POLLINATION AND DISPERSAL

OF THE NOXIOUS VINE *PASSIFLORA MOLLISSIMA*
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ABSTRACT

Banana passionfruit (*Passiflora mollissima*) is a noxious weedy vine that is problematic in coastal regions throughout New Zealand. I investigated the pollination and dispersal mutualisms that facilitate its spread, in the Marlborough Sounds. Flower observations revealed that visits were almost exclusively from introduced honey bees and bumble bees, indicating an invasive mutualism. I investigated the pollination system of banana passionfruit by comparing fruit set, fruit size, seed set, germination success and the effects of inbreeding depression on seedlings when grown in competition, between hand-selfed, hand-crossed, bagged and open flowers. Fruit set was significantly reduced when pollinators were excluded (3.0%) indicating reliance on pollinators for reproduction. While banana passionfruit is partially self-compatible, fruit set was significantly reduced in selfed flowers (17.5%) compared to crossed flowers (29.5%), and we found significant pollen limitation (natural [18.0%] was significantly lower than crossed). A significant degree of inbreeding depression was apparent in germination success but not in fruit size, seed set or seedling growth and survival. Surprisingly, open flowers had significantly lower germination success (10.1%) than the other three treatments (crossed = 16.5%, selfed = 15.0%, bagged = 25.3%). Overall, natural flowers produce more seedlings per flower (1.7) than bagged flowers (0.9), but less than hand-selfed (3.0) and crossed (5.3) flowers. To investigate dependence on dispersal, germination success was compared between hand-cleaned seeds, fleshy seeds and intact fruits in the field and glasshouse. Seeds germinated readily in all treatments, with no significant difference between cleaned and fleshy seeds, so seeds do not require frugivore handling to germinate. In the field, tagged fruits were removed quickly after ripening. Significantly fewer fruit were wholly removed from off-road locations than locations on road edges, but removal rate was high in both cases (93% and 70%) indicating dispersal by both humans and wild animals. I found no evidence of dispersal by birds. Infra-red camera evidence revealed possums and rats consuming fruits in the field. In addition, 2818 seeds were extracted from 1.5 kg of pig excrement collected from Te Weu Weu Bay in February 2006. Seeds readily germinated following extraction from pig and possum droppings with a final germination success, in the glasshouse, of 45.6% and 53% respectively. None of the few intact seeds excreted by rats germinated. Therefore, together with direct weed control, limiting the spread of banana passionfruit through the control of pigs and possums may improve management success and reduce the spread of banana passionfruit.

Chapter 1: GENERAL INTRODUCTION

1.1 BIOLOGICAL INVASION, MUTUALISMS AND LIANAS

1.1.1 Introduction to biological invasion

Humans have been introducing plant species to areas outside of their natural ranges for millennia (Henderson *et al*, 2006). The introduction of many of these species has led to the naturalisation of alien species in native ecosystems, sometimes becoming invasive. This has resulted in often detrimental effects on indigenous species and ecosystems. Invasion Ecology (largely initiated by C. S. Elton in 1958) is the study of the mechanisms and processes by which alien species become invasive (Henderson *et al*, 2006). The terminology used in invasion biology has been inconsistent which has caused some confusion, thus it is important to distinguish between several steps of the process, because not all introduced species become invasive. A *non-native*, *alien*, *adventive*, *introduced* or *exotic* species refers to any plant species that occurs in an area outside of its natural range, directly or indirectly through transport by humans (Henderson *et al*, 2006). If the plant is able to survive at the point of introduction, for example in someone's garden, it becomes *established* (Henderson *et al*, 2006). A species then becomes *naturalized* when it can effectively reproduce and thus is self-sustaining (Henderson *et al*, 2006). Species that become *invasive* are those that expand from the site of original introduction into intact or semi-intact vegetation and adversely affect the species in that ecosystem (Alpert *et al*, 2000). Invasive species are considered to be a serious threat to conservation and cause major modifications to indigenous biodiversity and ecosystem function. Isolated areas such as New Zealand that have evolved a high degree of endemism are particularly susceptible to invasive species (Williams & West, 2000), although undisturbed native vegetation can be quite resistant to invasion (Jesson *et al*, 2000).

Typically, multiple introductions of an exotic species are followed by a time lag before it becomes invasive. Thus, the probability a species will become naturalised increases with residence time, and with the number of individuals introduced and number of introduction attempts (Henderson *et al*, 2006). The mechanism by which species become invasive is not obvious. One of the leading hypotheses for why some exotic species become superabundant in their new ranges is the enemy release hypothesis which proposes that lack of coevolved enemies in the new range allow the exotic species to flourish (Parker *et al*, 2006). However, resistance by evolutionarily novel native enemies may limit the establishment or spread of most species so only some will become invasive; the biotic resistance hypothesis (Parker *et al*, 2006). Some species may evolve greater invasiveness in the period following introduction, for example by relocating resources from defence to growth following release from predation, which would account for the time lag (Alpert *et al*, 2000). Alternatively, some species may possess a 'general-purpose genotype' that allows them to proliferate when founded by small numbers of individuals through reproductive success systems, such as autogamy (selfing), that provide genetic assurance (Richardson & Pysek, 2006).

There is evidence for both, suggesting that some invaders are released from fitness constraints while others evolve invasiveness after colonisation (Richardson & Pysek, 2006). Generally, approximately 10% of imported species establish, 10% of those go on to naturalise, and 10% of naturalised species become invasive; a phenomenon known as the "Tens Rule" (Williamson & Fitter, 1996). In New Zealand, there are approximately 20,551 exotic plants. Of these, 2071 have naturalized (10% of existing exotic species) and 240 (11.6% of naturalized exotics) are recognized and managed as environmental weeds by the Department of Conservation (Williams & West, 2000). However, as with all trends in invasion ecology, this rule has variable applicability (Richardson & Pysek, 2006; Henderson *et al*, 2006). In addition, it may be an artefact of the time lag between introduction and invasiveness because many exotic species are likely to have similar residence times (time since introduction) and the heightened awareness of invasive species is fairly recent. Thus, the probability of a naturalised species becoming invasive increases with residence time, and many more exotic species are expected to become invasive, even in the absence of further introductions (Richardson & Pysek, 2006). In spite of this, the introduction of species that turn invasive is becoming increasingly common (Henderson *et al*, 2006). This is partially a result of a larger global population, increased wealth, and globalization (particularly of trade, which is associated with efficient global transport) accelerating anthropogenic dispersal. More importantly, environmental conditions are becoming increasingly conducive to non-native plant establishment and spread because human activities increasingly fragment landscapes, disturb remaining natural areas and modify atmospheric composition, resulting in further environmental stresses including changed precipitation and temperature regimes and modifications to the magnitude and frequency of extreme events (Henderson *et al*, 2006).

The effects of invasion, discussed at length in a review by Henderson *et al* (2006), are extremely far-reaching and occur on multiple scales. For instance, the economic impacts of biological invasions are estimated to cost the United States $138 billion each year and about 42% (but up to 80% in some areas) of indigenous species that require protection from the Endangered Species Act are declining predominantly as a result of invasive species (Henderson *et al*, 2006). At the species level, the most frequently reported impact of invasive species is loss of species-richness. This may be through the direct extinction of vulnerable native species, perhaps due to competition for resources or mutualists, or through loss of genetic diversity due to hybridization between native and introduced species (Henderson *et al*, 2006). For example, several studies have shown that introduced honeybees compete for resources with native insect and bird pollinators, decreasing their foraging success (Kearns & Inouye, 1997). In addition, the flowers of an exotic plant species may attract pollinator attention, resulting in significantly less visitation for native flowers, causing reduced fecundity (Henderson *et al*, 2006). Invasive plants also effect whole plant communities and are ultimately homogenizing global biota by causing a transition from unique community types to those dominated by a subset of highly vigorous generalists (Henderson *et al*, 2006).

Furthermore, invasive species may cause fundamental changes in resource availability and disturbance regimes that not only result in the extinction of some species, but cross the resilience threshold of some ecosystems causing unpredictable results and making restoration practically impossible. Even when invasive species are removed from a site they often leave a legacy through buried seed banks and permanent chemical or physical modifications (D'Antonio & Meyerson, 2002). However, humans rely on non-native plants in agriculture, forestry and medicine and many non-native invasions do not result in native decline and ecosystem disruption. Some exotics may even become useful successional species in ecological restoration (Henderson *et al*, 2006).

1.1.2 Invasiveness and Invasibility

In short, there are many ways in which invasive species impact ecosystems, though all appear to be site and species specific. Substantial research has been conducted in an attempt to find patterns from which generalisations and predictions can be made regarding the invasiveness (i.e. which species are likely to become invasive) and invasibility (i.e. which ecosystems are at risk of being invaded) of species and ecosystems (Alpert *et al*, 2000). This has proven extremely difficult because invasiveness depends not only on the invaders traits *per se*, but on the interaction of those traits with the recipient environment (Henderson *et al*, 2006). Thus, no rules hold true in all circumstances and invaders are likely to interact with the environment in unpredictable ways.

However, a number of factors are likely to contribute to the probability that an established species will become invasive (Henderson *et al*, 2006). Broad native range and rapid dispersal seem to best explain variation among species in invasiveness (Alpert *et al*, 2000). For example, a species with rapid growth and dispersal may have traits such as short generation time, long fruiting period, large viable propagule production, small seed size, prolonged seed dormancy, high rate of biomass production, efficient long distance dispersal (particularly by vertebrates), successful colonizer of disturbed or bare ground, short development time to reproductive maturity, and dependence on non-specific root symbionts, pollinators and seed dispersers (Alpert *et al*, 2000; Rejmanek, 2000; Williams & West 2000). Phenotypic plasticity is also associated with invasiveness probably because it allows the plant to survive in a wide range of environments and may allow the invader to more effectively respond to disturbance, grazing, and a low availability of water, light and nutrients (Alpert *et al*, 2000).

Traits that make particular habitats more susceptible to invasive species are slightly easier to identify. Research shows that riparian habitats and islands are high risk environments, although the latter might be the result of more alien species being transported and introduced to islands rather than because they are intrinsically more invasible (Alpert *et al*, 2000; Allen *et al*, 2006). For example, New Zealand is one of the most invaded countries in the world with 94% of terrestrial mammals, 40% of vascular plants and 31% of

terrestrial bird species being naturalised alien species (Allen *et al*, 2006). Evolutionary history, community structure, propagule pressure, disturbance and stress explain some of the differences between the invisibility of ecosystems, but they are difficult to separate as they are all closely linked and interact with one another (Alpert *et al*, 2000). At the community level, empirical studies have shown both positive and negative relationships between species diversity of resident plant ecosystems and their invisibility. However, functional diversity and trophic complexity may have a greater effect than species richness *per se* (Prieur-Richard & Lavorel, 2000). The effect of evolutionary history on the invasibility of an ecosystem with relation to resource stress is important. For example, species that have evolved in highly competitive environments are likely to be well adapted to competition and thus, unlikely to be displaced by exotic species. However, if resources are abundant, invasive species may dominate because of their faster growth rates (Alpert *et al*, 2000).

Disturbance is not required for invasion, however it often facilitates colonization by invasive species because it simultaneously increases resource availability and decreases competition, allowing invasive species to utilise their greater competitive ability (Prieur-Richard & Lavorel, 2000). It also exposes soil to higher light levels, greater temperature fluctuations and results in higher nutrient availability which can directly assist the germination of weed seeds from large, persistent soil banks (D'Antonio & Meyerson, 2002). Indeed in New Zealand, alien vines such as *Clematis vitalba*, *Lonicera japonica*, and *Passiflora mollissima*, typically invade disturbed areas such as small forest remnants, forest margins, canopy gaps and landslide scars (Wiser & Allen, 2006). Invasive plants that benefit from disturbance may develop a positive feedback loop in which disturbance permits invasion of a plant species which then causes more disturbance, facilitating further invasion (Henderson *et al*, 2006). For example, vines often connect the crowns of trees in a forest, thus when one tree falls others are damaged or dragged down by the vine, creating a larger gap which then facilitates further invasion by vines (Schnitzer & Bongers, 2002). However, it is probably deviance from the natural disturbance regime as opposed to disturbance *per se* that increases invasibility, as some native species specifically rely on disturbance for regeneration (Alpert *et al*, 2000). Combining the research on stress and disturbance, habitats should be invasible when stress is very low, when disturbance is significantly modified, or in particular when relatively low stress is combined with relatively large departures from typical disturbance regimes (Alpert *et al*, 2000). In New Zealand, proximity to towns in the most important predictor in determining the number of environmental weeds in lowland forests, reserves and coastal forest fragments (Sullivan *et al*, 2005). In addition, disturbed forests with fertile soils are typically associated with more alien species (Wiser & Allen, 2006). Mat-forming herbs and woody alien species, such as smothering vines, are considered most threatening to indigenous forests in New Zealand because they have the potential to alter forest structure, composition and functioning (Wiser & Allen, 2006).

1.1.3 Facilitative invasion – the role of mutualisms

There are many barriers that must be overcome in order for a non-native species to become invasive (Henderson *et al*, 2006). Until recently, the emphasis of invasion ecology has been on the negative interactions between invasive species and other resident species rather than the positive or mutualistic interactions which may enable a species to proliferate in an ecosystem (Richardson & Pysek, 2006). The relative frequency and importance of positive interactions versus negative interactions in plant invasion is unknown (Simberloff, 2006). However, in many cases, an invasive species is not able to invade and survive in an ecosystem in the absence of other species, whether they be native or exotic (Richardson & Pysek, 2006). For example, in New Zealand red clover (*Trifolium pratense*) is unable to set seed in the absence of introduced bumble bees (Richardson *et al*, 2000). Likewise, initially in any local invasion a non-clonal invader will always be seed and dispersal limited. Therefore, to understand what limits the spread of invasive species we must understand factors controlling reproduction (Parker & Haubensak, 2002) as many exotic plants depend on animal-mediated pollination and seed dispersal or symbioses between plant roots and microbiota. Thus the presence of mutualisms will facilitate exotic invasions (Richardson *et al*, 2000). Once exotic species reach a new area they will come into contact with novel species which will produce unpredictable ecological and evolutionary consequences (Henderson *et al*, 2006). In addition, the presence of exotic species in an ecosystem, for example generalist pollinators, may increase invasibility by facilitating the reproduction and spread of other exotic species through mutualistic interactions (Alpert *et al*, 2000). These mutualisms may reunite species that are mutualists in their native range, but very often different species are involved and many ecosystems are becoming more susceptible to invasion because they contain an increasing array of potential mutualistic partners. As a result, conditions conducive to alien/alien synergisms (invasive mutualisms) are becoming more abundant. There are many cases showing invasive mutualisms that facilitate invasion and enable species to invade areas that would be inaccessible in the absence of other exotic species (Richardson *et al*, 2000). For example, the abundance and spread of guava is extremely problematic in the Galapagos but would be much more restricted in the absence of introduced frugivorous mammals (Henderson *et al*, 2006). Similarly, Constible *et al* (2005) found that introduced bison on Santa Catalina Island, California were significant seed dispersers of native weeds. In the same way, in Australia blackbirds are well known for their role in spreading noxious weeds and contributing to invasiveness (Panetta & McKee, 1997).

The expansion in range and increasing abundance of generalist microbiota, pollinators and dispersers may have significant consequences for the number of invasive species in ecosystems (Richardson *et al*, 2000). For example, Parker *et al* (2006) conducted a meta-analysis of more than 100 exotic plant species and found that while native herbivores suppressed exotic plants, exotic herbivores facilitated both the abundance and species richness of exotic plants. As a result, the relative abundance of exotic plants was 52% higher in communities grazed by exotic herbivores than in communities grazed by native herbivores.

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Therefore, exotic plants may not become invasive through release from native enemies but rather through being introduced to areas that contain their enemies because they will have evolved resistance that the evolutionarily naïve natives have not, and thus have a competitive advantage.

Simberloff and Von Holle (1999) described a theoretical phenomenon they termed 'invasional meltdown' in which non-indigenous species facilitate one another's invasion, increasing the likelihood of survival and proliferation of the invader, and potentially leading to an accelerating number of introduced species with increased impacts. Invasional meltdown is more specific than an invasive mutualism which is a mutualistic interaction, such as pollination or seed dispersal, between two invasive species that increases the fitness and invasiveness of one or both of the parties, at least at the individual species level (Newstrom & Robertson, 2005). Rather invasional meltdown is a community level process and thus requires reciprocal population growth. It suggests that by aiding one another, introduced species could produce an auto-catalytic process that would accelerate the replacement of native communities, perhaps to the point of no return (Simberloff, 2006). Although the concept of an invasional meltdown has received a lot of attention by both scientists and the media, Simberloff (2006) argues that it has yet to be conclusively demonstrated at the population level. Similarly, Richardson *et al* (2000) failed to find any studies showing significant population impact of a mutualistic pollinator relationship between introduced plants and animals. However, this does not mean that the phenomenon doesn't exist (Simberloff, 2006) and mutualisms don't impart a reciprocal positive effect on each partner's rate of population increase (Buckley *et al*, 2006). Parker *et al* (2006) may be the first documented case provided convincing evidence in support of the invasional meltdown hypothesis. This phenomenon may be widespread and invasive mutualisms together with continued movement of species around the world and other anthropogenic disruptions of ecosystem functioning are probably causing ecosystems to become easier to invade (Richardson *et al*, 2000).

1.1.4 The ecology of lianas

The ecological importance of lianas and other vines has become widely recognized only in the last decade. Consequently, there is a lot of research still to be done (Schnitzer & Bongers, 2002). However, there is evidence to suggest that the presence of lianas have a significant impact on gap-phase regeneration, growth, forest composition and succession processes, often resulting in smothering and mechanical damage to their host trees. (Schnitzer, 2005). Furthermore, vines considerably affect whole ecosystem-level processes including forest transpiration, carbon sequestration and water budgets (Schnitzer & Bongers, 2002). Trees vary in their susceptibility to lianas so their presence may alter the competitive interactions within a community, disadvantaging slow-growing, shade-tolerant species and sometimes indirectly favouring pioneer species (Schnitzer & Bongers, 2002). In addition, lianas may compete with other plant species for mutualistic interactions such as pollinators and dispersers (Williams & West,

2000). However, in many ecosystems, their leaves, flowers and fruit are an important source of food for animals and they provide intercrown pathways which are an important means of avoiding predation for many canopy-dwelling animals (Putz, 2005).

Liana abundance varies with several key abiotic factors, including total rainfall, seasonality of rainfall, soil fertility and disturbance (Schnitzer & Bongers, 2002). For example, Laurance *et al* (2001) found that liana abundance was greater near forest edges and was significantly associated with higher levels of disturbance and soil-fertility. Vines are generally thought to be light-demanding as they thrive in areas with abundant light such as clearings resulting from natural disturbance or human activity (Baars & Kelly, 1996). In addition, lianas are often high in abundance in tree-fall gaps and young secondary forest (Schnitzer *et al*, 2005). Thus previously it was thought that they primarily out-competed host trees through intense competition for light (Schnitzer & Bongers, 2002). However, Schinitzer *et al* (2005) found that in disturbed tropical forests, lianas aggressively compete for below-ground resources such as water and nutrients (e.g. nitrogen) and consequently they significantly limited sapling growth over two years through below-ground competition. They found no effect of above-ground competition on the biomass of saplings from three native species. Rather, above-ground competition and mechanical stress resulted in shorter, thicker stems and a poorly developed crown. Therefore, it appears that lianas are capable of effectively competing for both above-ground resources such as light, and below-ground for water and nutrients, so the relative importance of each is likely to be determined by the most limiting resource (Schnitzer *et al*, 2005).

Little is know about the reproductive ecology of lianas, but most regenerate either from seed or as vegetative offshoots from roots and fallen stems of established individuals (Putz, 2005). Consequently, a large part of their success can be attributed to their ability to arrive at disturbed sites in high numbers, reproduce vegetatively and grow rapidly for long periods (Schnitzer & Bongers, 2002). There are several other factors that allow vines to flourish. Firstly, they use host trees for mechanical support reducing the investment of resources into thickening their stems, which enables rapid colonization and growth (Schnitzer, 2005). Consequently, they have high photosynthetic capacity and display their leaves above those of the host tree so are effective competitors for light (Putz, 2005). Secondly, they have an extremely deep and well-developed root and vascular systems, allowing them to rapidly take up resources from the soil and transport it efficiently to their crown, so are very effective below-ground competitors for water and nutrients and able access water and nutrients unavailable to other species in periods of drought (Schnitzer *et al*, 2005). Thirdly, most vines thrive in disturbances, which promotes colonization and growth from dormant seeds, formerly suppressed-seedlings, adults and vegetative propagation (Schnitzer & Bongers, 2002). However, lianas rely on other trees for support in order to access the canopy and as a result, the availability of suitable support is a major limiting factor (Baars *et al*, 1998). This may partially explain why vines are so abundant in the dense vegetation of forest edges where potential supports are

plentiful but light is not limiting (Putz, 2005). Typically lianas are unable to tolerate freezing temperatures due to their relatively wide vessel elements and thin, uninsulated stems which limits their distribution, particularly in temperate regions (Schnitzer & Bongers, 2002; Schnitzer, 2005).

In tropical forests, lianas are important structural parasites of trees (Laurance *et al*, 2001). Global increases in anthropogenic disturbance and forest fragmentation will increase the abundance and relative importance of lianas in forest ecosystems (Schnitzer & Bongers, 2002). In addition, it has been suggested that community composition is currently changing in old-growth Neotropical forests, perhaps resulting from increasing concentrations of atmospheric carbon dioxide, and lianas are becoming increasingly abundant (Schnitzer, 2005). For example, Phillips *et al* (2002) presented evidence that in tropical forests liana population size, basal area, relative dominance and size of individuals have all significantly increased. This is significant given the key functional role of lianas, which have a disproportionately large ecological effect, as they encourage tree mortality and influence the competitive balance of ecosystems (Phillips *et al*, 2002). Wright *et al* (2004) also presented evidence from Barro Colorado Island in Panama that lianas are increasing in importance in old growth tropical forests and suggested this may be the cause of recent increases in Neotropical tree mortality rates.

In summary, even in relatively low densities, lianas influence forest regeneration and competition not only through direct competition with plants, but also by differentially affecting tree species and consequently, the way they competitively interact with one another (Schnitzer & Bongers, 2002). Lianas may suffer less water stress in the relatively harsh conditions following disturbance and thus are better able to utilise the abundant light than many other species (Schnitzer, 2005). Lianas make relatively little structural support and while this requires them to rely on other species to reach the canopy, it enables a greater allocation or resources to reproduction, photosynthetic biomass, and stem and root elongation (Schnitzer & Bongers, 2002). While little study has been conducted on liana reproductive strategy and mutualisms, it appears that there is wide variation but large bees and beetles are disproportionately well presented as pollinators (Schnitzer & Bongers, 2002).

Lianas can become particularly aggressive weeds when they become naturalised. There are numerous examples globally of disturbed ecosystems that have been invaded by fast growing vines species such as *Cardiospermum, Ipomea, Macfadyena, Passiflora* and *Pueraria* spp (Baars & Kelly, 1996). In New Zealand, vines and creepers represent 14% of invasive species (Williams & West, 2000) and several species of vines are considered to be serious environmental weeds of native forest in New Zealand (Baars *et al*, 1998).

