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Benefits of seed dispersal for escaping seed
predation and examining the life history, host
suitability/preference and impact of the
polyphagous obligate seed predator

Cryptaspasma querula

A thesis presented in partial fulfilment of the requirements for the
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“The creation of a thousand forests is in one acorn”

- Ralph Waldo Emerson

Abstract

Cryptaspasma querula is an endemic moth (Family Tortricidae) that is the only known polyphagous obligate post-dispersal seed predator in New Zealand. It is suggested to have multiple hosts including tawa (*Beilschmiedia tawa*), taraire (*Beilschmiedia tarairi*), miro (*Prumnopitys ferruginea*), hinau (*Elaeocarpus dentatus*), karaka (*Corynocarpus laevigatus*), and acorns from exotic oaks (*Quercus sp.*). There have been few studies clarifying these claims and fewer still showing the effects *C. querula* has on the host plants' seeds' germination and establishment. Little is also known about the life history, host preferences, specialist predators/parasitoids and host cues of *C. querula*. The drilling damage *C. querula* does to its hosts has been noted in several studies but none have recorded the extent of this damage over the course of the season or what the end percentage of damaged seeds are.

Of the described hosts, the broadleaf tree tawa has had been studied the most. The apparent rarity of good germination and establishment under the parent canopy has been noted previously. It is unclear however, whether the impact of seed predators make dispersing seed away from the parent canopy (where fewer seed predators are in theory) more beneficial for tawa. If it is more beneficial, then the extent of dispersal through faunal influences will be important to know.

Life history and host cues (in the form of wind tunnel tests used to test olfactory attractants) of *C. querula* were examined in a controlled environment where rearing observations were taken and variables could easily be manipulated. The host preference and suitability for *C. querula* was examined in two settings: Laboratory and Field. The controlled environment utilised the seeds of tawa, taraire, hinau, and miro to see whether these were plausible hosts. The field experiments utilised the species miro, karaka, and acorns from exotic oaks. The examination of potential specialist predators or parasitoids took place in two settings: Field and Glass house (semi-controlled environment).

In captivity, mating of *C. querula* adults raised from field collected larvae frequently occurred and many eggs were produced which could be used in choice

tests and behavioural assays. Females lay a variable number of eggs and seemed to prefer ovipositing on smooth surfaces. The time spent in each instar and pupation varied greatly among individuals. Wind tunnel tests were inconclusive for both adult and larvae specimens. However, in choice tests and even when given no other hosts, many of the proposed hosts of *C. querula* appeared to be unsuitable and were not occupied by *C. querula* larvae in the lab. These included miro, hinau, and karaka where *C. querula* either ignored the fruits provided, or, if endosperm was utilised, the larvae did not complete their lifecycle. The only suitable and preferred hosts in these trials were tawa and taraire. However, it is highly likely acorns from exotic oaks are also suitable and preferred by *C. querula* since field trials showed frequent occupancy in acorns. Only one potential competitor (no specialist predators/parasitoids) of *C. querula* was discovered and it was a generalist facultative diptera (*Sylvicola sp.*).

In a factorial field experiment (repeated at three sites) in which fruit density, the presence or absence of fruit flesh, and the position either under or away from a tawa canopy were assessed, germination and establishment of tawa were highest in high seed density treatments showing potential predator satiation may be occurring. Seeds dispersed away from tawa canopy also had a higher establishment rate than seeds under the parent canopy, suggesting the importance of faunal dispersers. The effect of flesh removal was variable but generally favoured establishment. Several additional factors such as vertebrate seed predators/predators, abiotic influences, and site differences appeared to have influenced the results. Drilled/damaged seeds could still germinate/establish but their chances were much lower than undamaged seeds.

In these same three sites, plus an additional 8 sites added to widen the study, seed predation at the end of season was very high despite a very heavy fruiting season. As the season progressed, over 90% of tawa seeds ended up being drilled by *C. querula* suggesting the likelihood of very heavy influences on tawa regeneration. The proportion of bird dispersed seeds was also unexpectedly low (maximum of 8% of seeds beneath the canopy were bird dispersed). The surrounding floral species composition (and dominance) did not appear to influence the damage received to tawa seeds.

Information on *C. querula*'s life history, host preferences/suitability and the impact on hosts are better understood through this thesis. Seed dispersal and high seed density are shown to offer the best strategy for tawa seeds' establishment and survival. The extent of drilling damage on tawa fruits over the course of the season has been shown to be higher than expected and strongly detrimental and demonstrates the importance of predation escape. Thus, tawa is highly dependent on the remaining kereru populations and will be sensitive to further declines to dispersal service.

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Chapter 1

Introduction

1.1 Invertebrate Seed Predators

Studies of invertebrate seed predation in New Zealand are few compared with many other countries. A seed predator is an animal that sometime in its lifecycle consumes, partially or fully, seeds of host plants. Some are specialists that utilise only one or a few closely related host species, others are generalist targeting multiple host species. In general, seed predators have negative and damaging effects on the fecundity of host plants reducing successful regeneration. In extreme situations seed predators can cause collapse of host-species populations. The intense interactions of hosts and predators have direct impacts on reproductive fitness and thus have co-evolutionary implications.

One reproductive strategy expressed by many plants that is frequently interpreted as an adaptation to seed predation is mast seeding (Salisbury 1942; Janzen 1971; Silvertown 1980; Kelly 1994). The most commonly used and general definition of mast seeding is: ‘synchronous highly variable seed production among years by a population of plants’ (Kelly, 1994). This is a process whereby plants have irregular fruiting/seeding years or seasons. Thus in some years very few fruit or seeds are produced, resulting in low reproduction in a seed predator population because their resources are limited. In years when the host plants produce fruit/seed in vast amounts, the small population of seed predators is rapidly satiated themselves leaving many seeds to escape predation and to germinate and grow – this is called the predator satiation theory (Silvertown, 1980). The plants may use an environmental cue to synchronise masting among individuals. For some plants such as the tussock grass *Chionochloa sp.* it has been suggested that temperature is a trigger for masting (Kelly et al. 2008, Kelly et al. 2013) while other species may use a combination of climate cues and resource availability (Smaill et al. 2011). It is interesting to note that for plants to benefit from the predator satiation strategy, all potential host plants of a seed predator need to mast in synchrony. Otherwise seed predators can switch between host-species, so masting is most likely to be selected for in plants with specialist seed predators. An alternative explanation for

mast seeding is wind pollination effects (Nilsson & Wastljung 1987; Norton & Kelly 1988; Smith et al. 1990) that are independent of host-predator interactions.

Seed predators are not immune to predation. One particular group of animals, classified as parasitoids, can devastate seed predator populations. Parasitoids are insects that directly utilise host resources during their own larval stage. Usually the host is killed by the process while the parasitic larvae develops and emerge as adults that can repeat the cycle. There have been a number of studies on the complex dynamics of the plant–seed predator–parasitoid interaction (e.g. Gomez & Zamora (1994) studied the top-down effects of a guild of parasitoids (*Necremnus tidius*, *N. folia*, and *Aprostocetus sp.*), the seed predator (*Ceutorhynchus sp.*) and the host plant (*Holmaphylla spinosa*). Another study by von Zeipel et al. (2006) looked at the plant *Actaea spicata*, its lepidopteran seed predator the *Eupithecia immundata* and the guild of parasitoids affiliated with it.). In some cases, parasitoids can reduce the seed predator population to such an extent that the host-plants are released from the seed predation pressure. This could result in relationships between plants and parasitoids.

There are several key elements involved in seed predation, and often these are not well known in many cases: how do seed predators find the host seeds; where do the adults oviposit egg clusters; do adult seed predators remain where their host-plants are abundant or do they distribute randomly; are there multiple generations of the seed predator within one breeding cycle of the host; do the seed predators have a large impact on ecosystem communities?

Seed predators must first find the appropriate seeds that they can utilise. This could occur at either of two stages of their lifecycles. An adult female could lay an egg cluster randomly (near or far from a potential host seed) and emerging larva find the host seeds themselves. Larvae might navigate using visual cues or olfactory cues, or utilise a random walk strategy. Alternatively, adult seed predators could locate host seeds and lay eggs directly on them. This strategy enables larva to drill into the seeds and find nutrition immediately on hatching. Several studies have shown that adult herbivores and seed predators can find the hosts through smell and olfactory cues (e.g. Bruce et al. 2005; Tasin et al. 2011). These cues can stimulate females of some seed-predators to oviposit.

Species likely congregate in locations where resources they utilise are abundant. It would be interesting to see whether this is also the case for seed predators because at different stages of their lifecycle they may utilise different resources. If the adult seed predators remain in areas where they emerged (likely to be a place where host seeds are abundant) then ovipositing egg clusters freely may be a successful strategy because there will be a high likelihood that larvae will be able to find a host themselves. However, if the adult moths move away from their emergence sites into different communities of trees and forests, it is likely that suitable hosts for larva will be scarce (unless the seed predator is a very capable generalist). This means the adult seed predator would likely need to find small patches of suitable hosts before ovipositing.

Seed predators can be important players in shaping community assemblages of plants and vegetation distributions. As they can focus on one species of host (if they are specialists), the consequence is that the host plant may have difficulty reproducing and spreading in a given area (unless the seed predators do not kill the seedling and leave enough resources for the seed to germinate). Lewis & Gripenberg (2008) suggested that seed predators could, indeed, have a role in maintaining diversity in plant assemblages and structuring their composition by suppressing species that otherwise might outcompete other species. They focused primarily on a tropical setting. This is one of the motivations behind the Janzen-Connell hypothesis where natural enemies reduce the competitiveness of a naturally dominant species, thereby maintaining species coexistence (Janzen, 1970 & Connell, 1971). It would be interesting to see whether New Zealand seed predators could similarly influence plant assemblages and compositions in New Zealand's temperate forests.

It is also theorized that seed predators could be capable biological control agents on unwanted plants. Garren & Strauss (2009) showed that seed predators can potentially reduce already low plant densities. This supports the idea that seed predators can have potentially extreme impacts on the plants they utilise. Unprepared (not evolved to deal with seed predation) plants could possibly suffer such a loss in fitness that they become locally extinct or survive only at low population densities. Conversely, it is possible that despite the presence of seed predators, host plants are not affected significantly enough to be evolutionary influenced or to show selection for anti-feeding adaptations. A review

by Norton et al. (1988) explained this may be the case for Rimu (*Dacrydium cupressinum*) and its potential seed predators.

Seed size within plant species is often a relatively constant characteristic compared with other plant characteristics. However, among and within an individual plant, seed sizes can vary dramatically (Geritz, 1998). Several studies and models have looked at the correlation between seed predation their host seed size (Geritz, 1998; Honek et al. 2011; Espelta et al. 2009). Several of these studies showed that seed size directly influenced the type and size of the seed predator (Honek et al. 2011; Espelta et al. 2009). This suggests that selective seed predators may ignore a seed that is too small (or possibly too big) for it. This in turn could exert selection on host plants favouring individuals with smaller or larger seeds (depending on which are targeted). Geritz (1998) proposed a model that showed that not only do seed predators affect the host's evolutionary fitness but the host plant can also affect the seed predator's evolutionary fitness as well by producing the differently sized seeds.

1.2 Introduction to *Cryptaspasma querula*

The genus *Cryptaspasma* (Lepidoptera: Tortricidae) includes 35 or more species found all over the world including South America, Japan, Pacific Russia, East Africa, Malagasy and Indo-Australia (Horak, 2006). The Indo-Australian species (which includes *C. querula*) are all in the same morphologically defined sub-genus. All known hosts for this genus are big seeded and the larvae live inside the endosperm which they also feed upon. Some recorded hosts in the Indo-Australia area are species within the families Arecaceae, Lauraceae, Monimiaceae, Mytraceae and Proteaceae (Horak, 2006). One of the most renowned members is *C. sp. nr. lugubris* which is a pest of avocado in Mexico (Hoddle, n.d.).

In this study I examined the moth *Cryptaspasma querula* (see Figure 1.2.1 & 1.2.2) which is endemic to New Zealand. Larvae of *C. querula* target the seeds of several New Zealand trees, and is the only known polyphagous obligate seed predator in New Zealand (Sullivan et al. 1995). Its primary host is *Beilschmiedia tawa* (Beveridge, 1964), a podocarp found all over the North Island south of Northland and in South

Island, north of Westport. The larva of *C. querula* is also reported to utilise miro (*Prumnopitys ferruginea*) (Martin, 2001), hinau (*Elaeocarpus dentatus*) (John Dugdale, pers. comm.), taraire (*Beilschmiedia tarairi*) (Wotton 2007), acorns from exotic species (genus: *Quercus*), Kohekohe (*Dysoxylum spectabile*), Karaka (*Corynocarpus laevigatus*) and several other species but some of these need more support and clarification.



Figure 1.2.1. *Cryptaspasma querula* late stage larva and pupa.



Figure 1.2.2. *Cryptaspasma querula* adult male.
Scale is in mm (longer lines) and 0.5mm units (shorter lines).

Adult *C. querula* are small dark moths (Figure 1.2.3), sometimes with two small eye-spots on the dorsal surface of their wings. There is some slight sexual dimorphism in terms of size and patterning to the wings. Males (Figure 1.2.4) have reflective patterns on the dorsal surface of their wings (often near the two eye spots) and they are generally (not always) smaller than the females (greatly varies but around 2mm difference between genders). Female *C. querula* are generally longer and more robust than the males (though this may not always be the case) and have less decorative wings (plain brown sometimes has two tiny eye spots).



Figure 1.2.3. *Cryptaspasma querula* Adult.



Figure 1.2.4. *Cryptaspasma querula* Male.

The Egg sacs (Figure 1.2.5) of *C. querula* are very pale beige when first laid but slowly darken as the larva inside near hatching. Upon hatching the larva (Figure 1.2.6) are tiny in size (around 0.3mm thick and 1.5mm long). In a captive situation, larvae are seen to roam, apparently searching for a host seed to drill into. Once within a seed they are sustained by eating the endosperm. After an indeterminate number of instars they emerge from the host seed many times larger (around 15mm long) than when they entered (Figure 1.2.7). Upon leaving the host seed they search for a suitable location where they pupate (Figure 1.2.8). Hoddle (n.d.) observed that, more often than not, in laboratory rearing environments, larva would pupate (Figure 1.2.9) inside the paper towel layering the terrariums they were housed in.



Figure 1.2.5. *C. querula* egg sacs.



Figure 1.2.6. Tiny *C. querula* larvae.



Figure 1.2.7. *C. querula* larvae about to pupate.



Figure 1.2.8. *C. querula* pupae.



Figure 1.2.9. *C. querula* larva forming protective cocoon out of paper towel layers.

It is known that in some areas predation rates of *C. querula* by rodents (mice and rats) can be quite high. A pilot study by Jones & Toft (2006) found *C. querula* larva in 31% of mice stomachs examined. Horak (2006) found that the main diet of mice were various spiders and caterpillars. In one mouse stomach he found nine *C. querula* larva alone which suggests the rodents were targeting this insect food rather than the endosperm of the host plant *Beilschmiedia tawa*. It is also possible that during a rodent's attempt to get to a *C. querula* they may damage the seed irrevocably (larger holes; more likely to damage actual seedling inside) compared with the relatively little damage caused by seed predators (which may only eat the endosperm but leave the actual seedling unscathed).

Not much is known about whether *C. querula* find oviposition sites through olfactory cues or other means. Previous studies looking at lepidopteran species have shown response to various scents, pheromones and olfactory lures in wind tunnel experiments. von Arx et al (2011) showed that male *Lobesia botrana* respond to certain olfactory stimulating chemicals which help them find host plants. A similar study by Tasin et al. (2011) showed an oviposition response in female *Lobesia botrana* to the host plant the wine grape, *Vitis vinifera* which had certain sensory and olfactory cues.

An unpublished study by Robertson et al. (2010) looked at *Cryptaspasma querula* and the amount of seed predation in *Beilschmiedia tawa* seeds under tawa canopies and away from tawa canopies. They found there was more seed predation under tawa canopies than away, suggesting that for this particular species, adults may stay near host seeds despite the fact that they utilise a different resource to larva.

Often in the case of tropical seed predators, there will be multiple generations of the species within one year. This means in the course of one year the species of seed predator will go through multiple lifecycles (egg, larva, pupa, adult and back to egg). In New Zealand, our climate is largely considered temperate (newzealand.com, N/A) rather than tropical. It would be important to see whether seed predators in NZ can have multiple generations in one year or whether they only have one generation per annum. For certain species like *C. querula*, Dugdale (pers. comm.) believe there would be multiple generations per annum.

There has been little research on the lifecycle of *C. querula* or the host plants it utilises. There are lepidopteran seed predators in the tropics that have multiple generations over the course of one year. It is unknown as of yet whether *C. querula* follows this pattern in its lifecycle or whether it only has one generation per annum. John Dugdale (pers. comm.) believes *C. querula* may have multiple generations in one year. A related species, *C. sp. nr. lugubris*, has been shown to have multiple generations in one year (Hoddle, n.d.). If *C. querula* and *C. sp. nr. lugubris* are very closely related they may have similar lifecycles.

In *C. sp. nr. lugubris*, which utilises avocado stones, the larvae emerge from the stone when it is time to pupate (Hoddle, n.d.). They spend between 24 - 48 hours “wandering” before they form a silk cocoon between two layers of moist paper layering. It is in there that they pupate. They also noted that female *C. sp. nr. Lugubris* preferred laying egg clusters on smooth surfaces like glass or plastic than any other textures.

Seed predators can quite often have natural enemies in the form of parasitoids. Currently there is no information as to whether *C. querula* has any specialist or generalist parasitoids. The closely related *C. sp. nr. lugubris* has the hymenopteran *Pseudophanerotoma* sp. (Braconidae) as a parasitoid (Hoddle, n.d.). Parasitoids are an important part of any seed predator and host system. A seed predator can have devastating effects on the hosts they utilise to the point where there is no recruitment. A parasitoid can significantly lower the seed predator's population thereby increasing the host's chance of successfully reproducing. *C. querula* currently has no known parasitoid but this study will try identify whether any specialist or generalist parasitoids are, in fact using this species as a host.

1.3 Focal Host-Species

For the purpose of the present research, *Beilschmiedia tawa* (tawa) is regarded as the primary host plant. Tawa is a lowland, evergreen canopy tree growing up to 25m (Salmon, 1980) to 30m (Knowles & Beveridge, 1982) in height. It can be found from sea-level and up to 800m above sea-level (Salmon, 1980). In the North Island it is often the most common species found in forests and remnants (Salmon, 1980). The species is

known to undergo a degree of masting (Knowles, & Beveridge, 1982). This may be a response to seed predation (Kelly, 1994) or wind pollination (Smith et al. 1990) although *B. tawa* pollination is achieved through both insects and wind (Smale, 2008). Tawa fruits are large, oval, purple drupes (Figure 1.3.1) that can grow to 3cm long (Salmon, 1980). These fruits hold a single large seed which is targeted by *C. querula* (Figure 1.2.2).



Figure 1.3.1: Ripe *B. tawa* drupes.



Figure 1.2.2: *C. querula* drilled in a tawa seed.

Logging of many New Zealand forests removed tall emergent trees from forest communities and systems allowing smaller canopy trees like *B. tawa* to become the more dominant (Salmon, 1980). For instance, in the Manawatu it is said that *B. tawa* is likely much more dominant now than before the logging occurred (Esler, 1978). The process of prey switching which is where more common food sources are utilised more extensively by their consumers, could be occurring in tawa and its interaction with seed predators. A vertebrate seed predator study by Fletcher et al. (2010) showed that North American red squirrels switch food sources when one food source becomes scarce and another becomes more common. In this case *B. tawa* have become much more dominant than in the past thereby opening a niche in seed predation for the polyphagous obligate *C. querula*, which may have utilised multiple species more or less evenly. Due to the host-species increase, *C. querula* may be using or specialising on tawa more than it did in the past.

Another important question unanswered in the *B. tawa* – *C. querula* relationship is whether drilled seeds have a lower chance of germinating. If the location in which the seeds are drilled is relatively far away from the actual seedling inside, it is possible that some of the endosperm being removed/eaten will have no impact on the viability of the

seedling. However, if *C. querula* usually drill into the seed in a location that destroys the developing seedling then germination would not occur. This study will look at this situation in more detail to see whether seed predation is actually having an overall negative effect on germinating seedlings. Also if too much endosperm is being removed then the seedling may not have enough nourishment to reach a point where it can sustain itself. This will be briefly investigated as well.

An unpublished study (Robertson et al.) looked into the relationship of tawa stands and tawa-free stands and the amount of seed predation by *C. querula* occurring under each. *B. tawa* seeds were either cleaned (fleshless) or left whole (fleshed) and occurred at two different densities when placed either under *B. tawa* trees or away in non-tawa stands. The resulting data suggested that *C. querula* (and possibly other seed predators) preferred drilling into *B. tawa* seeds under *B. tawa* canopy than in non-tawa stands. They also preferred higher densities of tawa seeds and seeds that were whole, not cleaned. It is possible *C. querula* stay primarily around tawa stands as this is where their main food source is located. They also appeared to be density dependent and preferred fleshed seeds over cleaned ones. This leaves questions as to whether *C. querula* prefers staying primarily around tawa forests, whether they are, in-fact, density-dependant, and whether they require fleshed seeds for volatile cues as they may not be able to find cleaned seeds.

Chapter 2

Testing host species of *Cryptaspasma querula* & analysing its life history in a laboratory and field setting

2.1 Introduction

There are many different taxa of invertebrate seed predators each with differing life histories such as instar durations, pupation and egg incubation times. For this thesis, the lepidopteran, Tortricidae seed predator, *Cryptaspasma querula*, will be the focal species. There are few in-depth studies looking into *C. querula*, despite the interesting fact it is the only known obligate polyphagous seed predator in New Zealand (Sullivan et al. 1995). There have been a few studies briefly mentioning the occurrence of *C. querula* on different host plant species (Beveridge, 1964; Sullivan et al. 1995) but no in-depth examination of their lifecycles, emergence times from seeds or whether it has any generalist/specialist parasitoids. Fortunately there have been several studies on overseas species in the *Cryptaspasma* genus (Hoddle & Hoddle, 2008; Brown & Brown, 2004; Hoddle, n.d.). Hoddle (2011) examined specifically *C. sp. nr. lugubris*, which is a related seed predator that infects avocado in Mexico. The study included in-depth details of each stage of the *Cryptaspasma* species' lifecycle including the approximate time it took for each stage (egg-larva-pupa-adult).

It is unclear whether *C. querula* have multiple generations within a year like the related *C. sp. nr. lugubris* species and many other tropical seed predators (Hoddle, 2011) or whether it only has one generation in the year. In contrast to the climate experienced by *C. sp. nr. lugubris*, and other tropical seed predators, New Zealand's climate is classified as temperate rather than tropical, and the focal host species *Beilschmiedia tawa* (Martin, 2001) fruits only drop during one season in the year (February – April). Furthermore, tawa masting occurs once in every few years. This suggests that *C. querula* might be expected to have a single generation per annum, but because *C. querula* is polyphagous it might utilise multiple hosts when *B. tawa* is not producing seeds/fruits. Dugdale (pers. comm.) proposed that *C. querula* has multiple generations in one year. It is hoped this question (whether they have multiple generations in a year or only one) will be

answered in this chapter.

There is little detailed information about alternative hosts of *C. querula* but Wotton (2007) found grubs emerging from the large drupes of another tree Karaka (*Corynocarpus laevigatus*). This is logical as it has a very similar seed structure to *B. tawa* except that is slightly larger (possibly containing more utilizable resources) and could be an ideal host for *C. querula* (Martin, 2001). Other suggested host-species include Miro (*Prumnopitys ferruginea*) (Martin, 2001), and Hinau (*Elaeocarpus dentatus*) (John Dugdale, pers. comm.) and introduced oaks (John Dugdale, pers. comm.).

As a polyphagous obligate seed predator, *C. querula* individuals must sometimes have a choice of different host seeds to utilise. A study by Honek et al. (2011) found that certain ground beetle seed predators have preferences among the seeds of different host species. These preferences were often correlated with size of the beetles themselves and the size of the seeds. A study of polyphagous mammalian seed predators noted that the rate of seed predation on a particular host can decrease dramatically when fruits of another more desirable species become available (Hoshizaki & Hulme, 2002). In the case of the *C. querula-B. tawa* relationship, a similar situation would greatly relieve predation pressure on *B. tawa* if another host species was targeted or preferred by *C. querula*. Or the opposite could occur where another host species maintains high *C. querula* populations when there is little tawa seed available therefore making it harder for tawa to escape in both time and space.

Hoddle (2011) found that prior to pupation the larva of *C. sp. nr. lugubris*, left the avocado seed and wandered around for around 24 hours before building a small cocoon in the folds of the moist paper towels lining the terrariums. This could also occur in *C. querula* which would likely put them at risk to parasitism and predation in the field. Parasitoids and predators would likely have an easier time extracting or damaging *C. querula* larva while it is wandering around rather than when it is protected by the casing of a host seed. Another important question is whether *C. querula* will leave their currently parasitized seed for ‘greener pastures.’ If their current seed has been depleted of resources or is in a compromised area (waterlogged, near stationary predators etc.) then it would be interesting to see whether they leave to find other seeds of the same or

different species as their previous one. If this does occur there could be large impacts on other plant species that can be utilised by *C. querula*.

There have been several studies (e.g. Bruce et al. 2005; von Arx et al. 2011; Svensson et al. 2011) of various insects locating ‘targets’ (such as mates, flowers, oviposition sites, seeds & fruit etc.) using olfactory or pheromone cues. It would be advantageous for adult seed predators to be readily able to use such to identify cues to lead them to suitable oviposition sites. Currently it is unclear whether *C. querula* as a seed predator utilises any form of chemical cue from fallen *B. tawa* fruits.

Similarly, it would be interesting to identify whether any life stages of *C. querula* are subject to the allure of the chemical cues. There have been several experiments looking at adults of insects and their attraction to cues in wind tunnels (e.g. von Arx et al. 2011; Ichiki et al. 2010), but there have been relatively few looking at the larva of a particular species being attracted by chemical cues. *C. querula* adults may not react to any form of chemical cue that *B. tawa* fruits emit. If this is the case it would be beneficial to the larva if they could follow chemical cues instead. Alternatively, adult females may be attracted by a specific scent. This would result in them laying their eggs in proximity to fruit and by chance several of the young larvae manage to find suitable host fruits.

If *C. querula* are capable of reacting and following certain chemical cues (whether adult or larvae), the specificity of this response would be of interest. An example could be whether *C. querula* could tell the difference between fruits of different potential hosts.

C. querula utilises *B. tawa* seeds during their larval stage (Beveridge 1964) but appears to use several other species as well which can include Miro (*Prumnopitys ferruginea*) (Sullivan et al 1995), Karaka (*Corynocarpus laevigatus*) (Wotton 2007), and several species belonging to the genus *Quercus* (John Dugdale, pers. comm.). There have, however, been few studies actually supporting these claims.

Unlike laboratory tests, field tests are more similar to a ‘real world situation’ in allowing natural factors (such as temperature, soil pH, predatory & herbivore influences etc.) to affect the outcomes of various tests at the expense of controlling variables. *C. querula* seed predation of choice can be assessed using laboratory tests but, in a ‘real world’

situation, many other factors may influence a *C. querula*'s decision to drill into a particular species' seed.

All potential host species of *C. querula* will be tested in this section to see whether *C. querula* can utilise them as hosts.. The following sections will attempt to answer the questions of whether *C. querula* (adults and larva) are attracted to olfactory cues of differing host species, what species of plants are plausible hosts, and what host species are preferred?

2.2 Methods

2.2.1 In-Lab Rearing and Experiments

To establish a colony of *Cryptaspasma querula* in the laboratory, tawa seeds were collected in the field and searched for larvae. If larvae were present, the source of the seed was recorded and the larvae were placed in a small terrarium. (At first, oats were used as a food source to try keep the larvae alive but they seemed to be un-utilisable by them.) Undrilled tawa seeds were used instead. Adult moths were separated from pupa and larvae into separate terrariums by source location (Nga Manu, Turitea, Totara Reserve). Fleshed tawa fruit were placed in the terrariums to stimulate oviposition.

