Systematics of *Eucolaspis* (Coleoptera: Chrysomelidae) in New Zealand and ecology of Hawke's Bay lineage

A thesis presented in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

in

Ecology

at Massey University, Manawatu,

New Zealand

Prasad R.C. Doddala

2012

Abstract

Eucolaspis Sharp 1886 includes a group of native leaf beetle species, one or more of which infest exotic fruit crops. Economic losses suffered by organic apple orchards in Hawke's Bay prompt a revisit to ecological basics of the beetle. Taxonomic, behavioural and ecological knowledge gaps are addressed in the current research project. Phylogenetic analysis, based on cytochrome oxidase subunit 1 region of mitochondrial DNA, revealed that only one genetic lineage infests apples in Hawke's Bay and that there are only three putative species in mainland New Zealand with another separate species on Three Kings Islands. These findings are well supported by differences in male genitalia shape. Morphometric analyses also supported the phylogeny to some extent.

The current findings on host location show that *Eucolaspis* sp. "Hawke's Bay" beetles use plant odours to detect and discriminate host and non-host plants. The beetles were attracted to fresh leaf / fruit odour of apple and blackberry, but not to either clover or broad-leaved dock. The beetles were not able to distinguish between damaged and undamaged host plants and between closely related species of host plants just by olfaction. Irrespective of the geographical origin and ancestral host plant, beetles preferred to feed on blackberry over apple.

Emergence sex ratio in *Eucolaspis* sp. "Hawke's Bay" is found to be female-biased (0.35), whereas adult sex ratio in the active population on foliage was slightly male-biased (0.55) in organic apple orchards in Hawke's Bay. No evidence for a short-range sex pheromone was found through olfactometer bioassays. All the mating attempts in mating bioassays proceeded only after either antennal contact or licking of female's elytra by the male. Ablating antennae didn't impair mating, but significant delay was observed in locating the female. Males attempted to mate with intact and washed female cadavers, 45% and 35% respectively of the tested males, whereas no mating attempts were initiated towards male cadavers. Males of *Eucolaspis* sp. "Hawke's Bay" appear to utilize both contact sex pheromones and vision in locating potential female mates.

It was found in the current study that endogeic macro-invertebrates were more abundant in orchards that historically had high bronze beetle incidence, whereas epigeic macro-invertebrates were more abundant in orchards that had historically low bronze

beetle incidence. It may be that abundant surface-dwelling generalist predators in low bronze beetle orchards control bronze beetle from establishing in these orchards. However, this could only be confirmed by further research on specific predation of spiders and other generalist predators on bronze beetle.

A phenology model for adult emergence is proposed based on threshold temperature (4.69 ± 0.89 ^{0}C), degree-days (237 ± 22 ^{0}C days) and biofix date of September 11th. The model predicted adult emergence with a precision of ±4 days when tested with field data.

Acknowledgements

I would not have been able to finish my thesis without the constant support of my wife Manjulatha who relentlessly worked to take any pressure away from me and regularly motivated me throughout my study. I could not have come this far without the encouragement and support of my parents Linga Murthy and Parvathi Devi, who raised me with a sense of science and passion to the natural world. I have been taught wonderful values and self belief by them for which I am deeply indebted. I am also greatly thankful to my wonderful brother Rajasekhar, sister-in-law Hemalatha and my nephew Mourya & little niece Sofie for their affection. My only brother, Raja has always been an inspiration for me and was there whenever I needed him most. I am also indebted to my in-laws Venkaiah & Vijayalaksmi, Kishan, Anupama; bhav Ravindra, akka Haritha and their families, nephew / nieces Harsha, Siri, Abhi, Samhitha, and Hasitha for their affection and love for me.

I am greatly indebted to my chief supervisor, Dr Maria (Masha) Minor, for accepting me as her student and for all the help, advice and encouragement offered during the course of my PhD. Masha has always been accommodative, with ideas and with patience to answer all my (sometimes silly) questions. I would also like to thank Masha for collecting beetles for me, for teaching me soil ecology, digital imaging and statistics on numerous occasions.

I greatly appreciate the help and advice given by my co-supervisor, Assoc Professor Steve Trewick. Steve taught me from scratch all molecular techniques, both lab & analytic and was always energetic and friendly. Thanks for collecting beetles for me, for allowing me to use aDNA lab facilities and for paying my sequencing costs. Thanks for all your time, which I sometimes suspected to have taken too much of.

I would like to thank my co-supervisor, Professor Qiao Wang for helping with behavioural experiments and allowing me to use Y-tubes and laboratory space to conduct olfactometer bioassays.

I would like to thank my co-supervisor, Dr Dave Rogers for help in identifying orchards that were used as study sites and also for all the encouragement, ideas and help in planning experiments, for looking after my emergence traps and also for the hospitality whenever I was visiting Hawke’s Bay. I also greatly appreciate you organising collection of beetles from various places in New Zealand.

I would like to thank Richard Leschen, for lending valuable specimens from NZAC collection and for making my NZAC visits enjoyable. I would also like to thank John Marris for lending specimens from LUNZ collection and encouragement. I would also like

to thank Lyn Cole for looking after my emergence traps and kindly lending me pupae and larvae whenever possible. I would like to thank Peter Shaw and Lynda Hawes for collecting beetles for me. I would like to thank Chris Reid (Australian Museum) for his advice and encouragement, John Early (Auckland Museum) for beautiful photos and useful discussion.

Thanks are due to Dr Nigel Bell for help with nematode bioassays. Thanks are also due to organic apple growers in Hawke's Bay who allowed me to sample their orchards. I would like to thank Dr Alasdair Noble, for his valuable suggestions to get through perils of statistical analysis. I would like to thank Mary Morgan-Richards for collecting beetles for me, and for encouragement during regular lab meetings. I would like to thank Alastair Robertson for lending some beautiful photos and general help whenever approached.

I would also like to thank Ecology group staff in general for being generous, friendly and helpful. Especially, I would like to thank Paul Barrett, Cleland Wallace, Tracy Harris, Sharon Tougher, Barbara Just, Erica Dahya, Sharon Wright and Shaun Nielsen for logistic help and trying hard to reach my seemingly endless wants. I would also like to thank Trish McLenachan for general hospitality and help while working in the farside lab.

I would like to acknowledge fellow ecology students Amir Sultan, Hop Tran (I really enjoyed our numerous lunch time discussions), Nicole Schon, Arved Schwendel, Rashmi Kant, Rheyda Hinlo, Lizzie Daly, Sofie, Juan Carlos, for their assistance with field and / or lab work; Phoenixers for being kind (some of whose reagents and tips I definitely stole while working @ farside); Valter Cromberg who was a visiting scholar and numerous other students for their general help and friendship during the course of my study. I would like to thank ecology post-docs Simon Hills and Mariana Bulgarella for their help with phylogenetic analyses and general advice.

I would like to thank all my friends in New Zealand and elsewhere, some of whom have already been mentioned here, for their friendship, help and encouragement.

Last but not least, I would like to thank Massey University for various scholarships: DJ McGowan Scholarship, Helen E Akers PhD Scholarship (3X), Lovel & Berys Clark Scholarship and MU Doctoral Completion Bursary. I would like to thank New Zealand Plant Protection Society for their generous travel grants that enabled me participate in the society's annual conferences. I would also like to thank Institute of Natural Resources, Massey University for the resources and facilities provided for undertaking my doctoral research. I would also like to thank Plant and Food Research, Hawke's Bay station for the in-kind support for my research. I could not end without thanking, at the risk of reminding myself how big my current debt is, Study Link for lending money to pay my tuition.

Contents

List of tables

List of figures

Chapter 1: Introduction

1.1. Overview

A tiny nondescript native chrysomelid beetle, *Eucolaspis* sp., colloquially called "bronze beetle" *s.l.* is posing a significant threat to otherwise profitable New Zealand organic apple (*Malus domestica* Borkh.) industry, especially in the Hawke's Bay region. The beetle infestation is alarmingly spreading to more orchards, more regions of the country and more fruit crops (Rogers et al., 2009). The insecticide control measures available to organic growers so far are not effective against the beetle. Adult beetles damage developing fruitlets during New Zealand spring and early summer. Resulting yield losses reach up to 43% of the produce (Rogers et al., 2006). The beetle problem is becoming a plague in Hawke's Bay, with some of the organic orchards converting back to conventional production (D. Rogers, personal communication).

Currently, New Zealand has about 785ha under organic apple orchards, exporting ~$NZ35 million worth of produce annually (OrganicDirect NZ Ltd., 2011), but unless an effective control of bronze beetles is found sooner, the New Zealand organic apple industry is going to face serious consequences. Much research input is warranted to address the situation. Accordingly, this research sets out to address the knowledge gaps surrounding the bronze beetle. It focuses on various aspects, but is generally centered on the systematics and ecology of the bronze beetle.

1.2. Bronze beetle systematics

Knowing the taxonomic identity of a pest insect is crucial for application of any targeted control measures such as biocontrol. But, in this case, it is not yet known which species infest (s) apple orchards. Although *Eucolaspis brunnea* F. 1781 was previously implicated in the orchard infestations (Lysaght, 1930; Rogers et al., 2006), it is debatable (Rogers et al., 2007) and more species may be involved.

1.2.1. History and taxonomy

Eucolaspis is a complex containing several species, the taxonomy of which is unresolved (Kay, 1980; Dugdale & Hutcheson, 1997). The genus and all New Zealand species are native. The first description of the bronze beetle *s.l.* by Fabricius (1781) dates back to the late 18th century, from the specimens collected by Sir Joseph Banks who came to New Zealand in 1769 with Captain Cook. Fabricius (1781) named the beetle *Chrysomela brunnea.* White (1846) described another related species "*pallidipennis*", and changed the genus from *Chrysomela* to *Colaspis,* apparently being

unaware that *Colaspis* was already in use for another chrysomelid from North America (Lysaght, 1930). Broun (1880) translated the earlier descriptions of Fabricius and White, and added few more species. Sharp (1886) created the genus *Eucolaspis* for those species placed in *Colaspis* by White and Broun. Broun (1893b; 1893a; 1909) described more species, taking the species count to fifteen. Hutton (1904) modified the species name *brunnea* to *brunneus*; the former, however, is correct according to Lysaght (1930). Shaw (1957) revised the genus and reduced the number of species to five. However, the synonymy published by Shaw (1957) was rejected by authors such as Kuschel (1990) and Dugdale and Hutcheson (1997) who stated that Shaw (1957) did not consider genitalic characters and hence his review is best ignored. Due to the lack of further studies, the taxonomy of the genus remains unresolved at the moment (Rogers et al., 2007).

Eucolaspis is distributed throughout the North Island (including offshore islands such as Great Barrier Islands, Poor Knights Islands and Three Kings Islands) of New Zealand whereas in the South Island, beetles are generally rare South of Canterbury region (Figure 1.1) (NZAC specimen labels information and personal communications).

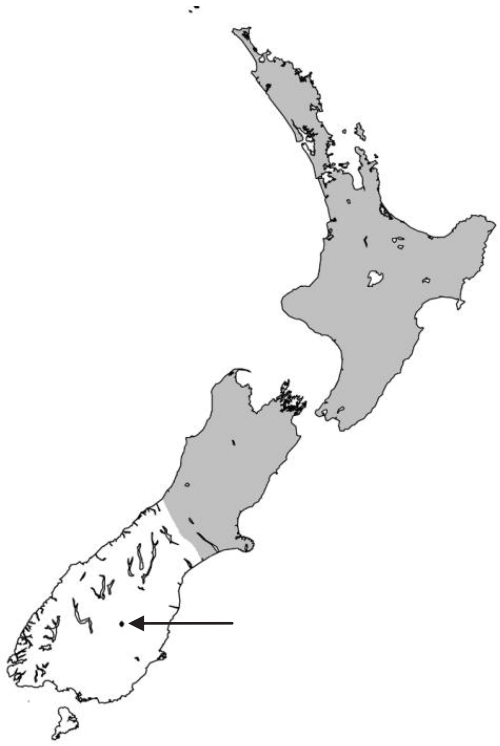


Figure 1.1 New Zealand map showing approximate distribution of *Eucolaspis* beetles; the area covered with grey colour with known / recorded instances and an isolated finding at a Roxburgh (Otago, New Zealand) orchard (apricot) is pointed with an arrow.

1.2.2. Adult morphology

Adults are oval-shaped, usually measuring 3-5 mm in length and 2-3 mm in width, shiny, with colour varying from yellowish brown to bronzy black. Antennae are thread-like with eleven segments. The pronotum is punctate and sometimes has two greenish-black spots. The elytra are broader than the thorax, extend beyond the end of the abdomen and are finely punctate and often dark to greenish-black along the mid-line (Kay, 1980). The hind wings are clear with light-brown chitinized areas, about three times as long as broad. The legs are robust for jumping and burrowing, and end in strong two-pronged claws (Kay, 1980). Whenever disturbed, the beetles jump characteristically, and fall to the ground. The abdomen resembles an inverted equilateral triangle in shape (Miller, 1926; Lysaght, 1930). Female beetles are larger in body size but have shorter antennae than male beetles. Male beetles possess a characteristic depression on the last abdominal sternum which is lacking in females (Shaw, 1957) (Figure 1.2).

