Regeneration of the native sand dune plant *Pimelea arenaria* in the lower North Island, New Zealand

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Abstract

New Zealand has 300,000 ha of coastal sand dunes in which many native species and introduced plant and animal species are established. One native plant inhabiting dune ecosystems is *Pimelea arenaria* (Thymeleaceae), the native sand daphne, which is gynodioecious with female and hermaphrodite flower types and listed as nationally declining. The cause of the decline is unknown, but anecdotal evidence suggests recruitment failure is occurring. This thesis looked at four *P. arenaria* populations in the lower North Island of New Zealand. The aims were (1) examine the population structure and establish whether recruitment failure was evident, (2) whether any failure was due to problems with pollination, and (3) whether house mice (Mus musculus) or birds had any impact on *P. arenaria* by removing fruit.

The *P. arenaria* populations ranged from 0.53-4.05 plants/ha, with female plants comprising the smaller portion of each population, the exact sex ratios varying between the sites. The standing crop of nectar of hermaphrodite flowers is modest and varies from 24 to 56 µg/flower. The pollen:ovule ratio (1987: 1), as well as casual observations, suggest that insects are the main pollen vectors. Recruitment failure in *P. arenaria* occurred with few or no seedlings found at any site, the maximum height above substrate and the surface area of substrate covered suggesting an adult biased population. Profuse flowering occurred (360-510 flowers/m^2), leading to viable seed from both female and hermaphrodite plants. Less than 50% of seed germinated from either flower type, regardless of pollination method (natural, hand out-crossed, or autonomously selfed). Female plants observed higher germination success in seeds in both hand out-crossed and natural pollination. *P. arenaria* seed weight remained constant between sexes and treatments, however the pulp weight was variable, with hermaphrodite autonomously selfed fruit having the smallest amount of pulp, but this was not significant (p=0.37).

Graded exclosures showed that fruit was removed but this was preventable by bagging the fruit (p<0.001). However, the results from the exclosures were unable to distinguish between mice and birds suggesting that both have similar, possibly cumulative, effects on fruit loss. Within the bagged fruit there were peaks of fruit loss at the start and end of the fruiting season suggesting that selective abortion of ovules is occurring, possibly because of low abundance of pollen sources or pollen vectors. Fruit loss occurred, but there was no direct field evidence of predation by mice and birds. A feeding trial with mice established that both female and male mice eat and destroy *P. arenaria* fruit and seed, and at a low dose rate (3 fruit/mouse) the fruit of *P. arenaria* was not poisonous.

Areas for future work are discussed for each section.

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Chapter 1

General Introduction

"To see what has never been seen before, look where no one has looked before"

- Cavendish Laboratories, Cambridge

1

1.1 Introduction

New Zealand has an extensive coastline with locally adapted flora and fauna. In the 1900's New Zealand had, as part of its coastline, approximately 300 000 ha of sand dunes (Hilton *et al.*, 2000). Current estimates are that 39 000 hectares remain; a reduction of 70 % in less than 100 years (Hilton *et al.* 2000). Many factors contributed to this loss but especially agricultural development, afforestation, and urbanization (Hilton *et al.*, 2000). The pressure of these factors on sand dune ecosystems continues to increase. The reduction of suitable habitat, the open growth pattern of native plants, increasing vehicle use, and the impact of introduced animals have all had adverse effects on the native vegetation of coastal sand dunes (Stephenson, 1999; Miller & Webb, 2001; Milne & Sawyer, 2002). Off road vehicles, for example, destroy vegetation which in turn can increase erosion rates (Stephenson, 1999). These effects can result in many native species becoming locally extinct, e.g. *Atriplex cinera* in the Wellington Conservancy (Milne & Sawyer, 2002). The open growth of some native dune plants also means that areas which native species occupy are reduced and possibly lost due to pressure from introduced plants such as marram grass (*Ammophila arenaria*). Marram was introduced from Europe (Esler, 1978) to many areas to promote dune stabilization and assist in forestation (Hilton *et al.*, 2000). This species rapidly invaded other areas and while it can be useful in some cases (Esler, 1978), it has proved to be an inefficient sand binder. Marram usually forming dunes that tend to collapse from blow outs caused by strong winds that undermine the marram, leaving behind dune turrets which quickly erode away (Elser, 1978), further reducing habitat availability for native plants (Hilton *et al.*, 2000).

In addition to the pressure from introduced plants, the native flora has had to contend with introduced fauna. This involves dune residents, such as rodents, and transitory animals that either feed in the dunes or gain beach access through the dunes (Stephenson, 1999). The impacts of animals may be considerable, both directly, through predation of seed (Miller & Webb, 2001), and indirectly, through predation of animals or competition with native fauna that could lead to adverse affects on regeneration by removal or suppression of dispersal agents and pollination vectors of native plants.

Coastal dune ecosystems, however, have been neglected in New Zealand conservation legislation, with dune systems falling between Parliamentary Acts dealing with the terrestrial environment and those dealing with the marine environment. However dunes are now legally recognized as being of national importance (Hilton *et al.*, 2000), and are 'one of the most threatened ecosystems and the least represented in the land administered by the Department of Conservation' (Milne & Sawyer, 2002). But lack of information has meant that identifying conservations needs has been difficult, as has determining trends in the decline of dune areas (Hilton *et al.*, 2000). These, together with a general lack of awareness of the threatened nature of dune lands, have resulted in many areas being lost (Hilton *et al.*, 2000), together with the species associated with them.

1.2 Sand daphne (*Pimelea arenaria*)

One native New Zealand dune plant is *Pimelea arenaria* (A. Cunn.) (Thymeleaceae), currently listed as "declining" (de Lange *et al.*, 1999). The term "declining" refers to "taxa that are numerically abundant but which are either under threat from serious adverse factors throughout their range, or occur as widely scattered, typically small populations, many of which are under going declines through loss of reproductive ability, recruitment failure, predation, or through other processes of often subtle habitat changes" (de Lange *et al.*, 1999). *P. arenaira* is one of 15 species of *Pimelea* found in New Zealand (Allan, 1961), and is the only one confined to sand dunes (Esler, 1978; Heads, 1994). It is typically found on the lee of the fore dune (Esler, 1978) but also extends inland along the arms of parabolic dunes at sites where these are present (pers. obs.). *P. arenaria* is a prostrate woody plant, often buried in sand, with small alternate leaves, (Allan, 1961; Milne & Sawyer, 2002) and is a gynodioecious species (Burrows, 1960) with two sexes: female and hermaphrodite. The hermaphrodite plants are distinguishable by two prominent yellow/orange anthers which insert just below the throat of the floral tube (Burrows, 1960). The flowering season of *P. arenaira* is from September to March, with the fruiting season from October to April (Milne & Sawyer, 2002).

While *Pimelea* has received attention in the literature, there is little on *P. arenaria*. The research on New Zealand *Pimelea* most directly related to this study is by Parlane (1925) and Burrows (1958, 1960). Parlane (1925) and Burrows (1958) dealt with the habitat, reproductive and vegetative variation between individuals and populations of four species of *Pimelea* in the Cass Valley, Christchurch, New Zealand. Burrows (1960) examined the breeding systems of *Pimelea*, and compared four species, also in the Cass Valley. Other studies looking at *Pimelea* species found in New Zealand have examined various toxic compounds and their properties (Adolf & Hecker, 1982; Zayed *et al.*, 1982; Petit *et al.*, 1983). The most recent published work reviews the biogeography of the *Pimelea* species in New Zealand (Heads, 1994). There appears to be no other literature on the population structure of *P. arenaria* in New Zealand or on the pollination biology of *P. arenaria*.

The cause of the decline of *P. arenaria* around New Zealand is unclear, although observations by Department of Conservation officers in the Manawatu (A. Dijkgraaf, C. Ogle, D. Ravine, pers comm.) suggest that the decline is due to a recruitment failure. The presence of fruit or seedlings in the wild is rarely observed (C. Ogle, D Ravine, G. Rapson, pers.comm.), despite the potential for a large fruit crop with *P. arenaria* flowering profusely. This anecdotal evidence suggested that either (1) *P. arenaria* plants were not producing fruit at all, (2) that seeds were failing to germinate or seedlings were failing to establish, or (3) that seeds were preyed upon before germination could occur. Since *P. arenaria* populations have existed till the present and have only been recently identified as declining it is likely that any recruitment failure, by seed or seedling predation, could be associated with introduced animal species.

1.3 Potential predators

Mice are one of four rodents introduced into New Zealand, all of which prey on a range of New Zealand native fleshy fruited species (Beveridge, 1964; Daniel, 1973; Campbell *et al.*, 1984; Williams *et al.*, 2000). However, the effects of mice on native species are the least known of all the rodents introduced into New Zealand (Miller & Webb, 2001). Mice have been established in New Zealand since 1830 (Miller & Webb, 2001), and inhabit coastal sand dunes (Miller, 1999; Miller & Webb, 2001; pers. obs.). Their diet, while omnivorous, is biased towards carnivory (Miller, 1999; Miller & Webb, 2001) with mice eating a wide variety of insects (Dugdale, 1996; Fitzgerald *et al.*, 1996; Miller, 1999; Ruscoe, 2001) and small reptiles (Newman, 1993, 1994; Ruscoe, 2001). Mice also eat plant material including vegetation (Miller, 1999; Miller & Webb, 2001), and fruit and seeds (Ryan *et al.*, 1989; Miller, 1999; Williams *et al.*, 2000; Miller & Webb, 2001). Mice are known predators, rather than dispersers, of seed (Williams *et al.*, 2000). Mice are suspected of affecting regeneration of other dune plants (Miller & Webb, 2001) and have the potential to impact on *P. arenaria* regeneration by fruit and/or seed predation. Indeed mice have been noted overseas as being responsible for regeneration failure in other plant species (e.g. Ryan *et al.*, 1989).

At coastal sites one can observe, in addition to indigenous gulls and shore birds, introduced species such as blackbirds (*Turdus merula*), starling (*Sturnus vulgaris*), house sparrow (*Passer domesticus*), chaffinch (*Fringilla coelebs*), greenfinch (*Carduelis chloris*), goldfinch (*Carduelis carduelis*), red poll (*Carduelis flammea*) and yellowhammer (*Embriza citrinella*), (see Appendix 1). All these introduced species are potential frugivores on *P. arenaria*.

1.4 Thesis aims

This thesis looks at four populations of *P. arenaria* in the lower North Island of New Zealand to:

(1) determine whether a recruitment failure is occurring. An absence of smaller size classes in an otherwise smooth size/frequency distribution might imply a failure of younger plants to establish and recruit into the population;

(2) to determine, by investigating pollination biology, whether any lack of fruit and seedlings is due to the inability of *P. arenaria* to produce fruit. Insufficient numbers of germinable seed, through for example pollen limitation, could result in any gaps in a size/frequency distribution;

(3) to measure by graded exclosures, the impacts of introduced mice and birds on fruit set by *P. arenaria.* Removal of large numbers of fruit by either or both of these predators could also result in a lack of seedlings able to be recruited into the population;

(4) to determine whether the fruit of *P. arenaria* are poisonous, and if not, whether the seed, if eaten, would remain viable. A poisonous fruit might imply a specialist disperser of *P. arenaria* fruit which itself might be in low abundance if gaps in a size/frequency distribution are present. Alternatively seeds may be eaten causing no harm to the predator, but resulting in a loss of seed viability.

1.5 Study areas

Three separate popualtions of *P. arenaria* were studied at Tangimoana, Himatangi, Castlecliff, on the west coast of the North Island, and the population at Castlepoint on the east coast (Figure 1.1).

The climate patterns for all the west coast sites are similar as the sites are reasonably close to each other. All are fairly windy places with northwesterly winds prevailing. The typical daily summer air temperature ranges from 19-24 °C, with approximately 900 mm of rain annually and an average of 2000 hours of sunlight (NIWA, 2003). There are few climatic extremes (NIWA, 2003).

The climate on the east coast is warm and dry with sheltered weather predominating, the typical daily air temperature in summer ranging from 20-

28 °C (NIWA, 2003). High temperatures are more frequent in summer and are often accompanied by strong, dry föhn winds from the northwest (NIWA, 2003). Masterton (Figure 1.3) averages 979 mm of rain, although heavier rainfall can occur, and the area averages 2200 hours of sunlight per year (NIWA, 2003).

Himatangi (40°23' S, 175°14' E)

This study site was located on private land (Figure 1.2) south of the Kaikokopu Stream, between the Manawatu and Rangitikei River mouths.

Tangimoana (40°19' S, 175°14' E)

The study site was in the Tawhirihoe Scientific Reserve located south of the Rangitikei River mouth (Figure 1.3).

Both Tangimoana and Himatangi are dune systems, comprising a fore dune running parallel to the surf line with lateral dunes running inland, similar to a parabolic dune system. Indeed Esler (1969) considers it to be a parabolic dune system that has been hindered in development by lack of sand and vegetation and so has only partly formed.

Castlecliff (39°56' S, 174°57' E)

This site is north of the Wanganui river mouth in the northern end of the Castlecliff reserve located on the edge of the Wanganui urban area. This 35 ha reserve comprises a small dune system where two dunes have formed parallel to the surf line at the base of the sea cliff which extends 30-45m high (Figure 1.4).

Castlepoint (40°54' S, 176°14' E)

This approximately 60 hectare reserve (Milne & Sawyer, 2002) is located on the east coast of the North Island in the Wellington Conservancy, 48 km east of Masterton (Figure 1.5), the nearest town to Castlepoint.

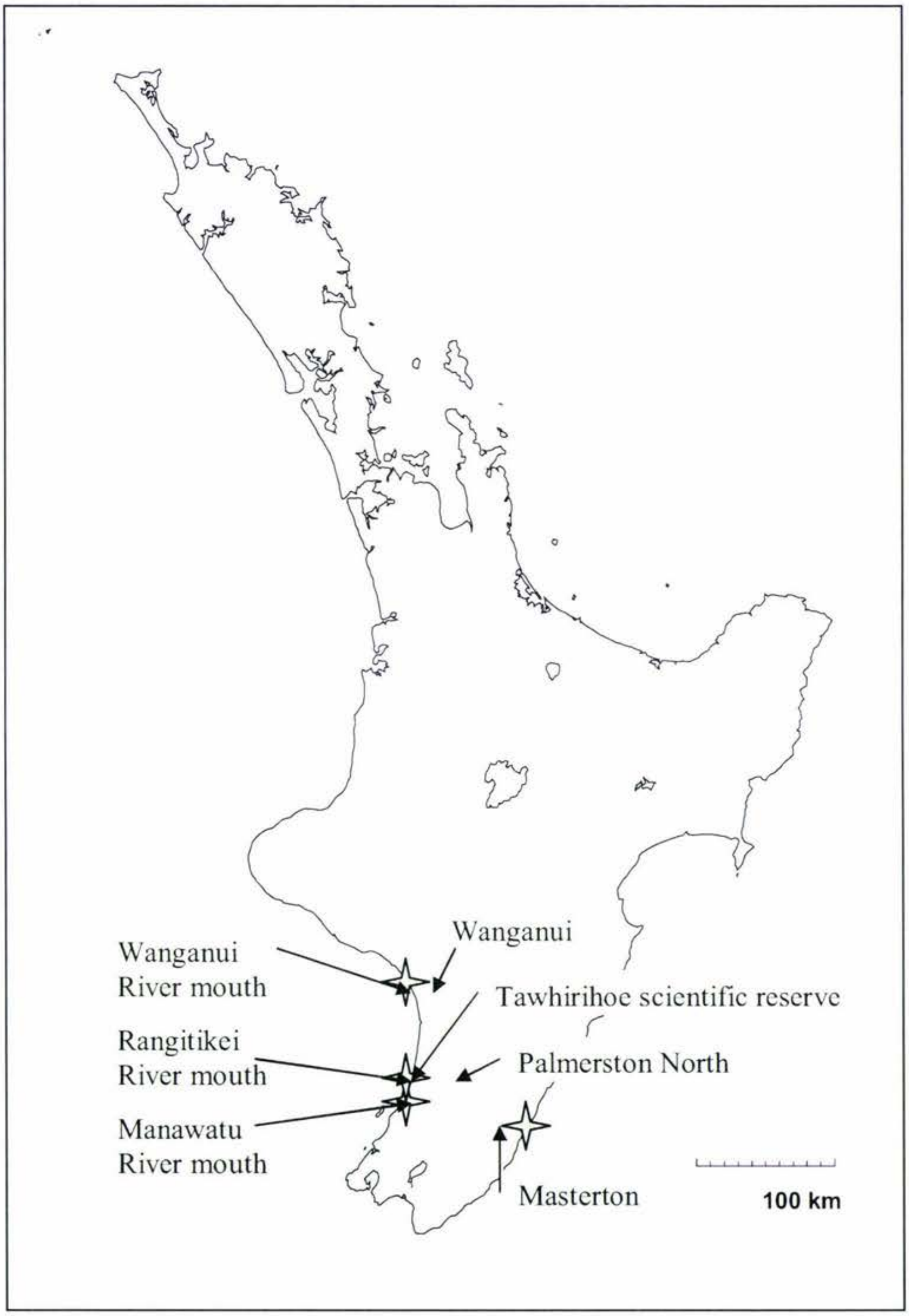

Figure 1.1. The North Island of New Zealand showing the four study sites of this thesis.

Figure 1.2. The sand dunes at Himatangi looking south-east from the fore dune. The plastic sheeting indicates some of the graded exclosure frames used in Chapter 3.

Figure 1.3. The sand dunes at Tangimoana looking inland from the fore dune over the study site. The plastic sheeting in the fore ground indicates some of the graded exclosure frames used in Chapter 3.

Figure 1.4. The environment of *P. arenaria* at Castlecliff; the sea cliff in the background, and the rearmost dune in the middle of the photograph. The plastic sheeting locates some of the graded exclosure frames used in Chapter 3.