1.2 INTRODUCTION TO BANANA PASSIONFRUIT

1.2.1 Subject species

The research in this thesis focuses on an introduced tendril-climbing, liana species, namely banana passionfruit (*Passiflora tripartita* var. *mollissima*; Subgenus – Tacsonia) (Heenan & Sykes, 2003), a species formerly known as *Passiflora mollissima* (Kunth) L. Bailey (Baars *et al*, 1998), which will be henceforth referred to as *P. mollissima* following Webb *et al* (1988). Banana passionfruit is from the Passifloraceae family which is comprised of 22 genera and over 600 species (Starr *et al*, 2003). Figure 1.1 shows the distinctive three lobed leaves, pink flowers and large, yellow, fleshy-fruits of *P. mollissima*.

Figure 1.1: *Passiflora. mollissima* leaves and large fleshy fruits and flowers. Left picture shows seeds inside fleshy capsules.

Banana Passionfruit is one of several introduced vines considered to be serious weeds in New Zealand. Presumably, similar to Hawaii, it was introduced into New Zealand gardens because of its large edible fruits and attractive flowers (LaRosa, 1992; LaRosa, 1984). This initiated its spread to native habitats. Banana passionfruit was first recorded as a naturalized in 1958 (Williams & Buxton, 1995), and has since become a serious weed in parts of New Zealand (Heenan & Sykes, 2003). Its invasiveness is widely acknowledged and consequently it has been classified as an 'unwanted organism', making it illegal to sell or propagate in New Zealand (Williams & West, 2000). This also enables management authorities to insist on weed control on private properties. With respect to management strategies, banana passionfruit is listed as a 'National Surveillance' pest species and is subject to Regional Pest Management Strategies in several regions of New Zealand, including Marlborough (Heenan & Sykes, 2003; see Appendix of Williams & West, 2000 for New Zealands policy framework for the management of environmental weeds). It is able to tolerate a wide range of environmental conditions (Warshauer *et al*, 1983) and has become widespread nationally, being particularly problematic in Nelson and Marlborough, but its range is probably still expanding (Williams & Buxton, 1995). Many locals in the Marlborough area, including Dave Miller of Marlborough Roads, emphasize the invasiveness of banana passionfruit (pers. comm.). In the last 20 years

they have watched small pockets along Queen Charlotte Drive develop into huge, widespread infestations, smothering entire gullies and forming archways over main roads throughout the Queen Charlotte and Keneperu Sounds.

In New Zealand, banana passionfruit typically occurs along roadsides and in moist, frost-free, lowland areas with disturbed indigenous or naturalized vegetation and forest gaps (Heenan & Sykes, 2003). As with many tropical lianas, banana passionfruit exhibits an efficient pattern of resource allocation, including staggered germination, rapid growth rates, early reproductive maturity, continuous growth and reproduction, and high seed output (LaRosa, 1992; Williams & Buxton, 1995). For example, in favorable conditions, plants may flower within a single year of germinating (LaRosa, 1992). Once dormancy is broken, seeds in a cohort tend to germinate fairly consistently over the following eight months, however some seeds remain dormant forming a seed bank in the soil that is released when favorable environmental conditions arise (LaRosa, 1992). In addition, flowers and fruit are copiously produced and can be found in all months of the year (Starr *et al*, 2003). Adult mortality is low and vines are estimated to survive for 15-20 years (LaRosa, 1992). The invasiveness of banana passionfruit is further exacerbated by the ability to utilize vegetative reproduction as stems as short as 5 cm that become detached or partially buried are able to re-root and continue growing (Williams & Buxton, 1995). This may become especially significant in allowing banana passionfruit to re-establish following mechanical control operations. Sites with thriving populations of banana passionfruit are generally associated with (but not restricted to) high soil pH, warmer and lower altitudes and predominantly early-successional vegetation (Baars *et al*, 1998). In addition, it is typically found in coastal environments which is probably related to its susceptibility to frost (LaRosa, 1992).

1.2.2 Limiting factors - light compensation and disturbance

Light availability is one of the restricting factors for seedling survival and consequently disturbance plays a significant role in the proliferation of *P. mollissima* at many sites (Baars & Kelly, 1996). Banana passionfruit seedlings reach their light-compensation point (at which growth ceases) at 2% of full-sunlight but seedlings from seed banks are able to rapidly exploit small forest gaps resulting from disturbance (Baars & Kelly, 1996). Plants are unlikely to germinate and survive in intense shade but shade tolerance increases in older seedlings (Williams & Buxton, 1995). This allows seedlings to remain in the understorey with limited growth until a disturbance, then respond quickly to canopy gaps with rapid growth. Growth and establishment increase proportionally to increasing light intensity, and under favorable conditions (above 7% full-sunlight) seedlings can grow very quickly (Baars & Kelly 1996). Consequently, small forest and scrub remnants are particularly vulnerable to infestation because they are typically high light, disturbed environments (Williams & Buxton, 1995). As a result, most wild infestations are concentrated in areas of human occupation. Anthropogenic disturbances, especially

adjacent to highways, camping grounds and baches will continue to aid the spread of this weed in the Marlborough Sounds (Williams & Buxton, 1995).

1.2.3 Why is *P. mollissima* problematic?

Once the canopy height has been reached, individuals are no longer restricted by light and can spread laterally along the canopy (LaRosa, 1992). Baars *et al* (1998) suggested that canopy height may constrain banana passionfruit success in New Zealand although there is evidence that it has reached canopies that are over 20 m high in Hawaii. Introduced vines that establish in New Zealand often result in a loss of species richness (Baars *et al*, 1998). Banana passionfruit is a structural parasite that grows on top of the canopy, forming dense foliage that excludes sunlight. This smothers the host tree by reducing photosynthesis, growth and reproduction, sometimes resulting in tree death (LaRosa, 1992). I have seen mature native trees that are dead underneath a blanket of banana passionfruit. Similarly, studies in Hawaii have found that in areas of advanced infestations the structural integrity and species composition of the native vegetation has been drastically reduced, which has far-reaching consequences for the flora and fauna, particularly for bird communities and other dependent animals (Warshauer *et al*, 1983). The weight of the plant may cause branch breakages, facilitating the entrance of pathogens to the host tree or forming gaps that may be further exploited by banana passionfruit (LaRosa, 1992). In addition, *P. mollissima* has an extensive root system so is also an effective competitor of water and nutrients. Consequently, banana passionfruit is threatening the integrity of native forests in New Zealand.

1.2.4 Native habitat

Banana passionfruit is indigenous in the upper montane forests of the Andean Highlands in South America (Starr *et al*, 2003). Its natural range extends from Venezuela to Bolivia at 2,000-3,600 metres above sea level (LaRosa, 1992) and into parts of Columbia and Peru (Starr *et al*, 2003). The climate of this area may be characterized as a cool, moist, tropical montane environment (LaRosa, 1992). In contrast to New Zealand, *P. mollissima* is not very common in its native range, averaging approximately 2-3 plants per hectare. This is probably because it is kept under control by a numerous species of co-evolved insect herbivores (Warshauer *et al*, 1983). It is pollinated by specialised birds (hummingbirds) as well as large bees and insects. In its native range, banana passionfruit is probably dispersed by monkeys and other frugivorous birds and mammals.

1.2.5 Research in Hawaii

Hawaii is one of the only places where *Passiflora mollissima* has long been considered a noxious weed and consequently, has been the subject of several studies. It was first reported in Hawaii in 1921, and had naturalised an area of more than 520 km^2 (190 km^2 of which were severely infested) by 1983(Warshauer *et al*, 1983; LaRosa, 1992). *P. mollissima* is the most aggressive of nine *Passiflora* vine species that have

become naturalised in Hawaii and its success is generally attributed to multiple factors including favourable environmental conditions (similar to those found in its native range), an abundance of disturbed habitats suitable for colonisation, numerous native and alien dispersal agents, a wide degree of environmental tolerance, a lack of damaging predators and pathogens, and general plant vigour (LaRosa, 1992; LaRosa 1984). No native or naturalised Hawaiian insects are known to attack the plant and restrict its growth (Warshauer *et al*, 1983). This release from predation and the large degree of environmental tolerance of *P. mollissima* has enabled it to invade many of the major upland wet and mesic *Acacia koa – Metrosideros polymorpha* forests on the islands of Hawaii and Kaua'i (LaRosa, 1992), where it grows between 600 and 2200 m elevation with annual rainfall being between 500 and 5100 mm (Warshauer *et al*, 1983). Infestations range from scattered individuals with low cover to areas of 100% cover, often inhibiting growth and reproduction of native forest species (LaRosa, 1992). Banana passionfruit is able to exploit light created by small disturbance events caused by tree-fall, windstorms, defoliation or dieback by climbing up through the understorey vegetation, exploiting the high-light environment and spreading along the canopy. As a result, often native vegetation components are eventually displaced and ecosystems are disrupted (Warshauer *et al*, 1983).

Research in Hawaii has shown that biotic pollinators play a major role in reproduction and the mating system combines outcrossing with selfing. While flowers are highly self-compatible, natural selfing is infrequent (LaRosa, 1992). The main dispersers of banana passionfruit in Hawaii are humans, who are the predominant long-distance dispersers, and approximately 20 primarily exotic species of birds and animals. The most significant animal is the feral pig (*Sus scrofa*) of which 32% of its diet is comprised of *P. mollissima* (Warshauer *et al*, 1983). Pigs were observed actively moving to areas of high banana passionfruit densities during periods of heavy fruiting (Warshauer *et al*, 1983). One area of very high density *P. mollissima* is associated with an average density of 63 pigs/km^2 though it is difficult to establish whether the plant abundance is the cause or result of pig densities (Warshauer *et al*, 1983). Other birds such as wild turkeys (*Meleagris gallopavo*) and Kalij Pheasants (*Lophura leucomelama*) may also be important dispersers as 82% of their crop and/or gizzard contents was found to be banana passionfruit seeds (LaRosa, 1992). Seeds are well adapted for dispersal from birds and mammals and survive following ingestion from many species. Hawaiian research shows that the spread of banana passionfruit can be limited by controlling anthropogenic disturbance and introduced animals (LaRosa, 1992).

In contrast, banana passionfruit has been the subject of few ecological studies in New Zealand. Birds and introduced mammals are likely to disperse banana passionfruit, although their relative contributions are unknown and the effect of ingestion on germination has seldom been investigated (Williams & Buxton, 1995). However, adventive birds have often been implicated in the spread of adventive plants (Williams & Karl, 1996) and research in Nelson has shown that blackbirds may play a role in the dispersal of several

weed species including *P. mollissima* (Williams, 2006). In order to effectively reduce and control the spread of weed species, an understanding of weed population dynamics and the ecological components affecting reproductive functioning is essential (Buckley *et al*, 2006). Thus, the purpose of this research is to determine the mutualistic interactions contributing to the proliferation of *P. mollissima* in New Zealand. In particular, given that banana passionfruit has such large flowers and fruit, how has it become so successful and widespread in New Zealand without the specialised pollinators and dispersers of its native range?

1.3 OBJECTIVES

Thus, the overall objective of this research is to gain a greater understanding of the ecology of banana passionfruit (*P. mollissima*) in New Zealand in order to determine potential weak points that may be targeted for more effective control.

More specifically, my research has two main aims:

1. To determine the extent to which biotic pollinators play a role in fruit production and subsequent seedling establishment and growth.
2. To investigate the primary dispersal vectors contributing to the rapid spread of banana passionfruit both within established areas and to new areas.

Chapter 2: POLLINATION
The role of biotic pollinators in seed production and subsequent progeny establishment

2.1   INTRODUCTION

2.1.1 Importance of pollination biology

Our understanding of the role of mutualistic interactions, and in particular of breeding systems and pollination biology, is very limited but extremely important in helping us to understand invasive species and the processes by which they become established (Richardson *et al*, 2000). Many species require mutualistic interactions to fulfil reproductive functions in their new environments or else they will not survive (Richardson *et al*, 2000). Schnitzer and Bongers (2002) suggested that more detailed studies on the pollination biology of lianas are needed and Newstrom and Robertson (2005) also stressed the need for, and importance of, more research in New Zealand focused on pollination, breeding systems and specifically, invasive mutualisms. Banana passionfruit, becoming increasingly widespread and problematic, is a prime candidate for such research. Several Passiflora species, such as *Passiflora alata*, *P. galbana*, and *P. speciosa*, have been found to be self-incompatible and consequently pollinators play an essential role in reproduction (Varassin *et al*, 2001). Souza *et al* (2004) suggested that cross-pollination is necessary in *P. edulis* because of self-incompatibility and flower morphology. However, the extent to which *P. mollissima* relies on out-crossing for reproduction is unknown.

2.1.2 Flower structure and native pollinators

Robertson *et al* (2005) discuss the distinctive features of one of the proposed pollination syndromes, namely ornithophily ('love of birds'). The structure of banana passionfruit flowers suggest that they are ornithophilous. They are large and brightly coloured, with no scent and five large, heavily laden anthers. The banana passionfruit flower itself is open and similar to *P. edulis* (Sousa *et al*, 2004) in that the anthers are located below the stigma facing outwards (Figure 1.1). This is characteristic of Passifloraceae and probably affects the ability of the flowers to pollinate autogamously which indicates a tendency away from self-fertilization toward out-crossing (Janzen, 1968). In addition, banana passionfruit flowers appear to be very specialized as they have an extremely elongated floral tube leading to a large nectar source. The floral tube has been reported as reaching up to 15 cm in length (Endress, 1994). This extended anther-nectary distance suggests evolution and adaptation to pollination by birds rather than bees. Several species of honeyeaters in Australia have long slender bills that enable them to drink nectar from the base of lengthy tubular flowers. To ensure effective pollination from bird visitors the plant must increase the length of the floral tube to greater than the bill length of the pollinator to ensure that the birds pollen-loaded facial feathers regularly contact the stigma whilst probing flowers and collecting nectar (Paton & Ford, 1977). In their native habitat, hummingbirds are the pollinator of banana passionfruit flowers (Janzen, 1968). Endress (1994) presents the pollinator-plant interaction of banana passionfruit as a striking

example of coevolution with one specific species of hummingbird, the Andean swordbill (*Ensifera ensifera*), whose bill length may reach more than ten centimetres in order to reach the nectar of banana passionfruit flowers.

'Ornithophilous' flowers may also be visited by many other pollinator groups. For example, native mistletoes *Peraxilla colensoi* and *P. tetrapetala* have ornithophilous flowers but are also pollinated by native short-tongued bees (Robertson *et al*, 2005). In New Zealand, banana passionfruit flowers have had to endure the loss of their specialised pollinator. The extent to which hummingbirds have been replaced by other pollinators and the ensuing effectiveness of their pollination has not yet been thoroughly investigated. This raises several questions. Firstly, do banana passionfruit flowers require pollinator visitation in order to reproduce? If so, which pollinator species are visiting these flowers in New Zealand, and are they effectively pollinating flowers?

2.1.3 Pollinators in New Zealand

In general, there is low biodiversity amongst native pollinators in New Zealand, particularly butterflies, with a complete lack of large long- and short-tongued social bees (Newstrom & Robertson, 2005). Insects are often ineffective pollinators of larger flowers because they tend to focus their activities below the stigma (Anderson, 2003). The most common and widespread of the native honeyeaters are tui (*Prosthemadera novaeseelandiae*) and bellbirds (*Anthornis melanura*) (Anderson, 2003). In addition to the native fauna, there are a small group of exotic pollinators, the most important of which are honey bees (*Apis mellifera*) and four species of large, social, bumble bees (*Bombus* spp.). The establishment of bees and bumble bees have had both beneficial and detrimental effects on agricultural and indigenous ecosystems (Kearns & Inouye, 1997; Newstrom & Robertson, 2005). Anderson (2003) studied the relative pollinator effectiveness and importance of birds and insects in New Zealand flora. She found that the seed set of eight diverse indigenous species was significantly increased with bird pollination compared with insect pollination, suggesting that bees may not be pollinating plants as effectively as birds, resulting in reduced reproductive success by pollen limitation.

2.1.4 Pollen limitation

Pollen limitation was defined by Ashman *et al* (2004) as being a reduction in plant reproductive success (of seed quantity or quality) due to an inadequate quantity or quality of pollen. For example, pollen quantity may be reduced by a decrease in the number of visitors to flowers or by pollinators depositing smaller pollen loads each visit. Pollen quality may be reduced if incompatible pollen (from the same or a different species) is delivered. It is very common for plants to suffer some degree of pollen limitation as demonstrated by a review of 258 species by Burd (1994), in which 62% of species were found to experience statistically significant pollen limitation. Similarly, Ashman *et al* (2004) reviewed the 85

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pollination studies conducted at the plant level and published between 1980 and 2003. Seventy-three percent of the studies showed significantly greater reproductive success where there was supplemental pollination. Not only was pollen limitation common but on average pollen supplementation resulted in a 42% increase in seed set per plant. There are several ecological causes of pollen limitation, one of which is the invasion of a plant species to a new area where native pollinators don't exist. This results in the plant receiving less pollen so is not able to produce as many seeds (Ashman *et al*, 2004). The availability of resources in the environment may also affect the degree to which a plant experiences pollen limitation. For example, a plant may be able to abort flowers with insufficient or incompatible pollen and redistribute its resources to focus on the production of fruit from flowers that are not restricted by pollen quality or quantity (Newstrom & Robertson, 2005).

2.1.5 Inbreeding depression and the evolution of mating systems

In the absence of sufficient pollen deposition some plants tend toward self-fertilisation, which presents certain advantages such as reproductive assurance following colonisation and the possibility of purging deleterious alleles (although there is considerable controversy regarding the extent to which purging effectively reduces genetic load) (Scofield & Schultz, 2006). However, self-pollination often results in inbreeding depression which can be defined as the reduced fitness observed by progeny from interbreeding among relatives (Byers & Waller, 1999). Inbreeding depression has been shown to reduce overall fitness of individuals and natural populations in plants across the entire life cycle including fruit set, seed set, seed size, germination success, survival, fecundity and resistance to stress (Byers & Waller, 1999; Keller & Waller, 2002). The degree to which inbreeding depression affects an individual or population varies depending on environmental conditions (Cheptou, 2006). For example, a meta-analysis of 34 studies by Armbruster and Reed (2005) confirmed that a reduction in fitness as a result of inbreeding depression is associated with increasingly stressful (relative to benign) environmental conditions.

Inbreeding depression is typically measured by comparing the fitness of out-crossed offspring with that of self-pollinated individuals. In order to determine the extent of inbreeding depression it is important to evaluate multiple fitness components to get an estimate of total fitness because some traits show higher levels of inbreeding depression than others and results often vary according to life-history stage, experimental habitat and environmental conditions (Keller & Waller, 2002).

While countless populations are unable to avoid inbreeding, many plants have mechanisms for avoiding self-pollination including dioecy, gynodiecy, monoecy, dichogamy and herkogamy (Byers & Waller, 1999). In addition, self-incompatibility, though it may vary in its expression with environmental conditions, eliminates inbreeding in some populations (Byers & Waller, 1999). Consequently there appears to be a trade off between availability of pollen (or the cost of outcrossing) and inbreeding

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depression, which plays a fundamental role in the evolution of mating systems (Cheptou *et al*, 2002). The selection pressures on mating behaviour are likely to change from the time populations are first colonised until when they become established. As a result, frequently mating systems have some degree of flexibility to provide resilience against disturbance, allowing them to shift from out-crossing to selfing (Brennan *et al*, 2005). For example, Cheptou *et al* (2002) found that self-incompatible *Crepis sancta* (Asteraceae) has a pseudo-self-incompatibility system in which there is a temporary partial breakdown of the self-incompatibility complex during colonisation and establishment, followed by recovery to full self-incompatibility system. This allows the plant to compensate for the lack of out-crossed pollen and/or pollinators. If inbreeding depression and genetic load are severe then selection will return the mating system to a full-self-incompatibility system when compensation for pollen limitation no longer increases fitness, as is the case with *Senecio squalidus* (Asteraceae) in Britain (Brennan *et al*, 2005).

2.2   AIMS

To determine the extent to which banana passionfruit relies on out-crossing from biotic pollinators for reproduction, or is self-compatible and capable of autogamy, and the effect this has on fruit production, germination success and seedling growth.

To establish the primary visitors to banana passionfruit flowers in New Zealand the extent to which they are enhancing reproductive success through effective pollination.

To gain a greater understanding of the breeding system of banana passionfruit and the degree to which reproduction is constrained by pollen limitation and inbreeding depression.

2.3   SITES

Two major sites in the Queen Charlotte Sound were used for pollination experiments. Each site had 10 replicate patches. Patches were used rather than separate plants due to the inability to distinguish one plant from another, and were separated by at least 15-20 metres of clear ground to avoid pseudoreplication. Site 1 was located on the uphill side of the segment of Port Underwood Road at 41°15.57'S, 173°58.97'E. Patches 1-7 were located on a dense stand of banana passionfruit on low broadleaf vegetation while Patches 8-10 were located below 'Marina Views' where the vegetation was mostly gorse rather than scrub and broadleaf species. Site 2 was located along Queen Charlotte Drive in Kaireperepe Bay (41°16.01'S, 173°58.97'E) on scrubby mid-successional native tree species regenerating on slips and the edges of broadleaf forest.

2.4   MATERIALS AND METHODS

2.4.1 Pollinator visitation observations

Personal observations

I observed insect visitations to flowers in different patches for eight, five minute periods between 9 January 2006 and 11 January 2006. The time at which the flowers were observed ranged between 9:52 am and 2:25 pm. Information regarding the visitor species, number of visits to flowers, total flower count, total time spent in the plant and on each flower and weather conditions was recorded.

Landcare Research data

Data showing visitations to banana passionfruit flowers were made available by The Landcare Research Community Pollination Project funded by Landcare Research Capability Funds (website http://www.landcareresearch.co.nz/research/biocons/pollination/). The data were collected at one site (Grampians, Nelson) between 17 November 2005 and 15 December 2005. Six flowers on four different plants were observed. Flowers were observed every two hours starting at 7:00 am or 9:00 am (i.e. 9:00, 11:00, 1:00, 3:00) and there were a total of 172 observation periods. Observation data were collected using 'near-instantaneous counts' of the number of individuals from each species visiting the six flowers when the observer approached. Weather conditions were also recorded. The methods used are described in more detail on the website.

2.4.2 First pollination experiment

Treatment effect on fruit set

Within each patch, 40 flowers were selected. Each flower was selected when the bud began to swell, prior to opening. The week that flowers were selected was recorded in order to allow for analysis of seasonal effects if required. Most were selected during a heavy flowering period in December 2005 and January 2006. The treatment application was interspersed to ensure results were not confounded by spatial or temporal patterns. Flowers were marked using a paper tag with cotton tie naming the Patch, flower number and treatment. Over 400 net curtain bags (nylon with 1 mm mesh size) measuring 19 x 23 cm were made to exclude pollinators from flowers. These were secured over a flower using a twist tie for selfed and bagged flower treatments. Each flower was tagged with a colour-coded wire tag to indicate the treatment.