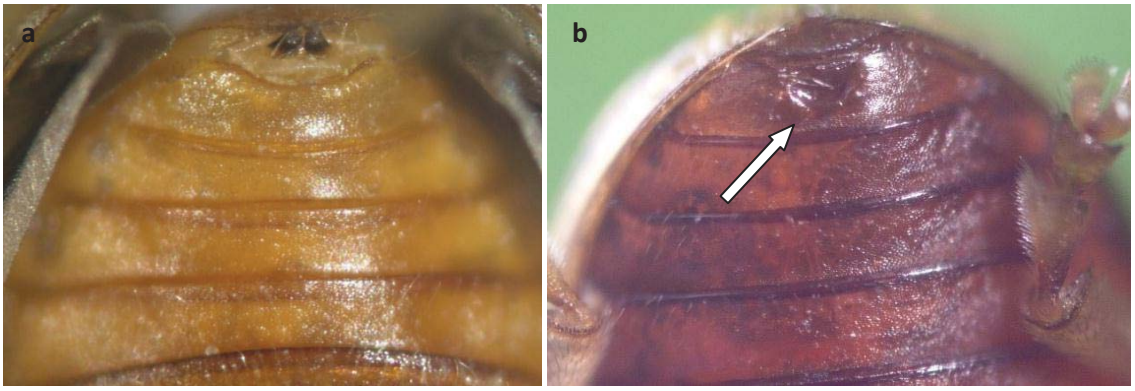


Figure 1.2 Ventral side of the abdomen of a) female and b) male *Eucolaspis* sp. beetles; males have a depression on last abdominal sternum as shown here (b) which distinguishes them from females that do not have any depression (a).

1.3. Economic importance and food plants

Bronze beetle was a serious apple pest into the 1930's, it was regularly mentioned in contemporary monthly advisory columns because of the serious and continual annual losses, and subject to detailed scientific studies (Miller, 1931; Cottier, 1935). After the World War II, dichlorodiphenyltrichloroethane (DDT) replaced lead arsenate as the major horticultural spray and provided good control of bronze beetle such that it was no longer an economic concern. After the decline of DDT usage during 1980's, bronze beetle damage to apples has re-appeared, especially in the Auckland

region, increasing pressure for the development of new control measures (Clearwater & Richards, 1984). Since the widespread introduction of integrated fruit production (IFP) management systems for apples, bronze beetle has remained a minor pest causing little economic damage in conventional production (because of effective pesticides such as thiacloprid). Recently, it reappeared as a serious threat to organic apple orchards with estimated annual losses of 12-15 million NZD (Mannering, 2006; Rogers et al., 2006). It was estimated that some of the organic orchards in Hawke's Bay experienced up to 43% fruit losses during 2005-06 season, and the damage appears to be increasing and spreading to more orchards (Mannering, 2006). Heavily infested orchards may have as many as 878 beetles emerging per m^2 of soil (Rogers et al., 2006).

The beetle damage intensities vary between orchards and within the same orchard, with some blocks more severely damaged than others, for reasons that are unknown. For instance, a previous study (Rogers et al., 2006) could not find any relationship between damage levels and any of the site characteristics, including type of shelter, soil, adjacent cropping activity, and physical features such as drains, creeks and rivers. It is assumed that the beetle is relatively localized with limited movement.

Eucolaspis spp. are polyphagous and originally lived on various native shrubs and trees. Initially, Fabricius (1781) described manuka *Leptospermum scoparium* (Myrtaceae), a New Zealand native shrub, as the host plant (in White, 1846). Subsequently, extensive damage by *Eucolaspis* has been reported on apples (Huntley, 1867; Miller, 1926; Lysaght, 1930; Clearwater & Richards, 1984; Rogers et al., 2006). It is also reported to feed on a wide range of indigenous plants including *Fuchsia excorticata*, *Myrsine divaricata*, *Alectryon excelsum*, *Coprosma* spp., *Pittosporum* spp., and *Podocarpus* spp. (Lysaght, 1930). Considerable damage by *Eucolaspis* was reported also on other fruit crops including pear, plum, peach, gooseberry, black currant, raspberry, blackberry, apricot, cherry, nectarine (Miller, 1926; Lysaght, 1930) and grape vine (Woodfin, 1927). The beetle has been observed on many garden and ornamental plants including *Camellia* (Royal New Zealand Institute of Horticulture, 2011), geranium, rose (Lysaght, 1930) and other exotic plants including acacia, hollyhock, hawthorn, laurel, clover, mallow, elm, violet (Lysaght, 1930), and forest trees, pine (Kay, 1980) and eucalyptus (Clark, 1938).

The damage inflicted on apple orchards and forest plantations was historically attributed to *E. brunnea* (Lysaght, 1930; Clearwater & Richards, 1984; Rogers et al., 2006), however, infestations may involve more than one species. It is not clear at this point whether all the species of the genus are pests or not.

Adult beetles feed on leaves, flowers and fruits of their host plants. Leaf damage typically results in a "shot hole" effect (Figure 1.3) and may sometimes cause severe defoliation (Lysaght, 1930; Kay, 1980). Beetles damage coniferous trees by feeding on young needles, and would sometimes give coniferous trees a scorched appearance (Kay, 1980).

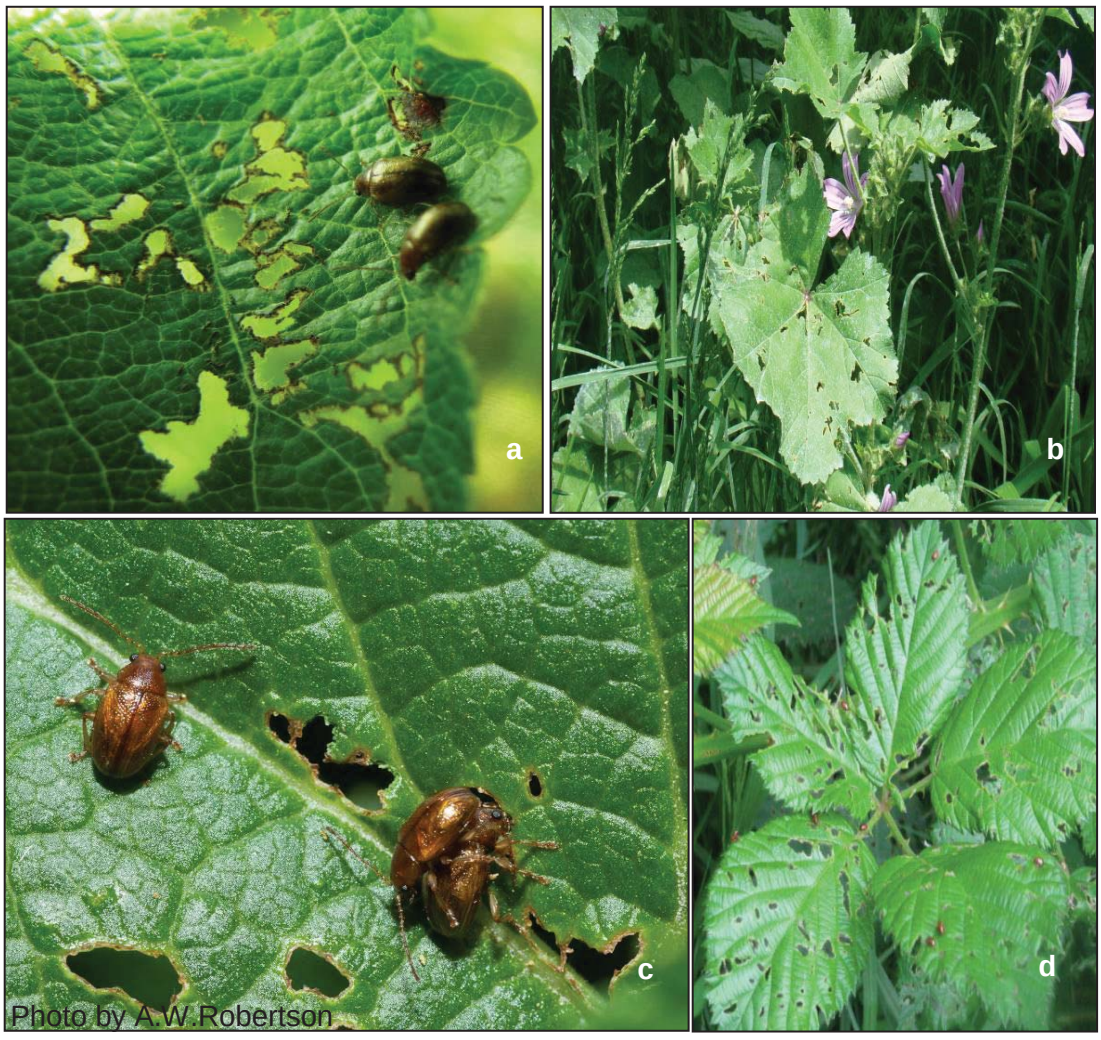


Figure 1.3 Leaf damage caused by *Eucolaspis* sp. adults on (a) apple, (b) mallow, (c) raspberry and (d) blackberry.

Fruits are also damaged; younger tender fruits are preferred, and usually attacked near the stalks or the stalk itself. The skin and underlying tissues of developing

fruits are eaten (Figure 1.4). Although the apple trees recover from this damage, the fruitlets often fall off because of damage to stalks (D. Rogers, pers. comm.), the remaining apples have large raised scabs and severe distortion, rendering them unfit for export (Miller, 1926; Lysaght, 1930; Miller, 1971).

Figure 1.4 Fruit damage caused by *Eucolaspis* sp. on 'Royal Gala' apples in Hawke's Bay, New Zealand.

The adult beetles prefer apple varieties with clusters of fruits, as these provide sheltered location with plenty of food. This was evident in the case of old varieties such as 'Cox's Orange', which produce clusters of fruits (Miller, 1926). Among modern apple varieties, 'Royal Gala', which produces fruits in clusters, is more severely damaged than other varieties such as 'Braeburn' (Rogers et al., 2006). In vineyards, the greatest damage was observed on vines grown on a horizontal support system (Woodfin, 1927).

1.4. Life cycle and biology

Bronze beetles have a single generation per year and overwinter in the larval stage (Miller, 1926; Lysaght, 1930). Adult beetles start emerging in New Zealand spring, late September to early October, and the emergence continues until mid-December. In Hawke's Bay adult beetles mostly emerge during November (Rogers et al., 2007). The beetles generally become scarce towards the end of February (Miller, 1926; Lysaght, 1930; Kay, 1980; Rogers et al., 2006; Rogers et al., 2007). Adult longevity is generally 6-8 weeks but differs between males and females; females being the shorter lived (Lysaght, 1930).

Egg-laying takes place between November and early March in the field (Lysaght, 1930). Eggs are laid in the soil in earthen capsules consisting of several egg compartments, with each egg mass containing 8-14 eggs (Lysaght, 1930; Miller, 1971). Dry lumpy soil is preferred for egg-laying (Lysaght, 1930). The beetles were observed to lay eggs more than once during their life time (personal obs.). Mated individual female beetles contain 11-14 mature eggs. Eggs are oval-shaped, cream-white coloured, very small and measure about 0.3 mm in diameter and 0.6 to 0.7 mm in length (Figure 1.5). The egg-hatching period varies from 15-22 days, depending on weather (Lysaght, 1930).

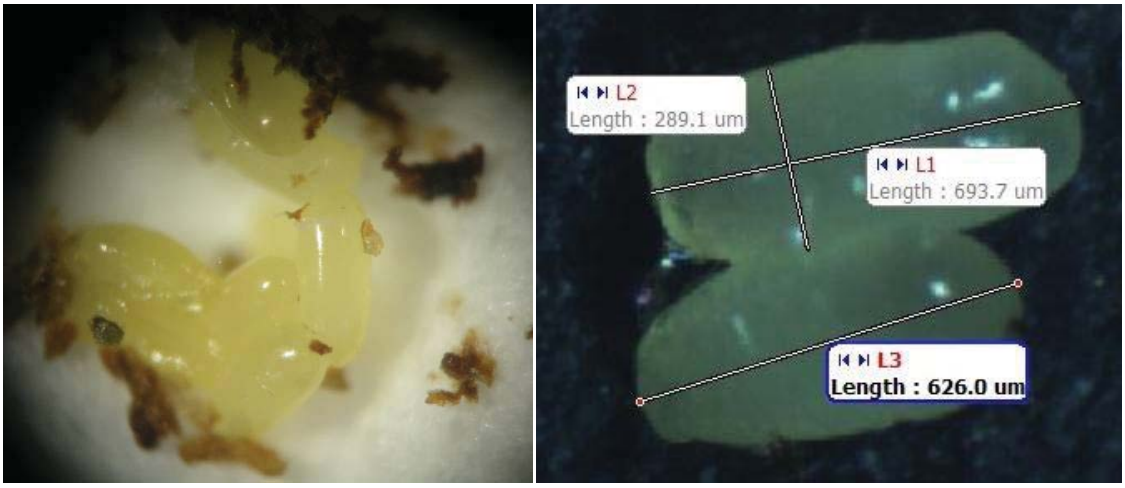


Figure 1.5 An egg mass of *Eucolaspis* sp.; eggs laid in a Petri dish in the laboratory.