Figure 1.5. The Castlepoint reef showing the location of *P. arenaria* plants used in Chapters 2, the plastic sheeting is for some of the graded exclosures in Chapter 3.

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Appendix 1

Birds that are potential frugivores on *P. arenaria*.
Adapted from Robertson & Heather (1999)

Common name	**Scientific name**	**Bird Weight (g)**	**Habitat**
Silvereye	*Zosterops lateralis*	13	Forest, scrub, orchards, parks, gardens
Blackbird	*Turdus merula*	90	Forest, scrub, orchards, parks, gardens, farmland
Song thrush	*Turdus philomelos*	70	Forest, scrub, orchards, parks, gardens, farmland
Starling	*Sturnus vulgaris*	85	orchards, parks, gardens, farmland, forest margins, beaches
House sparrow	*Passer domesticus*	30	Towns, farmland orchards
Chaffinch	*Fringilla coelebs*	22	Native and exotic forest, scrub, farmland, tussock
Goldfinch	*Carduelis carduelis*	16	Farmland, orchards, parks, gardens
Greenfinch	*Carduelis chloris*	28	Farmland, pine plantations, scrub/bush fringes
Red Poll	*Carduelis flammea*	12	Farmland, parks, gardens
Yellowhammer	*Emberiza citronella*	27	Open country from sea level to sub-alpine
Australian Magpie	*Gymnorhina tibican*	350	Open farmland, forest, parks and gardens

Chapter 2

Population structure and pollination in *Pimelea arenaria*.

2

2.1 Abstract

The population structure of *Pimelea arenaria*, a native sand dune plant, and pollination of four *P. arenaria* populations in the lower North Island are examined to identify reasons for observed declines in the species.

Line transects through patches of *P. arenaria* found no seedlings, and measures of individual plant sizes showed adult-biased populations at all four sites, suggesting recruitment failure.

The standing crop of nectar was modest, varying from 24 to 56 µg per flower. Observations suggest that insects are the main pollen vectors. Pollination manipulations were carried out, with fruit set measured from three pollination treatments: natural, hand out-crossed, and autonomous selfing. Hermaphrodite flowers produced fruit twice as often following natural pollination than did female flowers. Only hermaphrodite plants self autonomously to produce fruit, and nearly 100 % of the bagged flowers produced fruit. Hand out-crossing produced similar average proportions of fruit in both sexes, suggesting that female flowers, but not hermaphrodite flowers, may be pollen limited during natural pollination.

Results suggest that recruitment failure of *P. arenaria* is not due to failure of the plant to produce viable fruit, but might occur during seed germination or seedling establishment phases.

2.2 Keywords

Pimelea arenaria, sand dunes, regeneration, pollination, fruit production, population structure

2.3 Introduction

Since people arrived in New Zealand there has been a 70% decline in the area of active coastal dune lands (Hilton *et al.*, 2000). Hilton *et al.* (2000) estimate that the 39 000 ha remaining are coming increasingly under threat from urbanization, forestry, and agricultural development. These processes, combined with a former lack of recognition of the conservation needs of dune ecosystems (Milne & Sawyer, 2002), has meant that many native species, for example *Atriplex cinera* in the Wellington Conservancy, have fallen in abundance or become regionally extinct (Milne & Sawyer, 2002). In addition to anthropogenic influences, coastal ecosystems also experience the effects of introduced species further reducing available habitat (Hilton *et al.*, 2000), and increasing competition between species (Hann, 1990). Additionally the ecosystem is also affected by exotic species preying on native species (Stephenson, 1999; Miller & Webb, 2001). One native plant that is confined to coastal sand dunes (Esler, 1978; Heads, 1994) and is under threat (de Lange *et al.*, 1999) is *Pimelea arenaria* (A. Cunn.) (Thymeleaceae): the sand daphne. It is restricted to three main areas: Northland (south to Auckland on the west coast and Thames on the east coast), the southern quarter of the North Island, and in the South Island, in a small area around Dunedin (Heads, 1994).

Typically found on the lee of the fore dune (Esler, 1978), *P. arenaria* is a prostrate woody plant, with small leaves arranged in four rows at right angles to each other (Allan, 1961; Milne & Sawyer, 2002). It is a gynodioecious species (Burrows, 1960); i.e. it has two types of flowers, female and hermaphrodite. The hermaphrodite plants are distinguishable by two prominent yellow/orange anthers that extend from the floral tube (Burrows, 1958; Burrows, 1960). Because isolated flowers (Burrows, 1958; Burrows, 1960) or terminal branches (pers. obs.) are of the other flower type, this complicates the gender assignment for individual plants, although most of a plant will be one or other of the flower types (pers. obs). The flowering season of *P. arenaria* is from September to March, with the fruiting season from October to April (Milne & Sawyer, 2002). Currently this species is listed by de Lange *et al.* (1999) as nationally "declining", which is defined as "taxa are numerically abundant but are either [in] small, widely scattered populations or under threat from serious adverse factors…and that without some level of management…are destined to become the future threatened taxa of New Zealand".

Pimelea ecology has so far received little attention. The first reports on any New Zealand *Pimelea* species were two unpublished theses, one by Parlane (1925) and the other by Burrows (1958), dealing with *Pimelea* in the Cass Valley, Canterbury, New Zealand. Both reseachers examined the habitat and reproductive and vegetative variation between individuals and populations of four species of *Pimelea*. Burrows (1960) dealt with the breeding systems of the four *Pimelea* species in the Cass Valley. Other, more recent work, has looked at toxins, and their properties, in the Thymeleaceae, including *Pimelea* (Freeman *et al.*, 1979; Adolf & Heckler, 1982; Zayed *et al.*, 1982; Hafez *et al.*, 1983; Pettit *et al.*, 1983; Adolf *et al.*, 1988). The most recent work has described the biogeography of *Pimelea* species in New Zealand (Heads, 1994). Historical observations by Department of Conservation officers suggest that *P. arenaria* is in decline in several localities due to recruitment failure (D. Ravine, C. Ogle, A. Dijkgraaf, pers.comm.). The presence of fruit or seedlings in the wild is rarely observed (C. Ogle, G. Rapson, D. Ravine, pers. comm.) despite the potential for a large fruit crop because *P. arenaria* flowers profusely. While five regeneration strategies are recognized in terrestrial plants (Grime, 1979; Grime & Hillier, 2000), the particular regeneration strategy, or strategies, used by *P. arenaria* are unknown. An absence of smaller size classes in an otherwise smooth size/frequency distribution might imply a failure of younger plants to establish, hence a regeneration failure is occurring with no younger plants being recruited into the population. Further work can also investigate the possible causes of such a regeneration failure.

This chapter addresses four separate populations of *P. arenaria* with the aim of looking at

- (i) whether a lack of seedlings is apparent by looking at population structure and distribution,
- (ii) whether the plants are able to produce seed,
- (iii) if seed is produced whether it is able to germinate.

2.4 Methods

1 - Sites

This study was carried out at four sites in the lower North Island: Castlecliff, Tangimoana, Himatangi (west coast) and Castlepoint (east coast) (Figure 2.1). Castlecliff and Castlepoint are both scenic reserves and Tangimoana is a scientific reserve adjacent to a scenic reserve, all administered by the Department of Conservation. The study site at Himatangi was on private property.

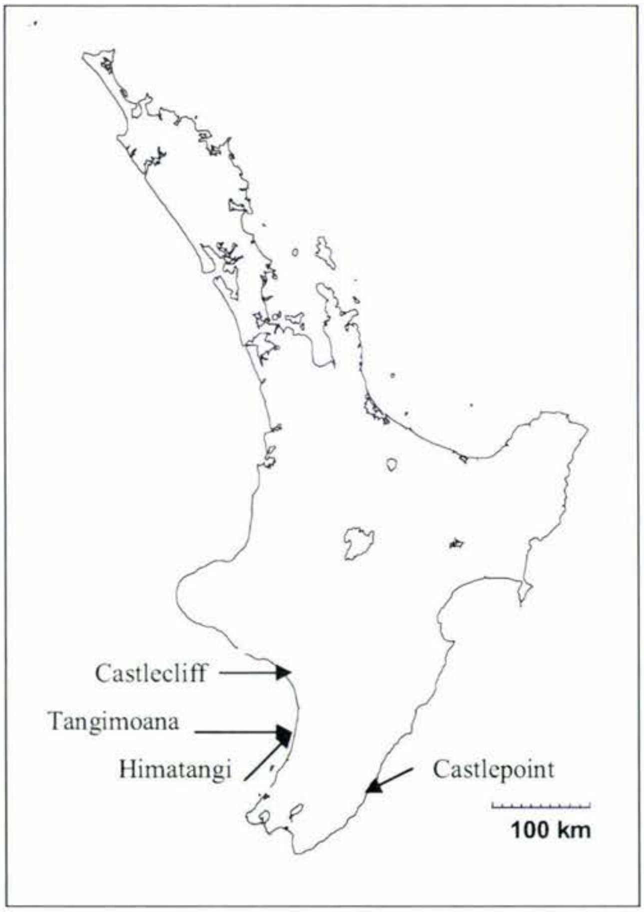

Figure 2.1. The four study sites around the lower North Island, New Zealand

2 - Population Structure

Between December 2002 and January 2003 a population count was made at four sites, with the total number of plants and sex of each plant being recorded. A separate search was made for seedlings. An estimate of the surface area of each plant was obtained by measuring (± 1 cm) the breadth (A), taken as parallel to the surf line, and width (B), taken at 90° to the breadth of each individual plant in the population. Both measurements were taken at the widest points of the individual. Maximum height above the substrate of each individual plant was also measured at the approximate centre of the plant. The approximate total surface area of substrate covered by the plants was calculated using surface area = $\pi*A*B$, assuming that the plants were elliptical in shape (Figure 2.2). As *Pimelea* species are unable to be aged by growth rings (Burrows, 1958), height was used as a positive correlate of age.

Figure 2.2. A *Pimelea arenaria* individual (arrowed), showing the typical half sphere growth shape of this species in sand dunes.

Because the functional sex of an individual can vary across the plant (Burrows, 1958; Burrows, 1960), in the present study the sex of an individual was taken from the sex of the flowers that were the overwhelming majority on the plant. Sex determination was not possible for some plants, because no flowers were present. Some plants which had fruit present were counted as adults, but excluded from the sex ratio calculation as it was not possible to distinguish female from hermaphrodite plants in the absence of flowers. A 50 m transect through the most abundant patches of *P. arenaria* at each site was conducted parallel to the surf line with sampling points every 5 m. At each sampling point a careful search was made for seedlings in a circle of 1m radius.

The approximate density of plants at each site was calculated using the population counts and the area of the reserves. Additionally, to investigate the spatial structure of the populations, the distance (m) to the nearest neighbour of 10 randomly selected plants at each site was also measured.

3 - Pollination biology

While no direct counts of floral density were made, estimates of floral density were obtained from photographs. Only photographs that showed an entire plant were used and all the flower heads were counted and then multiplied by four because each flower head normally contained four individual flowers. These counts were then combined with the approximate surface area of the plant to obtain an estimate of flowers/m^2.

The pollen/ovule ratio reflects the pollen vectors associated with a plant as there is a strong correlation between the pollination ecology of a plant and the amount of pollen produced (Cruden, 1977). Unopened flower buds were collected in the field and preserved in 3:1 ethanol : acetic acid. The anthers were then extracted and crushed in an ependorfer tube containing 0.3 ml mannitol solution, coloured with a little aceto-carmine. Pollen grains were then counted using a haemocytometer slide, and compared to the single ovule of each fruit, producing the pollen/ovule ratio. This followed the procedure of Kearns & Inouye (1993).

Nectar samples were taken from individual plants at Castlepoint and Castlecliff using filter paper wicks cut to a narrow point, which facilitated access to the base of the flower. Wet wicks were air-dried in the field, then placed in sealed containers for transport back to the laboratory. Nectar analysis used the anthrone colorimetric assay (Appendix 2.1) to determine total sugar concentration. The standing crop of nectar was then calculated following McKenna & Thomson (1988), in sucrose equivalents from a standard sucrose curve (Appendix 2.2).

Experimental manipulation of the flowers was also conducted at Castlepoint to assess fruit production under different pollination treatments. This site was chosen because of the large number of accessible female plants, allowing a more detailed comparison between female and hermaphrodite individuals. Twenty plants (10 hermaphrodite and 10 female) were selected at random from the study area on the western slope of the Castlepoint Reef. The ability of *P. arenaria* to set viable seed under different pollination regimes was assessed by randomly selecting three branches with flowers at the appropriate stage for treatment (see below) on each on the 10 plants of each sex, and randomly applying one of the following treatments:

(1) Natural pollination;
(2) Hand out-crossed pollination;
(3) Autonomous self-pollination.

(1) Natural pollination consisted of tagging inflorescences that had been pollinated via naturally occurring pollination vectors and were losing floral parts. (2) Hand out-crossed pollination used pollen combined from anthers collected from 10 other plants in the reserve. The anthers were crushed in a vial the same day the treatments were applied to release the pollen, which was then applied to the style on each of the flowers on the selected branches using a thin rod. The above treatments were bagged after the treatment was applied to prevent any agent removing developed fruit. (3) Autonomous self-pollination treatment used unopened flowers over which a mesh bag was also placed to prevent pollinator access.

These treatments were applied in early January 2003 and the resulting fruit harvested in March 2003. The fruits from each treatment and flower type were kept separate. In the laboratory the fruits of each treatment were weighed individually, the seeds extracted and weighed, and the pulp weight calculated. The seeds were then placed on water-soaked blotting paper in containers in a 20°C constant temperature room with a 12-hour light/dark cycle. The seeds from each treatment replicate and flower type were kept in separate containers. The total number of seeds that germinated was recorded to measure germination differences between the two sexes.

2.5 Statistical Analysis

The height and surface area data were analyzed using GLM models in SYSTAT (version 7) to compare between sites and sex, with site and sex as categorical variables.

Due to the small data set and there being only two possible outcomes: fruit set or not set, binomial ANOVA's using S-Plus were conducted to explore the data from the pollination manipulation experiments.

All significance levels were set at 0.05.

2.6 Results

The measure of individual plant heights showed few plants below 100mm in height (Figure 2.3) and transects from all four sites showed no seedlings. There was a significant difference in height between each site for hermaphrodite plants ($F_3 = 9.542$, $p<0.001$), but not for female plants ($F_2 = 0.667$, $p=0.523$). While there were few or no plants in the smallest height category, there were also few in the largest categories, >400mm tall. Most of the plants at each site were between 100 and 400 mm tall. All of the plants above 400 mm tall were hermaphrodite plants.

There were no differences in surface area of hermaphrodite ($F_3 = 2.322$, $p=0.075$) and female plants ($F_2=0.434$, $p=0.654$) compared between sites (Figure 2.4). The data are, however, biased towards plants with smaller total surface areas positively skewed towards plants with larger surface areas. The majority of the plants at each site were less than $9m^2$, although there were a few plants, both female and hermaphrodite, scattered between $9m^2$ and $20m^2$. There were a few plants with a surface area greater than $21m^2$ and those were all hermaphrodite plants (Figure 2.4). Castlepoint and Himatangi had the largest proportion of plants greater than $21m^2$ in surface area.

Width and breadth were highly positively correlated, but neither correlated with height. There was no significant difference in height ($F_2=2.091$, $p=0.125$) or surface area ($F_2=0.223$, $p=0.801$) between hermaphrodite and female plants at any of the four sites suggesting that female and hermaphrodite plants are very similar in appearance between populations.

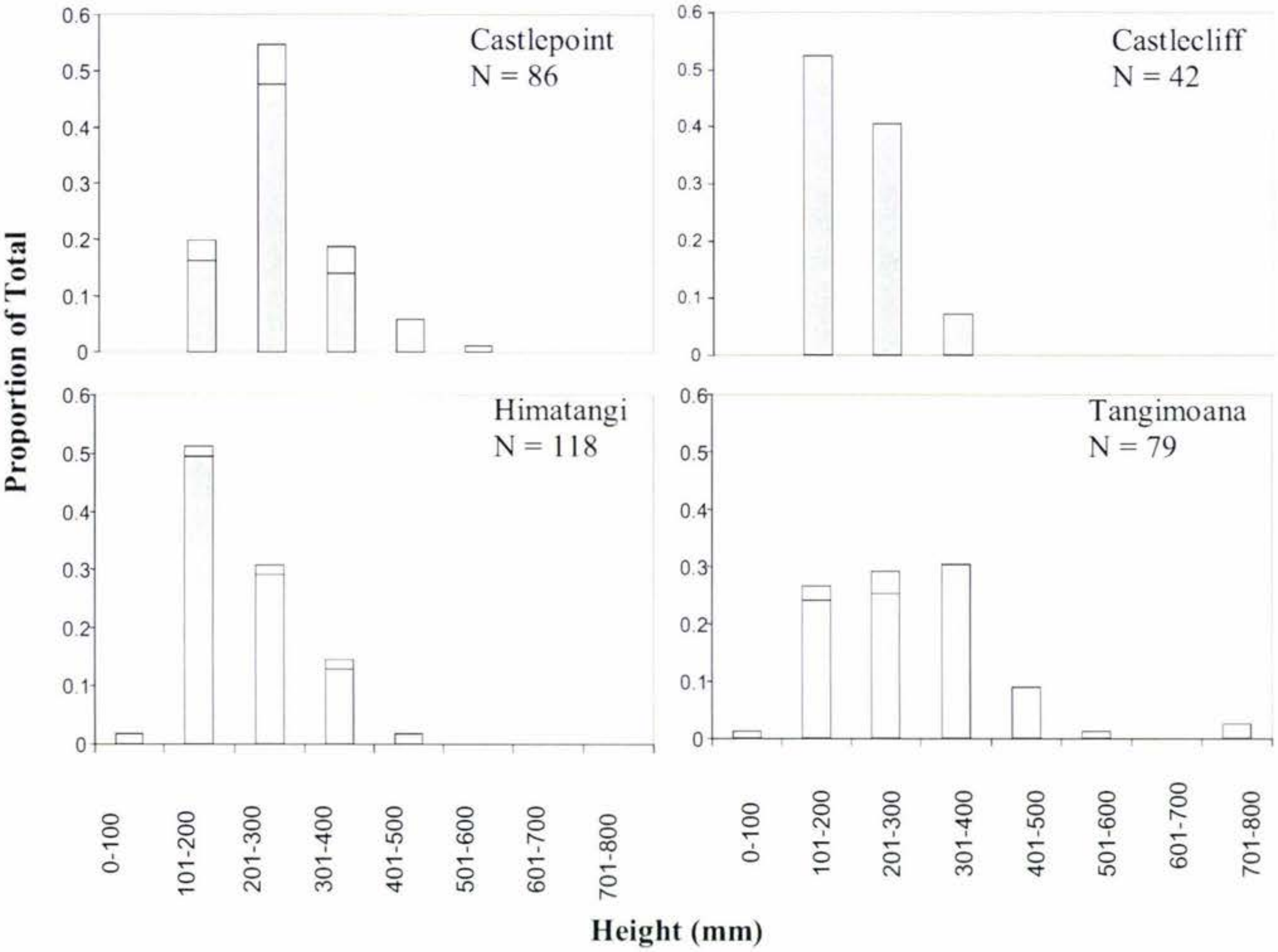

Figure 2.3. Height distributions of hermaphrodite (▨) and female (☐) *P. arenaria* plants at each of the study sites (December, 2002). N = number of individuals.

