The Role of Honeybee Pollination in Native New Zealand Plants

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For mum, whose support was unwavering, until the very end.

Abstract

Honeybees (*Apis mellifera*) fill a keystone role in the pollination of many plant species. Studies have found that honeybees visit native New Zealand plants, however, with exception to *Metrosideros excelsa*, little data on honeybee pollination has been collected. Therefore, the aim of my study was to gain an understanding of the role of honeybee pollination in native New Zealand plants which may aid conservation efforts. This was done by measuring components of pollinator performance, which encompassed flower visitation, visitor volume and pollen collection. Flower visitation was counted and timed from recordings. Visitor volume was measured live. Honeybee samples were collected whereby the number of pollen grains and pollen species was determined via acetolysis. My results showed that honeybees are likely good pollinators of many native plants. Honeybees often visited an ample number of flowers and consistently spent more time foraging than not. Furthermore, honeybees usually collected a large number of pollen grains in which most were conspecific. My study found that small plants with grouped flowers are likely to benefit the most from honeybee pollination. In contrast, plants with miniscule flowers, plants in cold, windy environments and the threatened *Muehlenbeckia astonii* are unlikely to benefit from honeybee pollination. My research fills part of the knowledge gap of honeybee pollination in native New Zealand plants which has implications in conservation. Research on the remainder of the honeybee pollinator performance components and cross-pollination by honeybees, particularly in natural environments, is needed. In addition, the pollinator performance of other pollinators needs to be investigated.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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1. Introduction

1.1 The structure of flowers and the distinctiveness of New Zealand flowers

Flowers are present on angiosperms in which they are specialised reproductive organs that produce seeds (Fogden & Fogden, 2018). Flowers have male and female sections that are preserved by petals (McGregor, 1976) which is usually the most colourful part of a flower, as the petals are used to attract pollinators by advertising rewards such as nectar and pollen (Fogden & Fogden, 2018). Petal colour can vary from black to white but is not often green (McGregor, 1976), and the perception of petal colour displayed varies within insects, mammals and birds (Fogden & Fogden, 2018). The number of petals (along with sepals, stamens and carpels) between flowers can vary (Fogden & Fogden, 2018). For example, most flowers have five petals, but flowers such as poppies have four petals (Fogden & Fogden, 2018). Flower symmetry also varies; flowers with radial symmetry, such as poppies and buttercups, provide a landing platform in which pollinators can land and orientate themselves in any position, which allows unspecialised pollinators to visit (Fogden & Fogden, 2018). Alternatively, in bilateral flowers such as orchids and pea flowers, the landing platform facilitates specialised pollinators to exhibit certain positions that increases the chance of successful pollination (Fogden & Fogden, 2018). Similarly, petal form can be simple or complex and the scent of the flowers can vary from fragrant or repugnant (McGregor, 1976). Stamens are male organs which consist of anthers (McGregor, 1976), whereby pollen develops on the stamens (Ketcham, 2020). Pistils are female organs which has an ovary that contains one or more ovules, where a style with a stigma protrudes from the ovary (McGregor, 1976). After pollen has landed on an ovule, a pollen tube is produced which fertilizes the ovule via penetration (Ketcham, 2020). The ovary produces fruit (McGregor, 1976) whilst the ovules form seeds within the fruit (Ketcham, 2020; McGregor, 1976). Normally, within the flowers there are one or more nectaries where nectar is excreted after flowering until fertilization (McGregor, 1976). There are numerous variations of sexual function of flowers. Most commonly, flowers with both stamens and pistils present are called complete flowers, whilst monoecious flowers have separate sexes but are on the same plant and dioecious flowers have male and female flowers on separate plants (McGregor, 1976).

In New Zealand, many native plant species have primitive features (Webb & Kelly, 1993). Firstly, they are usually small and inconspicuous (Heine, 1937). Secondly, native plants are often dioecious (Bawa, 1980) and are unspecialised in development (Butz Huryn, 1995) where they do not have morphological adaptions for attracting specialist pollinators (Lloyd, 1985) and the frequency of self-incompatibility is low (Webb & Kelly, 1993). This may result in a variety of pollinators visiting the plants and facilitate cross-pollination (Butz Huryn, 1995). However, not all native flowers have a simple form; *Parahebe* species have specialist structures such as flat dish bottom, spread anthers, exposed nectar and small corolla tubes (Lloyd, 1985). The flower colour of native plants is unique because there is a high proportion of white flowers and lack flowers of other hues, such as red, purple and blue (Godley, 1979; Lloyd, 1985).

1.2 Pollination

Pollination is the process of pollen being deposited or transported onto the stamen of a flower. This occurs when the stigma is receptive; it is covered with colourless stigmatic fluid in which pollen can adhere to (McGregor, 1976). The pollen germinates and a pollen tube descends down the style into the ovary and ovules (McGregor, 1976). Fertilization then occurs where a viable seed is created that can produce another plant (McGregor, 1976). The likelihood of successful fertilization increases the closer pollination occurs following flowering (Mayer et al., 2011). Pollination methods include self-pollination and cross-pollination. Self-pollination is when pollination occurs within the same flower or between flowers on the same plant, while cross-pollination is when pollination occurs between flowers of different plants (Fogden & Fogden, 2018). Self-pollination can occur across many plant species depending on the plant's ecology and life history, and self-pollination can be especially important if cross-pollination does not occur (Fogden & Fogden, 2018). However, cross-pollination is usually superior to self-pollination because it results in genetic diversity and across plants (Fogden & Fogden, 2018). Pollination can occur abiotically via water, wind and gravity, or biotically via animals and insects (Kevan, 1999), where insects are most commonly pollinators of plants (Newstrom-Lloyd, 2013). Most flowers require animals to spread their pollen (Fogden & Fogden, 2018). Visitors of flowers are called anthophiles (Kevan & Baker, 1983). Anthophile groups include: butterflies and moths (Lepidoptera), bees and wasps (Hymenoptera), true flies (Diptera), beetles (Coleoptera), bats (Chiroptera), flowerpeckers (Dicaediae), hummingbirds

(Trochilidae), honeyeaters (Meliphagidae), sunbirds (Nectariniidae), springtails (Collembola), lories (Loriinae) (Kevan, 1999) and primates (Kress et al., 1994). An anthophile is only considered to be a pollinator if a receptive flower is visited and viable pollen is carried from the anther and deposited onto the stigma. This depends on the compatibility between the flower and pollinator, where the anatomy, resources and floral advertisement of the flower and the behaviour of the pollinator must match (Kevan, 1999). For example, some visitors have body shapes that inhibits their contact with male and female reproductive structures, so they are considered to be "nectar thieves" (Inouye, 1980). However, plants have varying degrees of pollination specialization; generalist plants may interact with hundreds of pollinators whilst specialist plants may interact with only one pollinator (Aguilar et al., 2006). Most plants, however, are pollinated by multiple species of pollinators (Kevan, 1999) with 90 % of plants being pollinated by animal pollinators (Burkle et al., 2013). Though multiple pollinators may pollinate a plant, different floral visitors have different abilities to transfer pollen (Ivey et al., 2003). Pollinator preference also plays a role in pollination. These preferences can include flower characteristics, landing platforms, accessibility of pollen and nectar, reward value, energy spent whilst foraging on the flower and proximity between flowers (Newstrom-Lloyd, 2013). Pollination is a mutually beneficial interaction. Pollinators receive rewards and resources while depositing pollen onto the flower (Proctor et al., as cited in Newstrom-Lloyd, 2013). Pollen and nectar are rewards for pollinators (Fogden & Fogden, 2018). Nectar provides sugars, proteins, minerals, amino acids and antioxidants, while pollen provides protein, carbohydrates, vitamins and minerals (Fogden & Fogden, 2018). Other rewards include oils, resins, breeding sites and chemicals that are used to make pheromones and perfumes, though these are used by select species (Fogden & Fogden, 2018).

1.3 The ecology of *Apis mellifera*

Apis mellifera, the European honeybee, is part of the Apidae family comprising 20,000 species (Donovan, 1980).[1] Honeybees are eusocial, meaning that individuals are part of a caste system which consists of workers, drones and queens (Ketcham, 2020). The role of drones and queens is to reproduce (Ketcham, 2020). Workers are sterile females which perform services for the hive, including cleaning, tending to nurseries,

[1] Throughout the thesis, the terms '*Apis mellifera*' and 'honeybee(s)' are inclusive of subspecies.

guarding, and scouting and foraging for resources, though not all workers develop into foragers (Ketcham, 2020). There are two different types of workers that are involved in foraging; scout bees search for food sources and reticent bees receive information on foraging sources from scout bees (Abou-Shaara, 2014). They forage for nectar, pollen, resin and water (Abou-Shaara, 2014). Visited flowers are visually appealing and nectar is accessible (Ketcham, 2020). The timing of foraging and the flowers foraged on is determined from various clues, including flower colour, shape and scent, alongside the presence of competitors and predators (Ketcham, 2020). When a good foraging location is found, they return to the hive and indicate to fellow workers where the foraging location is by a waggle dance (Ketcham, 2020). The repetitive waggle dance directs other workers to a food source. Honeybees perform a waggle run, circle back to the start of the waggle run, perform another waggle run and circle back to the start of the waggle run on the opposite side (Grüter & Farina, 2009). The number of waggle runs coincide with the resource quality (Grüter & Farina, 2009). This cycle continues which results in a stream of workers travelling to and from the foraging location (Ketcham, 2020). This ends once the flower is pollinated and nectar is no longer produced (Ketcham, 2020). Honeybees have a proboscis which contains a tongue which is used to suck up nectar while foraging (Ketcham, 2020). The rear pair of legs features a cavity surrounded by hairs where pollen adheres to while foraging for food for the hive, which is referred to as a pollen basket (Ketcham, 2020), or corbicula. Because honeybees travel long distances quickly, they use their flight muscles, located between the wings, to produce fast wing beats (Ketcham, 2020). During summer, pollen and nectar are important resources because honeybee colonies rely on pollen and honey (produced from nectar) to survive through the winter (Donovan, 1980). Consequently, honeybees are most present outside the hive during summer and least present in winter (Donovan, 1980). Up to 20 kg of honey is needed through winter and the beginning of spring (Donovan, 1980). Foraging for resources is important to honeybee colony population increase – an excess in resources can result in brood rearing population increase (Donovan, 1980). Similarly, if enough food is present, the worker bee population can go from up to 2,000 workers present in the winter to up to 6,000 workers before summer (Donovan, 1980).

1.4 The role of honeybees in pollination

While foraging, honeybees unintentionally pollinate flowers. Honeybees are polylectic pollinators, whereby they retrieve pollen from a wide variety of plant species while foraging (Soper & Beggs, 2013). Honeybees are grouped within a category of species that fill a 'keystone role', where the species play a highly important function or role in the environment; however, not all species that play a keystone role are a keystone species, as they can easily be replaced (Modlmeier et al., 2013). Honeybees cannot easily be replaced as the extent of their function cannot be fulfilled by other species; hence, they are a keystone species, where a keystone species has a "disproportionally large effect on community dynamics relative to their abundance" (Modlmeier et al., 2013). For example, honeybees can significantly increase fruit set in fleshy fruits such as *Crataegus monogyna* (hawthorn) and *Vaccinium myrtillus* (blueberry), which makes up the majority of the diet of *Ursus arctos* (brown bear) following mating season and before hibernation, where their food intake greatly increases (Cayuela et al., 2011). Despite honeybees being a keystone species, there are differences in the pollinating ability between honeybees and other pollinators. Differences in pollinating ability between pollinators often depends on the plant species or environmental conditions, where differences in pollinator characteristics between species relates to their pollination abilities (Batra, 1995). Some characteristics that influence pollination abilities includes hairiness, flight range, cold tolerance and size (Batra, 1995). Hairiness may dictate how much pollen is collected by a pollinator. For example, in *Rubus idaeus* (raspberry), bumblebees (*Bombus*) were found to carry more pollen grains on their bodies and deposited more pollen grains on *Rubus idaeus* stigmas compared to honeybees (Willmer et al., 1994). It is likely that the difference between pollen collection and deposition was due to hairiness (Stavert et al., 2016). Bumblebees are hairier than honeybees, hence, they may collect and deposit more pollen during flower visitation. Additionally, bumblebees flew greater distances and moved around more than honeybees, which contributes to their ability to better pollinate *Rubus idaeus* (Willmer et al., 1994). Pollinators are limited by temperature, where different pollinators have different temperature tolerances. For example, the foraging activity of pollinators can be limited by low temperatures (Corbet et al., 1993). Low temperature tolerance relates to what time of day that pollinators start foraging and the environments in which they can forage in. For example, several bumblebee species were found to forage at lower temperatures than honeybees, therefore, they may contribute to more

pollination earlier in the morning and later in the evening (Corbet et al., 1993). Additionally, they may be better pollinators in cold environments. The difference in pollinator performance with relation to size had been seen between native bees and honeybees in *Metrosideros excelsa* (Pohutukawa). Native bees were found to contact the stigma of *Metrosideros excelsa* less often than honeybees, presumably because of their smaller size (Schmidt-Adam et al., 2009). The flowers of *Metrosideros excelsa* are relatively large, so the greater size of honeybees means that they were more likely to contact the anthers and stigmas, resulting in a greater chance of successful pollination. These examples indicate that, although a pollinator may be regarded as a keystone species, their pollinating ability compared to other pollinators varies across plant species and environmental conditions.

Pollination by honeybees in natural environments has been studied globally. Honeybees are widespread; they are found throughout Asia, America, Africa, Europe, Australia and New Zealand (Goulson, 2003). They are known to visit both native and introduced plants where they may have a positive, neutral or negative affect. It is widely perceived that honeybees are usually good pollinators of wildflowers (Goulson, 2003), which reflects their generalist nature. For example, a review found that honeybees are effective pollinators of most native plant species in Australia and North America (Butz Huryn, 1997); they were found to be floral parasites of a few plant species, however (Butz Huryn, 1997). In these plant species, pollination by native pollinators would be imperative. In native plants in Spain, Cayuela et al. (2011) found that honeybees significantly increase fruit set in *Crataegus monogyna* and *Vaccinium myrtillus*, which were two out of three plant species studied. In another study, honeybees were found to be as effective as a native bee, *Centris tarsata*, at pollinating native *Anacardium occidentale* (cashew) in South America (Freitas & Paxton, 1998). Although honeybees deposited significantly less pollen grains onto *Anacardium occidentale* stigmas compared to *Centris tarsata*, there was no statistically significant difference between the number of seeds set (Freitas & Paxton, 1998). Honeybees are also known to be pollinators of numerous introduced plants which are often problematic. For example, a Canadian study found that 75 % of pollen collected originated from introduced plants (Stimec et al., 1997). In another Canadian study, honeybees were found to be major pollinators of *Lythrum salicaria* (purple loosestrife) which is a major problem weed (Mai et al., 1992). In Australia, honeybees were found to pollinate *Lupinus arboreus* (yellow bush lupine), an introduced plant species; honeybees, alongside bumblebees, were the main pollinators of the introduced plant species (Stout et al., 2002).

Additionally, honeybees were found to be pollinators of *Phyla canescens* (turkey tangle frogfruit), a problematic introduced plant, native to South America (Gross et al., 2010).