Neonate larvae are very active and forage vigorously (personal obs.). Full-grown larvae are about 5 mm long and 2 mm in diameter, stout and slightly curved (Figure 1.6). The head capsule is light yellow tinged with brown. Short distinct legs and the shiny hard pronotum distinguish them from weevil larvae (which are legless), and grass grub larvae (which have legs but without a hardened pronotum behind the head). The larvae are able to fix their terminal segment firmly in the ground, while the rest of the body circles around feeding on surrounding small roots (Lysaght, 1930; Miller, 1971; Kay, 1980). Larvae feed on small roots of grasses, clover and various other understorey crops (Miller, 1926; Lysaght, 1930; Miller, 1971; Kay, 1980). Although little is known about larval feeding, extensive damage has not been reported. When larvae collected from apple orchards were provided with roots of common orchard understorey plants such as clover and mallow, no feeding was observed (Rogers et al., 2008), however this

may have been because the larvae used were already in the non-feeding diapause stage prior to pupation.

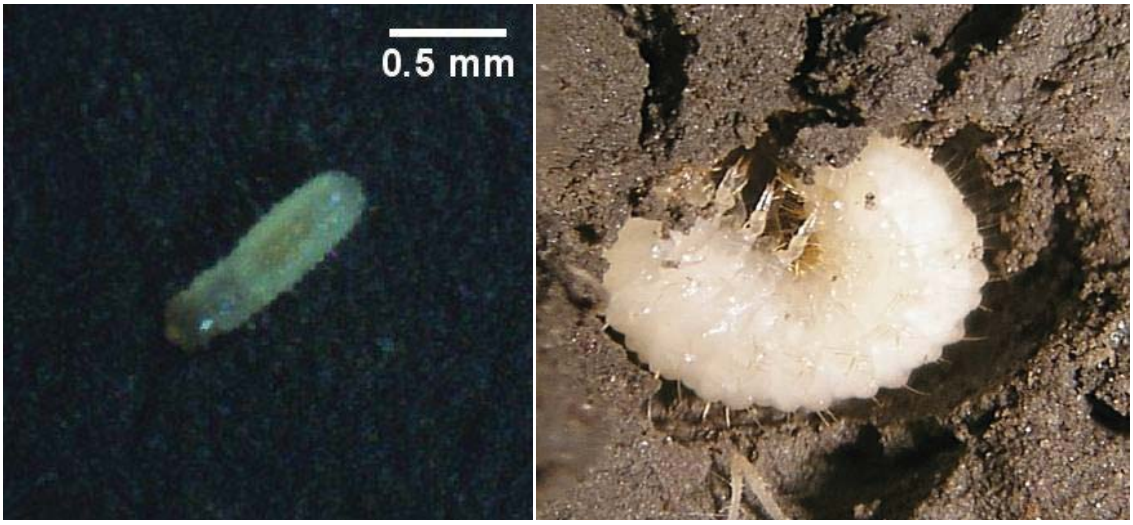


Figure 1.6 A first instar (left) and a last instar (right) larva of *Eucolaspis* sp., "bronze beetles", from Hawke's Bay, New Zealand.

Larvae occur in the field throughout most of the year, although a typical larval period may extend from early January to late September (pers. obs.). Larvae are found in the soil near any host plant where the adults have been abundant. In apple orchards, the larvae are abundant in the soil below any branch typically within 1 m radius from the tree trunk (Rogers et al., 2006). Larvae are found in various types of soils in different localities, but most abundantly in uncultivated soils. In winter larvae enter a diapause, occupy earthen cells in deep soil layers and are yellow in colour due to abundant fat reserves, that provide reserve material for the hibernating period (Lysaght, 1930; Kay, 1980). Between late winter to early spring, larvae become active again and move towards upper soil layers (Lysaght, 1930; Kay, 1980). In spring the larvae are mostly within 5-10 cm of the soil surface (Rogers et al., 2007; Rogers et al., 2008). A long pre-pupal period precedes the pupal stage (exact duration not known) and pupation occurs in small earthen cells that are finely plastered from the inside (Lysaght, 1930; Kay, 1980).

The pupae are creamy-white in colour and usually about 4 mm long and 2 mm wide (Figure 1.7). As they approach maturity, the eyes, wing cases and the tips of the mandibles become darker (pers. obs.). Pupae are found in the field from September or

early October (Rogers et al., 2007).The length of the pupal period varies from three weeks (Kay, 1980) to three and a half weeks (Lysaght, 1930).

Figure 1.7 Pupa of *Eucolaspis* sp., from Hawke's Bay, New Zealand, in its small earthen cell.

1.5. Control

Cultivation of the soil directly under the trees during spring has been found to be an effective control measure for reducing bronze beetle populations in apple orchards (Lysaght, 1930; Rogers et al., 2007) and vineyards (Woodfin, 1927). Multiple tillage was more effective compared to a single tillage (Rogers et al., 2007). This technique kills larvae and pupae by mechanical control resulting in reduced adult emergence, but it is costly and damaging to soil and plant roots. A less disruptive control measure is desired by the growers and scientists.

Previously, satisfactory bronze beetle control was obtained using chemical insecticides such as calcium cyanide (Miller, 1926), calcium arsenate (Miller, 1931), lead arsenate (Cottier, 1935), DDT and chlorpyrifos (Clearwater & Richards, 1984). The persistence, broad-spectrum nature and toxicity of these insecticides contributed to their removal from spray programmes. Today, Integrated Fruit Production (IFP) apple growers have several insecticide options, for example thiacloprid, which effectively controls bronze beetle (Rogers et al., 2006).

There are, however, no organically acceptable insecticide strategies currently to keep the beetle under check. Rogers et al. (2006) found that none of a suite of organic

insecticides tested, including Surround®, Entrust™, NeemaAzal™-TS and Pyradyne were effective against bronze beetle. However, pyrethrum, rotenone and Entrust™ treatments were observed to inhibit mating and feeding of beetles to some extent in laboratory bioassays (Rogers et al., 2006).

Recently, Hurst et al. (2011) found that a bacterial formulation of *Yersinia entomophaga* MH96 (Ye MH96) applied to apple leaves killed 42% of adult bronze beetles after a single application and 66% of adult bronze beetles after multiple applications. However, rapid loss of efficacy with no significant activity three days after application in field bioassays suggested that the bacterium is susceptible to environmental constraints such as desiccation and UV light (Hurst et al., 2011). An improved field performance of Ye MH96, if achieved, would provide an effective biopesticide option for the organic growers to control bronze beetles.

Predation of bronze beetle by ground beetles and robber flies was reported though not detailed (Lysaght, 1930; Kay, 1980). It was not clear from the literature which life stage of bronze beetle, these predators were feeding upon.

1.6. Knowledge gaps

The current biotic problems that persist in primary production occur at higher organization levels such as populations, communities and ecosystems, that are best addressed by the science of ecology (Weiner, 2003). The knowledge needed to build ecologically sound pest management systems will require research from molecular to landscape levels (National Research Council, 1996). The development of pest management systems rests on relatively few major ecological principles, such as genetic diversity of organisms, adaptation to environment, nutritional requirement, maintenance by reproduction, ecological plasticity, trophic interactions and interactions among various factors in the ecosystem (Huffaker, 1974). But, there are only few studies of the biology and ecology of New Zealand *Eucolaspis*, "bronze beetles" and several areas of knowledge are missing.

1. Unresolved systematics: New Zealand *Eucolaspis* appears to include a poorly defined and diagnosed number of species. It is unclear how many species are implicated in attacking exotic fruit orchards such as apples. There is no well accepted taxonomic key for classification of present day beetles.

2. Ovipositional behaviour: No published data exists on ovipositional details such as preferred habitat, fecundity and hatching temperatures.

3. Larval biology and behaviour: There is no information on the number of instars, duration of the larval stage (some indirect inferences exist but are inconclusive), larval feeding preferences, larval movement in the soil, diapause and host plants.

4. Adult mating behaviour: There is no information on sex ratio, mating behaviour, and existence of any close range or long range sex pheromones that mediate mate location.

5. Host plant location and preference: Bronze beetles appear to use a wide variety of native and introduced plants as hosts. However, there is no information available on host-finding behaviour of the beetles and on preference of the beetles for different host plants.

6. Natural enemies: Although two generalist predators have been named as predators of bronze beetle, no information exists on what life stage these predators prey upon. The existence of any other predators, parasites or pathogens on different life stages of the beetle is not known.

7. Spatial variations: The incidence of bronze beetle varies from region to region, from orchard to orchard, and even between blocks within the same orchard. No factor could currently be identified to explain these variations in damage levels.

1.7. Research objectives

Knowledge of the underlying ecology is crucial for formulating any successful pest control strategy (Zehnder et al., 2007) and successful strategies in organic systems are predominantly those that serve to maintain the ecological balance of agricultural pests and their enemies. Because pest management should be based on understanding of the agricultural and forest ecosystems, a better knowledge base of the interacting components and processes that characterize these ecosystems is pivotal (Shennan, 2008).

In order to formulate an organically feasible control measure for bronze beetle, a detailed knowledge base should be developed on its biology and ecology. The current research aims at filling the research gaps in the bronze beetle research with the objectives stated below. In doing so, this research contributes to autecology of the New

Zealand biota and our understanding of the species interactions arising from anthropogenic habitat modification.

1. How many species of *Eucolaspis* exist in New Zealand and how does the distribution of these species vary ecologically and geographically? Is a single taxon involved in infestation of apples in Hawke's Bay? How is this related to other lineages?

2. Do bronze beetles deploy olfaction in attraction to different host plants and is there any preference by the beetles for particular host plants?

3. What is the adult sex ratio of the beetles and is there any evidence for the use of close range and contact sex pheromones in mate location by the beetles?

4. How does the population structure of beetles in orchards vary in relation to other soil macro-invertebrates?

5. What are the thermal requirements of pupae and how adult emergence in the field can be predicted?

1.8. Thesis layout

Chapter 2, "Systematics and diversity", documents genetic diversity among New Zealand *Eucolaspis* populations and explicates phylogenetic relationships among different lineages of *Eucolaspis* in New Zealand, and their geographical distribution.

Chapter 3, "Host location and feeding preferences", expounds attraction of adult beetles to volatiles of different host plants, and feeding preference between apple and blackberry.

Chapter 4, "Adult sex ratios and mate recognition", documents sex ratio in the emerging population and in the active population, and the evidence of contact sex pheromones in mate recognition by male beetles.

Chapter 5, "Bronze beetle and other soil macro-invertebrates' assemblage", describes spatial and temporal dynamics of abundance and population structure of *Eucolaspis* sp., and community structure and dynamics of other endogeic and epigeic macro-invertebrates.

Chapter 6, "Phenology and modelling adult emergence", verifies degree days required for adult emergence from pupae and the minimum threshold temperature required for

pupal development, and uses the information to model adult beetle emergence in Hawke's Bay.

Chapter 7, "Synthesis", outlines the hypotheses tested, crucial outcomes and their implications for empirical and theoretical application.

Appendices include details of two small-scale experiments ("Entomopathogenic nematodes in organic apple orchards" and "Evaluation of mark-release-recapture techniques") that failed to produce any data. Raw data from Chapters 2 and 5 are also included in the appendix.

Chapter 2: Systematics and diversity of *Eucolaspis* in New Zealand

A version of this chapter has been presented at the Global Entomology conference (4-9 March 2011, Chiang Mai, Thailand) and the 3rd combined Australia and New Zealand Entomological Societies conference (28 Aug – 1 Sept 2011, Christchurch, New Zealand)

2.1. Introduction

The most frequently cited formal species name of the genus *Eucolaspis* in New Zealand is *E. brunnea* Fabricius 1781 and this species is commonly referred to as the "bronze beetle" (Miller, 1926; Ferro et al., 1977). The name "bronze beetle" has been also applied more generally to New Zealand *Eucolaspis* which are similar in size and colour (e.g., Rogers et al., 2009; Doddala et al., 2010; Hurst et al., 2011). Damage in apple orchards was attributed to *E. brunnea* until 2007 (Miller, 1926; Woodfin, 1927; Lysaght, 1930; Miller, 1971; Clearwater & Richards, 1984; Rogers et al., 2006), when *Eucolaspis* beetles from an organic apple orchard in Hawke's Bay were identified as *E. pallidipennis* (Richard Leschen, NZAC). However, due to the extent of variation in size and colour within beetle populations and ambiguity in the existing taxonomy, an assumption of uncertain identity was favoured (Rogers et al., 2007).

Unresolved taxonomy is an impediment to research on any aspect of insect biology, and in this case it also has implications for targeted methods of pest control, such as the use of biological control. Such poor taxonomy is an unusual situation for a pest insect that causes serious annual losses to New Zealand horticultural industry. The scanty knowledge on *Eucolaspis* is not limited to taxonomy; many aspects of biology are poorly studied (see Chapter 1 for various research gaps).

2.1.1. Background

Chrysomelidae (leaf beetles) is an important and diverse family in the order Coleoptera, comprising more than 50,000 extant species (Lopatin, 1984; Jolivet et al., 1994) placed in 11 (Reid, 1995) to 16 subfamilies (Seeno & Wilcox, 1982). Subfamily Eumolpinae represent one of the most diverse subgroups within Chrysomelidae, with more than 500 genera and 7000 species globally (Jolivet & Verma, 2008). It is estimated that there are 35 genera (Leschen et al., 2003) and 156 species (Leschen & Reid, 2004) of Chrysomelidae in New Zealand, among 5 subfamilies (Bruchinae, Galerucinae, Chrysomelinae, Eumolpinae and Cryptocephalinae). Subfamilies Criocerinae, Cassidinae, Spilopyrinae and Sagrinae are notably absent from New Zealand (Leschen & Reid, 2004). Subfamily Eumolpinae is represented by 4 genera in New Zealand, all of which are native (Leschen et al., 2003), and possibly a fifth undescribed genus (Leschen & Reid, 2004). Among the four described native genera of

Eumolpinae, genus *Eucolaspis* appears to be much more diverse and widely distributed than the other three (*Atrichatus*, *Pilacolaspis* and *Peniticus*).