In each patch, the 40 flowers were divided evenly among the four main treatments:
1. Selfed   ripe buds were bagged until open and were then hand-pollinated by removing the anthers using forceps and brushing them against the stigma. The bag was then replaced until the flower died or a fruit developed.

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2. <u>Crossed</u> flowers were emasculated (anthers removed before pollen release) prior to pollen production and hand pollinated using pollen of another flower located at least 100 m away. They were then left unbagged. Pollen was removed from open flowers very quickly by insects making it difficult to collect enough for hand-pollinations. Consequently, excess flowers were bagged on a number of plants from both inside and outside the patches and their pollen collected each morning by removing the anthers and putting them in a vial.

3. <u>Bagged</u> a bag was placed over the bud before it opened and left on till the flower parts withered to determine whether pollinators are required for fruit production or the flower is capable of autonomous selfing.

4. <u>Natural</u> these flowers were labelled and left from a bag to measure natural fruit set rates when accessible to all pollinators.

Overall, 200 flowers were selected for each treatment across patches and sites, giving 800 flowers in total.

In addition, 40 flowers (20 at each site) were selected to test for asexual (apomictic) fruit production. Buds were selected, emasculated prior to pollen production, and placed inside a bag to eliminate pollinators. The 40 flowers were scattered throughout the 10 patches at each site as flowers allowed (Table 2.1). This study included all of the five treatments specified by Newstrom and Robertson (2005) as being necessary to fully investigate the breeding system of a species.

Table 2.1: Distribution of banana passionfruit flowers used for apomictic treatment across two sites in Marlborough Sounds in Jan-March 2006.

Patch	Site 1	Site 2
1	2	0
2	1	4
3	3	3
4	0	2
5	5	2
6	1	2
7	5	4
8	0	3
9	3	0
10	0	0
Total	**20**	**20**

The flowers were revisited approximately a month later and the presence/absence of a fruit recorded. The fruit were then left to ripen on the vine. The identification number and treatment (e.g. 17S) of each flower was written on the fruit with a marker pen so it could be relocated and identified if it fell off the vine. Removal of fruit by animals was frequent, and consequently, bags were put back on all the fruit before they reached maturity. While this significantly reduced the number of fruit that disappeared, several of the

bags were found - often still on the vine - with a hole in the bag where an animal had chewed through it and stolen the fruit.

Collected Fruit

Fruit were collected when yellow and soft. The length, maximum width and number of seeds in each fruit were recorded. The sample sizes for each treatment were as follows: selfed = 23 fruit; crossed = 37 fruit; bagged = 4 fruit; natural = 18 fruit. Although some seeds were very small and seemed undeveloped they were included in total seed counts as it difficult to determine which seeds may be too immature to be viable. (See second pollination experiment below for more detail on this).

Analysis

Two binomial generalised linear models were run with the R package (v.2.1.1; The R development Core Team, 2005), to determine whether there were any treatment effects. In these, the response variable was fruit production or failure. Site or Patch were added as factors to the model before Treatment to determine whether they explained a significant proportion of the variance. Although treatments were applied over a 4 month period from November 2006 to March 2007, treatments were interspersed so seasonal effects were not included in the analysis. Significance was tested using an F test on the binomial GLM rather than a Chi-squared test because the residual deviance was more than 50% larger than the residual df. A Tukey's Honestly Significant Difference (HSD) test was used to determine which treatments were significantly different. Patch was included in the model rather than Site because it explained more of the deviance.

The degree to which the fruit length, width and total seed count were affected by the treatments were explored using ANOVA analyses with gaussian error distributions. The analyses used are the same as those used in a similar pollination experiment by Kelly *et al* (2004).

2.4.3 Second pollination experiment

A second smaller pollination experiment was carried out with treatments applied from 9 November 2006 to 16 November 2006. This was to determine whether selfed fruit have a higher proportion of non-viable seeds than crossed fruit because in the first pollination experiment the small seeds were included in seed counts but not selected for the germination experiment. Consequently, an effect of inbreeding depression may not have been detected if there was no difference in the germination success of large seeds between treatments but selfed fruit had fewer large seeds, reducing the ability of its progeny to establish. In this experiment, ten or more ripe buds were selected as available in ten of the 20 patches. Half the flowers received the selfed treatment and half were crossed as described in Section 2.4.2. The number of flowers selected in each patch were as follows: Site 1, Patch 1 (P1) = 10; P5 = 10; P6 = 10; P10 = 10; Site 2, P2 =

10; P6 = 10; P7 = 16; P8 = 10; P9 = 10; P10 = 12. This gave a total of 108 flowers (54 selfed and 54 crossed) with 40 being at Site 1 and 68 at Site 2.

Data on fruit presence/absence, fruit length and width, and total seed count were recorded when the fruit became ripe in March 2007 and analysed using the same methods described in Section 2.4.2. In addition, the number of large seeds and small seeds in each fruit were recorded. Small seeds were those that would float and didn't appear to be fully developed because of characteristics such as light colouring or no outer casing.

Eleven selfed fruit and 11 crossed fruit from a range of patches were used in the analyses on fruit length, width and seed count. An ANOVA comparing the number of large seeds in fruit between treatments was used to determine whether selfed fruit ripen fewer large seeds than crossed fruit and consequently give rise to fewer progeny.

2.4.4 Germination success

A germination experiment using fruit collected from the first pollination experiment was used to determine whether pollination treatments had any effect on germination success, which may be indicative of inbreeding depression. Where possible, 50 cleaned seeds from each fruit were planted in the glasshouse and 50 in the field. Some fruit that contained less than 100 seeds had seeds planted in either the field or the glasshouse. The methods of planting used in the glasshouse and field were the same as those described in Chapter 3.3.1. The number of replicate fruit from which seeds were germinated varied across treatments because flowers of some treatments produced a lot more fruit than others. Seeds were planted from as many fruit as possible (Table 2.2).

Table 2.2: Number of replicate fruit in each of four pollination treatments from which banana passionfruit seeds were planted in the glasshouse and field.

Treatment	Glasshouse	Field
Selfed	8	10
Crossed	17	20
Bagged	4	3
Natural	7	10

Glasshouse

Each tray was divided evenly into four sections for four different fruit. Seeds from fruit of various treatments were randomly distributed within and amongst the trays to ensure there was no confounding effect of tray on germination success. In each section of the tray, fifty seeds from one fruit were evenly scattered resulting in each tray containing 200 seeds from four fruit of randomly selected treatments. The

number of seeds that germinated each month were recorded and seedlings were removed to reduce competition effects and to ensure more accurate counts.

Field

In the field, four fruit were randomly selected from the fruits of all treatments for each 5 x 4 plot. Each fruit contributed 50 seeds which were randomly allocated to five different tubes (7 cm segments of downpipe to put in the ground to contain seeds) to control for environmental variation. Consequently, each tube contained ten seeds. The number of seeds that germinated each month were recorded and seedlings removed.

Analysis

Glasshouse and field data were analysed separately in R using a binomial Generalised Linear Model for which the response was the combined number of seeds that germinated and failed in each sample. Site from which the fruit was collected was added as a main effect to increase the accuracy of the model. An "F" test was used to test for significance as explained above.

2.4.5 Inbreeding effects on seedling growth with competition

Two trays of 25 selfed seedlings and 25 crossed seedlings of equivalent size and maturity from the germination experiment were planted with 2-3 handfuls of grass seed (a thin covering) sown on top to determine effect of inbreeding depression on seedling survival and growth under competition in the glasshouse. Seedlings were selected from a range of different fruit. Trays were arranged in rows of 10 x 5, alternating between crossed and selfed seeds. A small piece of red wire was tied around the selfed seedlings to assist identification between the treatments. The first tray was set up on 7 July 2006 and harvested after 118 days on 2 November 2006. The second tray was set up on 24 August 2006 and also harvested after 118 days on 20 December 2006. Each month the number of leaves on each plant, the length of the stem to the top leaf, and the length of the longest leaflet were recorded for each seedling.

When the seedlings were harvested only aboveground material was harvested; the stems were cut off at the soil level. Each shoot was dried individually in a paper bag for 48 hours in a $70^{o}C$ drying oven and then weighed. Shoots were put in a silicon drying jar after being removed from the oven until they were weighed to avoid inaccuracy from the re-absorption of moisture from the air. In addition, rubber gloves and forceps were used to transfer the shoots.

Analysis

All seedlings survived. Four variables were analysed using R to determine whether selfed and crossed seedlings showed a significant difference in their growth. The two replicate trays in this experiment were

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started on different dates so 'tray' was added as a main effect to all the analyses. The response 'number of leaves' produced count data and consequently was analysed using a poisson Generalised Linear Model. A Chi-squared test was used to test for significance because data were independent. Three separate gaussian ANOVA's were run for 'stem length, 'longest leaflet length' and 'dry weight'. The data for stem length and dry weight were not normally distributed and consequently data were log and square-root transformed respectively, prior to the analysis.

2.5   RESULTS

2.5.1 Pollinator visitation observations

In my study, typically buds ripened two days before the flower opened and remained open for about a week. However, this period was longer in rainy weather, particularly the bud ripening phase. During my 40 minutes of flower visitation observations I observed honey bees and bumble bees visiting the banana passionfruit flowers. Bumble bees often crawled right up inside the flower to get to the nectar source. In addition, while conducting other experiments I observed earwigs in flowers and occasionally bellbirds robbing flowers. Some flowers had large holes bitten into them at the base which may suggest that possums are also robbing nectar from banana passionfruit flowers.

Table 2.3 summarises the eight observation periods. On average, a flower was visited by 3.69 bees each hour. The mean period of time that all the bees will spend in that flower over an hour is 64.1 seconds. Therefore, each flower was potentially being pollinated for just over one minute each hour. If banana passionfruit flowers are open for 5-7 days and are visited by bees for approximately 8 hours everyday (probably conservative for summer months, as I have observed flowers being visited at 6 pm whilst doing other experiments and Landcare data shows flowers being visited before 7 am) they could be receiving over 50 minutes pollination time over the life of the flower, assuming that bees are effectively pollinating them and the stigmas stays receptive, throughout the time the flower is open.

Table 2.3: Summary of flower visitations throughout eight observation periods. States the mean and standard errors of the number of open flowers on the observed plants, the number of honey bees and bumble bees that visited the plant during the five minute observation period, the number of flowers that were visited within the five minutes, the number of bee visits per flower each hour and the number of seconds of flower visitation each flower receives in an hour.

	Mean	SE
Flowers watched	9.4	3.2
No. of bee visits (per 5 min)	3.6	2.2
No. of observed flowers visited (per 5 min)	3.1	2.0
No. visits/flower/hour	3.7	0.9
Time of visits (sec/flower/hour)	64.1	20.6

Landcare Research data

Introduced honey bees and bumble bees were regularly observed visiting flowers while native bees were only observed visiting banana passionfruit flowers on two occasions. Birds were not observed visiting flowers. Throughout the 172 observation periods 82 honey bees and 124 bumble bees were observed visiting flowers. This shows that introduced honey bees and bumble bees are the predominant visitor to banana passionfruit and likely to be the only potential pollinators.

2.5.2 First pollination experiment

Treatment effect on fruit set

Of the 40 flowers selected for the apomictic treatment, none ripened fruit which strongly suggests that banana passionfruit is not able to produce fruit using apomictic reproduction.

In the main pollination experiment, some patches failed to produce any fruit while others produced up to 19 fruit from the 40 selected flowers. Of the 800 flowers that were selected, 136 developed into fruit while 664 failed. Overall, a mean of 17% of flowers successfully produced fruit.

The results from the initial model (Table 2.4) revealed a highly significant treatment effect even after the variance due to Site had been accounted for as a main effect. Similarly, treatment remained significant when Patch was included as a main effect. There was no main effect of Site but fruit production varied significantly across patches (Fig. 2.1, and Appendix 1.1 for summary of fruit production in each patch).

Table 2.4: Analysis of the number of banana passionfruit fruits produced under natural, selfed, crossed and bagged pollination treatments across two sites with ten patches in the Marlborough Sounds during December 2005/January 2006.

Factor	Df	Deviance	F	Pr(F)
Patch	19	88.61	4.66	P < 0.001
Treatment	3	65.78	21.93	P < 0.001
Residual	57	64.62		

A post hoc Tukey's Honestly Significant Difference test revealed that bagged flowers produced significantly less fruit when compared to the other three treatments (Table 2.5). While there was considerable variability, crossed flowers were most likely to be successful while few bagged flowers produced fruit (Fig. 2.1). Therefore, *P. mollissima* does rely on visitation by biotic pollinators to effectively produce fruit. Selfed flowers were successfully able to produce fruit so are clearly self-compatible but were less productive than natural and crossed flowers. The Tukey test showed a significant difference in fruit production between natural and hand-crossed flowers ($P = 0.05$). This indicates that there is some degree of pollen limitation. Comparisons between the two hand-pollinated treatments, selfed and crossed, showed that significantly more crossed flowers successfully produced fruit than those that

were self-pollinated. Consequently, there appears to be some degree of inbreeding depression or self-incompatibility.

Table 2.5: Post hoc analysis comparing fruit set between pollination treatments using Tukey's Honestly Significant Difference comparisons. Results show confidence intervals and respective P values for comparisons between different treatments.

Treatments compared	Difference	Lower CI	Upper CI	P
Crossed cf. Bagged	26.5	14.99	38.01	< 0.001
Natural cf. Bagged	15.0	3.49	26.51	0.006
Selfed cf. Bagged	14.5	2.99	26.01	0.008
Natural cf. Crossed	-11.5	-23.01	0.01	0.050
Selfed cf. Crossed	-12.0	-23.51	-0.49	0.038
Selfed cf. Natural	-0.5	-12.01	11.01	0.999
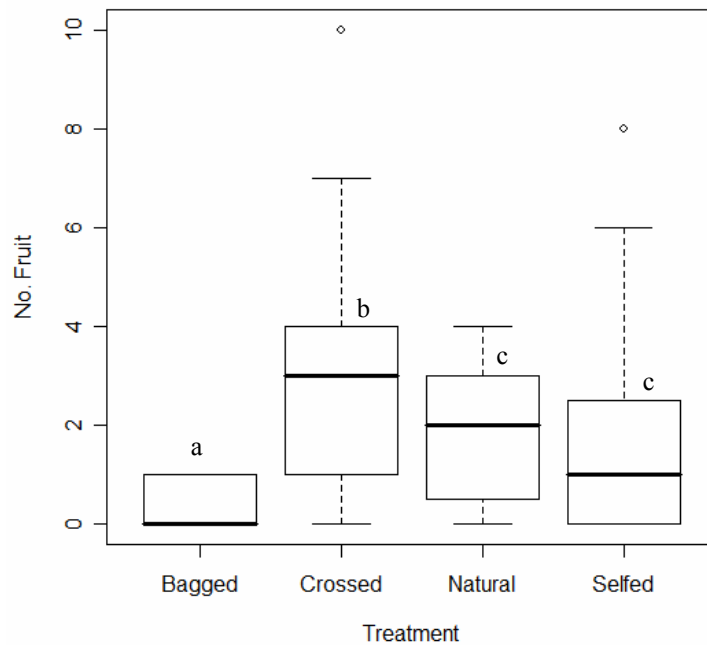
Figure 2.1: Average number of fruit out of 10 produced from flowers of each treatment. Circles represent outliers. The boxplot also shows the median and 95th, 75th, 25th & 5th percentiles. Treatments (means ± SE): B = bagged (3.0% ± 1.1%); C = crossed (29.5% ± 5.3%); N = natural (18.0 ± 2.9); S = selfed (17.5% ± 4.9%).

Site 2, Patch 4 was a little unusual because I had a lot of trouble getting flowers for the selfed treatment as many of the flower didn't produce any pollen. Of the ten flowers tagged originally, only three produced pollen and could be used. Several attempts using new flowers eventually enabled me to increase the sample size to ten. However, the Patch was destroyed by workmen before I was able to obtain the results from the last three flowers. Consequently, there were only seven flowers scored for self-pollination at this site.

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Collected fruit

A large proportion of the fruit set was unable to be collected as it was removed before it was ripe. Despite attempts to reduce removal through bagging fruit as they ripened and identification on the fruit itself, 48 fruit were removed overall, 12 of which were destroyed by a roadside mower. Others were probably removed by humans or animals. Several bags were found with holes bitten in them and the fruit removed.

There was a strong correlation between the length and width of fruits, as has been observed in other *Passiflora* species (Souza *et al*, 2004), with an r^2 value of 0.71. Fruit size and seed count were very variable (Appendix 1.2). Overall, fruit produced an average of 108.2 seeds and were 6.5 cm long and 3.1 cm wide. The maximum seed count was 213 while the minimum was 32. Lengths ranged from 4 cm - 9.1 cm and widths from 1.4 cm –4.4 cm.

Two ANOVAs showed no significant difference between the length or width of the fruit collected from flowers that had received different treatments (Table 2.6). Patch was included as a main effect but not found to be significant.

Table 2.6: ANOVA tests of the effect of pollination treatments on the length, width and seed count of banana passionfruit fruits from four pollination treatments, including Patch as a main effect.

	Df	SS	MS	F	Pr(F)
Length					
Patch	16	34.65	2.17	1.73	0.081
Treatment	3	0.69	0.23	0.18	0.907
Patch:Treatment	22	29.79	1.35	1.08	0.404
Residuals	40	50.12	1.25		
Width					
Patch	16	4.27	0.27	1.03	0.446
Treatment	3	0.98	0.33	1.26	0.301
Patch:Treatment	22	6.77	0.31	1.19	0.309
Residuals	40	10.35	0.26		
Seed Count					
Patch	16	64769	4048	2.41	0.012
Treatment	3	4395	1465	0.87	0.464
Patch:Treatment	22	42970	1953	1.16	0.331
Residuals	40	67237	1681		

The mean and median number of seeds was lowest in the natural treatment, however the number of seeds in each fruit was very variable (Fig. 2.2). The effect of treatment on total seed count was not significant suggesting that the number of seeds fruit produce are not affected by inbreeding depression or pollen limitation (Table 2.6). Seed count was significantly variable across patches so some plants produce fruit with more seeds than other plants.
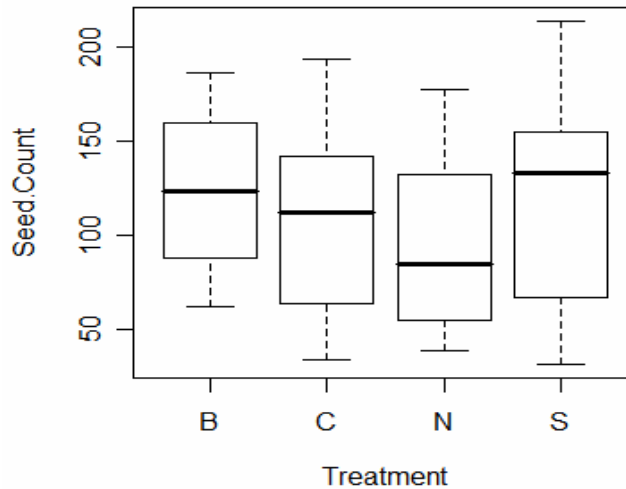
Figure 2.2: Number of seeds in fruit collected following four pollination treatments. Each shows median number of seeds and the 95th, 75th, 25th & 5th percentiles. Treatments (mean ± SE): B = bagged (123.75 ± 25.61); C = crossed (109.30 ± 7.50); N = natural (94.11 ± 10.28); S = selfed (114.57 ± 10.77).

2.5.3 Second pollination experiment

Overall, 35.19% of flowers successfully produced fruit in the second pollination experiment compared with 23.5% fruit set for selfed and crossed flowers in the first pollination experiment. Selfed and crossed flowers had 33.3% and 37.0% fruit set respectively. However, in contrast to the first pollination experiment there was no significant treatment effect on fruit set in the second experiment ($F_{1,9} = 0.181$, p = 0.671). Once again collecting fruit was problematic due to 11.1% of fruit being removed before harvesting.

The same analysis on length, width and total seed count as in the first pollination experiment was used on the fruit collected from the second experiment. The results obtained were similar to those found previously with no significant effect of treatment being found on the length and width of fruit or on total seed count (Table 2.7). Overall in the second experiment fruit were slightly larger (see Appendix 1.3 for individual means and SE). On average they had 139.9 seeds (compared with 108.15 from the first experiment) and were 7.9 cm long and 3.7 cm wide. The maximum seed count was 247 while the minimum was 49. Lengths ranged from 5.5 cm – 10.1 cm and widths from 2.7 – 5.0 cm. The increase in the sizes and seed counts for fruit is probably due to a seasonal effect because the second pollination experiment was conducted in the following summer which was not as dry as the previous year.

Table 2.7: ANOVA results showing comparisons of fruit length, width and total seed count between selfed and crossed fruit. The effect was of patch was tested as a main effect.

	Df	SS (2 dp)	MS (2 dp)	F (2 dp)	Pr(F)
Length					
Patch	8	10.44	1.31	1.58	0.229
Treatment	1	1.37	1.37	1.65	0.223
Residuals	12	9.92	0.83		
Width					
Patch	8	2.77	0.35	1.13	0.408
Treatment	1	0.40	0.40	1.31	0.274
Residuals	12	3.66	0.31		
Seed Count					
Patch	8	26438	3305	2.46	0.077
Treatment	1	1196	1196	0.89	0.364
Residuals	12	16093	1341		

Crossed fruit in the second sample had a mean number of 137.18 ± 16.05 filled (large) seeds while selfed fruit had 121.91 ± 12.82. An ANOVA showed the effect of treatment on the number of filled seeds in fruit to be not significant ($F_{1,8} = 1.33$, $p = 0.282$). Consequently, there do not appear to be fewer filled seeds in selfed fruit which shows that selfed fruit are not contributing fewer seeds to the seed pool.

2.5.4 Germination success

Overall, the mean germination success across treatments from the first pollination experiment was 39.9% in the glasshouse and 15.3% in the field. The binomial GLMs (Table 2.8) revealed significant treatment effects in both the glasshouse and field, even after the variance accounted for by site was removed, showing that the different pollination treatments did have an impact on the viability of seeds from the resulting fruit. In addition, there was a main effect of site and an interaction between site and treatment suggesting that the effect treatment has on seed viability is dependent on which site the fruit was from. However, treatments at Site 2 were applied later and consequently the fruit also ripened and were planted later so this is likely to be a seasonal effect or because of the small sample size as the sample included far fewer fruit from Site 2.

Table 2.8: Results comparing germination success of seeds from selfed, crossed, bagged and natural fruit. F test for significance used on binomial GLM.

Factor	Df	Deviance	F	Pr(F)
Glasshouse				
Site	1	24.96	24.96	$P < 0.001$
Treatment	3	158.33	52.78	$P < 0.001$
Site:Treatment	2	18.90	9.45	$P < 0.001$
Residuals	29	707.68		
Field				
Site	1	23.17	23.17	$P < 0.001$
Treatment	3	16.89	5.63	$P < 0.001$
Site:Treatment	3	11.76	3.92	$P = 0.008$
Residuals	35	256.81		

There was wide variation in germination success but both the results from the field and the glasshouse showed the same trend (Fig. 2.3). Crossed fruit had the greatest germination success on both accounts. Seeds from only three bagged fruit were able to be planted in the field, causing the data to be skewed. Surprisingly, germination was consistently lowest in seeds of natural fruits. In the glasshouse, germination success in seeds from natural fruits was only 46% of the total germination success of crossed seeds (Table 2.9). In the field, only 10% of natural seeds germinated. Inbreeding depression proved to have an effect on seed viability as bagged and selfed seeds had lower germination success than crossed seeds although the difference was small in the field. Some of the non-germinated seeds are likely to be dormant rather than dead.