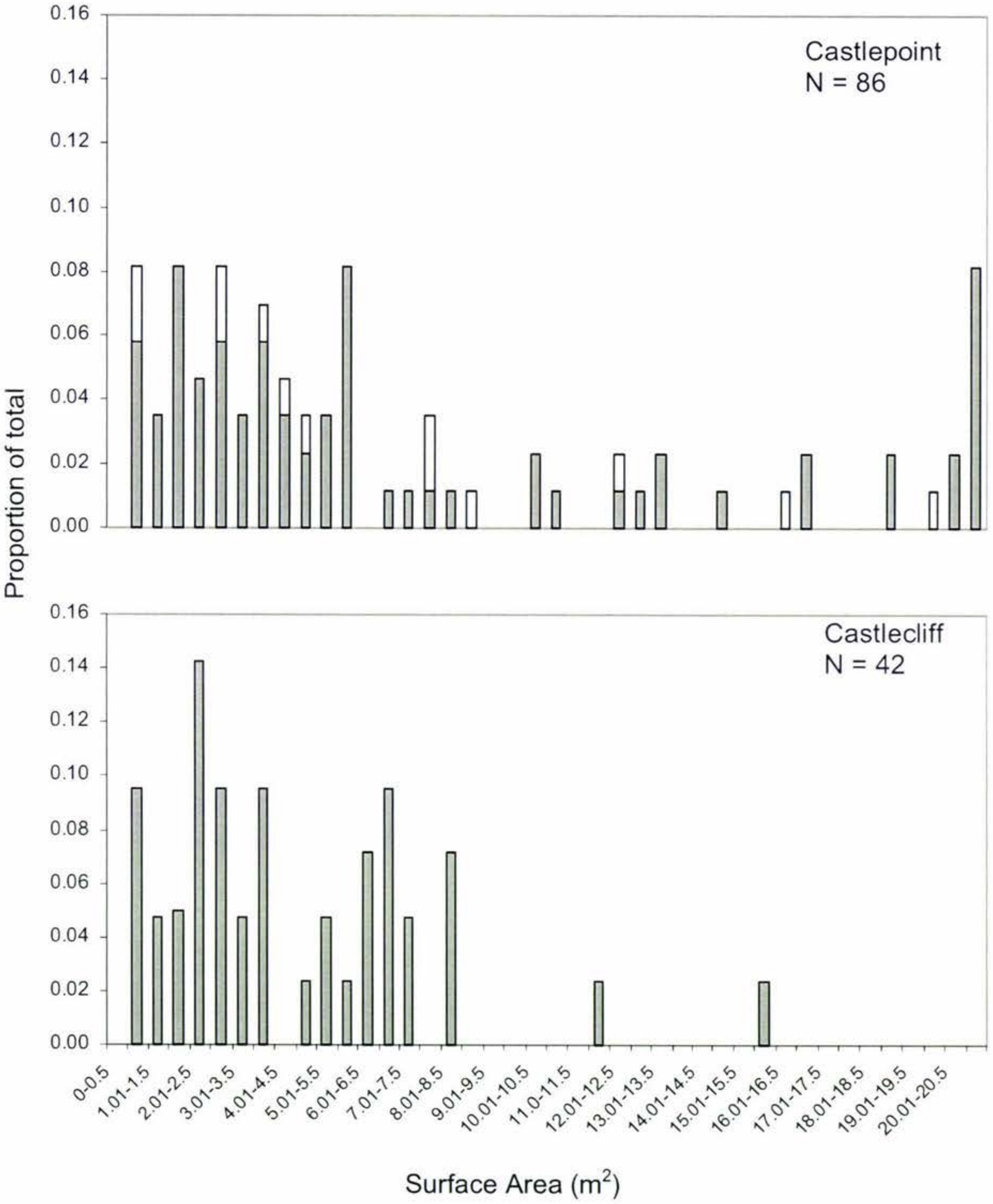

Figure 2.4.(a) Surface area distributions for hermaphrodite (▨) and female (☐) *P. arenaria* plants at Castlepoint and Castlecliff (December, 2002). N = the number of plants at each site

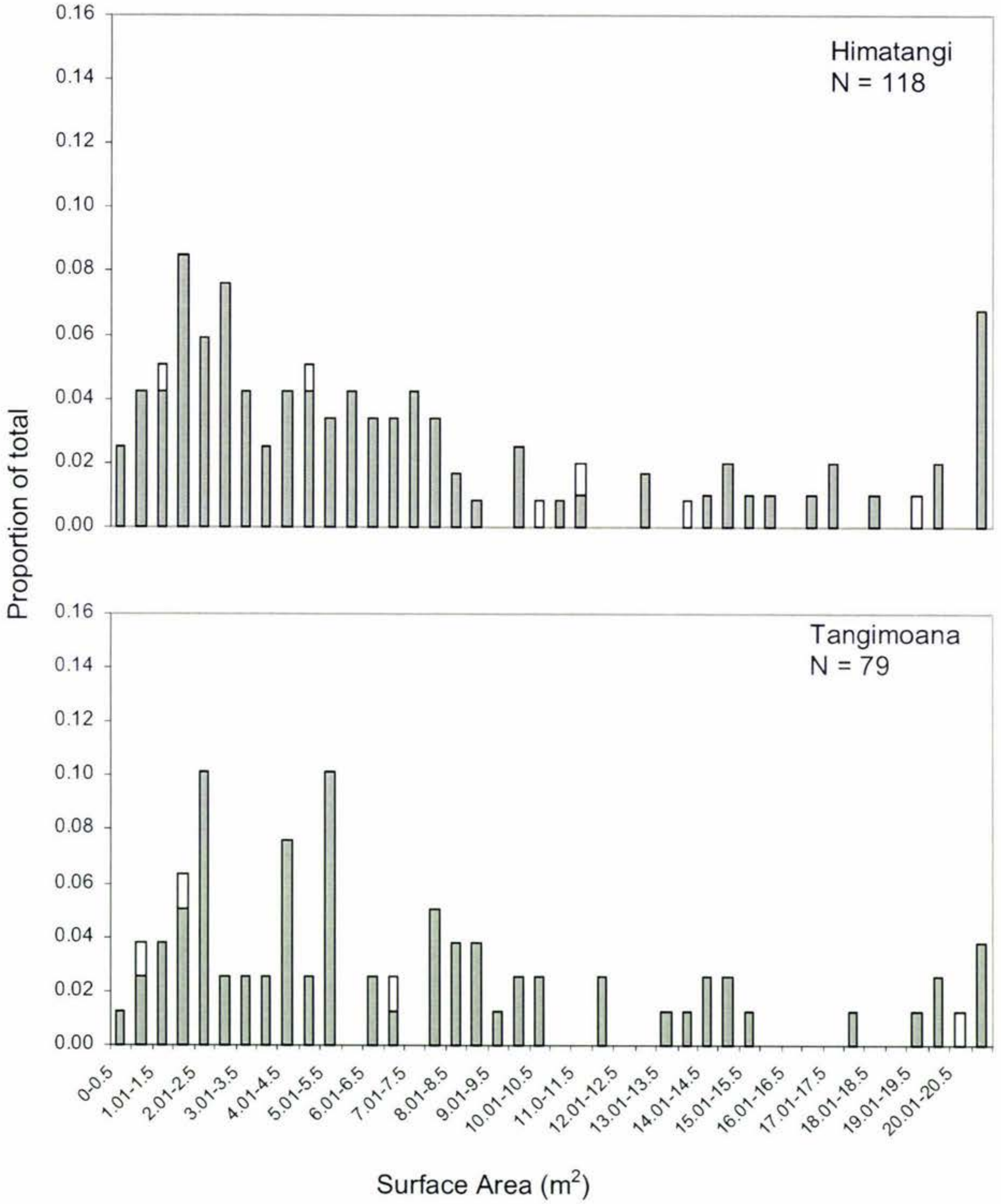

Figure 2.4.(b) Surface area distributions for hermaphrodite (▨) and female (☐) *P. arenaria* plants at Himatangi and Tangimoana (December, 2002). N = the number of plants at each site.

The density of *P. arenaria* plants varied at each site with the highest density being 4.05 plants/ ha (Castlecliff) and the lowest density 0.53 plants/ ha (Tangimoana) (Table 2.1).

Table 2.1. Estimated densities of *P. arenaria* individuals at the four study sites, December, 2002.

Site	Density (# plants / hectare)
Castlepoint	1.46
Castlecliff	4.05
Tangimoana	0.53
Himatangi	3.49

There was considerable variation in the distances between individual plants at all sites, except Castlepoint where nearest neighbour distances were extremely uniform, with approximately one metre between individuals. The other sites had larger variation around larger distances between individuals: 3m at Castlecliff, 4m at Tangimoana, and 2.5m at Himatangi (Figure 2.5).

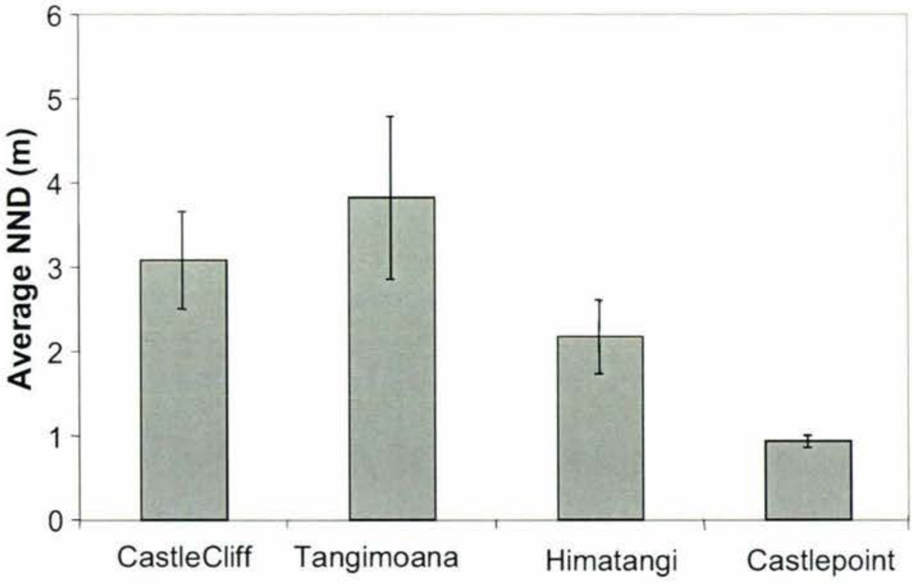

Figure 2.5. Average Nearest Neighbour Distance (NND) (± SE) of 10 randomly selected *P. arenaria* plants at each study site (September 2003).

Population sex ratios varied between sites, however in each population hermaphrodite plants were more common than female plants. Hermaphrodite plants only were found at Castlecliff. The other three sites had female plants present in varying numbers, Castlepoint having the largest number of female plants and Himatangi the least. Tangimoana also had few female plants with a sex ratio close to that found at Himatangi (Table 2.2).

Table 2.2. Population sex ratios of adult *P. arenaria* plants for each site (December, 2002).

Sites	Female Plants	Hermaphrodite Plants	Total number	Sex ratio (F : H)
Castlepoint	13	73	86	1 : 5.62
Castlecliff	0	42	42	all hermaphrodite
Himatangi	6	107	113	1 : 17.8
Tangimoana	5	71	76	1: 14.2

The Pollen / Ovule ratio was 1987.3 ± 172.3 : 1. Nectar was present in hermaphrodite plants at both Castlecliff and Castlepoint, the average standing crop of nectar being c. 57 µg sucrose equivalent/flower at Castlepoint and c. 24 µg/flower at Castlecliff, a factor difference between the sites of approximately 2.5 (Table 2.3). The amounts of nectar in female flowers were not recorded.

Table 2.3. Nectar reward available to pollinators, expressed as average amounts of sucrose equivalents present in *P. arenaria* flowers from Castlecliff and Castlepoint (January - March, 2003).

Site	Average Sucrose Equivalent (µg/flower)	Standard Error (µg/flower)	N
Castlepoint	56.903	4.088	12
Castlecliff	24.292	2.149	14

An estimate of *P. arenaria* flower density taken by counting flower heads from photographs suggests an average (± SE) of 423 (± 99) flowers / m^2, N (plant)=6.

As a percentage of the total number of flowers manipulated, hermaphrodite flowers produced twice as many fruit under natural pollination than female flowers (Figure 2.6).

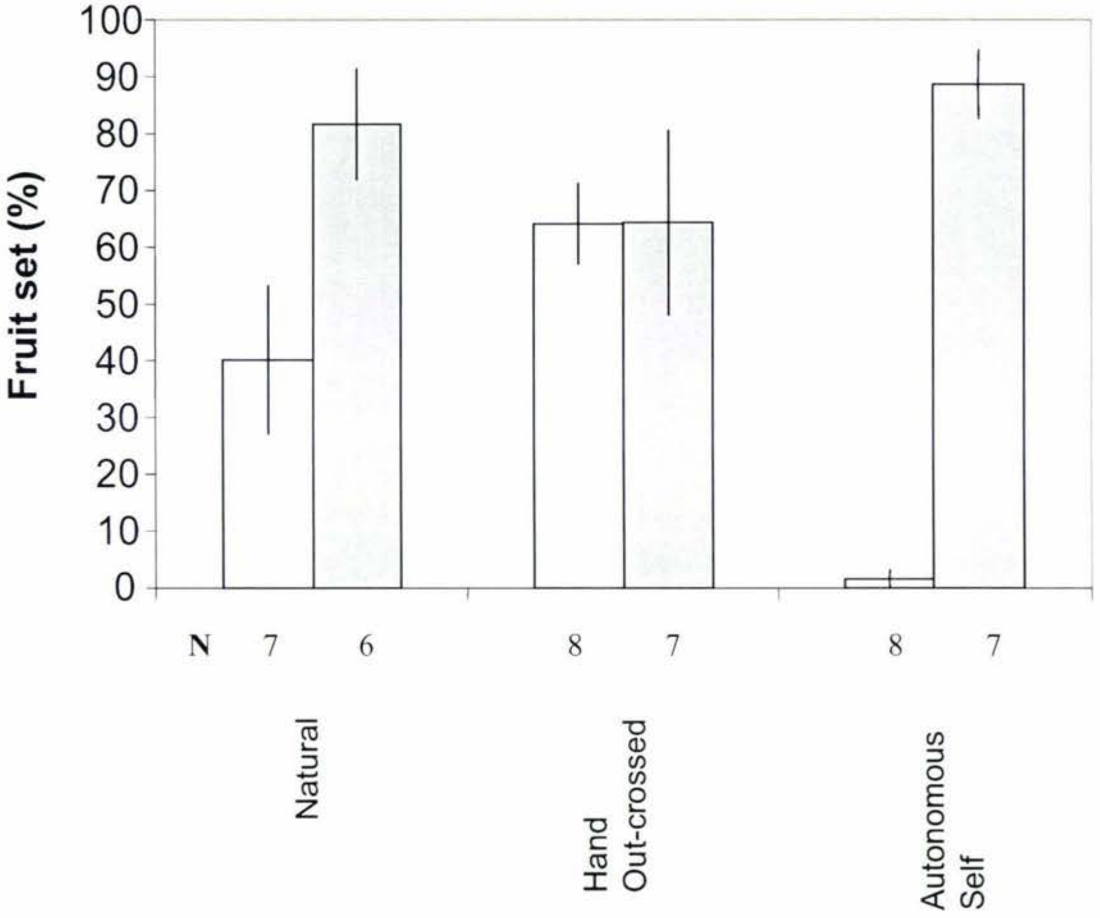

Figure 2.6. Average (± SE) percentage of fruit produced by each sex at Castlepoint (December, 2002) for each of the natural, hand out-crossed and autonomous self-pollination treatments. Female plants = ☐, hermaphrodite plants = ■ . N = number of inflorescences.

Hand out-crossed flowers produced the same percent of fruit in both sexes, although there was no significant difference in the average percentage of fruit produced by hand out-crossed female flowers compared to the percentage of fruit by naturally pollinated female flowers (F_2=1.19, p=0.34). Only hermaphrodite plants self autonomously to produce fruit. Female plants lack functional anthers, but a single female was recorded as autonomously selfing (Figure 2.6).

