

# **Low Fruit Set, Pollen Limitation and the Roles of Birds and Insects in Pollination of Native New Zealand Plants.**

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## Abstract

Pollination and fruit set of four species of native New Zealand flowering plant species were examined through two field seasons. Bird exclusion, pollinator exclusion, natural and supplemental pollination treatments were initiated on individuals of *Cordyline australis* (Cabbage tree), *Phormium tenax* (Flax), *Kunzea ericoides* (Kanuka), and *Pseudopanax arboreus* (Five-finger). The species differed in the self-compatibility as well as in their floral syndrome. No species showed any evidence of pollen limitation, and two species. *K. ericoides* and *P. arboreus* set fruit from more than 70% of their flowers. The response of fruit set to treatment in *C. australis* varied from season to season, with birds appearing important to pollination in the first but not the second field season, while birds were important in pollination across both seasons for *P. tenax*. *K. ericoides* was resilient to treatment, setting high fruit set in every treatment, compared to *P. arboreus* which set high fruit set when pollinators had access, but low when all pollinators were excluded. No trends relating to fruit set or PLI and self-compatibility or floral syndrome were found. That there was no evidence of pollen limitation for any species, despite variation in fruit set from some treatments, indicates that these species are performing well and not at risk of decreased population size due to pollen limitation.

## Chapter One: An introduction to pollen limitation

### 1.1 Introduction

Plants commonly produce fewer fruit than flowers, resulting in fruit set across species under natural conditions varying anywhere from less than one percent (0.015-0.064 in *Grevillea* species (Hermanutz et al., 1990)) to 100 percent (*Sanicula europaea*) (Lloyd et al., 1980), with variation often existing between individuals of the same species (Bawa and Webb, 1984). Four main explanations have been postulated to explain this range in fruit set. These are: bet hedging, the plant focusing its resources towards maximising some other component of fitness, resource or pollen limitation (Robertson et al., 1999). These hypotheses are not mutually exclusive, and which one is operating or the proportion to which the various mechanisms are operating in, can directly affect the population dynamics of the plant species, potentially affecting long term survival (Sekercioglu et al., 2004). Arguably, the most important stages in the sexual reproductive cycle of a plant are pollination, seed dispersal and seed recruitment. Inhibition at any one of these stages has the potential to restrict the long term survival of the population. Pollination is thought to play a role in fruit set for three of the four hypotheses listed above: pollen limitation, bet hedging and maximising another component of fitness. Each of these hypotheses will be discussed in further detail later in the chapter. Pollination is the first process out of the three, however, limitation in the fruits set can potentially lead to a reduction in the seed available for seed dispersal and recruitment (Turnbull et al., 2000). A theoretical example of this is a population which experiences a drought. This decreases the amount of resources available, in turn decreasing the number of fruit that are set within the season. This results in a subsequently lower seed set than under normal circumstances, which decreases the number of eventual seedlings that can be

produced and mature for the season. Thus, fruit set is an important factor in the population dynamics, as it has flow on effects.

## **1.2 The New Zealand situation**

New Zealand is a geographically isolated archipelago in the Southern hemisphere. Trees comprise 9.7% of flora with woody vegetation amounting to a total of 22.7% of flora (McGlone et al., 2010). A large proportion of the flowering species have small, dull, scented flowers, containing small amounts of pollen and typically considered to be insect pollinated (entomophilous) or generalist. In contrast, only a small proportion match the bird pollinated (ornithophilous) syndrome (around 4%) of large showy flowers that usually contain more nectar which is unscented and the flowers themselves are usually red, yellow or orange (Anderson et al., 2006, Castro and Robertson, 1997).

Analysis of sexual systems in New Zealand flora reveals a number of systems that indicate dependence on pollinators. One of these systems is gender dimorphism. Gender dimorphism has been documented in 23% of native New Zealand plant genera (Newstrom and Robertson, 2005). This is the separation of the male and female organs either within space or time, and can be present in the form of monoecy, dichogamy, dioecious or herkogamy. Monoecy is where flowers have either male or female function, but flowers of both genders are present on an individual plant. The alternative method of spatial separation is to be dioecious, where flowers are likewise of a single gender, but each individual plant only has flowers of a single gender. Consequently, dioecious plants can be considered either male or female depending on the gender of their flowers. Dichogamy is where male and female functions are separated through time rather than space, so that the two functions are not presented at the same time. Finally, herkogamy is a form of hermaphroditism where the male and female organs are on the same flower but they are spatially separated to avoid or decrease self-pollination.

A method of reproductive assurance in the absence of pollinators is autonomous self-pollination. The ability of a species to produce fruit from self pollen exists on two levels. Firstly, a species is either self-compatible or self-incompatible. If plants of the species are able to set fruit after artificial pollination of self-pollen only has occurred and all other pollen sources have been excluded, then the species is self-compatible. However, this does not necessarily indicate reproductive assurance. For self-compatibility to lead to the production of fruit in the absence of pollinators, the plant must be able to self-pollinate without any external assistance. This can be tested by bagging flowers prior to buds opening, and determining whether any fruit is set. Gender dimorphism makes autonomous self-pollination difficult, meaning the plant must rely more on external vectors such as birds and insects. Another important factor is self-incompatibility. While all autonomously self-pollinating plant species must be self-compatible, the reverse does not apply. There may still be some benefit derived from being self-compatible even if the species does not autonomously self-pollinate. For example, self-compatibility may allow plants to make use of more pollen delivered by birds and insects, as these vectors will often deliver both self- and cross- pollen. Newstrom and Robertson (2005) looked at three variables to determine sexual systems within New Zealand plants. They calculated the self-compatibility index (SCI) which is the ratio of fruit production for hand-pollinated self against hand-pollinated cross. In addition they calculated the autonomous selfing index (ASI), which is a ratio of the pollinator excluded fruit set against the cross pollinated ratio. Across all species the SCI averaged 0.64 (0.00-1.29). The self-compatible species were largely less pollen limited than both the partially self-incompatible and the fully self-incompatible species, but self-compatible species did sometimes show high pollen limitation. Autonomous selfing was low for all tree and shrub species, and averaged 0.36, which is considered a moderate value when compared to worldwide levels. There appeared to be little evidence to indicate that New Zealand species

are largely self-compatible in order to provide reproductive assurance in the case of unreliable pollinator service. This indicates that pollination in New Zealand is highly dependent on external vectors, making the status of the pollination within New Zealand, particularly the mutualism of plants with insects and birds important to monitor.

The immobile nature of plants means they must depend on a range of biotic and abiotic vectors to transfer the pollen required for pollination (Merrett et al., 2007, Newstrom and Robertson, 2005). Biotic factors include birds and insects, as well as less commonly examined vectors such as bats and lizards. Wind is an alternative pollen vector, and is common in grasses, gymnosperms, sedges and rushes. Newstrom and Robertson (2005) report 78 genera of plants as assumed to be wind-pollinated (29% of the total flora), while Webb et al. (1999) give 103 genera as wind pollinated. This makes it the pollen vector of highest prevalence, with bird pollination the next most common (Table 1.1). Water pollination also occurs, but it is much less common, with only two genera reported to use this mechanism (Table 1.1).

**Table 1.1:** Prevalence of pollination systems in New Zealand, table modified from McCann (1964) and O'Donnell and Dilks (1994).

	<b>Insect</b>		<b>Bird</b>		
<b>Pollination system</b>	<b>(Entomophilous)</b>	<b>Bat</b>	<b>(Ornithophilous)</b>	<b>Wind</b>	<b>Water</b>
<b>Number of genera</b>	235	4	14	103	2
<b>Percent</b>	65.64	1.12	3.91	28.77	0.56

### 1.2.1. Bird Pollination

A general principle is that there should be a match between the relative size of the blossom and the visitor. The size, shape and arrangement of flowers are all important in determining



both which animals will visit the flowers, and also which will be effective (Craig and Stewart, 1988, Robertson et al., 2005). Large visitors are unlikely to visit small flowers, except where it is energetically profitable to extract the reward, or where there are many small flowers clumped into tight inflorescences. The syndrome concept is a useful tool for describing how flowers can be adapted to different types of pollinators. A syndrome is a range of floral traits and rewards, used to define a flowering species into a class that is associated with the attraction of specific pollinator types. This is the basis of defining a species to be ornithophilous or entomophilous. Syndromes describe the physical shape and features of flowers and inflorescences, as well as traits such as their colour and scent. A subset of this is blossom classes; these are used to describe an inflorescence, a flower or part of a flower, whichever acts as the functional unit for the pollination of that species. The description includes the physical shape and features of the flower, but not other descriptors that are part of the syndrome. In general, syndromes are more descriptive, thus should have a higher predictive value than blossom classes alone, however colour and scent can have context dependant effects on pollinators. For example, if there is a shortage of blossoms in an area, pollinators are less likely to be concerned about colour and scent of the blossoms (Newstrom and Robertson, 2005). Many bird pollinated flowers correspond to the pollination syndrome of ornithophily, which means love of birds, however many birds visit flowers that do not correspond to this syndrome, and likewise, many insects visit so-called ornithophilous flowers (Anderson, 2003, Robertson et al., 2005). Ornithophilous flowers are generally considered to be large, yellow or red flowers with copious amounts of nectar. In contrast, entomophilous flowers are described as small, open flowers (Kelly et al., 2010). What potential pollinators visit flowers is only part of the process. Both the frequency and effectiveness of visitations to flowers are important, and observing visitation does not give sufficient information to determine the effectiveness of a particular pollinator (Larson and

Barrett, 2000). For example, small insects frequently visit large flowers; however, they do not always contact the stigmas or anthers, and thus are likely to be ineffective pollinators in most cases (Newstrom and Robertson, 2005).

There have been a variety of bird species observed as pollinators of native New Zealand species, Kelly et al. (2010) give a total of 12 native species and 5 introduced that have been observed visiting flowers of native plants. The native species were: tui (*Prosthemadera novaeseelandiae*), bellbird (*Anthornis melanura*), silvereye (*Zosterops lateralis*), kaka (*Nestor meridionalis*), stitchbird (*Notiomystis cincta*), saddleback (*Philesturnus carunculatus*), red-crowned parakeet (*Cyanoramphus novaezelandiae*), yellow-crowned parakeet (*C. auriceps*), kea (*Nestor notabilis*), whitehead (*Mohoua albigilla*), yellowhead (*M. Ochrocephala*) and kokako (*Callaeas cinerea*). The effectiveness of these species varies widely, for example the parakeets have been observed destroying flowers rather than pollinating. The five exotic species listed were: house sparrow (*Passer domesticus*), starling (*Sturnus vulgaris*), chaffinch (*Fringilla coelebs*), eastern rosella (*Platycercus eximius*) and myna (*Acridotheres tristis*). Based on observations of flower visitors, the majority of visits and thus pollination has been attributed to three species of native birds: tui, silvereyes, and bellbirds, with a total of 89% of observed visits being by these species, with no other species making more than 3.1% of total visits. While introduced birds play some role in the pollination of native plants, this role appears to be small. The main introduced birds observed to visit flowers were chaffinches and sparrows, but their contribution is rarely over five percent of the visits to the plant, which is less than that of even uncommon and range restricted native species (Kelly et al., 2006). Of these species, four have brush tongues, an adaptation for nectar feeding: tui (McCann, 1964, O'Donnell and Dilks, 1994), bellbird, stitchbird (Driskell et al., 2007) and kaka (O'Donnell and Dilks, 1994). Of these, only bellbird

and tui are widespread (Kelly et al., 2006), and they contribute a large proportion to pollination, as discussed above.

### **1.2.2. Insect Pollination**

Observations from New Zealand suggest that insect visitation to flowers is common (Anderson, 2003), partly due to the simple structures and apparent unspecialisation of the majority of New Zealand flowers (Lloyd, 1985). Large social bees are common worldwide as pollinators, but they are not present in New Zealand. Indigenous bees are small, generally solitary, all short-tongued, and often considered primitive compared to overseas species (Castro and Robertson, 1997, Kelly et al., 2006). 40 species of bees have been described in New Zealand, 32 of these are indigenous, with the remaining 8 naturalised. 36 of these belong to the family Colletidae while the remainder to the family Halictidae. The number of species is low when compared with the 630 species of bees in Australia, which represent a large range of families (Donovan, 1980). However, Australia has around 7,629,000 km<sup>2</sup> of land area compared to the 268,000 km<sup>2</sup> of New Zealand, giving Australia an average of 0.08 species per 1000 km<sup>2</sup> and New Zealand an average of 0.15 species per 1000 km<sup>2</sup>, indicating a comparable level of diversity between the two countries. Native bees have been observed collecting pollen from a range of both native and introduced flowering plants (Donovan, 1980). There have been seven species of the order Hymenoptera introduced into the New Zealand ecosystem. These are one species of honeybee (*Apis mellifera*), four species of bumblebees (*Bombus spp.*) and two species of wasp (*Vespula vulgaris* and *V. germanica*) (Kelly et al., 2006). These insects have had widespread success since their introduction, pollinating both native and introduced plants. Bees commonly pollinate introduced crop plants, such as red clover, but also visit a range of native flowering species (Donovan, 1990).

Butterflies are also poorly represented in New Zealand, with less than 30 species, of which around 13 are indigenous, despite butterflies being an important class of pollinators worldwide (Castro and Robertson, 1997). Diptera is also highly abundant in New Zealand, although little is known about their pollination role in New Zealand. Worldwide, diptera are thought to play a large role in pollination. However, there has been some debate as to whether, diptera are able to carry sufficient quality or quantity of pollen for pollination, and whether they visit flowers with sufficient constancy (Newstrom and Robertson, 2005). It has been suggested that dipterans may play a disproportionately strong role in pollination of New Zealand flowers, taking over the role of many overseas species that are lacking (Castro and Robertson, 1997), and the family has been noted to be the most numerous of the classes of flower pollinators. Newstrom and Robertson (2005) note that in New Zealand 'fly pollination can be especially important during cold rainy weather when other insects, such as bees, are inactive'. Moths are more highly represented, with over 1800 species, although they are not generally included in analysis of pollinator frequency. There are few reports of moth pollination, but the authors report that *Myosotis macrantha*, *Dysoxylum spectabil*, *Kunzea ericoides* and, *Olearia paniculata* are pollinated to some degree by moths. Hawkmoths are a specialised type of moth that feeds hovering, with a long tongue, which plays a large role in pollination overseas. Only one species of hawkmoth exists in New Zealand, *Agrius convolvuli*, which is exotic and found primarily in the North Island, but there have been no confirmed observations of it feeding on nectar. It has however, has been reported to feed on sweet potato, but it is unknown whether this feeding occurs at the larval or adult feeding stage (Newstrom and Robertson, 2005). There has been some evidence for beetle pollination and pollination by very small insects such as thrips, but few studies have looked at how effective this is.

There are a number of factors related to pollination that can potentially limit the population of a plant species. Quantity of pollen directly affects how many ovules can be fertilized. Plants that receive insufficient visitation may be limited in pollen compared to number of ovules, but pollinators also may not deliver sufficient pollen to each stigma to allow for all ovules to be fertilized. This can be caused by pollinators, such as diptera, carrying light pollen loads, so that even though the flowers are pollinated, the plant is still pollen limited. Mixed pollen loads can also hinder pollination in some self-compatible species. For example, in the species *Blandfordia grandiflora* presence of self-pollen renders many ovules unusable, as the ovules appear to reject the self-pollen due to inbreeding depression but not allow cross-pollen access to those same ovules. The timing of pollen arrival is also important, flowers open at different times on individual and different plants, and pollen will be ineffective if it arrives either before, or after the stigma is receptive (Wilcock and Neiland, 2002). Finally, too much pollen may decrease fruit set by mechanisms such as crowding of pollen tubes (Young and Young, 1992) or pollen clogging (Wilcock and Neiland, 2002).

An additional consideration is, even if the plant is limited in pollen, does this have an effect on plant demographics overall? Bond (1994) proposed three considerations for what effect the failure of a mutualism (such as pollination) would have on a plant population. The first of these considerations is does the plant have compensation mechanisms, or how dependant is the plant on the mutualism? This is something that can vary dramatically between species. Plants that reproduce asexually or regularly self pollinate have little reliance on external pollen vectors, while self-incompatible species depend more on pollination, and dioecious self-incompatible species even more again. The second consideration is whether dispersal limits seedling establishment. If dispersal is a limiting factor in how many seedlings establish, and thus how many offspring a plant produces in a season. Then, pollination

limitation is not relevant to population dynamics. This is because, even increasing the number of fruits, and thus seeds produced, would not change the number of seedlings that establish. The third consideration is whether the amount of seed is limiting. This is much like the example discussed for dispersal limiting establishment, if producing an excess number of seeds would not change the number of seeds recruited then the seed number is not the limiting factor, and thus decreasing pollination limitation would not affect population dynamics.

Pollen limitation is defined as where fruit set is limited by insufficient pollen receipt, arising from pollinator visits being too few (Kelly et al., 2006), or from a too low quantity of pollen being delivered (Wilcock and Neiland, 2002). Fruit set is often used as a measure for calculating pollen limitation, but another common measure is seed set (Young and Young, 1992). Both these variables are measures of reproductive success, and can collectively be referred to as female reproductive success (Ashman et al., 2004).

### **1.2.3 Other Vertebrates**

Pollination isn't limited to birds and insects, both lizards and bats have been proposed to play a role in the pollination of native New Zealand plants. There are two species of bat present in New Zealand: the short-tailed bat (*Mystacina tuberculata*) and the long-tailed bat (*Chalinolobus tuberculatus*) (Newstrom and Robertson, 2005). A third species, *M. robusta* is believed to have been present in New Zealand, but is now extinct (Lord, 1991). *M. tuberculata* appears to have adaptations for nectar feeding, making it a potential pollinator, although it is not specialised for this role as it has a broad diet. In contrast, *C. tuberculatus* is an aerial insectivore and is unlikely to have a role in pollination. *M. tuberculata* has been determined to be a frequent pollinator for a few floral species; with a few others it may also pollinate (Newstrom and Robertson, 2005). Bat pollination appears to be common in the

*Freycinetia* genera, but only two species appear to be adapted for bat pollination (Kelly et al., 2006).