Figure 2.3: Percentage of seeds that germinated in each treatment in the glasshouse and field. Circles represent outliers. Each shows the median germination and the 95th, 75th, 25th & 5th percentiles. Note that the graphs are not on the same Y-axis scale making the comparisons between treatments easier.

Table 2.9: Percentage germination success in seeds from the four pollination treatments planted in the glasshouse and field (mean ± SE).

Treatment	Selfed	Crossed	Bagged	Natural
Glasshouse	40.25 ± 11.52	49.53 ± 7.79	28.50 ± 10.94	22.61 ± 13.45
Field	15.00 ± 2.91	16.48 ± 3.61	25.33 ± 15.33	10.07 ± 3.51

2.5.5 Inbreeding effects on seedling growth with competition

The significance tests shown in Table 2.10 and 2.11 reveal that although the means for selfed seedlings were higher on all traits investigated (Table 2.12), there was no significant treatment effect in any of the four growth characteristics measured. The difference between treatments in the number of leaves was suggestive (P = 0.06) but not significant. Tray explained a significant amount of the variance in the number of leaves, stem length and leaflet length. There were also significant interactions between Tray and Treatment for stem length and leaflet length. The dry weight of harvested seedlings did not differ at all between trays or treatments. This experiment showed no evidence of inbreeding depression affecting the survival or growth of seedlings, even in the presence of environmental stress as a result of competition. One selfed seedling grew to 95.5 cm tall in the 118 days (approximately 4 months) with 21 leaves and a dry weight of 3.586 g, despite less than optimal conditions.

Table 2.10: Results from GLM using Chi squared analysis to determine whether there is a significant difference in the number of leaves on selfed and crossed seedlings.

Factor	Df	Deviance	P(Chi)
Tray	1	4.29	0.038
Treatment	1	3.47	0.062
Tray:Treatment	1	1.75	0.186
Residuals	96	94.29	

Table 2.11: ANOVA comparisons to determine whether there is a significant difference in stem length the top leaf and the length of the longest leaflet between selfed and crossed seedlings grown in competition with grass. Data for stem length and biomass have undergone log and square root transformations respectively to meet the normality assumptions of these analyses.

	Df	SS	MS	F	Pr(F)
Stem Length					
Tray	1	1.90	1.90	8.73	0.004
Treatment	1	0.27	0.27	1.25	0.267
Tray:Treatment	1	1.05	1.05	4.83	0.030
Residuals	96	20.93	0.22		
Leaflet Length					
Tray	1	13.62	13.62	9.98	0.002
Treatment	1	1.02	1.02	0.75	0.389
Tray:Treatment	1	8.94	8.94	6.55	0.012
Residuals	96	130.98	1.36		
Dry Weight					
Tray	1	0.03	0.03	0.44	0.509
Treatment	1	0.00	0.00	0.02	0.884
Tray:Treatment	1	0.21	0.21	2.65	0.107
Residuals	96	7.60	0.08		

Table 2.12: Means and standard errors comparing selfed and crossed seedlings grown in competition with each other and grass. No. leaves = the number of leaves on the stem; Stem length = the length of the stem to the top leaflet; Longest leaflet = the maximum length of the longest leaflet on the stem; Dry biomass = the dry weight of the stem and leaves above the soil.

Treatment	No. leaves	Stem length (cm)	Longest leaflet (cm)	Dry biomass (g)
Selfed	8.9 ± 0.5	45.5 ± 3.0	5.4 ± 0.2	0.95 ± 0.09
Crossed	7.9 ± 0.4	39.7 ± 2.5	5.2 ± 0.1	0.91 ± 0.06

Armbruster and Reed (2005) stressed the importance of combining the components of life stage into an index that measures overall fitness in order to accurately determine the effect of inbreeding depression on a species. The number of seedlings produced per flower (Table 2.13) determined by combining fruit set, seed set and germination success indicated that crossed flowers gave rise to nearly twice the number of seedlings' as selfed flowers. The trends were the same in the glasshouse and field with natural flowers producing fewer seedlings than both hand-pollinated treatments, but more than result from autogamous reproduction. Sample means for the number of seeds per fruit were used although they didn't differ significantly, because they are the best estimate we have.

Table 2.13: Summary showing the effects of pollination treatments on banana passionfruit progeny establishment by combining different life stages. Seedlings per flower gives an indication of the number of seedlings that will result from each flower – calculated by multiplying the proportion fruit set, the average number of seeds in each fruit, and the proportion of seeds that germinated.

Treatment	Proportion fruit set	mean seeds/fruit	% germination		seedlings per flower	
			Glasshouse	Field	Glasshouse	Field
Bagged	0.030	123	28.5	25.3	1.1	0.9
Crossed	0.295	109	49.5	16.5	15.9	5.3
Selfed	0.175	115	40.3	15.0	8.1	3.0
Natural	0.180	94	22.6	10.1	3.8	1.7

2.6   DISCUSSION

The main objective of this aspect of research was to determine the extent to which pollinators are necessary for successful reproduction of banana passionfruit. Many lianas are able to propagate effectively using vegetative reproduction (eg. rooting of stems, layering etc) which enhances their ability to exploit favourable conditions (Laurance *et al*, 2001). Banana passionfruit failed to produce any fruit from the 40 flowers selected to test for apomictic reproduction and had very low fruit set inside bags, showing it requires biotic pollinators for successful reproduction. I have chosen to focus this part of the discussion on mating systems, pollen limitation and inbreeding depression in banana passionfruit. The implications of these discoveries in the context of invasiveness and mutualisms will be addressed in Chapter 4.

2.6.1 Breeding system

The overall fruit production across treatments was very low with only 17% of flowers producing fruit in the first pollination experiment. However, this was compensated for by the fact that each fruit had on

average 108.15 seeds. Removing pollinator visitations to flowers significantly reduced the number of fruit that were produced. This indicates that while it is possible for *Passiflora mollissima* to produce a small number of fruit autogamously, fruit set is significantly reduced indicating that they rely strongly on pollinators for successful flower fertilisation and fruit production. This is consistent with research from Hawaii by LaRosa (1992) which shows that pollinators play a major role in the reproductive ecology of *P. mollissima* and significantly increase fruit production. There are two likely reasons for this. Firstly, the flower morphology is such that inflorescences are large and open with anther-stigma separation so there is a low chance the anthers will make contact with the stigma in the absence of external interference. Secondly, banana passionfruit pollen is large and sticky so probably unable to be wind-dispersed. On the rare occasion that flowers do produce fruit autogamously, the pollen is able to effectively fertilise the flower. However, there is some degree of self-incompatibility as selfed flowers were shown to produce significantly (40%) less fruit than crossed flowers. This may be due to partial self-incompatibility in banana passionfruit which, as mentioned in the introduction, has been found in several other *Passiflora* species. Alternatively, it may be the result of inbreeding depression where an increased number of homozygotes results in the expression of recessive deleterious mutations causing the flower to die or be aborted (Keller & Waller, 2002; Parker & Haubensak, 2002). Given that banana passionfruit seems to rely so heavily on pollinators, it is of particular interest that visitations to flowers were almost always from introduced honey bees and bumble bees.

2.6.2 How effective are pollinators?

We have established that pollination from insect visitors plays an important role in subsequent fruit production which raises the query of how effectively bees are pollinating flowers given that the flowers are large, specialised and pollinated by birds in their indigenous environment (Endress, 1994). Banana passionfruit is currently thriving in many areas around New Zealand, despite control efforts in many cases, which suggests fairly good pollinator performance. However, significantly fewer fruit were produced by natural flowers than those that were cross-pollinated indicating some degree of pollen limitation.

There are several characteristics that determine whether a flower will be effectively pollinated by visiting species. Several are outlined in Robertson *et al* (2005) such as the ability of visitors to pick up the pollen and to deposit it onto the stigma. The quality and quantity of pollen deposited as well as the frequency of visitation are also important (Anderson, 2003). There must be pollinators available to visit the flower whilst it is open and the stigma is receptive. During flower visitation observations, the periods when the greatest number of visitors were observed tended to be early in the day. For example the greatest number of pollinators were observed in the observation period commencing at 9.52 am, rather than later when rewards were depleted. In addition, I experienced difficulties collecting pollen for cross-pollinations even when freshly opened flowers were visited during early morning. This suggests that pollen is removed

promptly after the flower has opened. This was also found to be the case by Lange and Scott (1999) who observed that hummingbird visitation rate to *Penstemon pseudospectabilis* (Scrophulariaceae) was higher in the morning. In addition, Janzen (1968) observed banana passionfruit flowers and pollinator behaviour and concluded that flowers open very early in the day to match the activity period of hummingbirds.

According to the calculations made from flower observations, each flower has on average 3.69 bee visits an hour, which will stay for 64.1 seconds in total. Therefore, it may receive over 50 minutes pollination time from multiple bees whilst in bloom. These data were collected using similar methods and analysis to Robertson *et al* (2005) who observed visitors to native mistletoe flowers (*Peraxilla colensoi* and *P. tetrapetala*) over three years. They observed much lower visitation rates by native bees, the predominant visitors to flowers. In one year (l996/97) their visitation rates varied between 1.25 ± 0.69 and 9.40 ± 9.28 s $fl^{-1} hr^{-1}$ compared with the 64.1 ± 20.6 seconds per flower per hour that I observed. In addition, Lange and Scott (1999) observed hummingbird visitation rates to *Penstemon pseudospectabilis* to be only $0.30 \pm SD$ 0.23 visits per flower per hour and about 3.6 visits per day, so hummingbird visitations are likely to much less frequent. Consequently, banana passionfruit flowers do not seem to be limited by low pollinator visitation rates.

Pollinators are also required to deposit viable, compatible pollen onto the stigma in order to be effective (Ford *et al*, 1979). The amount of pollen that pollinators carryover may also be important especially for species, such as the skunk vine (*Paederia foetida L,* family Rubiaceae), that aren't self compatible (Liu *et al*, 2006). We have already established that banana passionfruit is at least partially self-compatible so any pollen deposited on the flower should be capable of producing fruit. Honey bees are active, constant foragers that are effective pollinators in many species of plant (Kearns & Inouye, 1997) and are capable of carrying large pollen loads (Buchmann & Nabhan, 1996). However, honey bees are only about 12 mm long and bees that are too small for a flower will transfer few, if any, pollen grains (Kearns & Inouye, 1997). Several studies have shown fruit set (number of successful flowers) and/or seed set (number of seeds per fruit) to be significantly lower when inflorescences of large flowered species were visited by insects rather than birds (Anderson, 2003; Celebrezze, 2004). In addition, Anderson (2003) observed that insect visitors tended to focus their activity below the stigma. Janzen (1968) observed large queen bumble bees (*Bombus medius*) visiting banana passionfruit flowers and 11 times the bees body failed to even touch the anthers en route up the floral tube to the nectar source. Indeed I have observed bumble bees flying up inside the elongated floral tube of the flower to the nectar source without touching the stigma. This would not be the case for the swordbill hummingbird that pollinates these flowers in their homeland. This suggests the primary reason banana passionfruit is pollen limited may be because the flowers are so big that bees are able to collect pollen and nectar without being forced to brush the stigma and consequently pollinate the flower.

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2.6.3 Inbreeding depression and the long term establishment of progeny

To gain a more full perspective on the effects of pollen limitation and mating systems on the establishment of progeny, multiple fitness traits from a range of life history stages must be examined (Brennan *et al*, 2005). In particular, it would be interesting to know whether banana passionfruit suffers inbreeding depression that may reduce seed viability and depress seedling growth. Studies such as Colling *et al* (2004) have found that selfed flowers not only produce less fruit but the fruit are of lower quality or seed count. However, while pollination treatments did have a significant effect initially on fruit set, this did not necessarily translate to other life history traits. On the whole crossed fruit tended to be longer and wider which would suggest a greater seed count, but there was a huge variation in fruit characteristics and no significant treatment effect was detected on any of these traits. The number of seeds in each fruit appeared to be more affected by what plant the fruit came from than by treatment, as patch explained a significant proportion of the variance. This variance in seed set across plants is probably a reflection of differences in plant health and resource availability as physical resources such as water or nutients may limit seed production (Byers & Waller, 1999; Souza *et al*, 2004). For example, Patch 3 in Kaireperepe Bay was particularly dry and located on a clay slip which may explain why it didn't produce any fruit in any treatment. It seems that inbreeding depression does affect whether a given flower will produce a fruit. However, once the fruit has begun developing it makes little difference whether the pollen originated from the same plant or was out-crossed in terms of the size and seed set of the fruit. Parker and Haubensak (2002) found the same result in an invasive species of broom (*Genista monspessulana*) which suffered a 50% reduction in fruit set in selfed flowers but no reduction in seed set. Furthermore, this has been demonstrated in a broad review of 258 species by Burd (1994) which showed that fruit set is more adversely affected by self-pollination than seed set, suggesting that plants don't produce a fruit unless it has a minimum number of seeds.

Although the total number of seeds didn't vary between treatments, some fruit had small seeds that were undeveloped and looked to be unviable. These were not considered whilst doing seed counts in the first pollination experiment. However, this would be a possible expression of inbreeding depression if there were to be a greater number of nonviable seeds in selfed fruit. In this case, selfed fruit may contribute fewer viable seeds and consequently progeny to the next generation, an effect that may be masked in the first pollination experiment. However this was remedied in the second pollination experiment which showed that selfed fruit do not contribute fewer large seeds to the environment than crossed fruit as there was no statistical difference in the number of large seeds between them. In addition, it reaffirmed that there was no significant difference in fruit size or seed count, between selfed and crossed fruit. Surprisingly, fruit set was not significantly affected by treatment in the second pollination experiment although selfed fruits still produced less seeds than crossed fruits. This may be related due to the limited sample size. Alternatively, it may be an exhibition of variability of response to inbreeding depression

depending on the stress of the environmental conditions (Armbruster & Reeds, 2005) because although the flowers were selected at approximately the same time of year, the summer of 2005/06 was much drier than 2006/07 (NIWA Science National Climate Centre, 2007).

On the whole, germination success was more than 50% greater in glasshouse (49.5%) than in the field (22.6%). This is common due to the ideal growing conditions of a glasshouse (Robertson *et al*, 2006). Byers & Waller (1999) stated that measurements of inbreeding depression may differ due to the stress, or lack there of, in different environmental conditions. However, the germination experiment was conducted in both the field and glasshouse as suggested by Armbruster and Reed (2005) and the significant treatment effect on total percentage germination success displayed the same trend regardless of environmental conditions (Fig. 2.3). Bagged and selfed flowers had slightly lower germination success suggesting some effects of inbreeding depression on germination success. However, the difference did not seem to be as pronounced as it was for fruit set, particularly in the field where only slightly fewer selfed seeds germinated (15.0%) compared with crossed (16.5%). Surprisingly, natural seeds were far less likely to germinate than the other three treatments. Unlike fruit set, this is not a direct effect of pollen limitation because the seed has already been produced and consequently presumably has been fertilised. The reason that natural seeds may be doing so badly is quite puzzling as we would expect selfed fruit to have the least-viable seeds. Colling *et al* (2004) conducted a study on *Scorzonera humilis* (Asteraceae) in which supplementary hand pollination not only increased the number but also the quality of the seeds produced. Consequently survival of offspring from hand-crossed flowers was nearly twice that of those that were naturally pollinated. They also found that germination of seeds in one population was increased by pollen supplementation. They suggested two potential reasons for this. Firstly, hand-crossing with pollen from further away decreased inbreeding depression because pollinators usually come from other plants in the immediate vicinity. Each mature banana passionfruit plant is large with a lot of flowers and consequently, bees may be delivering a high proportion of selfed pollen resulting in inbreeding depression in natural seeds. However, this does not explain why seeds from natural fruits are doing so much worse than selfed progeny. Secondly, they suggested that pollen saturation on the stigma in hand-pollinated treatments may increase selectivity among gametes during fertilisation or pre-fertilisation by increasing pollen competition and/or female choice. This results in seeds from pollen with high fitness and vigour that consequently have higher fitness and vigour themselves. A combination of these factors could explain the poor germination success of seeds from natural banana passionfruit flowers. We have already established that they are pollen limited which will reduce or eliminate pollen competition. If they receive mostly self-pollen from pollinators the effect of pollen limitation may be compounded by inbreeding depression explaining the low germination success we are seeing in seeds from natural fruits.

The final test for inbreeding depression looked at the growth of crossed and selfed seedling when put under competition with grass as stress often reveals the effects of inbreeding depression (Byers & Waller, 1999). The means by which environment affects the expression of inbreeding depression may be through intrinsic factors, such as the density of inbred individuals, or through extrinsic environmental factors such as lack of nutrients or water (Cheptou, 2006). Consequently, seedlings were transplanted close together in the trays with limited resources, however selfed and crossed seedlings all survived and there was no significant reduction in selfed seedlings in any of the traits measured. Furthermore, means were consistently higher for selfed seedlings on all accounts. This suggests that selfed seedling are able to exploit resources and grow just as quickly as crossed seedlings, even in a harsh environment so there does not appear to be any effect of inbreeding depression in seedling growth and survival at least within the first four months.

In short, inbreeding depression showed significant effects on fruit set and germination success but no effect on seed set or seedling survival and growth. During the colonisation of banana passionfruit in New Zealand the population would have undergone a significant bottleneck (Nielsen *et al*, 2007). In order to fully decipher the extent to which banana passionfruit in New Zealand suffers from inbreeding depression, comparisons between self-pollinated flowers and flowers crossed with pollen from its Andean native habitat would be required as the entire population may be affected by inbreeding depression (Keller & Waller, 2002). However, by combining results from the different traits we can gain a fuller understanding of the extent to which banana passionfruit is limited by inbreeding depression. This is important because its effect on one component of the lifecycle will have consequences in another by reducing competition or enhancing growth or fertility of progeny (Armbruster & Reed, 2005). Thus, the effects of inbreeding and pollen limitation are combined to determine the number of seedlings that will result from each flower (Table 2.13). In optimal conditions – i.e. when the stigma is saturated with crossed pollen and seeds germinated in a warm, light, hydrated, nutrient-rich environment - *P. mollissima* may produce over 15 seedlings from one flower. Out-crossed flowers whose seeds were germinated in the field on average produced over five seedlings per flower. It appears that wild banana passionfruit plants are currently reproducing at a rate of 1.7 seedlings per flower. This is quite high when compared with Colling *et al* (2004) who calculated the number of seedlings per flower head using the same components in *S. humilis*. Their most successful treatment between-population, hand-crossed produced only 1.57 seedlings per flower head. In contrast, in this experiment bagged flowers produced only 0.9 seedlings per flower which suggests that if banana passionfruit had to rely on autogamous reproduction plants would produce less than half the number of offspring. Consequently, in the absence of introduced bees, banana passionfruit may not have established or been able to proliferate to the extent to which it has now. Surprisingly, self-hand-pollinated flowers appear to have greater fitness (3.0 seedlings per flower) than natural flowers.

Byers and Waller (1999) believed that particular attention should be paid to male fitness components such as pollen number and viability. This was not directly studied in this research. However, the observable variation in the amount of pollen found on the anthers between patches and flowers would imply differences in male fitness between plants. Some anthers were very small and shrivelled with little pollen on them. Several flowers had no pollen at all. This was particularly problematic in Patch 4 in Kaireperepe Bay where it took several attempts to get flowers with enough pollen to complete self-pollinations. This could be significant in the maintenance of the reproductive system of banana passionfruit as the function of flowers in some plants may become predominantly maternal, potentially leading to gynodioecy. Alternatively, this could be due to lack of resources such as water and may just indicate an overall lower plant fitness considering Site 2 Patch 4 only produced one fruit from all the treatments combined. No conclusions in this area can be made from the research presented here but it may be of interest in future studies.

In indigenous populations of banana passionfruit, flowers reproduce predominantly by outcrossing and show a high degree of self-incompatibility (LaRosa, 1992). This may be in order to reduce geitonogamy as banana passionfruit vines become very large, or alternatively it may just be in response to inbreeding depression. In contrast, in New Zealand and Hawaii species appear to have a greater ability to self-pollinate. In order for an outcrossing mating system to persist, inbreeding depression must reduce the fitness of progeny by more than half (Brennan *et al*, 2005). Nielson *et al* (2007) suggested that new colonisers (particularly on islands), with a self-incompatibility system to prevent selfing may experience selection for a partially leaky system to enhance reproductive assurance. This may also be the case for banana passionfruit in New Zealand. A partial breakdown in the self-incompatibility complex would allow it to endure the small population size and loss of native pollinators. Inbreeding depression decreased the number of seedlings produced by approximately half. Therefore, because inbreeding depression is not extremely severe, the mixed mating system may persist in New Zealand although there will also be selection pressure toward predominant outcrossing.

2.6.4 Conclusions

Kearns and Inouye (1997) stated that the plants most at risk from loss of a pollinator are those that are dioecious and self-incompatible, those that have a single pollinator, and those that propagate only by seeds. While the structure of banana passionfruit flowers suggest a specific pollinator and it propagates only by seed, it is neither dioecious nor completely self-incompatible. In addition, community composition varies among habitats and what appears to be a specialized plant-pollinator relationship may change among communities (Kearn & Inouye, 1997). This appears to be the case with *P. mollissima* in New Zealand where hummingbirds have been replaced by honey and bumble bees. It is interesting that in New Zealand, visitations to flowers are predominantly by introduced bees rather than native bees. These

pollinators are important as banana passionfruit appears to rely on pollinators for fruit production because they don't often reproduce autogamously. Consequently, banana passionfruit may not have been able to survive and establish in New Zealand in the absence of these exotic pollinators.

Flowers are self-compatible though inbreeding depression significantly reduces fruit set, germination success and results in the production of fewer seeds per flower head. However, inbreeding depression does not appear to negatively affect seed set in fruits that do develop, nor the survival and growth of seedlings. There is some degree of pollen limitation so bees are not as effective as the hummingbirds with which it coevolved. Nevertheless, *P. mollissima* is still managing to produce numerous fruits which have many seeds. While banana passionfruit relies mostly on outcrossing in its native habitat (LaRosa, 1992), it seems in New Zealand it has developed a mixed mating system that combines out-crossing with selfing.