Seed weight was constant between all the treatments and between both sexes (Figure 2.7), but the weight of pulp varied, especially in the hermaphrodite fruit.

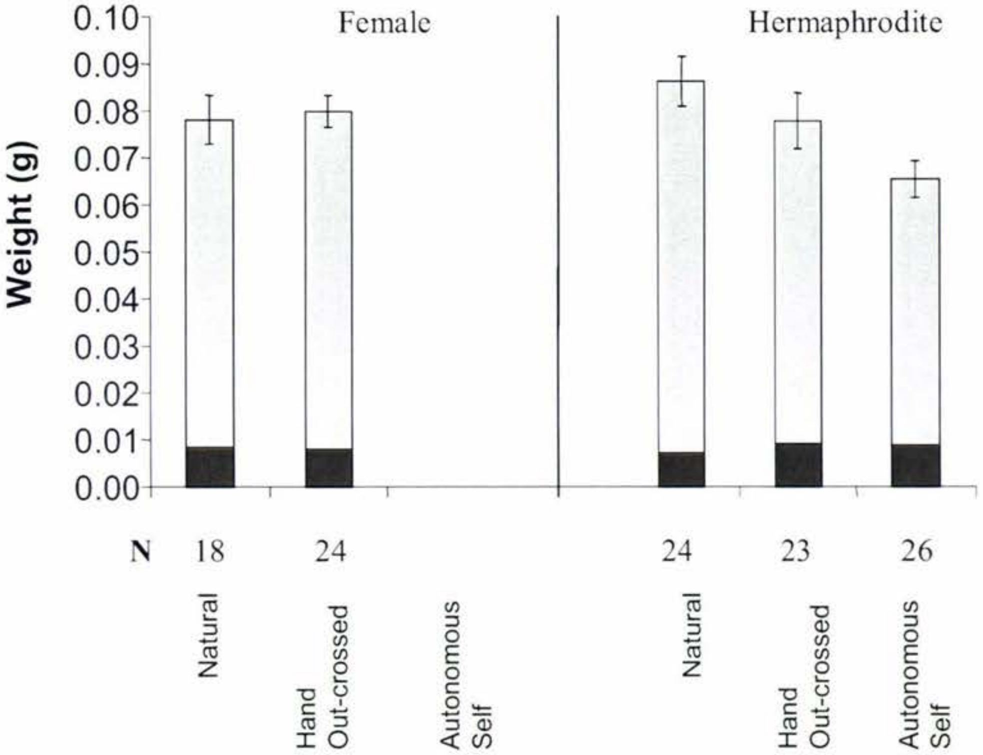

Figure 2.7. The average (± SE) weight of seed (■), and pulp (□) from the three pollination treatments (January - March, 2003). N = number of fruit.

Female plants produced fruit of approximately the same size and weight regardless of the pollination treatment. In contrast hermaphrodite plants produced varying weights of fruit depending on the pollination treatment. Natural pollination produced the most fruit pulp and autonomous selfed fruit the least (Figure 2.7).

Overall germination rate was low (Table 2.4), with less than 50 % germination from all treatments. There was higher germination success in seeds from female plants in both the hand out-crossed and natural pollination treatments (Table 2.4).

Table 2.4. The germination success, after 5 months, of seeds obtained through the pollination manipulation experiment (January - March 2003) with the seeds at a constant 20°C and in 12-hour light/dark conditions. Note: The single seed obtained from a female flower under the autonomous self treatment was included as a hand out-crossed fruit.

	Natural	**Hand out-crossed**	**Autonomous self**
Hermaphrodite	16.6% (n= 18)	34.7% (n= 23)	48% (n= 26)
Female	22.2% (n= 24)	48.1% (n= 24)	-

2.7 Discussion

This thesis looked at *Pimelea arenaria*, a native plant restricted to sand dunes and is listed as nationally declining (de Lange *et al.* 1999) despite profuse flowering. Anecdotal evidence indicating few or no seedlings suggested that this species was in decline due to recruitment failure.
This chapter looked at the population structure of four populations of *P. arenaria* to determine (1) if recruitment failure is occurring. If recruitment failure were evident (2) whether if it was affected by the pollination regimes in *P. arenaria* or by germination of the seed.

Results confirm that there is recruitment failure in the *P. arenaria* populations studied, as shown by the distribution of plant heights above substrate (Figure 2.3). Few or no plants were found in the smallest height category (Figure 2.3), and line transects failed to locate seedlings in any of the populations studied. The significant difference in height observed between the sites is likely to be related to the substrate, plants in sand dunes being repeatedly buried and uncovered by sand movement (Yanful & Maun, 1996; Maun, 1998; Milne & Sawyer, 2002). However, this difference may not be real as the measurements did not include the roots, and the height differences between the sites may simply reflect differing rates of sand accretion at each site, rather than a difference between the plants. The lack of a significant difference between female plant height and hermaphrodite plant height between sites is likely due to the small number of female plants.

The average nearest neighbour distance (NND) (Figure 2.5) varied considerably at all sites except Castlepoint, where the short very uniform average distance suggests clumping. This suggestion is further supported when the NND's were compared with the estimated population densities of *P. arenaria* (Table 2.1). Both Tangimoana and Castlepoint show signs of clumping of individual *P. arenaria* plants, both sites having low densities of plants per area but fairly small distances between individuals. This clumping could be a response to an environmental variable such as the position of the water table, or possibly the original distribution of seed from which the plants grew. Seed distribution is highly clumped in dunes systems due to seeds accumulating in unevenly spaced depressions (Zhang & Maun, 1994).

Population sex ratios were highly variable between the sites, although hermaphrodite plants were more common at all the sites (Table 2.2), being the only sex found at Castlecliff and in the lowest proportion at Castlepoint. Himatangi and Tangimoana had high sex ratios with large numbers of hermaphrodite plants and few female plants. The only other sex ratios recorded for *Pimelea* (Burrows 1958; Burrows; 1960) were different to the present study, all being close to 1:1 (female : hermaphrodite) throughout the flowering season (Burrows, 1960). *P. prostrata* has a ratio of 1:10 (female : hermaphrodite) in the early part of the flowering season (Burrows, 1960) and as such is the closest to the ratios recorded in this study. Only single counts of the sex ratio were made in the populations studied here and it is possible, given the results of Burrows (1960), that the ratio of female to hermaphrodite plants at the sites of this study would also change through the flowering season.

The pollen:ovule ratio indicates *P. arenaria* is an out-crossing species with a leaning towards facultative out-crossing (Cruden, 1977);Table 2.5.

Table 2.5. Comparison of the pollen:ovule ratios in different breeding systems. Modified from Cruden (1977).

Breeding System	Pollen /ovule ratio
Obligate selfer	1 : 30
Facultative selfer	1 : 170
Facultative outcrosser	1 : 800
Pimelea arenaria	**1 : 1987**
Obligate outcrosser	1 : 6000

The ratio of pollen grains to ovules further suggests that the pollination vectors are likely to be unreliable and unspecialized e.g. fly species (Cruden, 1977). As noted by Burrows (1960), nectar is produced by both female and hermaphrodite flowers; however Burrows (1960) gave no quantitative measurements, and no hermaphrodite plants were looked at in the present study. The quantity of nectar found in *P. arenaria*, c. 57 and c.24 µg (Table 2.3), is relatively small compared to other New Zealand plants (Table 2.6) however, the sample sizes were small, so no strong comparisons can be made.

Table 2.6. Pollination vectors of New Zealand plants and associated standing crops of nectar. Modified from Ladley *et al.*, 1996; Murphy, 1996; Castro & Robertson, 1997; McNutt, 1997; Perrott, 1997.

Pollination vector	Standing crop nectar (µg)
Birds and insects	220
Honey eaters	4140

Mechanisms of pollination for *P. arenaria* are present, and both female and hermaphrodite plants are capable of producing fruit (Figure 2.6). However, the amount of fruit produced by hand out-crossing *P. arenaria* compared to that from natural pollination (Figure 2.6), suggests that female plants are, currently at least, pollen limited, although relatively small sample sizes mean that this difference is not statistically significant. Under present conditions hermaphrodite plants are more productive because of their autonomous selfing and, therefore, their lack of reliance on pollinators.

Less than 50 % of the seeds collected at Castlepoint germinated successfully at 20°C (Table 2.4), a temperature at which germination should occur (Craig McGill, pers.comm) regardless of the pollination method. There was, however, a slightly better germination success observed in female seed in both natural and hand out-crossed pollination (Table 2.4). Despite these low germination rates, the results were contrary to Metcalf (1995), who suggested that only cold storage stratification and scarification could induce germination in *Pimelea* seed, except *Pimelea tomentosa*, and even then germination could not be expected within 12 months (Metcalf, 1995). In addition to the seed obtained from the pollination experiment, seed collected for a feeding trial (Chapter 4) germinated after 5 months through the pulp of fruit stored at 4°C (Figure 2.8).

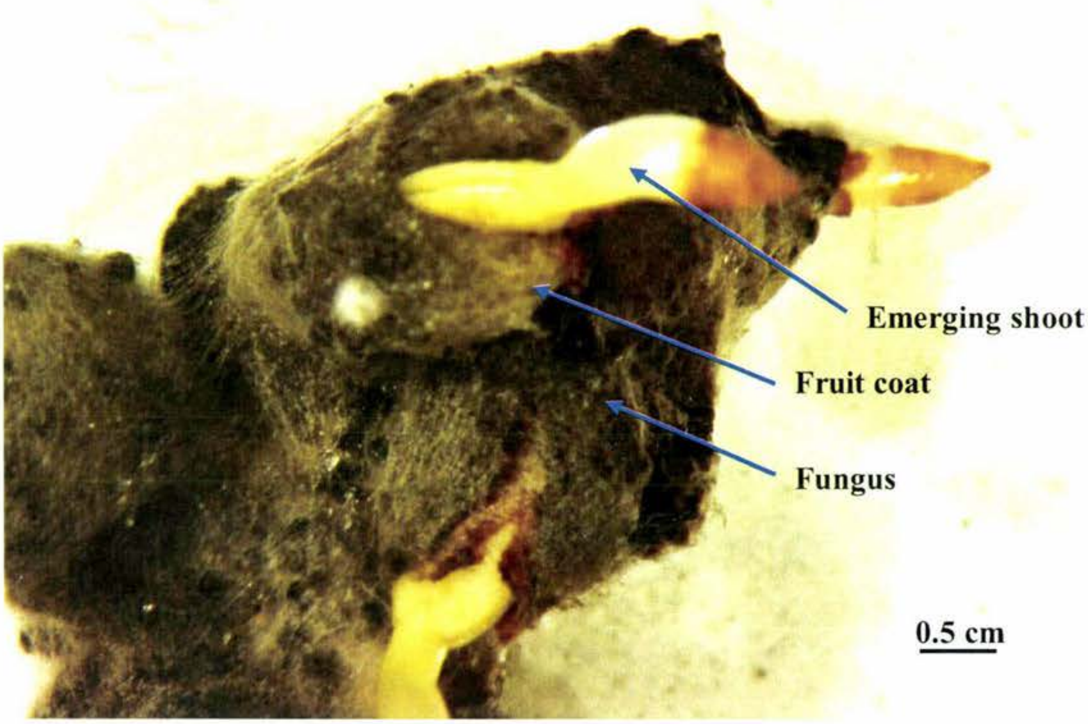

Figure 2.8. *P. arenaria* seed that germinated after a 5 month period through the fruit pulp while being stored at 4°C.

Grime (1979) and Grime & Hillier (2000) suggest that there are five regeneration strategies utilized by terrestrial plants. There is regeneration by (1) vegetative expansion, (2) numerous widely dispersed seeds or spores, (3) seasonal regeneration, (4) persistent juveniles, and (5) persistent seed or spore banks (Grime, 1979; Grime & Hillier, 2000). Nothing is known about whether **vegetative expansion** occurs in *P. arenaria*, although it could occur and be reflected in the clumping of individuals discussed above. It is possible that plants in close proximity to each other are exposed portions of the same individual, rather than genetically separate individuals, the point of attachment between the original plant and offspring being beneath the surface of the sand. *P. arenaria* seed do not fit the criteria for having **numerous widely dispersed seeds** as their seeds are not "exceedingly buoyant in air", one of the functional characteristics of numerous widely dispersed seeds (Grime & Hillier, 2000). Whether **seasonal regeneration** or **persistent juveniles** occur is unclear, due to the lack of seedlings found. *P. arenaria* plants however, currently produce large number of flowers (average 423 flowers/m^2, n(plant)=6) which results in substantial fruit production when protected from predators (Chapter 3). Persistent juveniles are more likely found in habitat which is relatively undisturbed (Grime & Hillier, 2000), but burial by sand is frequent in dune ecosystems, to which plants have adapted (Yanful & Maun, 1996; Maun, 1998). This suggests that *P. arenaria* has seasonal regeneration, although both seasonal and persistent juvenile strategies could be occurring with seedlings being eaten by herbivores such as rabbits which are abundant at all four sites. The current lack of seedlings (Figure 2.3) could indicate that *P. arenaria* has a spasmodic regeneration cycle with large numbers of seedlings being recruited into the population only in some years. Such a situation would be analogous to the mast seeding seen in several native species, such as *Chionochloa* (Webb & Kelly, 1993; Sullivan & Kelly, 2000) and in *Nothofagus* species forests (Kelly *et al.*, 2001). There are, however, no long-term data to assist further discussion of this with regard to *P. arenaria*.

There is little understanding of seed banks, either persistent or transitory, in sand dune ecosystems (Zhang & Maun, 1994). **Persistent seed banks** are defined as those having a proportion of seeds older than one year (Thompson &

Grime, 1979). Several authors have suggested that persistent seed banks are not present in sand dunes (Mack, 1976; Watkinson, 1978; Boorman & Fuller, 1984; Zhang & Maun, 1994), however many of these studies improperly adapted sampling techniques originally designed for forest use (Zhang & Maun, 1994). This resulted in samples being taken at too shallow a depth, and/or sampling not being intensive enough which, with the highly clumped distribution of seeds in the dunes (Zhang & Maun, 1994) may have resulted in no seed banks being found (Zhang & Maun, 1994). Zhang & Maun (1994) have also suggested that some plants may retain their seed on the inflorescences for a period, thereby avoiding the need for a buried seed bank. Several studies suggest that the persistence of a seed bank in sand dunes is due to the depth of burial of the seed (Permadasa & Lovell, 1975; Baskin & Baskin, 1985; Colosi *et al.*, 1988; Zhang & Maun, 1994). Germination is inhibited at greater depths, thus deeply buried seeds could be part of a persistent seed bank (Zhang & Maun, 1994). The high rates of sand movement, which can vary in space and time (Olsen, 1958; van der Valk, 1974; Maun, 1985), can result in a seed being buried deeply and then uncovered at a later time (Zhang & Maun, 1994) allowing subsequent germination of the buried seed.

One characteristic of plants in dune ecosystems is their ability to withstand burial by sand (Yanful & Maun, 1996; Maun, 1998), and to reproduce in such a dynamic, arid environment. The survival of adult dune plants after burial by sand depends on the species of plant, the rate of sand deposition (Maun, 1998), and many other environmental variables affecting survival, including atmospheric desiccation, root aeration, and root zone temperatures (Sykes & Wilson, 1990; Maun, 1998). If a *P. arenaria* fruit successfully ripens, and is not eaten, it will drop off the parent plant onto the substrate (Burrows, 1960). According to Zhang & Maun (1994) and Maun (1998) there are then four possible futures for the seed within that fruit; (i) germination and emergence of a seedling, (ii) germination but no emergence, (iii) no germination of the seed at all, and (iv) the entrance of a seed into a seed bank, as discussed above. The data presented so far indicate that *P. arenaria* seeds are produced and these seeds can germinate. However, the lack of seedlings in the study populations suggests that the seeds do not germinate, or they germinate but don't establish, or the seedlings establish but do not survive. Survival of seedlings, like that of adult plants, is affected by several environmental variables (Maun, 1998). The most important of which are sand movement in dunes, an important factor determining distribution and composition of the sand dune vegetation (Ranwell, 1958; van der Valk, 1974; Maun & Lapierre, 1984; Moreno-Casasola, 1985; Maun & Lapierre, 1986; Yanful & Maun, 1996), and desiccation (Maun, 1981; Maun, 1994; Yanful & Maun, 1996). Seedling emergence depth is positively related to seed mass (Buckley, 1982; Weller, 1985; Maun & Lapierre, 1986; Yanful & Maun, 1996; Leishman *et al.*, 2000), species being absent from areas where the sand accretion exceeds the maximum depth from which a seed can emerge (Maun & Lapierre, 1986). It is possible, therefore, that the rate of sand accretion has increased in areas that *P. arenaria* currently occupy, and that the rate is exceeding the emergence depth for seeds and/or the growth rate of seedlings. Alternatively, *P. arenaria* seedlings may emerge but are immediately eaten by herbivores such as rabbits that inhabit all the study sites (pers. obs.).

There is nothing in the literature about the germination requirements of *P. arenaria* seed in the wild and this was not investigated in this study. It is possible that the germination success of *P. arenaria* seed in the wild would be different because of a number of environmental variables not present in laboratory situation. These include substrate temperature, substrate moisture levels, and differing day/night temperatures. The micro-site requirements for *P. arenaria* seedling establishment are also unexplored, but it is possible that seedling micro-sites may need to be manufactured for *P. arenaria*, as has been found for other dune plants (Singers, 1997). The competitive ability of seedlings from female *P. arenaria* plants versus hermaphrodite plants is unknown.