Honeybees are an introduced species in New Zealand; they were introduced via transport of hives from England to Hokianga in 1839 (Hopkins, 1911, as cited in Donovan, 1980). Honeybees are known to visit and pollinate introduced plant species within New Zealand across a range of environments. In protected natural areas, honeybees have been recorded potentially utilising 68 of 158 (43 %) introduced species in New Zealand (Butz Huryn & Moller, 1995). The families with the most plant species that were visited by honeybees were Fabaceae (20.6 %) and Asteraceae (15 %) (Butz Huryn & Moller, 1995). The main types of introduced plants visited were shrubs (35 %), trees (32 %) and herbs (28 %) (Butz Huryn & Moller, 1995). Only about half of the introduced plant species visited by honeybees were considered as problematic (33 %) (Butz Huryn & Moller, 1995). The importance of introduced plant species was categorised as either 'Unknown', 'None', 'Low', 'Medium' or 'High' (Butz Huryn & Moller, 1995). Only one species (*Berberis darwinii*; Darwin's barberry), which was mainly pollinated by honeybees, was considered as high importance to them (Butz Huryn & Moller, 1995). This study concluded that it is unlikely that honeybees greatly contribute to the reproduction of introduced plant species in natural protected areas (Butz Huryn & Moller, 1995). However, there is a possibility that honeybees greatly contribute to the spread of *Ulex europeaus* (gorse), a problematic plant in New Zealand, though the importance of this plant species to honeybees was unknown (MacFarlane et al., 1992). Also, there were numerous influences that were not measured, such as honeybee density, hive proximity and density and synergistic effects, which are important to consider when measuring the impact of honeybees on introduced plants. They are also known to visit introduced plants in montane biomes. They have been found to visit *Echium vulgare* (blueweed), *Marrubium vulgare* (white horehound) and *Rosa rubiginosa* (sweet-briar) (Primack, 1983). However, it is unknown whether or not honeybees pollinate these species. Researchers are concerned that honeybees visiting introduced plant species may be damaging to native plant populations which is a possibility. However, there is a lack of data on honeybee pollination of introduced plant species in New Zealand (Butz Huryn & Moller, 1995; Goulson, 2003), so the role of honeybees in the proliferation of many introduced plants species is unknown.

As honeybees are an introduced species, the effects of honeybees on native plant reproduction may be either positive, neutral or negative and may vary between plant species. This has been seen in native Australian and North American plants (Butz

Huryn, 1997), as previously discussed. When the interactions between introduced species and native species are unknown, we do not know whether they will positively or negatively affect native populations of plants and/or animals which they interact with. This is especially true with threatened species; for example, if honeybees were found to be poor pollinators of threatened plant species and competed against native pollinators which are good pollinators of the plant, then management to promote native pollinator populations could be implemented. To inform such management decisions, we need an understanding of honeybee pollination of native New Zealand plants.

2. Literature review

Although there are studies on pollination of native plants by honeybees in other countries, little research has been done on honeybee pollination of native New Zealand plants. I have found eight studies that either specifically researched pollination of honeybees, provided some degree of information that contributed to honeybee pollination or reviewed the then-current literature.

2.1 Plant species visited by honeybees

In the earliest accessible study on honeybee flower visitation in New Zealand, Heine (1937) observed pollinators of native New Zealand flowering plants to gain a general understanding on the relationship between plants and insects in New Zealand. Heine (1937) recorded flower visitors on a range of native New Zealand plants. Honeybees were observed foraging on the flowers of *Myosotidium* and *Chathamica* species, as well as on *Astelia solandri* (perching lily) and *Gentiana patula* (gentian). Apparently, the pollen found on the body of honeybees was examined to identify if the pollen was collected from the flower it visited or from another flower, however, no methods on this were described and no data was provided. Because only four occurrences of flower visitation by honeybees were recorded and quantitative analyses were not used, this study does not provide much insight on the pollination mechanisms between honeybees and native plants. However, the study does provide some of the earliest recorded observations of native plant visitation by honeybees. These findings may have initiated conversation around honeybee pollination, sparking interest in other

researchers to further investigate honeybee pollination of native plants and ultimately, paving the way for further research.

Primack (1983) researched insect pollination among New Zealand montane flora because there was limited knowledge on this area. Primack (1983) mentions that up until then, only three surveys and one review of pollination systems in New Zealand had been done (one of the surveys was done by Heine, 1937). Researching pollination in montane flora would provide further understanding of pollination systems in New Zealand native plants (Primack, 1983). The study was done at four sites in the South Island; Cass, Craigieburn Mountains, Arthur's Pass National Park and Mount Cook National Park. The elevations across the sites was between 600-1,800 m. Honeybees were observed visiting one native plant species where the anther or stigma was contacted (*Leptospermum scoparium*; Manuka; captured at Cass: elevation 600-800 m). This study indicates that honeybees can forage in a wider variety of climate conditions than expected and could contribute to pollination in montane environments. However, the relative significance of this finding is unknown because ambient temperature and wind speed were not recorded. Furthermore, the methods to assess if stigma and anther contact occurred was not stated. It can be difficult to accurately assess if honeybees contact reproductive structures, so the reliability of this data is unknown. Additionally, only one replication was present. More replications were needed to derive statistical significance from this research. Despite this, this study indicates that honeybees may be able to forage on flowers in rather extreme environments, which provides a greater understanding of the potential of honeybee pollination within New Zealand. This may be especially important for the pollination of plant species in the South Island, where montane and alpine habitats dominate.

Later, Butz Huryn (1995) reviewed 37 New Zealand studies that observed honeybees visiting native plants. Honeybees were found visiting 67 families of native plants. These families consisted of 3 gymnosperms, 6 monocotyledons and 58 dicotyledons. Each family had at least 5 species of plants which honeybees visited and they were found to visit 13 rare or endangered plant species (Butz Huryn, 1995; Cameron et al., 1995); because this study is outdated, those numbers may have increased or decreased depending on the conservation status change of plant species. Although they visit a wide range of native plants, most of the foraging resources (nectar and pollen) come from 12 families (Butz Huryn, 1995), indicating that they may not significantly contribute to the pollination of numerous other visited native plants. Unfortunately, most of the original publications referenced by Butz Huryn (1995) could

not be located. Based on the titles alone, it appears that most studies only observed the occurrence of honeybees visiting flowers. However, the method and results sections provide further insight into the research referenced. Various studies listed numerous native plant species as pollen sources for honeybees, with two studies analysing the pollen found in honey (Harris & Filmer, 1948; Moar, 1985; as cited in Butz Huryn, 1995). Studies providing data on what plant species are a pollen source for honeybees is useful because it may help us understand flower preferences of honeybees, what influences these preferences and how they contribute to the pollination of these plant species. However, the studies that identified pollen from honey (Harris & Filmer, 1948; Moar, 1985; as cited in Butz Huryn, 1995) provides relatively weak evidence that the honeybees collected the pollen directly from the flower source. This is further explained by Butz Huryn (1995), who argued that although pollen found in honey can indicate what plant species the honey came from, however, abiotic factors such as wind and pollen disturbance in nectar can also influence what pollen is collected by honeybees. Therefore, pollen analyses in honey is not definite proof that the pollen was collected from the flowers it visited. Furthermore, most pollen species were identified on the genus level (Butz Huryn, 1995); this provides no insight to what plant species the honeybee collected the pollen from. Butz Huryn (1995) also stated that honeybees may be as or more effective at pollination than native pollinators because of their high floral constancy. This is an important literature review because it highlights how the knowledge of what native plants honeybees visit has expanded since Heine (1937). Furthermore, it indicates that the previous attempts of investigating what pollen is carried by honeybees produces inconclusive data and that the methods used to investigate this are unviable. This encourages researchers to devise other methods of assessing collected pollen. Ultimately, Butz Huryn (1995) concluded that future research needs to be done on the interactions between honeybees and native New Zealand plants and investigate the positive and/or negative impacts of honeybee pollination.

2.2 Flower visitation by honeybees

The first study known to me that investigated flower visitation rates of honeybees on native New Zealand flowers was done by Murphy and Robertson (2000). They did a preliminary study of pollination at Tongariro National Park. A 10-15 minute random walk was conducted where the number and type of insect observed visiting

native *Phormium tenax* (New Zealand flax) flowers was recorded. The results were stated as the number of flower visits per hour. They found that, across all the sites, the visitation rate of honeybees varied from 0.08-1.89 flower visits per hour. This indicates that honeybees have a low visitation rate of *Phormium tenax* in montane biomes. Using visitation rate is less insightful and accurate than count data (Reitan & Nielsen, 2016), so the data may not be as informative as it could have been. However, this research still contributes some insight on honeybee pollination within New Zealand. The greatest contribution of this study is that it is the first within New Zealand to use visitation rate measurements to investigate honeybee pollination of a native plant species. The use of this method highlights the change from gathering observational data to gathering quantitative data in honeybee pollination in New Zealand. Also, this study provided further insight into pollination of honeybees in montane areas and of the plant species *Phormium tenax*, expanding upon findings by Primack (1983).

Later, Burgess et al. (2006) conducted a literature review and a case study on bee visitation to *Peraxilla tetrapetala* (red mistletoe) flowers found on forest edges. The study sites were Lake Ohau and Craigieburn which are in central South Island, where fragmented forest is present. They created three edge categories according to each site which data was collected from (Ohau: interior, edge, isolated; Craigieburn: dense interior, light interior, edge) and, additionally, three weather categories (sun, wind and rain). The number of honeybee individuals visiting flowers was highest at the edge category of the Ohau site, followed by the isolated category; there were no cases of honeybees present at the interior category. At the Craigieburn site, the number of honeybees visiting flowers was highest at light interior category, followed by edge category and dense interior category. At the Ohau site, the visitation rate by honeybees was significantly negatively affected by wind, rain and visitation rate was significantly affected by edge category. At the Craigieburn site, rain had a weak significant effect. The most important finding of this study is that honeybees may contribute to the proliferation of plants at the forest edge and that honeybee visitation rate is significantly affected by wind and rain. This highlights the potential for honeybees to contribute to fragmented forest restoration and what weather conditions may affect visitation rate. This is an important research area as forest fragmentation is prevalent in New Zealand (Ogden et al., 1998). A weakness of this study is that the sample size of honeybees was small. This is probably because the study was not specifically focussing on honeybees. Therefore, those findings may not hold at the honeybee population level. Like the previous studies on honeybee pollination on montane and alpine native flora (Murphy &

Robertson, 2000; Primack, 1983), this study contributes to broadening the scope of honeybee pollination studies. Most importantly, this study utilises count data, which is more insightful and accurate than frequency data (Reitan & Nielsen, 2016) which was used by Murphy and Robertson (2000). The use of count data probably helped pave the way for extracting more insightful and meaningful data that can be compared and contrasted more easily between studies.

The most insightful and potentially most important study yet on honeybee pollination on native plants was done by Schmidt-Adam et al. (2009). They performed a study on pollinators of *Metrosideros excelsa*, including native birds, native bees and introduced bees. In relation to honeybee pollination, the aims of the study was to compare the foraging behaviour between native bees (*Leioproctus* sp.) and honeybees and, from this, assess which bees were more efficient pollinators of *Metrosideros excelsa*. Native bees were studied on Little Barrier Island and honeybees were studied on Whites Beach in the North Island of New Zealand. Pollination efficiency was assessed by measuring time spent per flower, if honeybees foraged for pollen or nectar and if the stigmas were contacted during flower visitation. Schmidt-Adam et al. (2009) found that, on one tree, honeybees only foraged for nectar, whilst on another tree they foraged for pollen and nectar equally. Native bees normally only foraged for pollen; exclusive nectar foraging only occurred 1.9 % of the time. Foraging behaviour of native bees did not appear disturbed by honeybees foraging. The time spent foraging on flowers was not significantly different between native bees and honeybees across four trees, but there was a significant difference on foraging time across two trees at Little Barrier Island. Honeybees contacted the stigma significantly more often than native bees, where honeybees contacted the stigma in 26.6 % of flower visits, whilst native bees contacted the stigma in 9.9 % of visits. In honeybees, the foraging activity is related to the occurrences of stigma contact; in pollen collection, the stigma was contacted in 60 % of flower visits on average, whilst in nectar collection, stigma contact occurred in 15.3 % of flower visits on average. Pollination efficiency was strongly influenced by body size and foraging activity. Native *Leioproctus* bees have a smaller body size (8.3-10 mm) than honeybees (13.5-15.4 mm); the differences in the occurrences of stigma contact is largely due to differences in body size (Schmidt-Adam et al., 2009). This is because honeybees are more likely to contact both the stamen and stigma whilst moving across the flower, whilst native bees often only contact the anthers, limiting pollination occurrence (Schmidt-Adam et al., 2009). However, when honeybees foraged for nectar, stigma contact occurred less often as they pushed through

the filaments to reach the nectar (Schmidt-Adam et al., 2009). One aspect that this study did not analyse was the number of pollen grains and pollen species that were deposited on the stigma. With this information we would have a more complete picture on the pollination of *Metrosideros excelsa* by honeybees. However, this detailed study helps us understand what native plants honeybees may be good pollinators of in native plants that have a similar flower morphology as *Metrosideros excelsa* and depending on what resource the honeybees are foraging for on the plant species. Furthermore, the comparisons between honeybee pollination and native bee pollination indicate that honeybees are better pollinators of *Metrosideros excelsa*; whether or not this is the case for other native plant species is unknown. Because of the level of detail of this study, it paves the way for future detailed research on honeybee pollination of native plants to be conducted. This study is stronger than previous research done on the occurrence of flower visitation, because flower visitation alone does not inherently mean that a plant is being pollinated or pollinated well; this is supported by a study by King et al. (2013), where they found that 40 % of flower visitors were ineffective pollinators. Therefore, it is important to assess other factors such as stigma and anther contact.

2.3 Pollen collection by honeybees

Only one study known to me has researched what pollen was collected during native flower visitation. This is the first known publication to research pollen carried by honeybees after visiting native plants. Pearson and Braiden (1990) investigated what pollen is used by honeybees through the year in the South Island of New Zealand. Pollen was collected via pollen traps. After acetolysis, the pollen species were identified under a microscope. Native and non-native plant species were found to be the main sources of pollen for honeybees in the study site. The authors state that 9 of the 21 species in which honeybees collected pollen from were native. Of these, the percentage of pollen collected from *Nothofagus solandri* (black beech) (7.9 %), *Discaria toumatou* (matagouri) (32.4-43.2 %), *Coprosma* sp. (81.9-83.7 %) and *Pimelea* sp. (0.9 %) was given. The nine native plant species provided 90 % of pollen collected between September and October. The quantity of pollen trapped in different weather conditions, such as temperature, windspeed, wetness and solar radiation, was provided. The relationship between these weather conditions and pollen collection was not analysed, though the authors did discuss some of the collected data. For example, Pearson and Braiden (1990) stated that, despite most days not being ideal for honeybee flight, there

were only six days where pollen was not collected. This means that the occurrence of honeybee foraging is rather flexible when in certain environments. The findings from this study gives us an idea of the host species preferences of honeybees in this environment and how weather conditions may affect pollen collection. Although pollination was not the focus of this study, it contributed to the knowledge of pollination of native plants by honeybees.

2.4 Current state of knowledge of honeybee pollination of native New Zealand plants

A review by Howlett and Donovan (2010) analysed the current impacts and potential impacts of introduced pollinators. This review points out that more research on honeybee pollination on native plants needs to be done as we have limited knowledge on this. The authors fail to acknowledge the study done Schmidt-Adam et al. (2009), however, this is probably due to the publication dates being close together. As previously discussed, past work encompasses what native plants honeybees visit (including in montane environments), flower visitation rates of *Phormium tenax*, honeybee visitation of *Peraxilla tetrapetala* at forest edges, honeybee pollination of *Metrosideros excelsa* and pollen collection from nine native species. It is clear that currently, with exception of *Metrosideros excelsa*, there are knowledge gaps in native plant pollination by honeybees, specifically with pollination measurements across plant species. This includes data on flower visitation counts, foraging behaviour, foraging time, stigma and anther contact, pollen collection, pollen deposition and seed set. These are vital components that need to be researched to understand pollination of a plant species.