Chapter 3: DISPERSAL
The role of introduced mammals in the spread of a noxious weed

3.1   INTRODUCTION

3.1.1 Dispersal mutualisms

Some of the most damaging invasive plants are dispersed by frugivores. Consequently, practical information describing the ways in which dispersal mutualisms are affecting the spread of weeds is extremely important for effective management and control (Buckley *et al*, 2006). The adverse effects of exotic mammals in New Zealand are widely acknowledged and frequently investigated (e.g. Sweetapple *et al*, 2004). However, the effect to which introduced animals may be responsible for enhancing the invasiveness and success of exotic weeds through dispersal mutualisms is not commonly addressed (Williams & Karl, 1996), despite the fact that invasiveness of exotic species in new habitats is strongly dependent on patterns and mechanisms of seed dispersal (Constible *et al*, 2005). Some invasive species are unable to reproduce and subsequently would not have established in the absence of frugivores (Panetta & McKee, 1997). Banana passionfruit continues to spread throughout the country and is becoming increasingly problematic in many areas. However, little is known about its dispersal ecology in New Zealand and in particular the effect of ingestion and gut passage on seedling survival (Williams & Buxton, 1995). As a result, this study seeks to gain a greater understanding of the frugivorous dispersal mutualisms of banana passionfruit in the Marlborough Sounds.

3.1.2 Benefits of dispersal

Seeds may benefit from dispersal because it allows colonisation of new areas, escape from predation and may enhance germination success (Kelly *et al*, 2004). Robertson *et al*, (2006) discussed three ways that frugivores can directly affect success in seed germination. Firstly, the pulp of some fruit contain chemical compounds that suppress germination so removal of seeds from the pulp may result in increased germination success (the deinhibition effect). Seed removal may also reduce the likelihood of microbial or fungal attack (Traveset, 1998).

Secondly, endozoochory is the mechanism of dispersal whereby frugivores ingest seeds and excrete them after a gut retention period (Constible *et al*, 2005). This form of dispersal has been well documented and is especially common in fleshy-fruited plants and grasses (Nogales *et al*, 2005). The effect of the gut passage on the seed may increase the permeability of the seed coat allowing water and gas through and consequently resulting in increased germination; the scarification effect (Robertson *et al*, 2006). Alternatively, ingestion of fruits by frugivores may have a negative effect or no effect at all on the likelihood that a seed will germinate and establish (Traveset, 1998). Whether a seed is positively, negatively or not affected by ingestion depends on both of the species involved and is unpredictable as no

pattern appears to exist, regardless of the genetic relatedness of plant species (Nogales *et al*, 2005). Traveset (1998) conducted a meta-analysis on 200 plant species and found that the germination speed and proportion of seeds germinating was affected by gut passage in 50% of plant species. Two thirds of the species that were affected experienced enhanced germination success. However, in the other third, seeds were damaged and thus germination was inhibited. Different animals groups appeared to have similar effect on germination, although non-flying mammals influenced germination slightly more often (Traveset, 1998). For example, Nogales *et al* (2005) found that seed-coat thickness decreased significantly in *Rubia fruticosa* (Rubiaceae), after passing through the digestive tracts of reptile, bird and mammal frugivores but mammal species damaged a greater proportion of seeds, probably due to a greater gut retention time. Dungan *et al* (2002) found that ingestion by possums resulted in a significant proportion of seed damage although the effect of gut passage varied widely between species. In contrast, it appears that at least some monkeys pass most to all seeds in viable condition (Schupp, 1993). The outcome of seed passage through the digestive tract may depend on the frugivore's morphological and physiological traits such as the length of the digestive tract, the presence of a gizzard and the digestive fluids in the gut (Nogales *et al*, 2005). In addition, seed-deposition patterns will also affect the probability that seeds are deposited in an environment suitable for germination.

Thirdly, there may be a fertilisation effect from faecal material that enhances germination success because seeds are deposited in nutrient- and microbial-rich faeces (Robertson *et al*, 2006). Buckley *et al* (2006) documented several cases where seedling growth was positively affected by the fertilisation effect, resulting in enhanced growth due to the shelter from desiccation or an increased probability of secondary dispersal, or negatively affected due to increased seed predation.

The relative contributions of the deinhibition effect, the scarification effect and the fertilisation effect need to be addressed in order to have a holistic view of the frugivorous mutualisms that are important for the survival of a particular species. In a review of 51 plant species, Robertson *et al* (2006) found that the deinhibition effect was larger than the scarification effect in all but seven cases. Still, it is very difficult to make generalisations about the contributions of frugivorous species to plant fitness because the characteristics that define an effective dispersal vector vary between species and depend on the ecology of the plant (Schupp, 1993). For example, gut passage time strongly influences the distances that seeds are dispersed but can vary for the seeds of different species, even when ingested by the same animal species (Buckley *et al*, 2006). Schupp (1993) discusses two main ways in which frugivores vary in the extent to which they contribute to plant fitness, namely dispersal quantity and dispersal quality. Firstly, effective seed dispersal depends on the number of visits made to the plant by a disperser and the number of seeds dispersed per visit. There are many variables that might affect the quantity of fruits being dispersed

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including frugivore density, gape size, dietary constraints and preferences as well as the size and availability of fruits (Buckley *et al*, 2006). Secondly, the quality of seed dispersal also affects plant fitness and depends on factors such as the treatment the seed receives in the mouth and gut of the frugivore, the distance the seed is dispersed from the parent plant, the level of competition with other seedlings and the probability that it will be deposited in a site suitable for germination (Schupp, 1993).

Kelly *et al* (2004) and Samuels and Levey (2005) both raised concerns about the ability of germination experiments to accurately distinguish between these different components of seed dispersal and their effects on seeds. They emphasised the importance of including intact fruits in experiments in order to accurately distinguish between deinhibition and scarification effects because direct comparisons between hand-cleaned seeds and excreted seeds combine the effects of these two mechanisms and do not take into account the fate of seeds that fall straight from the plant and hence remain in the fruit. They also stressed the importance of conducting experiments in different environmental conditions as this has been shown to have a significant effect on the effect of treatments. Robertson *et al* (2006) discuss similar concerns and also suggest that discrimination between dead and dormant seeds at the end of trials should be considered when interpreting results from germination experiments.

3.1.3 Banana passionfruit dispersal in New Zealand

Presumably in their home range, banana passionfruit are dispersed by monkeys although the indigenous disperser does not appear to have been documented. The relative contributions of various birds and mammals in the dispersal of banana passionfruit in New Zealand are unknown (Williams & Buxton, 1995). Little is known about whether seeds require removal from fruit in order to germinate and the effects of ingestion and gut passage on seed survival. Williams and Buxton (1995) reported that fruits were eaten particularly by possums (*Trichosurus vulpecula*) but also by Norway rats (*Rattus norvegicus*), ship rats (*R. rattus*), and kiore (*R. exulans*). However, it is not known whether seeds survive and germinate following ingestion. Similarly, pukeko (*Porphyrio porphyrio*), blackbirds (*Turdus merula*) and white-eyes (*Zosterops* sp.) have also been observed eating fruit and excreting the seeds, although none of the seeds ingested by white-eyes germinated (Williams & Karl, 1996; Williams, 2006). In contrast, Williams and Karl (1996) found that bellbirds (*Anthornis melanura*) and tuis (*Prosthemadera novaeseelandiae*) ignored the adventive fruits in their study (which included *P. mollissima*), despite their availability into winter when few indigenous fruits are available.

3.2   AIMS

To investigate and establish the relative contributions of frugivorous birds and mammals in the dispersal of banana passionfruit seeds. In particular, to test the hypothesis that seeds are primarily dispersed by introduced birds and mammals. More specifically, the main objectives are:

- To determine if seeds require removal from fruit in order to germinate
- To investigate the fates of fruit by identifying the proportion of fruit being removed compared with those that fall beneath the parent vine and the speed at which they are being removed.
- To observe which frugivores are the primary consumers of fruits and to establish whether consumption of fruits will result in effective dispersal by investigating the viability of excreted seeds.

3.3   METHODS AND MATERIALS

3.3.1 Germination – effect of fruit pulp removal on seed germination

Three main treatments (whole fruit, fleshy seeds and cleaned seeds) were used to determine whether removal of seeds from the fruit affects germination in banana passionfruit. Seeds collected from animal faecal material were also germinated using these methods and are described in more detail below. Each germination experiment was carried out in both the glasshouse and the field as results often differ significantly according to the conditions in which seeds are germinated (Robertson *et al*, 2006).

Glasshouse

Seeds were germinated in trays of potting mix in a glasshouse rather than Petri dishes in the lab because germination success tends to be adversely affected when seeds are grown in Petri dishes compared with the field, especially for intact fruits (Robertson *et al*, 2006). Each tray was half filled with fertilizer mix (containing 80% pH adjusted Horticultural Bark and 20% Bioblend of Blood and Bone – for details of fertiliser ingredients see Appendix 1.4) and segregated into four sections for different treatments. The seeds were scattered so as to be evenly distributed within each quarter. A thin layer of potting mix was then sprinkled over the seeds, followed by enough fine shingle to prevent seed desiccation and reduce the growth of mosses and liverworts. The trays were then placed in a glasshouse and heated from mid-March through winter. Summer temperatures ranged from approximately $15^{o}C$ to $32^{o}C$ averaging about $22^{o}C$ while winter temperatures ranged from $12^{o}C$ to $22^{o}C$. The seeds were watered every one to two days throughout summer and every four to five days during winter, but received no additional fertiliser.

Data recording the number of germination successes were collected monthly. Seedlings were removed as they reached 10 cm to reduce competition, assist accurate counting and so the trays were more manageable.

Field

An area of soil in the field was cleared of any vegetation and cultivated so the soil was loose in the Marlborough Sounds in area where fruit were collected. Segments of 65 mm diameter plastic down-pipe approximately 7 cm long were put in the ground to contain the seeds (similar to that found in Fig. 1 in Robertson *et al*, 2006). After the seeds had been scattered evenly in each pot (5 or 10 per pot depending on the treatment), a thin layer of soil was sprinkled overtop and some twigs were also used to provide cover from desiccation and the surrounding environment. The tubes of pipe were arranged in groups of 20 (5 x 4) and covered with pegged down wire mesh (6 mm) to stop disturbance or seed predation.

Main treatments

Fruit from nine patches (see Chapter 2) with large fruit sets were divided between two conditions, glasshouse and field. In each condition, there were three treatments: fleshy seeds, cleaned seeds and intact fruits. Each treatment had at least 10 replicate fruit (Table 3.1). Seeds were planted around two major fruiting periods. Firstly, between 15 and 27 February 2006 and secondly between 20 September 2006 and 1 November 2006. One group of tubes (containing two replicate cleaned/fleshy fruit) was found destroyed in the field reducing the sample size to ten fruit from eight patches.

Table 3.1: Number of replicate banana passionfruit in cleaned vs. fleshy and intact fruit treatments planted in the field and glasshouse in February 2006 and September-November 2006.

	Treatment	No. fruit	Replicate patches
Glasshouse	cleaned/fleshy	14	9
Field	cleaned/fleshy	10	8*
Glasshouse	whole	13	9
Field	whole	12	9

* began with nine but one plot destroyed part way through trial

Whole fruit were placed individually in a pot sitting on top of soil in the glasshouse or on the ground, covered by wire mesh to ensure they weren't disturbed by animals, in the field. The number of seeds that germinated each month were counted and seedlings removed to ensure accurate counting and to reduce competition effects. Descriptions of the fruits appearance as it decayed were also recorded. Fleshy seeds were simply separated from other seeds but left inside their fleshy, orange, capsule, while those for the cleaned treatment had all of their flesh removed. Twenty-five seeds were used in each of the fleshy and cleaned treatments. In the field these were divided amongst five tubes which alternated between treatments.

Analysis

Final germination success between cleaned and fleshy seeds were analysed using a binomial Generalised Linear Model. The response was the combined number of seeds that germinated and failed for each fruit. The source (i.e. which patch the fruit was from) and treatment were included as factors. Results from the

glasshouse and field were analysed separately. An F test was used to test for significance in the glasshouse as the residual deviance was more than 50% greater than the residual degrees of freedom. This was not the case for the field data so they were analysed using a Chi-square test for significance.

Because the starting number of seeds in each intact fruit was unknown, the counts of seeds that germinated from intact fruit were analysed to compare germination success in the glasshouse versus the field. An F test was used to test for significance on a poisson Generalised Linear Model for this analysis. In addition, the final percentage germination was estimated using the mean seed count of natural fruits in Chapter 2 (94 seeds per fruit).

3.3.2 Fruit removal rates (tagged fruit)

A group of 10 unripe fruit of similar size and maturity were selected, tagged and tracked in 10 of the 20 patches to investigate the dispersal of banana passionfruit. Groups of fruit were tagged across sites from December 2005 to February 2006 (Table 3.2). Five fruit in group C were removed by a roadside mower and consequently n = 5 for this group. Five of the patches were very close to the side of the road and consequently easily accessible by humans, while the other five were selected on steep banks and slips in areas that would be little affected by anthropogenic dispersal. Consequently, the accessibility of patches were recorded as on road or off road.

Table 3.2: Details showing the distribution of each group of 10 tagged fruit across sites and when the fruit were tagged. Data collection was completed when no fruit remained on the vine. Accessibility indicates whether fruits were directly adjacent to the road (on road) or inaccessible to most humans (off road).

	Location	Date Tagged	Date completed	Accessibility
A	Site 1	17/12/05	17/04/06	on road
B	Site 1	21/12/05	16/03/06	on road
C	Site 1	21/12/05	17/04/06	on road
D	Site 2	22/12/05	16/03/06	on road
E	Site 1	22/12/05	17/04/06	off road
F	Site 1	22/12/05	17/04/06	on road
G	Site 1	09/01/06	17/04/06	off road
H	Site 2	19/01/06	18/05/06	off road
I	Site 2	19/01/06	18/05/06	off road
J	Site 2	23/02/06	14/06/06	off road

Each fruit was tagged using three methods. Firstly, a labelled paper tag with cotton tie was attached where the stalk joins the vine. Secondly, a small length of blue wire was tied just above the fruit and thirdly, the identification number was written on the fruit itself using a felt pen. These three methods were used to try and ensure each fruit could be identified if it was found on the ground below the vine. The fruit were checked every 3-4 weeks and data collected recording whether each fruit was present on the vine, on the ground, wholly removed and/or partly eaten. In addition the colour of the fruit was recorded to give an indication of ripeness. Data were collected until all the fruit had been removed or fallen off the vine.

To determine whether there was a significant difference between the number of fruit dispersed on the road compared with off the road the fate of each fruit was summarized as either 'taken' or 'not taken'. A GLM using a Chi-squared analysis was run using 'taken vs. not' as the response with 'Accessibility' and 'Patch' as factors. Two fruit (H1 & G6) were found partially eaten on the ground so although some seeds were probably removed by the animal and dispersed they are entered as 'not taken' in this GLM.

3.3.3 Evidence of dispersal vectors

Many photos of partially eaten fruit (some still on the vine) were taken while out in the field. Some show teeth marks while others show distinct piles of fruit tissue that have been disassembled and sorted into piles on the ground.

An infra-red time lapse video camera was placed out in the field from dusk (18:00 hours) to dawn (07:00 hours) for three nights (15/6/06, 17/6/06, 19/6/06) overlooking fruit, to determine which animals are eating the fruit. The first night (15/6/06) two fruit that were nearly ripe were left uncovered but with wire through them so they didn't get pulled out of sight of the camera. The second and third nights, three fruit were placed underneath chicken wire (30 x 40 mm mesh) which had previously failed to keep predators out. Photos were taken before and after each night.

3.3.4 Ingestion effects on seed viability

Pigs

To collect droppings from feral pigs which had the opportunity to eat banana passionfruit, I talked to Ben Minehan of the Marlborough District Council who informed me of one of the largest areas of infestation in the Marlborough Sounds. Consequently, I visited Te Weu Weu Bay in Tory Channel on the 3rd of February 2006. Upon exploring the bush and scrub I discovered a lot of pig rooting and three patches of feral pig droppings. The pig droppings were found on the edges of rooted areas and were approximately 40-50 metres apart. Banana passionfruit seeds were visible in the pig droppings in the field. I collected the pig droppings and brought them back to the lab where I weighed and sieved them. I removed both the large debris and fine matter by placing the pig droppings in a 6 mm sieve on top of a 3 mm sieve and running water through them, catching the seeds and broken pieces in the 3 mm sieve. Once the seeds had been removed and counted, samples were planted in both the glasshouse (9 February 2006) and the field (16-18 February 2006) to determine whether they were still viable following ingestion by a pig and consequently whether pigs would be effective dispersal vectors. Eight hundred seeds were planted in the glasshouse (four trays with 200 evenly spaced seeds per tray) and 600 seeds were planted in the field (10 seeds per tube in three different groups of tubes). Data describing the number of seeds that had germinated were collected each month in the glasshouse and in the field for 14 months.

Possums

Seven fruit from different patches at Marlborough were fed to seven captive possums using animals from Pest Control Research in Christchurch on 24 November 2006. The possums consumed the fruit straight away and were even trying to get through the cage wall to steal the fruit off adjacent possums. The faeces were collected two days later. One hundred and eight seeds were found in the faeces of three possums. Of these 100 randomly selected seeds were planted evenly spaced in a tray in the glasshouse on 30 November 2006 and germination data collected each month for five months.

Rats

Six live traps baited with banana passionfruit were set up in the field in an attempt to collect ingested banana passionfruit seeds from feral rats. The rats proved to be very wary of the traps and only two were caught over six nights. The rats were kept in the traps two days then disposed of. I attempted to extract their excrement from the leaves and soil in and underneath the traps but this proved very difficult, especially due to some rain which made everything very soggy. It was also difficult to tell whether the seeds I found had been fully digested or were merely dropped during eating. Four seeds were extracted that appeared to have been ingested but it was difficult to be certain. Consequently, six captive Norway rats (also from Pest Control Research in Christchurch) were offered fruit originating from different patches in the Marlborough Sounds on 24 November 2006. Not all fruit were eaten although some were overripe and soft which may have been a contributing factor. In the faeces of one rat, four seeds were found and planted in the glasshouse 30 November 2006. The rest didn't appear to have any intact seeds or large seed fragments in their faeces. Germination data were collected monthly for five months as with the seeds from the possums and pigs.

Combined Analysis

A binomial Generalised Linear Model combining the germination success and failure of seeds from fleshy seeds, cleaned seeds, and the seeds collected from the droppings of pigs and possums germinated in both the field and glasshouse was run in R. An F test was then used to determine whether germination success differs significantly between these treatments and whether there is an interaction between germination conditions and germination success across treatments. Possum seeds were only planted in the glasshouse due to lack of seeds available. Two further binomial GLM's were run for glasshouse and field results that included the estimated germination percentage of whole fruits.

3.4   RESULTS

3.4.1 Germination – Effect of fruit pulp removal on seed germination

The mean percentage germination was higher in cleaned seeds than fleshy in both the glasshouse and the field (Table 3.3). Overall, a much higher percentage of seeds from cleaned and fleshy treatments germinated in the glasshouse (64.5%) compared with the field (7.7%). Similarly, more than four times as many seedlings emerged in the glasshouse from intact fruit than in the field. Whole fruits tended to break down and start germinating very quickly, which was surprising given the size of the fruit. In one fruit, the first seedling had germinated only one month after the fresh fruit was put in the glasshouse. In another, 85 seedlings germinated in under three months. Many of the seeds from the whole fruit in the field (and some in the glasshouse) had not germinated by the final data collection date but appeared to be dormant rather than dead.

Table 3.3: Germination success of seeds in cleaned, fleshy and whole fruit treatments. Means ± one standard error are shown for the glasshouse and field. Final % germination is given for clean and fleshy seeds. Actual seed count per fruit is given for whole fruit and percentage germination of whole fruit is estimated using a mean of 94 seeds per fruit.

	Cleaned (%)	Fleshy (%)	Whole (no per fruit)	Whole (%)
Glasshouse	65.93 ± 3.56	63.07 ± 5.66	47.85 ± 9.52	50.91 ± 10.16
Field	9.00 ± 5.49	6.40 ± 5.57	11.42 ± 2.08	12.13 ± 2.21

The two GLMs showed that there was significant variation in final germination success between fruit from different patches (Table 3.4). There was no treatment effect in the glasshouse suggesting that seeds did not germinate more readily when the pulp was removed. In the field, cleaned seeds were not significantly more successful although the results were suggestive of an effect (P = 0.076).

Table 3.4: GLM results comparing final germination success in cleaned and fleshy seeds. Glasshouse and field results analyses separately with using a F test and Chi square test respectively.

	Glasshouse					Field		
	Df	Deviance	F	Pr(>F)		Df	Deviance	Pr(Chi)
Source	8	50.08	6.26	P < 0.001		7	134.63	P < 0.001
Treatment	1	0.67	0.67	P = 0.412		1	3.16	P = 0.076
Source:Treatment	8	20.31	2.54	P = 0.009		7	5.10	P = 0.648
Residuals	10	23.45				4	2.81	

Figure 3.1 demonstrates the difference that environmental conditions make to the number of seeds that germinate from intact fruit. The maximum number of seeds that germinated from one fruit in the field was 22 compared with 87 in the glasshouse. The GLM showed this difference to be highly significant ($F_{(1,23)}$ = 297.87, p < 0.001).
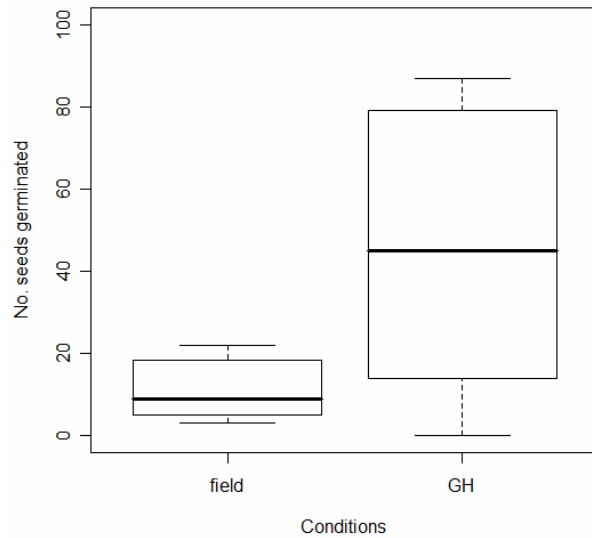
Figure 3.1: Seed counts showing germination success from intact banana passionfruit when left in the glasshouse or field. Graph depicts median and 95th, 75th, 25th and 5th percentiles of seeds that germinated from intact fruit.

3.4.2 Fruit removal rates (tagged fruit)

Table 3.5 gives a summary of the fate of each fruit across patches. Fruit took 2-3 months to ripen. On average it took 3.7 months before all ten tagged fruit in a group were no longer hanging on the vine. Of these 81.1% were not found on the ground around the vine from which they had disappeared. Two fruit (G6 and H1) were found on the ground and had been partially eaten (Fig. 3.2A). Only one fruit (H5) was found partially eaten on the vine (Fig. 3.2B) and it was later removed.

Table 3.5: Summary of fruit destination for tagged banana passionfruit in the Queen Charlotte Sound. Removed = wholly removed from the area; Ground = found on the ground; E (G) = found on the ground partially eaten; E (R) = partially eaten but later removed. The mean and standard error of each fate are also shown.