The genus *Eucolaspis* (Coleoptera: Chrysomelidae: Eumolpinae) was created by Sharp in 1886 for species described by Fabricius (1781), White (1846) and Broun (1880) under the genera *Chrysomela* and *Colaspis*. Fabricius (1781) and White (1846) each described one species, while Broun (1880; 1893b; 1893a; 1909) described thirteen species under the genus. By the end of 1909, the *Eucolaspis*, therefore, comprised fifteen species (Table 2.1). In addition to these 15 species, there may be another yet-to-be described species among New Zealand Arthropod Collection (NZAC) specimens collected from Three Kings Islands – this species is provisionally referred as *Eucolaspis* nov. sp. *triregia* (R. Leschen, pers. Comm.).

The earlier descriptions were brief and mostly based on colour and other highly variable characters, and it was not possible to compile a diagnostic key from the descriptions of Broun (see Table 2.1) (Lysaght, 1930). Tillyard (1926) suggested that among *Eucolaspis* species the larger and darker form was *E. ochracea* B. This appears unlikely, as both *E. brunnea* F. and *E. colorata* B. are larger than *E. ochracea* B. (Table 2.1). Apparently, Tillyard (1926) misunderstood Broun's (1880) earlier description of *Colaspis ochracea* B. 1880, which, in fact, was amended in 1893 by Broun as *Atrichatus ochracea*. The species *E. brunnea* F. 1781 and *E. pallidipennis* W. 1846 differ slightly from one another in colouration of the pronotum and also the latter is slightly smaller in size (Rogers et al., 2007).

Shaw (1957) revised the New Zealand species of *Eucolaspis* and concluded that some (12) of the species were obsolete, but added two new species: *E. hudsoni* Shaw 1957 and *E. antennata* Shaw 1957. He placed a total of 5 New Zealand species in the genus *Eucolaspis*, and devised a key for their classification (see Table 2.2). As Shaw (1957) appeared to have disregarded the differences in genital structures (aedeagus) between species, Kuschel (1990) recommended that the synonymy published by Shaw (1957) was best ignored, although the justifications for this were never published.

Bryant and Gressitt (1957) described two species of *Eucolaspis* outside New Zealand - *E. castanea* and *E. saltator* from Fiji; the two species differ from one another in terms of body colour and puncture density on head and pronotum. *Scaevola floribunda* (family Goodeniaceae) was reported as the host plant of *E. castanea*, whereas *Tarenna sambrucina* (family Rubiaceae) and *Commersonia bartramia* (family Malvaceae,) were reported as host plants of *E. saltator* (Bryant & Gressitt, 1957).

Table 2.1 Species separation characters as described by Broun[+] (1880, 1893a, 1893b & 1909).

Species	Length (mm)*	Width (mm)*	Body colour	Remarks	Distribution
E. brunnea F.	4.23-5.29	3.18	Variable	Two blackish green spots on thorax	NA
E. pallidipennis W.	3.70	NA	Deepish brown	Black band behind head	NA
E. puncticollis B.	3.70	~2.12			Tairua & Whangarei heads
E. jucunda B.	2.82	1.42		Purplish scutellum	Tairua
E. subœnea B.	3.54	1.69	Bronzy green		Tairua
E. sculpta B.	3.18	1.42		Pronotal punctures denser than *E. subœnea*	Tairua
E. mera B.	2.82	1.42	Pale brownish	Elytra more sparsely punctured than previous three	Whangarei heads
E. brevicollis B.	3.54	1.59	Testaceous	Thorax short and broad than *E. pallidipennis*	Whangarei heads
E. atrocerulea B.	3.54	1.59	Blackish blue		Whangarei heads
E. ochracea B.	4.23	2.38	Ochraceous	Pronotal punctures smaller than *E. atrocerulea*	Many North island locations
E. colorata B.	4.23	2.38	Variable	Pronotum explanate, shorter than *E. ochracea*	Waitakerei Range
E. montana B.	3.70	~2.12		Pronotal punctures larger than *E. puncticollis*	Mt Egmont
E. vittiger B.	4.23-4.76	2.38	Testaceous		Hunua Range
E. picticornis B.	4.76	2.65	Violaceous		Waitakerei Range, Pokeno
E. plicatus B.	4.23	~2.65		Resembles *E. subœnea*; pronotal front margins rectangular	Kaitoke, Pakuratahi

*Converted from line (an older, now obsolete measure of length in the Imperial system) to mm using an online conversion software (Edkins, 2009)

+No account of host plants was provided by Broun for the 13 species he described.

Table 2.2 Morphological characters separating the five species of *Eucolaspis* reviewed by Shaw, 1957.

Character	***E. brunnea*** F.	***E. jucunda*** B.	***E. puncticollis*** B.	***E. antennata*** Shaw 1957	***E. hudsoni*** Shaw 1957
♂ Size (mm)	3.5-5.8	3-3.6	3.0-3.4	3.8	4.6
♀ Size (mm)	4.6-5.4	3-3.8	3.4-4.2	4.5	NA
Pattern of pronotal punctures	Irregularly scattered	Irregularly scattered	Forming a dense regular pattern	Forming a dense regular pattern	Forming dense regular pattern
Pronotum size and shape	Pronotum distinctly transverse, half as long as broad	Pronotum less transverse, more than half as long as broad	NA	NA	NA
Pronotal punctures	Often elongate and never touching	Often elongate and never touching	Mostly oval to elongate, interspaces usually strongly alutaceous without or with only a few microscopic punctures	Mostly well defined round punctures, interspaces not or only slightly alutaceous with microscopic punctures	Mostly well defined round punctures, interspaces not or only slightly alutaceous with microscopic punctures
Pronotal punctures density	NA	NA	NA	More closely punctured, punctures smaller, interspaces with obscure microscopic punctures	Less closely punctured, punctures larger, interspaces with numerous microscopic punctures
Elytral striae	NA	NA	Elytral striae at apex clearly marked with slightly impressed serial rows of punctures	Elytral striae well defined at apex with un-punctured interspaces	Elytral striae at apex mostly obscured by strong interstitial puncturation
Hind coxa				More widely separated than that of *E.brunnea*	Less widely separated than that of *E.antennata*

In addition, two species from Australia were described under the genus *Eucolaspis* (Lea, 1915), but later the Australian species were assigned to genus *Eucolaspinus* (Lea, 1916). Close relatives of *Eucolaspis* might include Pacific (New

Caledonia, Norfolk Island and other Pacific islands) *Colaspoides* Laporte 1833 and *Dematochroma* Baly 1864 (C. Reid, 2011, personal communication). In this thesis all the information related to any "species" of *Eucolaspis* relates to New Zealand species only, unless stated otherwise.

Some of Broun's species appear to be sympatric, as four species each were described from Whangarei heads and Tairua (Table 2.1). Broun's initial descriptions included no specific delineation characters. However, as more species were described subsequently, Broun attempted to relate and differentiate these species. For example, according to Broun, *E. sculpta* differs from *E. subænea* by having denser pronotal punctures (Table 2.1). The main characters Broun used to delineate species appear to be body size, body colour, pronotum shape, size and density of pronotal punctures and density of elytral punctures. Shaw (1957), whose study was based almost entirely on reexamination of Broun's specimens at the British Museum of Natural History (BMNH), primarily used characters such as shape and density of pronotal punctures to synonimize and to delineate species (Table 2.2). However, the recent general consensus has been to treat the taxonomy of *Eucolaspis* as unresolved, partly due to the wide phenotypic variation among New Zealand populations (Kay, 1980; Hutcheson, 1992; Dugdale & Hutcheson, 1997; Rogers et al., 2007; Doddala et al., 2010).

2.1.2. Research objectives

In this Chapter, I address the issue of taxonomic complexity in the genus *Eucolaspis* Sharp 1886 with the following specific research questions:

1) How many species of *Eucolaspis* exist in New Zealand? How do these relate to each other and to other New Zealand and international Eumolpinae genera?
2) Which species of *Eucolaspis* occur where and on what host plants in New Zealand?
3) Which species of *Eucolaspis* infest(s) apples in Hawke's Bay, New Zealand?

2.2. Materials and methods

2.2.1. Fresh insect sampling

Adult *Eucolaspis* insects were collected from various New Zealand locations and host plants (Table 2.3 and Figure 2.1) by beating the plants' branches onto a drop sheet. The insects collected were preserved in 95% alcohol and transferred to the lab at

Massey University, Palmerston North. Some insects were brought alive to the lab, these were later frozen at -80 ^{0}C. Data on the host plant and geographic location (GPS coordinates) were recorded with these specimens.

Table 2.3 Details of fresh samples of *Eucolaspis* collected in New Zealand.

Collection locality	Host plant	New Zealand Ecological region*	Sample code	Latitude	Longitude
Ashley Clinton	Blackberry	Leeward Districts	AC2	-39.9327367	176.299615
Awaroa	Manuka	Windward Districts	Aw5	-40.85589	172.995186
Gisborne	Manuka	Leeward Districts	Gi5	-38.856333	177.903722
Golden Bay	Manuka	Windward Districts	Go5	-41.116667	173
Golden Bay	Wineberry	Windward Districts	Go9	-41.016667	173.016667
Hamilton	Blueberry	Northern North Island	Ha3	-37.783333	175.266667
Havelock North	Apple	Leeward Districts	HN1	-39.638678	176.790833
Havelock North	Linden	Leeward Districts	HN4	-39.627342	176.892972
Havelock North	Manuka	Leeward Districts	HN5	-39.627342	176.892972
Huka Falls	Kanuka	Central volcanic plateau	Hu7	-38.649363	176.089768
Kaikoura	Manuka	Leeward Districts	Kk5	-42.4	173.666667
Kerikeri	Blueberry	Northern North Island	Ke3	-35.216667	173.933333
Kuaotunu	Mixed+	Northern North Island	Ku8	-36.7547444	175.7278293
Kuaotunu	Manuka	Northern North Island	Ku5	-36.7547444	175.7278293
Manaia	Manuka	Northern North Island	Ma5	-36.8537239	175.452517
Mokoia Island	Blackberry	Central volcanic plateau	MI2	-38.066667	176.283333
Motutere	Kanuka	Central volcanic plateau	Mo7	-38.8938408	175.950552
Mt Ruapehu	Manuka	Central volcanic plateau	MR5	-39.183333	175.55
Nelson	Apple	Windward Districts	Ne2	-41.308374	173.121185
Omaio	Manuka	Northern North Island	Om5	-37.8484122	177.5887357
Orere	Kanuka	Northern North Island	O-M7	-36.9959616	175.2679749
Palmerston North	Apple	Windward Districts	PN1	-40.369705	175.601574
Palmerston North	Blackberry	Windward Districts	PN2	-40.413202	175.662503
Port Puponga	Manuka	Windward Districts	PP5	-40.566667	172.6
Rotoma	Blackberry	Central volcanic plateau	Ro2	-38.0571129	176.6438101
Tairua	Mixed+	Northern North Island	Ta8	-37.1721595	175.8498838
Tairua	Kanuka	Northern North Island	Ta7	-36.9774049	175.8391513
Torere	Mixed+	Northern North Island	To8	-37.9186089	177.5039606
Tuai	Blackberry	Axial Ranges	Tu2	-38.659778	177.06665
Waihau Bay	Kanuka	Northern North Island	Wa7	-37.6598527	177.8175447
Waikanae	Blackberry	Leeward Districts	Wk2	-40.866667	175.066667
Waikawa	Manuka	Windward Districts	Ww5	-41.2905398	174.0401655
Waitaia Bay	Kanuka	Northern North Island	Wt7	-36.774092	175.72134
Whiritoa	Manuka	Northern North Island	Wh5	-37.3149303	175.882591
White Pine bush	Blackberry	Central volcanic plateau	WP2	-38.0129026	176.9480455

*Data source for assigning ecological regions: http://www.teara.govt.nz/en/ecoregions/1/1 (McGlone, 2011)