3. Research aim

The role of honeybee pollination in native plants remains poorly understood. It is important to extend upon our vague understanding of native pollination performed by honeybees as the findings could contribute to the conservation of native New Zealand plants, whereby conservation planning and resource allocation can be guided more effectively. Therefore, the aim of my study was to gain a more detailed understanding of honeybee pollination in native New Zealand plants. To achieve this, I measured

components of honeybee pollinator performance, including flower visitation, visitor volume and pollen collected during flower visitation. These measurements will help us better understand the role of honeybee pollination in native New Zealand plants. Additionally, this will provide a stepping stone for future studies.

4. Methodology

4.1 Study site

My research was conducted at the Auckland Botanic Gardens (ABG) (37.0086 °S, 174.9058 °E). The Botanic Gardens provided an ideal study site because numerous native plant species were available in one, easily accessible area. My study sites within the ABG were located along the native plant I.D. trail and within the sections of the harakeke collection and threatened native plants. The area of the study sites covered approximately 9,685 m². Data was collected during November and December 2019; across this period, the average minimum ambient temperature ranged from 9.6-11 °C, while the average maximum ambient temperature ranged from 25-26 °C. The total precipitation ranged from 40.2-51 mm. These data were obtained from MetService (2019) in the historical data section, collected at the Manukau weather station (distance from ABG: 9 km).

4.2 Initial readings

Prior to beginning my study, I reviewed studies with methods and concepts of honeybee pollination to gain background knowledge on the topic, though not all of the final methods and ideas used in my study derived from the studies read here. There were many studies on flower visitation and foraging behaviour. The concepts and methods of visitation rate, visits per tree and stigma contact was used in several studies (Abrol, 1988; Bosch & Blas, 1994) which sparked the development of methods to measure flower visitation. Abou-Shaara (2014) summarised papers that measured foraging behaviour, such as how temperature affects honeybee foraging. This led me to include ambient temperature and, additionally, wind speed into my study. Similarly, numerous studies have assessed or outlined the concept of pollen load by honeybees (Free & Williams, 1972; Horskins & Turner, 1999). I modified this concept to focus on pollen collected on the body of honeybees, which was more relevant to my research. The use

of measuring pollen quantity was derived from Delaplane et al. (2013). All laboratory procedures involving pollen processing and identification followed methodology papers by Jones because of their great detail and clarity (Jones, 2012a, 2012b, 2014). Acetolysis was used to count and identify pollen grains. Acetolysis is the best method to use to recover and identify pollen as the chance of pollen loss from the honeybee sample is reduced and pollen identification characteristics are more easily identified (Jones, 2014). This is because acetolysis dissolves most organic debris and tissue and dissolves lipids, carbohydrates and proteins from the pollen surface (Erdtman, 1960; Low et al., 1989, as cited in Jones, 2014). Throughout my research, I did not use controls because I was researching the natural occurrence of honeybee pollination. This has been done in other studies (Adler & Irwin, 2006).

4.3 Understanding the concept of flower visitation

Pollination efficiency is a confusing term because it has been called numerous names in the scientific community and includes different measurements, depending on what researchers are investigating. For example, in a review by Ne'eman et al. (2010), 'pollinator/pollination efficiency' had over thirty different definitions which makes it difficult to compare data between studies. Therefore, Ne'eman et al. (2010) suggested a modular approach called 'pollinator performance', which focusses on visit frequency, pollen deposition and pollinator contribution to seed set. Because my study does not encompass all measurements of pollinator performance, I kept the terms simple and true to what was being measured so that the data can be combined or compared with data of future research. For example, I used the term 'flower visitation' rather than visitation efficiency, as the term 'efficiency' is not clearly defined in the existing literature. With this, I hope to encourage the use of the modular approach outlined by Ne'eman et al. (2010).

In my research, flower visitation refers to a honeybee intentionally contacting or foraging on a flower. Although the intention of a honeybee cannot be measured, for simplicity sake, incidents where bees were obviously knocked into flowers by wind or another force which do not appear to be purposeful were not considered as the occurrence of true flower visitation and hence not included in my study. Traditionally, flower visitation or equivalent is measured using a per unit time format. For example, Abrol (1988) used a pollination efficiency proxy which included flowers visited per minute, stigmas touched per minute and amount of time spent on each flower. Visitation

frequencies are commonly used, but a recent study comparing visitation frequencies and count data measurements found that visitation frequencies are less insightful and less accurate relative to count data (Reitan & Nielsen, 2016). The study states that count data increases the chance of detecting effects compared to frequency measurements (Reitan & Nielsen, 2016), therefore, I modified the Abrol (1988) pollination efficiency method. My flower visitation method included four categories: i) number of flowers visited per individual, ii) time spent at each flower type per individual, iii) if the stigma, anthers and stamens were contacted per individual and iv) behaviour exhibited during flower visitation per individual. It was important to determine if individuals were contacting the anthers and stigma as this is necessary information for assessing effective pollination (Shivanna & Tandon, 2014), while stamen contact indicates the likelihood that honeybees contacted the anthers. Contact occurrence was recorded as binomial data with levels 'likely' or 'unlikely'. These terms were used because it was difficult to ascertain definitive contact occurrence because of factors such as flower size, stigma/anther size and movement speed of honeybees. The behavioural data was collected during and between the flower visits and was assigned to one of the following categories: 'foraging', 'contact' or 'flying'. The term 'foraging' was used for instances when honeybees landed on the flower and moved around on the flower in a searching manner, presumably foraging for nectar and/or pollen. 'Contact' was used to describe when honeybees came in contact with the opening of the flower but did not land or move around on the flower. 'Flying' describes the honeybee movement between and around flowers without contacting flower organs. I introduced these categories to help determine the occurrence of honeybee pollination.

4.4 Understanding the concept of visitor volume

Visitor volume refers to the number of honeybees observed visiting the flowers of a plant species during a single time period. Plant species with less than 5 visitors per observation period were classed as having a 'low' visitor volume; species with 5-10 visitors per observation period were classed as having a 'moderate' visitor volume; species with more than 10 visitors per observation period were classed as having a 'high' visitor volume. Visitor volume was included as a metric because it enhances our understanding of what plant species honeybees are most attracted to, and therefore, what plant species may benefit the most from honeybee visitation.

4.5 Categorising flower and plant characteristics

Data on flower and plant characteristics was collected as additional information for the flower visitation and visitor volume measurements. This included flower form, flower colour, flower type, plant type, plant size, flower diameter, flowering and fruiting dates, plant reproductive system, known mode of pollination, distribution and natural or preferred habitat. Most of these are self-explanatory; however, I will expand on flower form, flower type, plant type and plant size. Flower form was categorised as 'flat' or 'tubular'. 'Flat' flowers had an open, relatively flat surface where the base of the inside of the flower was easily accessed by honeybees, whereby the anthers/stigma may be prevalent (Figure 1A). 'Tubular' flowers had an elongated, tube-like shape, often with prevalent anthers/stigma (Figure 1B). These distinctions may help us understand the likelihood of honeybees collecting or depositing pollen grains within a flower, as honeybees exhibited different foraging behaviours between these flower forms. Flower type was categorised as 'singular' or 'grouped'. During sampling, singular flowers (can include capitula) were easily distinguishable from one another and were not spaced closely together, while grouped flowers were not easily distinguishable from one another and were spaced very closely together, and were often very small, so it was difficult to determine when a honeybee contacted separate flowers (Figure 2). Therefore, grouped flowers were classified as one flower. Flower type helps us understand how many flowers they may forage on a particular plant species. For example, if the flowers were arranged in a group, then, from my observations, honeybees were more likely to spend time foraging and could move more quickly between individual flowers within the group. Plant type encompassed 'woody' and 'non-woody' plants. Woody plants included trees and shrubs and non-woody plants included herbs and flax. Including these categories in the data analysis helps understand pollination patterns of honeybees on different plant types. Plant size referred to the height of a plant; plants at or below eye level (approximately at or below 1.5 m) were classed as 'small' plants, whilst plants above eye level (above 1.5 m) were classed as 'large' plants. Plant sizes were specific to the time of data collection.

Figure 1. Examples of flower forms. ‘A’ is an example of a ‘flat’ flower (*Geranium solanderi* (native geranium)) and ‘B’ is an example of a ‘tubular’ flower (*Phormium tenax*).

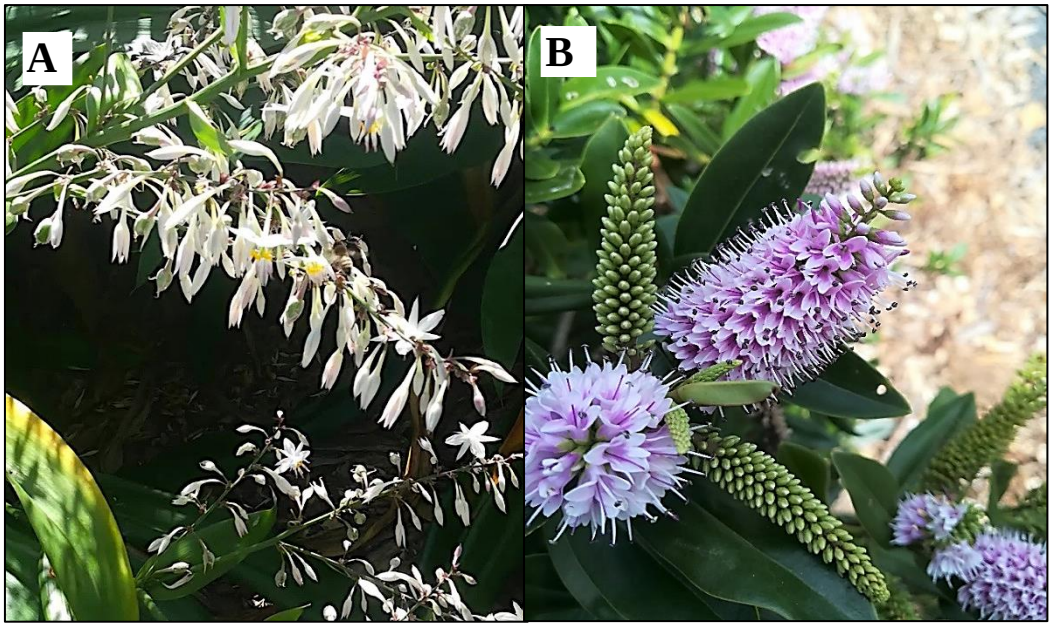

Figure 2. Examples of flower type distinctions used. ‘A’ is an example of ‘singular’ flowers (*Arthropodium cirratum* (New Zealand rock lily)) and ‘B’ is an example of ‘grouped’ flowers (*Hebe pubescens* subsp. *sejuncta* (Hebe)). Note the difference in photo proximity whereby individual flowers within each flower type are easily distinguishable from one another.

4.6 Understanding the concept of pollen collection

In my research, pollen collection refers to the pollen that was on the body of a honeybee whilst foraging on native plants. I focused on pollen species present and pollen quantity. I categorised collected pollen as either ‘conspecific’ or ‘heterospecific’, where ‘conspecific’ pollen was of the same species as the plant species honeybees were captured on and ‘heterospecific’ pollen was of a different species from the plant species honeybees were captured on. This can tell us how much contact honeybees had with the reproductive structures of flowers across plant species, and therefore, the chance of successful pollination. The basis for this extrapolation comes from a study by Howlett et al. (2011), where the pollen collected by honeybees (among other bees) was used to estimate the pollen deposition on the stigmas of *Brassica rapa* var. *chinensis* (bok choi). Howlett et al. (2011) found that the mean estimated body pollen and mean pollen deposition was highly correlated. Therefore, there is a high chance that collected pollen is deposited onto visited flowers.

4.7 Practicing and improving data collection methods

Data collection techniques were tested, practiced and improved before beginning the official data collection. Pollen identification and intertegular span measurements were practiced and improved over 3.5 weeks. Initially, I planned to use an anemometer for recording the ambient temperature and wind, however, the measurements were also constantly changing so it was difficult to get an accurate reading before each honeybee capture; therefore, MetService (2019), a national weather service was used, where the weather station was located in Manukau (distance from ABG: 9 km). Dummy honeybee samples were caught on wildflowers and a lemon tree near my house and were killed by freezing (Human et al., 2013). Trial and error showed that killing honeybees with ethyl acetate was faster and more accessible as freezing was not possible in the field. To minimise pollen loss during handling, I held the honeybee wing with forceps and removed the corbiculae and proboscis without touching any other body part. The honeybee was then placed directly into an Eppendorf tube (ET). In the lab, the LM5 acetolysis method by Jones (2012b) was applied to the dummy samples. Through trial and error, numerous modifications were made. For example, the original LM5 method by Jones (2012b) says to remove the wings and repeat the previously conducted ethanol washes, however, because honeybee wings did not cover the body and were relatively

small, this step was removed. I practiced the modified method until I felt comfortable in my ability to accurately perform it. At the ABG I practiced measuring flower visitation over two days. Originally, I planned to record all the observational data during the live observation periods on site, however, under field conditions this proved too difficult to achieve, so I recorded flower visitation measurements using the camera on my mobile phone. I then reviewed the dummy footage on my laptop and practiced recording the flower visitation measurements, which proved to be more reliable and accurate than the originally envisaged live method. This also made it possible to review the observations multiple times if necessary.

5. Methods and materials

5.1 Measuring flower visitation

A randomly selected honeybee visitor was observed by filming and following the individual until the individual was no longer on the plant or could no longer be followed. Immediately before each recording, and on the day of the recording, the wind speed (km/h) and ambient temperature (°C) was taken from MetService (2019) (in Manukau – distance from ABG: 9 km). Recordings were from 30 seconds to 9:30 minutes in length. Though recordings less than 30 seconds only occurred a few times, as revealed in pilot trials, recordings less than 30 seconds long yielded insufficient data on flower visitation. During my study, honeybees did not forage for longer than 9:30 minutes, hence the upper time limit. To minimise disturbance by human presence, I kept at the furthest distance possible from the honeybees I was observing. This process was repeated up to ten times on each plant individual where honeybees were found visiting. I recorded 138 visitations from 16 plant species. Next, I reviewed the footage on my laptop and measured the number of flowers visited, behaviour, length of behaviours, if the stamens, stigma, anthers and were contacted and flower and plant characteristics (flower form, flower type, plant type, plant size). The length of behaviours was measured using a stopwatch.

5.2 Measuring visitor volume

During each observation, I recorded the number of honeybee visitors at each plant species following the ‘low’, ‘moderate’ and ‘high’ criteria. Other data collected

includes flower and plant characteristics (flower colour, flower form, flower type). I recorded the visitor volume for 17 plant species.

5.3 Measuring pollen collection

Measuring pollen collected by honeybees was a two-stage process starting with the capture of the honeybee individuals followed by assessing the pollen in the lab. Honeybee capture occurred after flower visitation recordings were completed because honeybee capture could change their behaviour during flower visitation, influencing the accuracy of the results of the pollen collected. Honeybee individuals observed foraging on native flowers were captured in a jar with a cotton pad soaked in ethyl acetate at the bottom and a cotton pad on top (Carreck et al., 2013; Horskins & Turner, 1999; Howlett et al., 2011). Date and time of capture, the plant species they were captured on and wind speed (km/h) and temperature (°C) from MetService (2019) (in Manukau – distance from ABG: 9 km) was recorded. After 1-2 minutes, the dead honeybee was removed from the jar (Howlett et al., 2011) by the wing using forceps. To exclude any pollen material that bees may consume (Donovan, 1980; Jones, 2012b), the corbiculae and proboscis was removed before being stored (Jones, 2012b). Between each collected sample, the scissors and forceps were cleaned using individual ethanol soaked tissues and the cotton pad on top of the ethyl acetate soaked cotton pad was replaced. This was done to minimise cross-contamination between handling samples. This process was repeated up to ten times on each plant species where honeybees were found visiting. In total, I collected 93 samples from 13 plant species. I was not able to collect 10 samples from every plant because honeybees stopped visiting some plants throughout the data collection period.