After eggs were laid, they were left to develop on their own. Once the newly-hatched larva began to emerge they were separated and used in various experiments (see next sub-sections).

2.2.1.1 Monoculture Experiments

These experiments were designed to test whether the larva could adequately subsist and complete a life cycle on only one type of host species' seeds. *Beilschmiedia tawa* was not included in these experiments as it is already established that tawa is a host species for *Cryptaspasma*. However (see later section) there was a tawa experiment run later that consisted of twenty seeds (double the amount here) which can be used to compare. These monoculture experiments consisted of separate terrariums, each with ten seeds of a species. The three species used were Taraire, Hinau and Miro. Three replicates of each

monoculture were prepared. To each terrarium 10 newly-hatched larvae, from the same egg batch, were released as shown in Figure 2.1.

Each group of ten seed was weighed before being placed in the terrariums to get an estimate of how much they desiccated and how much of it was being eaten by the larva. The weights only give a very rough estimate of the amount of endosperm that was eaten since mass will have been lost by drying as well as consumption; therefore, seeds were also cut in half and scanned after 100 days exposure to larvae. The species seed scans were reviewed by eye and placed into percentile bins (0%; 1 – 25%; 26 -50%; 51 – 75%; 76 – 100%) according to how much endosperm was eaten (Figure 2.1). The analysis of the bin data was combined with the mass tawa experiments in the laboratory (which consisted of twenty seeds per replicate rather than the other species replicates which consisted of ten seeds each). These percentile bins were analysed using a Kruskal-Wallis test in the statistical computer program R.

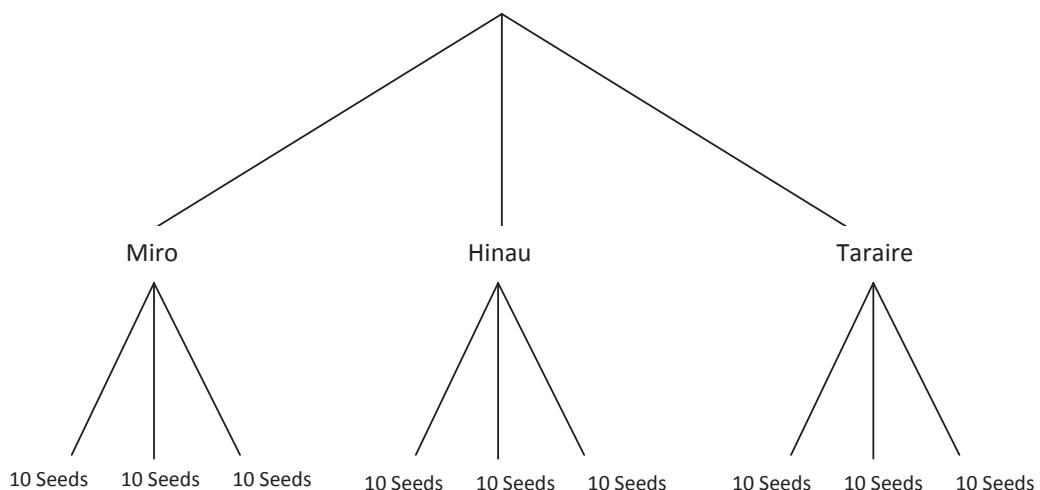


Figure 2.1 The end points are each one terrarium housing 10 seeds of a particular species and 10 *C. querula* larvae



Figure 2.2. Scanned seeds showing the damage done to the endosperm by *C. querula* larva.

Each terrarium was given 100 days, from when it was first prepared, to complete the larva's lifecycle. At the end of the 100 days the number of seeds drilled was recorded. Also the number of adults, pupa and larva were recorded. The presence of adults signified that the food source was sufficient for *Cryptaspasma* to survive on solely.

2.2.1.2 Tawa Predation Rates

Three replica monocultures of 20 tawa seeds were established in terrariums to see whether 10 newly-hatched larva (from the same egg batch) move between seeds after drilling. If 10 (of the 20) tawa seeds bore drilling holes at the end of the experiment then it was deemed possible for *Cryptaspasma* larvae to move around.

2.2.1.3 Choice Experiments

In a terrarium, five seeds belonging to four or three (Tawa, Hinau, Miro, and Taraire but miro seeds became limited part way through testing) species were placed as possible food sources for *Cryptaspasma*. 10 larvae were put into the terrariums for 100 days.

At the end of the 100 days, the species of seeds with drilled holes were recorded, as were the number of larvae, pupae and adults. To see whether if, given a choice, the larvae pick one species over another or do they select seeds randomly. Also if there were more holes in total than the number of larvae put in, it can be assumed the larvae may move around after utilizing the resources of one seed to find more beneficial resources to a particular instar stage in another species-host.

The stock of Miro seeds ran out before a large number of replicates of the experiments could be made so the last few replicates did not have Miro.

2.2.2 Behavioural Wind Tunnel Tests

To see whether larva or adult *C. querula* respond to volatile cues produced by bruised tawa fruits, two wind tunnel experiments were undertaken. The larvae and adults used for these trials were lab-reared. After each trial, the wind tunnel was wiped clean using 70% ethanol. A small two-minute break between each experiment was allowed so the ethanol would evaporate.

2.2.2.1 Larval Wind Tunnel Tests

The larvae were placed in a roughly 60cm long cylindrical y-tube which had a radius of roughly 3.5cm. The y-tube consisted of three ends, two where air was pumped in and one where the air escaped. On one end of the tube where the air was coming in, a bruised tawa fruit was placed. The wind should have carried the volatile cues down to the exiting end. The other wind entering end was left empty. The fruit were alternately placed in either of the two ends. Recently hatched larvae were placed in the end, where the wind exited, one at a time (Figure 2.2). The prediction was that volatile cues from the fruit should attract the larva as it searched for the host seeds.

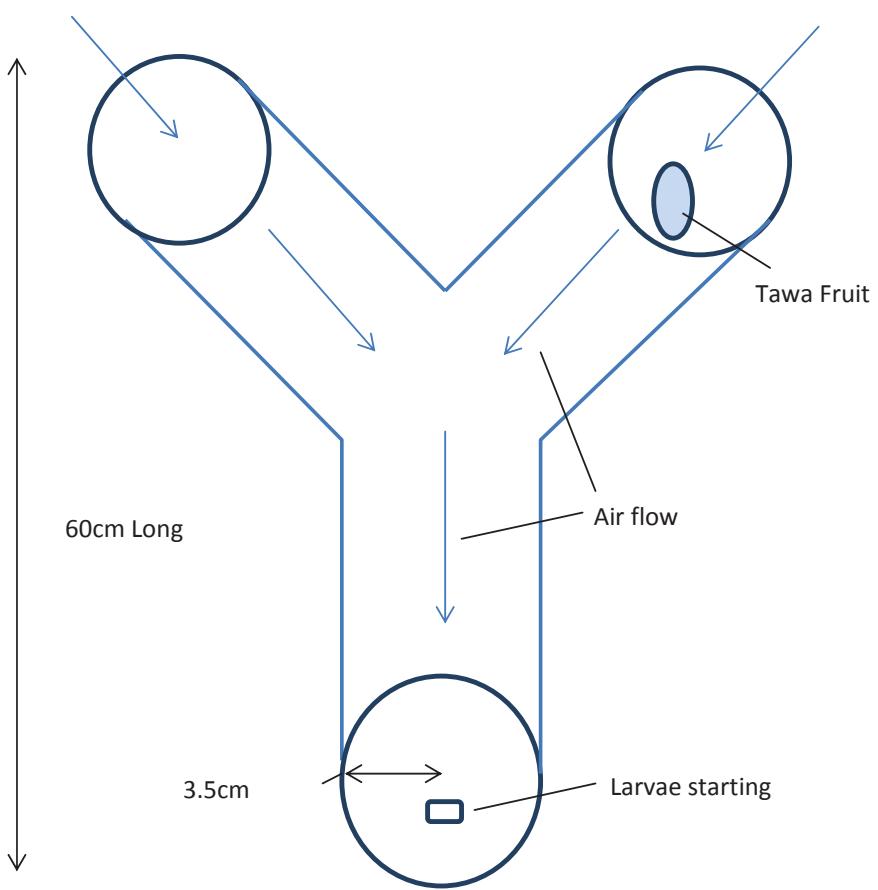


Figure 2.3. Diagram showing the y-tube used in larval wind tests.

Each larva (10 were initially used) was given two hours to complete the journey from their starting position to the tawa seed. A mid-point was also recorded to see how long it took them to reach halfway to the tawa seed. The y-tube was also moistened with a paper towel after every larva to prevent dehydration of the animals.

2.2.2.2 Adult Wind Tunnel Tests

Adult *C. querula* were assessed using a large wind chamber measuring 58.5cm tall, 58cm wide, and 172cm long. A bruised tawa fruit was placed in a petri dish upwind of the adult moth on one side of the wind tunnel. A second petri-dish with a blue marble was placed adjacent to the tawa seed but still upwind of the moth as a control. The mid-section of the wind tunnel was marked and when the moth flew over this point, the time taken for it to reach it was recorded. The prediction being tested was that if a *C. querula* preferred the bruised fruit, they are able to utilize olfactory or visual cues to actively search for oviposition sites. Males may also use the same cues to find females.

The wind speed was set to 30cm per second following von Arx's (2011) wind tunnel experiments dealing with European grapevine moths. Air entering the system was filtered through charcoal and the resulting air flow was roughly consistent in all places within the wind tunnel.

2.2.3. Field Host Tests

Beilschmiedia tawa is a known host of *Cryptaspasma querula* larvae but there are few other suggested host species. This experiment was designed to test whether other plant species like Karaka, Oaks and Miro are chosen as host species by wild *C. querula* in tawa forest in the Turitea Water Catchment (S40° 25.975', E175° 40.161').

50 seeds of the three particular species (Oaks (Acorns), Karaka and Miro) were placed in trays in an area underneath tawa canopy where it was assumed adult *C. querula* were common. The seeds were surrounded by a corflute circular ring (with a height of 6cm) and a 25mm chicken mesh net covering the top (refer to Figure 3.2.3 in Chapter 3) to exclude most rodents (not mice).

The plots were left out in the field for a total of six months to allow *C. querula* larva time to find and drill into these species' seeds. After the allocated time finished they were collected and placed in a chiller/fridge until the data were ready to be examined.

2.3 Results

2.3.1 In-Lab Rearing and Experiments

2.3.1.1 Monoculture Experiments

All the Taraire seeds suffered damage within the 1% - 75% range (Figure 2.3). No seeds were completely left alone nor were any completely eaten. After the 100 days, two adult *C. querula* and one larva were found in the Taraire experiment. The fact that adults arose from this experiment (despite the low amount of still living *C. querula* larva – 30%) clarifies that Taraire can be used as a host. Hinau seeds did not suffer much

endosperm damage (Figure 2.3). A vast majority of seeds were left untouched as indicated by the 0% percentile bin. The miro seeds did not suffer any damage (All seeds in the 0% percentile bin) and thus no graphs were included.

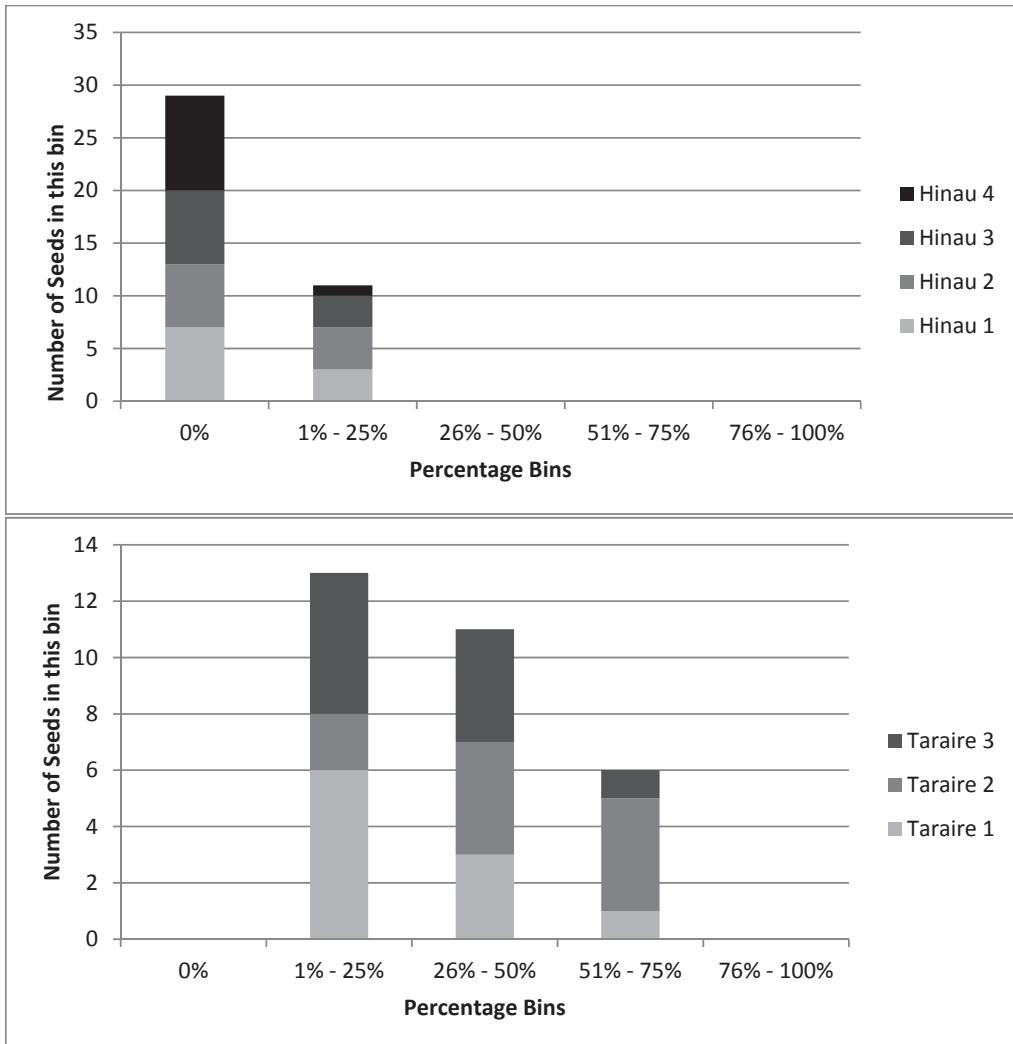


Figure 2.4. Stacked bar graphs showing the number of seeds in each bin category of endosperm damage/removal (0% = no endosperm removed or damaged; 76-100% = most endosperm removed from seed) for Taraire and Hinau (note: Miro did not suffer any damage and therefore was not included in the results). Each replicate is shown as discrete stacks in these graphs.

2.3.1.2 Tawa Predation Rates

The damage done to the tawa endosperms ranged greatly over the replicates and percentile bins (Figure 2.4). Many seeds (15 over all replicates) had nearly all of their endosperm removed (75%-100%) showing that this species can be utilised by *C. novaezelandiae*.

querula. Also 51 out of the 60 seeds (across all replicates) were drilled to some extent. This shows that some of the 30 larva, placed in these experiments, must have drilled into multiple seeds.

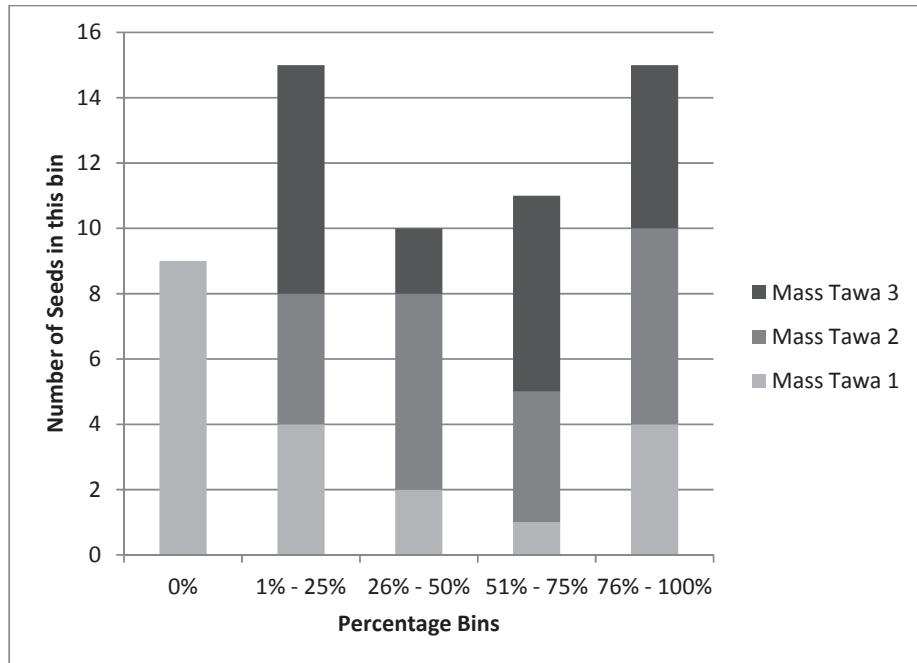


Figure 2.5. Stacked bar graphs showing the number of tawa seeds in each bin category of endosperm damage/removal.

2.3.1.3 Choice Experiments

The tawa seeds predominantly suffered a higher percentage of damage than the other seeds in the choice experiments (Figure 2.5) showing it is likely the preferred host species. The feeding rates differed significantly among the host species (Kruskal-Wallis chi-squared = 23.0028, df = 2, p-value = 1.012e-05)

Taraire's damage varied greatly but given the larger size of these seeds (i.e. more endosperm) it would be difficult to remove as much endosperm as compared with tawa's smaller seeds. Therefore Taraire was also a preferred host species. Hinau suffered minimal to no damage and is likely not an ideal host for *C. querula*. Miro was not included as they suffered no damage (0% percentile bin) and are not likely hosts.

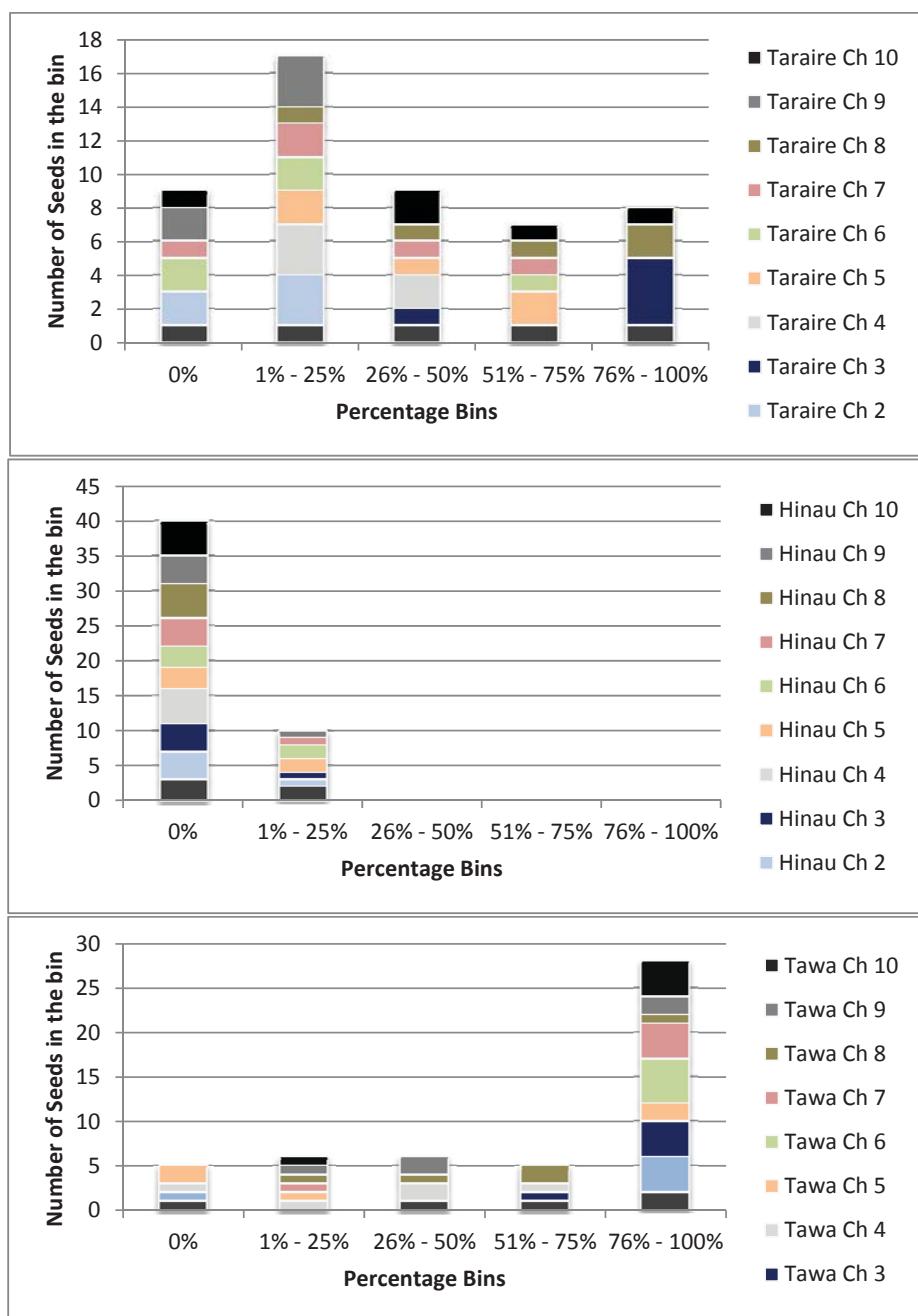


Figure 2.6. Stacked bar graphs showing the number of seeds in each bin category of endosperm damage/removal. Each colour represents a replicate (out of 10 replicates). Miro was not included in the results due to no damage to the endosperm.

2.3.1.4 Rearing observations

On average across all terrariums, the terrarium walls were a preferred oviposition site with 672 eggs; this was followed by the feeding tray which held 287 eggs (Figure 2.6). The plastic sheet held 167, the actual tawa had 66 seeds on them, and the paper sheets had 38 eggs. In total the average number of eggs per terrarium was 1230 with the number of female adults being 14 (roughly 87 eggs laid by each female).

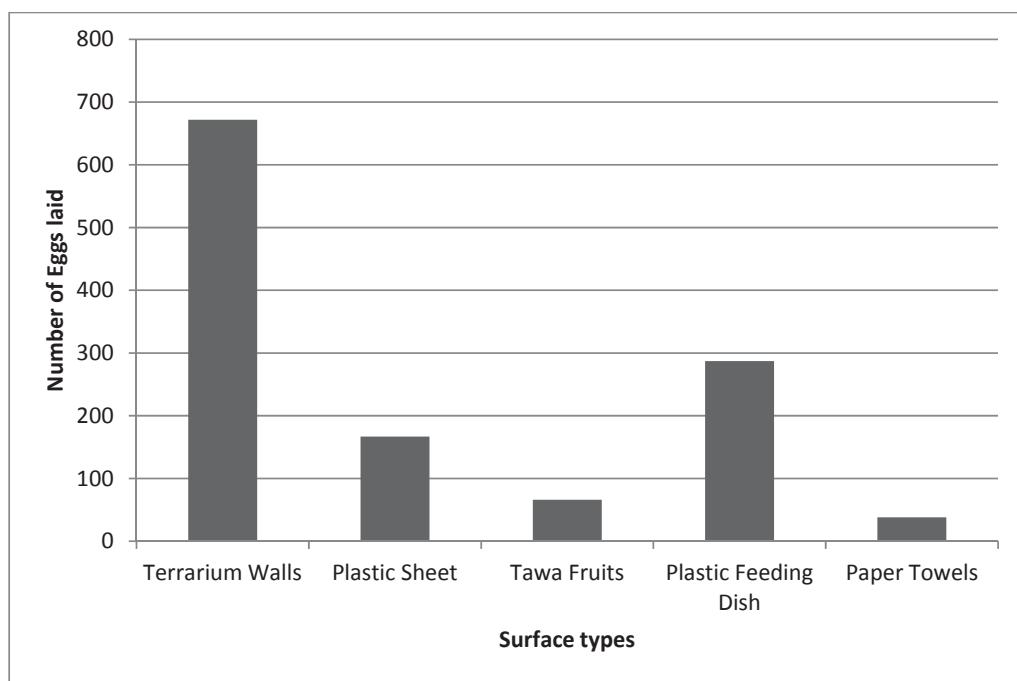


Figure 2.7. Bar graphs showing the preferential oviposition sites in a terrarium holding *C. querula*.

2.3.2 Behavioural Wind Tunnel Tests

The larval wind tunnel tests showed that none of the larva moved towards the tawa fruits but rather dehydrated or did not move far from the starting position. No larva reached the middle, let alone made a choice at either end of the y-tube.

The larvae were observed during their y-tube tests and often they dried out and died within 45 minutes of testing. As a result the inner tube was repeatedly moistened but the same results occurred. If the larva did dry out it would be considered the full 2 hours had elapsed as the individual larva would not move again. Often for the first 30 minutes

the larva would loop around their starting position but never go deeper into the tube. They often went to the top of the tube and ‘parasailed’ down on a thin line of silk.

Similarly, no adult moths made it either to the marble or to the tawa fruits but preferred instead to stay in the general vicinity of where it was placed initially. Each moth ran out of the allocated time to even reach the middle let alone make a choice.

2.3.3. Field Species-Host Tests

In the field trials, over 90% of the acorns suffered some form of drilling damage, likely making them sought after by *C. querula* (Figure 2.7). Less than 40% of the acorns germinated which may inversely correlate to the high drilling proportion. Karaka seeds suffered low drilling proportions and high germination (over 70%). Both species were scarcely affected by rodents. No damage or germination was recorded in the miro fruits

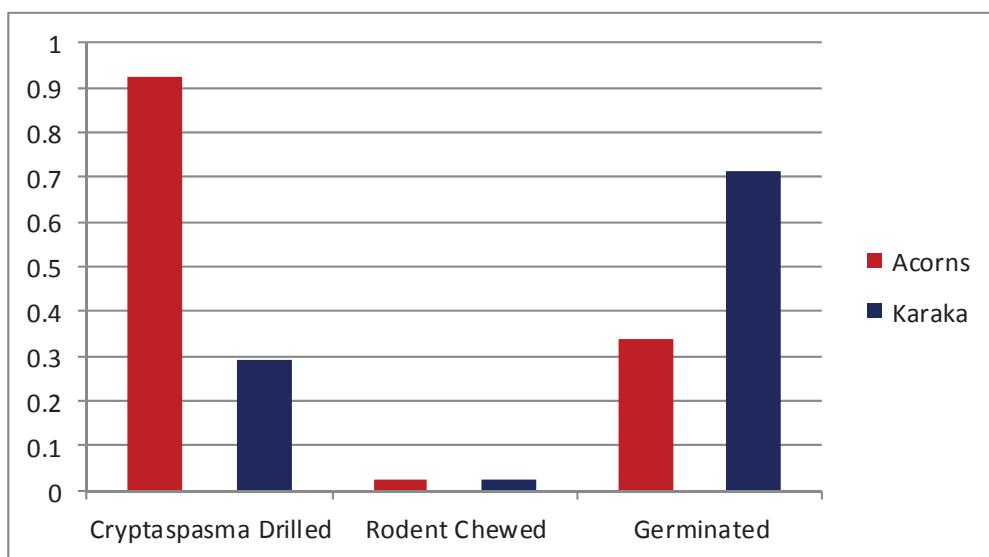


Figure 2.8. Proportion of the 50 seeds for each species affected by drilling, rodent-chewing and/or are germinated (note: No Miro were damaged or germinated in these trials).

Kruskal-Wallis chi-squared = 3, d.f. = 1, p-value = 0.08326.