As with other pollinators, lizards have suffered declines in density, as the result of human settlement. Members of the gecko genus *Hoplodactylus* have been observed to feed on nectar of a number of plant species. Geckos forage on nectar in the evenings, emerging after dusk, with the highest activity during the first 2-4 hours following their emergence. Geckos climb over the surface of flowers as they forage, lapping nectar by pushing their heads down between the stamens, resulting on pollen catching on their throats. This pollen is able to be carried for up to 12 hours, and across many metres. Around ten plant species have been observed to be pollinated by lizards; however, none of these appear to be adapted specifically for lizard pollination (Whitaker, 1987). On offshore islands, where the densities of lizards remain high, they have been observed to visit flowers, but they are limited in their ability to move between plants, and data has thus far failed to suggest a significant role of lizards in pollination (Kelly et al., 2006).

### **1.3 Distinguishing between the hypotheses**

As discussed at the beginning of this chapter, there are four main hypotheses for why plants produce fewer fruit than flowers. These are: resource limitation, bet hedging, for a fitness gain or pollen limitation. This section reviews the current evidence for each of these hypotheses.

#### **1.3.1 Bet hedging hypothesis**

The excess production of flowers may be a co-evolutionary adaptation for an environment where pollinator service is severely unpredictable. This strategy is often referred to as bet-hedging. It may be beneficial for the plant to produce excess ovules per flower to take

advantage of the receipt of occasional unpredictable pollen loads, such as from highly laden pollinators or the deposition of higher quality pollen. This theory can be extended to the number of flowers per plant. Here, plants produce an excess of flowers to enable it to take advantage of sporadic events, known as ‘boom’ years where a greater amount of pollination is received due to environmental changes, such as a larger than usual amount of pollinators, or higher activity of pollinators (Moody-Weis and Heywood, 2001), or when more resources are available to mature fruit (Sutherland, 1986). This strategy can be adaptive where the environment is unpredictable and where the cost of extra flowers is low (Ashman et al., 2004). In a similar way to the number of ovules per flower, in non-‘boom’ years the plants are likely to be pollen limited (Moody-Weis and Heywood, 2001).

An example of a collection of species which might undertake this strategy is plants that are pollinated by hawk moths. Hawkmoths are migratory species, that fluctuate in numbers and the time of their first brood varies from year to year. These factors create an unpredictable pollination environment, which is the situation where bet-hedging is thought to occur.

Experimental manipulations on one hawkmoth pollinated species, *Oenothera macrocarpa* found pollination limitation which could either be the result of bet hedging or of recent habitat degradation reducing pollinator numbers (Moody-Weis and Heywood, 2001).

If a species is operating under this bet-hedging strategy, then pollen limitation would be expected to be high, as many ovules would not get fertilized except where there was a high pollen load, or a ‘boom’ year. Knight et al. (2005) looked at this using a sample size of 148 species. The large sample size allows for overall trends to be easily recognised, as analyzing only a few species is problematic, as they may be experiencing an abnormal season at the time. The authors found that there was a significant increase in pollen limitation as the number of ovules per flower increased.



### 1.3.2 Fitness hypothesis

A plant's gender can be defined both phenotypically and functionally. Phenotypic gender is the emphasis that a plant puts on the male and female functions, while the functional gender is the relative contribution that pollen and seeds give to the next generation. For species that have both genders on a single plant, either monoecious, or some hermaphroditic plants which present the sexual organs at different times, the phenotypic gender can be measured by the proportion of female flowers that are open on a particular day. The phenotypic gender varies across days and seasons (Wells and Lloyd, 1991). Male fitness is obtained by pollen donation to sire offspring, and male function can be defined as the allocation of resources to the production and dispersal of pollen. Female fitness is obtained through seed and fruit set, and female function is the production of ovules, fertilization and maturation of seeds (Sutherland and Delph, 1984).

Sexual selection theory was initially developed to apply to animals, but it has since been extended to plants, and is referred to as Bateman's principle (Burd, 1994). Under sexual selection theory, female success from reproduction is limited by available resources, not access to mates (Knight et al., 2005), while male fitness is generally limited by the number of matings they are able to complete. As a consequence, the fitness of any plant that has both genders (i.e. is not dioecious) is derived from the individual fitness of the male and the female flowers combined. The 'equilibrium' fitness that a plant is at for a given level of resources is therefore a combination of these two components; however, there is no reason that at this equilibrium the number of male and female has to be equal. This is particularly the case in monoecious and dioecious plants which have the ability to adjust the number of male and female function flowers. In hermaphroditic plants however, the number of male and female function flowers must be the same, but the optimal ratio may not be equal of both. If this is

the case, then either the number of male function, or female function flowers is the limiting factor in the fitness of the plant (Sutherland and Delph, 1984).

If the optimal ratio is a higher number of male flowers than female, then a large number of flowers are produced that do not set fruit. In monoecious plants more males are produced, but this control is not possible in hermaphroditic plants. Thus, hermaphroditic plants produce an excess number of flowers that can potentially set fruit, but not. This occurs because the limiting factor for reproduction is the production, rather than the receipt of pollen, thus increasing the number of flowers is unlikely to increase the fruits set for that plant (female function), but rather it will increase the number fruits set on other plants (sired) by pollen from the males.

Experimentally, this has been studied by investigating whether a positive correlation exists between the number of flowers and the success of siring, as the hypothesis predicts that extra flowers are produced solely for their male function. This has been examined on a lily species *Zigadenus paniculatus*, where male function in larger plants was more successful than in smaller, but this effect was not true when relative size of plants was examined (Emms, Stratton, and Snow 1997). Another study looked at this theory by examining whether pollen donation increases more as flower number increases (male function) than seed set does (female function) for *Ipomopsis aggregata*, a species of self-incompatible herb. The author found that the results did not agree with the fitness hypothesis, in fact seed production and fruit set increased more than pollen donation (Campbell, 1989).

### **1.3.3 Resource and pollen limitation**

Resource limitation theory is based on Bateman's Principle, originally proposed in 1948. This looks at sexual reproduction of animals, but has since been directly applied to plants, and states that male fertility is unlikely to be limited by sperm (pollen), but rather than the number of females available, while female reproductive success is limited by resources (Burd, 1994). This predicts that resources are the only limiting factor for fruit and seed set in a population of plants. As such, resource limitation is often considered as an opposing hypothesis to pollen limitation, with many authors testing only the two hypotheses and using pollen supplementation experiments to determine between them (e.g. Ackerman and Montalvo, 1990). Pollen and resource supply are not independent factors. Some resources directly impact the pollen supply, such as carbon and mineral nutrients, as decreasing these decreases the resources a plant has available to attract pollinators (Ne'eman et al., 2006). Pollen limitation (PL) is a phenomenon where there is an inadequate quantity or quality of pollen is received for maximum plant fruit and/or seed set (Knight et al., 2005). The general suggestion of these observations is that if pollinators were more abundant or more effective, the plants would consistently have a higher reproductive success (generally measured in terms of fruit or seed set) (Calvo and Horvitz, 1990). This viewpoint sees pollen and resource limitation as a strict dichotomy. However, some authors have considered that this may be an oversimplification of reality. Haig and Westoby (1988) consider pollen and resource limitation to form an equilibrium, where the optimal level of resources invested into attracting pollinators is where female reproductive fitness is limited both by pollen and resources. In this situation, referred to as the Haig-Westoby equilibrium, neither additional pollen, nor additional resources will cause an increase in fruit set, as both factors are limiting (Knight et al., 2005).

Tests generally focus on either pollen or resource limitation, with evidence having been found for both (McCall and Primack, 1987). A handful of studies have manipulated both resources and pollination to try and determine whether the Haig-Westoby equilibrium is present. Campbell and Halama (1993) examined pollen supplementation and resource supplementation in *Ipomopsis aggregate*. They found that when both hand-pollination and fertilizing were applied to a plant the seed production was increased, while when hand-pollination was applied solely the number of seeds per flower alone increased, and fertilizing alone increased just the number of flowers produced. Brookes and Jesson (2007) investigated the effects of pollen supplementation, fertilizer addition and resource reduction (through removal of foliage). They found that when supplemental pollination was occurring, neither adding nor decreasing resources increased fruit set. One study examined simultaneous changes in resources and pollination, investigating both supplementation and reduction for both studies. This appears to be the only study that incorporates pollen reduction, and the authors found that supplemental pollination did not increase seed set, neither did supplemental resources. However, decreasing either resources or pollen resulted in a lower seed output. These results match the direction predicted by the Haig-Westoby equilibrium (Brookes et al., 2008).

Experimental evidence shows that despite predictions by Bateman's principle and the Haig-Westoby equilibrium, pollen limitation is widespread. For example, Ashman et al. (2004) found that out of 85 studies that involved studies at the whole –plant level (i.e. entire plants were used for hand-pollination and natural pollination), 73% showed significant pollen limitation. Another literature review, of 258 species, found that 62% of the species showed pollen limitation (Burd, 1994). There are a number of reasons why pollen limitation may be present even if the Haig-Westoby equilibrium exists. Ecological perturbations, like changes

in temperature, fragmentation of habitats, the presence of competitor plants or the loss of pollinators may disrupt the mutualism between pollinators and plants, resulting in lower pollination, and thus pollination. Alternatively, pollen limitation could, as explained in the bet hedging section above, be a strategy for reproduction in an unpredictable environment. Finally, pollen limitation could be a coevolved response to the selection on traits that promote outcrossing (Knight et al., 2005). An example of this would be self-incompatibility; this trait prevents the plant from pollinating its own flowers, greatly increasing cross-pollination but preventing self-pollination. Therefore, at times where cross-pollination is low, the plant would be pollen limited.

Pollen limitation has been measured by pollen supplementation experiments in a number of different studies, with varying types of response variable, and many studies investigating more than one response variable. A review of these found that the most common of these were percent fruit set (482/655) and number of seeds per fruit (182/655), and also included percent seed set (170/655), seeds per flower (94/655) and seeds per plant (87/655) (Knight et al., 2005). The unit of measurement used may be a branch or an individual plant (Bawa and Webb, 1984). In this type of experiment, liberal amounts of pollen are applied to the stigma; this pollen is from multiple compatible conspecifics. Reproductive success is compared between natural conditions and the hand pollinated treatments. In this type of experiment, liberal amounts of pollen are applied to the stigma; this pollen is from multiple compatible conspecifics. Reproductive success is compared between natural conditions and the hand pollinated treatments (Larson and Barrett, 2000, Robertson et al., 1999). If there is significantly lower reproductive success under natural conditions compared to hand crossed, then the reproduction of the plant is being limited by pollinator activity in some way (Bierzychudek, 1981). Reproductive success is commonly measured either in terms of seeds

per fruit (Burd, 1994), or proportion of flowers that set fruit (Robertson et al., 2008), while the unit of measurement may be a branch or an individual plant (Bawa and Webb, 1984). If pollen limitation continues persistently, it can have consequences on the evolution of the population, such as by favouring the evolution of mechanisms to decrease the cost of pollen limitation, such as self-compatibility or increased selfing, or stronger selection on traits that make flowers more attractive to pollinators (Knight et al., 2005). Long-term pollen limitation may indicate a breakdown in the mutualism between plant and pollinator, which can limit fruit set, and may ultimately have an effect on population persistence, population dynamics and even ecosystem-level processes (Ashman et al., 2004, Robertson et al., 1999, Sekercioglu et al., 2004).

Pollen supplementation experiments have associated empirical issues related to determining the level of pollen limitation. Pollen is added to stigmas by imprecise mechanisms, such as dabbing with a paintbrush, which makes determining the size of the effect difficult, as the amount of pollen deposited will differ between flowers, as will the quality of the pollen. Furthermore, the physical technique of artificially adding pollen may promote self pollination, or hinder pollination in some way, such as by damaging the flower (Ashman et al., 2004). Under natural circumstances, for a plant to receive a high amount of pollination they need to divert more resources towards attracting pollinators; this leaves a lower amount available for fruit maturation. However, with supplemental pollination the plant has not had to expend these resources, which leaves more available for fruit. Thus, resource limitation also plays a role here, as the plant has more available resources for the same level of pollination that it could have under natural pollination (Zimmerman and Aide, 1989). Plants are able to reallocate resources, such as to different flowers or seeds or at a different time in the season, or another season entirely. As such, pollen supplementation may increase the

reproductive success of the branch being tested, but decrease reproductive success of other branches or the reproductive success of the plant in later seasons (Ashman et al., 2004).

Reallocation of resources within the plant can be avoided by changing the level of experimental unit, and applying hand pollination to the entire plant. Similarly, reallocation across seasons can be examined by doing supplementation experiments on the same population over multiple seasons. Evidence of decreased reproductive success in the following season does not refute pollen limitation, as all forms of fitness needs to be examined to determine whether the increase in production in the first season came at an overall benefit or a cost to the plant (Calvo and Horvitz, 1990).

Resource limitation and pollen limitation are thus related factors, and a plants reproduction may be limited by one or the other or both. If the population is operating at Haig-Westoby equilibrium, then supplemental pollination will have no effect, likewise if the plant is resource limited. However, if pollen limitation is having an effect on reproduction, then pollen supplementation experiments would increase reproductive success.

## **1.4 Focus of study**

My research focuses on pollen limitation as the cause of low fruit-to-flower ratios in native New Zealand plant species. Pollination has been established as an important mutualism, with a number of recent reviews highlighting the risk of the mutualism failing (e.g. (Ashman et al., 2004, Burd, 1994, Kelly et al., 2010), particularly with the limited bird and insect abundance and range in New Zealand. I aimed to examine pollen limitation in a range of New Zealand plant species, focusing on those that were visited by both birds and insects, as the pollination of these is not easily predicted, due to the relative effectiveness of each type of visitor for that species being unknown. I focused on two approaches here; the first was to examine pollen limitation using traditional pollen supplementation experiments, as used in various studies

(Kelly et al., 2004, Nishikawa, 1998, Robertson et al., 2008), in some cases examining pollen limitation over multiple seasons. My second approach was to simultaneously set up caged and bagged treatments on the same plants, to look at the effect that the individual types of pollinators (i.e. insects versus birds) were having on pollination.

The key questions for this study were as follows:

1. Are unspecialized (open, small flowered) apparently insect pollinated plant species less pollen limited than ornithophilous (bird-dependent) species?
2. Are species that are able to self-pollinate and are hermaphrodites less likely to show pollen limitation than self-incompatible species or species with separate sexes?
3. On insect pollinated species that are also visited by birds, will excluding birds but not insects still give good pollination levels and fruit set?

To examine pollen limitation and the role of birds and insects in New Zealand, my study looked at four species of native plant species: *Cordyline australis*, *Phormium tenax*, *Kunzea ericoides* and *Pseudopanax arboreus*. The following chapters will examine methods, results and conclusions for each of the four species, and then examine how these relate to each other, to pollen limitation and to bird and insect pollination in New Zealand.



## Chapter Two: *Cordyline australis*

### 2.1 Introduction

*Cordyline australis* (Fig 2.1) has the earliest flowering season of the species I studied, and field work began in early November 2008. I choose it as it was an early flowering species with small flowers. It produces small hermaphroditic flowers (5-6 mm in length and 5 mm in width) in large inflorescences, with most inflorescences containing between 5000 and 10,000 flowers (Harris et al., 2006). Trees range in the number of inflorescences they produce, with some carrying a single panicle, and others having upwards of ten. The flowers are creamy white coloured and appear to match the entomophilous syndrome, with frequent insect visitors, although birds have also been observed visiting the species (Kelly et al., 2010).

Beever and Parkes (1996) did a pollination study on this species, investigating whether there was evidence of pollen limitation, and whether the species was self-compatible or self-incompatible. The authors looked at four trees established from cuttings grown in potting containers for their experimental treatment. They removed all open flowers from the ultimate panicle branches, and artificially opened 10 flowers and removed their anthers before pollen release, these were the experimental flowers. Flowers were then bagged in a paper bag, and then hand pollinated the following day, and left in the bag until fruit set where the number of fruits was counted. For each tree five treatments were initiated, no pollination, self-pollination, and pollination by each of the three other trees used for hand-pollination, one plant's pollen per treatment. Only three of the four trees had treatments, the fourth was used solely as pollen donor. The natural comparison was made on three mature trees within six metres of each other in Riverhead Forest in Auckland and a fourth mature tree on a roadside, 20 m from its nearest neighbour. The authors found that under artificial pollination where the pollen donor was another plant, around 66% of flowers produced fruit, while under natural

conditions fruit set averaged 44% (ranging from 28 to 53%). Only three flowers that were self-pollinated produced fruits, and these had only one or two seeds, leading to the authors' conclusion the species is self-incompatible. These data produce a PLI of 0.33, despite being referenced in a paper by Newstrom and Robertson (2005) which was summarising knowledge of pollination systems in New Zealand, including known pollen limitation values, as 0.90. A similar, more recent, paper which provided an updated version of pollen limitation values was Kelly et al. (2010). Here the authors did not include the pollen limitation data from the Beever and Parkes study, giving the reason that the natural and treatment plants were from different sites.



**Figure 2.1:** *C. australis* inflorescence in bud

The conclusions of the Beever and Parkes study should be treated with caution for several reasons. Firstly, as Kelly et al. observed the hand-pollination and natural treatments were at different locations, making the difference between hand- and natural pollination

indistinguishable from differences in location. Secondly, the flowers used for hand-pollination were artificially opened, emasculated, then bagged, and provided with pollen from a single donor (while natural flowers receive pollen from a range of different donors). This results in the hand-pollinated flowers being exposed to a set of different conditions than the natural flowers, making the effect of these indistinguishable from the effects of the artificial pollination. Finally, the number of replicates in the study was low; only three trees had treatments applied for the hand-pollination, and four for the natural comparison.