Next, the samples were assessed in the lab according to a method described by Jones (2012b), which was slightly modified, as previously described. Firstly, an acetolysis mixture (90 mL acetic anhydride: 10 mL sulphuric acid) was prepared. The samples were then placed onto a vortex stirrer for 30 seconds so that the ethanol repeatedly covered the insect. Using a micropipette, the ethanol was immediately transferred into centrifuge tubes (CT) that were to be used for external pollen recovery assessment. Following this, 1.5 mL of ethanol was placed back into the ETs after each wash. These steps were repeated three times. Next, the honeybee samples were stored in an ET so that intertegular span could be measured. The CTs were centrifuged (4,000 rpm for 5 minutes). I removed 4.5 mL of the ethanol from each CT and the remaining

0.5 mL of the ethanol and pollen mixtures were resuspended and transferred into ETs. Next, 1 mL of glacial acetic acid was added, vortexed (15 seconds) and centrifuged on a mini centrifuge (13,000 rpm for 2 minutes). One mL of glacial acetic acid and ethanol mixture was removed. Next, 1 mL of the acetolysis mixture was added and vortexed. The ETs were placed into a hot bath at 95 °C for 25 minutes, leaving the lids open. The mixture was mixed periodically with a micropipette. After acetolysis, the samples were mixed on a vortex stirrer followed by centrifugation. One mL of the acetolysis mixture was removed and 1 mL of glacial acetic acid was added; the samples were vortexed and centrifuged. One mL of the glacial acetic acid mixture was removed. Next, 1 mL of distilled water was placed into the ETs and vortexed and centrifuged; 1 mL of the mixture was removed and the process was repeated three times. Next, 1 mL of ethanol was placed into the ETs and the mixture was vortexed, centrifuged and removed. I added 0.15 mL of Safranin O and 0.85 mL of ethanol to the ETs; the mixture was vortexed for 15 seconds and centrifuged. One mL of the mixture was removed and 1 mL of ethanol was added. The mixture was vortexed and centrifuged. Then, 1.5 mL of the mixture was removed, leaving approximately 0.1 mL of the mixture to which 4 drops of glycerine were added and the ETs were then placed on a hot plate for 1.5 hours at 40 °C and were vortexed periodically. Finally, after vortexing the samples, one drop of the sample was placed onto a microscope slide with a cover slip, sealed with two layers of clear nail polish and labelled.

The pollen quantity and species abundance measured were relative measures because only one drop of each sample was analysed, and pollen quantity and species abundance was not calculated for the entire sample. Relative pollen quantity and relative species abundance was recorded using a Leica ICC 50HD camera and Leica Application Suite (version 3.4.0). I followed the microscope slide evaluation method described by Jones (2012a), with a few modifications: according to the computer screen, the slide was aligned so the left corner of the cover slip was in view. I moved the slide from left to right photographing all pollen in the field of view at 100 × magnification; pollen grains that had a different morphology were photographed in 400 × magnification. In the case of species with high relative pollen quantity, the pollen was photographed at 40 × magnification with pollen grains that had a different morphology at 400 × magnification. When I reached the right edge of the cover slip, a reference point at the bottom of the view on the computer screen was made and I moved the top of the view to that reference point, so that a new section of the microscope slide could be evaluated. I moved the slide from right to left and repeated the above method until

the entire slide was evaluated. For pollen grains that were partially at the top of the view, according to the above method, I positioned the camera so the entire grain was visible and then returned it to the original position. Other pollen grains that were partially in view were accounted for naturally because of the microscope slide evaluation method used.

Next, I grouped the photographs of pollen grains by similarity to form morphospecies (i.e. Species A, Species B…). The number of pollen grains per morphospecies was counted, giving relative pollen species abundance and, consequently, relative pollen quantity per sample. Pollen grains that were obscured from view were not included in the relative pollen quantity and relative pollen species abundance measurements. Pollen grains touching or were near the edge of the cover slip were included in the measurements. The most abundant morphospecies from each plant species in which honeybees were captured on was identified by an experienced palynologist from Massey University via photographic images. This was because in most cases, the most abundant pollen species was from the plant species visited. The only exception to this was with *Muehlenbeckia astonii* (shrubby tororaro), where all pollen grains were identified. This was because the most abundant pollen grains were not from *Muehlenbeckia astonii*.

5.4 Data analyses

5.4.1 Key data removal and manipulation

All statistical data analyses were done using the free statistical software and programming language R (3.5.2, R Development Core Team, 2018). Some data was excluded or slightly modified during data analyses. Data excluded from analyses included: flower diameter, flowering and fruiting dates, known mode of pollination, plant distribution, natural or preferred habitat of plants, flower form, plant type and contact occurrence of stigmas and anthers. Flower form and was excluded from the analyses because there were not enough replications of ‘tubular’ flowers (< 3). Similarly, plant type was excluded because there were not enough replications of grouped flowers in non-woody plants (< 3). The contact occurrence of stamens, stigma and anthers was not included in the analyses because it was ultimately too difficult to accurately determine the likelihood of stamen, stigma and anther contact in most cases, particularly because of small flower size in combination with the movement speed of

honeybees. Therefore, this was removed from the analyses to prevent inclusion of inaccurate data. However, the determination of the number of conspecific pollen grains is a strong indication that the anthers were contacted during flower visitation. Honeybees also have a high occurrence of stigma contact and pollen deposition (Howlett et al., 2011; Thomson & Goodell, 2001), so in most cases, there is a good chance that stigmas were contacted during flower visitation. The remainder of supplementary data was not included in the analyses because there was insufficient data available on these. The category names of foraging behaviour were changed during analyses because it provided more accurate representation of the data. Foraging behaviour was labelled as 'foraging' and 'not foraging', whereby 'not foraging' was inclusive of 'flying' and 'contact' behaviours.

5.4.2 Number of flower visits

To prepare the data, the number of flower visits where the honeybee was foraging was selected. As flower visits could only be measured on one plant individual per plant species, values were averaged per plant to avoid pseudoreplication. For the same reason, plant species could not serve as an explanatory variable. Because multiple species shared the same flower type and plant size, I used those variables as predictors to ensure sufficient replication. A generalised linear model (GLM) (R Core Team, 2020) with a Gaussian error distribution was used to analyse the average number of flower visits. The model contained flower type, plant size and their interaction as explanatory variables, whose significance was assessed using likelihood ratio tests (drop1() command in R). The standard error was calculated and plotted alongside the sample data.

5.4.3 Foraging and non-foraging time

A generalised linear mixed effects model (GLMM) was fitted with Template Model Builder (TMB) (R package *glmmTMB*, Brooks et al., 2017), with 'observation' as the random term to account for pseudoreplication. GLMM models with Poisson, negative binomial error ('nbinom2') (R package *glmmTMB*, Brooks et al., 2017) and Gamma (with 'log' link) distributions were run and compared using the Akaike's Information Criterion (AIC), which indicated that the model with the Gamma distribution was superior. The overall significance for the interaction between plant size,

flower type and behaviour (foraging and not foraging) was assessed using a likelihood ratio test (drop1() command in R). An additional GLMM model without the non-significant three-way interaction was fitted with TMB and a Gamma distribution to assess the overall significance of the following interactions: plant size × flower type, plant size × behaviour and flower type × behaviour, whose significance was assessed using likelihood ratio tests (drop1() command in R). There was a significant interaction between plant size × flower type and flower type × behaviour, which I followed up with a *post-hoc* test. I conducted pairwise comparisons between the variables and adjusted the resulting *p*-values for multiplicity using the Benjamini and Hochberg (1995) method (R package *emmeans,* Lenth, 2020). The standard error was calculated and plotted alongside the sample data.

5.4.4 The effects of ambient temperature and wind speed on foraging time

A generalised additive model (GAM) (R package *mgcv*, Wood, 2011) was fitted with a Gamma distribution to confine the lower bound of the mean model fit and its confidence interval to values above zero, as some values of the lower confidence interval bounds were below zero when assuming normally distributed errors. The ideal number of knots for the smoothing term was determined using the estimated degrees of freedom and the generalised cross-validation criterion as indicators (Wood, 2011). The significance of the smooth term for ambient temperature (°C) was obtained from the summary of the GAM model. The standard errors and model predictions were calculated and plotted alongside the sample data. The same method described was used to assess the relationship between foraging time and wind speed (km/h).

5.4.5 Flower visitor volume

Cumulative link models (CLM) (R package *ordinal*, Christensen, 2019) with different thresholds – ‘flexible’, ‘symmetric’, ‘symmetric2’ and ‘equidistant’ – were compared using an AIC-based comparison. The AIC values were within 2 units of each other, so a CLM model with the threshold set as ‘flexible’ was used. The overall significance of the interaction term between flower type and colour and their main effects was assessed using likelihood ratio tests.

5.4.6 Relative number of pollen grains collected

A GLMM was used to assess the relationship between the relative number of pollen grains collected by honeybees on plant species. Firstly, plant species that had less than three honeybee individuals captured on its flowers were removed (*Alectryon excelsus* subsp. *grandis* (titoki), *Carpodetus serratus* (marbleleaf), and *Pimelea prostrata* (Strathmore weed). The GLMM based on TMB was fitted with 'sample' as the random term to account for pseudoreplication and negative binomial error distribution (R package *glmmTMB*, Brooks et al., 2017). GLMM models with Poisson and negative binomial error distributions ('nbinom2') (R package *glmmTMB*, Brooks et al., 2017) were run and compared using the Akaike's Information Criterion (AIC), which indicated that the model with the negative binomial error distribution was superior. The overall significance for the difference in the relative number of pollen grains carried by honeybees whilst visiting different plant species was assessed using a likelihood ratio test. There was a significant interaction, which I followed up with a *post-hoc* test. I conducted pairwise comparisons between plant species and adjusted the resulting *p*-values for multiplicity using the Benjamini and Hochberg (1995) method (R package *emmeans*, Lenth, 2020). The model predictions and standard errors were calculated and plotted alongside the sample data.

5.4.7 The effects of ambient temperature and wind speed on pollen collection

A GAM (R package *mgcv*, Wood, 2011) was fitted with a Gamma distribution to confine the lower bound of the confidence interval to values above zero, as some values of the lower confidence interval bounds was below zero. The ideal number of knots for the smoothing term was determined using the estimated degrees of freedom and the generalised cross-validation criterion as indicators (Wood, 2011). The significance of the smooth term for ambient temperature (°C) was obtained from the summary of the GAM model. The standard errors and model predictions were calculated and plotted alongside the sample data. The same method described was used to assess the relationship between pollen collected and wind speed (km/h).

5.4.8 Relative number of conspecific and heterospecific pollen grains collected

A GLMM was used to assess the relationship between the relative number of conspecific and heterospecific pollen collected across plant species. Firstly, plant

species with less than three honeybees captured whilst visiting the plant individual was removed (*Alectryon excelsus* subsp. *grandis*, *Carpodetus serratus* and *Pimelea prostrata*). *Hebe* sp. was also removed because I was unable to obtain high quality images of the pollen grains due to unforeseen circumstances, so they were unable to be identified. Next, a GLMM based on TMB was fitted with 'sub.sample' as the random term to account for pseudoreplication (R package *glmmTMB*, Brooks et al., 2017). An AIC-based comparison of GLMM models with 'poisson' and negative binomial error distribution ('nbinom2') (R package *glmmTMB*, Brooks et al., 2017) identified the latter as the better model. There was a significant relationship between plant species and relative pollen species abundance which was assessed using likelihood ratio tests (drop1() command in R). I followed this with a *post-hoc* test, where pairwise comparisons of relative pollen species abundance and plant species were performed (R package *emmeans*, Lenth, 2020) and the *p*-values adjusted for multiple testing using the Benjamini and Hochberg (1995) method. The standard errors were calculated and plotted alongside the sample data.

6. Results

6.1 Flower visitation

6.1.1 Number of flower visits

The interaction between the average number of flower visits, plant size and flower type was not statistically significant (L = 0.56, df = 1, p = 0.45, n = 136) (Figure 3). The interaction between the average number of flower visits and plant size was statistically significant (L = 5.71, df = 1, p = 0.01), but the interaction between the average number of flower visits and flower type was not statistically significant (L = 0.46, df = 1, p = 0.49). On average, small plants had 2.7 times more flower visits than large plants. Honeybees visited between 5 ± 2 (mean SE) and 18 ± 5 flowers during each visitation session (Figure 3).

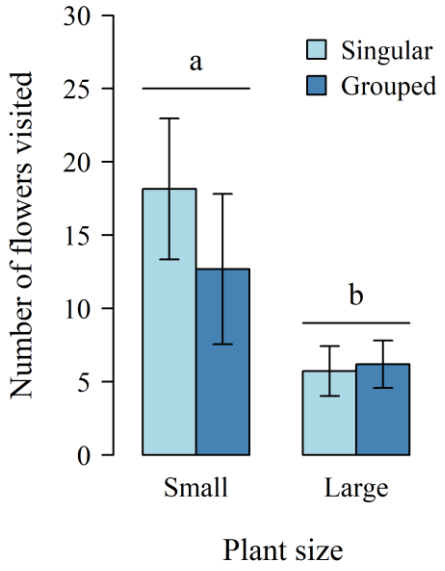

Figure 3. The interactive effect of plant size (small or large) and flower type (singular or grouped) across the average number of flowers visited by honeybees per visitation session (n = 136). The standard errors are indicated by vertical black bars. Different lower-case letters indicate statistically significant differences.

6.1.2 Foraging and non-foraging time

The interaction between plant size, flower type and behaviour was not statistically significant (L = 0.43, df = 1, p = 0.5; n = 133) (Figure 4). The interaction between plant size and flower type was

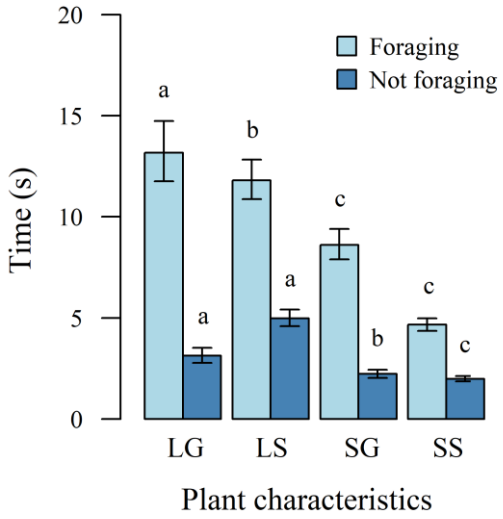

Figure 4. The interactive effect of plant size and flower type (LG = large, grouped; LS = large, singular; SG = small, grouped; SS = small singular) on the average time (s) honeybees spent foraging and not foraging on the flowers of native New Zealand plants (n = 133). The standard errors are indicated by vertical black bars. Different lower-case letters indicate statistically significant differences, which are independent of each other between foraging and not foraging behaviours.

statistically significant (plant size × flower type interaction; L = 9.462, df = 1, p = 0.002; n = 133), whereby the interaction between small plants and flower type was statistically significant (p < 0.005, df = 3509), but the interaction between large plants and flower type was not statistically significant (p = 0.19, df = 3509). Additionally, there was a significant interaction between flower type and behaviour (overall significance: L = 106.27, df = 1, p < 0.001), whereby the interaction between flower type and foraging behaviour was statistically significant (df = 3511, p < 0.001), but the interaction between flower type and non-foraging behaviour was not statistically significant (df = 3511, p = 0.30). The interaction between plant size and behaviour was not statistically significant (L = 0.314, df = 1, p = 0.57). Honeybees spent more time foraging than not foraging across all plant sizes and floral characteristics (Figure 4). On average, honeybees spent the most time (rounded to the nearest second) foraging on large plants with grouped flowers (13 ± 1.4 seconds, mean *SE*), followed by large plants with singular flowers (12 ± 0.9), small plants with grouped flowers (9 ± 0.7) and small plants with singular flowers (5 ± 0.3). The average time spent not foraging was similar across all floral characteristics and plant sizes (1-3 seconds), however, the non-foraging time was noticeably higher in large plants with singular flowers (5 seconds) (Figure 4).

