption of invertebrate flower visitors related to density of surrounding honey bee hives and differing land-management practices (Chapter 4). In addition, it presents an analysis of methods for identifying plant-pollinator interactions and suggests future pathways for research in this field (Chapter 5). Together, these contributions highlight key elements of the interactions between introduced honey bees and the other invertebrates and plants in the forest community.

Availability of floral nectar produced by *I. brexioides* and *W. racemosa* florets was found to be extremely variable between annual cycles in terms of sugar mass produced per floret, and the number of inflorescences produced per tree (Chapter 2). This characteristic has been observed in other studies (Enkegaard et al. 2016) but is seldom accounted for in floral resource inventory projections (Hicks et al. 2016; Ausseil et al. 2018). This is significant because of the flow-on effects of resource availability for the support of nectar-feeding flower visitor communities, and hence for seed set of flowering plants (Beard 2015). In addition, this has economic implications because it affects sustainability of apiculture, highlighting a need to improve stocking rate management by budgeting resource production and allocation across landscape scales (Paton 1990; Dicks et al. 2015; Arundel et al. 2016; Cane & Tepedino 2017; Ausseil et al. 2018).

Density of honey bee hives and pest-management strategies in areas surrounding study sites was shown to affect seed of *I. brexioides* and *W. racemosa* (Chapter 3). Seed set for *I. brexioides* was highest at Maungatautari where its predator-proof fence enclosures allowed for a greater abundance of nectar-feeding birds, indicating that bird pollination is likely more important for *I. brexioides* than previously thought (Thomson 2013). *Weinmannia racemosa* seed set was highest at high hive density sites, and lowest at low density sites. This was consistent with observations of flower visitor behaviour which demonstrated a lack of specificity in pollinator potential for *W. racemosa*, while *I. brexioides* pollination was suggested to be most successful by birds, beetles and native bees and least successful for spiders, wasps, ants and honey bees (Chapter 3). Reviews suggest that honey bees have the potential to compensate for pollination services provided by extinct or declining native species (Butz Huryn 1997; Beard 2015), but there are also costs (Young & Young 1992; Gross & Mackay 1998; Aizen et al. 2014). It was found that flower structure and flower-visitor behaviour are important factors determining whether flower visitation by honey bees will result in pollination compensation, and that small-flowered species similar to *W. racemosa* have the potential to benefit from increased pollination success in areas where honey bees are frequent visitors (Chapter 3).

Composition and structure of invertebrate communities of flower visitors were affected by honey bee hive density (Chapter 4). Honey bees were the key species contributing to differences between invertebrate communities at high hive density sites and other sites, and also acted as a key indicator species for the high hive density community. Network analysis demonstrated greater connectance and nestedness in low hive density networks, and greater a contribution of honey bees to pollination services in high hive density networks. Where density of honey bee hives was high, interactions with non-native species were found to be more frequent (Chapter 4), consistent with findings from Traveset et al. (2013). This study confirms other studies which have demonstrated negative correlation between density of honey bees and diversity of native pollinators (Chapter 4), with cascading effects on productivity and native flower visitation (Wenner & Thorp 1994; Kato et al. 1999; Roubik & Wolda 2001; Hansen et al. 2002; Kato & Kawakita 2004; Badano & Vergara 2011). This offers further evidence of how impacts of introduced pollinators on plant-pollinator networks are affected both by density of the invader

(Vázquez et al. 2007; Aizen et al. 2008; Giannini et al. 2015) and the modification of the surrounding environment (Didham et al. 2007; Giannini et al. 2015).