There are no reports in the literature on the effects of varying amounts of fruit pulp (Figure 2.7) on a seedling's survival and subsequent competitive abilities. Although the amount of fruit pulp seen in the autonomously selfed fruit of *P. arenaria* (Figure 2.7) is not significantly smaller (p=0.37069) than the amount of pulp in hand out-crossed hermaphrodite fruit, it is possible that is disadvantageous. The fruit pulp of some New Zealand plant species has been shown to have some inhibitory effect on seed germination (Burrows, 1989;

Partridge & Wilson, 1990; Bannister & Bridgeman, 1991; Fountain & Outred 1991; but see Clout & Tilley, 1992). In dune ecosystems one of the main physiological stresses for seeds and seedlings is desiccation (Permadasa & Lovell, 1975; Mack, 1976; Maun, 1981; Boorman & Fuller, 1984; Yamanaka *et al.* 2000). *P. arenaria* seedling survival could be affected by less fruit pulp that might inhibit germination for a shorter time. A shorter period of seed dormancy through germination inhibition could result in a seedling trying to establish at a time when seedling survival rates are lowest because of increased probability of desiccation. *P. arenaria* seedling survival could be improved by additional moisture released as the fruit pulp breaks down. Seeds surrounded by heavier pulp could be at a competitive advantage because more moisture and/or nutrients enter the seedlings' micro-site.

Nothing is reported about the levels of inbreeding of *P. arenaria* plants or how much autonomous selfing might contribute to this. Out-crossing is necessary to maintain genetic variability within a population, a lack of out-crossing eventually resulting in in-breeding and a loss of genetic variation. In *P. arenaria*, the insect pollination vectors that transfer pollen between individuals, often as a consequence of taking nectar (Burrows, 1960), affect out-crossing. For *Pimelea* the majority of pollination vectors are fly species (Burrows, 1960; pers. obs.) that appear not to distinguish between female and hermaphrodite plants in their foraging (Burrows, 1960). Flies were observed to move systematically from flower to flower within each head (Burrows, 1960; pers. obs.), which is likely to result in the transfer of pollen from a neighboring flower on the same individual. This behaviour suggests that levels of out-crossing may be low within *P. arenaria* populations.

Sand, like any other substrate, contains microorganisms that can affect adult plants, seedlings, and seeds in both positive and negative ways (van der Putten, 1993; Maun, 1998). According to van der Putten (1993) lack of sand movement may be more harmful to a plant than frequent burial because pathogens build up within the sand. It is possible that recruitment failure in *P. arenaria* is due to the activity of micro-organisms in areas adjacent to *P.*

arenaria plants where the sand is fairly static thereby allowing micro-organisms to build up.

Conclusions

All the populations in this study show a bias towards larger (older) plants with few or no seedlings, evidence that indicates recruitment failure. It is clear that the plants produce pollen that can fertilize ovules; that pollination vectors exist that can transfer the pollen; and that viable seed can be produced. Therefore seedling survival appears to be a vulnerable stage in the life history of *P. arenaria*.

Areas for future research

Heights of *P. arenaria* plants were used as a correlate of age as nothing is known about the age/growth relationship of *P. arenaria* and ageing by growth rings is not possible (Burrows, 1958). But is this a valid correlate of age? With no sure estimate of the age/growth relationship, no estimate can be made of the longevity of these plants, the age at which an individual can produce seed, or the amount of seed a plant might contribute to the population over its lifetime. While the estimated floral density on an individual plant averages 423 flowers/ m^2, the sample size is small and may not be an accurate estimate of floral density. Further more nothing is known about how many of these flowers result in viable seed. Consequently the total amount of fruit produced by an individual over a fruiting season can not currently be estimated and nothing is known about how total fruit production varies between individuals and between seasons.

The sex ratios of *Pimelea* species change through the fruiting season (Burrows, 1958; Burrows, 1960), however only single counts were made for *P. arenaria* in this study, so it is unknown whether the sex ratio for this species also changes. While there was no difference in height or surface area between hermaphrodite and female plants, a larger sample of female plants may show differences between sites that have not been detected in this study.

Larger samples of the standing crop of nectar and of the maximum amount of nectar produced, by both female and hermaphrodite flowers, should be taken all through the flowering season at as many sites as possible. Such sampling would indicate how nectar production changes through the season and the possible effects of this on fauna and flora associated with *P. arenaria*. Within an individual plant there may be both flower types (Burrows, 1958; Burrows, 1960; pers.obs.). It would be helpful to know whether the proportions of the flower types change through flowering season, and if so what effects this might have on fruit production.

Nothing is known about the requirements for seed germination of many New Zealand plant species (Fountain & Outred, 1991), including *Pimelea arenaria*. Whether seed banks of *P. arenaria* are present in the sand dunes is unknown. The presence of a seed bank might indicate that the recruitment failure currently observed might not influence *P. arenaria* populations indefinitely.

Nothing is known about the survival rates of seedlings in the wild, or what impact herbivores, such as rabbits, might have seedling establishment.

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Appendix 2.1

Total carbohydrate detection procedure using the anthrone colorimetric assay (modified from Kearns & Inouye 1993).

The total carbohydrate determination in the samples was conducted in four stages:

1. Extraction of sugar from wicks previously placed in plants. The filter paper wick from each sample was placed in a vial containing 5mL of distilled water, swirled and left for 30 minutes.

2. Determination of the appropriate dilution factor to use for each species. Sub samples of 1, 0.5, 0.1 and 0.01mL of the solution were removed from one of the vials and poured into boiling tubes previously labelled. Volumes were brought to 1mL using distilled water. 2mL of anthrone reagent (500mL of H_2SO_4, 25mL of distilled water and 1g of anthrone) was added to the tube and gently vortex to mix the reagents. Tubes were then placed for 10 min. in a water bath (80°C, using the Julabo 19 water bath).
When cool, tubes were compared with the standard sugar series previously prepared. The best dilution factor was selected based on the closest match with the standard near the lighter end of the scale.

3. Analysis of the replicate samples. The remaining samples were prepared as mentioned in the step 2 but using only the best dilution factor, in this case zero dilution. Sugar concentration was read in the spectrophotometer at 620nm (here the equipment used was the Jenway 6105 U.V./Vis. Spectrophotometer, Biolab Scientific Ltd.).

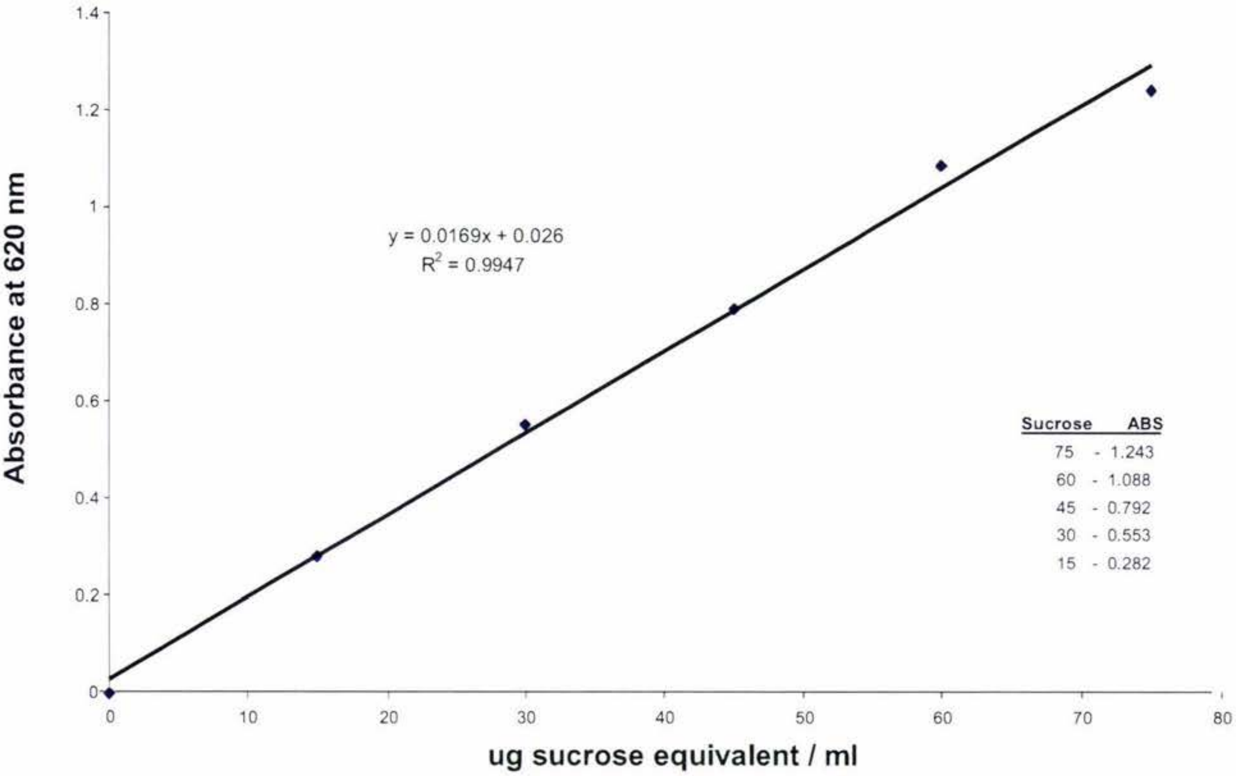
4. Sugar concentration in the sample was estimated using the calibration curve previously prepared. To calculate the actual sugar content, sugar concentration in the sample was multiplied by the dilution factor to give total amount of sugar in the vial.

Sugar standards

50mL of a stock solution of 75mg of sucrose (Analar) was prepared in a volumetric flask. Then dilutions of the stock were prepared taking 2.5, 2, 1.5, 1 and 0.5mL into 50mL volumetric flasks and top up with distilled water. A blank of distilled water only was also prepared.

Appendix 2.2

Standard glucose calibration curve



Chapter 3

Fruit set and fruit loss in *P. arenaria* – an exclosure experiment

3

3.1 Abstract

Pimelea arenaria is listed as a declining native dune plant due to a recruitment failure. Previous work suggests that *P. arenaria* plants are able to produce fruit, but that seedlings are completely absent from the population, possibly because the fruit are being removed and the seed destroyed by one or more predators. This study aimed to determine whether (i) fruit were being removed and (ii) whether any fruit loss could be attributed to the introduced house mouse (*Mus musculus* L.), or to various bird species. This was done by means of exclosures allowing selective access to potential predators, measuring fruit loss at four sites in the lower North Island of New Zealand. The results showed that bagging the fruit to prevent predator access had a significant effect on the rate of fruit loss ($p<0.001$), however there was no significant overall caging effect observed, although there was a significant difference between bagged and un-bagged fruit where birds had access ($p=0.023$), suggesting birds had a greater impact than mice.

There were no temporal changes in fruit loss between treatments or among sites for un-bagged fruit. There was however, a peak in fruit loss at the start and the end of the fruiting season within the bagged fruit which is attributed to selective abortion of ovules or embryos by the parent plant and not to predation.

These results suggest that both the house mouse and various undetermined bird species, appear likely to remove fruit which, coupled with ovule abortion, could be responsible for recruitment failure in *P. arenaria*.

3.2 Keywords

Graded access exclosures, *Pimelea arenaria*, mice, introduced birds, fruit predation, New Zealand

3.3 Introduction

Before the arrival of humans, New Zealand had very few species of land mammals (King, 1995, 2001). Since the arrival of man, a large number of species mainly, but not exclusively, from Europe, have become firmly established in the New Zealand landscape and have had devastating impacts on many native species (King, 2001). Among the introduced animals were three species of rats and one species of mouse (King, 1995). All of the rat species have had a huge effect on the New Zealand fauna and flora directly, by preying on native birds (Murphy & Pickard, 1995) and eating native plant fruit and seedlings (Williams *et al.*, 2000; Campbell & Atkinson, 2002), but also indirectly by supporting populations of larger predators (Alterio & Moller, 1997; Brown *et al.*, 1998; Murphy *et al.*, 1998) or via competition with native species for resources. Even though mice are found in a wide variety of habitats (Ruscoe, 2001), little is known about the effects of mice on local ecosystems (Miller & Webb, 2001). However, with the success of eradication programs against rats (Empson & Miskelly, 1999; Atkinson & Towns, 2001; Innes, 2001), some mice populations are periodically freed from the rat control that tends to keep their densities low. Thus, there is the potential for an increase in adverse effects of mice as mice populations increase (Ruscoe, 2001). So far, these effects have not been investigated (Miler & Webb, 2001). Most of the previous work on mice has concentrated on forested areas and on islands (Ruscoe, 2001), however mice also inhabit sand dunes (Miller, 1999; Miller & Webb, 2001; pers.obs) where several native species are under threat.

One species of plant is *Pimelea arenaria* (A. Cunn.)(Thymealeaceae), the sand daphne. *P. arenaria* is currently listed as declining (de Lange *et al.*, 1999), i.e. as 'numerically abundant but which are either under threat from serious adverse factors throughout their range, or occur as widely scattered, typically small populations, many of which are undergoing declines through loss of reproductive ability, recruitment failure, predation, or through other processes of often subtle habitat changes'.

P. arenaria is confined to sand dunes (Heads, 1994), typically being found on the lee of the fore dune (Esler, 1978). It is described by Milne & Sawyer (2002) as 'a low growing shrub with spreading branches often half covered in sand, with compact, leafy branchlets, with leaves arranged in four rows at right angles to each other'. Historical observations by Department of Conservation officers and local residents suggested that *P. arenaria* was in decline in several localities (C. Ogle, A. Dijkgraaf, pers.comm.) due to recruitment failure. The presence of fruit or seedlings in the wild is rarely recorded (C. Ogle, G. Rapson, D Ravine pers.comm.) despite *P. arenaria* flowering profusely with the potential for a large fruit crop. *P. arenaria* is able to produce viable fruit but no seedlings were found in the wild (Chapter 1) which suggests that recruitment failure could be due to fruit or seedling predation. *P. arenaria* fruit are c.10mm in length, contain a single large seed, and are comparable to those fruit fed to introduced rodents by Williams *et al.* (2000). *P. arenaria* fruit was not tested by Williams *et al.* (2000), however the size of the fruit make mice potential seed predators and/or dispersers of *P. arenaria*, although, as has been suggested by C. Ogle (pers.comm.), it is potentially poisonous. Other studies having looked at the toxins found within *Pimelea* and their properties (Freeman *et al.* (1979); Adolf & Heckler (1982); Zayed *et al.* (1982); Hafez *et al.* (1983); Pettit *et al.* (1983). There is little known about *Pimelea*. The earliest studies in New Zealand were carried out by Parlane, (1925) and Burrows (1958, 1960) in the Cass Valley, Canturbury, and the only other study on New Zealand *Pimelea* species by Heads (1994) deals with the biogeography of *Pimelea* species within New Zealand.

Mice are not the only potential predators or dispersers of *P. arenaria*. Birds are one of the most common dispersal mechanisms of New Zealand native plants (Clout & Hay, 1989; Willson *et al.*, 1989; Webb & Kelly, 1993; Burrows, 1994; Lee *et al.*, 1994; Williams & Karl, 1996; Lee *et al.*, 2001), which is reflected in the characteristics of many of the fleshy fruited native plants. Most are small (< 8mm)(Willson *et al.*, 1989; Burrows, 1994) and many are purple/black in colour when ripe, a common colour of bird-dispersed fruit, both in New Zealand (Burrows, 1994) and overseas (Turcek, 1963; Willson *et al.*, 1989; Willson & Whelan, 1990). The fruiting seasons of many New Zealand species are also spread over the course of a year thus providing a year round food supply.

P. arenaria has these characteristics and, given the land vertebrate history of New Zealand, this suggests that the original dispersal agent(s) of *P. arenaria* were birds or reptiles (Willson *et al.*, 1989). Among the possible bird predators are several introduced species - blackbirds (*Turdus merula*), starling (*Sturnus vulgaris*), house sparrow (*Passer domesticus*), and various finches such as chaffinch (*Fringilla coelebs*), greenfinch (*Carduelis chloris*), goldfinch (*Carduelis carduelis*).

The aims of this experiment were therefore;

(i) to establish whether fruit was being taken from *P. arenaria* plants in the wild and

(ii) to determine whether mice and/or birds were responsible for any fruit removal

3.4 Method

The experiment was carried out between December 2002 and March 2003 at four sites in the lower North Island - Castlecliff, Tangimoana, Himatangi and Castlepoint (described in Chapter 1). At each site, 16 plants were selected and four plants allocated to each of four treatments.
The treatments were;

(i) Total exclusion of mammals and birds, achieved with plastic sheeting around the sides and wire across the top of the cage.

(ii) Mice excluded but not birds; the top panel of the frame allowing unobstructed access by birds.

(iii) Birds excluded but not mice, which were able to access plants via narrow openings, approximately 2cm high, at the base of the frame.

(iv) Control plants which were surrounded by plastic sheeting on two sides only to control for the effect of the frames and the plastic on the plant but still allowing total access to the plant. The two sides left uncovered were continuous but selected at random.

Experimental plants were selected on the basis of (1) high numbers of flowers and flower buds, (2) ease of access and (3) ease of establishing a frame around the plant, i.e. steep slopes were avoided. Plants in areas frequently visited by vehicles were also avoided, as were plants that had other plant species growing in and around them.

Each treatment consisted of a frame surrounding the entire plant (Figure 3.1). This consisted of wooden stakes at the four corners of a plant, with industrial semi-transparent plastic wrapping, secured to the stakes, and modified for the specific treatments (Figure 3.1). Fishing line with small flags attached was strung across the top of the frame to prevent access by birds but minimize any effects on light levels or wind movements in the exclosures.

The treatments were then randomly allocated between selected plants at each site. Once a frame was placed around a plant it was examined for pairs of branches that had visible fruit of approximately the same age. Some were recently fertilized ovaries detectable by gently squeezing the base of the dead flower, where upon the fertilized ovule was identified as a hard lump (M. Merrett, pers. comm.). The branch pairs were then tagged with coloured wire and by random selection one branch was bagged while the other was left un-bagged. This was repeated for as many branch pairs as possible on the 16 plants at each site. Each plant varied in the number of pairs suitable for this study, the number of pairs ranging from 1 to 14 pairs per plant. The fate of the fruit of each member of the branch pair was then monitored approximately weekly. Throughout the experiment the plants were re-examined to check for additional fruit bearing branches that could be added as replicates.