6.1.3 The effects of ambient temperature and wind speed on foraging time

The interaction between average foraging time and ambient temperature (°C) was statistically significant ($F_{13,113} = 4.05$, $p < 0.001$, $n = 126$) (Figure 5). Most honeybees foraged above 20 °C (Figure 5A). Generally, honeybees foraged for less than 20 seconds on average; however, across the range of temperatures recorded, a small proportion of honeybees foraged several times longer (Figure 5A). Similarly, the interaction between average foraging time and wind speed (km/h) was statistically significant ($F_{13, 113} = 2.83$, $p = 0.003$, $n = 126$). Most honeybees foraged at wind speeds below 20 km/h (Figure 5B). Foraging time was highest when the wind speed was below 20 km/h and beyond this threshold, average foraging time dropped abruptly to less than 10 seconds (Figure 5B).

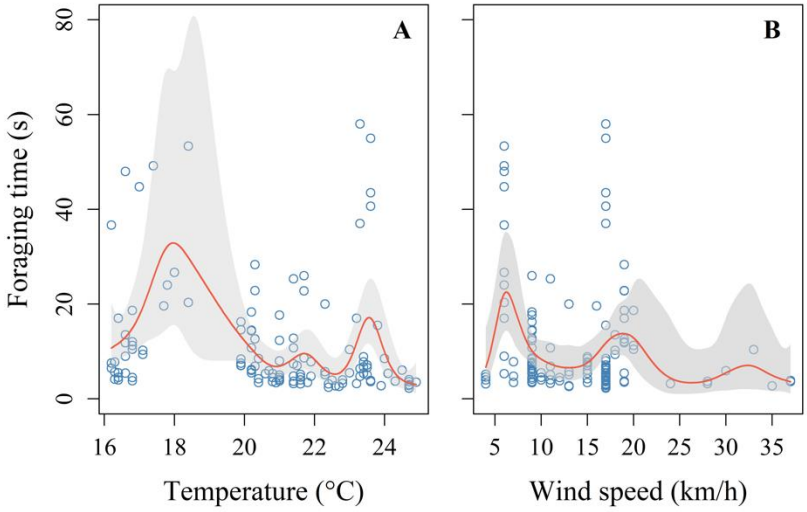

Figure 5. The effect of temperature (°C) (A) and wind speed (km/h) (B) on the foraging time (s) of honeybees per visitation session ($n = 136$). The standard errors are indicated by grey shading which was used to calculate the 95% confidence interval bounds. The model predictions are indicated by red lines.

6.2 Flower visitor volume

The interaction between visitor volume, flower colour and flower type was not statistically significant ($L = 0.04$, $df = 1$, $p = 0.84$, $n = 17$) (Figure 6). The interaction between visitor volume and flower type was not statistically significant ($L = 0.04$, $df = 1$, $p = 0.83$) and the interaction between visitor volume and flower colour was not statistically significant ($L = 0.58$, $df = 1$, $p = 0.44$). The model predictions only noticeably differ from the sample data in WS (white singular) and NWS (non-white

singular) flower groups (Figure 6B). In the sample data, there were no plant species with WS flowers that had a moderate volume of visitors but my model predicted a moderate volume of honeybee visitors for 23 % of plant species. In the model predictions, plant species with NWS flowers had a reduced occurrence of a moderate volume of visitors and an increase in the occurrence of high and low visitor volumes. When comparing the sample data and model predictions, plant species with NWS flowers appeared to have had the greatest number of visitors, though this was not statistically significant (Figure 6).

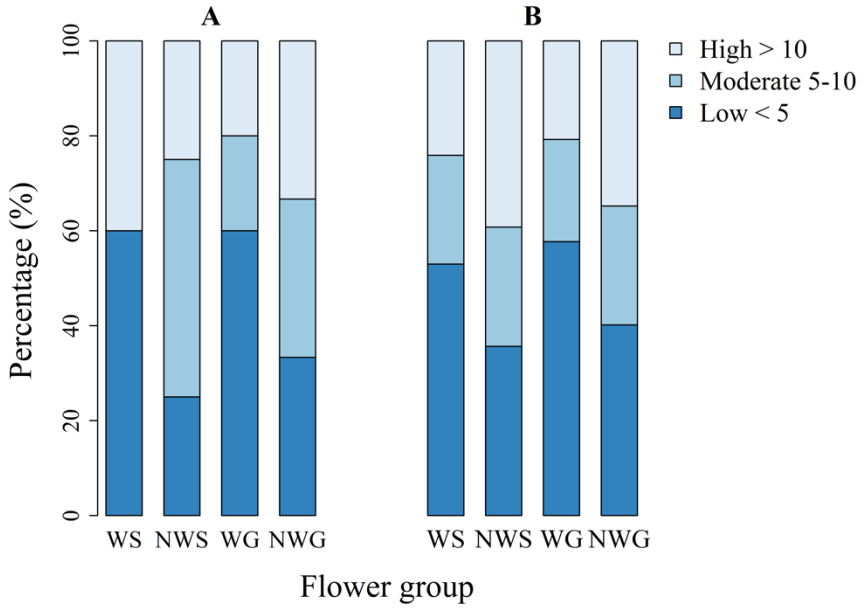

Figure 6. The interactive effect of flower group (n = 17) (WS = white singular, NWS = non-white singular, WG = white grouped, NWG = non-white grouped) on the visitor volume of honeybees (low < 5, moderate = 5-10, high > 10). 'A' is the original data and 'B' are the model predictions.

6.3 Pollen collection during flower visitation

6.3.1 Relative number of pollen grains collected

The interaction between the relative number of pollen grains collected by honeybees and the plant species in which honeybees were captured on was statistically significant (significant relative pollen density × plant species interaction; L = 30.4, df = 9, p < 0.001, n = 74) (Figure 7). Within the one drop sampled, on average, honeybees collected approximately 1,000 conspecific pollen grains from most plant species (Figure 8). Within the one drop sampled, honeybees visiting flowers of *Muehlenbeckia astonii* collected the lowest number of pollen grains (31 ± 8, mean *SE*, n = 10), whilst

honeybees visiting flowers of *Kunzea sinclairii* (Great Barrier Island kanuka) collected the highest number of pollen grains (3118 ± 713, mean *SE*, $n = 4$) (Figure 7A). For most plant species, the standard error increased in the model predictions, indicating that the true population mean occurs in a greater range than suggested by my sample data (Figure 7B).

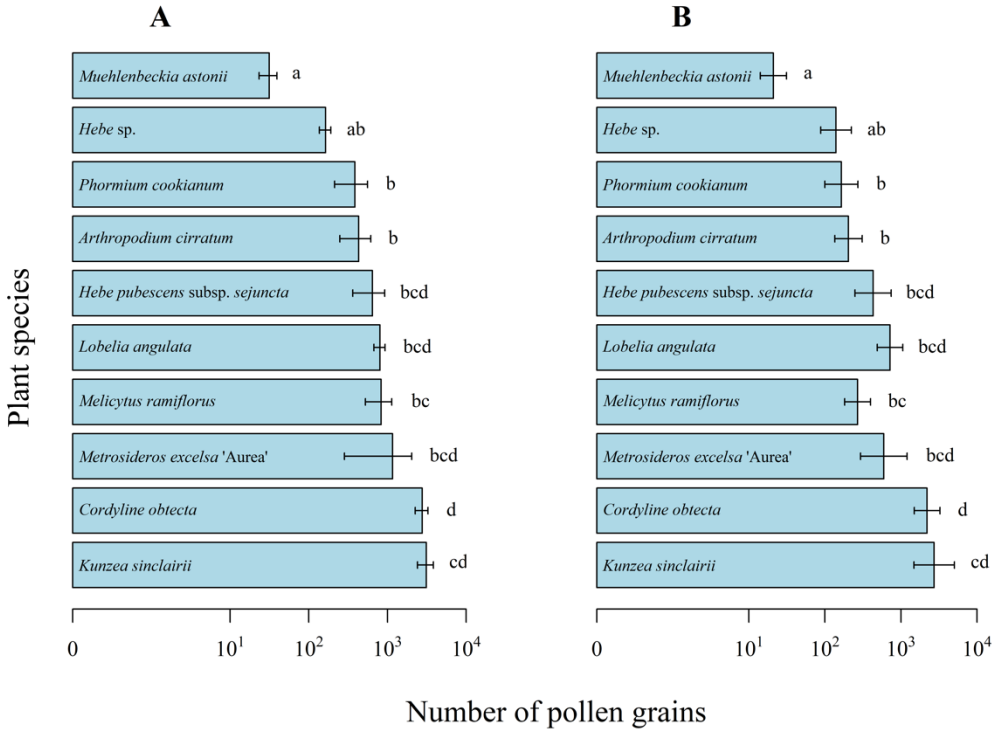

Figure 7. The mean relative number of pollen grains on honeybee samples (1 drop sampled) captured on native New Zealand plants during flower visitation ($n = 74$). The standard errors are indicated by horizontal black bars. The number of pollen grains is given on the log scale. Different lower-case letters indicate statistically significant differences. 'A' is the original data and 'B' are the model predictions.

6.3.2 The effects of ambient temperature and wind speed on pollen collection

The interaction between the number of pollen grains collected and ambient temperature (°C) was statistically significant ($F_{12,69} = 2.69$, $p = 0.02$, $n = 81$) (Figure 8). Honeybee body pollen increased with increasing temperature (Figure 8A). Within the one drop sampled, the number of collected pollen grains was greater when temperatures were at or above 22 °C (Figure 8A). Similarly, the interaction between pollen collected and wind speed (km/h) was statistically significant ($F_{12,69} = 4.33$, $p < 0.001$, $n = 81$). The model prediction shows that there was an increase in the average number of pollen grains collected at greater wind speeds, most notably in wind speeds above 15 km/h (Figure 8B). However, the confidence intervals show that, with wind speeds over 30 km/h, the number of pollen grains collected potentially increased (Figure 8B).

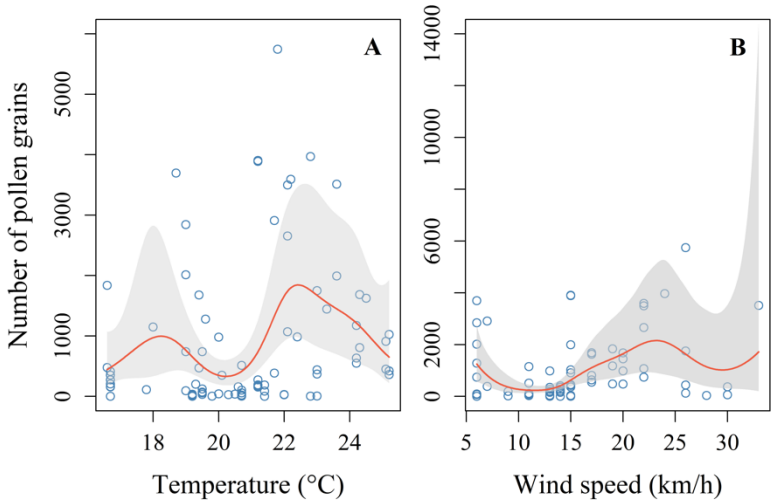

Figure 8. The effect of temperature (°C) (A) and wind speed (km/h) (B) on the pollen collected by honeybees (1 drop sampled) (n = 81). The standard errors are indicated by grey shading which was used to calculate the 95% confidence interval bounds. The model predictions are indicated by red lines.

6.3.3 Relative number of conspecific and heterospecific pollen grains collected

The interaction between the average number of relative conspecific and heterospecific pollen grains collected by honeybees and the plant species in which honeybees were captured on was statistically significant (significant relative pollen abundance × plant species interaction; L = 32.29, df = 8, p < 0.001, n = 67) (Figure 9). On average, honeybees collected 36 times more conspecific pollen than heterospecific pollen (in the drop sampled). Both plant species and from origin (yes = conspecific pollen, no = heterospecific pollen) were significant terms (L = 21.86, df = 8, p = 0.005; L = 163.92 df = 1, p = 0.001). Irrespective of plant species, honeybees mostly carried much more relative conspecific pollen than heterospecific pollen except for with *Muehlenbeckia astonii*, where more heterospecific pollen was collected (Figure 9). Honeybees that collected pollen from *Hebe pubescens* subsp. *sejuncta* showed the largest difference between the number of conspecific and heterospecific pollen grains (Figure 9). Similarly, *Arthropodium cirratum*, *Phormium cookianum* (mountain flax), *Metrosideros excelsa* ‘Aurea’ (Pohutukawa) and *Cordyline obtecta* (Three Kings cabbage tree) also showed a noticeably large difference between the number of conspecific and heterospecific pollen grains collected (Figure 9).

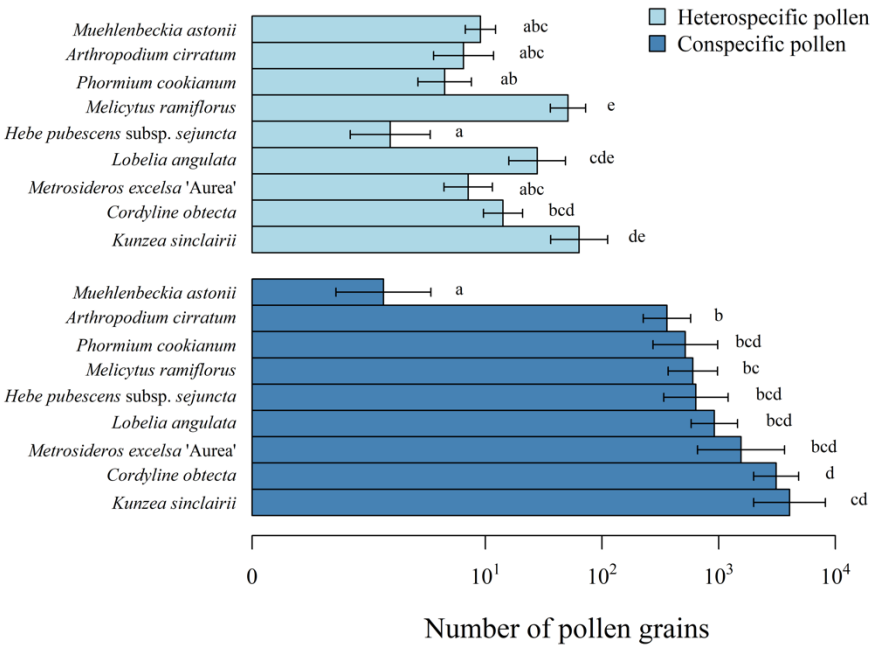

Figure 9. The mean relative number of heterospecific and conspecific pollen grains on honeybee samples (1 drop sampled) captured on native New Zealand plants during flower visitation (n = 57). The number of pollen grains are given on the log scale. The standard errors are indicated by horizontal black bars. Different lower-case letters indicate statistically significant differences, which are independent of each other between conspecific and heterospecific pollen.