Exploring methods for collecting, quantifying and identifying pollen loads carried by flower visiting invertebrates suggested that native bees were among those carrying the most dense pollen loads and this pollen was generally concentrated on head, abdomen and thorax, rather than on legs or wings (Chapter 5). There was also a marked difference between the variety of pollen species observed on the exterior surface of flower visitors, and those observed from digestion of internal tissues (Chapter 5). This highlights a need to tailor methods of pollen isolation to fit the research question, i.e. whether research is focused on pollination interactions or questions which are diet related. Comparison of methods for understanding plant-pollinator interactions concluded that identification of pollen by microscopic means identified a greater breadth of plant-pollinator interactions than that identified by field observations alone (Chapter 5), however DNA has the potential for greater specificity (Sickel et al. 2015), cost-effectiveness (Bell et al. 2017), and answering a range of questions not possible with traditional methods (Matsuki et al. 2008; Hasegawa et al. 2009; Ashley 2010; Isagi 2011). As a result of this review, funding has been granted to allow DNA barcoding approaches to be applied to samples collected during this research project to further validate methods and expand applications of DNA barcoding (Holdaway et al. 2017) in the study of plant-pollinator interactions.

6.2 Recommendations for management

Analysis and findings from this research support a case indicating that honey bees can affect seed set of native plants, and communities of invertebrate flower visitors in a number of ways. Prevention of permanent changes to flower visitor communities should be prioritised by preserving large areas of intact native forest where low levels of fragmentation create refuges for native flower visitors. In addition, seed set of *I. brexioides* at Maungatautari indicated the positive impact that pest-management can have for the longevity of bird-pollinated plant species. Increased pest-management regimes, fostering populations of native nectar-feeding birds, for example through the Predator-Free 2050 program (Predator Free NZ 2019) will also benefit species such as *I. brexioides* that are most suited to pollination by birds.

Measurements of nectar sugar production highlighted the dynamic nature of floral resource availability that should be reflected in decisions regarding apiary management on conservation land. Under current New Zealand stocking rates, the average amount of sugar required for honey production based on 2012-2017 average production ranged from 333 % of annual *I. brexioides* and *W. racemosa* nectar sugar production for a good year, to 2336 % of annual production for a bad year (assuming regular nectar replenishment). These figures call attention to the need to build greater flexibility into legislated stocking rates in native forest, despite the inherent difficulties, to minimise competitive effects on native biota during low production years.

6.3 Recommendations for further research

Maintaining sustainable native ecosystems in New Zealand will require increased awareness of the impacts of large-scale, managed introductions of non-native species through further research across a broader range of habitats and species. In addition, long-term effects from increasing honey bee presence in native forest should be assessed, such as alteration of forest composition and structure, impacts on plant genetic diversity, and effects on flower visitor population dynamics.

Floral resource availability data, compiled from both phenological recording and flower resource quantification, is needed to complete the picture of resource availability for forest ecosystems and to more accurately inform conservation efforts and management decisions for apiaries on conservation land. Developing global information databases on floral phenology and floral resource quantification, and effects of climate change on floral physiology will go a long way toward deepening our understanding of natural ecosystems from a resource dynamics point of view, enabling us to manage resource demands more appropriately.

This research demonstrated an observational approach to considering the effects of honey bee invasion on native flower visitor communities and further research is required to experimentally test these findings, as well as other potential impacts which have been identified in the literature. For example, experiments should include investigation into pathogen transfer to native bees, quantification of resource depletion by honey bees on New Zealand native plants, and effects of current advised hive stocking rates on population dynamics and fecundity of native flower visitors. Future research should also address the response of plant-pollinator networks to restoration and removal of invasive species to inform management

strategies and improve sustainability of native flower visitor communities in native ecosystems. In addition, this research represents a limited range of New Zealand's unique native forest. Future research should be structured to address a variety of different species and ecosystems to compare responses to pollination network invasion.

Work is currently underway to further develop DNA-based methods of identifying plant-pollinator relationships to ensure accuracy and suitability of results and to answer further questions about the effect of invasion on plant-pollinator interactions in the Kaimai-Mamaku Range, New Zealand. Additional funding has been successfully applied for from New Zealand's Biological Heritage National Science Challenge and work will commence in 2020. This research will be part of a greater effort to address barriers to the wide-spread and cohesive adoption of DNA meta-barcoding in ecology in New Zealand (Holdaway et al. 2017).