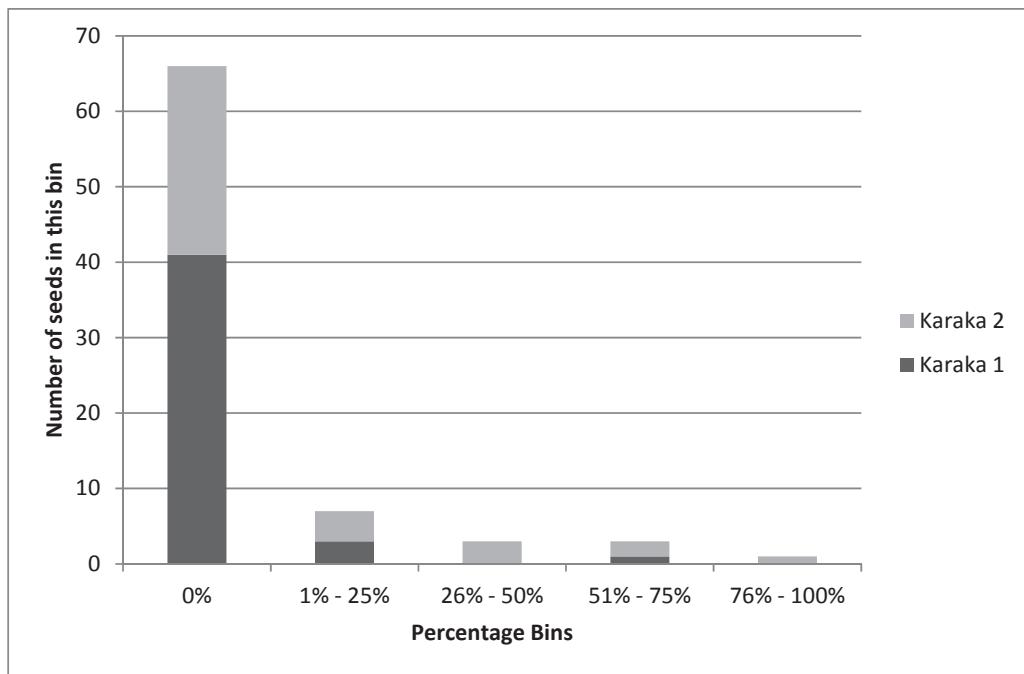


Figure 2.8. Stacked bar graphs showing the number of karaka seeds in each bin category of endosperm damage/removal.

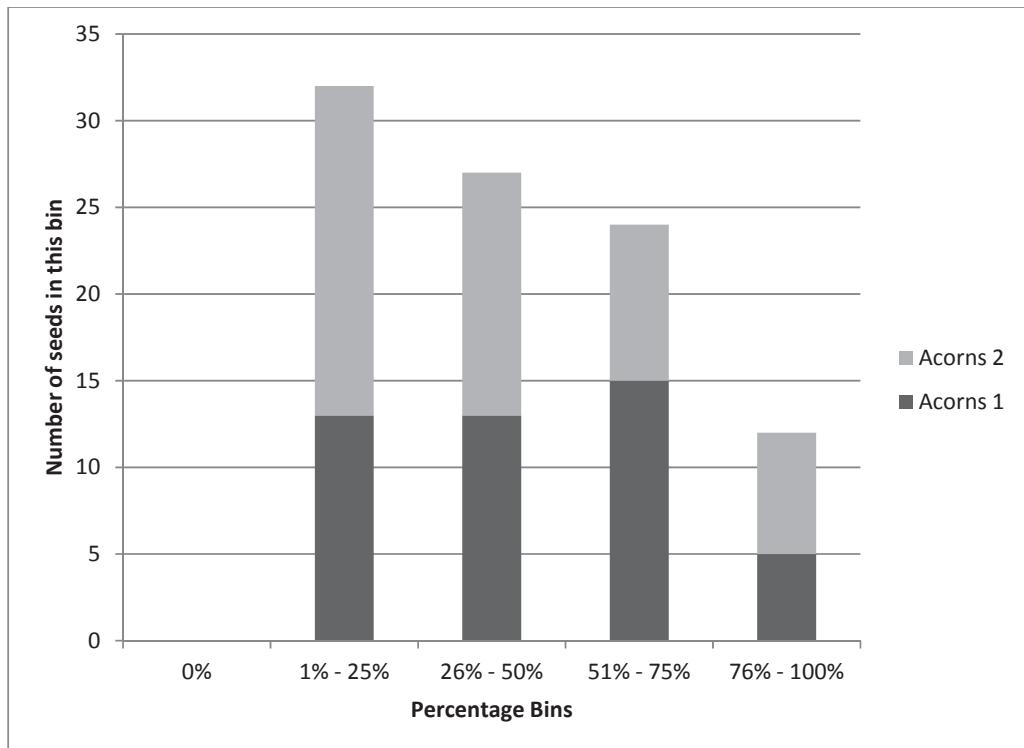


Figure 2.9. Stacked bar graphs showing the number of acorns in each bin category of endosperm damage/removal.

2.4 Discussion & Conclusions

2.4.1 In-Lab Rearing and Experiments

2.4.1.1 Monoculture Experiments & Tawa Predation Rates

While the Tawa experiment had double the amount of seeds than any of the other experiments, this in itself was a test to see whether larvae utilise multiple seeds before pupating. It also allowed for comparisons against the monoculture experiments. The three species (besides tawa) being tested in the monoculture experiments were Hinau, Taraire and Miro. Taraire showed the most drilling proportions followed by Hinau (which suffered very low drilling proportions). Miro suffered no drilling at all and was therefore excluded from the results.

The *Tawa Predation Rate* tests showed higher drilling rates than for Taraire and Hinau where two of three replicates showed at least some drilling in all of the seeds present. This suggests that either one larva from each experiment moved around to multiple seed or that multiple larvae moved among a few seeds. This experiment shows that *C. querula* larva will move from seed to seed and potentially cause higher damage to the regeneration of tawa (assuming that drilling into a seed causes significant mortality – see Chapter 3).

The majority of mass tawa experiment replicates showed that several seeds were fully or close to fully eaten (76-100%) which confirms that *B. tawa* are favoured hosts for *C. querula*. Interestingly a similar number of seeds was only lightly damaged (1-25%) which suggests that *C. querula* may sometimes drill into a seed to assess its quality before deciding to stay in the seed. This decision may depend on nutrient resources inside the seed (small seed) or inadequate conditions (damaged outer husk of seed allows predators easier access at reaching the larvae) or pathogen presence or these results may show random drilling and movement.

The Taraire seeds were all drilled to some extent in each replicate. However none of them were completely eaten which is likely due to Taraire's large seed size (which presumably means that there are more resources per seed than what is needed by *C.*

querula). Over 40% of the total seeds (across all three replicates) were only lightly drilled (1-25%) which could indicate a lack of satisfaction with those particular individual seeds or (due to the large seed size) a small percentage of endosperm in a Taraire seed is sufficient to feed *C. querula* until it pupates.

It is unlikely that Taraire acts as a substitute host when tawa is undergoing low fruiting years as the natural range of Taraire is the upper north island so *C. querula* cannot use Taraire to maintain its population size when tawa is not fruiting much (especially for lower North Island locations). The other replicates showed a much higher success of *C. querula* reaching adulthood or pupation (All the *C. querula* survived in the other replicates while only a proportion had reach pupation or adulthood).

Hinau seeds showed minimal damage from *C. querula*. The damage never exceeded 25% and coupled with the fact that the seeds are very small (less nutrients inside) it seems unlikely that Hinau could be a suitable host. This inference is supported by the fact that in two of the four replicates, all *C. querula* died while in the other two replicates only one larva was still alive (both were severely stunted compared to larvae in other experiments). Seeing as no *C. querula* made it to adulthood using only Hinau it is fairly safe to assume that Hinau is not an adequate host species for *C. querula*. Therefore, like Taraire, Hinau would not act as a substitute host when Tawa is undergoing low fruiting years.

In all three replicates of the Miro monoculture experiments, no *C. querula* survived which strongly indicates that Miro is not a suitable host for even subsistence.

2.4.1.2 Choice Experiments

The most frequently consumed seeds belonged to tawa and Taraire which both belong to the same genus (*Beilschmiedia*). Tawa seeds suffered higher damage in proportion to their overall seed size (76%-100% damage) compared with Taraire. However it is important to remember that Taraire is much larger than tawa and therefore the amount of the endosperm eaten from different species of seed does not represent a true preference. It is however evident that Taraire can successfully be utilised by *C. querula*.

Given that *C. querula* is a known polyphagous seed predator it can be assumed that

when tawa is not undergoing a heavy fruiting year, there are other seeds of different species that can be utilised. In the choice experiment the examined seeds, other than tawa and Taraire - Miro and Hinau, would likely not be one of those substitute species. Other species tested in field experiments were acorns, karaka as well as Miro. It is possible that karaka and oaks may act as a suitable substitute for tawa during low fruiting years – see section 2.4.3

2.4.1.3 Rearing observations

The first generation of adults raised in terrariums from a batch of tawa seed collected from a single specimen at Ruahine Forest Park ($S40^{\circ} 10.895'$ $E175^{\circ} 52.288'$) produced clutches of 20 – 35 eggs following their emergence from pupae and mating in the terrarium. The largest clutch size found was 69 eggs. Eggs were frequently deposited on the smooth terrarium walls which perhaps reflects the large surface area of the terrarium. Paper towels (which took up the entire bottom of the terrarium) appeared to be less desirable oviposition sites as did the tawa fruit placed in the chamber.

Despite being laid in clutches, the eggs appeared to mature at slightly different rates. It took roughly two and a half weeks (18 days) from when the eggs were first laid for the first eggs to hatch. It could take a further two days, for the last egg in a clutch to hatch. Once the larvae had hatched out, they appeared to disperse randomly around the terrarium (by this stage the adults had been removed). It was interesting to note that many larvae crawled to the roof of the terrarium where they produced a thin silk thread which they rode back down to the moist paper towels. This may be an effective method of reaching ground level if their parent laid them on a high up tree branch. It could also be a suitable dispersal strategy where the silk strands catch wind currents allowing the individuals to ‘ride’ the wind as seen in ‘ballooning’ spiders (Valerio, 1977). It was shown that preferred oviposition sites are on smooth surfaces; in nature leaves often have smooth surfaces. An inference could be made that wild *C. querula* adults oviposit on leaves in trees (possibly due to reduced predation risk) and the hatched larvae use silk threads to reach the ground level where they find suitable hosts.

Larvae, not used in experiments were reared on tawa seeds to keep the colony alive. The tiny first instar larvae appeared to have no problem drilling into tawa and reaching the endosperm. The subsequent amount of time spent within each seed before pupation

appeared to vary hugely between individuals (between 3 weeks to 6 weeks). This may be due to varying nutrition found in each seed (less nutritious seeds likely caused *C. querula* larvae to stay longer) or perhaps due to seed size (when one food source runs out they must leave and find another). When they emerged from the seeds they were much larger (compare Figure 1.2.8 and 1.2.9). They wandered around the terrarium for roughly 24 hours before forming silk cocoons in among layers of the paper towels as was seen in *Cryptaspasma* species in Mexico (Hoddle, 2011).

Within these silk paper towel cocoons, the larvae turned into pupae. This stage was prone to fungal infection and death in the terrariums (around 30% of pupa became infected). In the beginning of the pupal stage, the cases were light orange in colour, turning dark before emergence. This stage took between three and four weeks. Hoddle (2011) found that the gender of pupae could be determined by the presence of small protuberances near the posterior of the pupal case of males or a slit for females. This was not the case for *C. querula*, which all looked identical apart from slight size variances. Sex could only be discovered once the adults emerged.

Once the adults emerged (which again were at different rates), they were separated from the pupae/larvae terraria and taken to a breeding terrarium with other adult moths. The females appeared slightly larger (Figure 1.2.3), did not have wing patterns and had orange protuberances at the end of their abdomen (Figure 2.11), while males were (on average) smaller, bore small wing patterns (Figure 1.2.4) and had white hairs surrounding the tip of their posterior (Figure 2.10). Adults were fed a honey solution absorbed into pieces of paper towels. The moths were seen landing on these paper towels where using a thin proboscis, they drank the honey liquid. On average the moths lived two to five weeks (note there may have been a slight mortality difference between genders).



Figure 2.10. White-hairs of
C. querula Male.



Figure 2.11. Orange Protuberances of *C. querula* Female

2.4.2 Behavioural Wind Tunnel Tests

The wind tests were non-conclusive since either *C. querula* adults and larva did were not attracted to olfactory cues released by *B. tawa* fruits or they were uncomfortable behaving naturally in the wind tunnels. Given the timeframe for each test only one individual (adult female) completed the y-tube wind test but it chose the marble option and not the tawa fruit which may suggest that this species of seed predator are not influenced by olfactory cues.

Given that the control tests for both the Y-Tube and the Wind Tunnel showed there was little attraction to tawa fruits or olfactory cues for both larvae and adult *Cryptaspasma querula* there were no further wind tunnel experiments.

Given this result it may be that adults stay under tawa canopies and oviposit randomly without concern as to whether potential host seeds will be nearby for their young. Given that the larvae also do not appear to be attracted by olfactory cues it is likely the *C. querula* species employs a strategy where a large number of eggs are laid indiscriminately and that larvae stumble across tawa fruit.

The results shown here differs from that of several other studies (e,g, Svensson et al. 2011; Tasin et al. 2011; von Arx et al. 2011) which show that invertebrate herbivores and seed predators do find host plants for oviposition through olfactory cues. It is possible longer time intervals are needed for adult *C. querula* to find *B. tawa* fruits

through olfactory cues or that the testing chambers are too artificial for natural behaviours to occur.

2.4.3 Field Species-Host Tests

The results shown for the acorns revealed intense drilling (on average over 90% of the acorns were drilled) and the scans of the acorns showed on average that just under half (26-50%) of the endosperm was removed. There were multiple cases of an entire acorn being eaten which strongly suggests they could be suitable hosts for *C. querula*. While the field tests lose some degree of control over the experiments (abiotic factors more important, rodents small enough to fit through mesh can also damage seeds etc.), it is clear that *C. querula* can utilise acorns for nourishment as personal observations and dissections of the acorns showed multiple late instar *C. querula* living inside. This strongly suggests acorns are suitable hosts despite the *Quercus* genus being an exotic to New Zealand.

Acorns also showed some germination (on average 33%) despite the high rate of drilling which potentially shows some resistance to seed predation in *Quercus*. This creates a possible interesting situation where exotic *Quercus* species maintain high *C. querula* populations when *B. tawa* are not undergoing masting years particularly in forest fragments that are near to exotic plantings of oaks. This means that the presence of *Quercus* could indirectly have adverse effects on *B. tawa* populations by sustaining its natural seed predators. By maintaining high populations in *C. querula* it may reduce the effectiveness of masting as a predator satiation/starving mechanism. In the long run this could reduce tawa regeneration unless, like *Quercus* species, *B. tawa* has some tolerance to seed predation (i.e. still regularly germinates and establishes despite having been drilled).

Karaka also showed some drilling (just under 30%) in the field trial. When looking at the scans, however, it appears there is very little damage to the actual endosperm. There were a few seeds that had some drilling but the mode for both replicates was 0% damage. This suggests that *C. querula* may drill into karaka but the endosperm nutrition is not suitable for them and they either die or leave to find another food source (perhaps this is also what happens to hinau). Not surprisingly, due to the low damage to the

endosperm, karaka seeds on average showed very high germination (over 70% of seeds). This shows a different result to a study by Wotton (2007) where *C. querula* larvae were discovered in karaka fruits in Northland.

Both karaka and acorns showed very little rodent damage in these trials however it is important to note that both replicates had missing seeds and it is common knowledge that rodents like to carry their seed resources to small caches generally located away from resource patches. Therefore the represented rodent damage shown in the results may be substantially less if the rodents did, in fact, take the seeds out of the experiment (despite the mesh).

Overall it appears that *C. querula* prefer tawa, taraire, and potentially acorns (*Quercus sp.*) as hosts while avoiding (or barely using) miro, hinau, and karaka. Both adults and larvae do not appear to be attracted to volatile cues given off by tawa fruits (though this may be due to discomfort of specimens in controlled environments).

Chapter 3

The benefits of seed dispersal in *Beilschmiedia tawa*: a field experiment

3.1 Introduction

Seed predation is the process whereby an organism consumes and digests a type of seed for sustenance but it also includes parasitism of these seeds by invertebrate larvae (Science Reference, 2008). Invertebrate seed predators are often specialist feeders referring to their ability to utilise only one or a select few species of seeds (Science Reference, 2008) but there are multiple cases (Boieiro et al. 2010; Boieiro et al. 2012; & Ostergard, 2009) of generalist invertebrate seed predators which can utilise many different species of seeds that are not closely related.

Seed predation can occur at two stages: pre-dispersal & post-dispersal (Janzen, 1971); each potentially having significant influences on seedling survival. Pre-dispersal seed predation is the process where the seeds have been damaged/eaten/destroyed by an organism before the seed has undergone dispersal through whatever dispersal agent that species may use (Fedriani & Manzoneda, 2005). Pre-dispersal seed predation studies have shown severely declined seedling recruitment may result from this form of seed predation (Calvo-Irabien & Islas-Luna, 1999). However for this chapter the focus is on post-dispersal seed predation.

Post-dispersal seed predation is the predation of seeds after they have been dispersed by some dispersing agent the host plant may use (Hulme & Benkman, 2002). Several studies (Janzen, 1971; Fedriani & Manzoneda, 2005; & Hulme & Benkman, 2002) have discussed and compared the two forms of seed predation (pre- and post-dispersal) and while both can be devastating to recruitment, it appears that post-dispersal seed predation can be more severe (Hulme & Benkman, 2002).

The Janzen-Connell model is an important phenomenon identified independently by

Janzen (1970) and Connell (1971) which relates specifically to the effects of seed predation and their impacts on host plants' densities and distributions in tropical forests. It predicts that seed density and survival vary with the distance from the parent canopies in relation to seed predation. Seeds should be most abundant under parent plants (therefore higher density) and this density of seeds should decrease further away from the parent canopy. At the same time, seed predation should be higher under the parent canopy while lower further away from parental canopies; therefore the predictions suggest seeds further away from parental canopies should have higher survival rates. A study by Visser et al (2011) supported the idea that increased seed predation occurred in areas where there was a high density of host plants. This suggests that seed predators find and utilise hosts more readily in areas where host-density is a lot higher (Lambers et al. 2002). Various studies (Ruiz et al. 2010) have supported the hypothesis indicating that seed survival does increase further away from the parent canopy. This stresses the importance for plants, which suffer from seed predation, to increase their dispersal ability away from the parent plant and therefore away from the effects of their natural enemies. A study by Lewis & Gripenberg (2008) suggested that seed predators can have a role in maintaining diversity in plant assemblages and structuring their composition. Another study by Garren & Strauss (2009) showed that seed predators can potentially reduce already low plant densities. This supports the idea that seed predators can have potentially large impacts on the plants they utilise. These studies, therefore stress the fact that seeds must evolve a method of attempting to escape seed predation.

New Zealand has a temperate climate. This fact may influence the Janzen-Connell hypothesis as it is generally applied as a predictor of diversity in tropical rather than temperate forests (Clark & Clark, 1984). However, there have been relatively few studies (Hyatt et al. 2003; McCarthy-Neumann & Kobe, 2010) looking at the Janzen-Connell model in a temperate setting. It is often assumed that Janzen-Connell effects will not be common in temperate forests; however there are a growing number of studies which actually support the J-C hypothesis despite their studies being in temperate locations (Lambers et al. 2002; Pigot & Leather, 2008; Yamazaki et al. 2009; & Wang et al. 2010).

For the purpose of this experiment, the obligate polyphagous seed predator *Cryptaspasma querula* and its primary host *Beilschmiedia tawa* will be focussed on.

The torticidae seed predator *C. querula* is an endemic species to New Zealand and belongs to the order Lepidoptera. It is unique in that it is reported to use multiple host species' seeds for nutrition at the larval stage (it is generalist rather than specialist). It is the only known polyphagous obligate seed predator in New Zealand (Sullivan et al. 1995). Its primary host is *Beilschmiedia tawa*, a podocarp found all over the North Island south of Northland and in South Island, north of Westport, but it also uses other species including introduced oaks (see Chapter 2).

The genus *Cryptaspasma* contains at least 35 species with a very wide range including South America, Japan, Pacific Russia, East Africa, Malagasy and Indo-Australia (Horak, 2006). The Indo-Australian species (which includes *C. querula*) are all in the same morphologically defined sub-genus (*Allobrachygonia*). All known hosts are big-seeded. The larvae live inside the endosperm which they also feed upon. Many of the recorded hosts in the Indo-Australia area are in the plant families Arecaceae, Lauraceae, Monimiaceae, Mytraceae and Proteaceae (Horak, 2006). One of the most renowned species is *C. sp. nr. lugubris* which is a pest of avocado in Puerto Rico, Mexico, and Guatemala (Hoddle, 2011.). *C. bipenicilla* also affects avocado (*Persea Americana*), but is known to utilise aceitunillo (*Beilschmiedia pendula*). Brown and Brown (2004) suggest other highly probable hosts.

Several native birds of New Zealand such as the kereru and tui are known to be good distributors of seeds of tawa and other fleshy-fruited species (McEwen, 1978; Clout & Hay, 1989; & Kelly et al. 2010). Through this dispersal, vulnerable seeds may escape *C. querula* seed predation in space (J-C model) or time (given enough time to germinate and establish before they are found). However, it is unknown whether *C. querula* tend to stay around tawa dominant stands in forested areas or whether they move around unaffected by vegetation type. Many intact tawa seeds are found under tawa canopy (pers. obs.) as they drop straight from the trees to the ground (unless a bird like a kereru transports the seed to another location).

Birds can be important long-distance dispersal agents to flora in New Zealand (Young et al. 2012). A study by Wotton & Kelly (2011) has shown two large-seeded species taraire (*Beilschmiedia tarairi*) and karaka (*Corynocarpus laevigatus*) rely on only one type of avian frugivore the New Zealand Pigeon (*Hemiphaga novaeseelandiae*). If this disperser

were to disappear, recruitment would also drastically decline and increase the rate of seedling failure. It is likely that tawa will be equally vulnerable since it too depends principally on the NZ pigeon for dispersal (Kelly et al 2010). The concentrated seed resource under adult tawa may attract and enhance high densities of *C. querula*. Lewis & Gripenberg (2008) and Visser et al (2011) show that the density of seeds and host plants present can play a major part in attracting seed predators. Here I investigate whether tawa seeds that were deposited in an area that did not have tawa present were still found by *C. querula* and whether a larger more dense clump of seed were more or less likely to be attacked than a smaller, less dense clump.

In addition, plants have been known to release chemical pheromones and volatile chemicals which attract invertebrate pollinators (Bronstein, 1988; Svensson et al. 2011; von Arx et al. 2011) and possible seed distributors. It is possible, however that fruit dropped from canopy trees crack or split thereby releasing an alluring scent, in which possible seed predators could use to find hosts; therefore the smell released by the fruit may attract detrimental seed predators. Fleshless seeds (no fruit wall attached after passage through a bird) may have little to no scent and therefore attract less invertebrate seed predation. Flesh may also retard seed germination due to inhibitors within the fruit wall (Samuels & Levey, 2005; & Robertson et al. 2006).

Thus, we might expect that fleshed seed located under tawa canopies may be most affected by seed predation while cleaned seed dispersed at low density away from tawa stands, will be least affected by seed predation. These hypotheses will be tested in the following section.

3.2 Methods

Three sites in New Zealand's lower North Island were chosen based on the occurrence of tawa stands within the forest. The three sites (Figure 3.2.1) were: Nga Manu Reserve (Figure 3.2.2) in Waikanae ($S40^{\circ} 51.816'$ $E175^{\circ} 03.484'$); Totara Reserve (north of Ashurst, west of Pohangina) ($S40^{\circ} 08.907'$ $E175^{\circ} 50.459'$); and Turitea Water Catchment ($S40^{\circ} 25.975'$, $E175^{\circ} 40.161'$).

Within each site, suitable areas having both tawa-dominant stand and non-tawa stands were chosen. I used four areas with tawa stands and four areas with non-tawa stands at both Nga Manu and Totara Reserve but the Turitea Water Catchment had only two suitable tawa stands to use and so only two replicates were set up there. The aim was to have each tawa stands and its associated non-tawa bit of forest near or adjacent to each other to allow better comparisons but this wasn't always possible (e.g. at Nga Manu Figure 3.2.2).

Within each site, four replicate blocks (two at Turitea) were set up. Within each block and within each tawa stand and in its non-tawa pairing one replicate of each of four treatments were established:

- 10 Fleshed (flesh not removed) tawa fruits
- 10 Fleshless (cleaned of flesh) tawa seeds
- 50 Fleshed fruits
- 50 Fleshless seeds

To prevent rat interference (but not mice) a 6cm tall Corflute ring with a diameter of 36cm was covered with 25mm mesh chicken wire to enclose each batch of seeds or fruits.

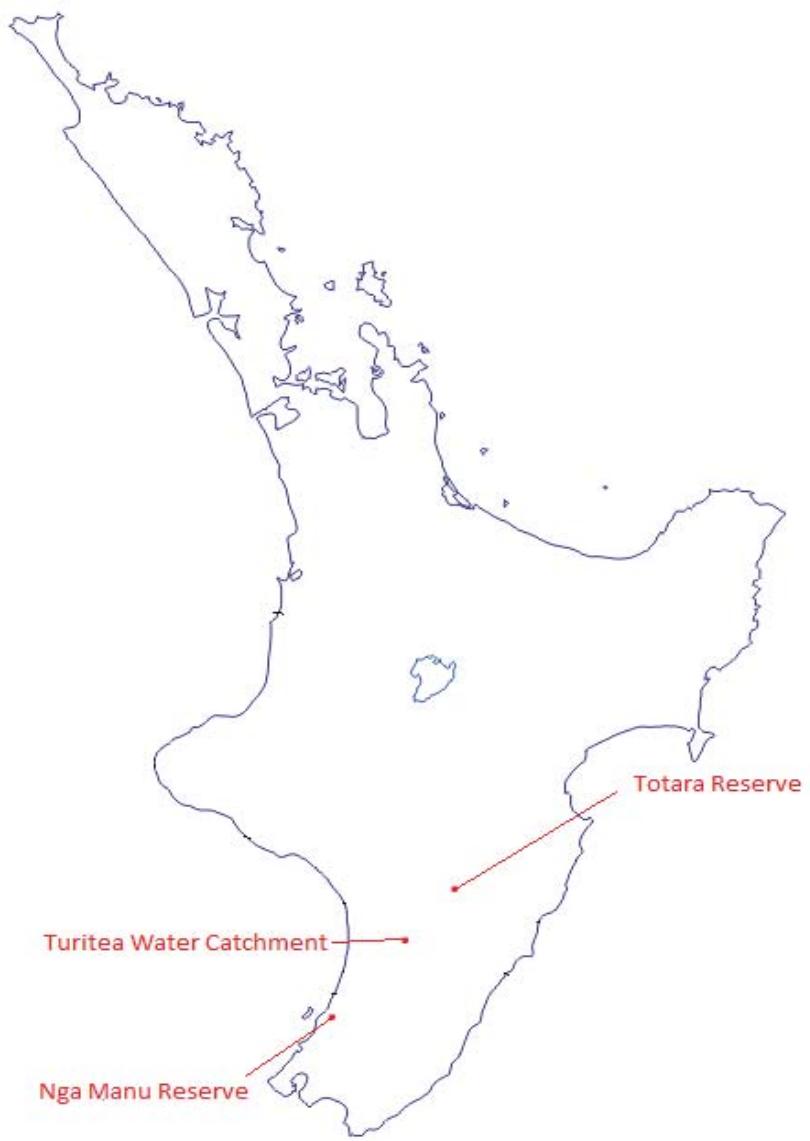


Figure 3.2.1. Map of New Zealand's North Island showing, in red, the sites in which the experiments were conducted.