The self-compatibility of *C. australis* has only been addressed in the Beever and Parkes paper detailed above. Their methods for determining self-compatibility are relatively similar to commonly used methods (flowers were emasculated, bagged, and pollinated with self pollen by hand), although their treatment numbers were low. *C. australis* is thus a native tree species that is widely distributed throughout New Zealand (Czernin and Phillips, 2005) that appears to be self-incompatible (Beever and Parkes, 1996).

*C. australis* begins flowering in mid to late November and can take up to six weeks to open all the flowers on a panicle. Flowers open for a single day, with tepals wilting the following day (Beever and Parkes, 1996). The amount of flowering can vary from year to year and new branches require at least two years before they can produce flowers (Harris et al., 2006).

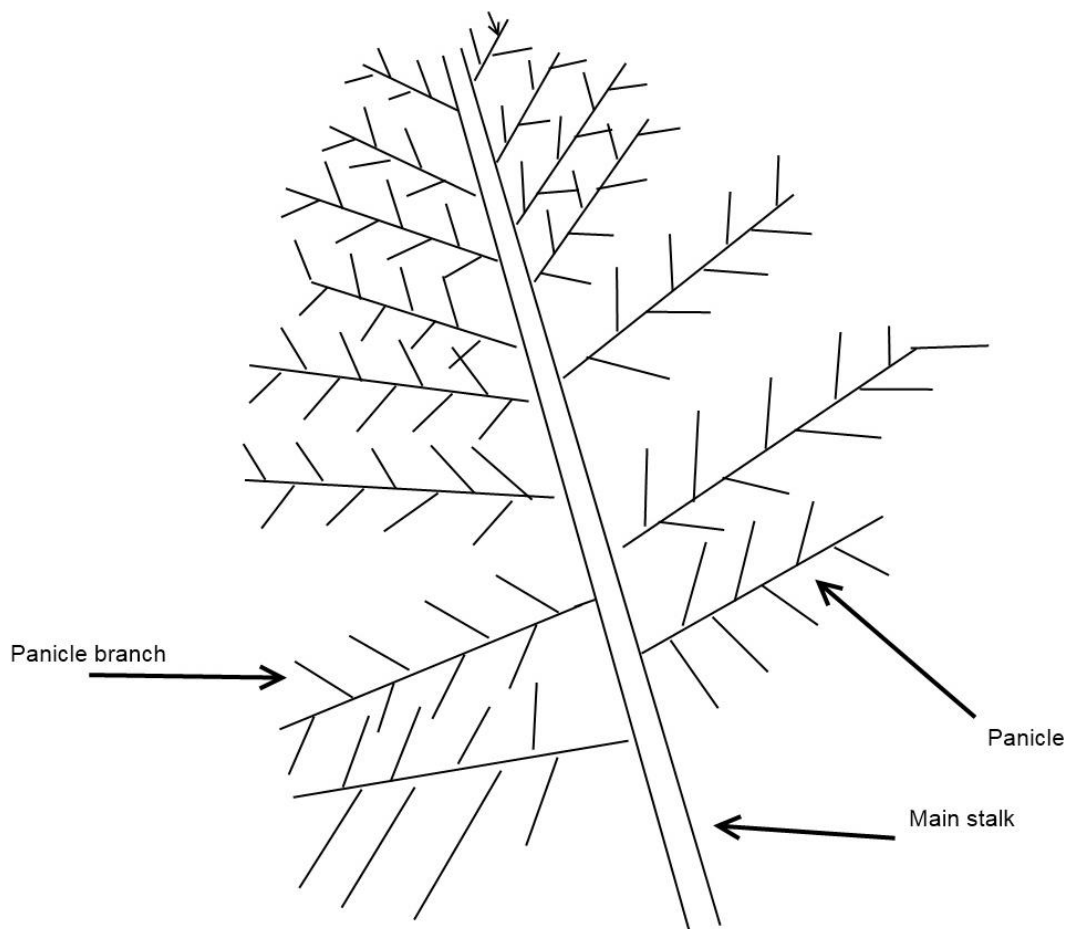
The entomophilous nature of *C. australis* flowers makes it likely that the main visitors and pollinators are insects rather than birds. For this study I aimed to examine the relative roles of birds and insects on fruit set for *Cordyline australis*, as well as whether the species is able to autonomously self-pollinate. I predict that excluding bird pollination from these flowers will result little or no change in the proportion of flowers that become fruit, but excluding both birds and insects from pollinating should result in very low levels of fruit set or none at all (as the species is reported to be self-incompatible).

## 2.2 Materials and methods

Field experiments were undertaken for two separate field seasons beginning 2008/09 and 2009/10. For the first season (Nov 2008 – Feb 2009) nine individual plants were chosen for study, and in the second season (Nov 2009 – Feb 2010) five individuals were chosen. All five individuals used in the second season were also used in the first season. Plants were chosen on the basis of having panicles that were lower than 3 m (to allow accessibility) and that were not easily accessed by the public.

The study for this species was in Victoria Park (43° 35' 23" S 172° 38' 37" E) on the Port Hills, Christchurch, New Zealand. Victoria Park is a reserve that is open to the public, the section I was working on had an altitudinal gradient of around 100 m. The Port Hills predominantly contains secondary scrub communities or native secondary forest remnants, where the dominant species is *Melicytus ramiflorus* (mahoe) or *Kunzea ericoides* (kanuka) (Partridge, 1989). Victoria Park is a managed nature reserve, containing many native species, and regenerating native forest.

Each inflorescence consists of a main stalk off which panicles are attached on alternative sides, and branching off these are panicle branches which are covered in flower buds, each containing around 50 buds (Fig 2.2). Flowers open at the bottom of the stalk first, continuing up the stalk with the flowers at the top opening last, up to several weeks after the first flowers open.



**Figure 2.2:** Schematic representation of a *C. australis* inflorescence.

For each treatment, a panicle branch was tagged and counted, with the number of buds per treatment ranging from 23 to 78 with an average of 47 buds per treatment. For this species three treatments were set up: bagged, caged and natural treatments. For each tree studied one bagged, one caged, and two natural treatments were setup. The second natural treatment was planned to be hand-pollinated, but the swift progression from flowering to fruit set prevented this.

Bagged treatment: the number of buds on a panicle branch was counted and tagged before buds opened. The entire panicle was enclosed in a cotton gauze bag which was tied onto the

panicle. The gauze bag had small holes <1mm to allow air to circulate around the panicle while preventing insect access to the flowers.

Caged treatment: like in the bagged treatment, the number of buds on a panicle branch was counted and tagged before flowering. Prior to bud opening a 2x2 cm wire mesh cage covered in plastic mesh with 2x2 cm holes was placed over the panicle and secured, giving a total mesh size of around 11mm. The cage was designed to prevent bird access to the flowers, while allowing insect access. 2x2 cm mesh cages were used for a similar exclusion experiment by Anderson (2003). She used three treatments on hermaphroditic species, caged, bagged and natural, and used caged and natural on non-hermaphroditic species. She found no evidence that the cage mesh obstructed insect movement. Robertson et al. (2005) used wire and netting cages with a mesh size of around 11mm. The authors tested the effect of the cages on insect visitation by watching bee and insect behaviour on hand-opened flowers both inside and outside cages. They found that insects passed through the mesh, but the visitation rate of native bees was decreased by 25% and other insects by 4% compared to outside cages. This difference was not significant, and was small compared with variation between factors such as sites, years and visitor type. Based on this evidence 2x2 cm mesh cages should not significantly impede insect visitation to study flowers.

Natural: the natural treatment was an unmanipulated treatment used to determine fruit set under natural circumstances. For this treatment, the number of buds on a panicle branch was counted and tagged, and then the panicle was left unmanipulated until fruit set was scored.

Plants were revisited once to twice weekly to determine when fruit set occurred. Fruit count was scored when fruit in all positions along a panicle branch was large enough to be reliably counted. The number of flowers that had set fruit on a tagged panicle branch was counted and recorded. For caged and bagged treatments, the cages and bags were removed prior to fruit

count. Across the two seasons three natural treatments were lost due to the disappearance of tags (two in 2009 and one in 2010), and thus were not counted and could not be included in analysis. In addition, treatments and tags from one entire tree in the 2010 had been removed by unknown means, and thus could also not be counted. Thus in total, fruit count from 49 treatments was counted and recorded.

Fruit set was taken as the difference between the number of buds when treatments were initiated, and the number of fruit that the plant set multiplied by 100 (fruit/flowers x100), giving an indication of the proportion of flowers that set fruit. Differences between the treatments were analysed using a Generalised Linear model (GLM) with binomial error distribution and a Chi square significance test. The response variable was proportion fruit set and the predictor variables were tree, season and treatment. The model used was affected by the order of factors, and so the treatment variable was last to allow for the determination of whether fruit set was affected by treatment when all other predictors had been taken into account. The tree variable was a unique identifier for each tree that was studied, irrespective of the season. This allowed for differences in fruit set between individuals to be accounted for. Analysis was performed using R, an open source statistical software package (version 2.9.2). To determine the statistical differences between treatments, pairwise *post hoc* comparisons were made, as standard *t*-tests, dividing the difference between the two means with the joint standard error.

## 2.3 Results

Trees progressed from bud to flower over around a month, while progression from flower to first fruit set occurred in little over a week. The number of flowering inflorescences varied between seasons, with more flowering occurring in the 2009 season. This effect was noted by Harris et al. (2006) who reported flowering being high in some years and low in others, like

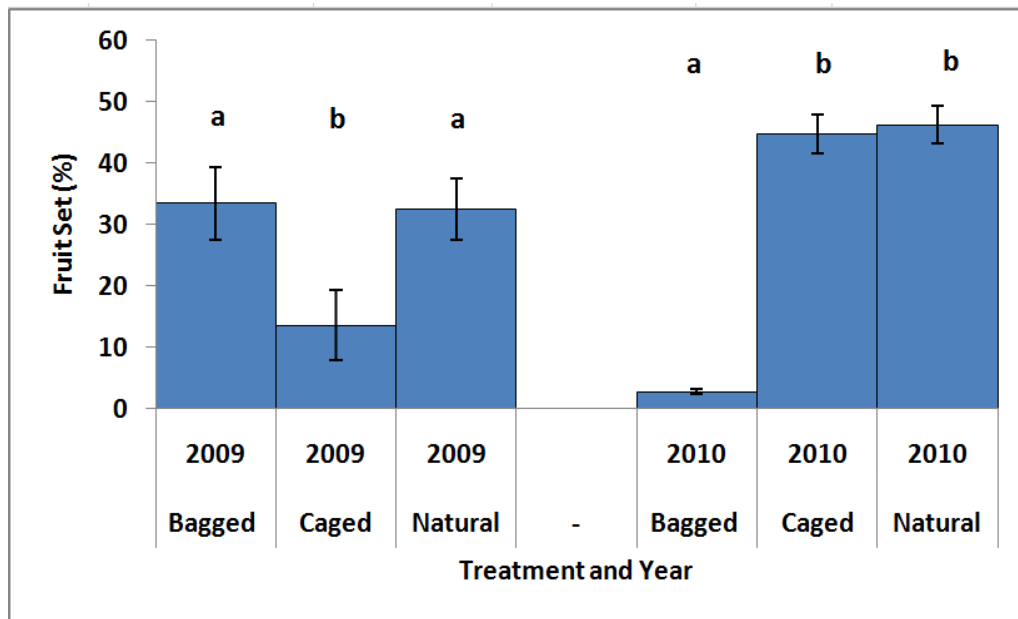
the mast flowering of other species such as *Phormium tenax* and *Nothofagus* species. When the whole data set was analysed the individual tree was the strongest predictor of fruit set (Table 2.2), with both season and treatment also significant. There was also a strong season by treatment effect, indicating that the treatments should be analysed separately for each season, however the mean fruit set across all treatments did not significantly vary between seasons (two-sample t-test;  $P = 0.3023$ ). I did no observation periods for *C. australis*, but did see both insects and birds visiting *C. australis* flowers as I was setting up treatments.

**Table 2.1:** Analysis of variance table for the fruit set of *Cordyline australis*. The model used quasibinomial error distribution and a Chi squared test for significance. Significance levels were: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

Model	df	Deviance	Residual df	Residual deviance	P(> Chi )	Significance
Null			48	511.46		
Tree	8	122.88	40	388.58	0.0002	***
Season	1	16.40	39	372.18	0.0436	*
Treatment	2	49.74	37	322.44	0.002	**
Tree:season	3	33.46	34	288.97	0.04	*
Tree:treatment	16	112.88	18	176.10	0.0314	*
Season:treatment	2	115.51	16	60.58	<0.0001	***
Tree:season:treatment	6	18.75	10	41.83	0.589	

Pairwise analysis showed that the caged treatment was significantly lower than the natural, and close to significantly lower than the bagged in 2009 (Table 2.3; Figure 2.2), with bagged and natural being similar. In 2010, caged and natural had similar fruit set, while bagged had a significantly lower fruit set.





**Figure 2.3:** Average fruit set for bagged, caged and natural treatments for 2009, 2010. Within a season two identical letters represents no significant difference, while different letters represent a significant difference between the pair. Error bars represent the standard error of the means.

**Table 2.2:** Season by season *post hoc* pairwise comparisons between treatments. Significance levels were: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$

Season	Comparison	<i>t</i>	df	P	Significance
2009			33		
	Bagged versus Caged	1.816		0.0785	
	Bagged versus Natural	0.120		0.906	
	Caged versus Natural	-2.069		0.0465	*
2010			14		
	Bagged versus Caged	-5.388		<0.0001	***
	Bagged versus Natural	-5.568		<0.0001	***

Caged versus Natural	-0.111	0.913
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## 2.4 Discussion

The aim of this chapter was to examine pollen limitation, and the relative roles of native bird and insect pollinators in determining fruit set. For *C. australis* I was unable to set up hand pollination treatments, and as such had no means of determining the maximum fruit set, and could not determine whether the species was pollen limited. The way treatment affected fruit set was significantly different depending on the season.

### *2009 Season*

In 2009 the average fruit set for both bagged and natural treatments was similar (33.4% and 32.5% respectively) while the caged treatment was lower, with 13.6% fruit set. There was a significant difference between the caged and the natural treatment, but not one between the caged and the bagged treatment. However, the P value for caged versus bagged was close to significant, and biologically similar, thus I assume that the significant difference seen between caged and natural also applies between caged and bagged. This species is thought to be entomophilous, receiving the majority of its pollination from insects. As a consequence, I predicted that the caging treatment would not have a significant effect on fruit set. The difference between my results, and predicted results could be caused by one of several factors. Firstly, the caging treatment may be dramatically reducing the number of insects visiting flowers, and thus reducing pollination compared to natural. Schmidt-Adam (1999) examined the effect of cages by recording the visits by bees (the largest pollinators in my study) both inside and outside cages for five minute periods, taking in total 18-20 scores. She found no significant difference in the number of visits to flowers, nor the time spent per

flower inside or outside of the cages. Robertson et al. (2005) found that their cages reduced visitation by 25% for bees and 4% for other insects. This difference was not significant, and the authors concluded it was negligible when compared to differences between season, treatment and plant. For my study, I observed insects still visiting caged flowers, and based on literature evidence I conclude that it is unlikely that the cages were the cause of the decrease in caged fruit set compared to natural. A second potential cause is that birds played a larger role in pollination for this species than anticipated. *C. australis* is considered to have entomophilous flowers that are frequently visited by birds. Their dull colour and small size contribute to the belief that they are adapted for insect pollination (Kelly et al., 2010). However, small clumped flowers may also be pollinated by birds. Rather than collecting pollen on their beaks when feeding, birds often collect pollen on their head and throat as they brush against flowers during feeding (Anderson, 2003). As such it is possible that the role of birds has been underestimated for this species.

A second effect seen in this season was the significantly higher fruit production of the bagged treatment compared to the caged treatment. A similar fact was noticed by Jennersten and Nilsson (1993) in *Viscaria vulgaris*. The authors found that inflorescences in the bagged treatments produced similar fruit set to those in the natural treatment. Therefore they concluded that the species showed no evidence of pollen limitation, and that fruit set was most likely influenced by resource limitation. My results are different from this in that I observed differences in fruit production with changes in treatment, rather than fruit set being unrelated. Therefore, it is likely that the high fruit set in the bagged treatment is not related to resource limitation. Bagging is a commonly used technique for excluding birds and insects, and is often used for testing whether a plant is self-incompatible. In contrast with my 2009 results, Beever and Parkes (1996) found that almost no fruit set occurred in their bagged

treatments, leading them to conclude that the species was self-incompatible. A possible cause of the inflated fruit set for the bagged treatment, is the bag encouraging self pollination by rubbing against the flowers. Experimental evidence of this has been shown in Kelly et al. (2004) where the authors used muslin bags for their bagging treatment. When they used small bags for the treatment the fruit set was around 15%, while when they switched to using small bags fruit set decreased to around 3%. Fruits produced from within the bagged treatments must be a result of self pollination as all in the bagged must be selfed fruits the flowers were unopened when the bag was put in place. A potential consequence of this is inbreeding depression, which can result in the quality of self-pollinated seeds being lower than that of cross pollinated seeds. Inbreeding depression doesn't always occur even if self pollination is occurring. For example, Robertson et al. (1999) looked at pollinator loss in *Peraxilla colensoi* and *P. tetrapetala*, and examined rates of germination and survival for self crossed and outcross fruits. They found no significant effect of pollination type, indicating that inbreeding was unlikely to be occurring for this species.

### ***2010 season***

In the 2010 season, a different treatment effect was seen. In this season, the caged and natural fruit set were not significantly different, with a mean fruit set of 44.7% and 46.2% respectively. The bagged treatment was significantly lower than both (2.8%). This matches my prediction that the removal of birds resulted in little change in fruit set, suggesting that the birds provided an incidental role in pollination for this species in 2010. The low fruit set under the bagged treatment also matches prediction, and furthermore agrees with data by Beever and Parkes (1996) determining that the species was self-incompatible.