Figure 3.1 A typical example of an exclosure frame. Note: This frame was designed to exclude everything. For control plots two sides of the frame were left open. Small flags (circled), approximately 5 flags/line, were attached to the fishing line and strung across the top of the frame, approximately 5 lines/frame, to exclude birds.

3.5 Statistical analysis

At each site plants were assigned to one of four treatments (exclusion type). For each plant there was a further treatment in which the fruit was either bagged or un-bagged, with a varying number of replicates. This experiment was therefore analyzed as a nested, split-plot design. Plants were nested within sites and pairs of bagged and un-bagged treatments were replicates (plot). All the ANOVA's were General Linear Models analyzed using SYSTAT version 7.01 with the significance level p=0.05. Percentage fruit losses were arc-sine transformed to improve normality, however there were minimal differences between the results of transformed and non-transformed data so only non-transformed data are presented here.

3.6 Results

There appeared to be high fruit loss from the bagged fruit at the start, and at the end of the season (Figure 3.2). Losses presumed to be due to fruit abortion as the ovules were fertilized and no ripe fruit had dropped off into the mesh bag. This peak in fruit loss/day at the start was also seen in un-bagged fruit (Figure 3.3) but there was no corresponding peak at the end of the season. Percentage fruit loss/day showed a similar pattern to fruit loss/day in the loss of bagged fruit (Figure 3.4). However, in contrast to the actual fruit loss/day, the % loss/day of un-bagged fruit (Figure 3.5) appears to increase towards the end of the fruiting season in a similar way to the bagged fruit. Comparing total % fruit loss between bagged and un-bagged fruit through the experiments duration showed that the total % loss in the un-bagged fruit varied from 75-100% and the rate of loss in the bagged fruit was much lower, 0-25%, although there was a slight increase in total fruit loss in the bagged fruit towards the end of the fruiting season (Figure 3.6).

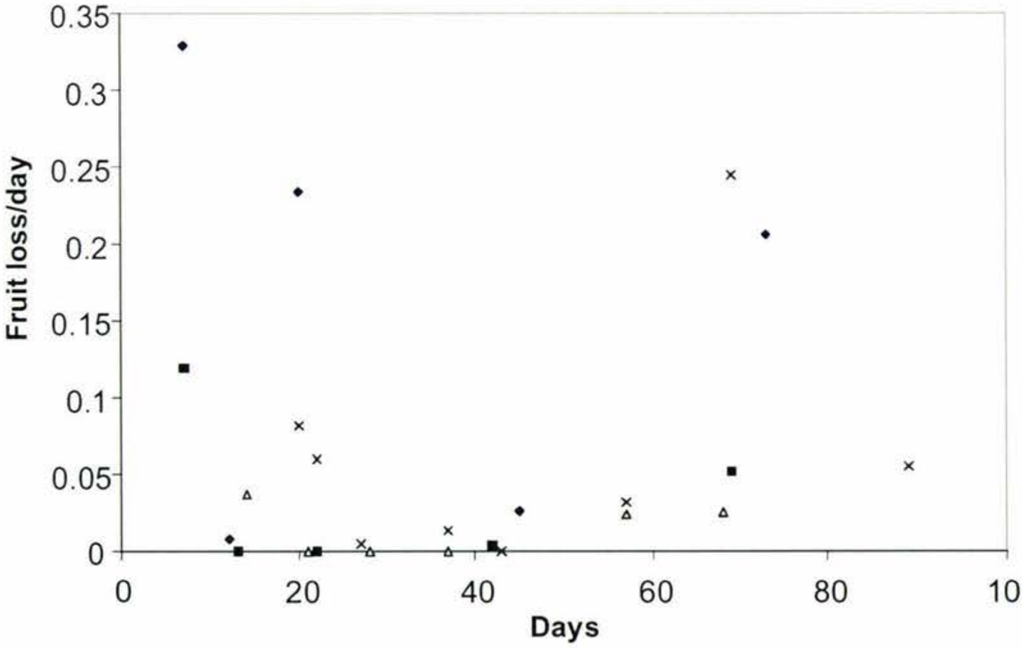


Figure 3.2 Fruit loss/day for bagged fruit at all sites. There was no treatment effect thus all the treatments were averaged and the replicates combined. Castlepoint = ▲, Castlecliff = ■, Himatangi = △ , Tangimoana = X.

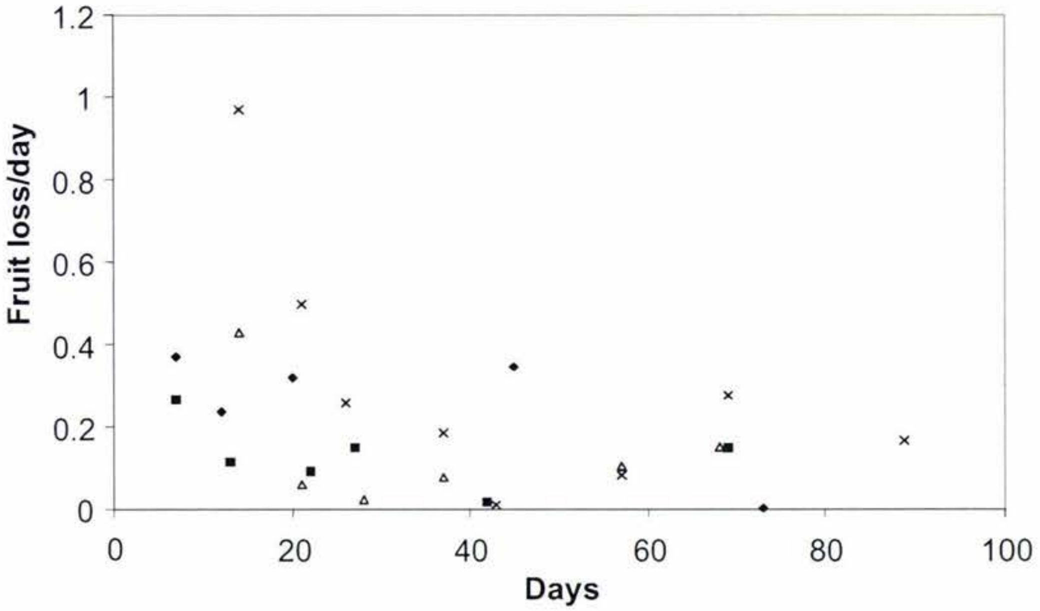


Figure 3.3 Fruit loss/day for un-bagged fruit at all sites. There was no treatment effect thus all the treatments were averaged and the replicates combined. Castlepoint = ▲, Castlecliff = ■, Himatangi = △ , Tangimoana = X.

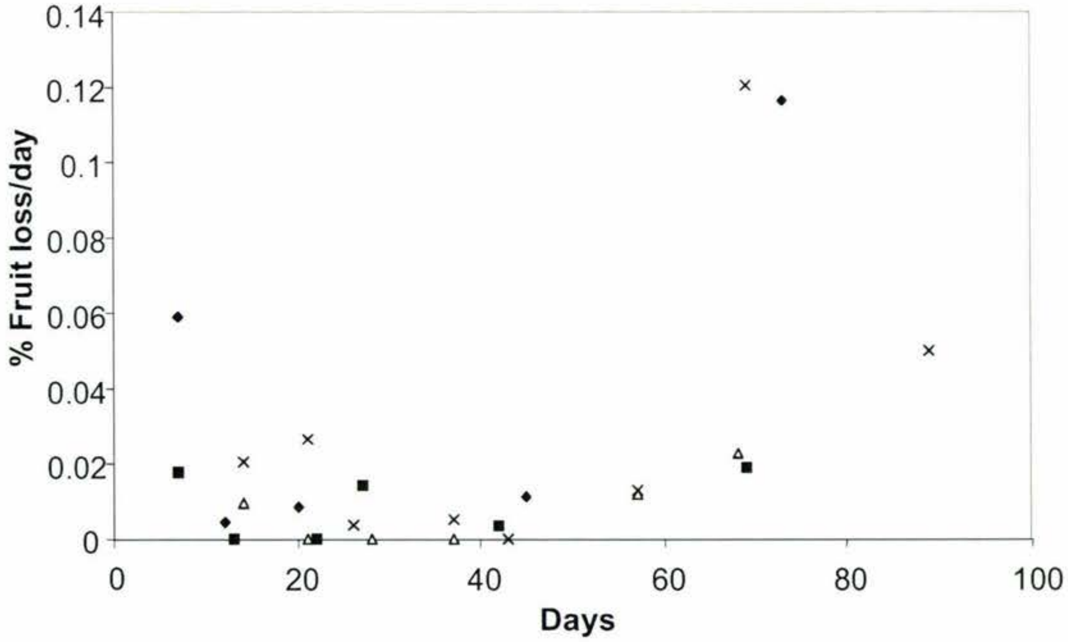


Figure 3.4 Percentage fruit loss/day for bagged fruit at all sites. There was no treatment effect thus all the treatments were averaged and the replicates combined. Castlepoint = ▲, Castlecliff = ■ ,Himatangi = △ , Tangimoana = X.

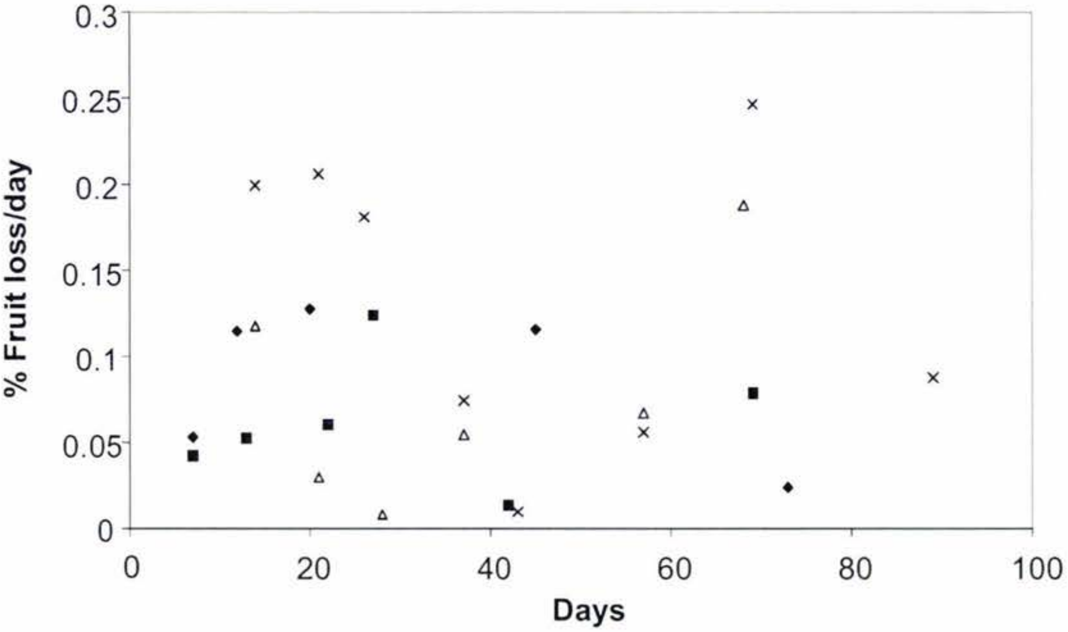


Figure 3.5 Percentage fruit loss/day for un-bagged fruit at all sites. There was no treatment effect thus all the treatments were averaged and the replicates combined. Castlepoint = ▲, Castlecliff = ■ ,Himatangi = △ , Tangimoana = X.

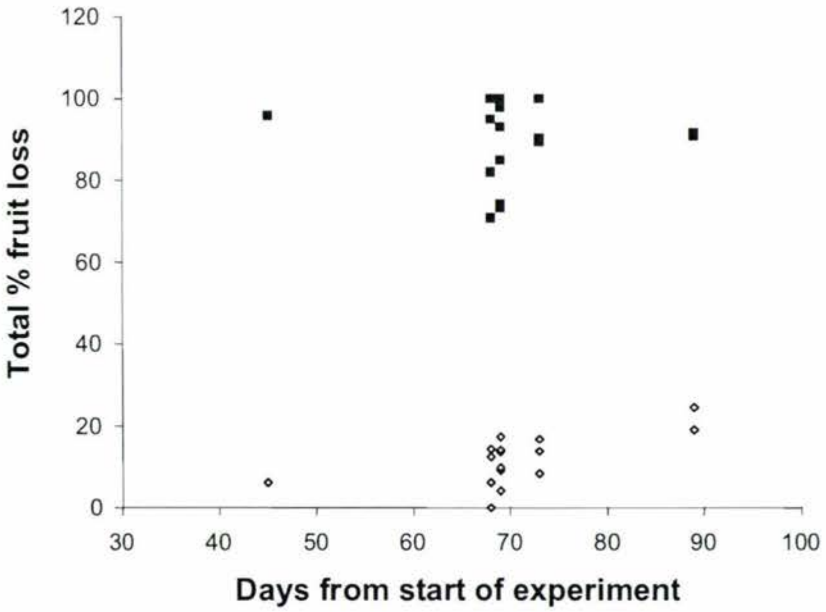


Figure 3.6 Total % fruit loss in each treatment at each site. Replicates are averaged for each treatment. Un-bagged fruit = ■, bagged fruit = ◇

There was no significant treatment effect in any measure of fruit loss. There were no significant effects on the rate of fruit loss by number of days in the experiment, and no temporal pattern was found between the treatments or between the sites. Overall the results showed that fruit was being taken from *P. arenaria* (Table 3.1).

Table 3.1 The effect of treatment on three measures of fruit loss at all sites combined. NS = no significant result

Factor	**Total % fruit loss**	**%fruit loss/day**	**Actual fruit loss/day**
Site	p=0.002	NS	p=0.001
Bagging	p<0.001	p<0.001	p<0.001
Site*bagging	NS	NS	p=0.031
Bagging*treatment	p=0.023	NS	NS
Block(trt*site)	p<0.001	NS	NS

Bagging the fruit had a strong significant effect in all measures of fruit loss ($p<0.001$). There was also a significant site effect observed - at Himatangi, total % fruit loss and actual fruit loss/day was higher than any of the other three sites. There was also a significant effect on the difference in fruit loss between the bagged and the un-bagged fruit in the bird treatment ($p=0.023$). There was also a significant effect from the treatment by site interaction when nested as blocks indicating that the plants acted independently of each other when total % fruit loss was measured.

3.7 Discussion

These results show that *P. arenaria* fruit is being taken, however it is unclear what removal agent or agents are responsible because there was no significant treatment effect and nothing is reported in the literature about the impacts of introduced species on *Pimelea* in coastal sand dunes. Mice and birds, the agents thought most likely in this study, both appear to remove *P. arenaria* fruit. Birds had a significant effect (p=0.023) on the difference between the bagged and un-bagged fruit loss when total % fruit loss was measured, however this was not noted in other measures of fruit loss. It should be noted however, that the lack of a statistical difference between the treatments could have been due to design faults in the exclosure frames. Fishing lines, with small flags attached, across the top of the frame were intended to prevent entry of birds. These lines may have been too far apart and, although no bird was ever seen inside these frames, it is possible that birds did have access to all frames, leading to fruit loss due to birds alone. Similarly, the small access holes cut in the base of some frames to give mice access to *P. arenaria* may also have allowed other small-bodied species to enter the exclosure.

It is likely that exotic and native bird species could remove fruit from *P. arenaria*. For instance Williams & Karl (1996) found that native birds fed almost exclusively on native plants, while introduced birds fed on native plants but also had a large proportion, sometimes100%, of introduced plant species in their diet (Burrows, 1994; Williams & Karl, 1996). Of the introduced birds only the blackbird (*Turdus merula*), song thrush (*Turdus philomelos*), starling (*Sturnus vulgaris*), silvereye (*Zosterops lateralis*) and chaffinch (*Fringilla coelebs*), are considered to be important frugivores by Williams & Karl (1996). Their importance is likely to differ between areas supporting different densities of birds because these species have varying amounts of fruit in their diets; chaffinches take "negligible" amounts of fruit (Williams & Karl, 1996) while silvereyes have the most varied diet which, in one Nelson study (Williams & Karl, 1996), included 29 native and 10 introduced plant species. It is important to note, however, that all the plant species considered so far in New Zealand have been forest species which may differ from dune plants in terms of fruit visibility, and fruit volume. The presence of other vertebrates and invertebrates leading to more competition, and environmental variables such as rainfall which can effect soil nutrients and moisture could also have flow on effects on fruit production (Willson *et al.*, 1989).

Mice, the other main potential predator considered in this study, are known to eat *P. arenaria* fruit and seed (Chapter 3) and to take several New Zealand fleshy fruited species (Beveridge, 1964; Daniel, 1973; Campbell *et al.*, 1984) as well as other dune plants (Miller & Webb, 2001; S Davidson, pers comm.) and generally act as predators rather than dispersers of fruit (Williams *et al.*, 2000). The lack of a treatment effect observed in the present study could relate to design faults discussed above. Another possibility is that both mice and birds have a similar impact on *P. arenaria* fruit and that their effects are indistinguishable and compensatory. There is a significant effect of birds on the difference in loss between bagged and un-bagged fruit ($p=0.023$) suggesting that birds have a slightly greater impact than mice, although the effects of both could be cumulative, leading to the observed recruitment failure (Chapter2).