7. Discussion

7.1 Flower visitation

Measuring flower visitation of honeybees is important for understanding how many flowers they can potentially pollinate and the chance of successful pollination in a plant individual. This helps us evaluate the pollinator performance of honeybees. The number of flowers visited varied, where honeybees visited between 5-18 flowers per visitation session (Figure 3). Though this may be perceived as a considerably large number of flowers visited, honeybees are capable of visiting many more flowers in a visitation session. For example, in *Prunus dulcis* (almond), honeybees visited between 22-54 flowers per visitation session (Bosch & Blas, 1994). Similarly, in *Lavandula latifolia* (lavender), the visitation rate (flowers/min) of honeybees was 10.5 (Herrera, 1989). Alternatively, in *Eucalyptus costata*, honeybees visited approximately 4-5 flower groups per visitation session Horskins and Turner (1999). These comparisons indicate that there is a large range in the number of flowers visited by honeybees, though honeybees usually visit an ample number of flowers in a visitation session.

Small plants may benefit more from honeybee pollination, as honeybees visited more flowers on small plants compared to large plants (Figure 3). There are three factors that could have influenced this pattern: flower proximity, foraging movement and abiotic factors. Generally, large plants have wider flower spacing, resulting in longer travel times between flowers, hence, fewer flower visits. This aligns well with the results of a recent study, where honeybees visited significantly fewer flowers with increasing distance between them (Hennessy et al., 2020). Honeybees have been shown to have particular foraging patterns; they were found to maintain the same foraging height, i.e. landing and departure occurred at similar heights (Faulkner, 1976) and do not visit much lower or higher positioned flowers (Waddington & Holden, 1979). However, within this rather narrow spatial range, honeybees do visit multiple flowers or flower clusters (Faulkner, 1974). Additionally, wind conditions worsen with increasing distance from the ground, hence honeybee flight performance may become impaired by turbulence more often. Large plants may be particularly disadvantaged because flowers may be spaced further apart and adverse wind effects may be great, resulting in a decrease in the number of flower visits by honeybees. However, some large plants had branches that reached relatively low to the ground, such as *Metrosideros excelsa*. These plants may benefit from a slightly greater number of flower visits compared to large plants with no low-lying branches because of the potentially reduced effect of wind. Although the average number of flowers visited per honeybee can be relatively low (as low as 5 in my study; Figure 3), this may be sufficient for successful pollination on the population scale. This idea is supported by a study on *Fragaria* × *ananassa* Duch. (strawberry) where at least four honeybee visits to one flower with a total foraging time of 40 seconds were needed for adequate pollination (Chagnon et al., 1989). Obviously, when the pollination rate approaches 100 %, subsequent flower visits and foraging are of no further benefit to the host plant. In *Fragaria* × *ananassa* Duch, a near 100 % pollination rate was reached at six flower visits (Chagnon et al., 1989). In my study, the minimum number of flower visits and foraging time required to attain 100 % pollination would certainly vary across plant species. However, without relevant information, we can only assume that there is a higher chance of successful pollination where more flowers are visited alongside a high foraging time. Therefore, in my study, although honeybees probably visited an adequate number of flowers on large plants, there is a greater chance that honeybees facilitated successful pollination across more flowers in smaller plants because more flowers were visited. This is especially true on a population

scale, where the difference in the chance of successful pollination across flowers would be much higher in smaller plants than larger plants.

Flower visitation alone is not indicative of the pollinator performance of honeybees as pollinator performance consists of other measures. This includes the duration of a visitation session and activities exhibited during a flower visitation session, such as foraging and non-foraging activity. Therefore, it is important to consider the number of flower visits with these factors to gain a deeper understanding in the pollinator performance of honeybees. In my study, visitation sessions varied in length between different flower types in small plants (Figure 4), meaning that flower type influenced the length of time spent visiting small plants. Additionally, honeybees were found to be reliable pollinators as they spent more time foraging than not foraging across plants (Figure 4). Comparing visit duration across different plant species between studies shows that visit duration varies strongly, probably due to differences in plant characteristics; additionally, studies on the pollination of different plant species show how honeybees consistently spend more time foraging than not. For example, in a study using singular artificial flowers, honeybees handled the flower for 6.42-11.92 seconds across different well depths and reward values, while the flight time (non-foraging time) was only between 1.69-1.85 seconds (Sanderson et al., 2006). In *Rubus idaeus*, which has singular flowers, honeybees spent between 10.49-12.42 seconds foraging and 3.27-4.35 seconds flying between flowers (Willmer et al., 1994). Another study, however, found a smaller difference between foraging and non-foraging time, where in *Lavandula latifolia* (grouped flower), honeybees spent an average of 3.6 seconds foraging and 2.1 seconds not foraging (Herrera, 1989). In my study, in grouped flowers, there was a greater difference between foraging and non-foraging time (Figure 4) than in *Lavandula latifolia*, implying that honeybees may be less effective pollinators in some plants. In this instance, this could relate to the size of the flower groups. In my study, grouped flowers were often larger than *Lavandula latifolia*. It is likely that honeybees spent more time foraging on grouped flowers in my study than in *Lavandula latifolia* due to the greater amount of time required to forage on each flower in a flower group. However, the non-foraging time was similar to my study, where honeybees exhibited non-foraging behaviour for 1-3 seconds (Figure 4).

Pollination occurrence probably varies between different flower types as the time honeybees spent foraging was variable across different flower types (Figure 4). Although plant characteristics were not the focus of these studies, other studies have found similar results, where foraging time varied across different plant species. On

average, honeybees foraging time was 5.94 seconds on *Malus domestica* (apple) flowers and 12.57 seconds on *Prunus dulcis* flowers (Thomson & Goodell, 2001). In *Asclepias incarnata*, honeybees foraged for an average of 3.7 seconds per flower (Ivey et al., 2003). In *Rubus* species, honeybees had a mean foraging time of 12.5 seconds (King et al., 2013). Honeybees were found to spend similar amounts of time foraging on *Brassica oleracea* (wild cabbage), *Brassica rapa pekinensis* (napa cabbage) and *Brassica oleracea* var. *gongylodes* (kohlrabi) flowers (5.39-6.82 seconds per uncaged flower) (Sushil et al., 2013). This highlights the different times honeybees spend foraging across different plant species. There is a high likelihood that plant characteristics, such as flower type, dictated the differences in foraging time in these species, as this was the determining factor in my study. For example, honeybees spent more time foraging on grouped flowers rather than singular flowers and the non-foraging time was relatively low compared to plants with singular flowers (Figure 4). Because the 'grouped' flower type consisted of multiple flowers within one cluster, it is clear that honeybees would spend more time foraging across these flowers; they did not need to fly between flowers to continue foraging, hence the lower non-foraging time. Also, there may have been differences in other floral characteristics, as well as rewards, across the plant species that resulted in differences in foraging time. For example, one study found that honeybee foraging time was significantly affected by stamen length, where longer stamens increased foraging time (Cakmak et al., 2009). This was predicted to be a result of the increased time that honeybees spent trying to access the nectar in flowers with longer stamens compared to flowers with shorter stamens (Cakmak et al., 2009). In native New Zealand flowers for instance, an increased foraging time may be seen in *Metrosideros excelsa*, which has very long stamens.

Although the lowest temperature encountered while recording foraging behaviour was 16 °C, foraging occurrence and high foraging time was greatest above 20 °C indicating a strong temperature preference in regard to foraging behaviour (Figure 5A). Joshi and Joshi (2010) reported virtually identical results showing that true honeybee flight occurred at 16 °C and foraging activity was at a consistently high level above 20 °C. Similarly, in another study, the foraging activity of honeybees peaked at 20 °C (Tan et al., 2012). The minimum honeybee foraging temperature seems to vary. In one study, little flight activity began at or below 10 °C, however, some flight activity began at 12 °C (Joshi & Joshi, 2010) and similar results were reported from apple orchards, where honeybee flight activity began at 12 °C (Vicens & Bosch, 2000). In another study, honeybees began foraging activity at 6 °C (Tan et al., 2012), though the

activity level was not stated. As ectotherms, honeybees are subject to low temperature-related metabolic limitations. The flight metabolic rate of honeybees is strongly linked to air temperature with a lower reported threshold of 16 °C (Heinrich, 1979), or more specifically, an ambient temperature range between 19-37 °C is required to sustain uninterrupted flight (Woods et al., 2005). Furthermore, wingbeat frequency increased with increasing temperature, while metabolic expenditure decreased (Woods et al., 2005). This indicates that honeybee flight becomes more economic with increasing temperature, which in turn increases foraging activity. However, with a lower limit comes an upper limit as the mean honeybee density has been shown to decrease with increasing temperatures (temperature range: 27-45 °C) (Blažytė-Čereškienė et al., 2010).

My findings further indicate more effective honeybee pollination on days with low wind speeds (< 20 km/h, Figure 5B). A New Zealand study found similar results, where visitation rate was significantly negatively affected by wind (Burgess et al., 2006). Another study found that foraging rate decreased with increasing wind speeds (Hennessy et al., 2020). The change in foraging time and foraging occurrence as wind speed decreases is probably related to a number of factors identified in bees, including hesitancy to take off (Hennessy et al., 2020), flight instability (Crall et al., 2017), and landing dynamics (Chang et al., 2016). Because wind speed varies in nature, it was suggested that honeybees may wait for a decrease in wind speed before taking off from a flower, as this has been seen in *Diachasmimorpha longicaudata* (ashmead) (Messing et al., 1997). Also, the body temperature of honeybees decreases in windy conditions (Hennessy et al., 2020); in the experiment, honeybees may have needed to warm up their flight muscles before lifting off the flower, which reduced the amount of time spent foraging due to the additional time spent on waiting for the opportune moment to lift off (Hennessy et al., 2020). Similar to bumblebees, bees struggle to fly in turbulent conditions (Crall et al., 2017). Interestingly, the bumblebees did not avoid foraging in periods of high turbulence intensities though (Crall et al., 2017). In my study, the flight challenge was obvious. Honeybees struggled to fly to target flowers during foraging and were often blown into or away from the plant (personal observation). Although honeybees did not avoid foraging altogether, the number of foragers and time spent foraging greatly decreased in wind speeds higher than 20 km/h (Figure 5B). This could at least partly be linked to greater energy expenditure attempting to fly in windy conditions, resulting from an increase in wingbeat frequency, as found in bumblebees (Crall et al., 2017). The landing dynamics also change with high wind speeds. For

example, Chang et al. (2016) found that bumblebees changed their approach pathways from multidirectional with no wind to unidirectional in windy conditions. Also, bumblebees maintained a high flight speed while approaching the flowers in windy conditions, compared to a smooth deceleration in non-windy conditions (Chang et al., 2016). This indicates that bees may expend more energy attempting to land when windy, potentially discouraging foraging occurrence. Also, it is clear that high wind conditions not only limit flight manoeuvrability but also landing control which may reduce movement efficiency between flowers, effectively reducing foraging time.

In conclusion, the number of flowers visited can vary widely. However, honeybees visited an ample number of flowers in my study, especially on the population scale. Small plants may benefit from honeybee pollination more than large plants as honeybees showed a clear preference towards small plants. Even though flower visitation alone is not the best proxy for pollination, the general consensus is that when more flowers are visited there is a greater chance of pollination. However, foraging time also plays a role in the chance of successful pollination. Plants with different characteristics probably benefit differently from honeybee foraging due to differences in foraging time, despite honeybees being reliable pollinators. Additionally, because honeybees spent more time foraging on grouped flowers compared to singular flowers, grouped flowers may benefit more from honeybee visitation. This suggests that plants with grouped flowers may have the highest chance of successful pollination. Plants that had a smaller difference in the amount of foraging and non-foraging time may not benefit from honeybee visitation as much as those with a greater difference between them. Foraging occurrence and time would be location-dependent because honeybee foraging is optimal in warm areas with low wind speeds.

7.2 Flower visitor volume

Honeybees did not show flower preferences in relation to flower colour and flower type (Figure 6A), so visitor volume is not dictated by these factors, but other cues must be decisive for host attraction. Additionally, though not statistically significant, it appears that visitor volume was variable and NWS flowers had the greatest visitor volume across flower characteristics. In these cases, the cause of variable visitor volume and the higher visitor volume of NWS flowers is unknown. However, because previous studies have found that factors such as floral traits and olfactory cues affect honeybee visitation, we can deduce that unmeasured factors such

as these affected visitor volume levels. For example, honeybees were found to prefer large flowers (Martin, 2004; Young & Stanton, 1990) and objects (which would be flowers in natural conditions) that matched the floral scents circulating in the hive (Arenas et al., 2008; Reinhard et al., 2004), increasing the number of visitors of these favourable flowers. Evaluating plant preference is important because floral trait preferences can influence what plant species are visited in a community (Gibson et al., 2012). Gibson et al. (2012) demonstrated an overlap in the number of flower visitors in flowers with floral traits similar to *Acacia saligna* (coojong), which the authors attributed to nectar availability, whereby honeybees that retrieved more nectar from flowers with particular floral traits are more likely to visit similar flowers where similar nectar rewards may be available. This may explain why honeybees appeared to visit plant species with NWS flowers more frequently in my study than plants with other floral traits. Alongside nectar rewards, we can expect that the energy expended to retrieve the nectar also plays a role in floral preference, i.e. flowers which have easy access to nectar are probably favoured. Although the reason for preference towards NWS flowers is unknown, we can suspect that it may be because of one of those factors. More studies with larger sample sizes are needed to have a greater understanding of visitor volume differences between plants, as indicated in my model predictions (Figure 6B).