### ***Comparing the seasons***

It is interesting that the relationship of both the bagged and the caged treatment to natural changes between the years. One possible reason for this is that the contribution of birds and insects to pollination for this species was different between the years, with birds playing a larger role in 2009 than 2010, while insects were more prominent in 2010. This could be caused by environmental factors such as temperature, as insect activity is connected to temperature, with higher activity as temperature increases (Anderson, 2003). Another possibility is that insects were higher in abundance in 2010 than in 2009, so they were able to make up for the decreased pollination that occurred when birds were excluded, while in 2009 they were unable to.

The bagged treatment produced low fruit set in 2010, which was predicted, implying the species is unable to autonomously self pollinate, although this does not exclude the species being self-compatible. This pattern may have been season specific rather than species specific. Insects could have been decreased in prevalence this year, or birds increased in prevalence, such that birds played a larger role than predicted. This could also be connected to mast flowering. In the 2009 season *C. australis* flowering was much higher than in 2010, both in terms of number of inflorescences per plant and number of flowering plants. Insects are more restricted in movement than birds due to differences in size, so birds may have been able to take better advantage of the mast flowering, having a stronger per capita effect on the flowers than insects. However, the low bagged fruit set in 2010 is contradicted by the high fruit set in the bagged treatment for 2009. The same bags and methods were used for both seasons, so it seems unlikely that the bags facilitated self-pollination in one season, but not in the following. Nine individuals were used in the first season, and five in the following (fruit count could only be obtained from four, see methods for details). All four trees used for the second season were also used in the first. If the low fruit set in the bagged treatment in 2010

was due to the tree selection, then those four trees in 2009 should have a lower fruit set than the other trees. However, this was not the case; the average fruit set for the four trees that were present both years was 41.0%, while the average fruit set for the remaining five trees that were only present in the first year was 31.7%. Therefore, the cause for the difference does not lie in the tree selection. Fruit set from bagged treatments will be entirely self-pollinated, and as such may be lower quality than fruits from the caged and natural treatments, which will have some proportion of outcross pollination. One potential cause of the variation in bagged fruit set between seasons is competition-dependant abscission of fruits. This has been commonly reported in *P. tenax*, and will be covered in further detail in the following chapter. It involves the plant preferentially aborting fruit of lower quality, commonly those produced from self-pollination in favour of maturing fruits of a higher quality. This has not been reported in *C. australis* previously, however, there is little literature on pollination biology of *C. australis*. Alternatively, wind pollination may be occurring through the mesh of the bags and providing conspecific pollen for fruit production. The fruit set data I have obtained gives no indication of the quality of the fruit that is set. It is possible then, that in the 2009 season, which appears to have a lower contribution by insects, and higher by birds, that a large portion of the fruit set were from self-pollen, or that there were ample resources, such that there was no abscission of fruit. In 2010, the role of insects appears to have been more major, and fruit set for both the caged and the natural treatment was higher. In addition, this was a low flowering year both in terms of number of plants that produced inflorescences and the number of inflorescences produced; the cause of this is unclear. Potentially, fruits produced in the bagged treatment in 2010 could have been abscised to allow the production of ‘better quality’ fruits in the caged and the natural treatments.

Finally, for the differences in the bagged and the caged treatments between seasons, it is possible that this is an artefact from the experimental technique. Specifically, the 2010 was a low flowering season, and only five trees were suitable for treatment, of which, one was interfered with, and produced no data. Therefore, only four individuals contributed to the data for the 2010 season. This low number of replicates may mask any true effect that is occurring, as fruit set may have represented extremes of ranges. Nevertheless, the results were significant.

### ***Conclusion***

*C. australis* responded differently to bagged, caged and natural treatments between the two studied seasons. The two seasons were notably different in the number of inflorescences produced by both study and non-study plants. In the high flowering season (2009), the role of birds in pollination appears to be much higher than in the low flowering season (2010). Pollen limitation could not be determined for this species, as no hand pollination treatments were undertaken.

## Chapter Three – *Phormium tenax*

### 3.1 Introduction

*Phormium tenax* is an endemic New Zealand plant, commonly known as New Zealand Flax, or harakeke. It has a strong cultural significance, having been used traditionally by native Maori for weaving items such as clothing, baskets and mats. In addition the species has been used in ecological restoration, to aid in erosion control and as hedging in farms. The species is widespread across New Zealand and is present in a wide range of habitats as well as being common in gardens as a decorative plant, and is thought to play a role in forest regeneration by playing the role of a nurse plant (Reay and Norton, 1999) *P. tenax* is currently included in the Phormiaceae (Wehi and Clarkson, 2007) but was once included in the Agavaceae (Newstrom and Robertson, 2005), partially due to its large, agave-like flower stalks (Becerra and Lloyd, 1992).

The flowers are tubular, usually 25-50 mm long (Wehi and Clarkson, 2007) and flower colour ranges from yellow-orange to deep red (Craig and Stewart, 1988), both of which are consistent with the ornithophilous syndrome (Robertson et al., 2005). The flowers are hermaphroditic and present the pollen and stigma sequentially (Craig and Stewart, 1988). Flowers produce a large amount of light, powdery orange pollen (Wehi and Clarkson, 2007). Flowers lowest on the inflorescence and closest to the stalk open first, and buds open following this pattern, with those at the terminal ends of secondary branches and at the top of the stalk opening last. The flowers on the bottom of the inflorescence have normally finished flowering before the top ones open (Craig and Stewart, 1988). Pollinated flowers mature into large seed pods, ranging from four to 10 cm in length with most pods producing between 50 and 100 seeds. Seeds are known to come in three distinct types, small with well-developed



endosperm, large with well-developed endosperm and large with little endosperm. Large and small seeds are easily discernable from each other by sight, with large seeds often being between eight – ten mm in length, and small seeds being below five mm in length.

Germination studies have shown that the small seeds are thought to be the result of resource competition (Tisch, 1996). Seed pods dry and open explosively, the seeds are light and are able to be carried by wind some distance, and seed dispersal via either wind or water appears to be likely, especially as the seed is able to float on water and germinate while floating (Wehi and Clarkson, 2007).

*Phormium tenax* has been primarily reported as bird pollinated and adapted to bird pollination (Newstrom and Robertson, 2005), with tui, bellbirds, stitchbirds, kaka and white-eyes (*Zosterops lateralis*) observed as collecting nectar from the flowers (Wehi and Clarkson, 2007). Tisch (1996) reported visitation by bellbirds, silvereyes and introduced starlings. Bellbirds and silvereyes contributed 49.5% of the visits each. On Kapiti Island where predators are controlled and bird presence more closely resembles pre-colonized situation, stitchbirds, bellbird and tui were observed frequently visiting *P. tenax* (Castro and Robertson, 1997). Wind, water and insects have also been proposed as possible pollen vectors (Wehi and Clarkson, 2007). However, the pollen of *P. tenax* does not resemble the dry and powdered pollen that is associated with wind pollination, instead the pollen clumps in a manner that is better associated with animal pollination (Craig and Stewart, 1988). Flower structure does not appear to promote pollination by insects, although observations have determined that they do pollinate the species (Craig and Stewart, 1988, Huryn, 1995). For example, the curled petals are separated from the pollen in a way that prevents the formation of a landing platform for insects (Craig and Stewart, 1988). Lizards have also been observed feeding on nectar on *P. tenax*. The role of lizards in pollination has never been examined in detail, and the rate at

which they transfer pollen is unknown, however they have been observed contacting the stigma and carrying pollen so they may play a role in promoting cross-pollination (Newstrom and Robertson, 2005) , if they move between plants with sufficient frequency (Kelly et al., 2006).

*Phormium tenax* reproduces both sexually and asexually. Asexual reproduction is achieved by producing ramets, which are attached to one another by underground rhizomes. Each ramet produces an inflorescence with flowers once in its lifetime, with the inflorescence able to be up to three meters tall (Becerra and Lloyd, 1992). A plant consists of offshoots from the original seedling and the offshoots from these (Craig and Stewart, 1988). A plant may consist of up to several hundred ramets, all of the same genotype as the parent (Wehi and Clarkson, 2007). As such, a plant is assumed to be of a single genotype (Becerra and Lloyd, 1992, Craig and Stewart, 1988).

Sexual reproduction involves the production of large inflorescences. Inflorescences are terminal (Craig and Stewart, 1988) (Craig and Stewart, 1988) and can be up to three metres tall consisting of a main stalk with between 8 and 15 secondary branches (penduncles). These penduncles produce up to seven tertiary branches, each containing one to four clusters of flowers (Wehi and Clarkson, 2007). Clusters of flowers generally contain between five and 10 flowers. An inflorescence can contain between 250 and 700 flowers (Tisch, 1996) , and there can be one or many inflorescences per plant.

Mast flowering is where a population intermittently produces large quantities of flowers or seeds in a manner that is synchronised to some degree between individuals. Synchronisation can extend beyond a population to plants thousands of kilometres distant. Flowering in *P. tenax* has been proposed to be connected to temperature in a ten-year period, however no correlation has been found (Schauber et al., 2002). Measurements have been made at

Wellington and Mt. Misery sites over a period of 41 years. Throughout this time plants flowered 10 times with an interval of three years, two times with intervals of four years, and one time with an interval of two years (Brockie, 1986). The CV (coefficient of variation for seed output among years) is used as a measure of how variable flowering is over time, with masting species generally having high CVs. For *P. tenax* the CV has been determined as between 0.97 and 1.20 (Webb and Kelly, 1993), while some other masting species have much higher CVs. This indicates that *P. tenax* does not mast to the same degree as some other masting species (Tisch, 1996). Some years many plants have been observed to have no inflorescences, other years every plant was observed to have at least one inflorescence, and some plants over 30 (Craig and Stewart, 1988).

### ***Competition-dependent abscission of flowers***

A number of studies have looked at self-incompatibility in *P. tenax*. Self-incompatibility is a mechanism to encourage cross-pollination by reducing or removing the ability of a plant's own pollen to produce zygotes. The incompatibility barrier varies in strength across species, from no self-incompatibly, through reduced self-compatibility to complete self-incompatibility. Cryptic self-incompatibility is a weak form of self-incompatibility that is where self- and cross- pollinations performed on different flowers have the same success in competition, but when they are performed on the same stigma the self-pollen is less successful (Becerra and Lloyd, 1992). For example, this can occur through self-pollen producing slower growing pollen tubes (Jesson et al., 2006). Cryptic self-incompatibility generally occurs at the level of the flower, but the allocation of resources can occur both between ovules within the same flower, and between different flowers. So, while there may be no difference in seed set between self- and cross-pollinated flowers, when they two are on

the same plant the seed set from cross-pollinated flowers may be higher (Jennersten and Nilsson, 1993).

*Phormium tenax* preferentially outcrosses, but some selfed flowers do produce fruit (Craig, 1989). It is common for *P. tenax* plants to abort a number of fruits in the days following pollination (Becerra and Lloyd, 1992). A number of authors have examined why this is occurring. Jenssen et al. (2006) looked at whether *P. tenax* was able to autonomously self-pollinate, what the effect of mixed pollination within a single flower was, and what the natural levels of cross- versus self-pollination were. By bagging peduncles, and removing all other flowers from the flower stalk, the authors determined that *P. tenax* was able to set seed when no pollinators were present. Seeds from self-pollination, however, were smaller and had less endosperm than seeds from open pollination, which may be an indication of inbreeding depression. They found no difference in the abscission of flowers with mixed pollination, compared with selfed flowers. However, flowers with completely outcross pollen were matured preferentially over both self- and mix- pollinated flowers. Becerra and Lloyd (1992) examined the success of fruit from self- and cross- pollinations at various distances from each other on the same and on different inflorescences. Self pollination was achieved by emasculating flowers, and applying pollen from the same inflorescence onto stigmas once they were receptive. They found that fruit set under natural conditions appears to be evenly distributed across the inflorescence and that flowers that were selfed were less likely to be retained if they were close to cross-pollinated flowers. This provides a strong indication that there is proximity-specific competition for resources within inflorescences. Tisch (1996) looked at the differences in how often flowers were aborted between different secondary branches, taking into account site, density, plant, stalk, pair and whether the plant was bagged as well as a range of interaction terms. Tisch found that higher levels of abortion occurred in

bags with open secondary branches. This indicates that the abortion was caused by competition with cross-pollinated flowers.

The ability of plants to abscise fruits based on the type of pollination presents the possibility for this to be used for reproductive assurance. If pollination levels are high the plant can preferentially mature the highest quality seeds, and if levels are low then the plant can mature selfed seeds. This was confirmed by Craig and Stewart (1988) who found that a plant appears to preferentially provide resources to cross pollinated seeds, with self-pollinated seeds only fully maturing when pollination is low. However, Becerra and Lloyd (1992) did not find any significant difference in fruit set between cross- and self- pollinated treatments, nor any difference in the number of seeds per fruit or the percent viable seeds produced.

### ***Seed size and germination***

Tisch (1996) examined the germination of the two seed sizes using 120 arbitrarily selected seeds from the two seed classes, and bagged and unbagged secondary branches. The seeds were placed in moist filter paper in petri dishes, which were kept at room temperature and watered every second day. Tisch was unable to test the germination statistically however, many large seeds germinated, while no small seeds did. There appears to be no correlation between high levels of self-pollination and the development of large seeds that had poorly developed endosperm, based on statistical analysis of the differences in seed weight between bagged and unbagged secondary branches. Large seeds were equally viable whether they were from self- or from cross-pollination. In a similar analysis Becerra and Lloyd (1992) found no significant differences in the seed weight between self- and cross-pollinated flowers.

### ***Hybridization***

Two species of *Phormium* are present in New Zealand: *P. tenax* and *P. cookianum*. There are two subspecies of *P. cookianum*: *P. cookianum* subsp. *cookianum* and *P. cookianum* subsp. *hookeri* (Houliston et al., 2008). Houliston et al. (2008) looked at whether there were differences in cross-compatibility between the three taxa as well as a population from Okiwi Bay, and a group of coastal plants, both of which are thought to be derived from hybrids. They carried out 368 crosses, determining that seed was produced from most cross combinations, excepting four of five cross combinations involving *P. cookianum* subsp. *cookianum* as the maternal parent, which set no fruit (Houliston et al., 2008). This work indicates that there are no strong barriers to hybridization between different *Phormium* taxa, which allows maintenance of genetic diversity even if seed set was restricting the number of *Phormium* seedlings produced in a generation.

### ***Aims of this study***

The large literature base for *P. tenax* made it a good plant to study, as unlike *C. australis* a lot has been determined about reproductive characteristics and pollination as examined above. This means the results of this study are able to be analysed with knowledge of various components of the species life history. No direct work has been done investigating pollen limitation in *P. tenax*, so my study will not be repeating past work. In addition, the large flower size of *P. tenax*, and the presence of copious amounts of visible orange pollen makes hand pollination easy, while the ornithophilous nature of the flowers provides a good comparison to the smaller, more generalised flowers of *C. australis*.

The large, ornithophilous nature of *P. tenax* flowers indicates that birds may be the predominant pollinators, with insects playing only a small role in effective pollination. As a result I predict that excluding birds from the flowers will dramatically reduce the fruit and seed set. *Phormium tenax* has been reported as partially self-compatible, it is able to

autonomously produce fruit from self-pollination, and under some circumstances preferentially allocates resources to mature fruits produced by outcross pollen.

For this species I aimed to:

- Examine the relative roles of birds and insects on fruit set
- Confirm whether the plants were able to autonomously self-pollinate
- Determine whether the population studied was pollen limited

For this, I set up four treatments on ten individual trees. The treatments were: bagged (with no artificial pollination), caged, hand-pollinated and natural (unmanipulated). I predict that both the bagged and the caged treatments will produce very few fruit, and potentially few seeds per fruit. Seeds per fruit and fruit set are both indicators of reproductive performance, and as such, I predict they will respond to the treatments in the same manner.

### **3.2 Materials and methods**

Field experiments were undertaken concurrently with *C. australis* as the two species set buds and flowers opened at similar times, although *P. tenax* flowers continued opening after *C. australis* had finished flowering, and fruit set was weeks later for *P. tenax*. The first field season ran from November 2008 until late February 2009, where 10 individual plants were selected. The second field season was from November 2009 to February 2010, as for *C. australis* there were less flowering individuals in the second season than the first, and as a result eight individuals were selected. Individuals were chosen on the basis of having an accessible flowering stalk that was either difficult for the public to access or to see. A localised collection of ramets was assumed to be an individual plant and all of the same genotype, as proposed by Wehi and Clarkson (2007). A single stalk was used for each plant.

**Table 3.1:** Treatments set up for *P. tenax* over two seasons at Victoria Park. A letter is given in the column if a treatment was set up for that plant in that season, if no letter is present then no treatment was set up.

Plant	November 2008 – February 2009	November 2009 – February 2010
F1V	X	
F2V	X	
F3V	X	X
F4V	X	X
F5V	X	X
F6V	X	
F7V	X	
F8V	X	X
F9V	X	
F10V	X	
F11V		X
F12V		X
F13V		X
F14V		X

Study for this species was undertaken at the Victoria Park, Christchurch. . For *P. tenax* four treatments were applied to each individual: bagged, caged and natural were set up using the same methodology as described for *C. australis*, with the exception that one natural treatment was set up per individual for *P. tenax*, compared to two per individual for *C. australis*.

Treatments were applied at the level of peduncles, with one tertiary branch tagged and counted for each treatment. The fourth treatment was hand pollination. This technique is often used for determination of whether a population or species is pollen limited. It involves the application excess viable pollen to the receptive stigmas of the chosen flowers. For this study I collected pollen from around 10 flowering individual plants (not treatment plants) in a



small container and then on the same day used a paintbrush to dab pollen onto the stigmas of the tagged flowers for the hand-pollination treatment.



**Figure 3.1:** Hand pollination of *P. tenax* flowers

Plants were revisited once to twice a week, and hand pollination was repeated until seed pod development was evident to ensure that no flowers in the hand pollination treatment missed receiving the pollination. Fruit count was scored once seed pods were sufficiently developed that counts could be made reliably. Across the two seasons, four treatments were lost due to the disappearance of bags (three in 2009 and one in 2010) and one due to the loss of the hand treatment.