Accessibility	Group	Removed	Ground	E (G)	E (R)
On road	**A**	9	1	0	0
	B	10	0	0	0
	C	4	1	0	0
	D	10	0	0	0
	F	9	1	0	0
Mean ± SE		8.4 ± 1.12	0.6 ± 0.24	0 ± 0	0 ± 0
Off road	**E**	7	3	0	0
	G	9	0	1	0
	H	7	1	1	1
	I	7	3	0	0
	J	5	5	0	0
Mean ± SE		7 ± 0.63	2.4 ± 0.87	0.4 ± 0.24	0.2 ± 0.2

Figure 3.2A: Fruit H1 found partially eaten on the ground.   **Figure 3.2B**: Fruit H5, partially eaten on vine.

A significantly higher percentage of fruit were removed from the roadside patches (Table 3.6). There was no significant effect of Patch. However, although fruit removal rates were significantly higher by the road, a large proportion of fruit are being removed regardless of accessibility, with a mean of 93% of roadside fruit being removed and 70% off-road.

Table 3.6: Results from binomial GLM analysis showing the effect of fruit location on the proportion of tagged fruit removed from the banana passionfruit vine.

	Df	Deviance	P(Chi)
On/off road	1	7.92	P = 0.005
Group	8	8.52	P = 0.384
Residuals	85	72.82	

Fruit were removed very quickly once they became ripe in both roadside and off road groups (Figures 3.3 and 3.4). The road side and off road groups have been separated to make each graph clearer. A lot of fruit were removed in the first and second months after ripening or even when they were still a bit green. It does not appear to be essential for fruit to be ripe before they are removed.
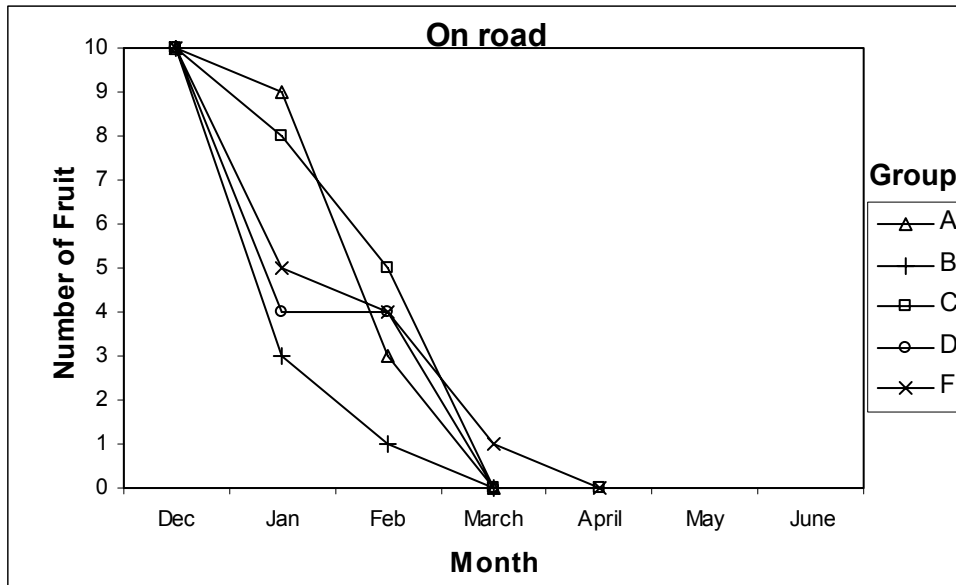
Figure 3.3: Number of tagged banana passionfruit still left on the vine when checked each month in the roadside groups. Fruit were tagged when not yet mature and ripe in December.
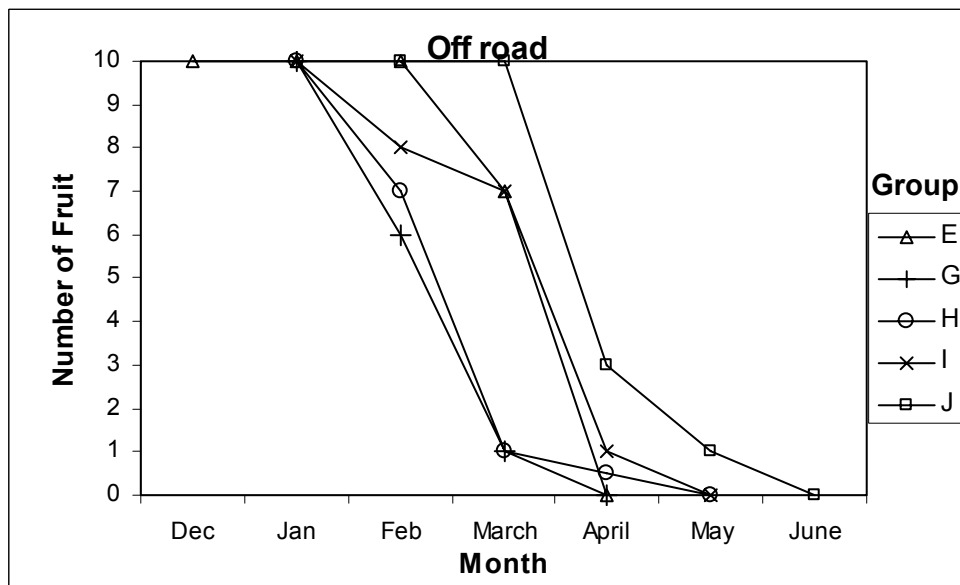
Figure 3.4: Number of tagged banana passionfruit still left on the vine when checked each month in the off road groups. Fruit were tagged when not yet mature or ripe (group J, particularly immature) in December (E), January (G, H, I) and February (J).

3.4.3 Evidence of dispersal vectors

No fruit were found with evidence of peck marks despite the hundreds of fruit seen and presence of birds in the area. This suggests that it is not a targeted food source for native birds, and they are unlikely to be dispersing the seeds.

Many fruit were found partially eaten, some with teeth marks on both the ground and vines (Fig. 3.5A). Patches of disassembled fruit, even when not properly ripe, were also found regularly on the ground (Fig.

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3.5B). Sometimes the peel, pulp and seeds of the fruit had been sorted into piles. While checking flowers for another experiment on 9 March 2006 I photographed four disassembled and partially eaten fruit within an area of approximately three square metres.

Fig. 3.5 A: Partially eaten banana passionfruit found hanging on a vine with teeth marks in it.

Fig. 3.5B: Fruit found on the ground in the bush that has been disassembled.

The infra-red video camera enabled us to determine whether our expectation that it was possums that were eating the fruit was correct. All of the fruit that were laid out in front of the infra red camera were eaten each night except for one which was half eaten. Fruit were not common in June so some of them were not completely ripe. The fruit that was left was particularly green. Two photos showing the fruit that were filmed on the night of 17 June 2006 are shown in Fig. 3.6. The first (3.6A) was taken when the fruit were set out and the second (3.6B) on the following morning. This allowed us to determine which animals were responsible for the distinctive piles of disassembled fruit which were commonly found.

Fig. 3.6A: Fruit set out underneath chicken wire in front of the infra red camera on 17/6/06.

Fig. 3.6B: Remains of the fruit on the morning of 18/6/06.

The summary of animals sighted in the infra-red footage showed rats to be regularly disassembling and eating the fruit and to be responsible for the distinctive piles thought to be caused by possums (Table 3.7). Rats disassembled fruits by first removing the skin and eating the seeds and fleshy capsules. Observations of caged rats suggested that they appear to ingest seeds as you could hear them crunching on them. On the night of 17 June 2006 rats spent over two hours and 12 minutes eating the three banana passionfruit and up to four rats could be seen within the scope of the video camera at any one time. On average it took slightly under 20 minutes for rats to completely disassemble fruits. One of the fruit was half disassembled after only four minutes. Rats tended to completely dismantle one fruit and eat significant proportion of it before moving onto the next. Rats also avoided being inside the chicken wire as much as possible so would drag the fruit to the side and attack it from outside the cage through the holes. This was typically done by a smaller rat which would sometimes be chased away by a larger rat once the fruit was more accessible. Multiple rats would feed on a fruit but typically not at the same time. Only on one occasion were a larger rat and a smaller rat feeding in close proximity at the same time. To avoid coming into contact with others the rats would often briefly visit the fruit to get a chunk and then take it away to feed and consequently spent quite a bit of time running to the fruit, spending a brief time period eating and then running off screen again. Rats also showed aggressive behaviour to other rats wanting to share the food source and physically fought one another twice. Although this evidence shows that wild rats definitely eat fruit on the ground in the field, it is difficult to say whether fruit that are found partially eaten on the vine were rats or another animal such as possums.

Two possums were also seen eating the banana passionfruit over the three nights the cameras were running. The camera evidence from the 15 June 2006 showed the possum eating the fruit that had already been disassembled by rats, for two minutes. Similarly, on 17 June 2006 a possum was seen four times, during two of which it ate fruit for a total of around seven minutes. In this instance, the possum appeared before the first rat at 18:16 hrs consequently was not able to get inside the cage so initially it sniffed the fruit and then kept walking. Later, the possum ate only the fruit that had been dragged outside and partially disassembled by rats. When this fruit was accessible, it continued to disassemble and eat the fruit.

On two nights a mouse was seen briefly eating the remains of the fruit or just running past after all the rats had left for the night at 06:45 am and 08:40 am. They are unlikely to have any affect on the dispersal of seeds.

Table 3.7: Summary of the animals sighted on the infra-red video tape and their behaviour. First sighted = the time at which the first animal was sighted; No. sightings = the approximate number of individual times that animal was sighted; Max. sighted at once = the maximum number of animals that were on the screen at any one time; Time eating = the approximate number of hours, minutes and seconds individuals of that species spent eating the fruit throughout the viewing period; % of night eating fr. = the percentage of the 13 hours between dusk and dawn that animals of that species spent eating banana passionfruit; Time disassembling each fruit = the time spent disassembling the fruit from when it was completely intact to when there were no large chunks left, listed in order they were disassembled.

Animal	First sighted	No. sightings	Max. sighted at once	Time eating (hr:min:sec)	% of night eating fr.	Time disassembling each fruit (min)
15/6/06						
Rat	20:33	31	2	54:30	7.0%	19; 16 min
Possum	22:20	2	1	2:00	0.3%	*na* – already done
Mouse	na	0	0	0:00	0.0%	*na*
17/6/06						
Rat	18:33	57	4	2:13:30	17.1%	21; 17; 27 min
Possum	18:16	4	1	7:00	0.9%	3 min of 1st fruit
Mouse	08:40	1	1	1:00	0.1%	*na*
19/6/06						
Rat	18:28	49	3	1:24:30	10.8%	25; 11; 4 min*
Possum	na	0	0	0:00	0.0%	*na*
Mouse	06:45	1	1	0:00	0.0%	*na*

* Not all fruit disassembled – about 1/3 still intact

Some highlights from the infra red footage can be seen on the CD included as Appendix 2.

3.3.4 Ingestion effects on seed viability

Pigs

A total of 1509.9 grams of feral pig droppings was collected from which 2818 intact seeds and 137 pieces of seed were removed (Table 3.8). This clearly shows that feral pigs are targeting banana passionfruit as a food source. Each fruit has 108 seeds on average which means that the seeds found in 1.5 kg of pig droppings are the equivalent to approximately 26 fruit. Table 3.8 shows the weight of each pile, the number of whole seeds and broken pieces of seed that were removed (the number of seeds the broken pieces would make if pieced together was estimated), the number of seeds from that pile that were planted in the glasshouse, and the number that were planted in the field. Two samples of 200 seeds from Pile 1 of the pig droppings were planted in the glasshouse and their percentage germination combined to give an average germination success from Pile 1.

Table 3.8: The weight of each clump of pig droppings collected and the number of seeds extracted. Pieces refers to broken bits of seed that were collected (the equivalent number of whole seeds was estimated and is written in the brackets). Glasshouse indicates how many seeds from the sample were planted in trays (200 seeds/tray) in the glasshouse. Similarly field indicates the number planted in tubes in the field to assess germination success.

	Weight	No. seeds	Pieces (~Seeds)	Glasshouse	Field
Pile 1	433.8 g	1250	63 (~20)	200 x 2	200
Pile 2	423.6 g	620	32 (~10)	200	200
Pile 3	652.5 g	948	42 (~14)	200	200
Total	1509.9 g	2818	137 (~44)	800	600

Seeds were viable following ingestion by pigs and many of the seeds collected from the pig droppings germinated successfully. Mean percentage germination was a lot higher in the favourable conditions of the glasshouse (45.6% ± 2.51%) compared with the field (7.5% ± 1.61%) although it is difficult to determine whether the seeds that didn't germinate are dead or merely dormant. One of the field plots was found destroyed, four months after it was planted and one month after the seeds started germinating (15 June 2006). Figure 3.7A is a photograph of one tray of 200 seeds just four months after the seeds were planted in the glasshouse and shows that seeds are able to germinate and increase in length and biomass very quickly. Figure 3.7B shows the length of one seedling after that time period.

Fig. 3.7A: Tray of 200 seeds from pig excrement, four months after planted (8/6/2006).

**Fig. 3.7B**: Length of one seedling from a seed extracted from pig droppings, four months after it was planted (8/6/06).

This experiment has shown that pigs are one dispersal agent of banana passionfruit seeds, as excreted seeds are still able to germinate readily, and consequently pigs may be responsible for the spread of banana passionfruit in areas that are currently infested and to new areas.

Possums

The captive possums readily ate the banana passionfruit when offered them. Some were trying to get through the cages to get to the fruit from their neighbour. There was a lot of crunching of seeds as they ate the fruit. One seed was found in the faeces of one of the animals, 49 from another and 58 from a third. Three of the possums had no broken pieces or intact seeds in their droppings.

After five months, 53 of the 100 seeds had germinated. Fig. 3.8 shows how quickly the seeds defecated by the possums germinated. Seeds are viable following ingestion and defecation by possums meaning possums may also important dispersal vectors.
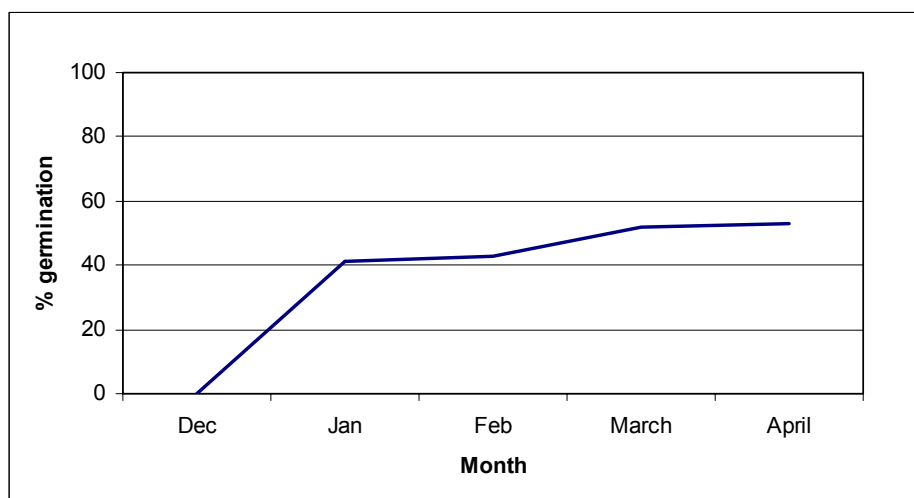
Figure 3.8: Cumulative number of seeds, defecated by possums, germinating in the glasshouse each month. Seeds planted 30 November 2006.

Rats

Of the four seeds defecated and planted, none germinated between November and April.

Combined Analysis

The binomial GLM showed a highly significant main effect on final percentage germination of both treatment and of the environmental conditions in which the seeds were planted (i.e. glasshouse or field) (Table 3.9). Furthermore, there was a significant interaction between germination conditions and treatment.

Table 3.9: Binomial GLM results comparing final percentage germination success of seeds in cleaned and fleshy treatments and extracted from possum and pig droppings in different environmental conditions (glasshouse and field). Doesn't include whole intact fruits.

	Df	**Deviance**	**F**	**Pr(F)**
Environment	1	686.84	686.84	P < 0.001
Treatment	3	50.20	16.73	P < 0.001
Environment:Treatment	2	6.56	3.28	P = 0.038
Residual	49	252.31		

The treatment effect remained significant when estimated germination percentage for whole fruits was included in both the glasshouse ($F_{4,41} = 14.43$, $p < 0.001$) and field ($F_{3,31} = 4.14$, $p = 0.006$). Percentage germination of all treatments was significantly lower in the field than in the glasshouse. In the glasshouse there was no difference between percentage germination of cleaned and fleshy seeds but significantly less seeds germinated from whole fruits (Figure 3.9). In the field there was a suggestive ($p = 0.076$) but not significant effect of pulp removal in cleaned and fleshy seeds, but whole fruits were the most successful of all treatments. Seeds from possum and pig faeces had significantly lower germination success than cleaned and fleshy seeds in the glasshouse so initially it appears that ingestion by animals has a negative effect on germination success. However, seeds from pig droppings have significantly greater success than both cleaned and fleshy seeds in the field. This is confirmed by the significant interaction between germination conditions and treatment as the trends in the other treatments do not differ between the field and glasshouse. Therefore, it appears that there is no adverse effect of scarification in seeds germinated in the field. Rather, ingestion by pigs seems to facilitate germination.
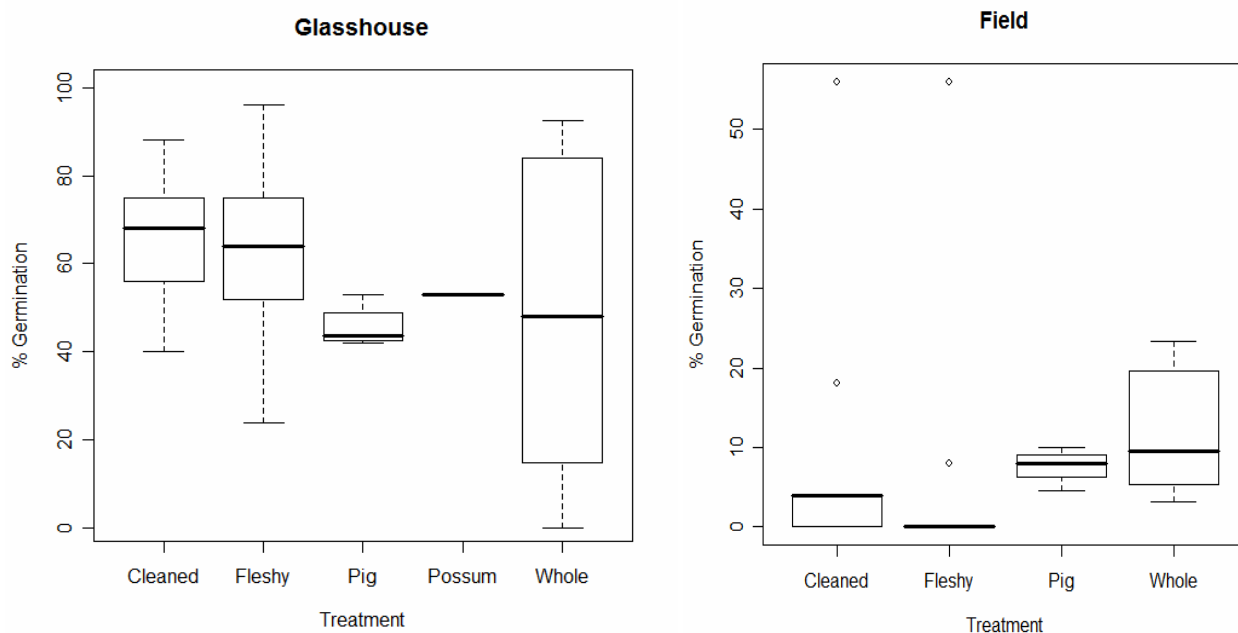
Figure 3.9: Percentage germination success of cleaned and fleshy seeds and those seeds found in the droppings of possums and pigs planted in the glasshouse and field. Also includes estimated germination success of intact fruits. Graph shows medians and 95th, 75th, 25th and 5th percentiles. Clear circles represent outliers.

3.5   DISCUSSION

In accordance with the suggestions of Samuels and Levey (2005) and Robertson *et al* (2006), germination was investigated in both the field and glasshouse and treatments included all three treatments stated to be necessary by Samuels and Levey (2005), namely hand-cleaned seeds, seeds retained in intact fruits and seed defecated by frugivorous dispersers. In addition, an intermediate treatment in which seeds remained in their fleshy arils but were separated from the fruit wall was included as this allowed direct calculation of percentage germination success rather than using an estimate as required for whole fruit. Any inhibition

compounds are likely to occur in the fleshy capsule rather than the fruit wall so comparisons between fleshy and cleaned seeds should indicate the extent to which deinhibition may occur through dispersal. Final percentage germination success was used to compare treatments but the speed of germination was ignored because germination following a period of dormancy may not be disadvantageous, but rather a mechanism of temporal dispersal (Kelly *et al*, 2004).

3.5.1 Environmental conditions

Robertson *et al* (2006) expressed concern regarding that fact that the effect of environmental conditions on the germination success of plants is often ignored in the literature. They cited several studies that have compared trials in lab, glasshouse and field conditions and results have been highly variable. In the germination trials conducted by Robertson *et al* (2006) on three New Zealand native plants species, they found that environmental conditions were particularly important for intact fruits where total germination success was zero for all species in Petri dishes but over 50% in the glasshouse and field. Consequently, in this study seeds were germinated in trays of potting mix in the glasshouse and not in Petri dishes because Petri dishes do not closely simulate field conditions and so results are unlikely to be directly applicable to the real world or to give us an accurate picture of what is happening in wild populations (Robertson *et al*, 2006). Comparisons between germination trials in the field and glasshouse show that significantly more seeds germinated in the glasshouse in all treatments ($F_{(1,23)}$ = 297.87, p < 0.001). This is due to the ideal conditions of a glasshouse as it is warm, sunny, fertile and regularly watered. There are also unlikely to be as many insect herbivores and mammals are excluded (Constible *et al*, 2005). In addition, there were significant interactions between environmental conditions and treatment, so the effect of treatment on the germination success of some treatments depended on whether seeds were planted in the glasshouse or field.

3.5.2 Deinhibition effect

No significant difference was found in the final germination success of cleaned and fleshy seeds in either the field or the glasshouse. Intact fruits decomposed and seeds germinated surprisingly quickly for such a large fruit. In one fruit the first seed germinated after only one month from fresh. In another fruit 85 seedlings germinated in under three months. This suggests that banana passionfruit seeds are able to germinate readily regardless of whether they are dispersed and are not likely to benefit from removal of flesh by frugivores in terms of total germination success. In addition, the estimated germination percentage for whole fruits showed that in the field, intact fruits have greater germination success than fleshy or cleaned seeds. Robertson *et al* (2006) suggested this may be because environmental conditions alter the ability of inhibitors to leach away and the availability of micro-organisms for decomposition of the fruit wall and pulp. Generally seeds from intact fruit have a significantly lower final germination percentage than cleaned treatments (Kelly *et al*, 2004), but in our experiment this was not the case. It is

possible that percentage germination may have been overestimated as the mean seed count (94 seeds) appeared quite low, especially considering *P. mollissima* fruits in Williams *et al* (2000) had a mean of 125.0 ± 18.0 seeds. However, a large proportion of the seeds that hadn't germinated by the final data collection date may have been dormant as more seedlings had germinated when observed two months later. Many of them may germinate over the following year or longer, especially since the summer 2006/07 which was a particularly cold year. Mean summer temperatures were well below average, making it the coldest summer since 1992/93, and average rainfall was also very low with parts of Marlborough receiving less that half its usual summer rainfall (NIWA Science National Climate Centre, 2007). As a result, water conditions may not have been conducive to breaking dormancy and consequently, total germination success may have been underestimated due to the time constraints of the experiment (Traveset, 1998). In addition, fruits may have a dormancy phase that allows them to abstain from germination until suitable environmental conditions arise. Research on banana passionfruit in Hawaii found that seed germination is staggered and while many seeds within a cohort germinate fairly rapidly once dormancy is broken, others are delayed for eight months or longer increasing the possibility of colonization in favourable conditions (LaRosa, 1992). Thus, there is no evidence to suggest that the fleshy pulp of banana passionfruit contains inhibition chemicals that when removed by dispersal increases germination success. In addition, seeds do not require removal from fruit in order to germinate.