Figure 3.2.2. Site Map of Nga Manu Reserve in Waikanae. Red numbers (and circles) correspond to non-tawa stands. White numbers (and squares) correspond to tawa dominant areas. The numbers are where the experiments were placed for one year. Unaltered map retrieved from the Nga Manu website.



Figure 3.2.3. Picture of experiment set up with corflute cylinder covered by 25mm chicken mesh.

The experiments were established on 20/02/2011 at the Nga Manu site, 19/03/2011 at for Turitea, and 25/04/2011 at Totara Reserve and collected on 06/12/2011, 08/12/2011, and 10/12/2011 respectively.

Upon collection each tawa seed was examined for *Cryptaspasma* drill holes, germination (root and/or stem showing but no leaves), establishment (at least some leaves evident) and for larger holes and gnaw marks likely caused by rodents (these holes were too large to be created by *Cryptaspasma querula*). It is possible that other animals like birds or large invertebrates (such as weta) could have also produced some of the large holes but the gnaw marks are reasonably distinctive. Rodent chewed and *Cryptaspasma* drilled seeds were considered separate results in the data as large holes created by rodents (or other species) often obscured holes drilled by the moths, so the proportions of seeds drilled by *Cryptaspasma* was evaluated on the unchewed seeds only (Figure 3.2.4).



Figure 3.2.4: Left photo – *Cryptaspasma querula* drilled holes in tawa seed. Right Photo – Rodent damaged tawa seed.

The data were analysed separately at each site using three-factor binomial ANOVAs on each of the conditions of the seeds (Drilled, Rodent Chewed, Germinated, and Established) using the statistical program R. The factors used in this analysis were Position (located under a tawa dominant stand or located in a non-tawa area), Density (50 versus 10 seeds), Flesh (whether the seeds were cleaned of flesh or left intact). The pairs of experimental replicates were entered first in the model as blocks to account for spatial variation in the fates of seeds independent of the treatment effects.

3.3 Results

The three sites (Nga Manu, Totara Reserve, and Turitea) were analysed separately to show more specific site effects. The results will be presenting by focusing on each of the conditions of the seeds (drilled, rodent chewed, germinated, and established) in turn. Only the significant ($P \leq 0.05$) effects of position (located either under a tawa dominant stand or in a non-tawa area), density (50 seeds or 10 seeds), flesh (cleaned of flesh or left intact) and interactions were graphed. The boxplot graphs did not include more than two interactions between the three variables Seeds that were established (presence of green leaflets) were also counted as germinated in the data. As mentioned in the methods, drilled seeds and rodent-chewed seeds were separate in these results.

The *C. querula* drilling proportions were highly variable at Nga Manu but it is clear that seeds away from the tawa canopy were more likely to be drilled than seeds under the tawa canopy. Seeds that were in a lower density setup also suffered higher drilling proportions than high density setups (Table 3.3.1, Figure 3.3.1). The significant result between the interaction of Position:Flesh is due to seeds being removed of flesh under tawa canopy but not away.

Table 3.3.1. One-way ANOVA Table showing the significant (P) results of drilling results at Nga Manu for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			29	177.632	
Replicate	3	43.621	26	134.011	<0.001
Position	1	31.472	25	102.540	<0.001
Density	1	6.593	24	95.946	0.0102
Flesh	1	1.868	23	94.079	0.1718
Position:Density	1	3.353	22	90.726	0.0671
Position:Flesh	1	27.653	21	63.073	<0.001
Density:Flesh	1	0.128	20	62.945	0.7210
Position:Density:Flesh	1	7.359	19	55.586	0.0067

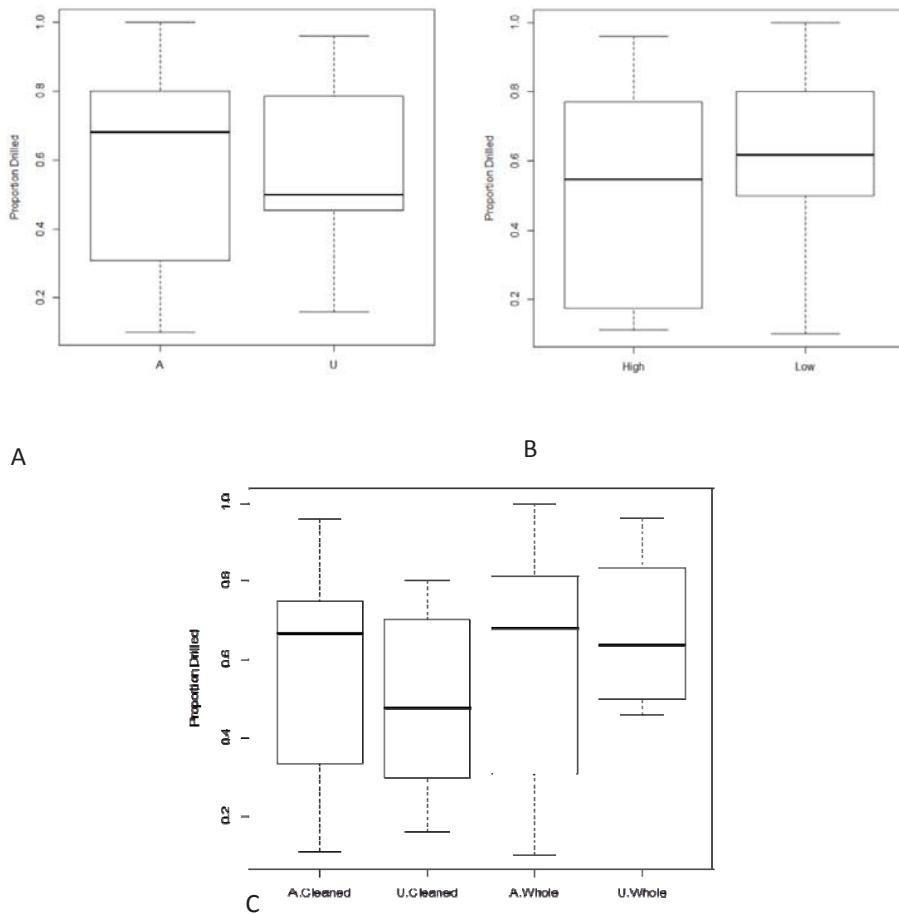


Figure 3.3.1. Boxplots showing the mean proportion of drilled seeds for A) the positional effect (away from or under tawa); B) the density effect (high = 50 seeds, low = 10 seeds); and C) the interaction between the positional effect and the seeds' flesh status (cleaned of flesh vs. whole) at the Nga Manu Site.

These boxplots use abbreviations for some variables (to make it easier to understand when showing the interaction of two variables). For boxplots with position: A= Away from Tawa; U = Under Tawa. For Density: High = 50 seeds; Low = 10 Seeds. For Flesh: Cleaned = Flesh was removed from the seed; Whole = Flesh remains on the seed. Boxplots showing an interaction of of variables (e.g. Position:Flesh) will have the shortened letters or terms separated by a full stop (e.g. Position:Flesh = U.Clean; A.Whole etc). Note: The boxplots do not all have identical y-axis ranges (e.g. some may go to 1.0 proportion while others only go to 0.8).

The rodent damage proportions were highly variable at Nga Manu where the damage was marginally higher in seeds away from tawa canopy. Seeds that were cleaned of flesh were more likely to receive less rodent damage than fleshed seeds. (Table 3.3.2, Figure 3.3.2). The significant result between the interaction of Position:Density is due to seeds being away from tawa and in a high density or under tawa and low density, which escape the most rodent damage. The interaction of Position:Flesh is significant where seeds under tawa that are cleaned of flesh, and seeds away from tawa that still have pericarp were damaged by rodents more than the other interactions. The final significant interaction (Density:Flesh) results from high density seeds that are cleaned of flesh and low density seeds with pericarp being damaged more than other density and flesh interactions (Table 3.3.2, Figure 3.3.2).

Table 3.3.2. One-way ANOVA Table showing the significant results of rodent chewed results at Nga Manu for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			29	282.29	
Replicate	3	17.8803	26	264.41	<0.001
Position	1	22.6320	25	241.78	<0.001
Density	1	0.0236	24	241.76	0.8779
Flesh	1	18.7856	23	222.97	<0.001
Position:Density	1	17.0340	22	205.94	<0.001
Position:Flesh	1	4.9985	21	200.94	0.0254
Density:Flesh	1	6.7710	20	194.17	0.0093
Position:Density:Flesh	1	3.0898	19	191.08	0.0788

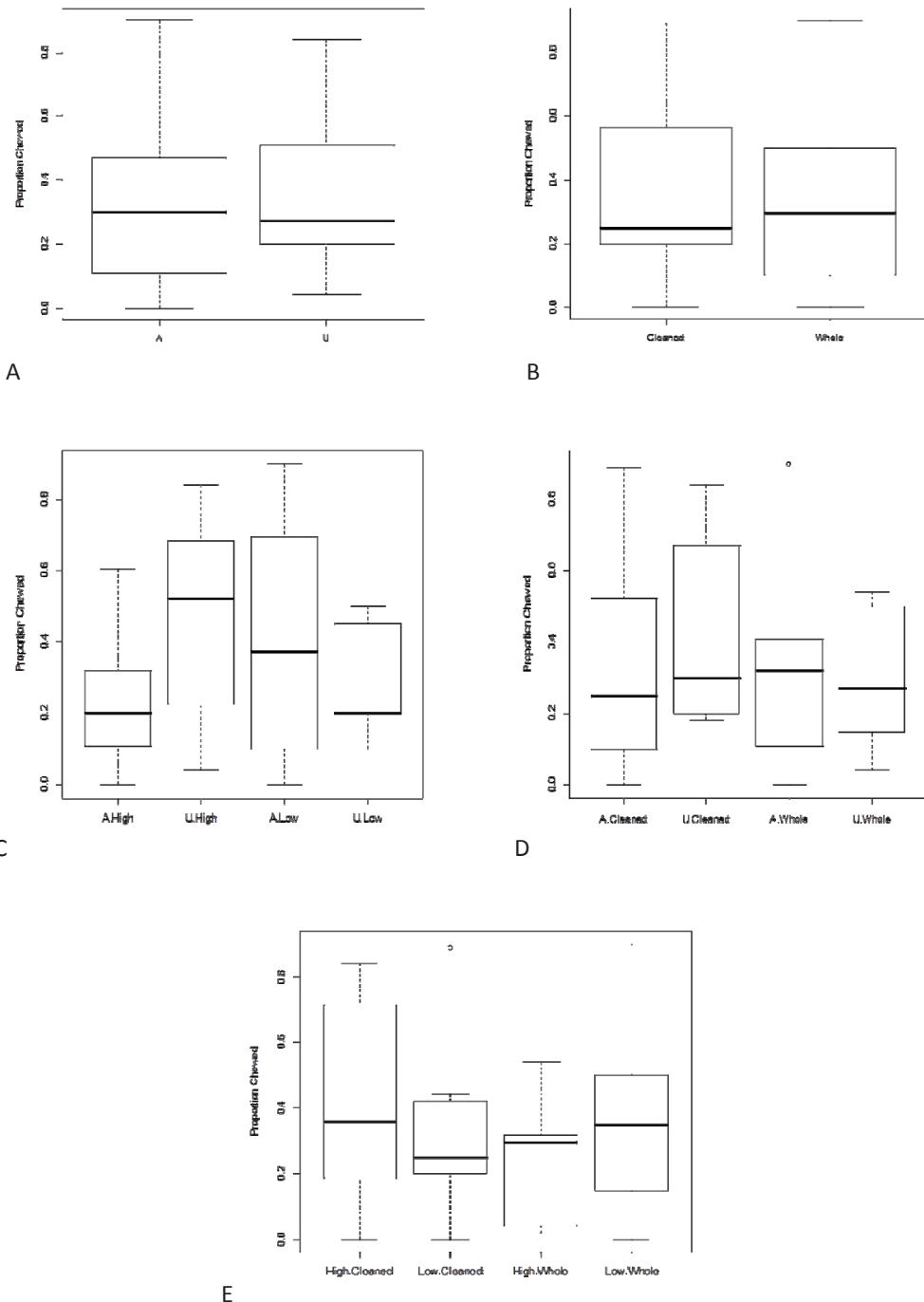


Figure 3.3.2. Boxplots showing the mean proportion of rodent chewed seeds for A) the positional effect; B) the seeds' flesh status; C) the interaction between the positional effect and density; D) the interaction between the positional effect and the seeds' flesh status; and E) the interaction between the density effect and the seeds' flesh status at the Nga Manu site.

The germination proportions were variable for seeds under tawa at Nga Manu but overall seeds away from the tawa canopy were more likely to germinate (Table 3.3.3, Figure 3.3.3).

Table 3.3.3. One-way ANOVA Table showing the significant results of germination at Nga Manu for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			29	241.99	
Replicate	3	36.275	26	205.71	<0.001
Position	1	68.063	25	137.65	<0.001
Density	1	1.808	24	135.84	0.1788
Flesh	1	1.959	23	133.88	0.1616
Position:Density	1	0.775	22	133.10	0.3785
Position:Flesh	1	3.517	21	129.59	0.0608
Density:Flesh	1	0.218	20	129.37	0.6403
Position:Density:Flesh	1	5.115	19	75.216	0.0237

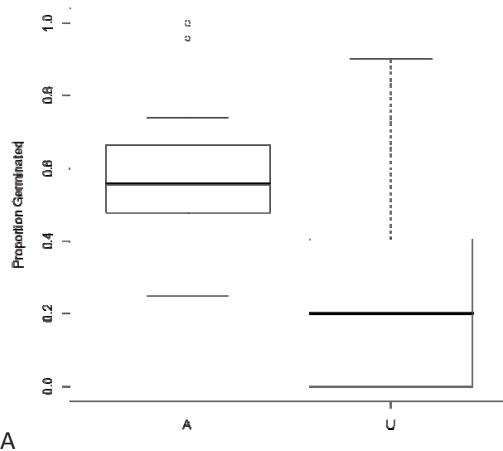


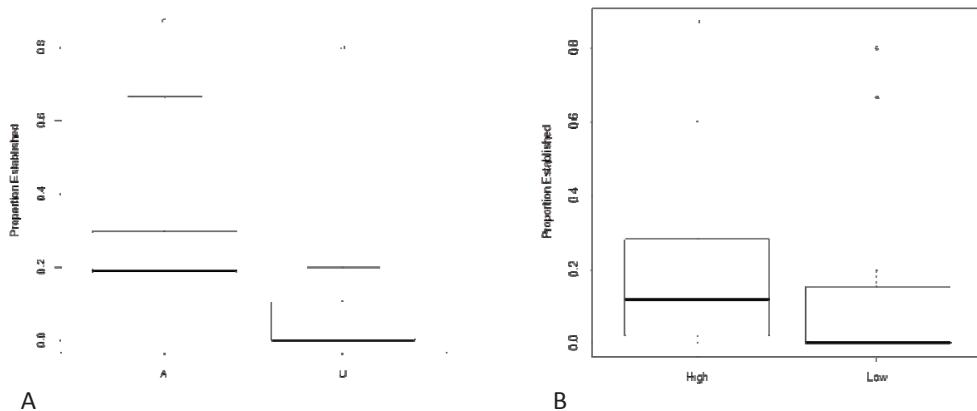
Figure 3.3.3. Boxplots showing the mean proportion of germinating seeds for A) the positional effect at the Nga Manu site.

The seed establishment proportions were mildly variable at Nga Manu where success

was more predominant in seeds away from tawa canopy or high in density (Table 3.3.4, Figure 3.3.4). It is very clear that seeds that are located away from tawa in high density are more likely to establish (Position:Density). Seeds that were away from tawa canopy that were either cleaned of flesh or still had their pericarp were more likely to establish than seeds under tawa canopy (Position:Flesh). The interaction of the density of the seeds and whether they still had a pericarp (Density:Flesh) was significant where seeds that were in high densities whether with or without a pericarp were more likely to establish (Table 3.3.4, Figure 3.3.4).

Table 3.3.4. One-way ANOVA Table showing the significant results of establishment at Nga Manu for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			29	312.843	
Replicate	3	87.672	26	225.172	<0.001
Position	1	90.030	25	135.141	<0.001
Density	1	4.491	24	130.650	0.0341
Flesh	1	0.542	23	130.108	0.4617
Position:Density	1	17.730	22	112.377	<0.001
Position:Flesh	1	7.203	21	105.175	0.0073
Density:Flesh	1	7.459	20	97.715	0.0063
Position:Density:Flesh	1	0.236	19	97.479	0.6272



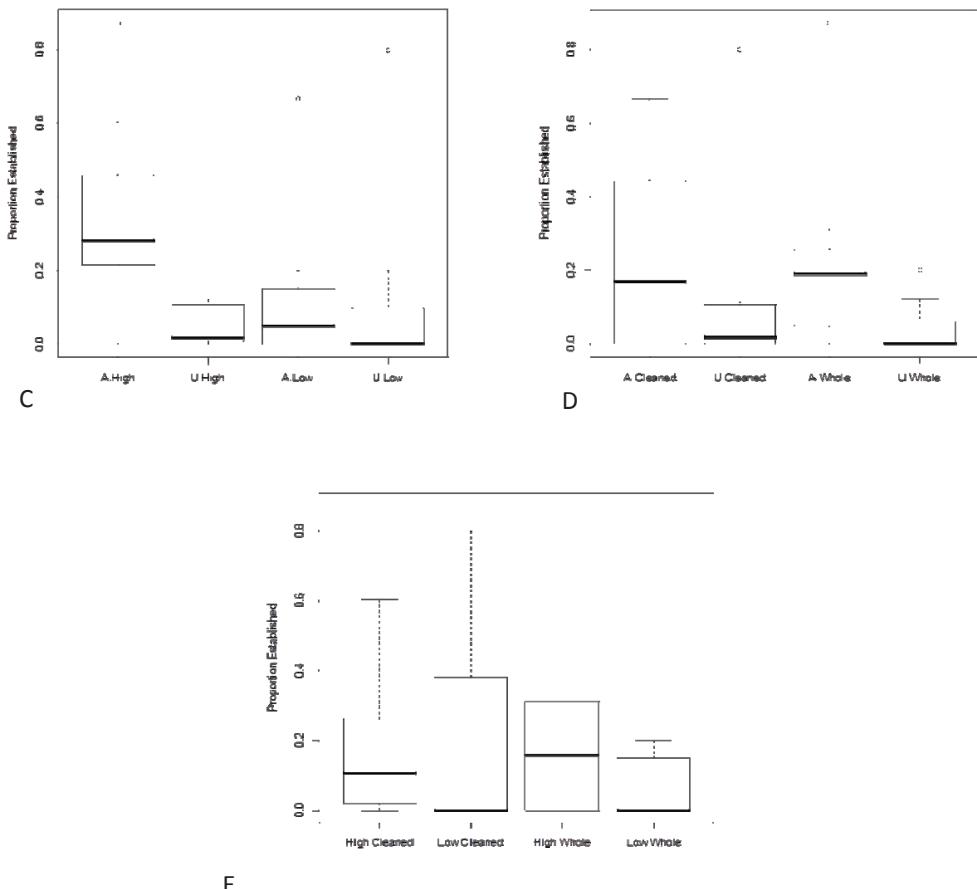


Figure 3.3.4. Boxplots showing the mean proportion of established seeds for A) the positional effect; B) the density effect; C) the interaction between the positional effect and density; D) the interaction between the positional effect and the seeds' flesh status; and E) the interaction between the density effect and the seeds' flesh status at the Nga Manu site.

The *C. querula* drilling proportions were highly variable at Totara Reserve where marginally more damage occurred to seeds away from tawa canopy than under it. Seeds that were in low density, however, were much more damaged than seeds in high densities (Table 3.3.5, Figure 3.3.5). The Position:Flesh interaction was significant at Totara Reserve where seeds that still had pericarp and were under tawa were much more likely to be drilled by *C. querula*. Seeds that were in low density whether fleshed or not (Density:Flesh) were also more likely to be proportionally drilled more than high density treatments (Table 3.3.5, Figure 3.3.5).

Table 3.3.5. One-way ANOVA Table showing the significant results of drilled seeds at Totara Reserve for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			30	328.11	
Replicate	3	138.635	27	189.47	<0.001
Position	1	6.019	26	183.45	0.0142
Density	1	19.305	25	164.15	<0.001
Flesh	1	1.661	24	162.49	0.1975
Position:Density	1	1.356	23	161.13	0.2442
Position:Flesh	1	4.002	22	157.13	0.0454
Density:Flesh	1	14.276	21	142.85	<0.001
Position:Density:Flesh	1	4.058	20	138.80	0.0420

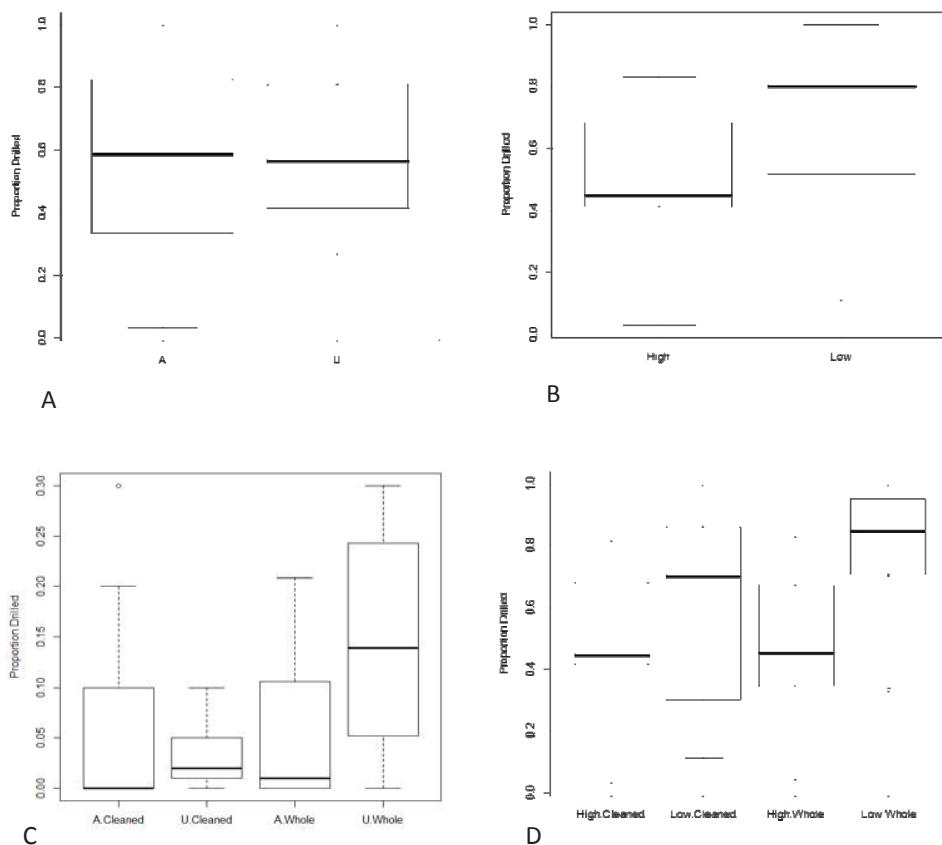


Figure 3.3.5. Boxplots showing the mean proportion of drilled seeds for A) the positional effect; B) the density effect; C) the interaction between the positional effect

and the seeds' flesh status; and D) the interaction between the density effect and the seeds' flesh status at the Totara Reserve site (please note the different Y-axis scales).

The rodent damage proportions were variable at Totara Reserve for seeds still having their pericarp, however it is still clear those seeds were damaged more by rodents than seeds cleaned of flesh (Table 3.3.6, Figure 3.3.6). Similarly with the drilling damage by *C. querula* (Figure 3.3.5), rodents targeted seeds that still had their pericarp and were under tawa canopy (Position:Flesh).

Table 3.3.6. One-way ANOVA Table showing the significant results of rodent chewed seeds at Totara Reserve for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			30	116.833	
Replicate	3	36.851	27	79.982	<0.001
Position	1	3.737	26	76.245	0.0532
Density	1	0.052	25	76.192	0.8189
Flesh	1	11.085	24	65.107	<0.001
Position:Density	1	0.099	23	65.009	0.7532
Position:Flesh	1	7.802	22	57.207	0.0052
Density:Flesh	1	0.032	21	57.175	0.8590
Position:Density:Flesh	1	0.293	20	56.883	0.5884

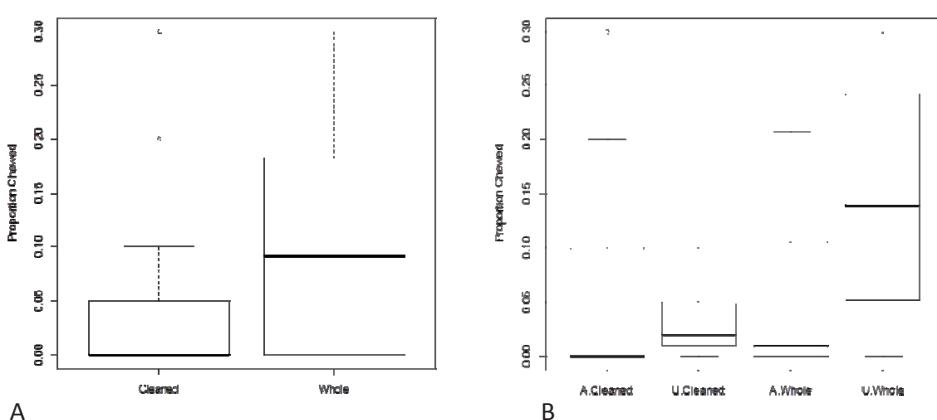


Figure 3.3.6. Boxplots showing the mean proportion of rodent chewed seeds for A) the

seeds' flesh status; and B) the interaction between the positional effect and the seeds' flesh status at the Totara Reserve site

The germination success was highly variable at Totara Reserve for seeds that were in low density treatments. On Average, high density treatments had marginally higher germination than the low density counterparts. Seeds that were in low density and not cleaned of flesh (Density:Flesh) did not germinate well compared with other treatments. A very high proportion of seeds germinated for the low density, cleaned of flesh treatment (Table 3.3.7, Figure 3.3.7).

Table 3.3.7. One-way ANOVA Table showing the significant results of germinating seeds at Totara Reserve for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			30	188.415	
Replicate	3	43.382	27	145.033	<0.001
Position	1	1.955	26	143.079	0.1621
Density	1	20.663	25	122.415	<0.001
Flesh	1	0.071	24	122.344	0.7898
Position:Density	1	1.383	23	120.961	0.2396
Position:Flesh	1	0.259	22	120.702	0.6106
Density:Flesh	1	26.041	21	94.661	<0.001
Position:Density:Flesh	1	1.695	20	92.965	0.1929

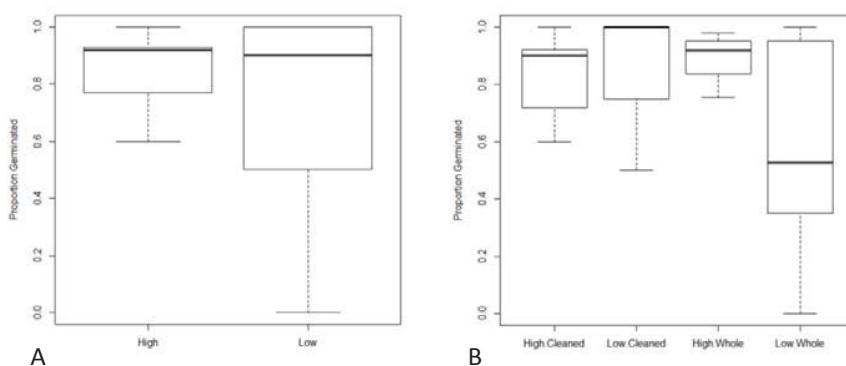


Figure 3.3.7. Boxplots showing the proportion of germinating seeds for A) the density

effect; B) the interaction between density effect and the seeds' flesh status at the Totara Reserve site.