One of the unresolved factors in this experiment is that the natural predators and dispersers of *P. arenaria* are not recorded. Possibly the rate of fruit loss seen here is influenced by other species leading to a cumulative increase in fruit loss that does not reflect the true effect of introduced species. The impacts of any natural agents are also likely to be effected by pressure from introduced species in the sand dunes via competition and predation. In particular any effects of the local lizards are unrecorded. Lizards are potentially important dispersers and predators of fruit (Olesen & Valido, 2003). They are known to eat several native New Zealand plant species (Whitaker, 1987; Lee *et al.*, 1994; Lord & Marshall, 2001; Wotton, 2002) including several species with ripe fruit of a similar colour to *P. arenaria* (Whitaker, 1987; Wotton, 2002). This suggests that lizards may also be predators or dispersers of *P. arenaria*, especially since *Pimelea prostrata* is recorded as being eaten by lizards (Whitaker, 1987). Lizards also have the potential to be important dispersers of *P. arenaria* because the seeds may be sufficiently small to be swallowed whole and remain undamaged (Whitaker, 1987). Additionally, consumed seeds are often defecated in areas that make suitable micro-sites for seedlings especially in arid environments such as sand dunes (Whitaker, 1987).

There were no clear changes in rate of fruit loss during the experiment. The high losses of bagged fruit at the start and, to some extent, the end of the fruiting season are probably due to the parent plant and not to predation. This is because mesh bags prevented removal of fruit by predators. Selective abortion of seed, a phenomenon common in plants (Niesenbaum, 1996; Niesenbaum, 1999; Kärkkäinen *et al.*, 1999) particularly in resource limited (Niesenbaum, 1999) and long-lived, outcrossing species (Kärkkäinen *et al.*, 1999) is a more probable explanation for the peaks in loss of bagged fruit. Selective abortion is related to resource availability, physiological activity within the seed, the genetic makeup of the seed, or a combination of these factors (Niesenbaum, 1996; Kärkkäinen *et al.*, 1999). Abortion of ovules can be due to lethal genetic combinations particularly as a result of increased self pollination (Kärkkäinen *et al.*, 1999) something which is likely to be quite high in these populations of *P. arenaria* given the high proportion of hermaphrodite plants. The quality of pollen produced by a plant can change between fruiting seasons (Copland & Whelan, 1989; Dudash & Fenster, 1997; Ramsey & Vaughton, 2000) and also through a 24 hour period (Tangmitcharoen & Owens, 1997). It is possible therefore, that pollen produced by *P. arenaria* at the start and end of the flowering season is of poorer quality than pollen produced at other times resulting in higher fruit abortion at the extremes of the fruiting season. Alternatively pollen quality may have been poor for several seasons prior to this study, or high quality pollen was being produced in only small amounts at a particular time of day. Both of these pollen factors could result in few high quality seed been set, the seedlings of which failed to establish giving the observed lack of seedlings at all the study sites. Studies in some plants have also found that an increase in the number of pollen grains and in the diversity of pollen on the stigma increases seed set (Niesenbaum, 1996; 1999). This suggests that low fruit set, or high abortion rates in *P. arenaria* at the start and end of the fruiting season could result from low numbers of pollen sources and/or low numbers of pollen vectors. Given the observations of some pollen vectors of *Pimelea* (Burrows, 1960; pers. obs.) it is likely that any pollen that a flower receives is from a neighboring flower on the same plant. This could potentially result in low fruit set, despite adequate pollination, with the pollen being self, or closely related, and lead to inbreeding depression affecting seed set (Ramsey & Vaughton, 2000) and eventually recruitment failure.

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Chapter 4

An experimental investigation into the palatability of *P. arenaria*.

4

4.1 Abstract

Pimelea arenaria is a declining native dune plant that has a regeneration problem; fruit are produced but seedlings appear not to be recruited into the population. One of the suggested reasons for the lack of seedlings is that the fruit are eaten and destroyed, or dispersed into areas where the seed are unable to germinate. This study looked at the consumption of *Pimelea arenaria* fruit and seed by adult mice (*Mus musculus* L.) and, if a seed was ingested, whether it was still able to germinate after passing through the digestive tract of the mouse. Eight male and eight female mice were divided into groups of four mice of the same sex and placed in a constant environment. The four groups of mice were then exposed for 18 hours to *P. arenaria* fruit with water *ad libitum* but with no other food sources present. The male mice destroyed 100% of the fruit, including fruit pulp and seed, and the female mice destroyed 92%. The results suggest that mice can act as seed predators of *P. arenaria*; however it is not known whether mice could aid the dispersal of *P. arenaria* by caching fruit and later failing to retrieve it. By acting as seed predators mice could affect the observed recruitment failure of *P. arenaria*.

4.2 Keywords

Mice, *Mus musculus*, *Pimelea arenaria*, seed predation, sand dune ecosystems, conservation.

4.3 Introduction

Of the introduced rodents in New Zealand, the house mouse (*Mus musculus*) is the least understood in terms of its impacts on Native flora and fauna despite mice being well established in New Zealand since 1830 (Miller & Webb, 2001). Of the work that has been done on mice in New Zealand, much of it has been carried out in forest areas. However mice also inhabit coastal sand dunes (Miller, 1999; Miller & Webb, 2001; pers. obs.) an area which has recently become an area of interest to the Department of Conservation (Stephenson, 1999; Hilton *et al.* 2000; Milne & Sawyer, 2002) with several species of animal and plant under (Milne & Sawyer, 2002), or potentially, under threat (Miller & Webb, 2001).

One native plant confined to the dunes is the sand daphne, *Pimelea arenaria* (A. Cunn.,Thymelaeceae)(Esler, 1978; Heads, 1994) which is typically found on the lee of the fore dune (Esler, 1978). It is distributed in three main areas: both coasts of the North Island from Auckland to Thames, the Wellington region and a small area around Dunedin (Heads, 1994). Currently the species is listed as nationally declining (de Lange *et al.*, 1999) which, as a category, is defined as "taxa that are numerically abundant but are either small, widely scattered populations or under threat from serious adverse factors....and that without some level of management.... are destined to become the future threatened taxa of New Zealand" (de Lange *et al.* 1999). The decline in *P.arenaria* is apparently due to a regeneration failure with fruit being produced but no seedlings present in the local environment (pers. obs.).

Milne & Sawyer (2002) describe *P. arenaria* as "a low growing shrub with spreading branches often half covered in sand, with compact, leafy branchlets, with leaves arranged in four rows at right angles to each other". The flowering season is from September to March, with the fruiting season from October to April (Milne & Sawyer, 2002). The fruit of *P. arenaria* in the lower North Island is fleshy, slightly flattened laterally, approximately 7 mm long and is a black/deep purple colour when ripe (Figure 4.1), although this varies from white to red of various shades (Allan, 1961; C. Ogle pers. comm.). The fruit is a drupe with a juicy pulp, similar to a grape, and a single hard seed (Figure 4.2).

The results from Chapters 2 & 3 suggest that the reason for the regeneration failure in *P. arenaria* is either predation of the fruit by some external agent, a failure of the seed to germinate, or a failure in seedling establishment. The recruitment failure is not due to the inability of *P. arenaria* to produce viable fruit.

Mice (*Mus musculus* L.) are potentially able to have a large negative impact on vegetation (Batzali & Pitelea, 1970) and have been noted overseas as being responsible for regeneration failure in many plant species (e.g.: Ryan *et al.*, 1989). Mice are known seed predators (Williams *et al.* 2000) and prey on a range of New Zealand native fleshy fruited species (Beveridge, 1964; Daniel, 1973; Campbell *et al.*, 1984). In one of a few papers on mice impacts in sand dunes, Miller & Webb (2001) found that much of the plant material eaten was unidentifiable, and the three plant species that were identified; Yorkshire Fog (*Holcus lanatus*), kneed foxtail (*Alopecurus geniculatus*), marram grass (*Ammophila arenaria*), are all introduced. However no browse damage was observed in *P. arenaria* populations in the field (pers. obs.) and fruit loss was observed (pers. obs.). In a study looking at dispersal of native and introduced plant seed by introduced rodents, including *Mus musculus*, Williams *et al.* (2000) found that mice acted as seed predators rather than dispersers. Mice have the potential, therefore to act as seed predators of dune plants like *P. arenaria* although none were tested by Williams *et al.* (2000).

A close relative of *P. arenaria*, *Pimelea prostrata* (Strathmore weed) is found in New Zealand and has been recorded as having killed cattle and horses (Connor, 1992). There is nothing in the literature to indicate *P. arenaria* is poisonous to animals however, because it belongs to the frequently poisonous Thymelaeceae, the Daphne family, *P. arenaria* is potentially poisonous (C. Ogle pers.comm.). Interestingly Colenso noted that Maori collected the berries of *P. arenaria* as a food source (Cheeseman, 1914). The berries were described as "white, fleshy berries" (Cheeseman, 1914) and so it is possible that the fruit were from another taxon of *Pimelea,* since all the fruits in the present study were deep red/purple in colour. As the *Pimelea* plants described by Cheeseman (1914) seem similar to those found in this study it is possible that mice do eat the fruit of *P. arenaria*. However the plasticity and similarity of many *Pimelea* species (Parlane, 1925; Burrows, 1958;

Burrows, 1960) suggests that several species of *Pimelea* may have been placed together by Cheeseman (1914) due to a lack of taxonomic resolution, thereby inadvertently combing poisonous and non poisonous species together.

This experiment investigates (i) whether laboratory mice (*Mus musculus*) will eat the fruit and/or seed of *P. arenaria* and (ii) if *P. arenaria* fruit is consumed, does it remain viable after passing through the digestive tract of the mouse and are there any adverse effects on the mouse from eating the fruit, and (iii) to examine seed cases in the wild to look for signs of seed predation.

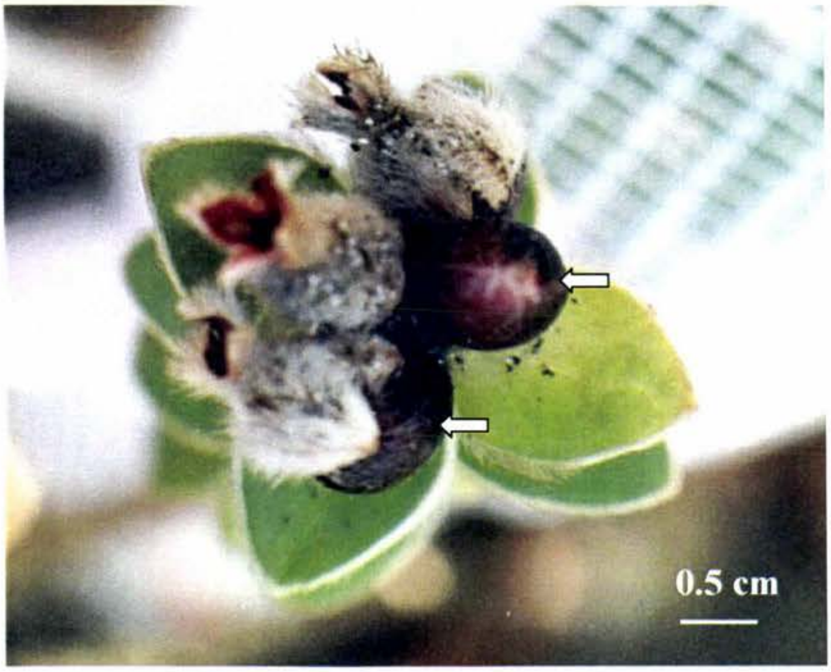

Figure 4.1. Ripening fruit (arrowed) of *P. arenaria*, Tangimoana, February 2003.

4.4 Method

Eight male and eight female laboratory mice were used, each sex was separated into two groups of four individuals. Males and females were tested on different nights with, in each case both groups left overnight in 0.5m x 0.3m plastic containers lined with paper towels and a metal wire lid at a constant 22°C and 54% relative humidity. Twelve *P. arenaria* fruit, collected in January 2003, were presented in a small bowl to each group of mice under these conditions with all the normal lab food pellets removed. Water was provided *ad libitum*. The fruit used for each group were gauged by eye to be as similar in size as possible. The mice were checked the following morning after between 16.5 - 17 hours and all the contents of the cages cleared and sorted into five categories; seed, intact fruit, fruit remains (skin and pulp), faeces, and unknown. The mice were then kept under observation for three days post-trial to observe any possible delayed adverse effects of the fruit or seed on the mcie. Faeces were collected during this time and assessed for seed remains. Any seed material found being germinated in the same manner as those in Chapter 2. Any uneaten seeds were germinated in the same manner as Chapter 2 by cleaning off the fruit pulp and placing the seed on moist paper in a sealed container at a constant 20°C.

The above procedures were approved by Massey University Animal Ethics Committee, MUAEC Protocol 01/88, signed 16 October 2001.

4.5 Results

The experiment confirmed that mice do eat *P. arenaria* fruit (Table 4.1). The male mice consumed all the fruit presented, the female mice eating all but 2 fruit which were left untouched. When the fruit was eaten no seed material was found, all seeds in the consumed fruits were destroyed by the mice with no seed material found in the cages or in the collected faeces of the mice. The two fruit not consumed (Table 4.1) did not appear damaged in any way; the skin was intact, and the seed contained within also assumed to be complete. Both seeds germinated successfully after the trial in the manner described in the methods.

The remains of the fruit consumed were scattered throughout the cages. Some fruit skin fragments were found; however all the pulp was eaten. The seed cases were broken into fragments of various sizes (Figure 4.4) and the seed had been removed. Some of the faeces collected from the trial were in a liquid or semi-liquid state, however after 3 days none of the mice tested exhibited any abnormal behaviour. Both sexes were observed eating, drinking and exhibiting other apparently normal behaviour.

Table 4.1. The number of mice with the number of *P. arenaria* fruit presented to each group and number of fruit and seed that remained after the experiment, together with the number of mice the died as a result of *P. arenaria* fruit consumption.

Sex	# of mice	# fruit presented	# fruit remaining	# seeds remaining	# mice dead
Male	8	24	0	0	0
Female	8	24	2	2	0
TOTAL	**16**	**48**	**2**	**2**	**0**

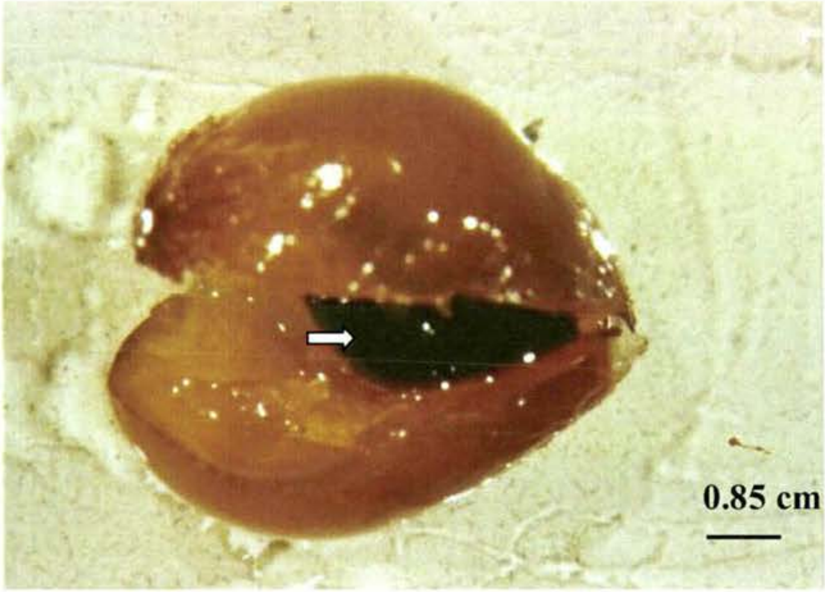

Figure 4.2. A ripe fruit of *P. arenaria* showing the fleshy pulp and single seed (arrowed). The fruit is slightly bleached from the alcohol preservative

The seed remains (Figure 4.4) left by the feeding mice had little resemblance to those found under *P. arenaria* plants at Tangimoana (Figure 4.5), other than the seed cases being of similar material and thickness. The fragments from this feeding trial were of various sizes and some showed definite teeth marks on the edges of the fragments and on the inside.

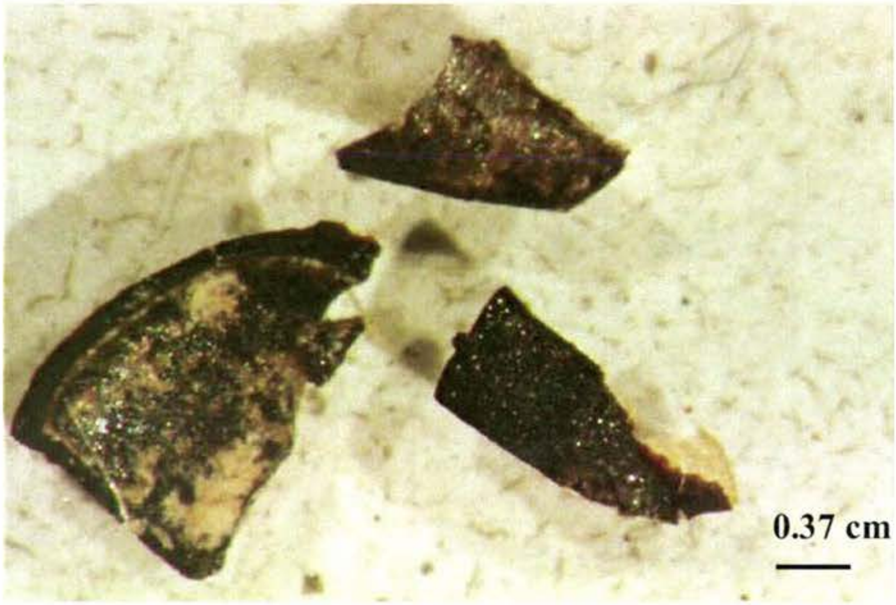

Figure 4.3. Seed case remains from the feeding trial. The yellow material seen in the fragments are the remains of the seed embryo.