The patch (or plant) from which the seeds for germination were collected had a significant effect on total germination success. Therefore, some plants appear to have a greater fecundity than others. There may be a number of reasons for this, but similar patterns emerged in pollination and inbreeding depression experiments. The reasons discussed in Chapter 2 are probably applicable here but it appears that some plants are just more fit than others and consequently produce more seeds with greater germination and reproductive success (Byers & Waller, 1999).

3.5.3 Fruit removal rates (dispersal quantity)

The number of fruits removed by frugivores is an important component in determining the number of seeds that will be dispersed (Schupp, 1993). By tracking fruit we found that 81% of fruit were not found on the ground beneath the vine but appeared to have been wholly removed. In addition, some fruit were removed before they were yellow and ripe. Inhibition may occur in the seeds of these fruits if seeds are not completely mature when ingested (Traveset, 1998). The majority of fruits were taken from the vine within two months after ripening so a large proportion of seeds are being dispersed away from the vine. This suggests that animals are targeting fruit.

Significantly higher numbers of fruit were removed from roadside patches compared with off road patches. There are several reasons why fruit removal may be higher from roadside sites. Many people

enjoy eating banana passionfruit and consequently collect fruit that are easily accessible on the side of the road. If the difference between roadside and off-road sites are merely due to the added effect of humans this is unlikely to have any significant ecological implications as most seeds consumed by humans will not result in seed dispersal unless fruits are half eaten and thrown over the bank. However, fruit removal rates were still high at off-road sites and Kelly *et al*, (2004) reported several cases where dispersal of seeds has been shown to be more effective from forest edges. Patterns of animal densities and foraging may differ in edge habitats compared with forest or scrub interiors (Kelly *et al*, 2004). Subsequently, animals may move along roadsides more commonly than through dense bush and as a result remove more fruit from roadside vines. This would be particularly likely for larger animals such as possums. In the infra-red video footage, possums typically entered and exited along the path the fruits were placed on. If dispersers do spend more time foraging along roadsides and paths it may increase the probability that seeds are deposited in high light, disturbed conditions, suitable for germination. Similarly, fruits on edges may be more visible to frugivores and consequently have a higher chance of being visited and removed (Kelly *et al*, 2004). In addition, the group where the highest proportion of tagged fruit were found on the ground (J – Table 3.5) was located on a vine that had a very large fruit set so the sheer abundance of fruit in that area may have resulted in a higher proportion of the tagged fruit falling on the ground rather than being removed.

As we have established already, seeds are able to germinate without removal from fruits so even those that fall below the vine will have a chance of surviving. In addition, deposition below the parent increases in the probably that environmental conditions will be suitable for germination and survival given that the parent plant has successfully matured and reproduced. However, conditions may change over time so this is not guaranteed. For example, banana passionfruit seedlings need at least 2% of the available sunlight in order to successfully grow (Baars & Kelly, 1996) but once on top of the canopy they are able to travel along it. In this case, fruits that were to fall into the bush below the parent plant they would not survive in the absence of secondary dispersal. In the fruit removal experiment, a low level of secondary removal was detected as only one fruit was found partially eaten on the ground. However, it is difficult to recognize identification numbers on fruits that have been on the ground for a month or more unless the exact location of the fruit is revisited, so secondary dispersal was probably more frequent than detected in tagged fruit. Furthermore, all fruits placed on the ground for the infra-red video evidence were disassembled and eaten by frugivores, and pigs forage on the ground. Consequently, just because a fruit is not taken directly from the vine does not mean that it will not be dispersed. Secondary dispersal probably plays a large role in the deposition of seeds in suitable germination sites.

Although the number of fruit removed from roadside groups (93%) was significantly higher than off-road groups (70%) both had a high percentage fruit removal so fruits are certainly targeted by animals. However, this only measures dispersal quantity but the plant will also be affected by dispersal quality as

seeds removed by destroyed but the frugivores will not result in the establishment of progeny (Kelly *et al*, 2004). Consequently, we now address the question of what animals are removing fruits and whether they will be effective dispersers.

3.5.4 Dispersal vectors and their relative significance in population growth

Birds

Throughout the entire research period, fruits were never seen with peck marks or any other indication that birds are consuming the them, despite viewing hundreds of fruits. The skin of the fruit is soft so although the fruits are too large to be swallowed whole, birds are able to access the flesh and seeds (Williams, 2006). This suggests that in the Marlborough Sounds birds are not the primary consumers of banana passionfruit. Williams and Karl (1996) have documented introduced blackbirds (*Turdus merula*) swallowing intact seeds in the Nelson region, however, blackbirds were not commonly seen in this part of the Marlborough Sounds (personal observation) and typically only consume whole fruits up to about 13 mm in diameter (Williams, 2006). We would expect to see some evidence of pecked fruits if blackbirds were frequently eating *P. mollissima* fruits, so they do not appear to be significant disperser of seeds in our study area. This is in contrast to Hawaii where birds were found to increase rates of spread and establishment of banana passionfruit (LaRosa, 1992).

Introduced mammalian frugivores

In contrast, numerous fruit were seen with mammalian teeth marks in the flesh (Fig. 3.5A), or disassembled on the ground (Fig. 3.5B). Furthermore, infra-red video evidence revealed possums and rats eating fruits in the field, even those that weren't completely ripe. On all three nights, all fruit placed on the ground were completely disassembled and significant proportions eaten except for one particularly green fruit which was only half eaten. This fruit was used because the cameras were set up in June which is not a heavy fruiting period for banana passionfruit and consequently, ripe fruit were hard to come by.

Rats took only 11-25 minutes to totally disassemble fruits. They appeared shortly after sunset and focused their foraging on fruits until these were largely eaten. Some rats would chase and fight other rats to defend the food source. We found no evidence of seed viability following ingestion by rats. This suggests that rats are probably normally seed predators rather than dispersers for banana passionfruit. This is not unusual as rats are predators of many species of seed (Price & Jenkins, 1986; Williams *et al*, 2000). However, only four intact seeds were able to be extracted from rat faeces collected in the feeding trials and planted to test for seed viability. While the limited number of excreted seeds suggest that most seeds do not survive rat ingestion intact, some of the fruit in our trials were not eaten (presumably because they were overripe) and all four seeds planted were collected from the faeces of one individual. The effect of ingestion on seeds may vary between plant and frugivore individuals within a population (Traveset, 1998). In addition, the

feral rats in this area tend to be ship rats (*Rattus rattus*) whereas the captive rats were Norway rats (*Rattus norvegicus*). Williams *et al* (2000) failed to find any intact *P. mollissima* seeds in the faeces of six ship rats following the ingestion of banana passionfruit pulp and seeds. The potential for rats to be a significant dispersal vector can not be ruled out though it appears that intact banana passionfruit seeds are not commonly deposited in rat faeces. Additional research with a larger sample size would more accurately determine seed viability following ingestion and hence whether feral rats are capable of seed dispersal or merely predation. If seeds are able to remain viable following consumption, rats may be an important dispersal vector given that they target banana passionfruit. One night rats spent over 17% of the night eating *P. mollissima* fruits when only three were available. If excreted seeds are not viable, rats may still contribute to short-distance dispersal. For example, rats were sometimes seen taking chunks of fruit away from scope of the infra-red camera to consume. In addition, rats are known to commonly hoard and cache seeds which may result some dispersal (Richardson *et al*, 2000).

Possums (*Trichosurus vulpecula*) were observed consuming *P. mollissima* fruits in both the lab and field. In addition, possums are likely to be primarily responsible for fruit removal in the tagged fruit experiment and for multiple sightings of half-eaten fruit still hanging on the vine. While significantly less seeds from possum defecation germinated in the glasshouse when compared with hand cleaned seeds, germination success was still 53% without considering probable dormancy in at least some of the remaining seeds. However, ingestion by possums does appear to have a negative effect on seed germination success. This is in contrast to Williams *et al* (2000) who also presented strong evidence that possums consume *P. mollissima* fruits but found no significant decrease in germination success of seeds ingested by possums. In their germination trials, 61% of seeds defecated by possums germinated. This difference is likely to be due to the environmental conditions of germination trials as their seeds were germinated on filter paper in a Petri dish rather than in a glasshouse. Our results show that seeds still germinate readily in conditions more representative of the natural dispersal environment. Either way, both experiments show that a large proportion of seeds remain viable following ingestion by possums. Consequently possums are likely to be significant contributors to the dispersal of banana passionfruit, especially considering they tend to favour fruit when it is available (Dungan *et al*, 2002). This may be particularly true when other food sources are limited (Buckley *et al*, 2006). For example, banana passionfruit has an extended fruiting period so fruit are available at the end of winter and early spring when other fruit sources are scarce (Williams & Karl, 1996).

Possums are reasonably big animals and so are capable of moving a large quantity of seeds (Nogales *et al*, 2005). They have large home ranges and are mobile (usually ranging over 1-3 ha but sometimes up to 30 ha), often moving 100-200 metres in a single night (Dungan *et al*, 2002). They also have a long gut retention period. Williams *et al* (2000) showed that on average, *P. mollissima* seeds take between two and

nine days to be excreted following ingestion by possums. In addition, the fruit tracking experiment suggests that possums tend to move along roadsides so are likely to deposit seeds in a high-light, disturbed environment conducive to successful germination. The combination of all these factors shows that possums are capable of dispersing a large quantity of seeds over long distances making them a very effective dispersal vector. Consequently, this invasive mutualism is likely to have accelerated colonisation and spread of banana passionfruit to new areas and will continue to increase its invasiveness in the absence of anthropogenic interference.

During periods of heavy fruiting, pigs come down to the bush just above Queen Charlotte Drive to eat the fruit (Tom Stein, personal communication). This is similar to behaviour observed in Hawaii where pigs congregated under *P. mollissima* vines during periods of intense fruiting, reaching densities of 63 pigs per square kilometre (Warshauer *et al*, 1983). The 2818 seeds removed from 1509.9 grams of feral pig droppings is compelling evidence that pigs are consuming large numbers of seeds. Germination experiments showed that seeds readily germinate following ingestion by pigs confirming that pigs disperse significant numbers of seeds. In the glasshouse, germination success was significantly lower in seeds excreted by pigs than in hand-cleaned, fleshy and possum treatments, suggesting that gut passage adversely affects seed viability. However, in the field there appears to be a positive scarification effect as seeds from pig faeces had significantly greater success than cleaned and fleshy seeds suggesting that ingestion by pigs facilitates germination in banana passionfruit seeds in wild populations. LaRosa (1992) found that defecation by pigs where does not enhance germination success in Hawaii. These differences could be caused by a number of uncontrolled variables such as differences between individual pigs, the maturity of the seeds they consumed, the environmental germination conditions or the age the pig droppings when collected. The important point is that pigs eating seeds and are likely to effectively disperse them.

In addition to scarification effects, the foraging behaviour of pigs may enhance their effectiveness as dispersers because seeds are likely to be excreted in areas of rooting, so competition from native plants will be reduced or removed (LaRosa, 1992; Starr *et al*, 2003). Thus, pigs may deposit seeds in areas that have been recently disturbed providing favourable conditions for banana passionfruit seed germination (Constible *et al*, 2005). In this experiment we did not test germination of seeds whilst still in the faecal matter, which has been reported to aid germination success (due to the fertiliser effect) in some species (Constible *et al*, 2005; Robertson *et al*, 2006). This may certainly be the case of banana passionfruit as naturally occurring seedlings have been sighted growing out of feral pig droppings in the field (Tom Stein, pers. comm.) and research by LaRosa (1992) indicated that feral pig droppings provides a fertile medium for germination in Hawaii. There has been discussion as to whether it is advantageous for seeds to be dispersed in the faecal matter of some frugivores as it is likely to result in the large numbers of seeds from

mixed species being deposited in clumps and in doing so, increase competition (Schupp, 1993; Traveset, 1998). However, only banana passionfruit seeds were found in feral pig droppings and they are effective competitors, as shown in Chapter 2 where all seedlings survived and grew, in close proximity and resource restricted conditions. Therefore, the advantage gained through dispersal to disturbed sites is likely to be greater than the cost of seedling mortality due to intraspecific competition. Furthermore, pigs are very mobile travelling up to 3.6 km a day, with home ranges varying from 0.7 to 11.7 km^2 (McIlroy, 2005). In addition, they are large animals so are likely to have a long gut retention period (Nogales *et al*, 2005). Therefore, they could very effectively carry seeds over long distances to new areas. For example, Phil Clerke from the Department of Conservation (Sounds Area Office) observed a few seedlings randomly located in the middle of dense bush on Mt Cawte, behind Anakiwa, far from where people usually go (pers. comm.). In some cases light availability may render such dispersal into bush ineffective. However, light levels in New Zealand lowland forest understories range from 1-5% full-sunlight (Wiser & Allen, 2006). As the light compensation point of banana passionfruit is 2% full-sunlight (Baars & Kelly, 1996), germination may be possible from understories in some areas. In addition, even if light levels are too low for seeds to germinate when deposited, they may persist in the seed bank and germinate in the event of disturbance (Wiser & Allen, 2006).

In short, similar to Hawaii (LaRosa, 1992), pigs appear to be greatly enhancing the dispersal of banana passionfruit seeds by depositing large numbers of viable seeds in fertile, disturbed sites ideal for germination. Therefore, feral pigs may be a significant contributor to the introduction of banana passionfruit in areas that aren't currently infested or are rarely visited by humans.

3.5.5 Conclusions

Humans play a hugely significant role in the invasion and establishment of exotic plant species into indigenous ecosystems (Sullivan *et al*, 2005). Certainly humans are responsible for multiple introductions of banana passionfruit into ecosystems in New Zealand. For example, in the Marlborough Sounds the abundance of banana passionfruit decreases as you drive along Queen Charlotte Drive away from Picton (personal observation). However, this study shows that invasive mutualisms between *P. mollissima* and introduced frugivorous mammals also has a significant effect on the proliferation of this pest species. Fruits are being rapidly removed and dispersed from both roadside and off-road sites confirming that humans are not solely responsible for fruit removal. Secondary dispersal by rats and pigs is likely to be common.

While the majority of fruit are removed, fruit that fall beneath the vine are certainly capable of germinating in the absence of dispersal vectors as whole fruit had the greatest germination success in the field. Therefore, any germination inhibitors in the flesh of *P. mollissima* fruit are rapidly leached or

broken down in field conditions, as cleaned seeds had significantly lower germination success than intact fruits. A scarification effect was detected in the field as seeds from pig droppings had significantly greater germination success than hand-cleaned seeds. In short, while whole fruits and defecated seeds had lower percentage germination success in the glasshouse, in feral populations of banana passionfruit germination success is greatest in these treatments. This research provides compelling evidence that introduced possums and pigs target fruits and play a significant role in the dispersal of banana passionfruit in the Marlborough Sounds because high numbers of seeds are removed, seeds are viable following ingestion, and dispersers are likely to deposit seeds in environmental conditions suitable for germination.

Chapter 4: GENERAL DISCUSSION AND CONCLUSIONS

4.1   The reproductive ecology and invasive attributes of banana passionfruit

The aim of this study was to investigate the pollination and dispersal mutualisms of banana passionfruit in New Zealand, and the extent to which these mutualisms are contributing to its invasiveness and proliferation. This research shows convincing evidence that banana passionfruit relies on introduced pollinators to produce fruit. Few fruit are produced autogamously and my results show that in the absence of pollinators, virtually all of which were exotic insects, each flower would only produce 0.9 seedlings per flower. Therefore, had honey bees and bumble bees not been introduced to New Zealand, the invasive capacity of banana passionfruit would have been severely reduced. Similarly, *P. mollissima* has formed dispersal mutualisms with a number of introduced mammals including possums, pigs, and introduced birds in some areas (Williams *et al*, 2006). In contrast, native pollinators and dispersers do not appear to contribute significantly to the reproduction of this noxious weed.

Although flowers are specialised, they may be able to be pollinated by other species when outside their natural habitat. "Baker's rule" suggests that plant species that become invasive will usually be clonal, self-compatible or apomictic (Baker, 1967). Recent research has shown that this is not always the case. For example, skunk vine (*Paederia foetida*) is an invasive liana that smothers vegetation. Although it has a self-incompatibility complex and is pollen limited in some areas, skunk vine has proliferated to the point where it is one of Florida's most noxious weeds and it continues to expand its range across the United States (Liu *et al*, 2006). Similarly, *Cytisus* and *Genista* (two species of exotic brooms) do not show a substantial level of any of Baker's forms of reproductive assurance, and yet are considered serious pests in California (Parker & Haubensak, 2002). Banana passionfruit shows neither autogamy nor apomixis. It is self-compatible although self-pollination results in a significant decrease in the number of fruit produced. However, once a fruit was produced inbreeding depression did not affect fruit characteristics or seedling competitive ability and vigour in my study.

While it appears that one of the primary dispersal vectors of banana passionfruit is humans, mutualisms with frugivores are certainly contributing to the dispersal of banana passionfruit in the Marlborough Sounds and presumably throughout New Zealand. The relative contributions of humans and frugivores to its dispersal are unknown but fruit removal rates showed that 93% of fruits were removed from roadside groups compared with 70% from off-road groups. This suggests that humans are removing at least 13% of fruit whereas in more remote areas animals remove 70% of fruits, though increased animal activity on edges could render this conservative. Intentional dispersal by humans is likely to be declining, particularly as propagation is now illegal and as peoples' awareness of the weediness of banana passionfruit increases.

However, unintentional dispersal incidental to eating the fruit could still be important. Educating the public of the risks banana passionfruit poses to native ecosystems may be a key way in which to slow further spread.

Aside from humans, banana passionfruit is being effectively dispersed by introduced mammals, in particular pigs and possums. Frugivore dispersers may be particularly important in areas where humans are scarce. Pig populations in the Marlborough Sounds are expanding (Tom Stein, pers. comm.) because wild pigs are no longer able to be exported as their livers may contain traces of broadifacoum, which has been used for pest control in the area (McIlroy, 2005). As a result, there has been a decrease in pig hunting in the Marlborough Sounds which has previously provided some pest control. Of the introduced mammals, pigs probably contribute the most to long distance dispersal due to the large numbers of seeds they consume, the viability of seeds following ingestion, and the favourable conditions in which pigs are likely to deposit seeds. In addition, their potential to disperse seeds over long distances (McIlroy, 2005) suggest that pigs are likely to contribute disproportionately to the rate of spread of *P. mollissima* (Buckley *et al*, 2006). Possums are also targeting fruit and very mobile dispersers. Thus, they will also assist the spread of banana passionfruit. Opportunistic generalist frugivores can contribute substantially to the invasiveness of fleshy-fruited species that are not otherwise capable of becoming naturalized (Panetta & McKee, 1997). They can profoundly affect invasion dynamics through long distance dispersal events that often lead to the formation of satellite foci of infestation which accelerates spread (Panetta & McKee, 1997). Williams and Buxton (1995) suggested that birds, mammals and humans are unlikely to transport seeds more than several kilometres from existing colonies. However, although long distance dispersal of *P. mollissima* is an infrequent event in Hawaii, it usually results in the establishment of a new population, exacerbating the problem (LaRosa, 1992). In addition, satellite populations are not exposed to intense intraspecific competition and therefore may expand rapidly if interspecific competition is not too severe (LaRosa, 1992).

Some short distance dispersal may occur as a result of rats and introduced birds but there is no evidence of native birds targeting fruits. Dispersal would have constrained population spread of banana passionfruit in the absence of introduced species, especially mammals. Hence, the role of monkeys in dispersal of banana passionfruit has been effectively replaced by other generalist mammal frugivores in New Zealand

Williams and West (2000) listed several characteristics of weedy species which are useful in determining additional reasons why banana passionfruit may have become so invasive (Table 4.1). *P. mollissima* stems that are broken off or partially buried are able to re-root and grow (Williams & Buxton, 1995). This provides protection against damage during tree-fall and may compromise the effectiveness of purely

mechanical control efforts (Warshauer *et al*, 1983). For example, the density of banana passionfruit is high along some roads that are flailed periodically. While this appears to remove banana passionfruit, in reality the plants may re-root and continue to grow. In addition, often trees are damaged in the process, sometimes severely so with whole tree crowns being cut off at the trunk, which may allow banana passionfruit to displace some plants by gaining a competitive advantage. *P. mollissima* is also is a successful colonizer of bare ground and consequently, is facilitated by disturbance (LaRosa, 1992).

Table 4.1: Characteristics of plants which indicate a potential for weediness in native ecosystems from Williams & West (2000).

High input of viable propagules.
Short (< 2 years) development time to reproductive maturity
Seed or other reproductive units with prolonged (>5 years) periods of dormancy.
High rate of aerial or subterranean biomass production, particularly under conditions of low light, water or nutrient availability.
Dense and spreading foliage canopy.
Efficient long distance (>1 km) dispersal capabilities.
Presence of interspecific allelopathic properties or absence of intraspecific allelopathic properties.
Successful colonizer of disturbed or bare ground.
Reproductive strategies that facilitate survival in fire prone environments.
Broad distribution over a range of distinct climatic types.
Low susceptibility to attack by phytophagous organisms.

Typically lianas are more abundant on edges of fragments than in the forest interior (Laurance *et al*, 2001). Most infestations of banana passionfruit are concentrated in areas of human occupation, for example near coastal camping grounds, so increased activity in these areas will increase the chance of long dispersal events (Williams & Buxton 1995). As the Marlborough Sounds become more heavily populated, disturbance caused by increased numbers of baches and driveways may affect the future spread of banana passionfruit. The high-light environment on the edge of shrubby and forest fragments, with some shrubs and smaller trees to 'climb', will provide favourable conditions from which banana passionfruit can access the canopy (Ogle *et al*, 2000). Slips are also areas of heavy infestation, particularly below Queen Charlotte Drive (personal observation). Rapid growth rates and structural support from other species allow vines to accumulate biomass quickly, forming a dense foliage cover over the canopy exploiting the high light environment, but smothering other species (Putz, 2005). Banana passionfruit has an extensive root network (pers. obs.) as is characteristic of lianas, which also allows effective competition for water and nutrients (Schnitzer *et al*, 2005). Similarly, banana passionfruit has many reproductive traits typical of invasive species. For example, flowers are long-lived with high visitation rates, and are at least partially self-compatible with limited inbreeding depression once a fruit has been produced. This may have significant implications for the spread of banana passionfruit to new areas because even if only one viable seed is deposited in an area that is free of *P. mollissima*, given suitable environmental conditions and available pollinators, a new population and further infestation could

potentially result. Furthermore, this is a plausible scenario considering how widespread honeybees and bumble bees are in New Zealand. It is conceivable that a single seed in the faecal matter of a blackbird or possum could result in another population, not to mention the hundreds of seeds found in one pig dropping.