7.3 Pollen collection

To determine the chance of pollination across plant species, it is important to measure how much pollen is collected from each plant species during flower visitation. In my study, according to the drop sampled, honeybees collected a significantly different number of pollen grains across native plants (Figure 7). However, in most cases, the number of pollen grains collected by honeybees was ample. Commonly, there was at least 100 pollen grains that adhered to a bee body but often they were in the thousands. Because only one drop was sampled, it is likely that some honeybees carried many more pollen grains. This wide range in the number of adhered pollen grains is in line with previous findings. One study found that honeybees visiting *Brassica rapa* var. *chinensis* flowers collected 10,940 pollen grains on average (Howlett et al., 2011). In another study, honeybees that were captured while visiting crop plants were carrying at least 1,602 pollen grains on average (visiting *Trifolium pratense*; red clover), with the highest number of pollen grains collected being 53,911 on average (visiting *Taraxacum*

officinale; common dandelion) (Free & Williams, 1972). In a study by Thomson and Goodell (2001), across four apple varieties, honeybees collected almost 40 % of pollen grains available during flower visitation. However, the number of pollen grains that adheres to bee bodies varies in a species-specific fashion. In my study, honeybees only collected 31 pollen grains from *Muehlenbeckia astonii*, on average. Similarly, Adler and Irwin (2006) found that honeybees collected small amounts of pollen grains from *Gelsemium sempervirens* (yellow jessamine). It is evident that honeybees do not always collect large amounts of pollen from visited plant species. The variability in the number of pollen grains collected by honeybees likely pertains to body size, flower size, foraging behaviour and the amount of pollen produced. Honeybee body size in relation to flower size can influence the chance of anther contact. Honeybees having a large body size relative to a flower can mean that the anthers are not inevitably contacted during foraging as the body surface is naturally positioned above the anthers during foraging. Though honeybees are generalist pollinators (Adler & Irwin, 2006; Butz Huryn, 1997; Olesen et al., 2002) where they successfully pollinate many plant species, anther avoidance probably occurs with very small flowers. The flowers of *Muehlenbeckia astonii* are minuscule (2-3 mm diameter), so anther avoidance may occur frequently. Also, honeybees can 'side-work' flowers (Delaplane et al., 2013; Thomson & Goodell, 2001), where the anthers can be avoided whilst foraging for nectar. It is likely that this occurs more easily in flowers that have a large opening to the nectar. When looking at the relative size of *Gelsemium sempervirens* used in the study by Adler and Irwin (2006), it appears that honeybees would be able to easily avoid the anthers of these flowers while nectar foraging by skirting around on the petals. This indicates that honeybees may be better pollinators of some species over others, depending on flower characteristics. However, when foraging for pollen, honeybees would contact the anthers and stigmas more frequently, so pollen collection would probably only be limited in nectar foragers. In a New Zealand context, flower size would probably be the most dominant influential factor in pollen collection because many native plants have small flowers (Butz Huryn, 1995), suggesting that honeybees may avoid anthers relatively frequently. However, the point at which flower size becomes truly limiting in terms of pollination is unknown; based on my results, despite small flower size, honeybees usually collected sufficient amounts of pollen grains from flowers. Considering only one drop was sampled, it is likely that much more pollen was actually collected by the honeybees. It is likely that truly subpar pollen collection only occurs in miniscule flowers. However, because honeybees have the ability to collect up

to tens of thousands of pollen grains in other plant species (Free & Williams, 1972; Howlett et al., 2011), it is evident that the ability of honeybees to collect pollen grains in small-flowered native plants is relatively limited and there is a good chance that smaller insect are more effective pollinators. The amount of pollen produced by each plant species is also likely to be a large contributing factor in how much pollen is collected by honeybees. In crops, plant species produce varying amounts of pollen; in plants that produce little pollen, the collection of pollen is unlikely (Free & Williams, 1972). This is supported by a study in *Raphanus sativus* (radish), where the more pollen produced, the more pollen that was removed by honeybees during a single visit (Young & Stanton, 1990).

In my study, there is a high chance that anther contact, hence pollen collection, occurred by honeybees as indicted by the presence of conspecific pollen. Though this does not necessarily mean that honeybees deposited pollen onto the stigmas, we can assume that the chance of pollen deposition, of any viable proportion, was high. Honeybees have been shown to deposit an ample amount of pollen grains onto the stigmas of visited flowers. In a study by Howlett et al. (2011), insect body pollen and pollen on *Brassica rapa* var. *chinensis* stigmas, following a single visit, was counted. In honeybees, there was a mean body pollen count of 10,940 and a mean stigma deposition pollen count of 123. In another study on honeybee pollination of apples cultivars, honeybees only failed to contact the stigma one out of thirty times while foraging, where honeybees deposited a median of approximately 60 pollen grains on *Malus domestica* 'Rome' (rome apple) stigmas and 170 pollen grains onto *Malus domestica* 'Golden Delicious' (golden delicious apple) stigmas (Thomson & Goodell, 2001). However, the number of pollen grains deposited is not always high across plant species. Adler and Irwin (2006) found that honeybees were found to deposit approximately 50 pollen grains on average on the stigmas of *Gelsemium sempervirens*. Similarly, in *Prunus dulcis*, honeybees deposited a median of approximately 18 pollen grains across total visits (Thomson & Goodell, 2001). The difference in pollen grains deposited probably relates to body contact during foraging. If the honeybees have limited contact with the anther and stigmas, then there is a low chance of large quantities of pollen being deposited onto the stigmas. Determining anther and stigma contact depends on foraging activity and body size. This can be seen in a New Zealand flower, where the chance of stigma contact is variable. A study found that honeybees contacted the stigma of *Metrosideros excelsa* in 26.6 % of visits (Schmidt-Adam et al., 2009). However, the chance of stigma contact depends on foraging activity (nectar or pollen foraging)

(Schmidt-Adam et al., 2009). When nectar foraging, stigma contact occurred only 15.3 % of the time on average, but during pollen foraging, stigma contact occurred 60 % of the time on average (Schmidt-Adam et al., 2009). When looking at the flower morphology of *Metrosideros excelsa*, the difference of stigma contact occurrence between behaviours makes sense. *Metrosideros excelsa* has long, protruding filaments and styles which, from my personal observations (though observing the 'Aurea' variation), honeybees have to scrabble through while foraging. When nectar foraging, I often did not observe stigma contact. This is because the stigma was easily avoidable due to the large flower size and the fact that honeybees mostly scrabbled downwards to the nectar from the edge, rather than across and down, in which stigma avoidance occurrence was confirmed by Schmidt-Adam et al. (2009). When pollen foraging, I observed honeybees crawling across the filaments where the chance of anther contact was high, and consequently, the chance of stigma contact was also high. Schmidt-Adam et al. (2009) stated that body size was also a determining factor in stigma contact; honeybees are larger than native bees (that honeybee stigma contact was compared to), so they are more likely to contact the stigma on *Metrosideros excelsa*. However, the difference in stigma contact between foraging behaviours seen in *Metrosideros excelsa* is probably not very common in other native species because most native flowers have a simple form (Butz Huryn, 1995) and are thus readily accessible to generalist pollinators such as honeybees, increasing the chance of stigma contact (Adler & Irwin, 2006; Butz Huryn, 1997; Olesen et al., 2002). On the other hand, like anther contact, there is a low chance of stigma contact in miniscule flowers or flowers where the honeybee body is naturally positioned well above the stigma while foraging. Because stigma contact usually differs between nectar foraging and pollen foraging behaviours, stigma contact also depends on the proportion of time honeybees spend on different foraging behaviours. However, this is not the case for all plant species. For example, on apple cultivar trees, pollen deposition was not different between foraging behaviours (Thomson & Goodell, 2001).

The number of pollen grains collected by honeybees increased with greater temperatures and wind speeds (Figure 8). As discussed earlier, honeybee foraging occurrence and foraging time was highest above 20 °C (Figure 5). It is likely that increased foraging time and pollen collection at higher temperatures correlated, due to an increase in movement on flowers. This was also suggested by Synge (1947), whereby similar results were found. Alternatively, or in conjunction with this, honeybees may forage for pollen over nectar at higher temperatures, as this was found

in bumblebees (Peat & Goulson, 2005). Additionally, Peat and Goulson (2005) found that bumblebees foraged for pollen over nectar in windier conditions, which potentially explains why honeybees collected slightly more pollen at higher wind speeds (Figure 9B). Peat and Goulson (2005) suggested that greater pollen collection at higher temperatures and wind speeds may be due to the resulting drier conditions, whereby there would be less dew on the flower; pollen collection may be inhibited by water droplets (Peat & Goulson, 2005). Because my study found that honeybee foraging occurrence and duration was greatly reduced with higher wind speeds, this may influence the occurrence of pollen collection and deposition. Additionally, this study also found that low humidity contributed to this (Peat & Goulson, 2005), therefore, we can expect that humidity may play a role in this relationship.

Collecting a large amount of pollen and having a high chance of deposition onto stigmas is a good indicator of the chance of successful pollination and vice versa. However, it is important to assess what proportion of conspecific pollen is collected during flower visitation to accurately determine the likelihood of successful pollination. In my study, honeybees collected thirty-six times more conspecific pollen than heterospecific pollen from the flowers of native plant species, based on the drop sampled (Figure 9). Similar results have been found in other studies. One study found that in *Anacardium occidentale*, honeybees collected 1,241 of conspecific pollen grains, on average (Freitas, 1997). Another study investigated the pollination of crops and found that the percentage of the non-predominant pollen species on honeybees was generally below 26.4 %, though this was not the case in *Trifolium pratense* (Free & Williams, 1972). This indicates that in most cases, a large proportion of collected pollen was conspecific (Free & Williams, 1972). The amount of conspecific pollen collected is not always high, however. In my study, surprisingly, honeybees did not collect a large proportion of conspecific pollen in *Muehlenbeckia astonii*. In fact, honeybees had more heterospecific pollen on their bodies than conspecific pollen. The low number of conspecific pollen grains carried probably relates to the reproduction system of *Muehlenbeckia astonii*. *Muehlenbeckia astonii* is gynodioecious (Wotton, 2018), whereby plants either have female flowers or are hermaphrodites (Asikainen & Mutikainen, 2005). Hermaphrodite flowers produce pollen which pollinates female flowers. The pollen production of hermaphrodite flowers (Asikainen & Mutikainen, 2005), the proportion of female flowers in a population (Lewis, 1941) and the distance between hermaphrodite and female plants (Widén & Widén, 1990) determines the occurrence of reproduction in gynodioecious plants. In the case of *Muehlenbeckia*

astonii, the low number of conspecific pollen carried by honeybees probably pertains to at least one of these factors. Though not gynodioecious, a similar outcome was found by Adler and Irwin (2006), as they found that the proportion of *Gelsemium sempervirens* (conspecific) pollen collected by honeybees was only 8 %, whereas the proportions of conspecific pollen collected by other pollinators was between 57-69 %. Similarly, in *Trifolium pratense*, only 32 % of conspecific pollen was present on honeybees (Free & Williams, 1972). My findings and those from other indicate that in some plant species, honeybees do not collect much conspecific pollen during flower visitation, so honeybees may not be good pollinators of these plants. However, in the majority of cases there is a high chance of successful pollination due to the high proportion of conspecific pollen collected across different plant species. It is important to note, however, that because the data was based on a singular drop, there is a chance that there were less or more conspecific pollen that the honeybees collected. Like with pollen density, the differences in conspecific pollen collected across plant species can be explained by body size, flower size, foraging behaviour and the amount of pollen produced. Although conspecific pollen grain collection and deposition can help us determine the chance of pollination, other factors, such as stigma receptivity (Galen et al., 1986; Yi et al., 2006) and pollen viability (Shivanna et al., 1991), influence pollination probability. For example, the percentage of pollen grains germinating on *Clintonia borealis* (blue-bead lily) increased between first opening and bell (stages of flower development) (Galen et al., 1986). This indicated that stigma receptivity increased with increasing flower age until the bell phase was reached. Similar results have been found in *Prunus dulcis*, where germination increased with flower maturation (Yi et al., 2006). However, the period of optimal stigma receptivity varies between species, where receptivity can occur for as little as a few hours (Tangmitcharoen & Owens, 1997) or for several days (González et al., 1995; Kalinganire et al., 2000). This means that the floral stage or age in which honeybees deposit pollen onto stigmas determines pollination success; if honeybees deposit pollen too early into flower development and not during the time of optimal stigma receptivity, the chance of successful pollination decreases. Pollen viability occurs when a pollen grain can successfully complete fertilization of the ovum (Shivanna et al., 1991). Temperature can affect pollen viability as it was shown for example in *Mangifera indica* (mango), where temperatures below 10 °C reduced pollen viability (30-40 %) and temperatures between 15-33 °C provided conditions for optimal pollen viability (70-85 %) (Issarakraisila &

Considine, 1994). It is important to consider these factors in determining the pollination success of honeybees.

In conclusion, honeybees usually collect a large amount of pollen and in most cases, a large proportion of the pollen is conspecific, meaning that the chance of successful pollination is often high. This is further supported by the common occurrence of anther and stigma contact. However, my results imply different host-plant compatibilities and preferences, making honeybees more reliable pollinators of certain plant species. Additionally, pollen collection and deposition varies across different weather conditions.

7.4 The role of honeybee pollination in native New Zealand plants

We can understand the role of a pollinator by assessing their pollination performance, which can be estimated from measurements of the visitation frequency, pollen deposition, and the pollinator contribution to seed set. In my study, I assessed components related to visit frequency and pollen deposition of honeybees in native New Zealand plants, which has provided a greater understanding of honeybee pollination in New Zealand. For the majority of the native plant species used in my study, honeybees were found to be potentially good pollinators since they visited an ample number of flowers during a visitation session and spent most of their time foraging, i.e. in close contact to the anthers and stigma(s). This is further supported by the fact that, in most cases, honeybees collected a large number and proportion of conspecific pollen, meaning that pollination of native plants is likely. A comparatively large proportion of conspecific pollen was found most notably in *Hebe pubescens* subsp. *sejuncta*, *Arthropodium cirratum*, *Phormium cookianum*, *Metrosideros excelsa* ‘Aurea’ and *Cordyline obtecta*. However, my findings suggest that pollination success is linked to plant size and floral traits and thus widely varies among native plant species. Because honeybees visited more flowers on smaller plants and foraged for longer on grouped flowers, we can deduce that small native plants with grouped flowers are likely to benefit the most from honeybee pollination. Alternatively, it is unlikely that honeybees would facilitate pollination of *Muehlenbeckia astonii* in most scenarios, as indicated by the lack of conspecific pollen carried. It is likely that this pertains more to the fact that *Muehlenbeckia astonii* is gynodioecious, however, because of flower-pollinator size incompatibility, there is a chance that the pollinator performance of honeybees in this species contributes to this. The low chance that honeybees are good pollinators of

Muehlenbeckia astonii can be concerning, due to the low reproduction rate (Given, 2001) and endangered status of this species in natural environments.

Though there is evidence that honeybees are likely to be good pollinators of many native plants, a number of environmental elements and factors identified in my study influence pollination efficiency. Firstly, temperature and wind had a strong impact on honeybee pollination of native plants. Cooler temperatures limited foraging time, foraging occurrence and pollen collection. In January (middle of summer), the average temperature ranges from 14.2-19.9 °C, with the South Island exhibiting the lower temperature limit and the North Island exhibiting the higher temperature limit. Therefore, honeybees would be more important pollinators in the North Island compared to the South Island. Additionally, the temperature drops approximately 0.6 °C per 100 m of increasing altitude (MetService, 2019); therefore, honeybees are unlikely to be good pollinators in mountainous areas, which is common in the South Island. Although foraging activity is limited in windy environments, a greater number of pollen grains is likely to be collected, and therefore deposited onto the stigma, during flower visitation. However, because flowers require several visits and a minimum foraging time for successful pollination (Chagnon et al., 1989), the low foraging occurrence and duration would remain an inhibiting factor in pollination in windy environments. Therefore, in windier parts of the country, pollination of native plants by honeybees would be reduced. For example, Wellington, located in the lower North Island, is the windiest city in New Zealand, where wind gusts reach over 60 km/h over an average of 173 days in a year (MetService, 2019). Clearly, the pollinator performance of honeybees would be greatly limited in the Wellington area compared to locations further inland with less severe winds, such as Rotorua (central North Island), which receives the same wind gusts for 30 days in a year (MetService, 2019). Similarly, in summer, many coastal environments receive sea breezes, which could potentially limit the pollination of coastal plants, such as *Metrosideros excelsa*. Therefore, it is likely that inland environments with lower winds and warm temperatures are likely to benefit the most from honeybee pollination, however, we can expect some flexibility in the foraging ability of honeybees in windy and cold environments, as deduced from the results presented by Pearson and Braiden (1990). Secondly, honeybees are known to have plant preferences, in which this was also apparent in my study with regard to vastly differing visitor volumes, translating into differing pollination probabilities. The underlying reasons require further exploration.