6.4 References

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Appendix: Glossary of species names - Plants

Glossary of scientific names, common names and life forms of species mentioned in the thesis. Naming was based on the New Zealand Plant Conservation Network conventions.

Scientific name	Common name	Life form
Alseuosmia macrophylla	Toropapa	Shrub
Asplenium bulbiferum	Hen and chicken fern	Fern
Beilschmiedia tawa	Tawa	Tree
Carex uncinata	Hook sedge	Sedge
Coprosma grandifolia	Kanono	Tree
Cyathea dealbata	Silver fern	Tree fern
Dicksonia squarrosa	Wheki	Tree fern
Freycinetia banksii	Kiekie	Liane
Geniostoma ligustrifolium var *ligustrifolium*	Hangehange	Shrub
Hedycarya arborea	Pigeon wood	Tree
Ixerba brexioides	Tāwari	Tree
Knightia excelsa	Rewarewa	Tree
Leucopogon fasciculatus	Mingimingi	Shrub
Lomaria discolor	Crown fern	Fern
Melicytus ramiflorus	Māhoe	Tree
Microlaena avenacea	Bush rice grass	Grass
Parablechnum novae-zelandiae	Kiokio	Fern
Weinmannia racemosa	Kāmahi	Tree

Appendix: Glossary of species names - Mammals

Glossary of scientific names, common names and life forms of species mentioned in the thesis. Naming was based on the New Zealand Plant Conservation Network conventions.

Scientific name	Common name	Life form
Capra hircus	Goat	Mammal
Cervus spp.	Deer	Mammal
Erinaceus europaeus occidentalis	Hedgehog	Mammal
Felis cattus	Cat	Mammal
Lepus europaeus occidentalis	Hare	Mammal
Mustela erminea	Stoat	Mammal
Mustela furo	Ferret	Mammal
Mustela nivalis vulgar	Weasel	Mammal
Oryctolagus cuniculus cuniculus	Rabbit	Mammal
Rattus norvegicus	Norway rat	Mammal
Rattus rattus	Ship rat	Mammal
Sus scrofa	Pig	Mammal
Trichosurus vulpecula	Possum	Mammal

Appendix: Glossary of species names – Flower visitors

Glossary of scientific names, common names and life forms of flowers visitors collected by sweep netting at field sites in the Kaimai-Mamaku Range, New Zealand. Asterisks indicate if the species is native to New Zealand.

Order	Family	Genus/Species
Acari	BDELLIDAE	
	CROTONIIDAE	*Crotonia sp.**
Araneae	ARANEIDAE	
	CLUBIONIDAE	*Clubiona sp.**
	LINYPHIIDAE	
	SALTICIDAE	
	THOMISIDAE	*Diaea sp.**
		Sidymella sp.
Blattodea	BLATTIDAE	*Celatoblatta sp.**
	CANTHARIDAE	*Asilis fulvithorax**
		*Asilis sp.**
	CERAMBYCIDAE	*Calliprason sinclairi**
		*Navomorpha sulcata**
		*Oemona hirta**
		*Spilotrogia maculata**
		*Spilotrogia sp.**
	CHRYSOMELIDAE	*Adoxia sp.**
		Bruchidius villosus
		*Eucolaspis sp.**
		*Trachytetra rugulosa**
	CLAMBIDAE	
	CLERIDAE	*Phymatophaea sp.**
	COCCINELLIDAE	*Halmus chalybeus*
		Harmonia axyridis
		*Rhyzobius sp.**
	CORYLOPHIDAE	*Arthrolips sp.**
		Sericoderus sp.
	CRYPTOPHAGIDAE	*Paratomaria sp.**
	CURCULIONIDAE	*Chaetoptelius mundulus**
		*Hoplocneme sp.**
		*Nyxetes bidens**
		*Psepholax sp.**
		*Rhopalomerus sp.**
		*Scolopterus aequus**
		*Scolopterus penicillatus**
		Sitona lepidus (a.k.a. Sitona obsoletus)
		Sitona obsoletus (=S. lepidus)
		*Stephanorhynchus lawsoni**
	ELATERIDAE	*Metablax acutipennis**