For this species the number of buds tagged per treatment ranged from 8 to 66, with an average of 33 buds per treatment. In addition to fruit set, in the 2009 season seed set was also examined. Once seed pods had matured, so that they were dry and beginning to split open, up to three seed pods were collected per treatment, with fewer collected if the treatment did not have sufficient intact seed pods. Pods were collected only if seeds had not been released, which could be determined with sufficient accuracy by whether the pod showed small splits

or whether the splits were large. For each pod collected the plant and treatment was recorded as well as the length and width of the pod; the pods were then split open and the number of small and large seeds were counted. Seed set was analysed using GLMs, through the R statistical software package. Each GLM tested a single predictor variable (Table 2), using the appropriate error distribution for the data type, and a Chi square test for significance.

**Table 3.2:** Variables used in Generalised Linear model tests of significance for seed set predictors.

Response	Predictor(s)	Error distribution	Link
Proportion large seeds	Plant, Treatment	Quasibinomial	Logit
Pod length	Plant, Treatment	Gaussian	Identity
Pod width	Plant, Treatment	Gaussian	Identity
Number of large seeds	Plant, Treatment	Quasipoisson	Log
Area of seed pod	Plant, Treatment	Gaussian	Identity
Number of seeds per pod	Plant, Treatment	Quasipoisson	Log

Fruit set was taken as the percentage difference between the number of buds when treatments were initiated, and the number of fruit that the plant set. Statistical differences between predictors were analysed using a GLM with quasibinomial error distribution and a Chi square significance test. The response variable was proportion fruit set and the predictor variables were plant, season and treatment. The statistical relevance of differences in fruit set between treatments was analysed using pairwise *post hoc* comparisons using standard *t*-tests, as used for *C. australis*.

Pollen limitation was determined by comparing the natural treatment to the hand pollinated to produce a pollen limitation index (PLI). The PLI is determined by the equation  $1 - (\text{natural fruit set} / \text{hand crossed fruit set})$ . PLI is a commonly used measure of pollen limitation, generally truncated at 0, with a PLI of 1 representing complete pollen limitation, while a PLI

of 0 represents no pollen limitation (Kelly et al., 2007, Larson and Barrett, 2000).

Determining the PLI for a species or a population is not limited to using fruit set; seed set is another means of determining pollen limitation. For *P. tenax* the percentage of flowers that set fruit (fruit set) was used to determine one measure of PLI, and the absolute number of seeds that were set was used to determine a second PLI.

Where fruit set for an individual plant was 0% calculation of PLI was impossible as it involved division by zero. Following methods used by Robertson et al. (2006) 0.5% was added to each zero value, meaning that the final PLI determined is a conservative estimate.

### 3.3 Results

#### *Fruit set*

Progression from bud to flower occurred over a period of several weeks, likewise from flower to fruit. Flowering followed the same trend as observed in *C. australis*, with individuals producing more flowering stalks in 2009 than 2010, and more individuals in 2009 flowering than in 2010. I did around an hour of observation of flowering plants, 30 minutes observing birds and 30 minutes observing insects. During this time I observed bees and wasps frequently visiting *P. tenax* flowers both inside and outside cages, although the species of insects could not be determined. I observed no other insects visiting the flowers, and wasps frequented flowers more often than bees (413 seconds versus 206 seconds). I didn't observe birds visiting the flowers during any of the observation periods, however at several stages while setting up treatments saw birds visiting non-tagged *P. tenax* plants in the distance but was unable to identify species.

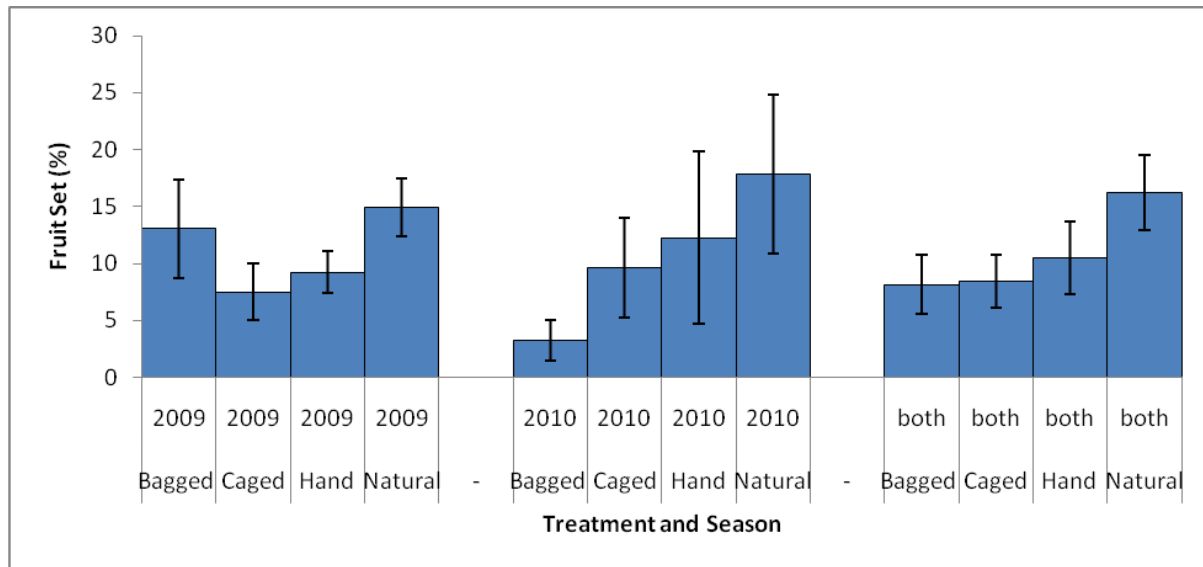
Analysing the entire data set showed a strong significant effect of the individual plant on fruit set, but no significant effect of season, treatment or the season by treatment interaction,

although both the treatment and the season by treatment interaction were close to significant (Table 3.3). Consequently, analysis was done with both seasons in the same data set, rather than separated out as was done with *C. australis*.

**Table 3.3:** Analysis of variance (ANOVA) for fruit set of *Phormium tenax*. This model used a quasibinomial error distribution with a Chi squared test for significance. This model used fruit set as the response term, with plant, season, treatment and the plant by season interaction as predictors. Significance levels were: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

Model	df	Deviance	Residual df	Residual deviance	P(> Chi )	Significance
Null			68	329.28		
Plant	13	139.20	55	190.09	2.775e-06	***
Season	1	0.02	54	190.06	0.93026	
Treatment	3	18.06	51	172.00	0.08919	
Season:Treatment	3	18.43	48	153.57	0.08408	

The pattern of fruit set response to treatment, varied widely both between plants, and between seasons. When the data is separated out into seasons (Fig 3.2) there is a high amount of variance (as shown by the black error bars), especially in the 2010 season. The variance is much lower when the two seasons are combined into a single data set.



**Figure 3.2:** Average fruit set for the four treatments for 2009, 2010 seasons, and the seasons combined. Error bars represent the standard error.

Despite the ANOVA showing significant marginally treatment effect ( $P=0.089$ ), pairwise analyses showed that the caged treatment was significantly lower than the natural treatment, and that the bagged treatment was nearly significantly lower than natural.

**Table 3.4:** Pairwise *post hoc* comparison of fruit set means for the two seasons combined. Degrees of freedom were 68 for these comparisons. Significance levels were:  $.P \sim 0.05$  \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ ,

Comparison	<i>t</i>	P	Significance
Bagged versus Caged	0.2438	0.4040	
Bagged versus Hand	-0.7957	0.2147	
Bagged versus Natural	-1.9518	0.0552	.
Caged versus Hand	-1.1754	0.2441	
Caged versus Natural	-2.5217	0.0141	*
Hand versus Natural	-1.2885	0.2021	

The main change across the seasons was in fruit set of the bagged treatment, so to determine whether this was driving the season effect, and if so, whether removing it would increase the significance of the treatment effect, an ANOVA was run containing only the caged, hand and natural treatments,

**Table 3.5:** Analysis of variance (ANOVA) for fruit set of *Phormium tenax*; with bagged treatment excluded. This model used a quasibionomial error distribution with a Chi squared test for significance. Significance levels were: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

Model	df	Deviance	Residual df	Residual deviance	P(> Chi )	Significance
Null			52	237.998		
Plant	13	111.775	39	126.222	0.00027	***
Season	1	1.945	38	124.227	0.41494	
Treatment	2	13.870	36	110.407	0.09347	
Season:Treatment	2	0.782	34	109.625	0.87487	

Significant of the treatment remained essentially unchanged when bagged treatment was excluded (Table 3.5) as when it was included, indicating that the season effect was not masking a treatment effect.

### *Seed set*

Testing individual seed pod variables revealed that the pod length and area were significantly affected by the treatments (Table 3.5). The number of seeds per pod bordered on being significant, with a P value of 0.05091.

**Table 3.5: Significance tests for the effects of treatments on factors related to seed set and seed pod size, including the plant by treatment interaction term. Each GLM uses plant and treatment as predictors. Error distribution and link functions are described in the methods.**

	Model	df	Deviance	Residual df	Residual deviance	P(> Chi )	Significance
Number of seeds	Null			38	752.83		
	Plant	1	35.14	37	717.69	0.17389	
	Treatment	3	147.73	34	569.97	0.05091	.
	Plant:Treatment	3	19.2	31	550.77	0.79872	
Number large seeds	Null			38	725.88		
	Plant	1	60.74	37	665.14	0.05266	
	Treatment	3	84.41	34	580.73	0.15652	
	Plant:Treatment	3	9.9	31	570.83	0.89363	
Proportion large seeds	Null			38	1364.31		
	Plant	1	23.96	37	1340.35	0.4093	
	Treatment	3	77.59	34	1262.76	0.5311	
	Plant:Treatment	3	38.71	31	1224.05	0.7771	
Length	Null			38	9984.4		
	Plant	1	229.2	37	9755.2	0.31518	
	Treatment	3	2175.2	34	7580	0.02254	*
	Plant:Treatment	3	538.1	31	7041.9	0.49948	
Width	Null			38	249.59		
	Plant	1	0.272	37	249.318	0.8465	
	Treatment	3	14.12	34	235.198	0.5834	
	Plant:Treatment	3	10.419	31	224.778	0.6969	
Area	Null			38	2.99321		
	Plant	1	0.07932	37	2.91389	0.28117	
	Treatment	3	0.59459	34	2.3193	0.03347	*

Plant:Treatment	3	0.20213	31	2.11717	0.3979
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General analysis found that the length of the seed pod and area were significantly affected by treatment. Pairwise analysis found a significant difference in pod length between the hand treatment and both the bagged and the natural treatments, with the hand being lower in both cases. Despite a significant treatment effect for the pod area, pairwise analysis didn't reveal any significant differences. The closest to being significant were hand versus bagged and hand versus natural comparisons, in the same direction as for pod length. As there was an almost significant treatment effect for the number of seeds per pod, I ran pairwise analysis for this which showed a close to significant difference between the bagged and both the caged treatment and the caged treatment with the bagged being higher.

**Table 3.6:** Means for seed pod variables, error shown is the standard error of the mean.

Response	Treatment	Mean
Length		
	Bagged	69.8 ± 2.6
	Caged	64.3 ± 5.9
	Hand	51.5 ± 5.1
	Natural	72.9 ± 4.8
Area		
	Bagged	904.8 ± 55.3
	Caged	769.6 ± 104.7
	Hand	705.1 ± 106.8
	Natural	922.7 ± 38.5
Seed number		
	Bagged	165.7 ± 19.6
	Caged	113.4 ± 11.6
	Hand	100.2 ± 20.1



Natural	129.2 ± 11.5
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**Table 3.6** Pairwise *post hoc* comparison of seed pod variables for the 2009 season in *P. tenax*.

Degrees of freedom for these tests were 38. Significance levels were: .P ~ 0.05 \*, P ≤ 0.05; \*\*, P ≤ 0.01; \*\*\*, P ≤ 0.001.

Response	Comparison	<i>t</i>	P	Significance
Length				
	Bagged versus Caged	0.5152	0.6094	
	Bagged versus Hand	2.0713	0.0452	*
	Bagged versus Natural	-0.2425	0.8101	
	Caged versus Hand	1.423	0.1629	
	Caged versus Natural	-0.7163	0.4783	
	Hand versus Natural	-2.2334	0.0315	*
Area				
	Bagged versus Caged	1.2123	0.2329	
	Bagged versus Hand	1.84	0.0736	
	Bagged versus Natural	-0.0694	0.9454	
	Caged versus Hand	0.6066	0.5477	
	Caged versus Natural	-1.2459	0.2204	
	Hand versus Natural	-1.8603	0.0706	
Seed number				
	Bagged versus Caged	1.8063	0.0788	.
	Bagged versus Hand	1.9678	0.0564	.
	Bagged versus Natural	1.1705	0.2491	
	Caged versus Hand	0.2586	0.7973	
	Caged versus Natural	-0.07763	0.9386	
	Hand versus Natural	-1.0079	0.3203	

### ***Pollen limitation***

Pollen limitation was determined using the PLI, separately for each of fruit set and number of seeds produced per pod

**Table 3.7:** Natural and hand pollinated fruit set for individual trees in percentages, with calculated PLI and years separated. Where no PLI is given, the PLI was incalculable due to a zero hand pollinated fruit set.

Year	Tree	Natural	Hand Pollinated	PLI
		Fruit Set	Fruit Set	
2009	F10V	22.6	17.6	-0.28
2009	F11V	15.0	6.9	-1.18
2009	F1V	12.0	3.8	-2.12
2009	F3V	11.1	14.3	0.22
2009	F4V	15.8	4.6	-2.47
2009	F5V	6.7	2.7	-1.47
2009	F6V	13.2	8.8	-0.51
2009	F7V	6.4	5.1	-0.25
2009	F8V	13.0	14.3	0.09
2009	F9V	33.3	14.3	-1.33
2010	F13V	51.9	34.6	-0.50
2010	F14V	4.2	0.0	
2010	F15V	0.0	0.0	
2010	F3V	4.3	0.0	
2010	F4V	18.4	47.2	0.61
2010	F5V	0.0	4.0	1.00
2010	F8V	24.0	0.0	

Across all trees the calculated PLI was mostly negative (Table 3.7), in the 2010 season; four trees produced no fruit on the hand pollinated treatment and in three of these cases (F3V, F14V and F15V) fruit set for the bagged treatment were likewise low. In only two cases (FV4 and F5V both 2010) was the fruit set in the hand treatment higher than that of the natural treatment. The PLI for 2009 alone was  $-0.9 \pm 0.3$ , while the PLI for 2010 was  $-8.5 \pm 6.6$ .

In order to calculate a PLI 0.5% was added to all zero value from the hand treatments. This resulted in a PLI of  $-4.1 \pm 2.8$ . Because only undamaged, unopened seed pods were used for seed counts and up to three pods per plant were used, there were no direct pairs of hand pollinated to natural. For each plant and treatment combination where more than one seed pod was collected the seed set was averaged across pods. Only plants which had at least one pod collected for both natural and hand treatments were included in the PLI calculation. The average PLI for number of seeds was  $-0.5 \pm 0.3$ . A negative PLI indicates that the natural treatment produced more seeds/fruit than the hand-pollinated treatment, thus there was no evidence of pollen limitation.

### 3.4 Discussion

All four treatments were successfully initiated for both seasons in *P. tenax*. In addition, fruit set results were measured in both seasons, and seed set data for the 2009 season. This gives a comprehensive data set for statistical analysis. The inclusion of the hand pollinated treatment allows the level of pollen limitation to be determined, providing a measure that can be used to compare to other populations or other species.

#### *Fruit set*

The only significant predictor of fruit set was the individual plant; neither season nor treatment was significant. Despite this pairwise analysis show a significant difference between the caged and natural treatments, and a marginally significant different difference between the bagged and natural treatments. Fruit set decreasing as a result of caging, but not further decreasing through bagging, matches my predictions for this species. *Phormium tenax* is visited by insects (Kelly et al., 2010), but is generally viewed as a bird pollinated species (Robertson et al., 2005). The most likely explanation for these results is that excluding birds from pollinating (caged treatment) caused a decrease in fruit set as birds are the main pollinators for this species. Excluding both birds and insects (bagged treatment) had no additional impact, as insects were not having a significant role in pollination. Insects were observed visiting the flowers, but this is not the only component for successful pollination, the visitor must also collect pollen, and deposit it on the receptive stigma of a conspecific (Newstrom and Robertson, 2005). Craig and Stewart (1988) noted that the structure of *P. tenax* flowers means that bees were only likely to pollinate the flower when it first becomes female, which agrees with the low role of insects in pollination seen in my results. A second potential cause for this pattern is that the caged treatment deterred insect visitation. This is unlikely as insects were observed visiting flowers within cages, the caged treatment showed a different response than the bagged for *C. australis* and tests show that cages are not likely to significantly impair insect visitation levels (Anderson, 2003, Robertson et al., 2005).

### ***Seed set***

The amount of fruit that is set is only relevant if this difference is carried across into differences in seed set. I examined two sets of related variables to determine this, the number of seeds, number of large seeds and the proportion of large seeds was the first set, while the second set comprised of seed pod length, width and area. Treatment significantly affected

pod length and as a result the area of the seed pods. Here, the treatment means were similar across all treatments, but the hand pollinated treatment was lower than both the bagged and the natural, significantly for pod length and non-significantly for pod area. This is interesting, as it is the same pattern that is reflected in fruit set, although it is not significant in the case of fruit set while the number of seeds per pod was marginally significantly affected by treatment. There was a marginally significant effect of treatment on the number of seeds per pod, with bagged having a higher number of seeds than all other treatments, although not significantly higher. Biologically, this result is unexpected. When the bagged treatment was set up, none of the flowers were open, and the treatments were monitored to ensure none of the bags came loose. As such, any pollination that a bagged treatment receives is entirely self pollen. If bagging somehow facilitates self-pollination, then it is reasonable that more seeds could be produced under bagged conditions than under other treatments. However, the seeds produced in this manner are likely to be of lower quality than those produced through cross pollination. For this species, the most evident sign of seed quality is seed size, yet there was no significant effect of treatment on the number or proportion of large seeds. Seeds were not weighed in this study, so it is possible that the amount of endosperm for seeds differed between treatments. Endosperm provides nutrients, and differences in this would indicate a difference in seed quality. One of the problems with using fruit or seed set to determine plant reproduction is that these do not take into account the quality of offspring. Looking at seed pods variables and seed numbers, I have attempted to examine whether quality changes, as fruits do not necessarily have the same number of seeds, and small seeds have been determined to be infertile (Tisch, 1996). The fact that small differences were observed in the number of seeds, and none in the proportion of large seeds, does not indicate that the seeds are identical. For example, the quality of seeds across different treatments may differ by quantities such as the amount of nutrients that are present or the amount of endosperm

(Zimmerman and Aide, 1989). This wouldn't be detectable by counting fruit or seed set, but the difference in size of seed pods may be an indication of a change in quality. If this is the case, the fact that the pod size was lower for the hand treatment than any other treatment may indicate that the quality of the hand pollinated seed is lower; however there also may be no difference in quality across treatments.