Establishment was highly variable at Totara Reserve but it is evident that seeds in high density treatments had a higher chance of establishing than seeds in low densities. Seeds that were under tawa that were also cleaned of flesh (Position:Flesh) had the best establishment proportions while seeds under tawa that still had their pericarp had the lowest establishment success. The Density:Flesh interaction showed clear establishment success in seeds that were in high densities whether they were fleshed or not (Table 3.3.8, Figure 3.3.8).

Table 3.3.8. One-way ANOVA Table showing the significant results of established seeds at Totara Reserve for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			30	290.60	
Replicate	3	88.511	27	202.09	<0.001
Position	1	1.750	26	200.34	0.1859
Density	1	57.803	25	142.53	<0.001
Flesh	1	1.231	24	141.30	0.2671
Position:Density	1	0.021	23	141.28	0.8843
Position:Flesh	1	12.785	22	123.50	<0.001
Density:Flesh	1	4.722	21	118.77	0.0298
Position:Density:Flesh	1	0.000	20	118.77	0.9915

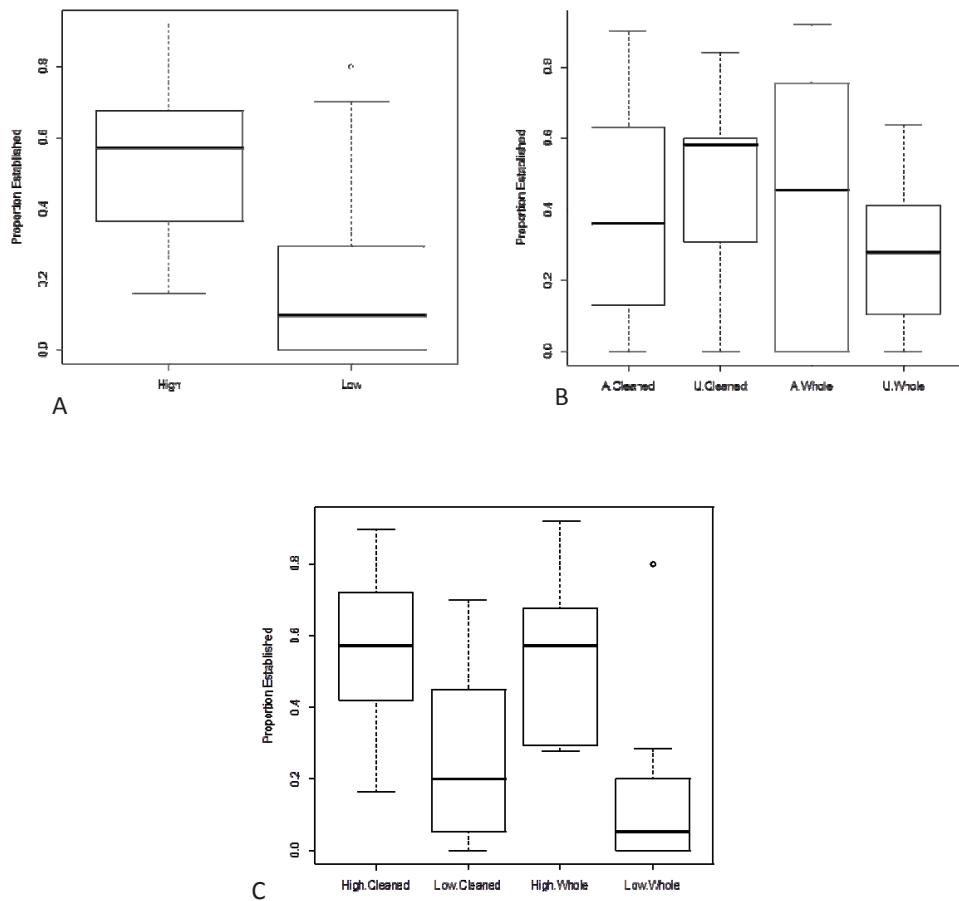
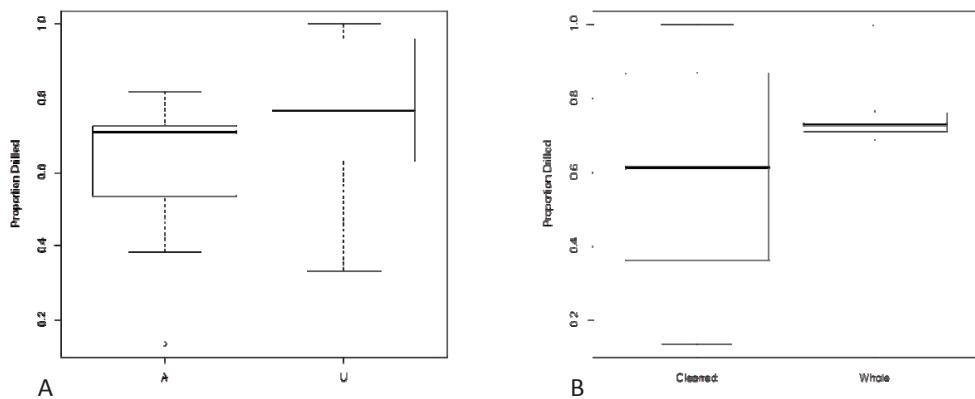


Figure 3.3.8 Boxplots showing the proportion of established seeds for A) the density effect; B) the interaction between the positional effect and the seeds' flesh status; and C) the interaction between the density effect and the seeds' flesh status at the Totara Reserve site.

C. querula drilling proportions varied at Turitea Water Catchment with the exception of seeds still having pericarp. Seeds under tawa canopy were marginally drilled more proportionally than seeds away from tawa canopy. Fleshed seeds were also drilled more than cleaned seeds (Table 3.3.9, Figure 3.3.9). Seeds that were under tawa canopy and in low densities (Position:Density) were drilled a lot more proportionally than the other interacting treatments.

Table 3.3.9. One-way ANOVA Table showing the significant results of drilled seeds at Turitea Water Catchment for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			14	110.578	
Replicate	1	11.8712	13	98.707	<0.001
Position	1	20.9287	12	77.779	<0.001
Density	1	0.9225	11	76.856	0.3368
Flesh	1	18.0157	10	58.840	<0.001
Position:Density	1	4.1702	9	54.670	0.0411
Position:Flesh	1	2.6544	8	52.016	0.1033
Density:Flesh	1	2.3643	7	49.651	0.1241
Position:Density:Flesh	1	3.0921	6	46.559	0.0787



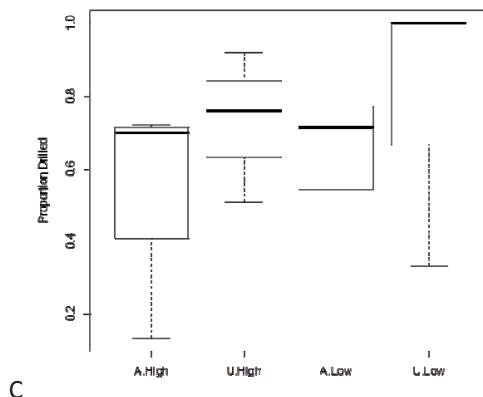


Figure 3.3.9. Boxplots showing the proportion of drilled seeds for A) the positional effect; B) the seeds' flesh status; and C) the interaction between the positional effect and density at the Turitea site.

Rodent damage proportions varied somewhat at Turitea Water Catchment where seeds under tawa canopy were subject to higher damage than seeds away. Seeds under tawa canopy that were in high densities (Position:Density) also suffered high damage from rodents than other interacting treatments. The safest option (though also highly variable) were seeds under tawa canopy that were in low densities (Table 3.3.10, Figure 3.3.10).

Table 3.3.10. One-way ANOVA Table showing the significant results of rodent chewed seeds at Turitea Water Catchment for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			14	69.623	
Replicate	1	12.3257	13	57.297	<0.001
Position	1	21.6023	12	35.695	<0.001
Density	1	0.8466	11	34.848	0.3575
Flesh	1	0.1759	10	34.672	0.6749
Position:Density	1	6.7383	9	27.934	0.0094
Position:Flesh	1	2.2174	8	25.717	0.1365
Density:Flesh	1	1.7614	7	23.955	0.1845
Position:Density:Flesh	1	3.4863	6	20.469	0.0619

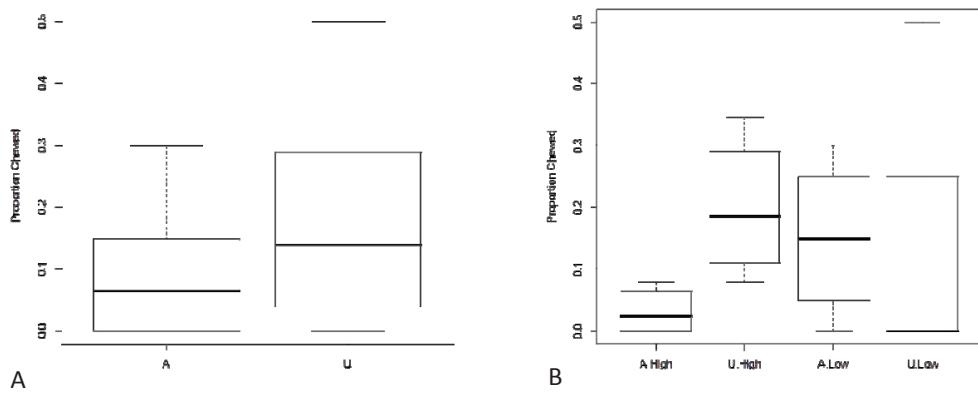


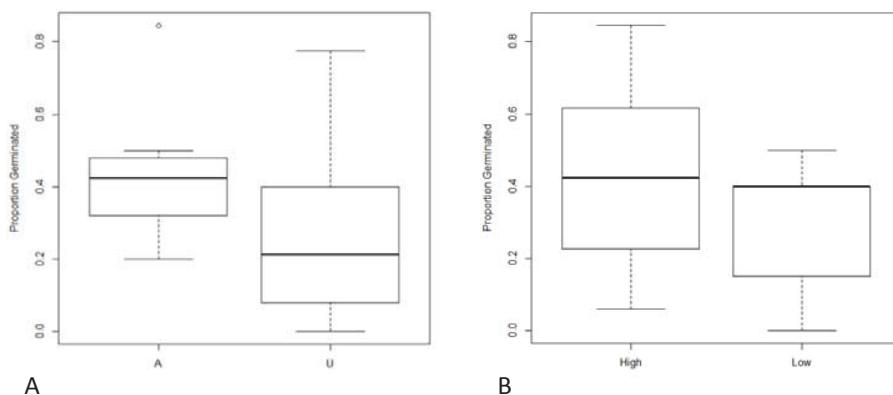
Figure 3.3.10. Boxplots showing the proportion of rodent chewed seeds for A) the positional effect; and B) the interaction between the positional effect and density at the Turitea site.

Germination proportions varied for multiple treatments at Turitea Water Catchment. Seeds that were away from tawa canopy had a higher germination success. Similarly seeds in high density treatments had marginally higher germination proportions than low densities. Cleaned seeds also had much higher germination than fleshed seeds (Table 3.3.11, Figure 3.3.11). The interacting treatments Position:Flesh was significant where seeds that were away from tawa canopy and cleaned of flesh had much higher germination proportions while seeds that were under tawa canopy and were fleshed had the lowest germination success.

Establishment at Turitea Water Catchment was variable for high seed density treatments and seeds cleaned of flesh. Seeds away from tawa canopy established better than seeds under tawa canopy. Seeds in high densities also established proportionally better than low density treatments. Similarly with germination (Figure 3.3.11) seeds that were cleaned of flesh had a much higher establishment chance than seeds still having pericarp (Table 3.3.12, Figure 3.3.12).

Table 3.3.11. One-way ANOVA Table showing the significant results of germinating seeds at Turitea Water Catchment for these variables: Position, Density, Flesh and the interaction between them).

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			14	123.933	
Replicate	1	9.972	13	113.962	0.0016
Position	1	7.991	12	105.971	0.0047
Density	1	6.466	11	99.505	0.0110
Flesh	1	59.357	10	40.148	<0.001
Position:Density	1	1.968	9	38.179	0.1606
Position:Flesh	1	5.290	8	32.889	0.0214
Density:Flesh	1	1.234	7	31.655	0.2666
Position:Density:Flesh	1	0.107	6	31.548	0.7432



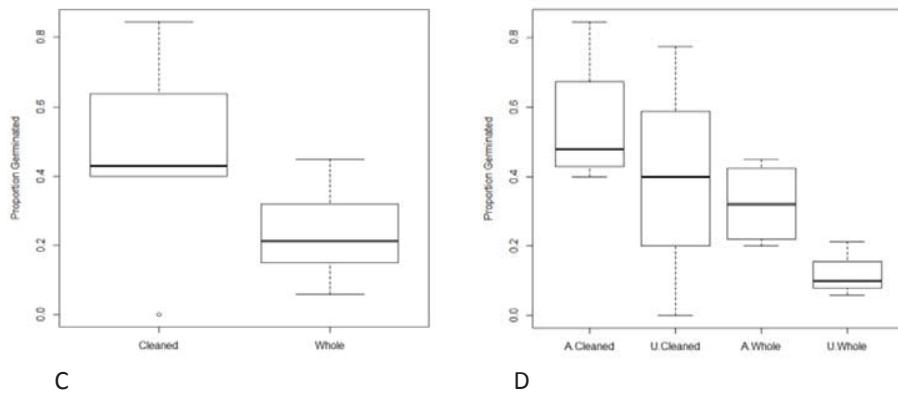


Figure 3.3.11. Boxplots showing the proportion of germinating seeds for A) the positional effect; B) the density effect; C) the seeds' flesh status; and D) the interaction between the positional effect and the seeds' flesh status at the Turitea site.

Table 3.3.12. One-way ANOVA Table showing the significant results of established seeds at Turitea Water Catchment for these variables: Position, Density, Flesh and the interaction between them.

	Df	Deviance	Residual Df	Residual Deviance	P
NULL			14	131.215	
Replicate	1	12.061	13	119.154	<0.001
Position	1	28.227	12	90.926	<0.001
Density	1	9.108	11	81.818	0.0025
Flesh	1	40.069	10	41.749	<0.001
Position:Density	1	3.283	9	38.466	0.0700
Position:Flesh	1	0.821	8	37.646	0.3650
Density:Flesh	1	0.368	7	37.277	0.5440
Position:Density:Flesh	1	0.000	6	37.277	0.9999

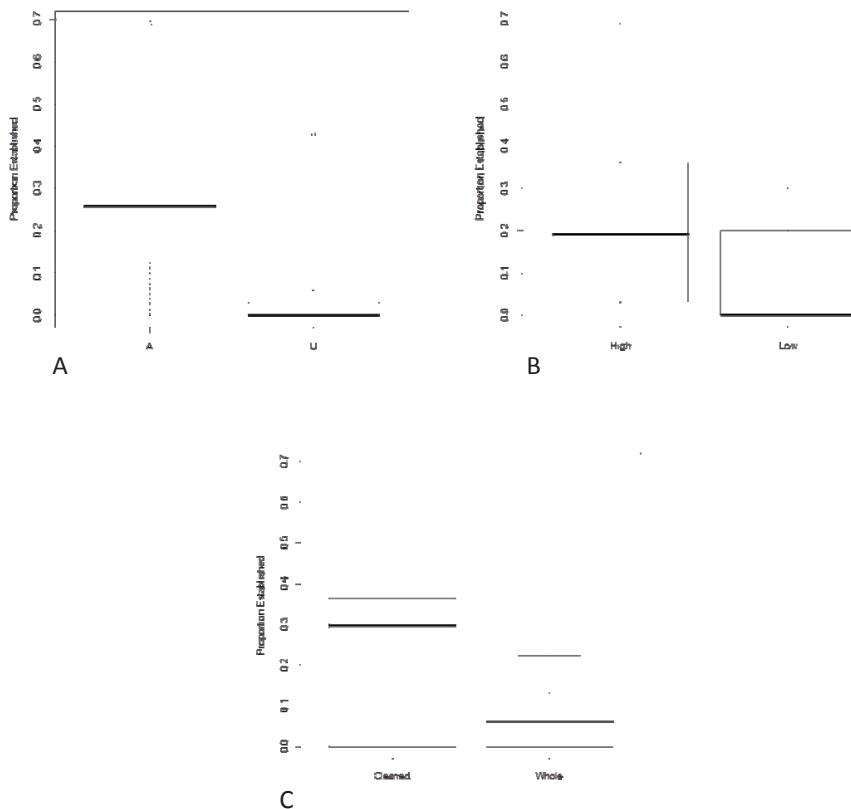


Figure 3.3.12. Boxplots showing the proportion of established seeds for A) the positional effect; B) the density effect; and C) the seeds' flesh status at the Turitea site.

Table 3.3.13. Summary table showing the significant factors (position, density, flesh) and interactions influencing the four main factors (rodent damaged, drilled, germination, establishment) at three sites (Nga Manu, Totara Reserve, Turitea Water Catchment). In each case, the riskiest and safest combination of treatments and interactions is shown. Where two combinations of the interactions were more or less equal in effect size, both are shown, one above the other.

Condition	Site	Position (P)		Density (D)		Flesh (F)		PxD		PxF		DxF	
		Riskiest	Safest	Riskiest	Safest	Riskiest	Safest	Riskiest	Safest	Riskiest	Safest	Riskiest	Safest
Drilling Risk	Nga Manu	A	U	L	H					A/W A/C U/W	U/C		
	Totara Reserve	A	U	L	H					A/W A/C U/W	U/C	L/W	H/C H/W
	Turitea	U	A			W	C	U/L		A/H A/L A/H U/L			
Rodent Risk	Nga Manu	A	U			W	C	U/H		A/W	A/C U/W	H/C L/W	L/C
	Totara Reserve					W	C			U/W	A/W A/C		
	Turitea	U	A					U/H		A/H U/L			
Germination Chance	Nga Manu	U	A										
	Totara Reserve	U	A	L	H								
Establishment Chance	Turitea	U	A	L	H	W	C						
	Nga Manu	U	A	L	H			U/H U/L		A/H		L/W	L/C
	Totara Reserve			L	H	W	C			U/W	A/C	L/W	H/W
	Turitea	U	A	L	H					U/W	U/C	L/W	H/W H/C

Position: A = Away from Tawa; U = Under Tawa

Density: L = Low (10 seeds); H = High (50 seeds)

Flesh: W = Whole; C = Cleaned

At all three sites it is evident that seeds that were not drilled had a much higher proportion of germinating seeds. The proportion of germinating seeds that were drilled is highly variable across all sites as well (Figure 3.3.13).

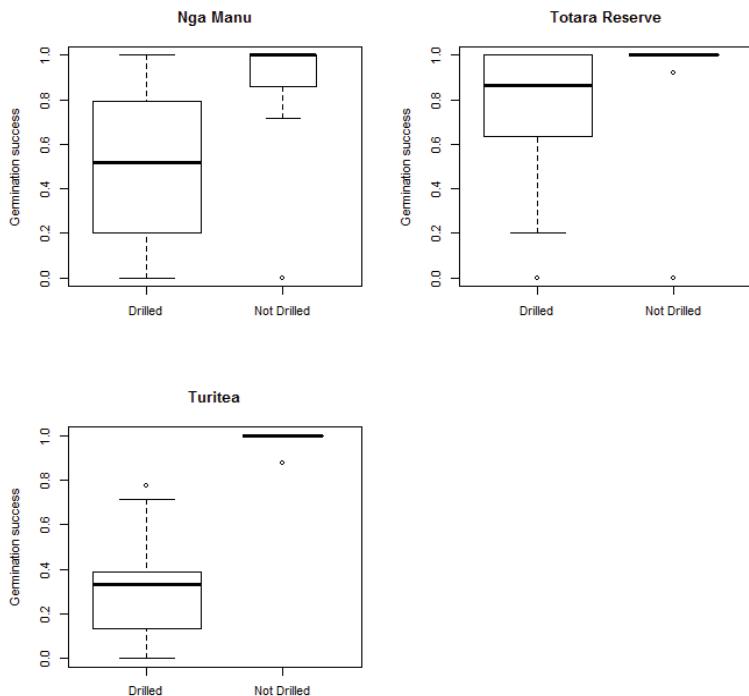


Figure 3.3.13. Boxplots showing the proportion of germination success for drilled and non-drilled tawa seeds at three sites. In these box plots, the width of the box is proportional to the number of seeds in each group (drilled or not-drilled).

For Nga Manu the establishment of seeds that were not drilled was highly variable but the average follows the trend shown across all three sites where non-drilled seeds establish much better than drilled seeds. Nearly polar opposite results are seen at Turitea Water Catchment where a good majority of undrilled seeds establish while virtually none of the drilled seeds establish (Figure 3.3.14).

Germination success was highly variable for seeds that were damaged by rodents (Chewed) but there was little variability for undamaged seeds. Similarly with drilling damage (Figure 3.3.13) non-chewed seeds experienced much higher germination success than seeds that had suffered damage from rodents (Figure 3.3.15).

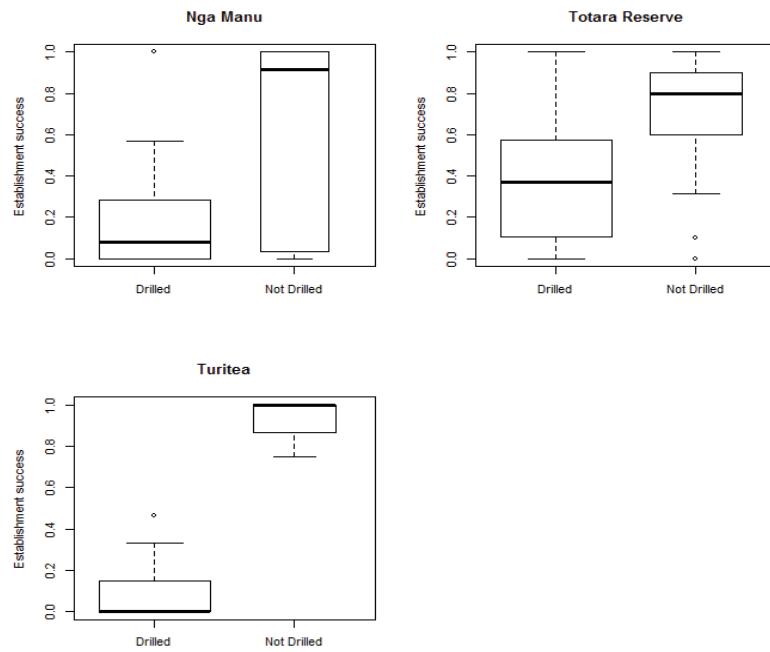


Figure 3.3.14. Boxplots showing the proportion of establishment success for drilled and non-drilled tawa seeds at three sites.

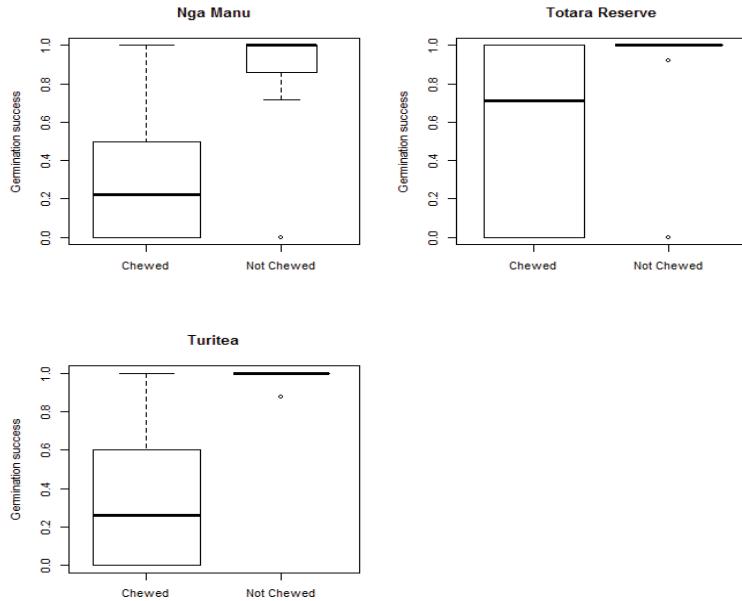


Figure 3.3.15. Boxplots showing the proportion of germination success for rodent damaged and undamaged tawa seeds at three sites.

Seeds that were not damaged by rodents had a lot of variability in their establishment at

all sites especially at Nga Manu. The results are very similar to the drilling damage by *C. querula* (Figure 3.3.14) where undamaged seeds had a much higher establishment success than the rodent damaged seeds. Average establishment for rodent damaged seeds was very low indicating that these seeds may not be able to adequately establish after damage by rodents (Figure 3.3.16).

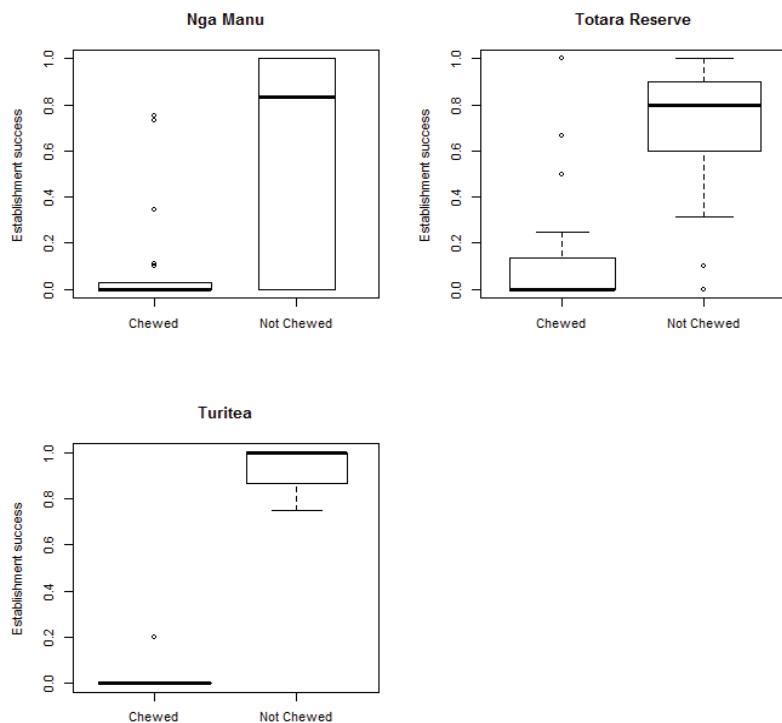


Figure 3.3.16. Boxplots showing the proportions of establishment success for rodent damaged and undamaged tawa seeds at three sites.

3.4 Discussion & Conclusions

3.4.1 Drilling & Rodent Damage effects on Germination & Establishment

This experiment has demonstrated that *Cryptaspasma* drilling and rodent damage both have a strongly detrimental effect on the germination and establishment of tawa seedlings. Germination success means were nearly all approximately 100% when not subjected to drilling or rodent damage. A similar effect was seen in establishment proportions (though not all were as high as 100% success without damage) where seeds were much more likely to establish if they had not suffered any form of drilling or rodent damage.

Despite the lower chance of germination and establishment that the drilled and rodent chewed seeds suffered, there are still a number of seeds that do still germinate and establish, however, it is unclear whether, in these cases, the drilling/rodent damage occurred before or after the seed had germinated/established. If the damage did occur after the seed had already established then there would be very little effect, however if the damage occurred before the seeds germinated then it shows that while drilling damage and rodent damage have clear adverse effects on tawa seeds they may not necessarily kill every seed they damage. This may show some form of evolutionary resistance of the seedlings to dealing with external damage.

3.4.2 Drilling Proportions & Invertebrate Seed Predators

The analysed individual variables (position, density, flesh) across all three sites (Nga Manu, Totara Reserve, Turitea Water Catchment) showed interesting trends relating to the resulting factors (Drilling, Rodent-attack, Germination, and Establishment). Over all three sites, the variable ‘position’ was significant suggesting that whether a seed is drilled or not strongly correlates to whether the tawa seed is located under tawa canopy or whether it is located under non-tawa stands.