Order	Family	Genus/Species
		*Panspoeus guttatus**
	HYDROPHILIDAE	*Rygmodus sp.**
	LATRIDIIDAE	
	MELYRIDAE	
	MORDELLIDAE	*Stenomordellaria neglecta**
	SALPINGIDAE	
	SCARABAEIDAE	*Pyronota festiva**
		*Pyronota sp.**
	SCIRTIDAE	*Amplectopus sp.**
		*Atopida sp.**
		*Mesocyphon sp.**
		*Veronatus sp.**
	SCRAPTIIDAE	*Nothotelus nigellus**
		*Nothotelus sp.**
	STAPHYLINIDAE	*Anotylus sp.*
		*Ischnoderus sp.**
		*Sepedophilus sp.**
Collembola		
Diptera	AGROMYZIDAE	*Cerodontha angustipennis**
		Cerodontha australis
		*Cerodontha sp.**
		Liriomyza sp.
		Phytomyza sp.
	ASILIDAE	
	BIBIONIDAE	*Dilophus alpinus**
		*Dilophus nigrostigma**
		*Dilophus sp.**
		*Dilophus tuthilli**
	CALLIPHORIDAE	*Calliphora sp.*
		*Calliphora sp.**
		Calliphora vicina
		Calliphoridae
		*Pollenia sp.**
		*Xenocalliphora sp.**
	CANTHYLOSCELIDIDAE	*Canthyloscelis sp.**
	CECIDOMYIIDAE	
	CERATOPOGONIDAE	
	CHIRONOMIDAE	
	CHLOROPIDAE	*Conioscinella sp.**
		*Tricimba tinctipennis**
	CULICIDAE	*Culex sp.**
	DITOMYIIDAE	*Nervijuncta sp.**
	DOLICHOPODIDAE	*Australachalcus sp.**
		*Chrysotimus sp.**
		*Parentia sp.**

Order	Family	Genus/Species
	EMPIDIDAE	*Chelipoda sp.**
		Cladodromia sp.
		*Cladodromia sp.**
		*Hilara sp.**
		*Hilarempis sp.**
		*Homalocnemis sp.**
		*Phyllodromia falcata**
		*Phyllodromia flexura**
		*Phyllodromia sp.**
	EPHYDRIDAE	*Ditrichophora flavitarsis*
		Hydrellia sp.
		Hydrellia tritici
		Scatella nitidithorax
		Scatella sp.
	HELEOMYZIDAE	*Allophylopsis sp.**
		*Fenwickia sp.**
	HELOSCIOMYZIDAE	*Scordalus femoratus**
	HOMALOCNEMIDAE	*Homalocnemis sp.**
	HYBOTIDAE	*Oropezella sp.**
		*Platypalpus sp.**
		*Pseudoscelolabes sp.**
	KEROPLATIDAE	
	LAUXANIIDAE	*Poecilohetaerus punctatifacies**
		*Sapromyza neozelandica**
		*Sapromyza sp.**
		*Trypetisoma sp.**
	LIMONIIDAE	*Toxorhina sp.**
	LONCHOPTERIDAE	*Lonchoptera bifurcata*
	MUSCIDAE	*Calliphoroides antennatis**
	MYCETOPHILIDAE	*Aneura sp.**
		*Mycetophila sp.**
		*Tetragoneura sp.**
	PERISCELIDIDAE	*Cyamops sp.**
	PHORIDAE	*Megaselia sp.*
	PSYCHODIDAE	
	RANGOMARAMIDAE	*Rangomarama sp.**
	SCATOPSIDAE	
	SCIARIDAE	
	SIMULIIDAE	*Austrosimulium sp.**
	SPHAEROCERIDAE	
	SPHAEROCERINAE	
	STRATIOMYIDAE	*Australoberis sp.**
		*Odontomyia sp.**
		*Zealandoberis sp.**
	SYRPHIDAE	*Allograpta sp.**