In summary, banana passionfruit has many of the traits Alpert *et al* (2000) and Williams and West (2000) suggested were typical of weedy species with rapid dispersal. Firstly, plants are capable of quickly reaching reproductive maturity. Secondly, flowers and fruit are copiously produced throughout the year and fruits contain large numbers of seeds. Thirdly, reproduction is facilitated by mutualistic interactions. Finally, seeds have prolonged viability allowing them to form seeds banks (LaRosa, 1992) and they germinate following excretion by efficient long distance dispersers. While frugivore dispersal aids reproduction, even in the absence of seed removal by dispersers, fruits are able to germinate readily from intact fruits. All these factors have contributed to the proliferation of banana passionfruit in New Zealand.

Banana passionfruit has coped well despite the loss of its native mutualists by forming mutualisms with other exotic generalist pollinators and dispersers in New Zealand. Mutualisms with honey bees and bumble bees are responsible for the large numbers of seeds produced by banana passionfruit which are being effectively dispersed by introduced mammals, enhancing population growth. However, as shown by our experiments, banana passionfruit is not reproducing as fast as it could. This is demonstrated by a 68% decrease in the number of seedlings produced per flower in the field between hand-crossed and natural flowers. Therefore, while *P. mollissima* has adapted to New Zealand ecosystems by forming mutualisms with introduced pollinators, bees are not pollinating flowers as effectively as they could. Nevertheless, banana passionfruit produces a lot of flowers and still appears to be producing scores of viable seeds per fruit. In addition to these acquired mutualisms, release from the large number of herbivorous insects in its native habitat may have allowed banana passionfruit to flourish, reaching much higher densities than in South America. Low light levels, particularly in forest interiors, and freezing temperatures are likely to be limiting its distribution as it effectively competes with other species for water and nutrients (Schnitzer *et al*, 2005).

This research provides evidence that banana passionfruit is a double invasive mutualist because it relies on invasive exotics for both pollination and seed dispersal. Therefore, in the absence of other introduced species, banana passionfruit would have much lower seed output and little seed dispersal so would not have been able to spread as effectively in New Zealand, perhaps never becoming invasive.

4.2   Ecosystem processes - are New Zealand ecosystems becoming more invasible?

We have shown that invasiveness of banana passionfruit is augmented by facilitative plant-animal interactions. Research regarding the effects of introduced animals on indigenous ecosystems have typically focused on predation and herbivory rather than on the role of exotic species in the spread of noxious weeds, so the extent to which invasive mutualisms are required in order to develop invasiveness is not known (Richardson & Pysek, 2006). It is possible that the apparent 'lag phases', occurring before an introduced species undergoes seemingly explosive spread, could be partly attributed to the slow adoption of a new resource by dispersal vectors (Panetta & McKee, 1997). Regardless of the mechanism, there are many documented cases where exotic animals have facilitated weed reproduction through dispersal mutualisms. For example, introduced bison (*Bison bison*) facilitate the dispersal of weedy grasses on Santa Catalina Island, California (Constible *et al*, 2005).

Given that introduced pollinators and dispersers appear to be facilitating invasiveness in some species in New Zealand, the question of whether New Zealand ecosystems are becoming progressively more invasible remains. Is it plausible that introduced keystone species are affecting ecosystem processes and thus substantially modifying the composition of our native forests? By definition, a keystone species has an influence on the community that is disproportionately large relative to its abundance (Power *et al*, 1996). The significance of a keystone species in an ecosystem is so great that the addition to, or removal from, a community would have major consequences for the persistence of other species and ecosystem processes (Cowan, 2005). Lianas could be described as keystone species because they alter the structure of, and competitive interactions within, forests and significantly affect ecosystem-level processes (Schnitzer & Bongers, 2002). For example, Ogle *et al* (2000) found that old mans beard (*Clematis vitalba*) reduced species richness and the abundance of native New Zealand species, eliminated many native understorey trees and shrubs, and caused local extinction of some species, in an indigenous forest remnant.

Similarly, the widespread ecosystem effects of adding or removing possums from communities, often demonstrated through effects of eradication on offshore islands, have been well documented (Clout, 2006). Consequently, possums are prime candidates to be classified as a keystone species. They cause extensive defoliation of native forest and prey on nests of native birds (Clout, 2006). For example, Sweetapple *et al* (2004) scored canopy condition in three forests in South Westland within which possums had been present for 10, 20 and 30 years and found that catchments with a longer history of possums were in worse condition. Some preferred species suffered population decline, to the point in fuschia of local extinction. In addition, overall abundance of native birds was lower in catchments where possums had been present for longer. Therefore, podocarp-broadleaf ecosystems in the Marlborough Sounds are likely to be modified due to the presence of possums alone.

However, this may be exacerbated by banana passionfruit. Fruits typically have greater nutritional value than foliage and consequently, may have a disproportionately large effect on possum reproductive success and local possum carrying capacity (Cowan, 2005). Furthermore, banana passionfruit tends to have a period of heavy fruiting during late winter and early spring (pers. obs.) when other food sources are scarce (Williams & Karl, 1996). Therefore, *P. mollissima* fruits could be an important component of possum diets during periods when food is limiting. As a result, the presence of banana passionfruit may have reciprocal positive effect on possum populations. In addition, as a liana, banana passionfruit may be a keystone species, affecting forest structure and altering competitive interactions between species (Warshauer *et al*, 1983). Therefore, both possums and banana passionfruit are likely to exert strong modifying pressures on native vegetation, having significant repercussions for community competition. Possums also consume a wide range of fleshy fruits but often destroy the seeds of native species such as nikau (*Rhopalostylis sapida*), hinau (*Elaeocarpus dentatus*) and tawa (*Beilschmiedia tawa*) rather than dispersing them (Clout, 2006). This destruction of native propagules whilst dispersing exotic seeds may give banana passionfruit further competitive advantage over native species. In this way, the mutualistic interaction between possums and banana passionfruit may facilitate the invasiveness of both species.

Pigs also eat and destroy large numbers of native seeds, including hinau and tawa and also matai (*Prumnopitys taxifolia*), but there are currently no data regarding the viability of excreted seeds (Kelly *et al*, 2006). However, in our sample, only *P. mollissima* seeds were found in pig droppings and we have shown that seeds from banana passionfruit remain viable following excretion by pigs. In addition to their role in spreading this noxious weed, pigs could have a significant effect on long-term ecosystem process including nutrient cycling and plant species composition, through disturbing the ground and removing soil and litter invertebrates (McIlroy, 2005). In Hawaii, pigs actively move to areas with high densities of *P. mollissima* during heavy fruiting to take advantage of the fruit (Warshauer *et al*, 1983). While it is not possible to conclude whether the very high cover of *P. mollissima* is the cause or result of the very high densities of pigs in some areas, the connection between the two is considerable (Warshauer *et al*, 1983). Similarly, we have shown that in New Zealand, pigs significantly contribute to the spread of banana passionfruit. The extent to which banana passionfruit may support greater densities of pigs than would otherwise be possible is unknown.

In New Zealand there has been a serious decline of indigenous keystone vertebrate pollinators and dispersers since invasion by exotic animals, particularly mammalian predators (Kelly *et al*, 2006). It is becoming apparent that the loss of these mutualists is having an effect on the reproduction of native plants (Kelly *et al*, 2006). Exotic birds have done very little to functionally replace declining endemic birds and the combined contribution of all introduced animals to pollination and dispersal mutualisms of native plants appears to be surprisingly small (Kelly *et al*, 2006). In contrast, my research has shown that these

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generalist exotic species may instead form mutualisms with exotic plants. Indeed for banana passionfruit, invasiveness may not have been possible in the absence of introduced species.

In the Marlborough Sounds there are many interactions that may result in a fundamental change in forest species composition. Introduced mammals prey on native birds reducing their abundance, and hence their ability to perform pollination and/or dispersal to native vegetation. Consequently, the fecundity of native plants species is decreased, and invasive weedy species like banana passionfruit gain further competitive advantage. Native species will be differentially affected by competition and thus, some species may be displaced by banana passionfruit. Furthermore, herbivory will compound the adverse effects of mammals on indigenous plant reproduction by reducing tree health, while dispersal by introduced mammals continues to aid invasiveness in banana passionfruit. In addition, disturbance may form a perpetuating cycle that gives banana passionfruit the upper hand as structural parasitism causes further breakages to already weakened trees, resulting in tree fall gaps that may drag down surrounding individuals, and form high light environments from which more *P. mollissima* seedlings can access the canopy. Therefore, uniting the negative effect of exotic animals on native species, with their facilitation of invasive species, we might conclude that the abundance of generalist pollinators and dispersers threatens the integrity of New Zealand's ecosystems by making them more invasible, indicative of an invasional meltdown.

This idea is supported by a meta-analysis conducted by Parker *et al* (2006) of more than 100 exotic plants which revealed that native herbivores suppressed exotic plants, whereas exotic herbivores facilitated both the abundance and species richness of exotic vegetation. Overall, the relative abundance of exotic plants was 52% higher in communities grazed by exotic herbivores than in communities grazed by native herbivores. In short, Parker *et al* (2006) concluded that the widespread replacement of native with exotic herbivores eliminates indigenous ecosystem service, facilitates plant invasions, and triggers an invasional meltdown. They suggested that the mechanism for this was the competitive advantage of evolutionary history with herbivores, resulting in adaptations to avoid herbivory. However, the same principle could apply to facilitative mutualisms. The replacement of specialised pollinators and dispersers with exotic generalist mutualists appears to be resulting in declining specialised ecosystem service to native plants (Kelly *et al*, 2006) and, as suggested here, may also increase service to invasive plants.

Therefore, it could be inferred that ecosystems in New Zealand are undergoing invasional meltdown, as suggested by the invasive mutualisms of banana passionfruit. However, as with many studies, the evidence provided by my research is not conducted at the community or population scale (Simberloff, 2006). The invasive mutualisms of banana passionfruit may certainly result in population growth of both species, but this must be confirmed by further research at the population level in order to determine whether ecosystems are undergoing a full-scale invasional meltdown.

4.3   Implications for management

Gaining a greater understanding of the ecology of invasive plant species, as I have for banana passionfruit, provides valuable insight for management attempts to prevent the spread of weeds (Constible *et al*, 2005). However, the value of this information is severely reduced if it does not help us understand particular areas that could be targeted for control (Williams & West 2000). In the last 20 years, *P. mollissima* populations have undergone a population explosion in the Marlborough Sounds, moving from small isolated pockets to widespread infestations, particularly in areas with high anthropogenic activity. Banana passionfruit will continue to spread through lowland ecosystems in the absence of intervention. For example, along Queen Charlotte Drive there are dense infestations along and below the road, particularly near baches, but the hillside above the road does not yet appear to be infested. However, there is a lot of pig rooting toward the ridge and some clearings where large pine trees have fallen down (pers. obs.). In addition, as previously reported, pigs from the hillside congregate beneath vines just above the road to eat *P. mollissima* fruits. It is just a matter of time before seeds are transported back up the hill by the pigs and deposited in these rooted clearings, thus allowing banana passionfruit access to the canopy and spread throughout this more remote bush. Therefore, control of banana passionfruit now, while it is still largely confined to housed and disturbed areas, rather than overrunning the entire hillside, would seem a wise and cost-effective solution (Hulme, 2006).

There are several different ways in which banana passionfruit could be targeted for control. At present, the extent to which banana passionfruit damages native bush does not appear to be widely recognised by the general public. A large proportion of people enjoy eating the fruit and are aware of banana passionfruit, but don't realise that it is a pest and some still purposely grow it in their gardens (personal communication). In contrast, the threat of old mans beard (*Clematis vitalba*) is widely known due to an extensive campaign in the 1980's (Williams & West, 2000) and consequently, many community weed groups have been involved with control on a local scale (Ogle *et al*, 2000). Educating the public of the threats banana passionfruit poses and mobilising volunteer control efforts may be a key way in which to maximise resources available for control (Hulme, 2006). We need to raise awareness of the seriousness of the problem and try to gain the support of the communities and land owners so people manage it on their own land and control attempts are not solely conducted by management authorities. This is already happening in some areas of the Queen Charlotte Sound, for example Te Ranui Bay, where land owners are working with an independent contractor to control banana passionfruit and mammalian predators. Such initiative would be encouraged if the Department of Conservation were able to provide or recommend contractors at minimal cost and perhaps subsidise control as cost is likely to be a barrier for some people.

Previous research by Williams & Buxton (1995) has shown that cutting vines alone is not sufficient to kill banana passionfruit as it will simply re-root. Therefore, care is needed if using mechanical control or it

will be ineffective. For more mature plants, cutting the stem and painting each side of the severed stem with a herbicide (such as Roundup, Escort or Vigilant) will usually kill the plant and cause limited non-target impacts if applied carefully (LaRosa, 1992). Below-ground competition is a key interaction between trees and lianas so isolated cutting of lianas in the crown of a tree does not guarantee release from competition for the host. Vines competing below ground must also be severed and poisoned (Schinitzer *et al*, 2005). Furthermore, timing of control may reduce reproductive spread. There appear to be two heavier fruiting periods during the year, firstly after summer between about February and April and secondly, in early spring around September. Cutting and spraying vines in winter when fruits are still underdeveloped may be the most effective for reducing the number of offspring produced, because plants will be killed before fruits mature and the harsher environmental conditions of winter may reduce the likelihood of recovery from poisoning. In addition, fruits are likely to be the most important in the diet of invasive frugivores at the end of winter. The labels on some herbicides, such as Escort, recommend that they be used when plants are growing most actively between late spring and early autumn. However, others such as Roundup, are able to be used all year. The Department of Conservation website (www.doc.govt.nz) recommend that young banana passionfruit vines are cut in winter and the regrowth sprayed in spring. With larger vines, herbicide should be applied to stumps immediately after they are severed and reapplied when the plant actively begins to grow again in spring.

Targeted control in high risk areas, such as outlying populations which are not only easiest to control but have the greatest potential for spread, is an effective strategy for controlling banana passionfruit (Starr *et al*, 2003). Similarly, removal from along roadways may limit anthropogenic dispersal, and forest edges are important canopy access points for banana passionfruit so would be logical areas for control (LaRosa, 1992). In addition, control or elimination is most likely to be successful if implemented early so recently established plants and small populations should be targeted and removed (Cowie & Werner, 1993). Searching for small or recently established populations could be facilitated by identifying clearings or areas of rooting where pigs are likely to have deposited seeds.

Propagule supply, determined largely by pollination and dispersal, is critical in determining invasion success for plants (Richardson *et al*, 2000). Therefore, because mutualisms are very important in the reproduction of banana passionfruit, integrated pest management will be an effective way of slowing its spread (LaRosa, 1992). Integrated pest management utilises several complimentary measures to control a pest species, which in this case corresponds to the management of both banana passionfruit and its mutualists (Hulme, 2006). This is widely regarded as being an important determinant in whether control efforts will be a success (e.g. LaRosa, 1992; Williams & West, 2000; Constible *et al*, 2005).

Many of the predominant mutualistic partners of banana passionfruit are pests themselves, meaning mutualist control is a viable option (Hulme, 2006). Because of the importance of rare long-distance dispersal events for population spread, species with large home ranges may be a useful focus for management as they contribute disproportionately to the rate of spread (Buckley *et al*, 2006). Pigs are particularly important in the Marlborough Sounds because their populations are increasing and they are likely to transport large quantities of seeds further into the bush, providing a major opportunity for spread. Therefore, control of pigs may be an effective way of reducing the probability of new populations emerging. This has been recognised in Hawaii where the control of feral pigs and birds that disseminate the seeds is used in conjunction with control of *P. mollissima* itself (LaRosa, 1992). Pigs also have adverse effects on other aspects of the ecosystem, as mentioned in previous sections, so reductions in pig densities may generally contribute to the long-term conservation of the ecosystem (McIlroy, 2005). However, this may not receive the support of pig hunters. Previously, the populations of pigs in New Zealand have been constrained by recreational pig hunters and damage by pigs has not been considered severe enough for official control operations (McIlroy, 2005). As a result, there is currently no national pest management strategy for pigs (McIlroy, 2005). Due to recent increases in population size in the Marlborough Sounds, control of pigs may now be warranted, particularly prior to periods of heavy *P. mollissima* fruiting.

Possums are also probably important dispersal vectors for banana passionfruit in addition to many other ways in which they adversely affect indigenous ecosystems. Control of possums may also be warranted especially whilst undergoing a major control operation of banana passionfruit. While rats are not dispersing large numbers of seeds long distances, they may be contributing to success of banana passionfruit through short distance dispersal and caching. However, only four seeds were excreted by rats and none of these germinated, suggesting that rats may actually be helping prevent the spread of banana passionfruit by rapidly destroying seeds. Thus reduction in their abundance will not be critical for reducing the spread of banana passionfruit (as reducing pig and possum densities may be), but may result in release for banana passionfruit from an important source of seed predation. This represents one of the management dilemmas raised by Simberloff (1998) whereby management of one species conflicts with management of another, because rat predation can reduce the abundance of native birds in New Zealand (Innes *et al*, 2004). For example, possums and rats have been targeted in some private bays along Queen Charlotte Drive over the last two years and already there is a noticeable increase in the number of birds, with the return of bellbirds and perhaps even kaka. Therefore, while allowing rats to flourish may help reduce the spread of banana passionfruit, other species will be adversely affected by their presence.

Although banana passionfruit also forms invasive mutualisms with generalist pollinator species, bee species provide pollinator service to many native plants (Kelly *et al*, 2006). Removal of honey bees from

conservation land through the relocation of hives will significantly reduce the reproductive capacity of banana passionfruit, but the extent to which native plants would suffer in the absence of honey bee pollination would need to be determined to evaluate whether bee removal was appropriate (Hulme, 2006). In addition, it is possible that the spread of the varroa bee mite may reduce feral bee populations in the Marlborough Sounds. However, many of the visitors to banana passionfruit flowers were feral bumblebees which are likely to be much harder to control or remove.

In conclusion, the integrated management of banana passionfruit through cutting and poisoning vines and reducing densities of pigs and possums in particular, in combination with increased public awareness and private control may be capable of reducing the adverse effects of banana passionfruit in the Marlborough Sounds. However, a lot of time, resources and commitment will be required in order to have a sustained significant impact on banana passionfruit populations.

Biological control may be the only management option that will have a long term effect on the abundance of banana passionfruit. Having evaluated the status of *P. mollissima* in Hawaii, LaRosa (1992) warned that delaying control measures would be costly. Sure enough, infestations in Hawaii became so dense and widespread that mechanical and chemical control were impractical (Starr *et al*, 2003). For example, the Division of Forestry and Wildlife spent $90,000 per annum on up-rooting vines at the Hilo Forest Reserve, but to no avail (Trujillo, 2005). Thus, biological control was the only viable long-term control option (Causton *et al*, 2000). After several attempts at identifying and successfully introducing an appropriate biological control agent *Septoria passiflorae* was introduced, resulting in a 40-60% reduction in year following inoculations and a more than 95% reduction after four years (Trujillo, 2005). Subsequently, the use of this pathogen saved millions of dollars (the total cost of research was approximately $2.5 million but benefits exceed $100 million) in weed control, and *P. mollissima* populations in areas such as Hilo Forest Reserve have almost disappeared, resulting in forest regeneration and rejuvenation of old trees that were dying (Trujillo, 2005).

Until a suitable candidate is found and extensive testing has been conducted biological control of banana passionfruit is not an option in New Zealand. In the event that resources were made available for a major control operation, the integration of cutting and spraying vines with the control of mammalian dispersal vectors may prove most effective.

Control should aim to bring about a long-term reduction in banana passionfruit to an abundance that no longer significantly threatens the structure and composition of indigenous vegetation (Hulme, 2006). Further research will be required to determine the density at which banana passionfruit will have minimal impact on our native ecosystems, because even in relatively low abundance, lianas such as *P. mollissima* decrease the growth, fecundity and survivorship of trees and forests (Schnitzer & Bongers, 2002).

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APPENDIX 1

Appendix 1.1: Total number of fruit (out of 10*) produced in each Treatment.

Site 1: Beeches Bay						Site 2: Kaireperepe Bay				
	Selfed	Crossed	Bagged	Natural	**Total (/40)**	Selfed	Crossed	Bagged	Natural	**Total (/40)**
Patch 1	1	4	0	2	**7**	2	1	1	1	**5**
Patch 2	1	5	0	2	**8**	0	2	0	1	**3**
Patch 3	0	3	1	2	**6**	0	0	0	0	**0**
Patch 4	4	2	1	2	**9**	0	1	0	0	**1**
Patch 5	0	1	1	2	**4**	2	3	0	4	**9**
Patch 6	1	3	0	3	**7**	8	7	1	3	**19**
Patch 7	2	3	0	3	**8**	3	4	0	2	**9**
Patch 8	1	1	1	0	**3**	0	1	0	0	**1**
Patch 9	1	1	0	0	**2**	3	3	0	3	**9**
Patch 10	6	10	0	3	**19**	0	4	0	3	**7**
Total	**17**	**33**	**4**	**19**	**73**	**18**	**26**	**2**	**17**	**63**

* Except for Site 2, Patch 4 where n=7

Appendix 1.2: Means ± one standard error of fruit from first pollination experiment collected, measured and seeds counted in each treatment. All values taken to two decimal places.

	Selfed	**Crossed**	**Bagged**	**Natural**
Length (cm)	6.39 ± 0.24	6.65 ± 0.20	6.40 ± 0.58	6.43 ± 0.31
Width (cm)	3.00 ± 0.12	3.25 ± 0.09	3.20 ± 0.11	3.03 ± 0.11
Seed Count	114.57 ± 10.77	109.30 ± 7.50	123.75 ± 25.61	94.11 ± 10.28

Appendix 1.3: Summary of fruit characteristics in second pollination experiment. Table shows means with one standard error for fruit length, width, total seed count, number of viable seeds and the number of nonviable seeds.

	Length (cm)	**Width (cm)**	**Total Seeds**	**Viable seeds**	**Nonviable seeds**
Selfed	7.4 ± 0.3	3.4 ± 0.1	133.3 ± 12.8	121.9 ± 12.8	11.4 ± 3.5
Crossed	8.4 ± 0.3	3.9 ± 0.2	146.5 ± 15.0	137.2 ± 16.1	9.3 ± 5.9

Appendix 1.4: Fertiliser Ingredients for potting mix used in glasshouse germination experiments

Ingredients	**kg per 2 m^3**
Dolomite	2.50
Gypsum Screened	3.00
Ag Lime	2.00
Enduro Short	0.70
Trace Element Mix	0.60
Nutri Start	2.00
Nutricote 3/4 Mth Total 13%	1.00
Nutricote 5/6 Mth Total 13%	2.00
Nutricote 8/9 Mth Total 18%	3.00
Penetraide	3.00