+Mixed vegetation of manuka, kanuka and blackberry

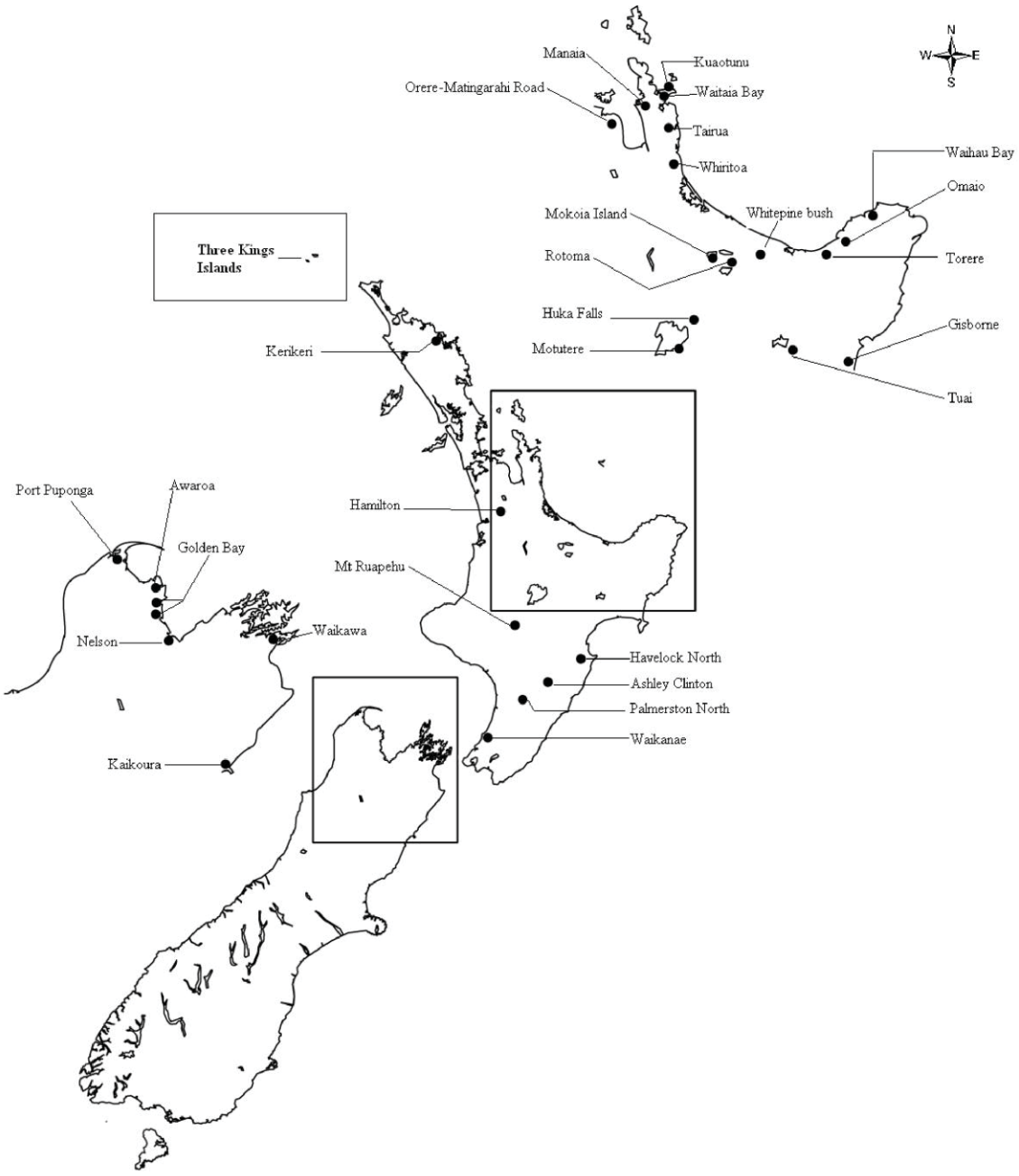


Figure 2.1 New Zealand collection localities of *Eucolaspis* beetles used in this study. Each locality is represented by a dark circle on the map. Inset maps show details of more intensely sampled regions. Collection locality for NZAC specimens of *E.* sp. n. *triregia*, i.e., Three Kings Islands is also shown (enclosed in a rectangle).

2.2.2. Museum specimens

Extensive collections of *Eucolaspis* specimens held by the New Zealand Arthropod Collection (NZAC, Landcare Research Ltd., Auckland) were also utilized in this study. Representative individuals of each of the named species in the collection including type specimens (whenever available) were loaned from NZAC and studied at Massey University, Palmerston North. Specimens of *Atrichatus ochraceus*, *A. aenicollis*, *Peniticus* sp. and *Pilacolaspis* sp. were loaned from Entomology Research Museum (LUNZ) at Lincoln University, Lincoln, NZ to use as outgroups and to study relationships among New Zealand Eumolpinae genera.

2.2.3. External morphology

A minimum of 1 and a maximum of 10, but usually ~5 insects (fresh and / or museum) were randomly selected from each location sample to study morphology. Measurements of 10 different external morphological characters (Table 2.4) were recorded for all selected beetle specimens by photographing with a digital camera (Moticam 2000 2.0 MP USB 2.0; Motic Group Co., Ltd.) fitted to a dissecting microscope (Zeiss Stemi 2000-c; Carl Zeiss, Inc.). Motic® Images Plus v.2.0 (Motic Group Co., Ltd.) was used to record measurements from the photographs (e.g. Figure 2.2).

Table 2.4 External morphological characters measured for morphometric relationships among *Eucolaspis*.

Character code	**Character description (units of measurement)**
LEN	Body length (mm)
WID	Body width (mm)
AN-LEN	Antennae length (mm)
EL-LEN	Right elytron length (mm)
EL-WID	Right elytron width (mm)
PR-LEN	Pronotum length at the median line i.e., above scutellum (mm)
HD-PUN	Puncture density on head (number / mm^2)
PR-PUN	Puncture density on right side half of pronotum (number / mm^2)
AEL-PUN	Puncture density on anterior half of right elytron (number / mm^2)
PEL-PUN	Puncture density on posterior half of right elytron (number / mm^2)

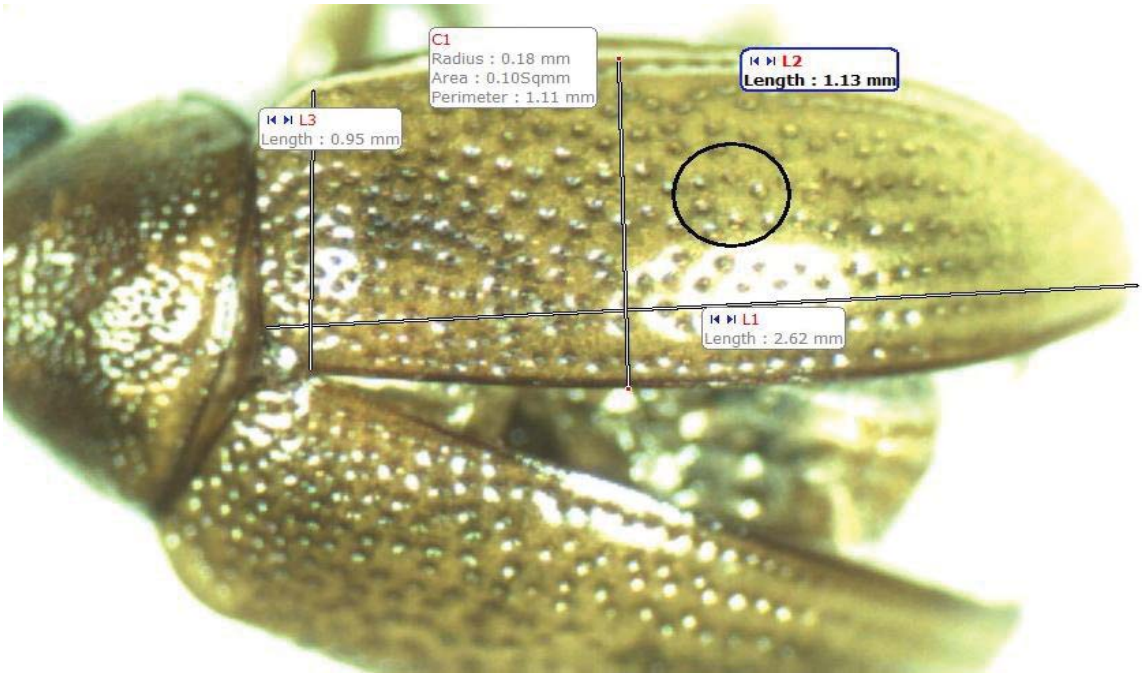


Figure 2.2 *Eucolaspis* beetle showing the measurements of the elytron. The left vertical grey line indicates elytron width; the horizontal grey line indicates elytron length; the right vertical grey line indicates the line of separation between anterior and posterior elytron; the dark circle represent a 0.1 mm^2 area of posterior elytron within which punctures density was recorded.

2.2.4. Internal morphology

The morphology (structure, shape and size) of internal genitalia was studied for the male beetles among the randomly selected fresh insects used for external morphology. Individual beetles were soaked in cold 10% potassium hydroxide (KOH) for 12 hours, rinsed thoroughly in 70% ethanol followed by further rinsing in dH_2O, and then soaked for an hour in hydrogen peroxide (H_2O_2) (Gurney et al., 1964). The cleared specimens (Figure 2.3) were then observed under a dissecting microscope and measurements were taken as described in the above section using Motic® Images Plus software. The procedure was repeated for the specimens that were not sufficiently cleared for observations of genitalia. For some of the insects, particularly from samples in which fewer than 5 individuals were collected at a location, clearing of genitalia was achieved by tissue digestion using a DNA extraction protocol (see DNA extraction).

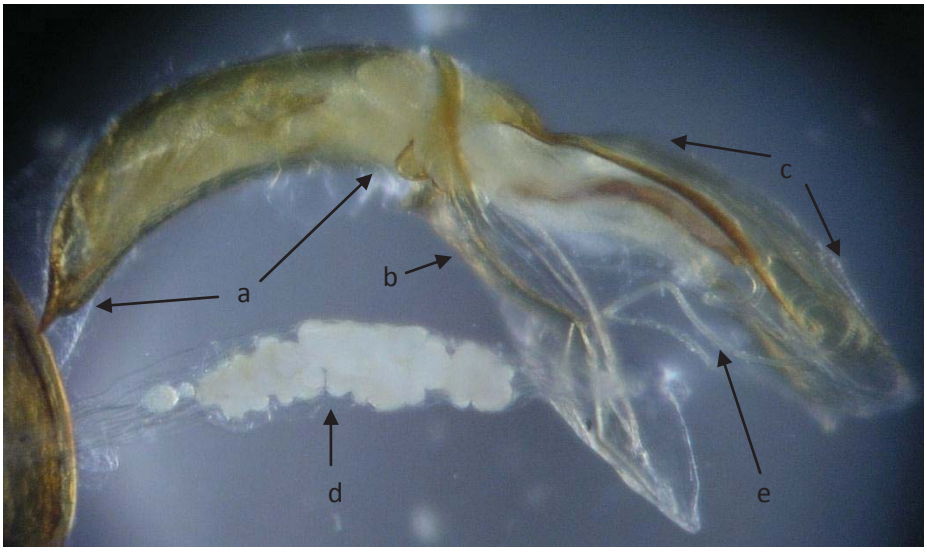


Figure 2.3 Aedeagus from a male *Eucolaspis* beetle collected on apple in Hawke's Bay, New Zealand (a-aedeagus proper; b-tegmen; c-basal hood; d-ejaculatory sac; e-median ejaculatory duct).

2.2.5. DNA extraction

Total genomic DNA was extracted from the randomly selected individuals from each of the freshly collected samples and also from some museum (NZAC and LUNZ) specimens. For fresh samples in which there were more than 5 insects in the sample, 3 legs from each selected individual were excised using fine scissors and used for DNA extraction. For all other samples (both fresh and museum), entire individuals were used for DNA extraction. From beetle legs DNA was extracted using a Salting-out extraction method (Sunnucks & Hales, 1996). The excised legs from each insect were put in separate 1.5 ml micro-centrifuge vials, crushed in 10 µL of protinase K enzyme using a plastic pestle, 300 µL of TNES buffer added [20 mM EDTA (ethylene diamine tetra acetic acid), 50 mM TrisHCl, 400 mM NaCl (sodium chloride) and 0.5% SDS (sodium dodecyl sulphate)] and incubated at 55 ^{0}C and 200 rpm in an oven for at least 24 h. The samples were checked and vortexed every few hours. The tubes were unloaded after ~24 h, and 85 µL of 5 mM NaCl was added to each tube, mixed well for 15 seconds and centrifuged at 13000 rpm for 5 minutes. The supernatant from each of these centrifuged tubes was carefully (without any precipitate) individually pipetted into new tubes. Then, 400 µL of ice cold 100% ethanol was added and allowed to stand at -20 ^{0}C for ~6 h to overnight. The tubes were centrifuged at 14000 rpm for 30 minutes, the ethanol was removed and the pellet was washed with 400 µL of 70% ethanol. The tubes were then centrifuged for 5 minutes at 13000 rpm, the ethanol was removed by pouring off and the

pellet was dried by keeping the tubes on a heat block set at 37 ^{0}C for up to 1 h. Once the pellet was completely dried of ethanol, the DNA was re-suspended in ~30 µL of Milli-Q® H_2O (Millipore corp.) and stored at -20 ^{0}C.

The QIAGEN® DNeasy® blood and tissue kit (QIAGEN N.V.) was used for DNA extraction from entire specimens following the manufacturer's protocol except that, at the elution step, only 30-50 µL (instead of 200 µL) of buffer AE (a proprietary elution buffer of QIAGEN®) were used, to avoid over-dilution of the final DNA. Extraction of DNA from dry museum specimens was carried out in a separate dedicated Ancient DNA laboratory, at Massey University, Palmerston North.

DNA extracted was checked for quantity and quality by gel-electrophoresis and spectrophotometry (NanoDrop; Thermo Fisher Scientific Inc.). One percent agarose gels with SYBR® Safe DNA gel stain (Life Technologies Corp.) in TAE buffer (Tris, glacial acetic acid, EDTA and H_2O) were used for electrophoreses. Two µL of each whole genomic DNA extract along with 2 µL of loading dye was loaded from ice into the agarose gel, run for about 40 minutes at 100 V and 400 A of AC electricity. DNA samples that had more than 10 ng/µL of DNA concentration were diluted before amplification, so that only 5-10 ng/µL concentration of DNA was used.