The Janzen-Connell model suggests (in combination with many other effects like pathogens and natural enemies) that seeds should be more successful the further away they are from the parent plant/canopy. The Janzen-Connell model does state that natural

enemies cause this higher success and it is thought that they do contribute to the driving of this pattern. This was supported by the Turitea results where there was severe seed predation under tawa canopy but substantially less away from tawa. There are many studies (Visser et al. 2011; Ruiz et al. 2010;) that find that seed predation does decline further away from parent canopies, thereby supporting Turitea's results and the Janzen-Connell model. Nga Manu, however, found the opposite result, where there was greater seed predation away from tawa. Totara Reserve acted as the intermediate result where the proportion of drilled seeds, both away and under tawa, were relatively similar. Possible reasons for this are that, at Nga Manu, *C. querula* adults may utilise their resources away from tawa stands, where the ‘away’ seeds are located; this allowed easier oviposition on the seeds away from tawa than travelling back into tawa forest to oviposit. Another explanation could be that other utilizable seeds (i.e. non-tawa seeds as *C. querula* is polyphagous) were present in non-tawa areas and therefore the population of *C. querula* already existed there.

The three sites varied slightly with their species compositions. Nga Manu and Totara Reserve both had predominantly naturally occurring species (not introduced by people) in both positions (away and under tawa) while at Turitea tawa was dominant in all the native forest and the “away” forest was predominantly pines. This means there are not likely to be pre-existing populations of *C. querula* at the pine forest unlike at Nga Manu and Totara Reserve away sites, which still had native species some of which are possible alternate hosts for *C. querula*. This is further supported by a study undergone by Nopp-Mayr et al (2012), who showed that seeds do suffer different levels of seed predation in different forest types. This may partially explain the results shown.

The variable ‘density’ (10 seeds = low density; 50 seeds = high density) was shown to significantly impact whether a seed is drilled or not for two of the three sites (Nga Manu and Totara Reserve) where seeds with lower densities suffer higher drilling proportions than seeds in dense groups. This supports the idea of protection in numbers, where predators will become satiated or occupied with some of the seeds while undrilled seeds are given the time to germinate and establish. Several studies (Vieira et al. 2011; Boudreau, & Lawes, 2008; Takeuchi & Nakashizuka, 2007) support these findings and the theory of predator satiation where seeds are dense in number, especially when located under parent trees (Takeuchi & Nakashizuka, 2007). These results, however, do

not support the Janzen-Connell model where seeds, that are dense in number (from being under parent plants/canopies), should suffer higher seed predation. The opposite of this was found at the two sites.

The variable ‘flesh’ (Clean = No flesh around seed; Whole = Flesh remains around seed) was shown to only significantly impact whether a seed is drilled or not at the Turitea Site (graph 30) where the seeds that still had flesh had a higher drilling proportion than cleaned seeds and much less variability. This result possibly suggests that olfactory cues appear to attract adult *C. querula* adults which oviposit near these seeds; or removed flesh may stimulate early germination therefore making the time the seed can be drilled lessened before they become less attractive to *C. querula* as the endosperm will be steadily depleted. At Totara Reserve and Nga Manu there was no significant overall effect of flesh removal. Various studies (von Arx et al. 2011; Tasin et al. 2011; Bruce et al. 2005) have shown that invertebrate species may find hosts through olfactory cues however there are limited studies that show invertebrate seed predators finding ideal oviposition sites (i.e. near hosts) for their young through olfactory cues.

Drilling proportions were significantly impacted at the Turitea site (but not at Totara Reserve or Nga Manu) by the interaction of the variables: ‘position’ and ‘density’. The two combinations of ‘away from tawa’ and ‘high density’ or ‘away from tawa’ and ‘low density’ were the safest strategy for the tawa seeds. This interaction of variables is the closest representation of the Janzen-Connell model which suggests seed density should be highest under parent canopies and as a result there should be greater seed predation. As expected, experiments that were located under tawa canopies (irrespective of density) experienced higher drilling proportions than those away from tawa. Interestingly, seeds that were under tawa canopies and low in density suffered extremely high drilling proportions (nearly 1.0 – i.e. all of them). This goes with the expectations that *C. querula* adults would stay around tawa canopies and any low density groups of seeds would suffer tremendous amounts of predation through drilling. The Janzen-Connell model would be fully supported by the results if the seeds away from tawa, which were low in density, were the lowest in seed predation proportions, however this was not quite the case. High density seeds that were away from tawa suffered the least drilling proportions. This could be explained by a combination of the Janzen-Connell hypothesis (Clark & Clark, 1984) and the localised predator satiation theory (Karban,

1982; & Molles, 2002) where seeds that escape from the parent canopy must also be in a dense seed environment so that the odd passing seed predator is satiated.

The interaction of the variables ‘position’ and ‘flesh’ significantly altered the drilling rate at both the Nga Manu and Totara Reserve sites. However, the two sites showed very different results where at Nga Manu, seeds that were away from tawa (irrespective of their flesh status) would have higher drilling proportions and more variability, but at Totara Reserve the opposite was true, where seeds under tawa (irrespective of their flesh status) were drilled more than away from tawa. The flesh doesn’t have as strong a relation with drilling as position at the two sites but a slight trend appears that whole tawa fruits are drilled more than cleaned seeds. Overall, the results of the interactions between these two variables are too inconsistent to give firm conclusions.

For the Totara Reserve site, the interaction of the variables, ‘density’ and ‘flesh’ were significant in impacting drilling rates. Irrespective of flesh status, seeds in low density experiments suffered much higher proportions of drilling than higher densities. The combination of low density and whole seeds resulted in a very high proportion mean of drilling and very little variability. This supports the idea that olfactory cues may attract *C. querula* and with too few seeds to cause ‘predator satiation’, a high number of seeds will be attacked. Studies by Vieira et al (2011) and Boudreau & Lawes (2008) both support that high densities appear to protect some seeds through predator satiation, however, few studies appear to simultaneously consider both densities and flesh status (olfaction). The opposite of the above variables appears to show appropriately opposite results as seeds that were in dense (high density) and cleaned experiments suffered the lowest drilling proportions (note: the mean was very similar to seeds that were in high density and whole experiments) supporting further the predator satiation hypothesis and somewhat less that a lack olfaction cues (i.e. cleaned) will result in lower drilling proportions. An MSc thesis by Sidney (2005) supported the idea that volatile cues and certain olfactory (such as the flesh of hard unripe fruits) do attract invertebrates (in this case lepidoptern twig borers).

The last factors pertaining to drilling proportions is the three-way interaction of all the variables. At both Nga Manu and Totara Reserve were the three-way combinations significant on impacting drilling proportions. There are multiple situations these

combinations represent: low density, cleaned, away from tawa represents seeds that have been transported away, cleaned and excreted by a bird while the combination of high density, whole seeds and under tawa mimics a high masting year. While, it was not shown in a boxplot (as the data becomes too convoluted to do so) the highest drilling proportions at Nga Manu were seen in the high masting year equivalent (under tawa, high density, whole flesh) which is an expected result. The expected result for bird transportation was not seen however, as the result was much higher which suggests that even if a seed does escape from the parent canopy, it might still suffer high seed predation. There were no remarkable findings at the Totara Reserve site for the three-way interaction of variables (both high masting year and bird transportation representations were intermediate in terms of seed predation).

Seeds, therefore, that have passed through a bird and have been taken far away from the parent plant may still have a chance of being drilled. There have been many studies (Guerrero & Tye, 2009; Christianini & Oliveira, 2010; Guerta et al, 2011) supporting that birds can act as excellent seed dispersers (if the fruit of the seed is removed in the gut of the bird while the husk/endosperm is unaffected) but there have been very few studies showing whether successful bird dispersal helps/hinders seeds escape invertebrate seed predation. The results shown previously do not support the idea that bird dispersers consistently help seeds escape seed predation.

3.4.3 Rodent Attacked/Damaged Seeds

As was the case with drilling proportions, the likelihood of rodent attack varied with the positioning of seeds in the experiments (under or away from tawa canopies) at two of the three sites. The site Turitea had relatively low rates of rodent attack (between 0.01 and 0.2) but sites under tawa suffered higher proportions of rodent damage than those moved away. Jones & Toft (2006) found that rodents seem to specifically target *C. querula* larvae as a food resource (breaking open seeds to eat the *C. querula* larvae rather than the endosperm); if this were true the results for rodent damaged seeds should show a similar pattern as the proportion of drilled seeds (as rodents would prefer to break open drilled seeds to find *C. querula*). Interestingly this was the case for the Turitea site. For Nga Manu there was a suggestion that this was the case here too. At Nga Manu, seeds placed away from tawa experienced marginally higher mean rodent

damage proportions than seeds under tawa. The mean proportions of rodent damage was also higher (0.25 to 0.3) than the other sites suggesting that while some rodents likely attacked seeds containing *C. querula* some rodents seem to attack non-drilled seed (either to get endosperm as their main reward or in an attempt to find *C. querula*).

The density of seeds at all three sites did not significantly impact whether they would be attacked by rodents or not. The presence of fruit flesh, however, was important at two of the sites (Nga Manu and Totara Reserve) but did not seem to play any role (even when interacting with other variables) at the Turitea site. Whole seeds were attacked more often by rodents at both Nga Manu and Totara Reserve. At Nga Manu the mean proportion of attacked seeds with whole flesh was only slightly higher than those with cleaned flesh status; however for Totara Reserve, cleaned seeds suffered a mean proportion of nearly zero rodent damage while whole seeds suffered a higher damage proportion of 0.1. This could indicate that rodents prefer the fruit of tawa seeds and ‘damage’ the husk of the seed without intention; however the fact that cleaned seeds (completely devoid of flesh) were also damaged at some sites suggests that they still do damage seeds for a reason other than the outer fruit. Perhaps rodents can detect the drilled seeds and preferentially attack them but this would require some rodent choice experiments to test. There have been few studies looking at rodent’s choice to attack seeds based on the presence of boring invertebrates or not. One study by Nano et al (2003) showed the dietary preference of the rock rat. Its primary food source was seeds, followed by leaves and at a very low percentage invertebrates – it is possible rodents in this thesis have similar diet priorities and therefore do not actively search for *C. querula* (especially when there are easier-to-obtain invertebrates around).

The interaction of the variables ‘position’ and ‘density’ was significant in impacting proportions of rodent attacks on tawa seeds for two (Nga Manu and Turitea) of the three sites. Both sites showed nearly identical patterns (Nga Manu however had higher overall proportions than Turitea) where seeds that were under tawa and high in density suffered a slightly higher mean proportion of rodent damage, followed by seeds that were away and in low density experiments. Seeds that were placed either: under tawa and low in density, or away from tawa and high in density both experienced roughly the same mean proportions of rodent damage (which was substantially lower than the other two combinations). The expected results of higher proportions of damaged seeds at

experiments located under tawa and with high density can be explained by several possibilities. The rodents have possibly learned that resources (in the form of either endosperm or *C. querula* larvae) are predominantly under tawa canopies and over the course of one year have steadily damaged the seeds (coming back when no longer satiated – i.e. making predator satiation hypothesis redundant under tawa canopies). Various studies support that rodents use specific resource patches more commonly than others which can be indirectly shown in these results (Fraschina et al 2009; Fanson et al 2008). Seeds, however, that were far away from tawa but are few in number (low density) also suffer relatively high proportions of rodent damage as they do not have the protection of predator satiation via dense seed numbers. The seeds in experiments that are either away and high in number benefit from the escape hypothesis (less predator further away from parent plant) and the predator satiation hypothesis (any rodents chancing upon the area will be sated). Seeds that are under tawa but are low in density likely represent a poor resource patch and are likely not worth the risk and effort of foraging compared with more resource-rich patches.

The interaction of ‘position’ and ‘flesh’ significantly altered the rate of rodent damage for two sites (Nga Manu and Totara Reserve). The results for each site were vastly different where Nga Manu’s rodent damage mean proportions were relatively similar across all four combinations of variables, however seeds that were away and whole exhibited slightly higher proportions of rodent damage while seeds away and cleaned were the lowest in terms of damage. At Totara Reserve seeds that were under tawa exhibited higher rodent damage proportions but whole seeds were still targeted more than cleaned seeds. The difference in results for the position part of the interacting variables at each site is unclear. Possibilities could be different forest types (though both were in natural flora), different species of rodents at each site etc. Excluding Nga Manu’s under and cleaned results it appears that if a seed is whole, irrespective of position, they will more likely be targeted by rodents, which suggests the rodents are either attracted by volatile cues released by the flesh of the fruit or they are actively searching for flesh to feed on (as opposed to endosperm/*C. querula*).

At the Nga Manu site, all the two-way interactions, were significant predictors of rodent attack. Given the low proportions of rodent damage at the other sites, Nga Manu is perhaps the most informative about rodent preferences. The final interaction of

variables was ‘density’ and ‘flesh’. The mean proportions of damage were very similar except for the experiments that were high in density and were cleaned of flesh, and the experiments that were low in density and were cleaned of flesh. The two intermediate treatments of high & cleaned, and low & whole both suffered higher rodent damage than the opposite treatments (high:whole & low:cleaned). The high density, whole fleshed experiments were likely protected slightly by the predator satiation hypothesis while the low density and cleaned experiments were likely so few and without scent that they were rarely found and preyed upon.

3.4.4 Germination

The proportion of germinating tawa seeds were higher overall at Totara Reserve (0.79) followed by seeds from Nga Manu (0.43) and lastly Turitea (0.36). The variables affecting the proportion of germinating seeds were relatively similar across all three sites though some were not significant. Judging by the summary table (Table 2.3.13), seeds placed under a tawa canopy had less chance of germinating than seeds placed away from tawa and that seeds at high densities had a higher chance of germinating than seeds in low densities. A study by Yoko-o & Tokeshi (2012) contradicts the positional finding when they showed that *Quercus glauca* seeds (an evergreen broadleaf tree) under *Pyrrosia* and *Trachelospermum* forest cover were more likely to survive until they germinated (then other factors played more important roles during the establishment phase) which was not the case for these results. Possible reasons for this result (position) are that seeds under tawa canopies must compete with their own parent plant for sunlight. Various studies have shown that various light types (red, blue green etc) stimulate germination in seeds through their photoreceptors (Baeza & Roy, 2008; Kan & Song 2008; & Dissanayake et al. 2010) and the seeds under their parent canopy cannot receive enough of this essential light to germinate, however in seeds away from thick tawa canopies they do get a sufficient amount of light to germinate. Another possibility is that drilling occurred quicker under tawa canopy giving the tawa seeds less chance of germinating before they were attacked. It is also interesting to note that at Totara Reserve seeds that were under tawa were less drilled but suffered higher rodent damage. These factors likely played a role in the germination of seeds. Rodent damage may be more fatal to a seed despite that various studies (Aref et al. 2011; Celedon-Neghme et al. 2008) have shown abrasion (though the seed damage by rodents may be

more damaging than abrasion (e.g. partial consumption)) can greatly assist in germination.

The variable ‘density’ was also significant in impacting germination at the Totara Reserve and Turitea sites but had no significant impact at Nga Manu. Seeds that were in high densities had much higher germination success than seeds in low densities, which suggests that there might be a benefit of being clustered in high densities (predator satiation). A contradicting study by Augspurger & Kelly (1984) showed that seeds congregated in high densities can suffer from higher attacks of pathogens which was not directly shown or supported for the above results at Totara Reserve and Turitea.

The ‘flesh’ variable significantly impacted the proportion of germinating seeds at the site Turitea, however it did not impact seeds by itself (but it did when combined with other variables) at Totara Reserve. The study by Robertson et al (2006) found varying results with the retention of fruit flesh (pericarp) on the germination of differing plant species. Whole seeds there had a lower chance of germinating than cleaned seeds. Studies (Bannister & Bridgman, 1991; Otani, T. 2004; Kobayashi et al. 2010) have shown that fully fleshed seeds suffer lower germination than seeds cleaned of their outer fruit layer. This appears to be the case for *B. tawa* seeds at Turitea. Different abiotic factors may account for the difference in significant results between the sites or perhaps differences within tawa at a local level (i.e. minimal gene flow might cause slight germination differences). This should be investigated further.

The two interacting variables ‘position’ and ‘flesh’ were significant in impacting germination for one of the three sites (Turitea). Seeds that were under tawa canopies and had whole flesh showed the lowest germination proportion out of the four possible combinations. This makes sense given that the results of the individual variables: Position and Flesh. Being under tawa had lower germination as did being whole fruited. It therefore also makes sense that the opposite combination (away from tawa and cleaned) would have the highest germination success. There are many different possible reasons for this result: combination effects of light levels and outer fruit inhibiting germination, escaping predators by going into different forest types combined with lack of olfaction cues to attract natural enemies (though olfaction attraction was not seen in laboratory wind tests in Chapter 5).

The site Totara Reserve showed significant results for affecting germination with the interacting variables of ‘density’ and ‘flesh’. It showed relatively low germination rates in experiments with low densities and whole flesh, and high germination in low density and cleaned seeds. The result of lower germination in seeds with whole flesh and in low densities is supported by a study which suggests that an outer fruit layer actually hinders germination (Jordaan et al. 2011). The result here shows that low density seeds’ germination success depends on whether they are fleshed or cleaned.

The last interaction of variables to significantly impact germinations was the three-way interaction of each individual variable (position:density:flesh) and it only occurred at the Nga Manu site. While there is no visual representation of these interacting variables, the results will still be discussed. Interestingly the bird transportation representation (away from tawa, low density, cleaned flesh) had quite high proportions of germination (compared with other factors) across two of the sites (Nga Manu and Totara Reserve although the results at Totara Reserve were not significant). This suggests that bird dispersers, while unable to affect seed predation directly, may give seedlings enough time to escape seed predation until they are ready to germinate. Studies (Guerrero & Tye, 2009; Christianini & Oliveira, 2010; Guerta et al, 2011) have shown that bird dispersers can facilitate germination and that appears to be further supported by the results of this thesis. The other representation (high masting year – under tawa, high density, whole fruit) had very low proportions of germination at Nga Manu and Turitea while relatively high proportions of germination at Totara Reserve.

3.4.5 Establishment

The average (mean) proportion of established seeds was lower at Nga Manu (0.16) and Turitea (0.17), while much higher at Totara Reserve (0.39). This suggests that abiotic or biotic factors differ among the sites. The variable ‘position’ was significant in impacting establishment for Nga Manu and Turitea but not for Totara Reserve. Both significant sites show very similar results where experiments away from tawa had much higher establishment means than experiments located under tawa. This could be due to the factors given in the *germination* section. Higher light levels (Augspurger & Kelly, 1984; Vranckx & Vandelooy, 2012) outside tawa canopies (though this was not directly tested

and is given only as a possible explanation) have allowed higher proportions of establishment while experiments under tawa have a mean of nearly zero establishing seeds. The very similar results at the Nga Manu and Turitea sites (even having similar outliers) suggests there are similar acting factors here that do not significantly influence Totara Reserve.

Interestingly at all three sites, the variable ‘density’ was strongly significant in impacting establishment success. While Totara Reserve had a higher overall proportion of establishing seeds, all three sites showed very similar patterns. Experiments that had higher densities of seeds had a much higher establishment rate. For all sites, experiments with low densities had low establishing proportions. This consistent pattern at all three sites shows strong evidence that factors that produce low seed density will be greatly inhibitory to establishment. The possible reasons for this are that seeds in high concentrations are more likely escape predation and damage due to predator satiation (Vieira et al. 2011; Boudreau & Lawes, 2008) allowing them time to establish (which by that time they are fully self-sufficient and no longer require the endosperm of the seed to survive).

The variable ‘flesh’ was only significant by itself (as opposed to combined with other variables) in impacting establishment at the Turitea site but the results there showed vast differences between the experiments with clean and whole seeds. Seeds that were cleaned of flesh showed much higher establishment rates than whole seeds. As mentioned in the *germination* section, fruit pulp/flesh is theorized to inhibit germination and therefore in the long run establishment (Jordaan et al. 2011). This appears to be the case for differently fleshed seeds in experiments at Turitea.

The interacting variables of ‘position’ and ‘density’ were only significant (but strongly significant) in impacting establishment at the site Nga Manu. Nearly all of the combinations had similar mean establishment proportions except for experiments that were away from tawa and high in density, which had a much higher establishment success (over triple the proportion of establishing of other variable combinations). An explanation for this would be that this experimental set up gets benefits of higher light levels and the protection of high density levels (predator satiation). The studies mentioned previously (Augspurger & Kelly, 1984; Vranckx & Vandelooy, 2012; Vieira

et al. 2011; Boudreau & Lawes, 2008) support these findings and hypotheses. The earlier germination thus escaping seed predation is also a potential explanation. Different abiotic factors at the non-significant sites may explain why these variables do not seem as influencing/important for establishment.

For the two sites, Nga Manu and Totara Reserve, the variables ‘position’ and ‘flesh’ are significant in determining establishment in seeds. Both sites show similar results except for Totara Reserve’s experiment with clean seeds, under tawa which was substantially higher at this site than the same variable experiment at Nga Manu Reserve. Despite this, the next highest experimental setups with germination proportions are seeds that are whole and away from tawa canopies. This can be possibly explained the light levels mentioned earlier, however the fact that the seeds still bear fruit goes against Jordaan’s et al. 2011 predictions and results. The experimental setups with the lowest establishing seeds were under tawa with whole flesh status. This further supports the escape hypothesis and Jordaan’s et al. 2011 predictions and results.

The final interactions of variables are between ‘density’ and ‘flesh’. These were significant at both the Nga Manu and Totara Reserve sites. Any experiments with low densities had significantly less establishment regardless of flesh status. At Nga Manu the highest establishing seeds belonged to the experiments with a high density of seeds and whole flesh status; it was nearly the same for Totara Reserve except high density and cleaned seeds had slightly higher (though the means for both were very similar). Again, this suggests that flesh status can vary the success rate of establishment in seeds marginally, where in other sites (and previous sections) whole seeds have had low establishment and germination success, this result suggests whole seeds can actually benefit more from containing flesh than being cleaned first. The main conclusion drawn, in this result, is that higher densities of seeds appear to create better conditions for establishment (which is the opposite to Augspurger & Kelly’s (1984) findings).

3.4.6 Conclusions

Overall the Janzen-Connell hypothesis does appear to affect the rates of *B. tawa* establishment, in part because of differences in the rate of *C. querula* predation at a majority of the sites. Two of three sites (Turitea & Nga Manu) supported the hypothesis

while Totara Reserve was not significant. There were also cases where the results supported a combination of predator satiation and the Janzen-Connell hypothesis. It appears that in this seed predator-host relationship, a mix of factors, and theories likely play a large part in establishing seed survival and escape.

The main conclusion shown from these results are that each site (Nga Manu, Totara Reserve, & Turitea Water Catchment) can and do vary greatly with respect to similarly set up experiments. All factors being tested (Drilling proportions, Rodent-attacked proportions, Germination, Establishment) and the variables (Position, Density, & Flesh status) showed at least some variation across the three sites. There was the odd result that showed very similar trends or patterns such as the variable density and its impact on establishment, across all three sites but by far a majority of the results differed. This suggests that untested factors (e.g. abiotic factors) that differed at each of the sites played a role in influencing each of the effects (Drilling proportions, Rodent-attacked proportions, Germination, Establishment). The study by Tuomisto et al (2003), while looking specifically at plant species, showed that site heterogeneity played an important part as to what species would be found and where. Another study by Stevens et al (2012) suggested that site heterogeneity may greatly impact success of restoration projects focussing on plant species ravaged by invertebrate seed predators. These articles back up the findings of this thesis showing that every site is unique.

Another important note is that the block effect (replicate) was strongly significant for almost every test (drilling, rodent-attacks, germination, establishment) for every site with the exclusion of germination at Turitea. This suggests there was a strong spatial variation at each site and that it is therefore important to replicate across a wide spatial scale.

In conclusion, tawa could partially avoid seed predation by undergoing better dispersal and as a result, achieve higher seedling establishment. However given that the Kereru (native wood pigeon) is the only natural disperser of tawa seeds, and is now less common, in a heavy fruiting year like the one measured (2011), too few seeds are actually dispersed (see chapter 4). The rate that seeds currently naturally escape seed predation through dispersal is now appears to be a strong limiting factor preventing frequent tawa recruitment.

Chapter 4

Seasonal progression of seed predation and fruit degradation in fallen *Beilschmiedia tawa* fruits at three lower North Island sites

4.1 Introduction

Seed predation is an often common process occurring in many systems, arguably as important as the process of pollination (Lewis & Gripenberg, 2008). It involves an animal (vertebrate or invertebrate) that feeds upon a host plant's seeds to gain sustenance (Science Reference, 2008). For this chapter, the focus will be primarily on post-dispersal (after the seed has been dispersed, away from the parent plant) invertebrate seed predators and a brief look into the effect vertebrate (rodents) generalist feeders may have on seeds.

The focal seed predator for this chapter (and thesis) is *Cryptaspasma querula*. It is an endemic species to New Zealand but the genus it belongs to can be found all over the world (Horak, 2006). It is also the only polyphagous obligate seed predator in New Zealand (Sullivan, 1995) making it especially unique and worthy of research. It belongs in Tortricidae, Lepidoptera. The best known host for *C. querula* is the podocarp *Beilschmiedia tawa* (Martin, 2001) which is found all over the North Island and the Northern areas of South Island (Knowles & Beveridge, 1982).

B. tawa seeds start ripening and dropping in February (Beveridge, 1964) and by late March most fruits will have ripened and dropped (Knowles & Beveridge, 1982). The seed will often lose viability (and rot) within three months in moist conditions (Knowles & Beveridge, 1982). The time in which seeds can be seen germinating varies greatly between locations but the latest month recorded was November (Knowles & Beveridge, 1982). It is between these times (February and November) that *C. querula* can negatively impact *B. tawa* regeneration through post-dispersal seed predation. Several studies have shown most post-dispersal seed predation occurs with 28 days (Osunkoya,

1994) or after 40 days (Blate et al. 1998) depending on the host species and seed predator species' involved but this will depend on the phenology of the predator and the time taken for the fallen seed to rot.

It is unknown whether *C. querula* are univoltine (having one generation per year) or whether they are multivoltine (having multiple generations within a year). Multi-voltine seed predators are perhaps more likely in tropical environments and rarer in temperate locations (Poyry et al. 2011) though a study by Corbet et al. (2006) found no relationship between voltinism and latitude. Given that New Zealand is mostly temperate, it might be assumed that *C. querula* would be univoltine. However John Dugdale (pers. comm.) believes they probably have multiple generations per year (multi-voltine). If *C. querula* are multi-voltine this could prove more detrimental to *B. tawa* regeneration as any seeds that managed to escape predation from the first generation of moths may be subject to attack from the second or subsequent generations.

Seed predators, such as *C. querula*, often suffer from parasitoids (an insect species whose larvae utilises arthropod hosts, and the host is ultimately killed (Eggleton & Gaston, 1990)). In most cases a parasitoid is extremely specialised and utilise only one host species. There are many cases of seed predators, in particular, being hosts to parasitoids (Gomez & Zamora, 1994; von Zeipel, 2006). No parasitoids have yet been reported for *C. querula*. Further investigation into whether *C. querula* does suffer from parasitoids could reveal certain evolutionary and behavioural traits in *C. querula* during their lifecycle. A study (Gomez & Zamora, 1994) looking into parasitoids and tritrophic interactions showed that their particular parasitoids had an effect on both the density (by direct predation and through death during oviposition) and the behaviour of *Ceutorhynchus sp. nov*, a weevil seed predator. It is possible a similar situation may be occurring to the *C. querula* species. Parasitoid adult females often actively seek potential hosts for their young through various cues such as olfaction or sight. They can also specifically target host species during a certain stage in the host's life cycle such as eggs, larva, and/or adults (Cônsoli et al. 2010). In the case of *C. querula* there are certain times in a year where they are more abundant or clustered; this may make finding them easier/harder at certain stages. *C. querula* eggs (Figure 1.2.7) are not protected after being laid potentially making them ideal targets for parasitoids. The larva

(Figure 1.2.8 and 1.2.9) could be targeted except they have powerful jaws for defence and are often hidden inside the protective shell of their host seeds such as *B. tawa*. They do leave however after they reach the stage before pupation where they actively search for an ideal position to pupate (Figure 1.2.11). It is at this stage and the pupal stage (Figure 1.2.10) where they are also vulnerable to parasitism and predation.