### ***Pollen limitation***

For both seed and fruit set, the hand pollination treatment behaved in a different manner than anticipated. Pollen supplementation is a common method of testing whether a species or population is pollen limited, as if there is a lack of pollen, providing excess pollen should result in increased reproductive success, often seen through fruit or seed set. My manipulations resulted in a decrease in production for the hand pollinated treatment compared the natural treatment, although in both cases these differences were not significant. This resulted in a PLI of  $-4.1 \pm 2.7$  for fruit set and  $-8.6 \pm 0.3$  for seed set. Pollen limitation studies generally truncate PLI at 0, because the theoretical basis for the procedure is to test whether pollen supplementation increases or causes no change in female reproductive success (Young and Young, 1992). This leaves no allowance for negative pollen limitation; any instances of this would be truncated to zero and considered evidence of no pollen limitation. A second approach, taken by some authors, is to consider negative response as an indication of flaws in the pollen supplementation experiment (Anderson, 2003). Negative responses to pollen supplementation experiments are more common than generally believed. Out of 99 cases reviewed in 2002, 42.4% found a significant increase of female reproductive success as a result of pollen supplementation, 40.4% found no significant effect and 17.2% found significantly reduced female reproductive success (Young and Young, 1992). My results for

this species fall in the middle category, no significant effect. The implications of negative pollen limitation will be examined in further detail later in this thesis.

### ***Conclusion***

*Phormium tenax* did not respond strongly to pollen supplementation or exclusion treatments. Differences in fruit set showed that the natural treatment was significantly higher than the caged treatment, and non-significantly higher than the bagged treatment, indicating that birds played the dominant role in pollinating this species, with insects appearing to have only a minor role. Seed production did not appear to change across treatments, although differences in pod size suggested that there may be an unseen change in quality between the hand and the natural treatments, indicating that the hand pollinated treatment was possibly of lower quality than the natural. This reinforces the non-significant trend seen in fruit set that the hand pollinated treatment had lower reproductive output than the natural treatment. With a PLI of -0.5 for fruit set and -0.3 for seed production I conclude that this population was not pollen limited, however I will examine negative pollen limitation in further detail in a later chapter.

## Chapter Four – *Kunzea ericoides*

### 4.1 Introduction

*Kunzea ericoides* (Kanuka) is a native New Zealand plant that forms a small tree, which grows up to around 15 m in height (Burrows, 1996b). It is present in native forests, but establishes readily on bare ground or lightly grazed pasture, resulting in the formation of close to monospecific stands in cleared forest sites or pasture (Allen et al., 1992). It flowers around January to February (Huryn, 1995) producing small, creamy white flowers around 10 mm in size (Webb and Lloyd, 1986). The flowers are hermaphroditic, and occur either singly or in groups; the male and female parts of the flower are separated spatially, with the stamen filaments bending away from the style after the presentation of pollen. The flowers have a strong sweet scent, which is thought to attract insects (Beardsell et al., 1993), in addition, the dish shape of the flowers provide easy insect access (Newstrom and Robertson, 2005). Kelly et al. (2010) classify *K. ericoides* flowers as entomophilous but occasionally visited by birds. *K. ericoides* is self-compatible, able to set viable seed even when flowers are bagged, and isolated plants are able to form colonies (Burrell, 1965).

*Kunzea ericoides* is thought to act as a pioneer species by establishing readily on bare ground or lightly grazed pasture, resulting in the formation of close to monospecific stands in cleared forest sites or pasture. By its presence *K. ericoides* is thought to provide an environment that facilitates other species establishing (Allen et al., 1992). A study on deer grazing under a canopy of *K. ericoides* and *Leptospermum scoparium* found that small, broad leaved species like *Melicytus ramiflorus* and *Myrsine australis* would grow and over a period of 10 years replace *K. ericoides* in the canopy. However, when deer were allowed to graze, the broad-leaved species were repressed and the canopy remained unchanged (Coomes et al., 2003).



This has relevance in two ways, firstly it indicates that species are able to establish under a *K. ericoides* canopy and receive sufficient light to fully mature, and secondly that *K. ericoides* seedlings are not able to establish under a canopy. Allen et al. (1992) looked at whether *K. ericoides* is able to act as a pioneer species, by its presence altering conditions in a way that facilitates the establishment of later successional species, leading to it eventually being replaced by vegetation commonly present in primary forest. They looked at nine Otago sites in which *K. ericoides* was the dominant woody vegetation. In quadrats within these sites they examined aspect, slope and which vascular species were present, as well as the basal diameter of all stems greater than 10 cm in diameter. Soil profiles were taken, as well as number and height of woody stems less than 2 m tall and the estimated cover of all those species with more than 5% cover. The authors found that while tree seedling establishment can occur within *K. ericoides* stands; these are only able to progress past the sapling stage if there is a substantial reduction in the stem density of *K. ericoides*. This was true of both *K. ericoides* seedlings, and those of other species. This reinforces the determination that *K. ericoides* cannot regenerate in the shade, and also provides an example of where *K. ericoides* is not acting as a pioneer species, as it is inhibiting the succession process rather than facilitating it.

I chose *K. ericoides* as a study species, primarily because the small, creamy white flowers appear adapted for insect pollination, yet birds have been observed visiting them. This indicated that caging and bagging treatments were likely to find some interesting interactions. In addition, it is a common species present in large numbers at many sites.

## **4.2 Materials and methods**

*Kunzea ericoides* flowers later and for longer than either *C. australis* or *P. tenax*, so field work for this species began in early December 2008. In the first season (December 2008 – March 2009) populations of plants were studied at two locations, Victoria Park, and Hinewai

Reserve (43.81687°S, 173.02254°E), near Akaroa. Ten individuals were chosen at each site for study. In the following season (December 2009 – March 2010) just the Victoria Park site was used. Hinewai is a 1000 hectare reserve on the Banks Peninsula that is being managed in order to facilitate restoration and protection of native vegetation. The reserve is being managed by a technique of ‘minimum interference’, which involves removing or reducing introduced mammals on the reserve such as goats, sheep and possums, checking for establishment of introduced plants that are regarded as deleterious to regeneration and removing these while introduced plants considered neutral are left alone, and ensuring there are precautions in place against fire. Aside from this natural processes are left alone (Wilson, 1994).

For each individual plant studied, four treatments were set up on tagged branches: bagged, caged, hand and natural using the same methodology as for *P. tenax* and *C. australis*. In *P. tenax* hand pollination was achieved by removing pollen laden anthers, and collecting, then applying this pollen to the stigma of the treatment flowers. The smaller size of the *K. ericoides* flowers made this difficult, so instead entire flowers were collected and rubbed onto the stigma of treatment flowers. Hand pollination was repeated to ensure that no flowers were missed, and then plants were visited regularly to monitor progress. Once fruit were sufficiently matured success could be determined, fruit set counts were made from each individual. Across all treatments and individuals, 16 replicates were lost due to the disappearance or loss of tags, bags or cages, leaving a total of 104 treatments. The number of buds that were tagged per replicate, ranged from 2 to 154, with an average of 58.

As for the previous species, fruit set was taken to be the number of fruit matured divided by the number of buds when treatment was initiated multiplied by 100 to give a percentage. A GLM, using quasibinomial error distribution, and Chi squared significance test was used to

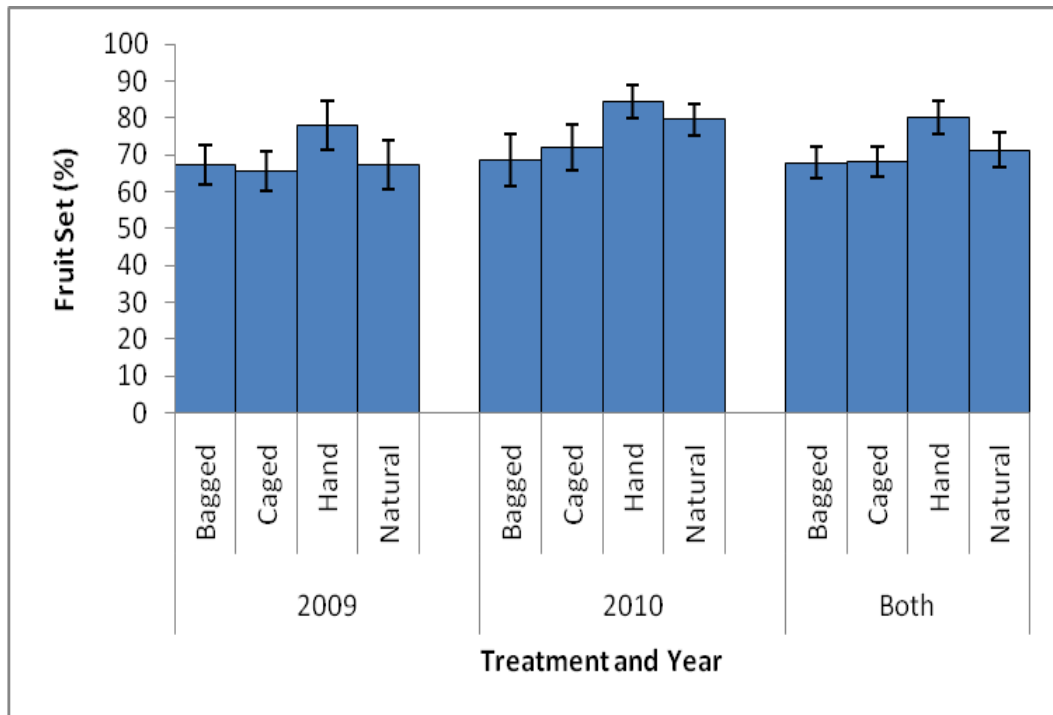
test treatment effects. Fruit set was used as the response variable, while season, treatment and location (Victoria Park versus Hinewai) were the predictor variables. Pollen limitation was determined by using the equation  $PLI = 1 - (\text{natural fruit set} / \text{hand crossed fruit set})$ .

### 4.3 Results

*Kunzea ericoides* progressed from bud to flower and flower to fruit slowly, with each stage taking upwards of a month. Number of flowers produced, and number of flowering plants appeared consistent across seasons. No observation periods were done for this species, but I frequently saw insects visit flowers as I was working with the species, but no birds were observed. Across all treatments and seasons fruit set was high, with a mean total fruit set of  $71.6\% \pm 2.2\%$ . A broad analysis of the data set showed no significant predictors for fruit set, with only season being close to significance (Table 4.1). Examining the differences for the seasons combined, or separated out (Figure 4.1), showed no additional differences across the treatments.

**Table 4.1:** Analysis of variance table, using quasibinomial error distribution and a Chi squared test for significance for *Kunzea ericoides*.

Model	df	Deviance	Residual df	Residual deviance	P(> Chi )
Null			103	1638.51	
Season	1	43.18	102	1595.33	0.0859
Location	1	0.1	101	1595.23	0.935
Treatment	3	15.04	98	1580.2	0.795
Season:Treatment	3	36.63	95	1543.57	0.475
Location:Treatment	3	55.44	92	1488.13	0.285



**Figure 4.1:** Average fruit set for bagged, caged, hand and natural treatments for the seasons separated and combined. The 2009 data is for both sites combined. Error bars represent the standard error.

As well as no differences being significant across treatments, the differences across the treatments were not biologically large. The largest difference was in the 2010 season between the hand (84.4%) and bagged (68.5%), a difference of 15.9%.

**Table 4.2** Pairwise *posthoc* comparison of mean fruit set for all treatment data from *K. ericoides*.

Degrees of freedom were 103. Significance levels were: . $P \sim 0.05$  \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

Comparison	<i>t</i>	P	Significance
Bagged versus Caged	0.102	0.919	
Bagged versus Hand	-0.253	0.802	
Bagged versus Natural	-0.497	0.620	
Caged versus Hand	-0.305	0.761	

Caged versus Natural	-0.519	0.605
Hand versus Natural	-0.155	0.439

Even though not significant, the most important difference here is the difference between fruit set for the hand and the natural treatments, as this provides the best estimate for the PLI. To look at this I ran a model incorporating only the hand and natural treatments (Table 4.3). Under this model treatment was still non-significant.

**Table 4.3:** Analysis of variance table for natural and hand treatments on *Kunzea ericoides*.

The model used quasibinomial error distribution and a Chi squared test for significance.

Model	df	Deviance	Residual df	Residual deviance	P(> Chi )	Significance
Null			51	952.76		
Season	1	68.89	50	883.87	0.0401	
Location	1	5.23	49	878.64	0.572	
Treatment	1	2.99	48	875.66	0.669	
Season:Treatment	1	9.59	47	866.07	0.444	
Location:Treatment	1	6.81	46	859.26	0.519	

PLI was calculated from the individual PLI values per plant, and most PLI values were near zero. Mean PLI was -0.003, this was not significantly different from zero (Standard deviation = 0.512, mean/standard deviation = -0.006). Analysis on per plant PLI's showed there was no significant difference in PLI between the locations ( $P = 0.489$ ), or between the seasons ( $P = 0.706$ ).

**Table 4.4:** Hand pollinated and natural fruit set values for individual *Kunzea ericoides* trees and the calculated PLI scores.

Tree	Location	Season	Hand	Natural	PLI
K10H	Hinewai	2009	19.4	11.8	0.39
K1H	Hinewai	2009	95	30.3	0.68
K2H	Hinewai	2009	50	99.1	-0.98
K3H	Hinewai	2009	100	75.5	0.24
K4H	Hinewai	2009	82.4	80	0.03
K6H	Hinewai	2009	83.3	96.4	-0.16
K9H	Hinewai	2009	95.5	40.5	0.58
K1	Victoria Park	2009	48.0	65.5	-0.36
K10	Victoria Park	2009	100	43.4	0.57
K2	Victoria Park	2009	100	94.6	0.05
K3	Victoria Park	2009	31.8	87.4	-1.75
K4	Victoria Park	2009	82.7	69.9	0.16
K5	Victoria Park	2009	100	100	0
K6	Victoria Park	2009	100	93.5	0.07
K8	Victoria Park	2009	78.9	100	-0.27
K9	Victoria Park	2009	80	60.9	0.24
K1	Victoria Park	2010	97.7	98.8	-0.01
K10	Victoria Park	2010	91.5	73.7	0.19
K2	Victoria Park	2010	93.9	73.2	0.22
K3	Victoria Park	2010	76.2	93.9	-0.23
K5	Victoria Park	2010	95.8	63.9	0.33
K6	Victoria Park	2010	83.3	65.6	0.21
K8	Victoria Park	2010	61.3	73.3	-0.20
K9	Victoria Park	2010	75	81.6	-0.09

## 4.4 Discussion

*Kunzea ericoides* had a large number of treatments with 104 for which fruit set was scored across two seasons and two locations. Potentially this gives the ability to assess with high accuracy the effects of treatment on this species. Interestingly, there were no significant effects of treatment, season or location

Pairwise analysis showed that there were no significant differences between any of the treatments. Trends showed that the bagged, caged and natural treatments were all very similar in fruit set, while the hand treatment was a little higher. This gives an indication that treatment had little effect on fruit set. This is a surprising result, as bags and cages were strongly secured, and regular checks confirmed that they were working as intended.

Therefore, the result is unlikely to be an artefact of the experimental procedure. That fruit set was high in the bagged treatment indicates not only that the species is self compatible, but also that it is capable of autonomously selfing, as buds were bagged prior to opening and no further manipulation was applied to them. Hand pollination caused no increase in fruit set (PLI = 0.003), but this difference was not significant. A PLI of 0.003 would generally be considered as evidence of low to no pollen limitation, for example, Newstrom and Robertson (2005) in a review of pollen limitation, takes a PLI of over 0.75 as evidence of high pollen limitation, a PLI of between 0.75 and 0.25 as evidence of moderate pollen limitation and a PLI of less than 0.25 as evidence of low pollen limitation.