2.2.6. DNA amplification and sequencing

A fragment of mitochondrial DNA of ~700 base pairs (bp) length at the Cytochrome Oxidase I (COI) locus was amplified by polymerase chain reaction (PCR) using universal insect primers, LCO1490: 5'-GGTCAACAAATCATAAACATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Figure 2.4) (Folmer et al., 1994).

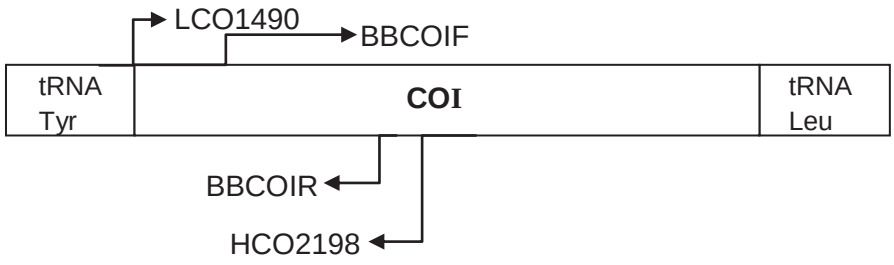


Figure 2.4 Cytochrome oxidase subunit I gene showing approximate positions and extension directions of the primers used in this study.

For some of the DNA extractions from museum specimens where longer fragments could not be amplified, a smaller fragment (~350 bp length) was also targeted using specially designed primers, BBCO1F (5'-TGACTRCTRCCCCCGTCATT-3') and BBCO1R (5'-GGRTCWCCWCCTCCKGCAGGRTC-3') (Figure 2.4). These taxon-specific PCR primers to target shorter fragments were designed in Geneious Pro v.5.5 (Biomatters Ltd., Auckland) using alignments of the COI nucleotide sequences obtained from modern specimens.

A 10µL reaction protocol was employed for polymerase chain reactions. Each 10µL reaction consisted of 3.3µL of Milli-Q® H_2O, 1.0µL of dNTPs mix (0.2 µL of each 2mM dNTP), 1.0µL of 10x PCR buffer, 0.8µL of 25mM $MgCl_2$, 0.4µL of each 10µM primer, 2.0µL of betaine, 0.1µL *Taq* DNA polymerase enzyme (500U) (F. Hoffmann-La Roche Ltd.) and 1.0µL of the extracted DNA. PCRs were carried out in a Biometra T3000 thermocycler (Biometra GmbH) using the conditions: 94 ^{0}C for 2 minutes (initial denaturation), 40 cycles (50 cycles for some of the smaller fragments of ancient DNA) of 94 ^{0}C for 30 s (denaturation), 52 ^{0}C for 30 s (annealing), 72 ^{0}C for 1 minute 30 s (primer extension) and 72 ^{0}C for 8 minutes (final extension). The quantity and quality of PCR products were checked using 1% TAE agarose gel electrophoresis with the Invitrogen™1 kb plus DNA ladder (Life Technologies Corp.).

Prior to sequencing, PCR products obtained through amplification of desired fragments were purified using the SAP (Shrimp Alkaline Phosphase) / EXO1 (Exo nuclease I) digest protocol. Incubation of PCR products, SAP and EXO1 enzymes was at 37 ^{0}C for 30 minutes followed by a denaturation at 80 ^{0}C for 15 minutes. Cleaned PCR products were sequenced from the 5' end using one of the forward primers (either LCO1490 or BBCOIF). Sequencing used Bid Dye Chemistry and an ABI3730 genetic analyser (Applied Biosystems Inc.).

In addition to these sequences, I obtained COI mtDNA and 18S rDNA sequences for other Eumolpinae genera from GenBank® (National Centre for Biotechnology Information – NCBI, USA) database.

2.2.7. Data analysis

Morphometric data were tested for normality using multivariate procedures. Differences between the two sexes in morphometrics were tested using t-test. Stepwise

discriminant analysis was used to identify the significant variables (observations) contributing to delineation of sample classes. Subsequently, canonical discriminant analysis was performed using the variables found by Stepwise discriminant analysis, to verify clustering of samples. Classification of samples was analysed by grouping data according to location, ecological region, host plant, genetic clade or genitalia shape. A 95% level of confidence was used as a significance level for all the statistical analyses. All analyses were performed using SAS v.9.2 (SAS Institute, 1992).

DNA sequences were checked using SEQUENCHER v. 4.2 (Gene Codes Corp., Michigan); ambiguous base calls were corrected manually and ambiguous end regions were trimmed. Sequences were aligned using Se-Al v2.0a11. Unique haplotypes were identified and sequence divergence was measured using DnaSP v.5 (Librado & Rozas, 2009); the haplotypes were aligned by Clustal-W using Geneious™ Pro (version 5.5) (Biomatters Ltd., Auckland) (Drummond et al., 2011). Phylogenetic analyses were conducted using MEGA (version 5.0) (Tamura et al., 2011) and Geneious™ Pro (with plugins for MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), PHYML(Guindon & Gascuel, 2003; Guindon et al., 2010) and PAUP*(Swofford, 2003)). The optimal models of nucleotide substitution were identified using jModeltest (version 0.1.1) (Posada, 2008). Evolutionary distances between lineages were calculated in MEGA 5. Species delimitation analyses were conducted in Geneious Pro (using the species delimitation plugin (Masters et al., 2011)).

2.3. Results

2.3.1. Morphology of adults in fresh samples

Body length varied from 2.69 mm to 4.45 mm (mean 3.56 mm) whereas body width varied from 1.54 mm to 3.16 mm (mean 2.14 mm) (n=135). Punctures were denser in the pronotum region than on elytra or on head regions in all the insects. Pronotal punctures density varied from 160 to 810 per mm^2, whereas head punctures density varied from 20 to 320 per mm^2. Elytra were rather sparsely punctured at 50 to 180 punctures per mm^2.

There was noticeable sexual dimorphism; in general, male beetles were significantly smaller and more slender than female beetles, but possessed longer antennae (Table 2.5). There was no significant difference between male and female beetles in the density of punctures on head, pronotum and elytra.

Table 2.5 Sexual dimorphism in *Eucolaspis* beetles collected in New Zealand (fresh samples), measured in terms of different morphological characters.

Morphological character	**Mean (S.E.) for ♀**	**Mean (S.E.) for ♂**	***t***	***p***
Body length (mm)	3.65 (0.0373)	3.44 (0.059)	**3.16**	**.002**
Body width (mm)	2.22 (0.0334)	2.03 (0.0443)	**3.44**	**<.001**
Elytra length (mm)	2.88 (0.0392)	2.61 (0.0561)	**4.10**	**<.001**
Elytra width (mm)	1.00 (0.0147)	0.93 (0.021)	**2.79**	**.006**
Antennae length (mm)	2.13 (0.0326)	2.66 (0.0576)	**-8.48**	**<.001**
Pronotum length (mm)	0.998 (0.0119)	0.95 (0.0193)	**2.11**	**.037**
Head punctures density (num. / mm^2)	185.7 (5.34)	171.1 (7.0)	1.69	.093
Pronotal punctures density (num. / mm^2)	418.8 (15.35)	417.5 (22.86)	0.05	.959
Anterior elytral punctures density (num. / mm^2)	116.5 (3.38)	114.4 (4.04)	0.40	.692
Posterior elytral punctures density (num. / mm^2)	100.8 (2.48)	99.6 (2.76)	0.31	.758

Morphological variation among different samples: All morphological characters measured except elytra length (EL-LEN) and anterior elytral puncture density (AEL-PUN) were found to contribute significantly to variation among different site samples. Density of pronotal punctures varied most among the samples (F (33, 100) = 7.98, p <.001). Morphometric relationships among individuals from different sites did not show any clear clustering (Figure 2.5). The greatest Mahalanobis distance between any two site samples was observed between sample HN1 (Hawke's Bay apple) and sample Ke3 (Northland blueberry) whereas the least Mahalanobis distance between any two site samples was observed between sample HN1 (Hawke's Bay apple) and HN4 (Hawke's Bay linden).

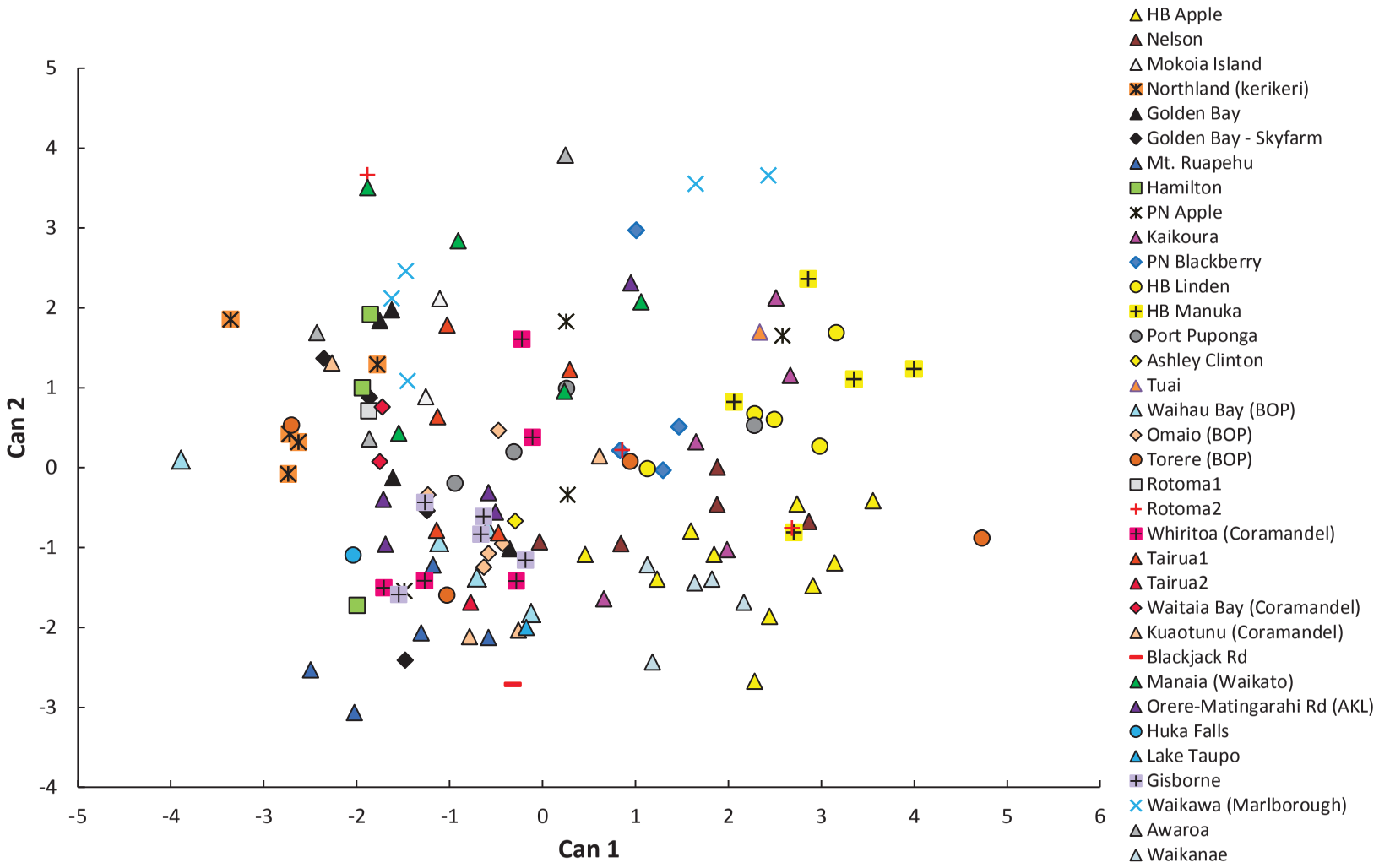


Figure 2.5 Morphometric relationships among specimens of *Eucolaspis* collected in New Zealand, coded by their collection locality. Data subjected to Canonical Discriminant analysis; Can1 and Can2 denote the two canonical variables that best describe the variation among samples.

Morphological variation among different ecological regions: "Axial ranges" ecological region was represented by a single sample locality in this study and hence this ecological region was removed from the analyses. The density of punctures on the pronotum (PR-PUN) and head (HD-PUN) were the only characters contributing significant variation among beetles of different ecological regions of New Zealand (STEPDISC procedure - stepwise discriminant analysis). Can1 (canonical variable 1: X-axis in Figure 2.6) explains about 91% of variation among the ecological regions. PR-PUN contributed most to separation along Can1, while HD-PUN contributed most to Can2.