C. querula may compete with other seed predators for host seeds. Plants can often have multiple post-dispersal seed predators that compete for the seed's resources (e.g. many South Island flora in New Zealand shown by Sullivan & Dugdale (1995)). Dugdale (pers. comm.) believes other unidentified seed predators have been reared from tawa seeds. A study by Boivin et al. (2008) showed that various traits and life history of two competing seed predators can both have strong influences on one another. Other factors like whether the seed predator is host-specific or whether they can utilise resources other than seeds will play a big part in the ability of the seed predators to compete with one another. *C. querula* is a polyphagous obligate seed predator meaning it can potentially utilise multiple species' seeds but it cannot use other food resources (i.e. foods other than seeds) during its larval stage (adults likely feed on nectar or sugar water which is completely different from the endosperm which the larva utilise).

The process of masting is a temporal event where there is synchronised seed production among a population of plants in one particular year while missing out several subsequent years (Kelly, 1994). The two most widely supported explanations for mast seeding are those of seed predator satiation (Salisbury 1942; Janzen 1971, 1976; Silvertown 1980; Kelly 1994) and increased wind pollination (Nilsson & Wastljung 1987; Norton & Kelly 1988; Smith et al. 1990) in years of heavy seed set. Both explanations may be plausible for *B. tawa* in particular as they may be pollinated by wind and possibly by insects as well (Smale, 2008). These two theories are associated with masting because they explain the benefits of plants undergoing masting. The predator satiation theory causes the small population of seed predators to satiate themselves on the plentiful seeds of a masting year leaving many undamaged seeds that escape predation and are able to germinate and establish. The benefit of wind pollination during masting comes from higher fertilization and seed set during heavy flowering, due to a pollination economy of scale (Kelly et al. 2001).

The time and intensity of a masting event could vary between locations (some areas produce more seeds and may occur later or earlier than other sites) and this is more likely if the specific plant species that is undergoing masting uses temperature as an environmental cue to start. This is due to locations varying in average temperature because of latitudinal or altitudinal differences. However Schauber et al. (2002) states that there can be synchronous masting within a species over hundreds or thousands of kilometers suggesting that the timing and intensity of the masting events are sensitive to regional climate rather than microclimate variations.

It would be interesting and useful to know whether the location of the investigated species will affect the amount of seed predation, rodent attacks, and the decay rate of the fruits. This may be due to varying intensities of seed fall (masting), or at different times, or due to factors specific to each location (e.g. one site may have rodent control which thereby reduces rodents which could increase seed predator populations, or there may be alternate hosts for *Cryptaspasma* at some sites that allow resident population to reside that otherwise may be starved by masting in tawa).

Over time, fruit that has fallen from the adult will undergo stages of decay. When it first falls it will likely be largely fleshed possibly giving off volatile cues for seed predators to find them unless it has been cleaned by gut passage through a frugivore. Over time, these fleshed fruits will lose their outer fleshed layer exposing the hard stone within. It is important to know whether these fruits become unattractive to *Cryptaspasma* as they age, so that new drilling become less common with age. Alternatively, the larva may find the fruit a hindrance to actually getting inside the seed and so later drilling may be favoured.

Many studies look at a single point or stage of seed predation influences on host species seeds. This is like taking a photograph of a movie and expecting it to tell the full story. Studies that look at seed predation rates over an extended timeframe are much more likely to see the full story (Klinken, 2005). In the case of *C. querula*, examining its effects on *B. tawa* over the course of one year at regular intervals will likely show the growing or dying influences it has over its host plant (e.g. seeds at the beginning of a masting event would likely be unscathed and undamaged by seed predators but as the year progresses the amount of undamaged seeds would like decrease while damaged

seeds would increase if *C. querula* is multi-voltine). Klinken (2005) states that most studies use a single well timed survey to estimate the effects of seed predation but this may not always be suitable. This could be due to multiple reasons. Interesting trends may occur over multiple points within a season and a year and only taking one survey during this will not show all the other trends or may underestimate the final predation rate.

In this chapter, fruits will be collected every month to score for gut passage, *C. querula* and rodent predation, and to measure the decay state of the fruit wall. At the same time, possible competitors and parasitoids of *C. querula* will be inspected in fruits collected from the field and the fruits are then brought into a glass house environment for monitoring for emerging insects. *B. tawa* seeds that carry *C. querula* but there may also be competitors and/or parasitoids within fruits. The surrounding leaf litter could also potentially have infected *C. querula* eggs/larva/pupa or unharmed competitor eggs. Field trials can often end up damaged or destroyed through unforeseeable events such as storms or falling trees etc. These glass house experiments were also to be used as a backup to inspect drilling rates, germination, establishment and rodent-attacked seeds if the experiment in Chapter 3 and the transect experiments in Chapter 5 did get damaged during the course of the year.

There have been relatively few studies in New Zealand looking at the effects of a polyphagous seed predator and other factors on host seeds over the course of a year. *C. querula* are best known to utilise *B. tawa* but the extent of this seed predation is not known over a one-year time period. This study answers a number of questions about the tawa-*Cryptaspasma* system: when do the *B. tawa* seeds start to get attacked by *C. querula* after fruit fall?; when do *B. tawa* fruits start to become fleshless and does this coincide with increased drilling rates?; and do other invertebrates or rodents have an influence in the success of the *B. tawa* seeds

4.2 Methods

4.2.1 Monthly Surveys

At the three sites used in the experiments described in Chapter 3 (Nga Manu, Turitea, and Totara Reserve), monthly samples of tawa seeds were collected at a different plot each time to monitor the appearance of *C. querula* over the course of the season. Nga Manu collections started on 16/02/2011 and occurred every month on the 16th (or roughly around the 16th) from then on until 16/07/2011. Turitea collections occurred every month from 19/03/2011 until 19/07/2011. Totara Reserve collections occurred every month from 10/04/2011 until 10/07/2011.

Roughly 200 fallen tawa fruits and leaf litter were collected in four areas within Nga Manu and Totara Reserve; and two areas were collected from within Turitea. The area of collection was a 1.5m radius plot. If 200 fruits could not be collected then the radius was steadily increased until the full 200 fruits were collected. In addition, a small sample of leaf litter for the rearing trays was also collected (See PGU Experiment Section). In total 200 fruits per area, and 800 fruits per site (400 at Turitea) and 2000 fruits over all the sites (Nga-Manu, Turitea, Totara Reserve) were collected monthly. Each area was chosen in a tawa-dominant area. In the earlier months of collection overly squishy and hollow seeds were assumed to be last year's fruit fall and were ignored in the collection. Later in the year the difference between last year's fall became difficult to differentiate and thus all seeds were collected.

Roughly 100 of the fruits were set aside for the PGU tray experiments (see below section). The remaining fruits were inspected for different categories and classified into



Figure 4.2.1.1. Picture of trays in a PGU (Plant Growth Unit) greenhouse
– picture taken by Alastair Robertson

(1) fresh undamaged fruits; (2) partially damaged/rotten fruit flesh; (3) fully decomposed fruit flesh. In addition, seeds were classed as drilled or non-drilled. Other categories were Rodent chewed and passed through a bird. Rodent chewed seeds were identified by either a gnawing pattern on the flesh or by a hole in the seed far too large to be created by *Cryptaspasma* larvae (6mm or larger). Bird-cleaned seeds were easily identified in the initial months as they were completely without flesh. However, bird-cleaned seeds and uneaten seeds became steadily more difficult to distinguish as time progressed.

Some of the undrilled/undamaged seeds collected in these surveys were subsequently used for food in laboratory rearing experiments (See Chapter 2).

4.2.2 Plant Growth Unit (PGU) Setups

The leaf litter and the separated 100 fruits were placed into a seed tray covered with a mesh bag to trap flying insects and prevent other invertebrates entering the trays after they had been placed into the glasshouse (Figure 4.2.1.1). These trays were placed in an unheated glasshouse where they received regular overhead watering too. Every month the trays were checked for *Cryptaspasma* emergence and other possible seed predators or parasitoids.

4.3 Results

4.3.1 Monthly Surveys

Across all sites there was a steady increase in drilling proportions over the course of the season (Figure 4.3.2.1). By July for all sites (June for Nga Manu) 90% of all seeds were drilled. As expected stage 1 & 2 seeds disappeared fairly quickly and stage 3 became the most dominant over the rest of the months. The proportion of rodent-damaged seeds peaked in later months and a small proportion in the beginning months for Nga Manu. The number of bird-excreted seeds was higher in earlier months and not seen in later months but overall was only a small proportion of the fruit crop.

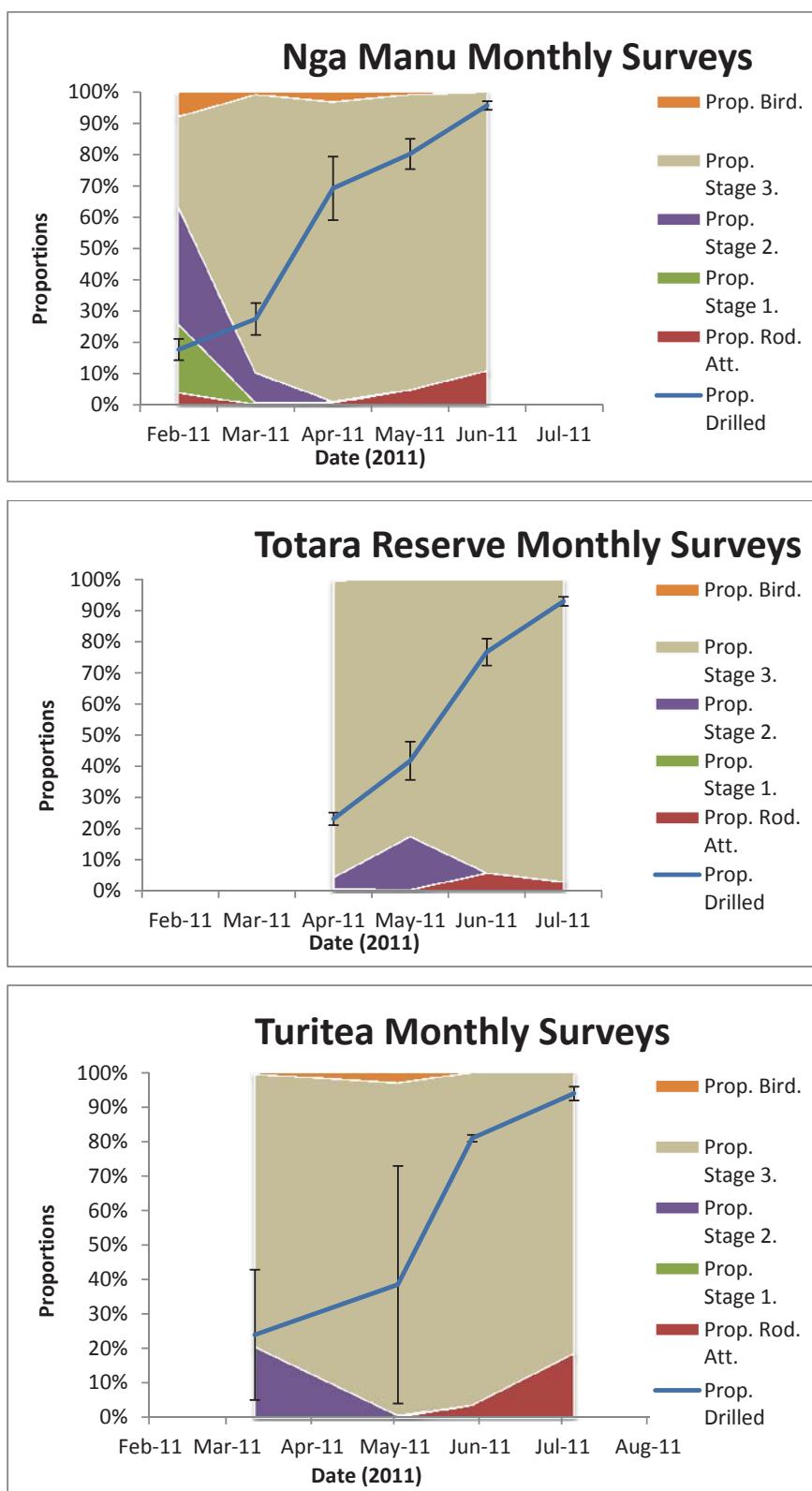


Figure 4.3.1.2. the proportions of drilled seed is superimposed on the state of pulp degradation with the rodent damaged bird cleaned fruit also noted.

4.3.2 Plant Growth Unit (PGU) Setups

The PGU trays found one possible competitor of *C. querula* which was the generalist outhouse fly (*Sylvicola sp.*). It appeared to utilise both the fruit and possibly the endosperm of the tawa seeds. Empty pupal cases and larvae were found in around 30% of the tawa fruits, surrounding leaf litter, soil, and inside the tawa seeds' husk. The outhouse fly was first seen in the Nga Manu trays (which were collected on 16/02/2011) in May, 2011. Turitea and Totara trays were seen several months later. It took roughly three months after collection before the outhouse fly were seen. Due to the fine mesh over the trays, it would be very improbable the outhouse flies managed to enter the trays after placement and therefore their eggs must have been present in the litter surrounding the tawa seeds on the forest floor. Several individuals were captured and identified to genus. After discovering their generalist nature and the improbability of them being competitors or specialist predators of *C. querula*, no further action was taken.

C. querula larvae, pupa and adults were also common within each tray showing that the environment in the PGU plant house was adequate for survival. Several small arachnids (likely being transported through the leaf litter) were also present but were not further investigated as they were likely generalist predators rather than parasitoids/competitors.

No parasitoids or any other competitors were found.

4.4 Discussion & Conclusions

4.4.1 Monthly Surveys

The trends across all three sites are relatively similar for all factors being tested/investigated. The rate at which fruits degrade was consistent across all three sites. The proportion of stage 1 fruits (undamaged, not rotting at all) declined rapidly at the Nga Manu site and were not seen at all at Totara Reserve and Turitea but the surveys started later at these sites compared with Nga Manu. The stage 2 fruits (partially damaged/rotten) were present in the early months at all three sites. They lasted about 1.5 – 2 months before degrading into stage 3 fruits (almost completely fleshless and rotten).

In all sites, the fruits decayed into stage 3 by the last few months of surveying.

The proportion of bird-cleaned seeds was unexpectedly low (A maximum of 0.08 proportion of seeds at Nga Manu passed through a bird, 0.003 at Totara Reserve, and 0.03 at Turitea). Given tawa's relatively poor dispersal ability (Dawson, 1992), it was assumed the kereru had an active role in transporting tawa seeds around (Wotton & Kelly, 2011) and cleaning flesh off them (thereby potentially stimulating germination). Given the low number of bird-cleaned seeds, this assumption was not supported.

The rodent-chewed seeds were most commonly seen in later months (with the exception of Nga Manu). This is likely due to the rodents actively hunting *C. querula* in the seed husk rather than foraging for endosperm. Jones & Toft (2006) and Horak (2006) both found *C. querula* in mice stomachs and showed that they can make up a substantial part of their diet. This supports that mice will actively hunt *C. querula* thereby making it more probable that rodent-damaged seeds will become more predominant in later months.

The proportion of drilled seeds steadily accumulated over the season, taking roughly 4 months to complete. It could have potentially continued longer meaning more than 90% of the seeds would have ended up drilled. This shows particularly high seed predation rates, which bodes poorly for Tawa's regeneration since the previous chapter showed that drilled seeds have little chance of germinating or establishing..

A thesis by West (1986) also looked into the seed predation rate by *C. querula* in Pureora, North Island, New Zealand. She observed relatively low drilling/predation rates of around 11.7% which is substantially smaller than what has been found in the three sites in these results (also look at Chapter 5 which included Pureora as a site). A possible reason for this, is that West performed her surveys early in the year (when the fruit were still falling or had recently completed falling) and therefore did not get the full predation percentage which is likely to have been substantially higher (>90%) by the end of the year/fruiting season (see Chapter 5 where I measured seed predation rates at 99% in the same sites on the 27th September, 2011). The benefit of the monthly surveying is it shows the steady increase in drilling rates over the course of the year and thus does not suffer from the underestimation of surveys early in the season such as

those of West (1986).

Predation of the seeds continues to occur over the course of four months, which implies there must be a reason behind the extended period of predation. There are three possibilities: Extended periods of egg hatching, slow development of some larvae, or multiple generations of *C. querula* within a season.

Extended periods of egg hatching refers to larva emerging from their egg clusters at different times – sometimes the difference being weeks or in this case potentially months. Rearing observations in Chapter 2 showed that at most, eggs within the same clutch, could only hatch two days apart. Different clutches of eggs could take much longer to hatch which could suggest a genetic difference on an individual scale (or that *C. querula* are multiple sub-species).

Slow development of larvae refers to larva progressing and reaching pupation at different rates despite potentially hatching from the egg case at the same time. This may be due to different resource availability (one larva finds a resource-rich seed while another finds a much poorer one), or through differing resource use. Another possibility is that different egg clutches may hatch at the same time though genetically they are different (i.e. different sub-species) meaning one may be faster at developing than another. This was seen in rearing observations in Chapter 2 where *C. querula* larvae that were newly hatched were placed in terrariums with choice or monoculture food sources. For both experiments the difference in larva reaching pupation varied wildly, sometimes reaching two or three weeks.

The explanation considered most likely to explain the accumulating seed predation seen in *B. tawa* would be multiple generations (multi-voltine) of *C. querula* within a season. Dugdale (pers. comm.) believes this is the case for *C. querula*. Rearing observations in Chapter 2 have shown that there are multiple generations of *C. querula* within 4 months in a room at 14 Degrees Celsius. Multi-voltinism would presumably be beneficial to *C. querula*, especially when food is plentiful (heavy masting years).

It is unknown whether *C. querula* undergo diapause when their main resources (tawa seeds) are no longer in season or rather they undergo multiple generations within the

year potentially using multiple hosts or sufficing on the numerous tawa seeds (though this would only work on masting years). It is likely *C. querula* go through multiple generations in one year in a natural environment given they were seen to do some in controlled environments. Given they are polyphagous (Sullivan, 1995) it is possible they will utilize the resources of other species' seeds if tawa seeds become scarce.

There can be some quite profound effects of seed predators in tropical settings and while New Zealand is temperate, *C. querula* may act more like a tropical seed predator thereby having similar influences tropical seed predators have on plant communities. Lewis & Gripenberg (2008) suggested that seed predation in tropical forests can help maintain diversity in plant assemblages. *C. querula* may reduce tawa regeneration thereby giving other species a chance to survive there. They are also a likely contributor to Janzen-Connell patterns (Connell, 1971; & Janzen, 1970; & Janzen, 1971) where the likeliest chance of seed survival is away from the parental canopy.

The presence of bird-eaten seeds declined with the stage 1 & stage 2 seeds, which is understandable as birds such as the kereru eat the seeds for the outer flesh not the endosperm (Wotton, 2007). However, even early in the season before fruits are losing flesh, the number of bird transported seeds overall was low. The results showing a low proportion of ingested seeds could be due to one factor. The seeds were only considered ingested by a bird if they were completely devoid of outer flesh. However Steve Trewick, Massey University (pers. comm.) suggested some partially digested seeds maybe regurgitated resulting in some seeds still having some flesh attached. If this is the case, the above results may underrepresent the amount of seeds that are ingested by a bird.

If the number of bird-digested seeds is accurate in the above results, this poses potentially negative consequences for tawa regeneration as uncleaned seed generally have a lower establishment success than cleaned seed (Chapter 4) and if germination does not happen quickly over 90% of the seeds end up being eventually drilled. If the percentage of seeds that escapes through bird dispersal is low, then tawa will have a hard time regenerating and dispersing. The highest percentage of seeds to pass through a bird was just below 10% on the first monthly survey at Nga Manu. This is a very low number of dispersed seeds. The kereru is the only known avian disperser of tawa (West,

1995) and this may indicate problems with kereru populations or other preferred seed-species. Kelly et al. (2010) showed that all but the smallest fruits of the three largest fruited species (Taraire, Karaka and Tawa) completely rely on kereru for effective seed. With this disperser removed, dispersal and recruitment will decline and would likely be even lower when coupled with the damaging effects of seed predators (Wotton & Kelly 2011).

Rodent-damaged seeds (presence of holes larger than 6mm or in early months the presence of gnawed fruit flesh) were predominantly seen in later months of the survey. This is an interesting finding as it is likely at this time that *C. querula* are present within many of the seeds. The rodent damage seems to first appear when about half the seeds have been drilled and increase once 75% or more of the seeds are infected (or have been infected) by *C. querula*. This supports suggestions that rodents may specifically target tawa seeds for their seed predators rather than the seeds' endosperms (Jones & Toft, 2006; and Horak, 2006).

C. querula clearly have a high impact on the number of seeds attacked through seed predation. However it is entirely possible that some seedling are still able to survive even after some of the endosperm has been removed by larva (see chapter 2). If a proportion of damaged seeds are still able to germinate and establish, the high incidence of drilling (90%) may not be as detrimental to tawa regeneration as it first appears. Nevertheless, the high proportion of drilling will still work against tawa as many seeds will not be able to establish if the actual seedling or embryo is damaged or if too much endosperm has been removed (or the drilling hole allows access to pathogens). As *C. querula* acts like a tropical seed predator in that it likely has multiple generations in a year or season, it will play a part in shaping forest communities and structure (Lewis & Gripenberg, 2008).

Further research should focus on the number of tawa seeds that survive until the seedling stage has been reached, taking particular notice as to whether drilled or rodent damaged seeds are disadvantaged in any way. This could show how much *C. querula* actually reduces tawa regeneration.

4.4.2 Plant Growth Unit (PGU) Setups

The complete lack of parasitoids found in the PGU experiments suggests that *C. querula* are not commonly attacked by parasitoids (and indeed no one has previously reported finding a specialist parasitoid of *C. querula*). This is supported by a complete lack of common parasitoids orders (diptera, hymenoptera etc.) observed over all experiments and surveys conducted for this thesis. Other species in the *Cryptaspasma* genus have suffered from one known endoparasitoid which is the *Pseudophanerotoma* sp. which belongs to Hymenoptera: Braconidae: Cheloninae (Hoddle, n.d.; & Hoddle & Hoddle, 2008). Further testing may be required to find out if generalist parasitoids target *C. querula* but the overall likelihood of specialist parasitoids affecting *C. querula* populations is low.

Competition for tawa seeds is unlikely to be happening between *C. querula* and *Sylvicola* sp. as both are generalists in their own way. While *C. querula* young are bound to seeds for nutrition (obligate), they can utilise multiple species' (polyphagous) seeds (which was found in Chapter 5). The *Sylvicola* sp. takes it one step further and appears to utilise many different kinds of resources (Clunie, 2004) as long as it is decaying vegetation (leaves, roots, seeds etc.). For two generalist species it is likely there is no competition over resources as both can easily switch to other forms of nutrition when the need arises.

Chapter 5

High seed predator damage found in *Beilschmiedia tawa* across the North Island of New Zealand in a multi-area survey

5.1 *Introduction*

Ecological processes differ across varying spatial and temporal scales (Rastetter et al. 2003). Although short-term, small plot studies may adequately describe the processes occurring in one particular environment but they do not sufficiently identify processes occurring on a larger scale. Seed predation is a highly structured pattern that occurs over a wide range of temporal and spatial scales (Janzen, 1971). However, the number of seed predation studies looking at different spatial scales is relatively low especially for invertebrate seed predation (Lewis & Gripenberg, 2008). Van Klinken, & Flack (2008) found relatively low seed predation rates in their continental-scale surveys. Within their survey, predation rates varied regionally apparently for several reasons (egg parasitism, larval/pupal mortality etc.). Another study by Villacide & Corley (2008) showed pre-dispersal seed predation varied depending on the spatial composition of canopy species. Mixed stands suffered higher seed predation rates than pure stands.

The results from Chapter 4 showed that over the course of the first few months of the season at three sites more than 90% of the tawa seeds suffered drilling damage. This amount was much higher than expected and it ultimately suggests that nearly all tawa seeds will be eventually drilled. Given this result, it was important to see whether this degree of predation occurs on a greater scale encompassing multiple regions within New Zealand. These locations in New Zealand often vary greatly in their microclimate. Some areas have higher rainfall, and vary in their average temperature (altitudinal, latitudinal differences or heat islands caused by nearby urban settlements). These differences in abiotic factors can have strong influences in biotic ecosystems and communities (Loreau et al. 2001). Due to anthropogenic effects and to natural patterns within the landscape, many of New Zealand's forests are also highly fragmented (Ewers, 2004). Lewis & Gripenberg (2008) have suggested that the effects of invertebrate seed predation in these artificially altered environments (such as New

Zealand's highly fragmented systems) may be under estimated due to the low amount of studies. This section will look at seed predation, germination and rodent damage in these differing areas. Coupled with abiotic factors, specific areas may have varying biological life as a part of their ecosystems. Some areas may have more rodents present than another site which in turn may influence seed predator populations. Tilman et al. (1997) looked at the influences of diversity and composition on ecosystem function. In particular they looked at different species of plants but the presence of mobile animals likely has similar effects. This will be examined further but in context to seed predation. There have been relatively few studies looking at the effect of abiotic factors, different sites, combined with predator influences on seed predation and their damage to their hosts. In this chapter, tawa seed predation rates were measured at a total of eight sites were investigated in the North Island of New Zealand each with likely differing microclimates and forest composition and animal assemblages.

In this section we are not investigating microclimates themselves but rather getting a collection of data on seed predation and their hosts at multiple sites where microclimates might be causing different results. Increasing the sample size (i.e. number of locations/sites investigated) will hopefully show a clearer picture of the consistency of *C. querula*'s seed predation of *B. tawa*. It would also be interesting to see if there will be significantly different results among and within the sites. Factors being investigated are drilling rates, rodent attacks on seeds, germination rates and successful establishment of seedlings. Holl (1999) found that microclimate can have an effect on seed germination. Another study by Simkin et al. (2005), however, found that mound microclimate did not influence factors like seedling survival or germination.

Each site will likely have similarities and differences in the composition of the plant species present. The more *B. tawa* dominant areas will likely have more *C. querula* individuals present as there should be more food sources around to be utilised (more seeds from the masting event) unless the seed fall was negatively influenced by microclimate. This could possibly lead to higher drilling rates (unless predation satiation applies) which in turn could increase the amount of rodents (which possibly utilise the *C. querula* and *B. tawa* seed endosperm as resources). However more species-rich environment may not necessary have less *C. querula* as the seed predator is apparently polyphagous (Sullivan et al. 1995) though Chapter 2 suggests they may not be polyphagous (only the *Beilschmiedia* genus was actively used). As long as the plant

species' seeds are capable of being infected and utilised then there may be equal if not more *C. querula* individuals present.

The purpose of this chapter is to see whether the seed predation rates of tawa were as high all over the North Island of New Zealand as the results shown in Chapter 3 of this thesis. It was also of interest to see whether the canopy species composition of these surveyed sites will play a part in the intensity of seed predation.

5.2 Methods

Sites were researched and chosen based on whether they likely had tawa or not. DOC sites and personal sources were used primarily to identify these sites. Overall, seven sites were chosen: Tararua Forest Park, Pureora Forest Park, Mohi Bush Scenic Reserve, Kitchener Park, Horse Shoe Bend Recreational Reserve, Mount Egmont National Park, Ruahine Forest Park, Manawatu Gorge. At some of the larger sites multiple replicate surveys were conducted to better represent the range of landforms and vegetation types (Table 5.2.1). The field collections were done over the course of 11/10/2011 – 23/10/2011 in the season when the drilling rates of the seed predations on *B. tawa* had peaked rather than over the course of the year as had been done in chapter 4 which looked at seed predation over the period February to July).