## 4.5 Conclusion

*K. ericoides* showed no significant differences in fruit set across any treatments, locations or seasons, and what differences were present were small, and unlikely to be biologically relevant. The relatively high fruit set for bagged treatment indicates that the species is able to autonomously self pollinate, and this is likely to be the reason that the PLI (0.003) is low for

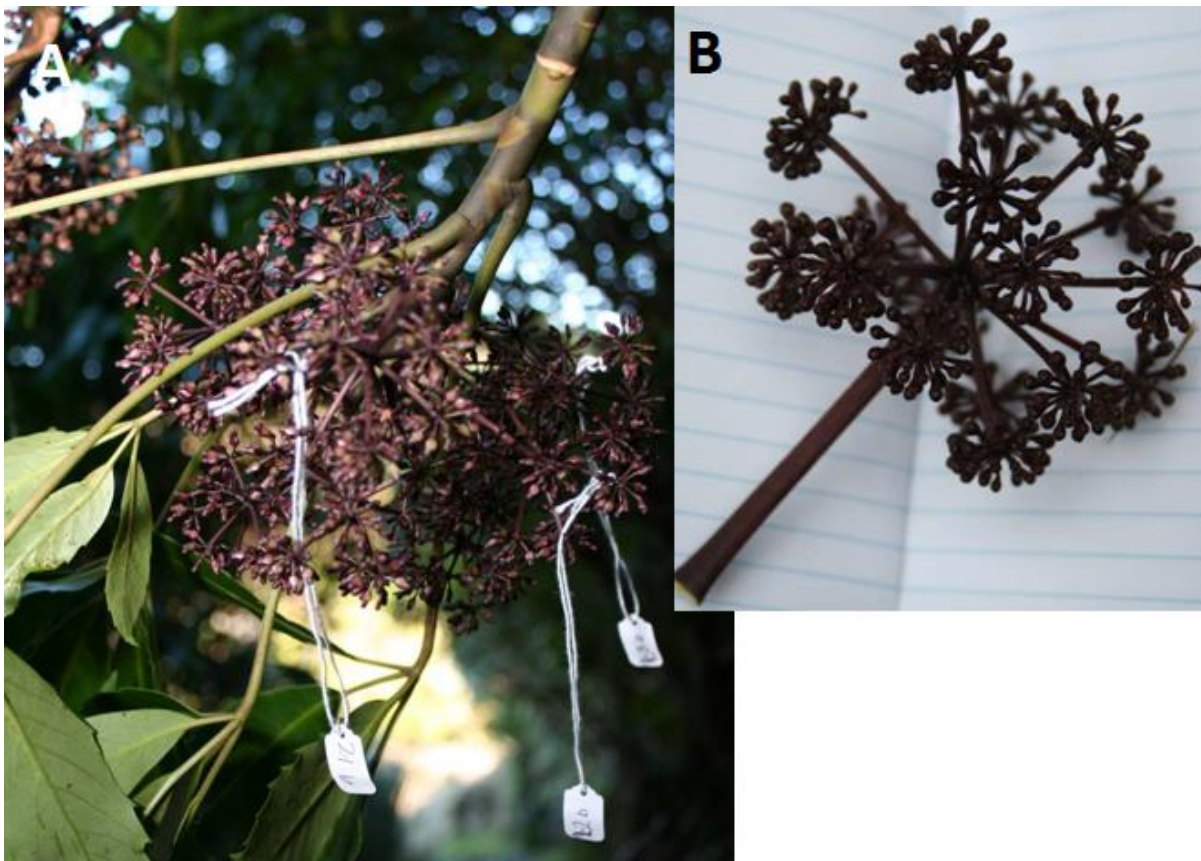
this species. Any effect of the changes in visitation across the treatments is not apparent through fruit set, so the question becomes whether inbreeding depression is occurring, rather than whether pollen limitation is occurring.



## Chapter Five – *Pseudopanax arboreus*

### 5.1 Introduction

*Pseudopanax arboreus* (Araliaceae), commonly known as five-finger produces tightly packed inflorescences of flowers each smaller than 3 mm in diameter. The species is dioecious (Anderson, 2003), with male and female plants coexisting in close proximity. Flowering occurs from the end of June through to December (Castro and Robertson, 1997, Godley, 1979) ; meaning this winter and spring-flowering species is likely to be subject to different conditions and pollinator availability during flowering than the other three, summer flowering, species studied. Inflorescences consist of many-flowered umbels (Fig 5.1), are terminal on branches and may contain some hermaphroditic flowers or flowers of the opposite sex to the majority (Burrows, 1996a)



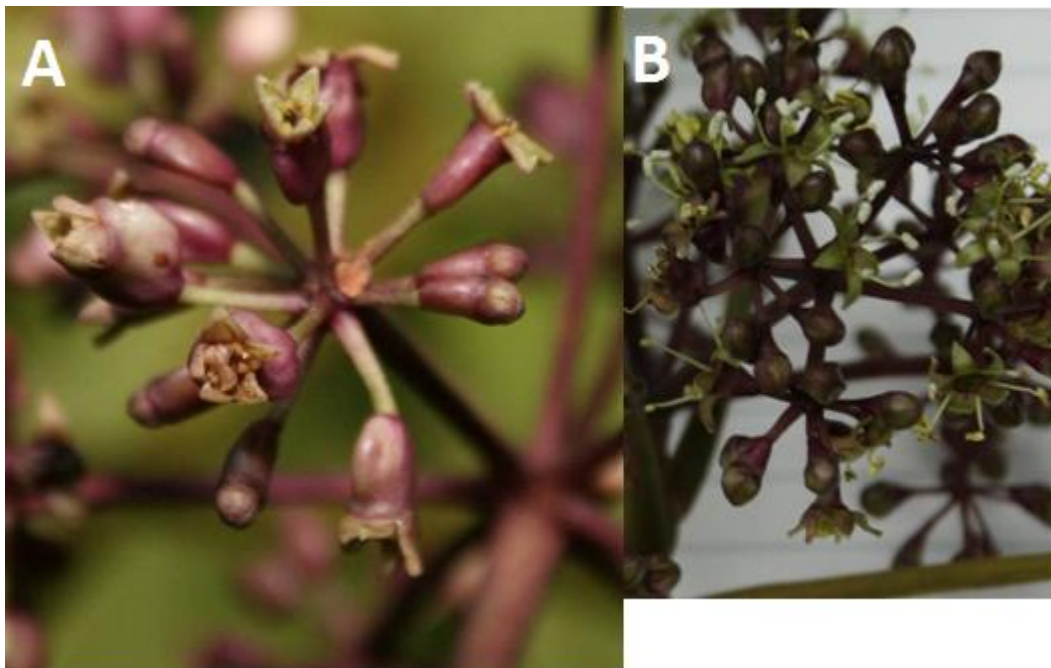
**Figure 5.1:** *Pseudopanax arboreus* inflorescence structure. A: A single inflorescence tagged for treatment. Each tag was on a separate umbel. Branch was being held down for the photo. B: A single umbel, with branching off umbellules.

Flowers of this species are considered generalist (Kelly et al., 2004) with the size of the stigma and flowers indicating that all potential pollinators are likely to contact the stigma during visitation. This structure has been referred to as ‘knob’ structure under syndrome classification, with the term ‘lollipop pollination’ also used, due to the manner in which birds lap up nectar from the tightly packed clusters of flowers (Newstrom and Robertson, 2005). Observations have shown that this species is visited by members of the Coleoptera, Diptera, Hymenoptera and Hemiptera families of insects, as well as tui, bellbird, hihi, silvereye and whitehead, with bellbird then tui being the prominent bird visitors. In addition, observational scans of number of birds and insects per plant per scan found that birds were present significantly more often than insects (Anderson, 2003). *Pseudopanax arboreus* flowers are likely to provide a concentrated energy resource for honeyeaters, despite their small size, due to flowers on an inflorescence being presented simultaneously, in compact clusters and in high numbers (Castro and Robertson, 1997). The flowers also play an important role in bee nectar gathering, particularly between October and November (Godley, 1979).

Anderson (2003) examined the relative roles of birds and insects for this species by exposing unopened buds to two treatments, a caged treatment involving 2x2 cm mesh cages, and an unmanipulated treatment where buds were counted then left for pollination to occur naturally. Cotton gauze bags were used on unopened buds to form a third treatment, but this was only done for hermaphroditic species, and thus was not undertaken for *P. arboreus*. Anderson found a significant effect of the caging treatment, with more fruit being set in the open treatment ( $82.7\% \pm 0.2\%$ ) than in the caged ( $35.0\% \pm 0.4\%$ ), indicating a strong role of birds

in the pollination of the species. However, no pollen supplementation experiments have been undertaken for this species, and no other literature that could be found looked at the pollination of *P. arboreus*.

The species is diecious (Burrows, 1996a), with male and female flowers being morphologically distinct prior and following flowering. Female buds are elongated, opening at the tip when flowering (Fig 5.1, A). Buds of male flowers are more rounded, and anthers are clearly visible, extending away from the flower (Fig 5.1, B).



**Figure 5.2:** Flowers and of female (A) and male (B) *P. arboreus* near the beginning of flowering.

## 5.2 Materials and methods

Field work for this species began for this species in early June 2010. At this stage all trees had buds but no flowers, and treatments were set up on ten trees tentatively identified as female. Work was undertaken along Mitchell's track (43°59'42"S 172°64'70"E), on the Port Hills, Christchurch, New Zealand, approximately 1.6 km from the Victoria Park study site.

Mitchell's track is a dirt tract that weaves through native regenerating bush. Male and female trees of *P. arboreus* were common, and were not spatially separated according to gender, with male and female trees often growing side by side.

As for the previous species, four treatments were set up on each individual tree: bagged, caged, natural and hand pollinated. For this species however, an additional level of replication was implemented. Treatments were initiated at the level of inflorescence, for each treatment three separate umbellules were tagged, each on a separate umbel. The number of buds per treatment on each tree ranged from 14 to 52, with an average of 28. Only female trees were used for treatments, as males do not set fruit.

Treatments were set up and checked on a weekly basis until fruit had sufficiently developed for determination of the number of fruit to be made. During fruit counting I noticed that for some treatments all fruit within the treatment appeared desiccated and almost dead. It is likely that these fruit will not germinate, or not germinate as effectively as healthy fruit. To take this into account I ran two analyses, one including desiccated fruits as successful fruit, and the other excluding them. For statistical analysis, the fruit count from the three umbellules per treatment were added together to provide a single count for each tree-treatment combination. Differences between treatments were tested with a GLM using quasibinomial error distribution and a Chi square test for significance. Pollen limitation was determined by the substituting into the equation  $1 - (\text{natural fruit set} / \text{hand crossed fruit set})$  to give the PLI.

During fruit counting I observed that for three bagged treatments, and one caged treatment fruit had partially developed, then the branch supporting the fruit had detached from the main tree, leaving the branch within the bag, which was still attached to the tree. In all cases, the

point where the branch detached was clean with no indication of any force being applied.

These were excluded from analysis.

### 5.3 Results

*Pseudopanax arboreus* progressed slowly from bud to flower and from flower to fruit. Buds were initially tagged in early May, with the first flowers opening in late May. Most flowers were open by late August, with fruit set occurring in early December. Analysis of the data set showed a strongly significant treatment effect, with an almost significant tree effect and no tree by treatment interaction (Table 5.1).

**Table 5.1:** Analysis of variance (ANOVA) table for *Pseudopanax arboreus*. The GLM used a binomial error distribution, based on whether a flower did or did not set fruit, and a Chi squared test for significance. Significance levels were: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

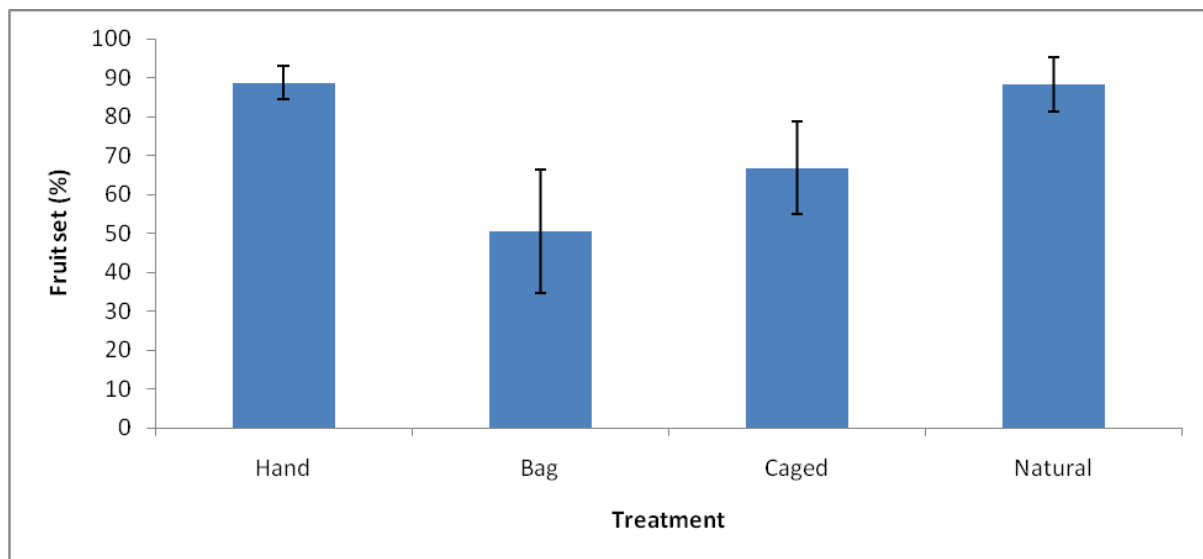
			Residual	Residual	
	df	Deviance	df	deviance	P(> Chi )
Null			27	431.07	
Tree	1	3.26	26	427.81	0.563
Treatment	4	170.07	22	257.74	0.00159 **
Tree:Treatment	4	42.2	18	215.54	0.364

Pairwise analysis showed no significant difference between the hand and the natural treatments (Table 5.2) with the hand treatment setting an average of  $88.7\% \pm 4.3\%$  and the natural treatment setting  $88.4\% \pm 7.0\%$ . The bagged treatment set a significantly lower percentage of fruit than its flowers than both the hand and the natural treatments, with a fruit

set of  $50.5\% \pm 15.7\%$ , while the caged fruit set ( $66.8\% \pm 11.9\%$ ) was not significantly different than any other treatment.

**Table 5.2:** Pairwise *post hoc* comparison of fruit set means. Degrees of freedom was 27, and significance levels were . $P \sim 0.05$  \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .

Comparison	<i>t</i>	P	Significance
Bagged versus Caged	-1.534	0.137	
Bagged versus Hand	-2.203	0.0364	*
Bagged versus Natural	-2.0670	0.0485	*
Caged versus Hand	-0.576	0.5701	
Caged versus Natural	-0.685	0.499	
Hand versus Natural	-0.184	0.856	



**Figure 5.3:** Average fruit set for the four treatments for *Pseudopanax arboreus*, error bars represent the standard error of the mean.

When desiccated fruit set were excluded for the second analysis, the same trend was observed, but it was more pronounced. All desiccated fruit occurred within the bagged or caged treatments, with only one bagged treatment setting any non desiccated fruit. Thus, both

bagged and caged treatments had a lower average value than when the desiccated fruits were counted as normal fruits (Table 5.3). As with the previous analysis, the bagged treatment was significantly lower than both the hand ( $P = 0.036$ ) and the natural ( $P = 0.049$ ) treatments, with no other differences being significant.

**Table 5.3:** Mean and standard error of the mean fruit set for each treatment for two models.

In ‘All fruit’, fruit was scored regardless of apparent quality, while for ‘Sound fruit’ any fruit that appeared severely desiccated was counted as zero.

	All fruit	Sound fruit
Hand	$88.7 \pm 4.3$	$88.7 \pm 4.3$
Bag	$50.5 \pm 15.7$	$14.3 \pm 14.3$
Caged	$66.8 \pm 11.9$	$31.5 \pm 15.7$
Natural	$88.4 \pm 7.0$	$88.4 \pm 7.0$

#### *Pollen limitation*

*Pseudopanax arboreus* had the longest progression from bud to fruit of any of the species I studied, and in addition was subject to the harshest weather conditions due to it flowering over winter. As such, many of the tags on the more exposed hand and natural treatments became illegible or detached from the treatment, meaning counts were not obtainable in all cases. The PLI for this species was calculated from the individual trees for which tags for the natural and the hand pollinated treatments enabled the two treatments to be differentiated (Table 5.4).

**Table 5.4:** Individual hand pollinated and natural fruit set values and corresponding PLIs for *Pseudopanax arboreus* trees for which both hand and natural treatments were discernable.

	Natural	Hand Pollinated	
Tree	Fruit Set	Fruit Set	PLI

P1	95.5	87.0	-0.01
P2	95	100	0.05
P4	96.6	85	-0.14
P5	93.1	89.2	-0.04
P8	53.3	66.7	0.2
P9	96.7	97.9	0.01

For all trees the pollen limitation was around zero, with the mean being  $0.14 \pm 0.15$ . This mean indicates that no evidence of pollen limitation was found, confirming the statistical analysis which found no significant difference between fruit set in the natural and in the bagged treatment.

## 5.4 Discussion

*Pseudopanax arboreus* was unique of the species I studied in that it was diecious. Diecious species can be considered, by definition, self-incompatible, as the male flowers that produce the pollen have no capacity for fruit set. This leads to the prediction that no fruit set should occur within the bagged treatment.

Analyses showed that the individual tree was not a significant predictor of fruit set, but treatment was. The bagged treatment produced fruit from 50.5% of its flowers, however, only the fruit from one tree appeared healthy, the remainder was desiccated, giving the impression that nutrient supply had been cut to the fruit part way through development.

Only one tree set healthy fruit inside the bagged treatment, and this tree set fruit from 100% of its flowers. Biologically, this is unusual as the species is diecious it has no pollen on the female flowers to enable self-pollination, although the presence of hermaphroditic or flowers



of the opposite gender within an inflorescence has been reported (Burrows, 1996a), and if this was occurring in this case, the bag may have facilitated the transfer of pollen across flowers. As this was the only bagged treatment that this occurred in, it is unlikely that it is a result of wind pollination. This could have occurred through the bag not being secured correctly so that a pollinator got into the flowers, given the physical hindrance created by the bags, if this was the case, the pollinator is likely to be an insect. The fact that some bagged treatments produced no fruit while others produced desiccated fruit suggests that females may take a while to abort unpollinated fruits. Fruit set in this treatment may be an example of the female taking a longer time than most to abort. Finally, some individual females may be capable of producing seeds without pollination (parthenocarpy).

The flowers of *P. arboreus* are considered a generalist, in part due to its small, plain flowers. However, these are thought to provide significant nectar resources for bird species, as the flowers are clustered close together, allowing gathering nectar from these flowers to be energetically favourable for birds (Castro and Robertson, 1997). As a consequence of this I predicted that excluding birds from pollinating, but allowing insects access (caged treatment) would result in a decrease in fruit set compared to natural. While this trend was observed, it was not strong enough to be significant, indicating that insects appear to be playing the primary role in pollination compared to birds, as even with cages in place, the treatment set 67% fruit from flowers, with a 32% fruit set of healthy fruit.