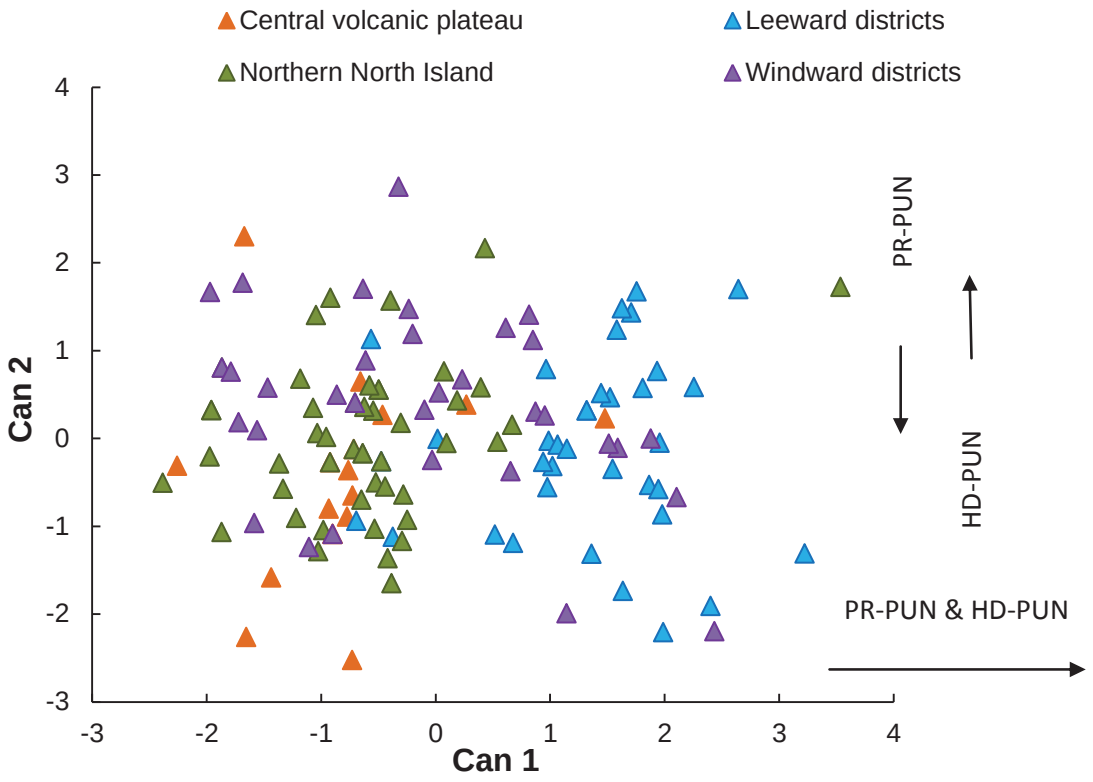


Figure 2.6 Morphometric relationships among *Eucolaspis* beetles collected from different ecological regions across New Zealand. Data subjected to Canonical Discriminant analysis; Can1 and Can2 denote the two canonical variables that best describe the variation among different samples. PR-PUN and HD-PUN represent puncture density on pronotum and head respectively; the direction of arrows indicates progression of values for these two characters.

Northern North Island and Central Volcanic Plateau samples were morphometrically similar to each other (p =.197). Leeward districts samples were more distant from any of the other regions (p <.001) (Figure 2.6).

Among host plants: Pronotal puncture density, pronotum length, body length and anterior elytral punctures density were the only characters, among the ten measured characters, that significantly delineated beetles from different host plants. Pronotal punctures density contributed most to variation than the other three characters (F (7, 123) = 12.3, p <.001). Can1 and Can2 together explain about 89% variation among the beetles from different host plants. Samples from apple and blackberry appeared to cluster together while samples from manuka were very diverse with no clustering (Figure 2.7).

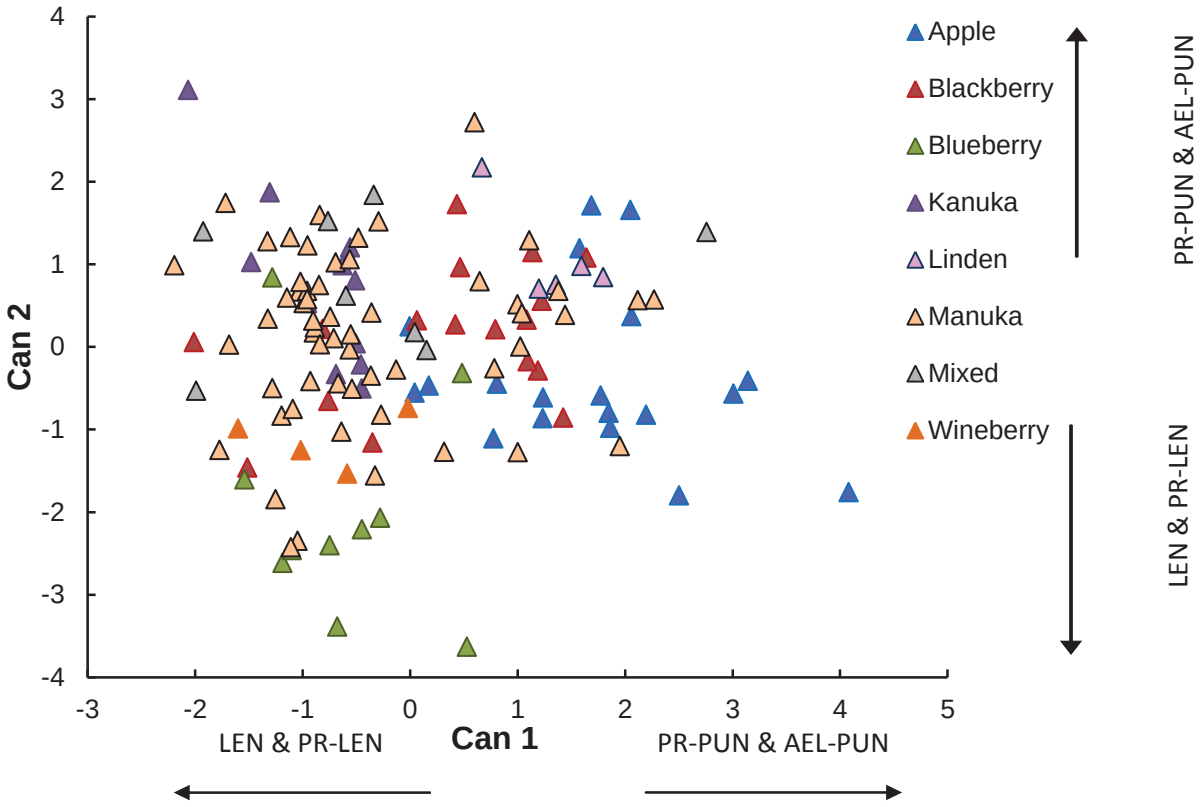


Figure 2.7 Morphometric relationships among *Eucolaspis* beetles grouped according to host plant they were collected on. Can1 and Can2 denote the two canonical variables that best describe the variation among different samples. LEN= body length; PR-LEN = pronotum length; PR-PUN = punctures density on the pronotum; AEL-PUN= punctures density on the anterior elytral region. Direction of arrows indicates progression of values for the characters along the X-axis.

Morphology of museum samples: Morphometric relationships among identified museum specimens (NZAC) showed no distinct clusters. Different species, such as *E. vittiger*, *E. colorata* and *E. brunnea* tended to overlap, suggesting poor phenotypic separation of "species" as currently recognised (Figure 2.8). Individuals assigned to *E. picticornis* appear to be highly variable morphologically and did not

cluster together. The four *E. montana* paratypes from Broun's collection varied considerably, highlighting the existing taxonomic instability (Figure 2.8).

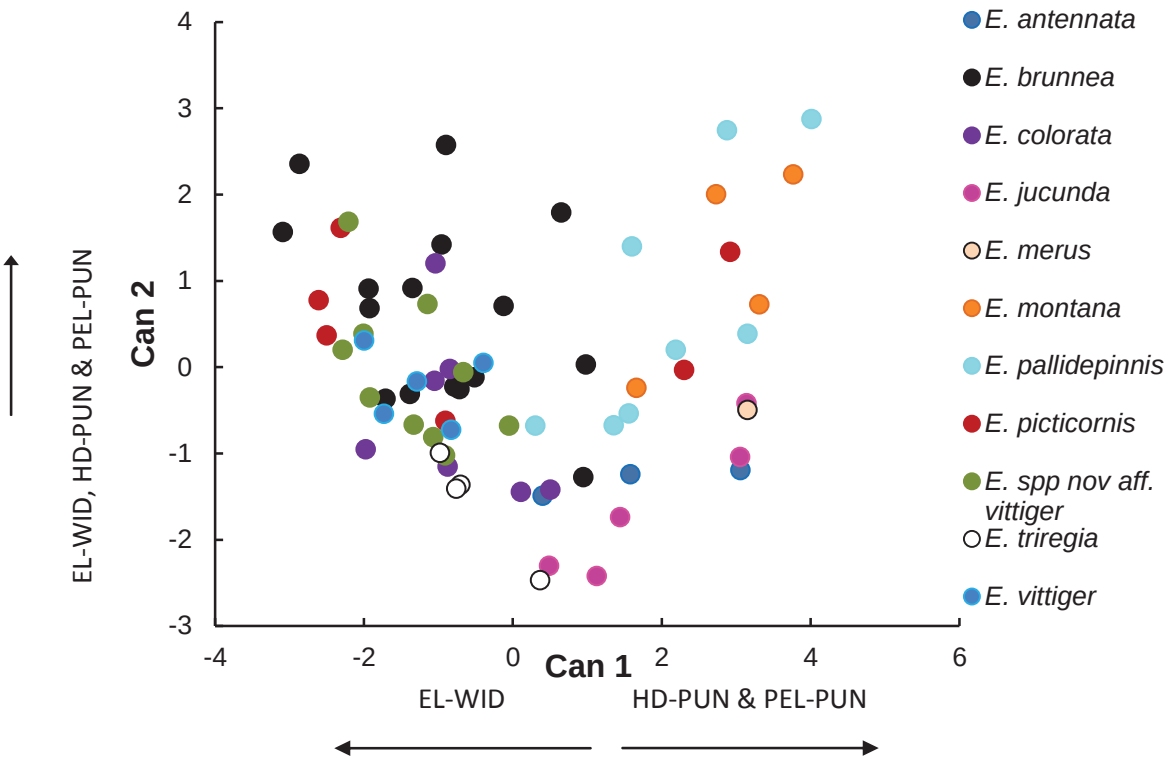


Figure 2.8 Morphometric relationships among identified voucher *Eucolaspis* specimens in NZAC collection. Can1 and Can2 denote the two canonical variables that best describe the variation among the samples independently diagnosed to species. EL-WID = Elytral width; HD-PUN = punctures density on the head; PEL-PUN= punctures density on the posterior elytral region. Direction of arrows indicates progression of values for these characters along the X and Y axes.

Among the ten morphological characters measured, only elytral width (EL-WID), puncture density on the head (HD-PUN) and puncture density on the posterior elytral region (PEL-PUN) contributed to significant variation among "species". PEL-PUN contributed to greater variation (F (10, 58) = 11.77, p <.001) than the other two characters. Can1 (X-axis in Figure 2.8) explains about 73% variation, whereas Can2 (Y-axis in Figure 2.8) explains about 19% variation among "species".

2.3.2. Molecular analysis

Mitochondrial DNA sequences were obtained from a total of 74 fresh *Eucolaspis* specimens. In addition to these, 2 sequences were obtained from *E.* sp. nov. *triregia* (NZAC) and 1 sequence each was obtained from *Atrichatus ochraceus, A. aenicollis* and *Peniticus* sp. (LUNZ).

The 74 mtDNA aligned COI sequences (617 bp) from mainland New Zealand *Eucolaspis* revealed 40 haplotypes. Haplotype diversity (Hd) was 0.97 and the standard deviation (S.D.) of Hd was 0.011. Nucleotide diversity per site (Pi) was 0.06335 and the S.D. of Pi was 0.0046. The final alignment (617 bp) contained 129 variable positions and 97 parsimony informative sites (see Appendix for complete list of variable positions). Reconstructed phylogeny of these haplotypes, using *E. triregia* as an outgroup, showed three well supported distinct lineages in the ingroup. Trees constructed by different methods (Minimum Evolution, Maximum Likelihood and Bayesian inference) yielded the same results in terms of these three clades, with minor variation in the placement of haplotypes within clades (Clade1: Yellow; Clade2: Orange; Clade3: Green) (Figures 2.9 and 2.10).