4.6 Discussion

This study addressed the question of whether the fruit and seed of *P. arenaria* are eaten by mice, whether the seeds, if consumed, survive, and whether the mice survive after consumption of the fruit. While this trial used only 16 mice the results clearly showed that laboratory mice eat the fruit and seeds of *Pimelea arenaria*, destroying 96% of the fruit and seed presented, and suggests that feral mice could do the same. The clear result made further experimentation with a larger number of mice unnecessary. This result is comparable to that of Williams *et al.* (2000) who found that feral mice acted as predators eating, and by doing so destroying, all the seeds they ate of fleshy fruited native species of a similar size and in some cases colour, to

P. arenaria. In contrast to the present experiment where mice consumed and so destroyed 92 -100% of the fruit and seed presented, Williams et al. (2000) found that feral mice ate only 66% of fruit and 50% of seeds of the various species presented, ignoring many species. Although the mice in the study by Williams *et al.* (2000) had a choice of several species of fruit, in the present study only *P. arenaria* was presented. While the results from this study give no indication of whether *P. arenaria* is a preferred food; they do show that hungry mice will eat *P. arenaria*.

Figure 4.4. Seed cases found under *P. arenaria* plants at Tangimoana, January, 2003.

P. arenaria, at least in the quantities consumed here (0.01497 g fruit/g male mouse and 0.016 g fruit/g female mouse, assuming each mouse ate 3 fruit) appears non-poisonous despite *P. arenaria* being a member of Thymeleaceae, although the semi-liquid faeces suggest that the fruit may have a laxative effect. None of the mice tested died or exhibited abnormal behaviour during the three days following the trial.

Figure 4.5. Seed cases obtained from seeds germinated in the laboratory, October, 2003.

This suggests that the fruit of *P. arenaria* contains none or low levels of the toxic compounds found in other *Pimelea* species, such as those responsible for skin irritations (Adolf & Heckler, 1982; Zayed *et al.*, 1982; Hafez *et al.*, 1983). However, no conclusions can be drawn from these results with regard to the longer term effects of low doses or the effects of higher dose rates on mice or any other predator of *P. arenaria* fruit and seed. It should, however, be noted that many of the effects, such as the tumour promoting activity of some Thymelaeaceae species, including *Pimelea* (Zayed *et al.*, 1982; Hafez *et al.*, 1983; Adolf *et al.*, 1988), are only recorded for aerial portions of the plant (Freeman *et al.*, 1979; Hafez *et al.*, 1983; Pettit *et al.*, 1983)

While the difference between the sexes in this trial was negligible, it is possible that the rate mice consume *P. arenaria* fruit in the wild varies with the stage of the fruiting season and the reproductive status of the mouse. Nothing is recorded about the behaviour of mice when feeding on *P. arenaria* fruit either in captivity or in the wild where fruit are exposed at all stages of development up to and including ripeness, although there are no data on whether the fruit at an earlier development stage are edible. No feral mice were tested in this study, but it is assumed that they would probably have the same effect on *P. arenaria* seed as laboratory reared mice. However two aspects of behaviour are potentially different between lab mice and feral mice. For instance feral mice could cache the fruit then later fail to relocate them which, in a suitable substrate, could result in seed germination. No caching was observed in this study, however caching of other New Zealand native fleshy fruits has been observed with *Mus musculus* (Williams *et al.* 2000). In addition, the foraging methods of feral mice on *P. arenaria* are unknown and, given the openness of the landscape, the mice may consume fruit some distance from the plant. Both of these factors could result in fruit dispersed across the landscape with the potential to germinate and produce seedlings that could then be recruited into the *P. arenaria* population. No response to changing density of fruit could be tested because of the national conservation status of *P. arenaria* that allowed for only a small numbers of fruit to be collected from each of the study areas. However work on Pingao (*Desmoschoenus spiralis*), another dune plant, suggests that when more seed is available the more mice eat it (S. Davidson, pers.comm.). The diet of feral mice in coastal dunes does vary with season (Miller & Webb, 2001) and with the reproductive status (Miller & Webb, 2001) and so the prevalence of *P. arenaria* fruit in the diet of mice could vary especially with the availability of *P. arenaria* fruit compared to other foods from year to year.

The conditions required by *P. arenaria* seeds to germinate in the sand dune environment are unknown. The seeds from this study were germinated on filter paper in a constant temperature room and it is possible that even if the fruits are dispersed by feral mice, there may be no net benefits to the plant if seeds are buried too deep, buried in unsuitable substrate, or otherwise rendered unviable by mice.

The fragments from the feeding trials (Figure 4.4) are very different to the seed cases found under *P. arenaria* plants in the field (Figure 4.5), and the seed cases from the laboratory germinated seed (Figure 4.6). When the seed cases from the field (Figure 4.5) are compared to those from the lab germinated seed (Figure 4.6) they appear very similar. In both cases the seed case has split length-ways into two equal halves, although the sides of the seed cases from the field have coalesced. This is most likely due to the desiccating environment although there is no certainty as to how long the cases had been under the *P. arenaria* plant. It would therefore be difficult to assess from the samples taken from the field whether mice had been responsible for the presence of those seed cases under the *P. arenaria* plants or whether they are due to the fruit falling off the plant and the seed subsequently germinating but the seedling failing to establish. Using seed cases from the wild as an index of mouse activity is impractical until further data are gathered on whether seed cases are common in the wild, on the foraging habits of mice on *P. arenaria*, and whether there are any other agents that may prey on *P. arenaria* seed.

Recommendations for future research

This study suggests that *P. arenaria* is probably not toxic to laboratory mice, at least in low dosages but direct observation of mice eating *Pimelea* fruit could clarify their behaviour towards fruit. If the fruit of *P. arenaria* does have a laxative effect, this could modify mice behaviour and alter their relative preference for *P. arenaria* and other plants such as Pingao. If the behaviour of mice does change towards *P. arenaria* in the presence of other fruit types, it would be useful to identify whether other dune plant species are more at risk of predation (spatially or temporally) because they have similar fruiting times to, or are associated with, *P. arenaria*. It would also help to know whether the behaviour of mice towards *P. arenaria* changes in the presence or absence of other potential fruit predators such as birds.

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Chapter 5

General Discussion

"…..when you have eliminated the impossible , whatever remains, *however improbable*, must be the truth."

Sherlock Holmes, in *The Sign of Four* (1888)

5

This thesis looked at *Pimelea arenaria*, a native plant restricted to sand dunes (Esler, 1978; Heads, 1994). It is found in three main areas in New Zealand: both coasts of the North Island from Auckland to Thames, the Wellington region, and a small area around Dunedin (Heads, 1994). It is currently listed as nationally declining (de Lange *et al.*, 1999). *P. arenaria* is a plant with dark brown/ black bark and small close set, alternate leaves (Allan, 1961). The fruit of *P. arenaria* are drupes that range from white to red when ripe (Allan, 1961), although in this study all the fruit seen was dark purple/black in colour when ripe (pers. obs).

The aims of this thesis were (1) to establish whether recruitment failure was occurring by examining population structure, (2) if such a failure was detected whether it was because *P. arenaria* did not produce viable fruit, (3) whether viable fruit was being produced but subsequently removed by an external agent, and (4) whether the fruit or seed of *P. arenaria* was poisonous if consumed. Four populations were studied in the lower North Island of New Zealand, at Castlecliff, Tangimoana, and Himatangi (all west coast), and at Castlepoint (east coast).

Recruitment failure may be occurring in wild populations of *P. arenaria*, which would explain the young individuals it appears are not being recruited (Chapter 2). Measurements of all the individuals in the population at each site showed a gap in the smallest height category, where one would expect to find a number of seedlings, assuming equal duration times in each size category, at least the number of plants in the next size category. Fifty-meter transects with 1m radius sampling points every 5m through existing patches of *P. arenaria* also failing to find any seedlings. So a lack of seedlings apparent in the dunes, suggestive of a recruitment failure.

Possible causes for this recruitment failure are in two areas: intrinsic or extrinsic factors. The investigation into the possible intrinsic factors contributing to this recruitment failure focussed on the pollination of *P. arenaria*, as well as in the production and germination of seed. Within the four populations the sex ratio is variable ranging from 14% to 100% hermaphrodite plants (Table 2.2). This differs from ratios reported for other *Pimelea* species that are close to 1:1 (Burrows, 1958, 1960). One cause of recruitment failure is lack of seed set. This may be due to pollination failure. If there is any nectar present, it would indicate possible faunal pollination. The standing nectar crop in hermaphrodite flowers was c.57 µg at Castlepoint and c.24 µg at Castlecliff. Compared with other New Zealand plants, these results suggest that the main pollination vectors of *P. arenaria* are insects, and casual observation suggests that flies are the main pollinators. An estimate of floral density was obtained (423 flowers/m^2) however no measures were made of the proportion of flowers that successfully produced fruit and so the total number of fruit produced by an individual through the fruiting season can not be estimated.

The pollination experiments showed that both female and hermaphrodite plants in all four sites are able to produce fruit containing seed that is viable in the laboratory. However, less than 50% of the seeds produced by female and hermaphrodite plants germinated. Female seed had higher germination success than hermaphrodite seed, except for autonomously selfed seed, which is produced only by hermaphrodite plants. For conservation purposes, therefore, general habitat, including germination micro-sites, may need to be provided or constructed for *P. arenaria*, as has been found for other dune plants (Singer, 1997).

Attention to possible extrinsic factors centred on the effects of feral house mice and various bird species on fruit loss in *P. arenaria*. These species were thought to be the most likely predators of *P. arenaria* fruit, given its similarity to the fruit of other plant species eaten by mice and birds, both in New Zealand (Beveridge, 1964; Williams & Karl, 1996; Williams *et al.*, 2000; Miller & Webb,

2001) and overseas (Turcek, 1963; Willson *et al.*, 1989; Willson & Whelan, 1990). The graded exclosure experiment (Chapter 3) to test whether fruit was being removed by either birds and/or feral mice showed that fruit was being taken from *P. arenaria*, and that this loss was significantly reduced from approximately 90% to 15%, by bagging the fruit ($p<0.001$) (Figure 3.6). There was no difference in fruit loss between exclosures testing for the effects of birds and mice, suggesting that mice and birds might have similar impacts on fruit loss, impacts which may also being cumulative with the effects of mice and birds combining together. The bird treatment did, however, have a significant effect on the difference in total percentage fruit loss between the bagged and the un-bagged fruit ($p=0.023$) suggesting that of the two, birds remove more fruit than mice do. When fruit was protected from potential predators there were peaks of fruit loss at the start and end of the fruiting season. Selective abortion of seed is common in resource-limited and long-lived, out-crossing plants (Niesenbaum, 1996, 1999; Kärkkäinen *et al.*, 1999), and is a more probable explanation for the peaks in loss of bagged fruit. Selective abortion is related to resource availability, physiological activity within the seed, the genetic makeup of the seed, or a combination of these factors (Niesenbaum, 1996; Kärkkäinen *et al.*, 1999).

Fruit loss also differed between sites. For instance at Himatangi there was more total loss ($p= 0.002$), and rate of fruit loss/day ($p=0.001$) than any of the other three sites, suggesting that either fruit predators are more active at Himatangi, or that there were greater levels of ovule abortion. There were, however, some design faults with the exclosures so that birds gaining access to exclosures of some treatments may have increased fruit loss.

Measures of fruit loss at the study sites may, therefore, be overestimated.

In this study the potential role of lizards in fruit loss was unmeasured. Lizards are known to eat the fruit of several native New Zealand plants (Whitaker, 1987; Lee *et al.*, 1994; Lord & Marshall, 2001; Wotton, 2002), including several species with ripe fruit of a colour similar to that of *P. arenaria* fruit (Whitaker, 1987; Wotton, 2002). The seeds of *P. arenaria* are small enough to be swallowed whole by lizards with little damage to the seed (Whitaker, 1987). Seed eaten by lizards are often defecated in areas that provide suitable micro-sites for seedlings, especially in arid environments such as sand dunes (Whitaker, 1987). A recruitment failure in *P. arenaria* being possibly related to loss of abundance of lizards at the study sites. Lizards which possibly act as dispersers of *P. arenaria*.

Despite these results, there was no direct field evidence that either mice or birds ate the fruit or seed of *P. arenaria*. Members of the Thymelaeceae contain several toxins which in animals can cause skin irritation or which have tumour promoting activity (Adolf & Heckler, 1982; Zayed *et al.*, 1982; Hafez *et al.*, 1983; Adolf *et al.*, 1988). So it is possible that *P. arenaria* is also poisonous. *P. prostrata* is poisonous and sufficiently toxic to kill cattle (Connor, 1992). Birds were not tested due to the restricted number of seed that could be collected and the large number of potential bird species. In a feeding trial (Chapter 4) 16 mice were separated into a male group, which ate 100 % of the fruit presented to them, and a female group, which ate 92 % of the fruit presented to them. Both groups destroyed all the seed eaten. None of the mice died or showed abnormal behaviour during the three days of observation after the trial, suggesting that either *P. arenaria* fruit, at this dosage, is not poisonous to mice, or the fruit do not contain the toxins associated with other species of Thymelaeceae. No conclusions can be drawn as to any adverse effects at higher dose rates of fruit, or the effects of consuming other parts of *P. arenaria* plants.

5.2 Areas for future research

This study has identified a recruitment failure in *Pimelea arenaria* that is probably due to a combination of intrinsic, such as ovule abortion rates and low germination rate of seed, and extrinsic factors, such as fruit predation or seedling herbivory. There are, however, many unresolved areas for possible future investigation.

Nothing is known about the longevity of *P. arenaria* but in this study height was used as a correlate of age due to the impossibility of aging this species by growth rings (Burrows, 1958). However height-age correlations in plants are problematic. In general a very large *P. arenaria* plant is likely to be older than a very small plant, but a new approach to aging multi-trunked, partly buried plants would assist ecological research in sand dunes. While an estimate was obtained for the density of *P. arenaria* flowers, nothing is known about how many of these lead to viable seed, or how much this varies between individuals and between seasons. Also unknown are: the age at which an individual plant is able to produce flowers and seed, and the total amount of seed a plant might contribute to a population over its lifetime. Furthermore, it is unknown how typical this season was of the long term average of *P. arenaria* fruit production and the dynamics of fruit predators.

The sex ratios of *Pimelea* species change through the fruiting season though no reason is obvious (Burrows, 1958, 1960); however, only one count was made for *P. arenaria* in the present study, and so it is unknown whether the sex ratio for this species also changes. Similarly the ratios of flower types on an individual plant could change through, and between, seasons.

The genetic structure of any *P. arenaria* population is currently undescribed. As a result nothing is known about the levels of inbreeding within these populations, or the effect this might have on fruit and seed production. Niesenbaum (1999) found that both pollen load size and diversity affect the production of fruit by a plant, fruit production being greatest with large numbers of pollen grains, and with multiple donors. Kärkkäinen *et al.* (1999) suggest that increased self-pollination can also result in greater abortion of ovules as a result of lethal genetic combinations. It is possible, therefore, that in populations containing closely related individuals, with a large proportion of these being hermaphrodite and capable of self-pollination, that fewer fruit are produced because of a lack of pollen diversity and the genetic consequences of inbreeding. Burrows (1960) noted that in some species of *Pimelea*, hermaphrodite plants have a lower fruit set than females, possibly related to incompatibility, and this further reduces the number of establishing seedlings. The conditions required for germination and seedling establishment of *P. arenaria* in the field are unknown and it is possible that artificial micro-sites are needed for *P. arenaria* seedlings to establish. Potentially there are differences in the competitive abilities of *P. arenaria* seed from female and hermaphrodite plants. However, studies of this would be complicated by the selective abortion of ovules occurring in *P. arenaria* because, as noted by Niesenbaum (1999); the fitness differences between seeds are likely to be low with all the seeds of lower quality being aborted before maturation.

Whether seed banks of *P. arenaria* occur in the sand dunes is also unknown. The presence of seed bank might indicate that the recruitment failure currently observed may not in the long run be detrimental to the continued persistence of the *P. arenaria* populations. The temperature range that a *P. arenaria* seed can germinate in is not recorded, other than germination can occur at 20 °C.

Little is known about the interactions between *P. arenaria* and the local fauna. The natural predators and dispersers, especially the local lizard fauna, as mention above, are unrecorded. While some insect browse damage was observed on *P. arenaria* leaves in this study, it was not investigated. There are several species of moth, *Ericodesma areodana*, *Meterana pictula* (rare, possibly extinct in the North Island; B. Patrick, pers.comm.), and *Notoreas* spp., all of whose larvae are known to feed on *P. arenaria* (B. Patrick, pers.comm). The effects of these animals on individual plants and on fruit set are unknown.

Rodent eradication and control programmes have been successful in other environments of New Zealand (Newman, 1994; Empson & Miskelly, 1999; Atkinson & Towns, 2000; Innes, 2000), but such programs have yet to be implemented in coastal sand dunes. The impacts on the sand dune ecosystem of introducing rodent control measures are at present unquantified, although there are potential benefits to several native animal and plant species. Firstly predation pressure on fruit and seed is likely to be reduced, not only for *P. arenaria* but also for other plants species (e.g. Pingao and sand tussock) eaten by mice (Miller, 1999; Miller & Webb, 2001). Other benefits would flow on to other native animals in sand dunes, particularly the invertebrate community, which are under predation pressure by mice (Miller, 1999; Miller & Webb, 2000). The removal of this control mechanism on the invertebrate community might also be further reflected in the plant community by increasing the number and diversity of pollinators, predators and dispersers of *P. arenaria* fruit.

This thesis investigated the native sand daphne *Pimelea arenaria* and found that, as suggested by anecdotal evidence, recruitment failure was occurring with few or no seedlings present in the environment. Further work into possible causes for this failure suggested that a problem might be occurring in the germination of seed or in establishment of seedlings. Additionally not all flowers produced fruit and seed and agents, which are not as yet identified in the field, removed much of the fruit that was produced. A laboratory feeding trial established that mice eat and destroy *P. arenaria* fruit and seed. However the coastal sand dune ecosystem is poorly understood and this research, while answering some questions, posed many more giving direction to other avenues of future research.

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