Diecious species are limited in pollen receipt compared to hermaphroditic species, as they cannot use self-pollen to produce fruit, and they require pollinators to have been to the correct gender of plant as well as species, whereas this is not a requirement for hermaphroditic species. As such, I predicted that this species would show pollen limitation, however, statistical analysis shows clearly that this is not the case. The difference in fruit set between

the hand and natural treatment was less than one percent, and the PLI determined was  $0.14 \pm 0.15$ , indicating no evidence of pollen limitation for this species.

Despite its diecious nature, *P. arboreus* was not pollen limitation at Mitchell's track, Christchurch. Supplemental pollination neither increased nor decreased fruit set. Excluding birds from pollinating did not significantly reduce fruit set, even when desiccated fruits were counted as not being fruit, thus indicating that insects play a strong role in pollination for this species. Within the bagged treatments, only one set healthy fruit, with the of the trees either aborting the branches and fruits on them prior to fruit count, or the fruits appearing desiccated. Thus, *Pseudopanax arboreus* appears to rely heavily on pollinators to set healthy fruit, and at this site pollinators are providing adequate service.

## **Chapter Six: Discussion**

### **6.1 Introduction**

The aim of this thesis was to evaluate pollination mutualism in native New Zealand plant species, investigating the relative roles birds and insects play in the pollination of plants with a range of sexual systems, and whether pollen limitation played a role in low fruit to flower ratios. This is particularly important in New Zealand, as the country has suffered a substantial loss of native bird and insect pollinators as a consequence of European and Polynesian settlement.

### **6.2 Self-pollination**

#### **6.2.1 Fruit set in bagged treatments**

The bagged treatment involved securing cotton gauze bags onto inflorescences prior to buds opening. The bags had small holes less than 1 mm in size to allow air circulation while preventing all pollinator access to the flowers. Bagged treatments of this type are common in pollination studies, primarily as a method of determining whether a plant is able to autonomously self pollinate. Some studies have used fine and coarse mesh bags, the coarse bags allowed pollen transfer through wind pollination (Ge and Sun, 1999). The rubbing of stigmas against the gauze bags has thought to potentially promote self pollination, as has been observed through the decrease in fruit set with the increase in bag size (Kelly et al., 2004). Some authors have used plastic cones (Jesson et al., 2006) or plastic frames (Blanche et al., 2006) to support the bag in order to prevent rubbing. For my study gauze bags were secured directly onto branches, with no cones or frames for support, and the holes in the bags

were likely too small for pollen to transfer through wind pollination. The effect of bagging on fruit set varied between the species I studied.

For the hermaphroditic *C. australis*, fruit set varied between seasons. In the first season (2009) the bagged treatment set fruit from 33.5% of flowers, not significantly different to the amount set from the natural treatment. However in the second season (2010) there was almost no fruit set (2.8%), while the natural fruit set was 46.2%. Wind pollination could explain the high fruit set in the bagged treatments during the first season of study, if pollen was in fact able to pass through the small holes in the gauze bags. This doesn't explain why high fruit set in bags was seen in only one of the two seasons. The species has been reported as self-incompatible, based on experiments where bagged flowers were pollinated with self-pollen only. This was done by Beever and Parkes (1996), however their tests only used four trees, so the result could have been a result of natural variation. The results determined by Beever and Parkes agree with results I saw for the second season of study, where fruit set was low. The dichotomy between these two years is difficult to explain biologically, yet persisted both with bagged fruit set and caged fruit set.

*Phormium tenax* has been reported as partially self-compatible while it is able to produce fruit from self-pollen; resources are frequently preferentially allocated to the production of cross-pollinated seeds (Becerra and Lloyd, 1992). Fruit set was similar across all treatments ranging from 8.2% (bagged) to 16.2% (natural), with none of the differences being significant. That the species was able to set fruit with no pollinator activity indicates that either wind pollination was occurring, or that the plant was able to autonomously self-pollinate, perhaps through the bag rubbing against the stigmas. The first is unlikely as the pollen of *P. tenax* does not match the form suitable for wind pollination, it clumps rather than being in a dry and powdered form that allows for easy dispersal via wind (Craig and Stewart,

1988). This makes the second option more likely as experimental evidence for two mistletoe species showed that small bags produced fruit set of around 15%, while when larger bags were used the physical contact between the stigmas and the bags decreased and likewise the fruit set decreased to 3% (Kelly et al., 2004). Seed pod size and number of seeds per pod did not appear to vary in any predictable manner across the different treatments, with the bagged treatment not being significantly different than another treatment across these factors.

*Kunzea ericoides* has been reported as self-compatible and previous work has shown that it is able to set fruit at high levels in bagged treatments where no cross-pollination is occurring (Beardsell et al., 1993). My experimental work on this species found no significant variation in fruit set across treatments, with an average fruit set of 71.6% ( $\pm 2.2\%$ ). This indicates that the species is able to autonomously self-pollinate when no pollinators are present.

Winter flowering species *Pseudopanax arboreus* showed a strong treatment effect, with fruit set within the bagged treatment being significantly lower than the hand and the natural treatments. Fruit quality appeared to be relevant for this species, as many fruit that were set within the bagged or caged treatment were desiccated, appearing that resources had been withdrawn from them prior to the full maturation of the fruits. The species is dioecious, thus self-pollination is unlikely, however the presence of some male or hermaphroditic flowers on female trees has been reported (Burrows, 1996a). This may have driven the apparently healthy fruit set in one of the bagged treatments.

Across most species fruit set in bagged treatments was comparable to natural treatment. However, in the 2010 season the fruit set in *C. australis* for bagged treatments significantly lower than the natural. This also occurred for *P. arboreus*, with fruit set of 50.5% in the bagged treatment, and 14.3% set of healthy fruit. In the remainder of the cases these native species were able to autonomously self-pollinate in the absence of pollinators. This is

important from the conservation perspective, as it indicates that if pollination service is low, the species may be able to survive by producing seeds from self pollen. However, one caveat to this is that we have no indication of the quality of these seeds. For *P. tenax* factors relating to seed pod size, seed size and seed number were examined, but no significant relation was detected in any of these and the differences between the bagged and the natural treatments. Self-pollination can lead to inbreeding depression, potentially leading to a reduction in the quality of the seeds produced from self-pollen compared to those produced from cross pollination (Robertson et al., 1999). As such, although the species may be able to maintain seed production in the absence of pollinators through self-pollination, offspring produced in this manner may be of lower quality, such as having a decreased chance of germination, lower rate of growth or decreased survivability. A second caveat is that the bags used for the bagged treatment may have facilitated self-pollination at a level that would not be present in normal circumstances. My data cannot distinguish whether this is occurring, however the high level of fruit set in bags, particularly for *K. ericoides* indicates that this is most likely not the only cause of the high fruit set.

### **6.2.2 Self-compatible versus self-incompatible species**

All four species studied fell in different positions along the self-compatibility to self-incompatibility spectrum. Studies have indicated that *C. australis* is self-compatible (Beever and Parkes, 1996), *P. tenax* is partially, or cryptically self-incompatible (Becerra and Lloyd, 1992), and *K. ericoides* is fully self compatible (Beardsell et al., 1993). *P. arboreus* is dioecious (Burrows, 1996a), and thus cannot self-pollinate, thus being effectively self-incompatible.

The proportion of flowers that set fruit varied across the different species. With only one replicate of each compatibility type, these differences cannot be attributed to compatibility, as

they may be differences between species. Theory suggests that self-compatible plants will set more fruit (and thus seed) than self-incompatible as they are able to use their own pollen for fertilization, while self-incompatible plants are not able to do this (Burd, 1994). While fruit set itself has not been tested against compatibility, a meta-analysis of studies consisting of in total, 224 species of animal pollinated plants, found self-incompatible plants to be significantly more pollen limited than self-compatible plants (Larson and Barrett, 2000).

The highest natural fruit set was in *K. ericoides* where non-manipulated treatments set fruit from 71% of their flowers. Fruit set for *K. ericoides* was consistent across all treatments, with an overall mean fruit set of 72%. Biologically it is predicted that self-compatibility allows for reproductive assurance, allowing the plant to set fruit even when pollinators are low in abundance or ineffective. As such, it is not surprising that *K. ericoides* had a high fruit set under all conditions. Fruit set for *C. australis* and *P. tenax* did not follow such strong patterns. In *C. australis* average natural fruit set in 2009 was 32%; in 2010 it was 46%, while for *P. tenax* natural fruit set averaged at 16%. Theory predicts that *P. arboreus* would have the lowest fruit set, as it is unable to reliably set fruit from self-pollen, however this was not the case. Natural fruit set for *P. arboreus* was 89%, while the overall mean was 73% fruit set, and 56% healthy fruit set. As such, for the species studied there was no clear trend linking self-compatibility to fruit set.

### **6.3 Variation across seasons**

Changes in PLI from one year to the next were able to be studied in two species: *P. arboreus* and *K. ericoides* were studied in two consecutive seasons, during which hand-pollinated and natural pollination were examined. For both species, season had no effect on fruit set within any treatments and as a consequence no effect on the PLI.

In *C. australis* the effect of treatment on fruit set varied significantly between the seasons, but not in a predictable manner. In 2009 the bagged treatment had a high fruit set, not significantly different than the natural fruit set, despite the species being reported as self-incompatible. For the same season the caged treatment had a low fruit set. However, in 2010 this was reversed with bagged having very low fruit set, and the fruit set of caged being not significantly different than natural.

One concern about pollen supplementation experiments is that the artificial increase in fruit set through the addition of excess pollen comes at a cost to another component of fitness. The ability of plant to reallocate resources among flowers and seasons means that an increase in fruit set beyond normal levels (such as by a season of high pollinator activity, or by artificial pollination) may cause a reduction in survivability or fruit set in following seasons (Ashman et al., 2004). This does not appear to be the case with the species I studied, as pollen supplementation did not increase fruit set.

## **6.4 Low flower-to-fruit ratios**

I proposed four hypotheses for why the ratio of flower to fruit may be low: bet hedging, fitness, resource limitation and pollen limitation.

### **6.4.1 Bet hedging**

This hypothesis predicts a high number of flowers compared to fruit for most years, so the natural fruit set in a given year would be low, unless that year was a ‘boom’ year. This makes data from one or a few years difficult to analyse, as it cannot be determined whether the years analysed contained a ‘boom’ year. Bet hedging occurs often in unpredictable pollinator environments; where the plant produces excess flowers so that in years where there is



abundant pollen deposition the plant can capitalize on this having a large number of ovules available to be pollinated. This strategy can also extend to environments where resources are sporadic, and thus in this situation the plants are able to mature more seeds in years where resources are abundant than in seasons where resources are scarce.

As such evidence of either pollen limitation or resource limitation can suggest the possibility of bet hedging, but does not confirm it. The strongest indication of bet hedging behaviour would be high pollen limitation one year and low another, or alternatively low fruit set one year and high another. With only two seasons studied this is difficult to determine with accuracy, however some indication can be gained. *C. australis* was the only species which showed variation across seasons. As no hand pollination was done for this species PLI couldn't be determined, however, the number of fruit produced in each season was examined and there was no significant difference between the seasons.

#### **6.4.2 Fitness**

The fitness hypothesis predicts the over-production of male or hermaphroditic flowers for the purpose of performing the male function by providing pollen to conspecifics. As these flowers, particularly male flowers, are produced for the male function, this predicts that the plant is likely to have a low ratio of flowers to fruit. However, 'low' is a relative term, and does not readily allow the prediction of whether a population or species is producing 'male' flowers for the purpose of male function based on the percentage of flowers that produced fruit. Species producing excess male function in this manner are attempting to overcome the production of pollen, rather than the receipt, limiting fruit set.

This hypothesis is difficult to test, and whether it is occurring is indeterminable from the treatments used in this study. It is likely that the plant will appear to be pollen or resource

limited, as male flowers will not produce fruits, and in the case of hermaphroditic flowers, there are likely to be too many for high fruit set.

#### **6.4.3 Resource and Pollen limitation**

Resource limitation and pollen limitation are often thought of as two mutually exclusive, alternative theories. Often tests for pollen limitation use a null hypothesis of resource limitation. Standard tests resource limitation involve the addition of nutrients such as through fertilizer to determine if this increases fruit set, or the removal of resources such as by removing leaves to look for a decrease in fruit set. Pollen limitation is generally tested by the addition of supplemental conspecific pollen to treatment flowers, often by dabbing the pollen on with a paintbrush.

However, if pollen and resource limitation are not operating in a strict dichotomy, but rather in the Haig-Westoby equilibrium, then adding supplemental pollen or resources will have no effect on reproductive success, as reproduction is simultaneously limited by both.

For my research I tested pollen limitation by supplemental pollination experiments. If supplemental pollination does not increase reproductive success, then either the population is not pollen limited, or it is limited by both pollen and resources.

#### **6.4.4 Conclusion**

None of the species studied showed any evidence of pollen limitation. In addition, two species, *K. ericoides* and *P. arboreus* had fruit set higher than 70%. This is a strong indication that these species are faring well despite the pollinator environment in New Zealand, and it is likely that none of the above hypotheses apply to these species, as their fruit set is not low and they may be limited by other reasons such as biological limitations.

## 6.5 Original questions

### 6.5.1 Are unspecialised apparently insect pollinated plants less pollen limited than bird dependant species?

The species I studied had a range of different pollination syndromes. *C. australis* and *K. ericoides* both conformed to the entomophilous, with the former having 5mm hermaphroditic flowers, and the latter having creamy white hermaphroditic flowers around 10 mm in size. *P. arboreus* is diecious, with flowers of a single gender, less than 3mm in width, which have been described as matching the generalist syndrome. *P. tenax* exhibits the ornithophilous syndrome, with large (25-50 mm in length), brightly coloured flowers.

No pollen limitation was found for any species; however *P. tenax* was the only ornithophilous species studied, and also had the lowest fruit set. With no pollinator syndrome replication it is difficult to tell whether this is the result of the species being bird pollinated, or something specific to *P. tenax*.

### 6.5.2 Are species that are able to self-pollinate less likely to show pollen limitation than self-incompatible species or species with separate sexes?

My data suggests that this is not the fact, at least for the species studied. There was no pollen limitation detected for any of the species studied, and in addition the highest fruit sets came from a self-compatible species (*K. ericoides*) and a diecious species (*P. arboreus*). There was little replication of the sexual types of the species, so it cannot be reliably determined whether these levels of fruit set are a consequence of species, or of sexual type.

### 6.5.3 On insect pollinated species that are also visited by birds, will excluding birds but not insects still give good pollination?

For *C. australis* excluding birds but not insects gave good pollination in the second study season (2010) but not the first indicating that the insects are playing a significant role in pollination. However, the large decrease in the fruit set in the caged treatment compared to the natural in the first season indicates that the major role was played by birds. This result is surprising, as what mechanisms could change the relative roles of birds and insects in pollination from one season to the next are unclear. In both seasons the caged treatment set fruit, in 2009 fruit set was 14% ( $\pm 6\%$ ) of flowers, while in 2010 fruit set was 45% ( $\pm 3\%$ ).

In contrast, fruit set in *K. ericoides* appeared resilient to treatment, with all treatments producing a similar percent of fruits, with no significant difference between treatments. This may indicate a low role of birds in pollination, with the majority of pollination being carried out by insects; however, the bagged treatment also set fruit at the same level. In the bagged treatment all pollinators were excluded, this it appears likely that the species is able to autonomously self-pollinate. The data gives no indication of the quality of the fruits, thus there is no way of determining whether there is any difference in fruits set in the caged treatment (which may be from insect pollination, or self pollination) and the bagged (most likely self pollinated).

*Pseudopanax arboreus* is considered to have generalist flowers, so could be considered under this category. Excluding birds but not insects from flowers for this species did give a decrease in fruit set, but this was not strong enough to be significant, indicating a low role of birds in pollinating this species.

## 6.5 Conclusion

Four native New Zealand species were examined for evidence of pollen limitation, and for insight into the relative role that birds and insects played in pollinating. The species differed both in flower morphology and self-compatibility. *Cordyline australis* and *Kunzea ericoides*

both exhibited the entomophilous structure in their flowers and literature suggests that they are primarily insect pollinated, in contrast *Phormium tenax* is ornithophilous and *Pseudopanax arboreus* flowers are considered generalist, as the tight bunches allow for birds to use the species as an energetically favourable food source. The species exhibited a range of compatibility types: *K. ericoides* is self-compatible, *P. tenax* shows cryptic self-incompatibility by preferentially aborting self-pollinated fruits, *C. australis* is self-incompatible and *P. arboreus* is diecious, and as such generally considered to be self incompatible.

This thesis examined four hypotheses as to the cause of plants producing fewer fruits than flowers: bet hedging, fitness, pollen limitation and resource limitation. Of these, data found suggested against the bet hedging and pollen limitation hypotheses, and also to an extent the fitness hypothesis. None of the tests done under this project could determine whether resource limitation was occurring, or whether fruit set was being limited by another factor, or simultaneously by resource and pollen limitation, as some biological theory suggests.

Fruit set in *C. australis* varied significantly with bird exclusion (caged treatment) and exclusion of all pollinators (bagged treatment) and this varied between the two seasons. During the 2009 field season, the caged treatment set significantly lower fruit than both the bagged and the natural treatment, however in 2010 the caged and the natural treatment were not significantly different, while the bagged treatment was significantly lower than both of them. This implies that in the 2009 season birds played a large role in pollination for this species, as excluding them significantly decreased fruit set, while in the 2010 season they played little role in pollination. The potential biological mechanism behind this trend is unknown.

Excluding birds from pollinating flowers in *P. tenax* resulted in a significant decrease in fruit set compared to natural, while bagging caused no further decrease. This provides a strong indication that birds were responsible for most of the pollination for this species.

Fruit set in *K. ericoides* was resistant to treatments, with no significant treatment effect.

Finally, caging had no significant effect on fruit set for *P. arboreus*, indicating a strong role of insects in pollinating this species, despite common and frequent observations in literature of bird pollination.

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