Order	Family	Genus/Species
		Eristalis tenax
		*Helophilus antipodus**
		*Melanostoma fasciatum**
		*Orthoprosopa bilineata**
		*Platycheirus sp.**
	TABANIDAE	*Scaptia sp.**
	TACHINIDAE	*Protohystricia sp.**
		*VORIINI**
	TIPULIDAE	*Aurotipula clara**
		*Sapromyza sp.**
Ephemeroptera		
Hemiptera	ACANTHOSOMATIDAE	
		*Oncacontias vittatus**
	ACHILIDAE	*Agandecca annectens**
	APHROPHORIDAE	*Philaenus spumarius*
	CALOPHYIDAE	*Atmetocranium myersi **
	CICADIDAE	*Notopsalta sericea**
	CIXIIDAE	*Cermada sp.**
		*Koroana rufifrons**
	MIRIDAE	*Chinamiris indeclivis**
		*Chinamiris sp.**
		*Diomocoris sp.**
		Stenotus binotatus
		*Xiphoides sp.**
	PSYLLIDAE	*Arytainilla spartiophila*
	TRIOZIDAE	*Trioza sp.**
Heteroptera	RHYPAROCHROMIDAE	*Targarema stali**
Hymenoptera	APIDAE	*Apis mellifera*
	BRACONIDAE	*Aphidius sp.*
		Ascogaster sp.
		*Chorebus rodericki**
	COLLETIDAE	*Hylaeus agilis**
		Hylaeus sp.
		*Hylaeus sp.**
		*Leioproctus sp.**
	CRABRONIDAE	*Rhopalum sp.**
	DIAPRIIDAE	*Neurogalesus carinatus*
		*Spilomicrus sp.**
		*Stylaclista sp.**
	ENCYRTIDAE	
	EULOPHIDAE	
	FORMICIDAE	*Monomorium sp.**
		*Prolasius advena**
		Technomyrmex jocosus

Order	Family	Genus/Species
	GASTERUPTIIDAE	*Pseudofoenus sp.**
	HALICTIDAE	*Lasioglossum sp.*
		*Lasioglossum sp.**
	ICHNEUMONIDAE	*Aucklandella sp.**
		*Degithina sp.**
		*Lissonota sp.**
		Xanthopimpla rhopaloceros
	MEGASPILIDAE	
	PLATYGASTRIDAE	
	POMPILIDAE	*Epipompilus insularis**
		*Sphictostethus nitidus**
	PROCTOTRUPIDAE	
	PTEROMALIDAE	
	VESPIDAE	*Vespula germanica*
		Vespula vulgaris
Lepidoptera	CRAMBIDAE	*Deana hybreasalis**
		*Glaucocharis auriscriptella**
		*Glaucocharis chrysochyta**
		*Glaucocharis sp.**
		*Orocrambus flexuosellus**
		*Orocrambus sp.**
	ELACHISTIDAE	*Elachista sp.*
	EREBIDAE	*Rhapsa scotosialis**
	GELECHIOIDEA	
	GEOMETRIDAE	*Ischalis gallaria**
		*Poecilasthena pulchraria**
	GLYPHIPTERIGIDAE	*Glyphipterix simpliciella*
		Glyphipterix sp.
	MNESARCHAEIDAE	*Mnesarchaea fusilella**
	TORTRICIDAE	*Apoctena sp.**
	XYLORYCTIDAE	*Izatha sp.**
Mantodea	MANTIDAE	*Orthodera novaezealandiae**
Neuroptera	HEMEROBIIDAE	*Micromus tasmaniae*
Odonata	LESTIDAE	*Austrolestes colensonis**
Plecoptera		
Psocoptera	CAECILIUSIDAE	
	ECTOPSOCIDAE	*Ectopsocus sp.**
	ELIPSOCIDAE	*Euryphallus stigmaticus**
	PERIPSOCIDAE	*Peripsocus sp.**
Trichoptera	HYDROPTILIDAE	