Different breeding systems of plants can influence the pollinator performance of honeybees. Honeybees may be good pollinators of self-compatible monoecious and hermaphroditic native plant species as they often visit many flowers on an individual plant during a visitation session (Figure 3) and usually collect a large amount of pollen (Figure 7). For example, *Metrosideros excelsa*, a partially self-compatible hermaphrodite (Schmidt-Adam et al., 1999), may benefit from honeybee pollination when individuals are self-compatible, as I found that a fair number of flowers were visited per visitation session (Figure 3) and a high number of pollen grains were collected during a visitation session (Figure 7). Similarly, although hermaphrodite flowers are capable of self-pollination, autonomous self-pollination is not a common occurrence in native plants and heavily rely on pollinators as vectors (Newstrom-Lloyd & Robertson, 2005), hence, honeybees may be important vectors in some hermaphroditic native plant species, particularly in unspecialised flowers. My study does not reveal whether honeybees greatly contribute to the pollination of dioecious plants species, or plants which are self-incompatible. Dioecy is relatively common in native New Zealand flowering plants, with 12-13 % plants being dioecious (Godley, 1979). Honeybees have been found to contribute to the pollination of several dioecious plant species. Some examples include *Ceratonia siliqua* (carob) (Ortiz et al., 1999, as cited in de Jong et al., 2005) and *Actinidia deliciosa* (kiwifruit) (Testolin, 1991). However, the chance of successful pollination can vary. Regarding these examples, successful pollination was limited by distance between female and male plants in *Actinidia deliciosa* (Testolin, 1991), but not in *Ceratonia siliqua* (Ortiz et al., 1999, as cited in de Jong et al., 2005). This indicates that, although honeybees contribute to the pollination of dioecious plants, factors such as female and male plant distance can potentially limit pollination. Other factors that can limit pollination success is the reward which honeybees are foraging for (pollen or nectar) (de Jong et al., 2005) and flower sex, where honeybees and other pollinators can show preference towards male flowers and vice versa (Kay et al., 1984). Reproductive success in dioecious plants depend on pollinating agents, resulting in a relatively high rate of reproductive failure (Dötterl et al., 2014); the previously described limiting factors of insect pollinators would influence this. Plants which are self-incompatible face the same pollination limitations that dioecious plants do (de Jong et al., 2005). Because of the many factors that can influence honeybee pollination in self-incompatible and dioecious plants, we can expect that honeybees are less effective pollinators of native plant species exhibiting these breeding systems, compared to native plants which are hermaphrodites or self-

compatible monoecious plants. Because my study did not measure different breeding systems in the results, we cannot assume that honeybees are good pollinators across all breeding systems in native plants; however, we can assume that they are likely to be good pollinators of hermaphroditic or self-compatible monoecious native plant species.

It is important to consider how honeybee pollination compares to pollination by native species as to ascertain the impact of honeybee pollination of native plants. In *Metrosideros excelsa*, tui (*Prosthemadera novaeseelandiae*) and bellbird (*Authornis melanura*) have been found to visit a large number of flowers per hour (bellbird: 17.5 ± 5.4, tui: 24.2 ± 5) (Anderson, 2003). Though not a one-to-one comparison, when comparing this data to honeybee visitation in the same plant species in my study, honeybees visited less flowers than native birds (5.4 flowers visited, on average). However, the number of pollen grains collected by honeybees in my study in *Metrosideros excelsa* (Figure 7) matched the same ranking as pollen loads on tui, bellbird and kakariki (*Cyanoramphus novaezelandiae*) (Anderson, 2003); honeybees collected more pollen than Coleoptera (Anderson, 2003). This indicates that honeybees may collect just as much pollen, or more pollen, on *Metrosideros excelsa* as native pollinators. Although this does not consider other aspects that lead to successful pollination (for example, in this study, native bees contacted *Metrosideros excelsa* stigmas more often than honeybees (Anderson, 2003)), this shows that honeybees may have the potential to be an important contributor to pollination of native plants. This has been found in other introduced species, where invasive rats and colonist birds (*Rattus rattus*; *Zosterops lateralis*) have been found to partially compensate for the absence of native pollinators of three forest species, including *Metrosideros excelsa*, which supports the idea that introduced pollinators can support and maintain ecosystem function (Pattemore & Wilcove, 2012). There are also differences in the pollinating ability between native pollinators which is important to consider. Studies have found that bird pollinators are important for pollination. In one study, across eight native plant species, fruit set was significantly higher in plants that were visited by native birds (and a few introduced birds) compared to those not subject to bird visitation (Anderson, 2003). Bird visitation was high, with tui and bellbird being the most ubiquitous visitors (Anderson, 2003); large pollen loads were carried by these birds, as well as other bird visitors (Anderson, 2003). Only tui and bellbird visited enough plants to facilitate potential cross-pollination, where they were highly likely to visit another plant of the same species following a visit (Anderson, 2003). Pollen transfer was variable across bird species (Anderson, 2003); kakariki were destructive visitors in the case of

Pittosporum umbellatum and *Geniostoma ligustrifolium*, meaning that effective pollen transfer was unlikely (Anderson, 2003). Birds were likely to contact the stigma of small flowers, but in some larger flowers, stigma contact depended on how birds approached the flowers (Anderson, 2003). From this, the author found that native honeyeaters, especially bellbird and tui, were found to be reliable pollinators of native plants (Anderson, 2003). However, the chance of successful pollination was variable across bird species, meaning that plants did not always benefit from pollination from every bird species. Similarly, native insects were found to be important pollinators of native plants, however, pollination success varied. In the same study, insects were frequent visitors of native plants with the majority being Hymenoptera, Diptera and Coleoptera. Stigma contact was low for native bees, however, this was compensated by high visitation rate, where pollination was comparable with birds (Anderson, 2003). Insects were found to contribute to the pollination of some native plants but were not important pollinators of four plant species (Anderson, 2003). In another study researching pollination of New Zealand alpine, *Hylaeus matamoko*, a small, short-tongued bee, was found to be the best pollinator of this species despite the pollination by other pollinators (Bischoff et al., 2013). For example, *Hylaeus* bees deposited 10 times more germinating pollen on *Ourisia glandulosa* stigmas than *Allograpta* flies in single visits (Bischoff et al., 2013). *Hylaeus matamoko* was estimated to perform 90-95 % of pollination of *Ourisia glandulosa* and *Wahlenbergia albomarginata*, causing them to be critical pollinators of these species (Bischoff et al., 2013). There are also differences between the ability of native birds and native insects to pollinate plant species; in *Phormium tenax*, birds were more likely to facilitate cross-pollination whereas bees facilitated the deposition of self pollen (Howell & Jesson, 2013). In another study, birds and bees (including natives) were found to be pollinators of *Peraxilla tetrapetala* (New Zealand mistletoe) (Robertson et al., 2005). Bees were found to be able to partially replace bird pollinators (Robertson et al., 2005). Hence, birds are likely to be better pollinators of this species. It is clear that there can be disparities between pollinator importance within and between native bird and native insect species; like native birds and native insects, it is reasonable to assume that the role of honeybees in native plant pollination can vary between plants and there may be instances where honeybees play a greater role in pollination of a plant species than some native pollinators, and vice versa. Hence, when comparing to native pollinators, the role of honeybee pollination should be considered based on plant species and native pollinators in which they are interacting with. Additionally, interactions between honeybees and native pollinators should be

considered. Honeybees are known to adversely affect other pollinators. Although there is little information on the interactions between honeybees and native pollinators in New Zealand (Howlett & Donovan, 2010), there is a chance that honeybees negatively impact the foraging behaviour of native pollinators in some species. For instance, competition between honeybees and native pollinators would inevitably occur due to resource overlap. This was found in *Leptospermum scoparium* and *Hebe stricta* (koromiko), where there was an inverse relationship between honeybee abundance and native insect diversity, in which flies were affected the most (Murphy & Robertson, 2000). I also observed this inverse relationship during data collection, though this was not recorded. Furthermore, honeybees have been found to impact the foraging behaviour of native pollinators in other countries (Butz Huryn, 1997). However, another study found that across native and introduced plant species, introduced bees (honeybees and bumblebees) and native pollinators had little resource overlap and different foraging preferences, so in most cases, the magnitude of competition was low (Iwasaki et al., 2018). More studies are needed to investigate the relationship between honeybees and other pollinators. Nevertheless, it is evident that there is the potential for honeybees to negatively impact the conservation of native plants when other pollinators are better suited to pollinate them. Overall, to understand whether honeybees are good pollinators of native plants or not, this should be done for each individual plant species as pollination success of plant species varies across pollinators. Not only should visitation rates and pollen deposition be considered, but also fruit set and other measures of pollinator performance, to get an accurate understanding on how honeybee pollination of native plants compares to pollination by native pollinators.

Although my study was not designed to investigate the role of honeybees in the conservation of native plants in particular, my findings imply that honeybees may aid the conservation of threatened indigenous plants depending on plant and floral characteristics. However, because they are not good pollinators of every plant species, conservation planning of native plants involving honeybees should be done in a case by case basis. For example, my research has shown that honeybees are likely poor pollinators of the naturally threatened *Muehlenbeckia astonii*, rendering them unfit for conservation planning of this plant species. Breeding systems and pollinating ability of other pollinators also needs to be considered here.

8. Research limitations

My research had five main limitations. Firstly, across most plant species, there was only one individual per plant species to sample from. In order to overcome this limitation and to obtain replication, I used plant characteristics such as flower type to pool plant species. Although this is insightful in understanding drivers of honeybee flower visitation, my data analyses would have been more informative for conservation decision-making by comparing flower visitation across different native plant species. Secondly, I could not determine the number of flowers visited per cluster in the 'grouped' flower type because of the small size and close proximity of the flowers. This means that, although fewer numbers of flower groups may be visited, the number of individual flowers visited within a cluster remains unknown. This prevented me from drawing a direct comparison of the number of flowers visited per plant between the grouped and singular flower types. Thirdly, I lacked expertise to correctly identify pollen species and I was unaware of the stringent identification procedures. I was only able to group the images of pollen species according to morphology. A highly experienced palynologist from Massey University assisted me by identifying the most abundant grouped pollen species across all samples from each plant species (except in the case of *Muehlenbeckia astonii*, where all pollen grains were identified). Nonetheless, occasional incorrect identification may have occurred because, rather than physical samples, I provided images, in which pollen is can be difficult to identify (K. Holt, personal communication). However, due to the high likelihood that the most abundant pollen species present on a honeybee derived from the previously visited flower, I am confident that the great majority of identifications were correct. To prevent similar issues in the future, seeking advice from a palynologist in the early research planning stages will allow the provision for contingencies. Fourthly, only one drop of the pollen mixture per honeybee was analysed. Because of this, the true number of pollen grains and pollen species abundance collected by the honeybees is unknown. This could have influenced the results. For example, if in the one drop sampled, little pollen was present but a greater proportion of pollen was present in the remainder of the mixture, then some analyses on the pollen density and pollen species abundance may be misleading. Additionally, it is more difficult to compare the data to other studies. Finally, my research does not encompass all measurements of pollinator performance. The partially answered or excluded components are pollen deposition and pollinator contribution to seed set. Pollen deposition was partially answered, because it can be

argued that a portion of the pollen grains acquired during flower visitation by honeybees is likely to be deposited onto the stigma. However, to confirm this assumption, research on actual pollen deposition onto the stigma was needed. Pollinator contribution to seed set was not assessed because a different sampling set up was required and this was not within the scope of my thesis as there was not enough time to record the life cycle progression of visited flowers. Therefore, we cannot gain a full understanding of the role of honeybee pollination on native plants without these measurements.

9. Future research

I have identified four key areas where further research could help in completing the picture of honeybee pollination of native New Zealand plants. Firstly, research should hone in on a single or small number of native plant species of conservation concern, whereby pollen deposition onto the stigma and seed set should be assessed. This is suited for a greenhouse setting, where the influence of abiotic factors, such as wind, can be controlled. Secondly, the occurrence of cross-pollination and its effects should be investigated, especially in dioecious species. This can also be done in a greenhouse setting which inhibits the influence of abiotic factors. Thirdly, my research was done at a site under unnatural conditions where native plants were present in one small area and were placed according to human design. Future research should sample from natural plant populations. This will help us understand the relative importance of honeybees in natural environments and assess whether honeybee pollinator performance is consistent across various settings. Fourthly, in the scope of pollination webs, pollinator performance is relative; it is important to further assess the pollinator performance of common pollinators of native plant species to understand if there are better pollinators than honeybees across native plant species. Pollinator interactions should also be considered here.

10. Conclusion

My thesis aimed to provide a greater understanding of honeybee pollination of native New Zealand plants by assessing components of their pollinator performance. Numerous flower visitations with emphasis on foraging and little time spent non-foraging along with extensive transport of (conspecific) pollen suggest that honeybees

are good pollinators of some native plants. My research further showed that honeybees may be particularly good pollinators of small native plants with grouped flowers but might be poor pollinators of plants with very small to miniscule flowers. Additionally, plant species in low-wind, warm environments are likely to benefit the most from honeybee pollination. Because the remaining components of pollinator performance and the comparison of pollinator performance between honeybees and other pollinators is unknown, we still do not have a full understanding on the role of honeybees on native plants, as well as the relative importance of honeybee pollination. Therefore, future research should focus on assessing the pollen deposition, seed set, cross-pollination and the pollinator performance of honeybees and other pollinators.

11. References

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12. Appendix

Table 1. Native plant species included in each relevant statistical analyses with the corresponding results section number.

Plant species	**Analyses**						
	6.1.1 Number of flower visits	6.1.2 Foraging and non-foraging time	6.1.3 The effects of ambient temperature and wind speed on foraging time	6.2 Flower visitor volume	6.4.1 Number of pollen grains collected	6.4.2 The effects of ambient temperature and wind speed on pollen grains collected	6.4.3 Number of conspecific and heterospecific pollen grains collected
Alectryon excelsus subsp. *grandis*	✓	✓	✓	✓			
Arthropodium cirratum	✓	✓	✓	✓	✓	✓	✓
Carpodetus serratus	✓	✓	✓	✓			
Cordyline obtecta	✓	✓	✓	✓	✓	✓	✓
Geranium solanderi	✓	✓	✓	✓			
Kunzea sinclairii	✓	✓	✓	✓	✓	✓	✓
Kunzea toelkenii	✓			✓			
Lobelia angulata	✓	✓	✓	✓	✓	✓	✓
Lophomyrtus obcordata	✓	✓		✓			
Melicytus ramiflorus	✓	✓	✓	✓	✓	✓	✓
Metrosideros excelsa 'Aurea'	✓	✓	✓	✓	✓	✓	✓
Muehlenbeckia astonii	✓	✓	✓	✓	✓	✓	✓
Phormium cookianum	✓	✓	✓	✓	✓	✓	✓
Phormium tenax				✓			

Table 1. (continued)

Plant species	**Analyses**						
	6.1.1 Number of flower visits	6.1.2 Foraging and non-foraging time	6.1.3 The effects of ambient temperature and wind speed on foraging time	6.2 Flower visitor volume	6.4.1 Number of pollen grains collected	6.4.2 The effects of ambient temperature and wind speed on pollen grains collected	6.4.3 Number of conspecific and heterospecific pollen grains collected
Pimelea prostrata	✓	✓	✓	✓			
Hebe pubescens subsp. sejuncta	✓	✓	✓	✓	✓	✓	✓
Hebe sp.	✓	✓	✓	✓	✓	✓	

Table 2. Plant species and their corresponding flower type and plant size.

Plant species	**Flower type**	**Plant size**
Arthropodium cirratum	Singular	Small
Cordyline obtecta	Grouped	Large
Kunzea sinclairii	Singular	Small
Hebe pubescens subsp. *sejuncta*	Grouped	Small
Hebe sp.	Grouped	Small
Lobelia angulata	Singular	Small
Melicytus ramiflorus	Grouped	Large
Metrosideros excelsa ‘Aurea’	Grouped	Large
Muehlenbeckia astonii	Singular	Small
Phormium cookianum	Singular	Large