Table 5.2.1. Sites and sub-sites for the transect surveys. The total number of tawa seeds at each site/sub-site was used as a threshold for analysis. The number of tree species within 10 metres (5 metres either side of the transect line) is also shown.

Site	Sub-Sites	Latitude	Longitude	Total	No. of tree
				Seeds	species
Pureora	1	S38° 27.368'	E175° 32.057'	204	4
	2	S38° 27.134'	E175° 32.805'	295	3
	3	S38° 24.222'	E175° 32.293'	121	3
Ruahines	1	S39° 57.936'	E176° 17.006'	124	5
	2	S39° 55.898'	E176° 17.989'	165	6
	3	S40° 11.099'	E175° 52.178'	255	5
Egmont	1	S39° 19.437'	E174° 06.205'	5	4
	3	S39° 14.200'	E174° 06.418'	29	5
	1	S40° 14.799'	E175° 32.282'	209	4
Manawatu Gorge	1	S40° 20.347'	E175° 49.073'	244	5
Mohi Bush	1	S39° 51.393'	E176° 53.706'	403	5
Horseshoe Bend	1	S40° 29.351'	E175° 31.608'	251	3
Tararuas	1	S40° 52.357'	E175° 13.820'	106	5

At each of these sites, a 50m transect was laid out in an area within the forest that had at least some tawa canopy. A 0.5m band each side of the transect was surveyed and all tawa seeds within it were collected. If the transect bisected a swampy or man-made area (i.e. gravel path etc) then seeds were not collected from that part of the transect. Seeds were collected along the 50m transect until either the collector reached the end of the 50m or roughly 400 seeds were collected. It was felt that a minimum of 100 seeds were needed at each laid out transect to give representative data. If roughly 100 seeds were not collected at the transect then another transect was laid out somewhere else within the same main site, repeating as many times as necessary to get to at least 100 seeds. If 100 seeds were not reached after multiple transects, the location was considered a poor representative of tawa stands and was not included in further analysis.

Within 5m either side of the transect line, all canopy trees (except tree ferns) with a diameter more than approximately 25cm were counted to indicate which were the dominant species on each transect.

Once the tawa seeds were collected they were returned to the lab where they were examined for drilled holes (HS), rodent-chewed holes (RC), and for germination (G).

5.3 Results

The percentage of canopy tree species showed that at all sites Tawa was the dominant canopy tree (indicated by the underlined percentage) with the sole exception of Horseshoe Bend where Mahoe was dominant (Table 5.3.1). It appears as though only Tawa and Mahoe were highly prevalent in all the areas surveyed.

Table 5.3.1. Percent composition showing the relative dominance of canopy tree species at all surveyed sites. The most dominant species at each site is underlined and species with more than 20% frequency are shown in bold.

	Tawa	Rimu	Mahoe	Matai	Totara	Kahikatea	Lemonwood	Red beech	Kamahi	Titoki	Lancewood	5 Finger
Pureora	81.8	6.1	6.1	3.0					3.0			
Ruahines	46.1		11.8		7.9	18.4	5.3	1.3	2.6	6.6		
Kitchener Park	66.7	14.3	9.5		9.5							
Manawatu Gorge	55.2		10.3		13.8					17.2	3.4	
Mohi Bush	60.0		15.0				5.0			15.0		5.0
Horseshoe Bend	26.3		68.4						5.3			
Tararuas	33.3	16.7	25.0		16.7				8.3			

The drilling damage showed similar results to Chapter 4 as there was a high proportion (over 90%) of drilled seeds most sites (Kitchener Park and the Ruahines were somewhat lower). Drilled seeds and rodent damaged seeds share a similar trend to that discussed in the previous chapter where when drilling proportions are high, rodent damaged seeds increase as well (Figure 5.3.1). Germinated seeds show an almost identical inverse pattern where increased drilling or rodent damage coincides with less germination.

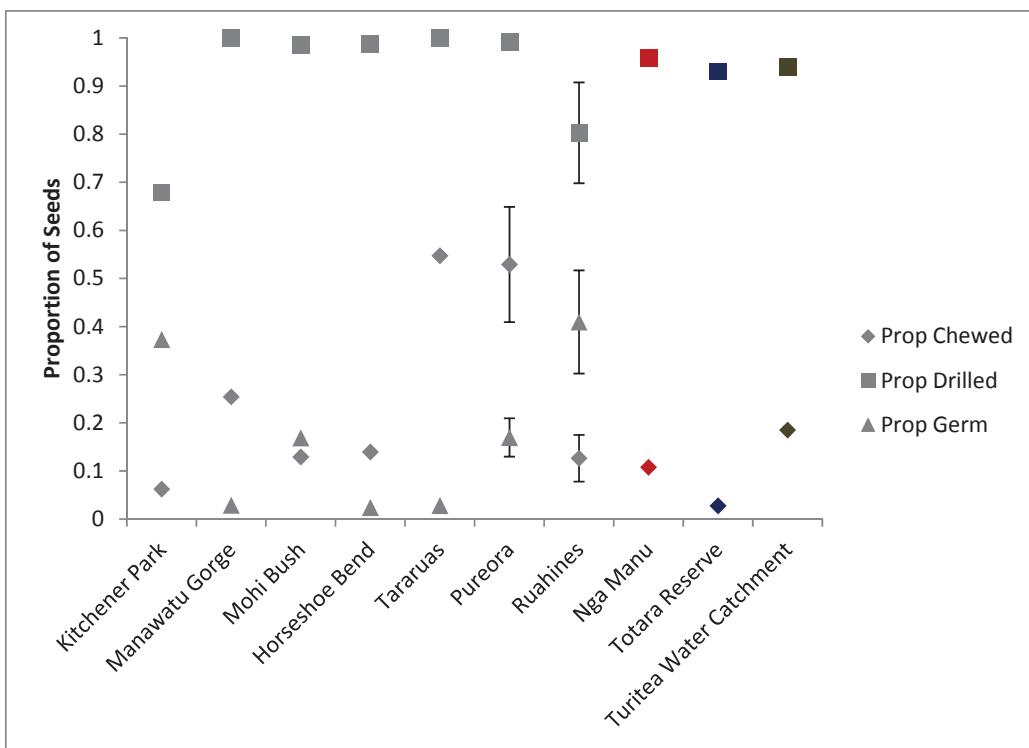


Figure 5.3.1. The seeds that were rodent damaged (chewed), drilled by invertebrates, or whether they germinated at each of the sites where the transect surveys were done. Error bars are added to sites where there were multiple surveys within one site. The data on seed fates from the monthly surveys at the three sites from Chapter 4 are also shown for comparison.

The non-drilled seeds had a higher germination rate than drilled seeds at five of the seven sites (Figure 5.3.2). In many sites, if a seed was drilled it had less than half the chance of germinating than a non-drilled seed. Table 5.3.2 shows that drilling damage has a significant effect as to whether the seeds will germinate. The sites also appeared to vary in their germination rates. However the interaction between drilling damage at the various sites did not indicate significant site-level differences in the chance of germination following drilling.

Table 5.3.2. Binomial One-Way ANOVA Table showing the significant results (bolded) of germinated seeds for the two variables, Site and Drilled Seeds (and the interaction between them).

	Df	Deviance	Residual Df	Residual Deviance	P
Null	18	346.75			
Site	6	228.624	12	118.13	<0.001
Drilled	1	54.541	11	63.59	<0.001
Site:Drilled	4	3.860	7	59.73	0.4253

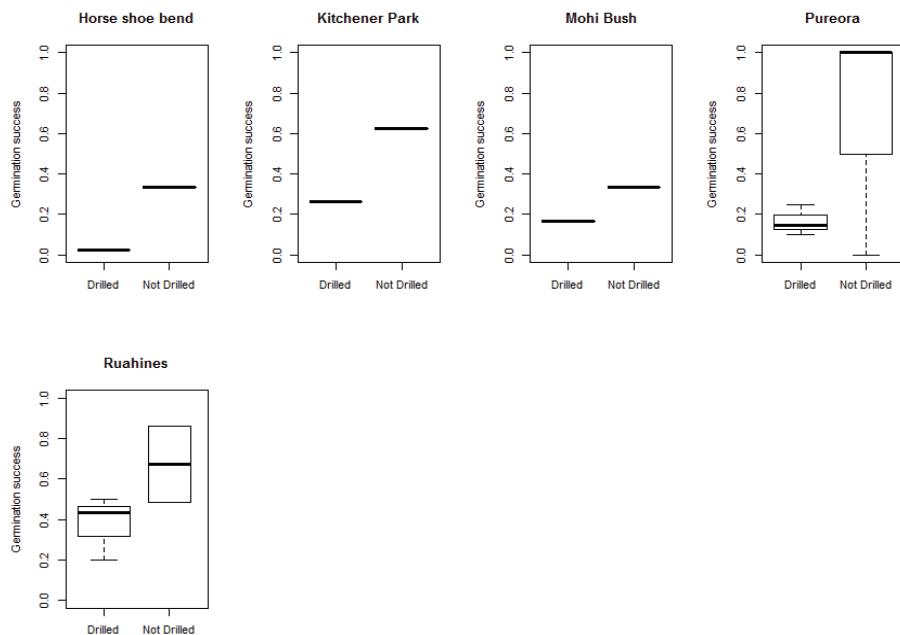


Figure 5.3.2. Boxplots showing the proportion of germination success for drilled and non-drilled tawa seeds at five different sites. Two sites: Tararuas and the Manawatu gorge were not included as they had all drilled seeds (no non-drilled seeds).

Five out of the seven sites also showed non-chewed seeds to have higher germination than chewed seeds (Figure 5.3.3.) but the effects of rodent chewing appears to be much less harmful than *Cryptaspasma* drilling. The two sites (Pureora and Mohi Bush) showed the opposite result although the difference in germination was very small. Table 4.3.3 shows that similarly to the drilling influences above, that both site and rodent damage by themselves influence germination in tawa seeds (though not the interaction between site and rodent damage).

Table 5.3.3. One-Way ANOVA Table showing the significant results (bolded) of germinated seeds for the two variables, Site and Rodent damaged/chewed (Rodent) (and the interaction between them).

	Df	Deviance	Residual Df	Residual Dev.	P
Null	21	368.120			
Site	6	280.902	15	87.22	<0.001
Rodent	1	4.427	14	82.79	0.0354
Site: Rodent	6	5.416	8	77.38	0.4917

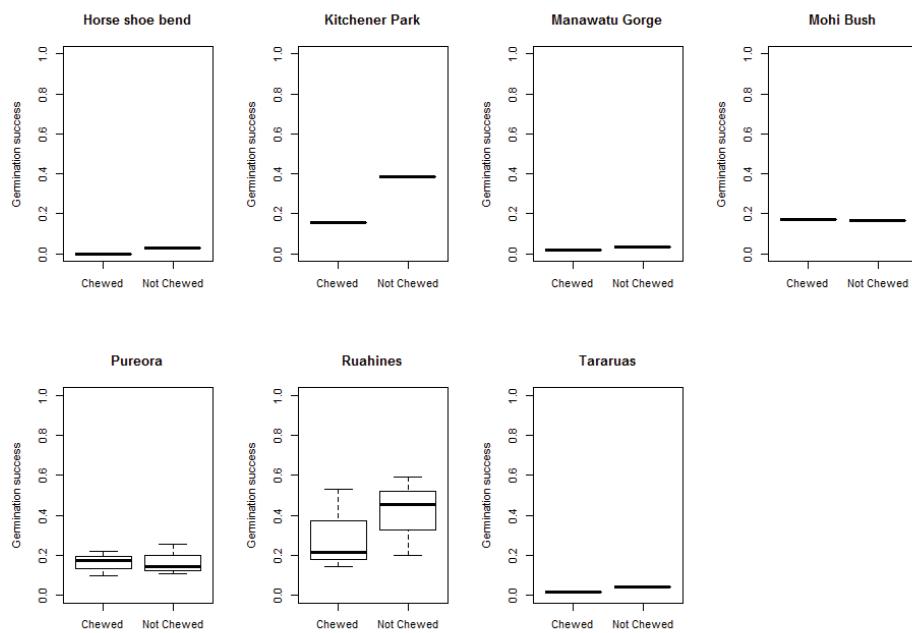


Figure 5.3.3. Boxplots showing the proportion of germination success for rodent chewed and non-rodent damaged tawa seeds at seven different sites.

5.4 Discussion & Conclusions

The eight sites surveyed here in late winter showed similar results to those shown in the Monthly Survey Chapter (Chapter 4) where most sites reached over 90% in drilling proportions of seeds by the spring. Only two sites were different: The three sites in the Ruahines, and Kitchener Park. This is an extremely high number of seeds that do not escape drilling under tawa (partial or dominant) canopy which potentially signifies future consequences for tawa regeneration all over the North Island of New Zealand.

The year these surveys were taken was a masting year for tawa where the fruit fall appeared to be much higher than that of a non-masting year. It is proposed that plants could undergo masting to help their seeds escape seed predation through the predator satiation hypothesis (Salisbury 1942; Janzen 1971, 1976; Silvertown 1980; Kelly 1994). However, the results shown here (and in Chapter 4) show that most sites eventually suffer over 90% drilling damage. Nevertheless, chapter 2 shows despite this high proportion of drilling damage, having a locally dense patch of seeds offers protection and helps germination. The high proportion of drilling damage would be less consequential if many drilled seeds were still able to germinate, however the results show that most drilled seeds are killed before they germinate.

The results, here show a similar result to a Brazilian study (da Silva et al. 2012) showing the predation of seeds of the palm *Syagrus romanzoffiana* where they hypothesized that the rates of seed predation by *Curculionidae Revena rubiginosa Boheman*, *Anchylorhynchus aegrotus Fahraeus*, and *Anchylorhynchus variabilis Gyllenhal* are smaller in years of higher fruit production. However they found that predation rate did not relate to the number of seeds produced per reproductive plant and roughly 60% of the seeds escaped predation whether the fruits were heavy or lightly produced.

A thesis by West (1986) looked at the seed predation rates of *C. querula* at Pureora (one of the sites surveyed here) and found considerably less (11.7% drilled seeds) than what the value was here (99% across three Pureora Sites). The likely reason for the large difference was that West surveyed the seeds near the beginning of the fruiting season while the survey in this section was after winter when seeds had had more time to be colonised. The results in Chapter 4 show the gradual increase in drilling damage

proportions over the course of the season. It is likely West's thesis represents a data point early in the fruiting season rather than at the end. Therefore the 11.7% result doesn't show the final proportion of drilled seeds as *C. querula* haven't had time to drill into them.

Interestingly, it would appear as though there is little evidence of a trophic cascade (plant – herbivore – predator. i.e. Tawa – *Cryptaspasma* - Rodents) occurring in this tawa-*Cryptaspasma* relationship. There is also no evidence of parasitoids (See chapter 4). Rodents show a very similar pattern to the drilling proportions of seeds, however they likely do not relieve tawa from predation pressure (especially for this year) as they potentially do more damage to the seed in the attempt to remove larva inside. They also do not seem to be able to stop or reduce *C. querula* populations before they infect the seeds (except perhaps in the year prior); therefore it is hard to infer any benefits tawa gets from this interaction.

Future studies should focus on whether there are differences in survival in tawa on a mast year and a non-mast year and whether the seeds are able to reach establishment (not just germination). This should be done in similar sites to this section and more to give a broad picture of tawa's potential to regenerate when it has extremely high seed predation pressures, which actually reduce the seeds' chances of germinating, along with rodent damage and potentially poor vectors of dispersal (the kereru - see Chapters 3 and 4).

6. Overall Discussion and Conclusions

The polyphagous, invertebrate seed predator *Cryptaspasma querula* has been shown to impact *Beilschmiedia tawa*'s regeneration and overall fecundity (even in a masting year). By drilling into the seeds *C. querula* create three negative consequences for that seed's chances of establishing. The larvae may eat through the seedling inside thereby rendering the whole seed unviable, or the larva may devour so much of the endosperm inside that the germinating seedling cannot get enough nutrition to establish. The final consequence of drilling is that by creating a hole in the outer husk, pathogens or generalist parasites may be able to enter more freely. Another possible detrimental result of drilling is that rodents appear to actively hunt larva in tawa seeds. Rodents are much more destructive than *C. querula* and while the larva inside may not have killed the seedling or eaten enough of the endosperm to be detrimental; the damage of a rodent may be enough to prevent establishment (40% higher chance of germinating if not drilled; 70% higher chance of establishing if not drilled).

Seeds that are subject to drilling or rodent damage may still be able to establish as there have been some seeds that still germinated and established despite the presence of *C. querula* holes and rodents' larger holes. It is unclear however whether the seedlings managed to establish prior to the damage as this would no longer be detrimental. However, if the damage occurred before germination and the seed still managed to establish, this may show some evolutionary resistance to seed predation (e.g. packaging seeds with more endosperm than what is required). A study by Shiels & Drake (2011) showed that large-seeded species in Hawaii were mostly destroyed when targeted by rodents but there was a very small amount (partially damaged seeds) that managed to survive. Future studies should include a controlled environment where non-germinated tawa seeds are subject to drilling and rodent damage to see the success proportion of establishment after the damage has occurred.

Facets of the Janzen-Connell hypothesis can be seen in the *C. querula-B. tawa* relationship where germination and establishment are more likely in seeds that have been dispersed away from the parental tawa canopy. Many studies support this finding (Ruiz et al. 2010; Visser et al. 2011). This, however, does not correlate exactly with the

drilling rates. There were mixed results where tawa were drilled more if they were dispersed in some sites while in other sites drilling proportions were higher in seeds under parental canopies. This inconsistency paired with the germination results show that while in certain sites (away or under) seeds may be drilled more, this may not mean they are unable to germinate and establish. Overall seeds will have a higher chance of success if they are dispersed away from their parental canopies. This is in-sync with the Janzen-Connell hypothesis though only contributes to a facet of it (Janzen, 1970 & Connell, 1971). This further illustrates the importance of New Zealand's only natural large-seed disperser, the kereru (Wotton & Kelly, 2011). Density appeared to be a more consistent factor in depicting whether a seed would germinate or not. A high density of tawa seed resulted in higher germination proportions than low densities of tawa which suggests that predator satiation may play a role in the *C. querula* – *B. tawa* relationship.

New Zealand is a temperate landscape rather than tropical. The Janzen-Connell hypothesis is primarily used to predict or explain patterns in tropical environments. However, the results seen in this thesis show that the J-C effects are seen in *B. tawa* forests. There have been several other articles (Lambers et al. 2002; Pigot & Leather, 2008; Yamazaki et al. 2009; & Wang et al. 2010) that also report similar J-C effects in their temperate experiments/studies. Further studies of forests with tawa present should include all components of the J-C hypothesis (pathogens, predation, dispersal etc) to see whether this model/hypothesis is also capable of predicting/explaining patterns in temperate environments (perhaps in the presence of certain conditions i.e. polyphagous invertebrate seed predators).

Apparent predator satiation was seen in several sections of this thesis but also negated by other sections. Seeds that were clumped into large congregations (50 seeds) stood a better chance of germinating (2% better germination in high density) and establishing (23% better establishment in high density) than seeds in smaller groups (10 seeds) and were also more likely to escape drilling from *C. querula* (Chapter 2). This is interesting as monthly surveys (Chapter 3) of the similar sites (to Chapter 2) and one-off transect surveys (Chapter 4) of different sites showed that seeds in most areas eventually reach over 90% drilling damage. As predator satiation (Silvertown, 1980) is a possible explanation for masting, it appears this evolutionary trait in masting is not working as many of the seeds end up drilled anyway. However if the seeds manage to establish and

create its own resources from sunlight before they are drilled then any later damage to the seed becomes irrelevant. Predator satiation, in this case, may allow a proportion of seeds to escape predation until they establish, in which any later drilling does not harm them. If a seedling has successfully established by the time their seed is drilled, the detrimental effects may be minimal as the individual plant may no longer require the endosperm to survive.

A possible explanation for the high number of drilled seeds is that larvae may ‘migrate’ from seed to seed. There would be many benefits of this in the wild. An individual *C. querula* larva may run out of food in its current seed (i.e. all endosperm removed) or living conditions may no longer be ideal in the current seed thereby causing them to move out and find another seed. A *C. querula* larva may inspect a seed by drilling into it and check its suitability. This could also explain the high proportion of drilled seeds seen in Chapter 3 & 4. While it is possible that some tawa seeds may germinate and establish even after drilling damage, their chances of survival will be reduced. The consequence of this is that multiple seeds may be utilized by only one larva. Results from Chapter 2 clarify this, where only 10 larva were placed in a terrarium where 20 tawa seeds were placed. More than 10 seeds were drilled thereby representing movement by *C. querula* larvae. If this is predominant in nature then the consequences could be serious for tawa regeneration and fecundity. Even if larvae do not eat a lot of the resources inside before moving, it still creates an access point (the drilled hole) for pathogens and generalist seed predators.

Several authors (Samuels & Levey, 2005; & Robertson et al. 2006) have commented that the retention of flesh outside the seed may inhibit germination. This is potentially due to inhibitors within the flesh wall. If this is the case, it may be particularly detrimental to tawa, as discussed above, tawa seeds that are delayed in any way are likely to be drilled and/or suffer rodent damage before they have a chance to germinate, severely reducing the likelihood of establishing. The results in this paper support this idea as all seeds cleaned of flesh had a higher chance of germinating and establishing while it was riskier to have flesh left on. This also signifies the potential importance of the kereru, the only large-seed disperser in New Zealand (Wotton & Kelly, 2011). When the kereru ingests the seed, it removes (or partially removes (Trewick, pers. comm.)) the

outer layer of flesh thereby increasing its chances of (and speeding up) germination when they are dispersed.

Other than rodent predation, *C. querula* does not suffer from any specific/specialised predators or parasitoids that could be found during experiments in this thesis. Another species, *Sylvicola sp.* were also seen utilizing the endosperm of tawa seeds however according to Clunie (2004), they are generalist plant feeders and are likely more opportunistic feeders than a true competition for *C. querula*. Both species are generalist in their own ways (*C. querula* is polyphagous and *Sylvicola sp.* can rely on any dead plant material) and therefore are unlikely to present a huge competitive advantage over one another. The presence of *Sylvicola sp.* in tawa seeds is likely the result of easy access through *C. querula* drilled holes.

Dispersal of tawa seed appears to be one of the few ways that tawa can maximise its seeds' chances of survival and establishment. It is, therefore, important that tawa have agents of dispersal during its fruiting season. The only known natural avian disperser of tawa is the Kereru (*Hemiphaga novaeseelandiae*) which can utilise and disperse most of New Zealand's large-seeded species (Wotton & Kelly, 2012). The problem however appears that the number of bird-dispersed seed in experiments/surveys in this thesis, was very low. The only time dispersed seeds were seen were during the initial fruiting phase (which is understandable as the birds eat the fruit not the endosperm (Wotton, 2007)). This low figure may be under-represented however as it became increasingly difficult (as time went on) to decipher the difference between a seed that passed through a bird, and a seed that had decayed so much that it had lost its flesh. Trewick (pers. comm.) also commented that he observed some bird-passed seeds (i.e. ingested and excreted from a bird or ingested and regurgitated) with some flesh still remaining on the seed (partially fleshed). If this was the case, these seeds would have been considered rodent-attacked (thereby also over-representing rodent prevalence in early sampling). Kereru populations are slowly declining which may also explain the reduced number of cleaned seeds. As they are the only natural disperser of tawa, the reduction of their populations will leave a severe lack of suitable dispersers for tawa thereby reducing their chances of regeneration (50% of tawa seeds underneath parent canopies have passed through a bird according to a study by Kelly et al. 2010).

Many locations around the North Island of New Zealand with *B. tawa* present (dominant or otherwise) show similar trends in drilling rates (except for two sites). Rodent damage and germination proportions appear to vary from site to site though with the same germination-damage relationship (i.e. higher proportion of seeds damaged by rodents means less germination in seeds and vice versa). The different sites had different species composition where some were tawa-dominant while others merely had tawa present. A patchy composition of tawa trees should allow Janzen-Connell conditions to occur and result in less predation than pure tawa stands. The number of sites, in this case, wasn't enough to see whether this pattern was occurring. A study of seed survival of central European old-growth mixed-species forests by Nopp-Mayr et al (2012) showed that there are varying levels of seed predation in different forest types. In this thesis however, drilling proportions were fairly consistent across sites (thereby refuting this article) though the experiment in Chapter 1 supported Nopp-Mayr's study. Two other studies by Tuomisto et al (2003) which looked at the distribution of plant species and the species compositions of different amazon sites; and Stevens et al (2012) which looked at the impact of post-dispersal seed predation on plant populations in degraded and intact Florida habitats, both illustrate the difference site heterogeneity have on the species living there and the impact of seed predation and similar factors. This could potentially explain the difference of rodent damage seen at each site. It is also important to note that the transect surveys occurred later in the year (long after all tawa fruit had fallen) thereby giving seed predators ample time to drill into most tawa seeds around (hence the high proportion of drilled seeds).

The two species: Miro (*Prumnopitys ferruginea*), and Hinau (*Elaeocarpus dentatus*) do not appear to be suitable host-species for *C. querula* as during the experiments in Chapter 5 they did not drill Miro and only lightly drilled Hinau (no pupae or adults emerged from these species). However it is clear that Acorn (*Quercus sp.*) and Taraire (*Beilschmiedia tarairi*) species are potential hosts as many signs of drilling and occupation were seen (frass, pupal cases, removed endosperm etc). Pupa and adults were reared from Taraire confirming that it would make a viable host for *C. querula*. Naturally however it is most commonly found in the upper North Island of New Zealand and occasionally scattered throughout lower areas of the North Island (NZ Plant Conservation Network, 2010).

The field experiments in Chapter 5 showed acorns to be a possible host-species for *C. querula* as many drilling holes were evident as well as the presence nearby empty pupa cases. Dugdale (Per. Comm..) believes *C. querula* readily use *Quercus* species as hosts. He found many adult *C. querula* present in an oak dominated canopy where few/no tawa were present. Through these observations it is reasonable to assume *C. querula* can utilise acorns as well as tawa as resources. This creates further problems for tawa where masting in the past, could possibly have been used to reduce *C. querula*'s carrying capacity, now has become redundant as during periods of low tawa seed fall, *C. querula* can 'swap' to acorns, thus maintaining a high population size.

An inference could be made about potential problems with tawa regeneration due to a combination of factors shown from multiple chapters in this thesis. Tawa has become much more dominant in systems since human colonisation as competitive emergent trees were often removed by loggers (Esler, 1978). Given tawa's sudden dominance in many areas they may not have developed evolutionary defences from certain seed predators. *C. querula* is a polyphagous seed predator meaning it may have more readily utilised other species until tawa's sudden dominance, which may have encouraged a form of specialised prey-switching. It is known tawa undergoes masting which may be an evolutionary adaptation to seed predators however given the introduction of exotic species such as oaks, *C. querula* may be able to maintain its carrying capacity during tawa's low mast years by utilising these species. Coupled with these observations, the only natural known disperser of tawa is the kereru which is slowly going into decline. Furthermore a study looking into residual forest fragments by Burns et al (2011) has shown very little tawa regeneration despite animal control. Due to these reasons, it is possible that future tawa regeneration may be problematic.

The wind/olfactory experiments tested in chapter 2 showed that within the allocated times given, *C. querula* were not attracted to the smell of tawa fruits at any stage of their lifecycle (larva, adult). Given this result, it would seem adult *C. querula* lay their eggs at random and the emerging larvae must find tawa seeds through some other method.

Further research into *C. querula* could include DNA based work where *C. querula* adults from different locations in the North Island are compared to see whether this is

just one species or many closely related sub-species. If *C. querula* is actually multiple species this could have very different impact on tawa regeneration (i.e. some may only be able to utilise tawa, while others could utilise both etc). Further study into *C. querula* could prove to be beneficial to the understanding of *C. querula*'s relationship with *B. tawa*.

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