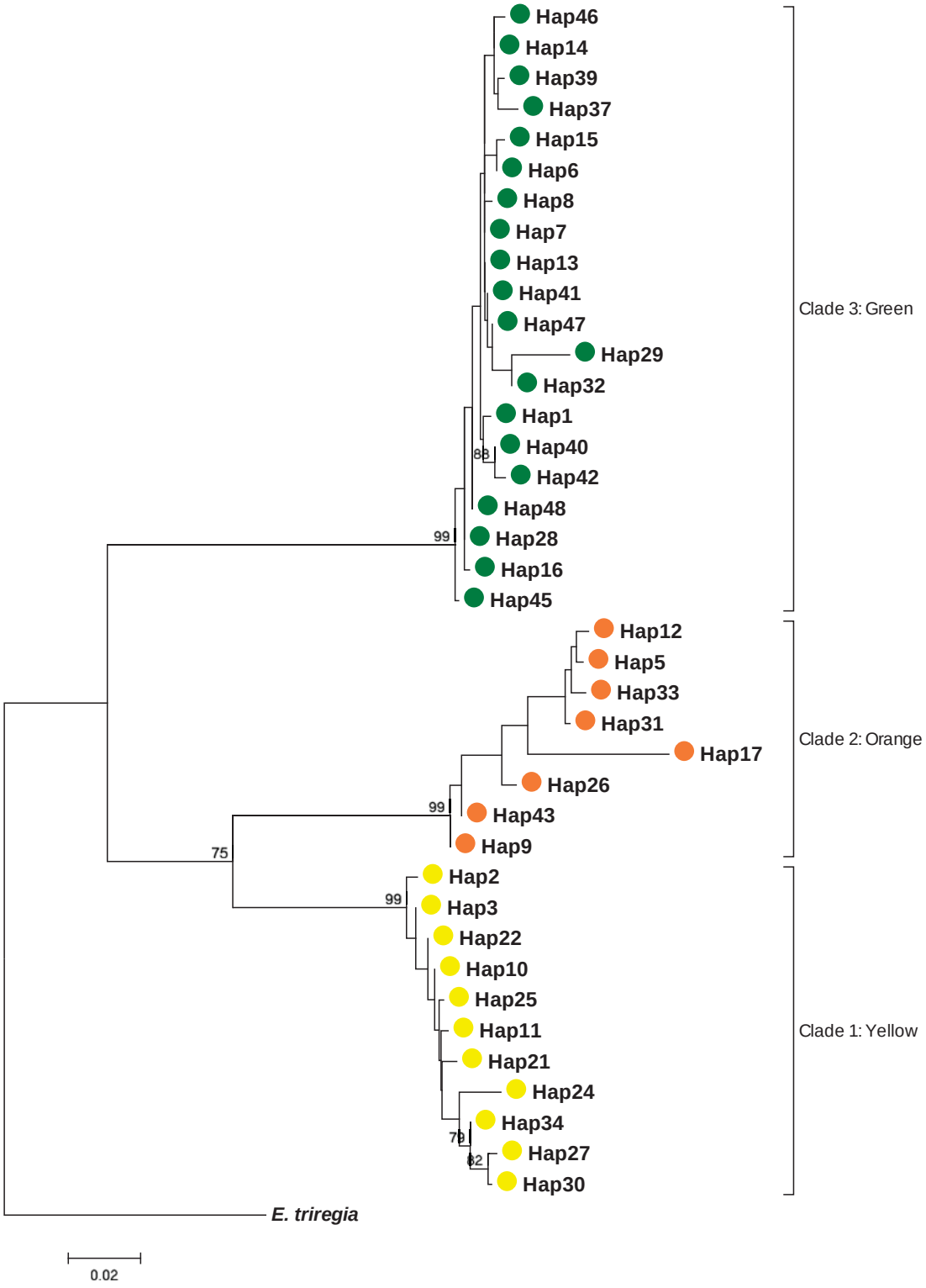


Figure 2.9 Bootstrap consensus tree of New Zealand *Eucolaspis* COI sequences constructed by Minimum Evolution method in MEGA5 using K2+G model. SBL = 0.50061533. Branch labels denote proportion (%) of tree occurrences in total of 500 replicates (consensus support values <75% are not shown). Tip labels indicate corresponding haplotypes and the colouring of nodes indicates respective lineage.

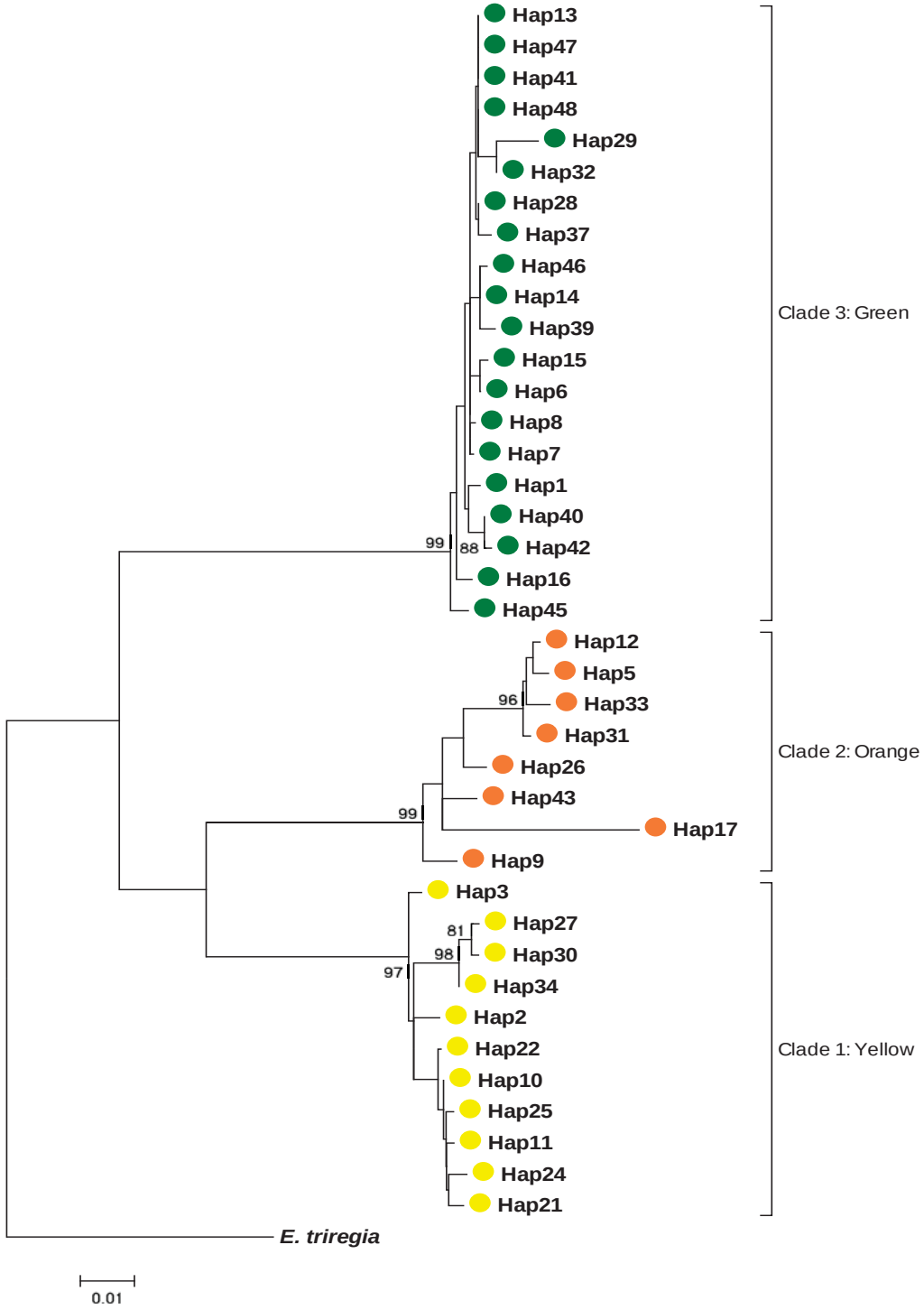


Figure 2.10 Bootstrap consensus tree of New Zealand *Eucolaspis* COI sequences constructed by Maximum Likelihood method in MEGA5 using GTR+G+I model. Log Likelihood=-2056.46; SBL=0.38881496. Branch labels denote proportion (%) of tree occurrences in total of 500 replicates (consensus support values <75% are not shown). Tip labels indicate corresponding haplotypes and the colouring of nodes denotes respective lineage.

Overall, the mean genetic distance (p-distance ± Standard Error) among haplotypes was 0.062 ± 0.006. Clade 2 (Orange) had the highest within-group mean genetic distance (0.018 ± 0.004) compared to the other two clades (Clade 1: 0.011 ± 0.003; Clade 3: 0.006 ± 0.001). Intra-clade pairwise genetic distances ranged from 0.1% to 4.3% whereas inter-clade pairwise genetic distances ranged from 7.9% to 13.7% (Figure 2.11) (see Appendix for complete list of pairwise P-distances). Mean genetic distance between clades varied from 7.4% (clades 1 and 2) to 10% (clades 1 and 3) (Table 2.6). Clades 1 and 2 were genetically nearer to each other than to clade 3.

Table 2.6 Estimates of net evolutionary divergence between groups of sequences: measured as the number of base differences per site between groups of sequences.

Clade	**P-distance ± S.E. (lower) and ML distance* ± S.E. (upper)**		
	1	**2**	**3**
1(Yellow)		0.133 ± 0.028	0.199 ± 0.045
2(Orange)	0.074 ± 0.010		0.213 ± 0.048
3(Green)	0.096 ± 0.012	0.100 ± 0.012	

*using K2+G model.

Species delimitation analysis conducted on a Bayesian inference tree, confirmed the monophyly of the three lineages. Mean intra-clade (intra-species) distance was 0.014, whereas mean inter-clade (inter-species) distance at closest point was 0.92 (Table 2.7).

Table 2.7 Species delimitation results of phylogenetic tree constructed by Bayesian inference method. Intra Dist = mean pairwise tree distance between member of the focal species.

"Species"	**Closest "species"**	**Intra Dist**	**Inter Dist closest**	**P ID (strict) (95% CI)**	**Av (MRCA-tips)**	**P (randomly distinct)**
1(Yellow)	2	0.013	0.108	0.94 (0.87, 1.0)	0.0089	0.67
2(Orange)	1	0.021	0.108	0.86 (0.75, 1.0)	0.0139	0.63
3(Green)	1	0.009	0.129	0.97 (0.92, 1.0)	0.0060	0.05

Inter Dist closest = mean pairwise tree distance between the members of the focal species and members of the next closest species; P ID(strict) = mean probability of correctly identifying an unknown specimen of the focal species using placement on a tree sequence; Av (MRCA) = mean distance between the most recent common ancestor of a species and its members; P (randomly distinct) is the probability that a clade has the observed degree of distinctiveness due to random coalescent processes.

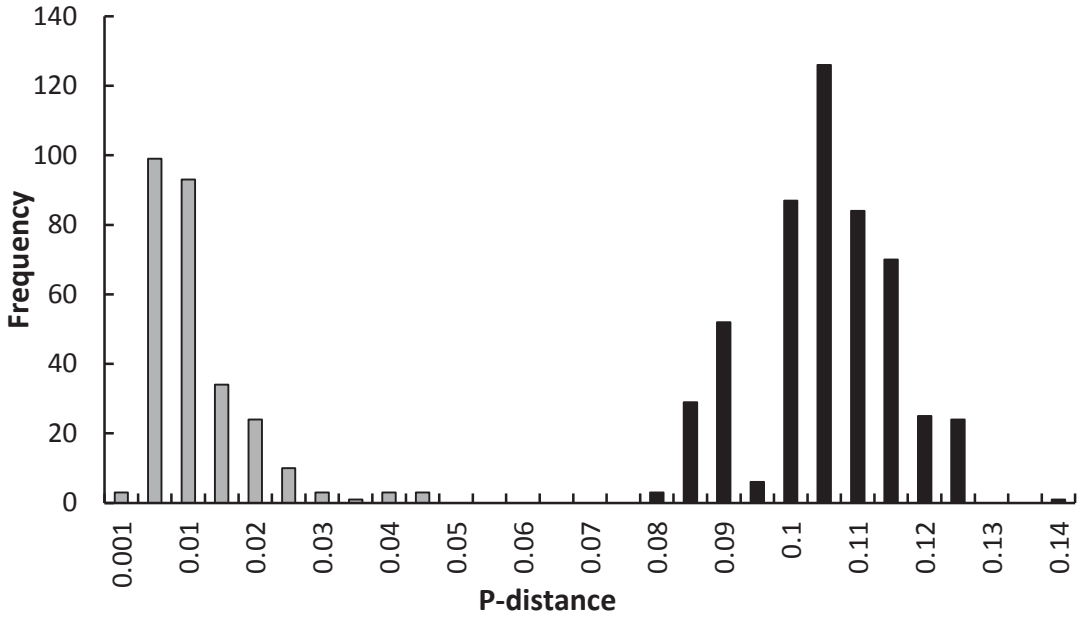


Figure 2.11 Frequency distribution of pairwise genetic distances (number of nucleotide base differences per site between sequences) among New Zealand *Eucolaspis* haplotypes; grey bars represent pairwise distances for sequences within a clade whereas black bars represent pairwise differences for sequences between clades.

2.3.4. Male genitalia

Three forms of aedeagei were found in a sample of 55 male beetles. The three types differed primarily in the shape of the tip of aedeagus proper (Figure 2.12).

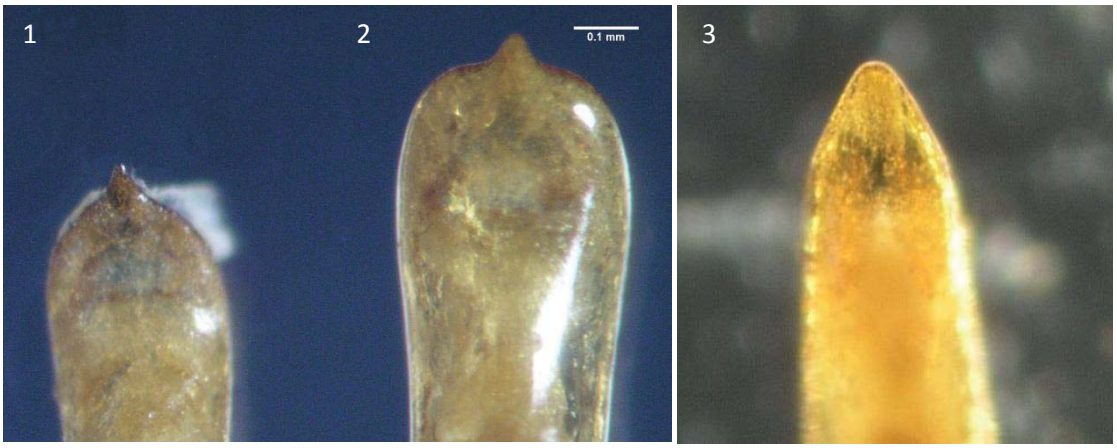


Figure 2.12 Three forms of aedeagus found among mainland New Zealand *Eucolaspis* (1-pointed, 2-shouldered, 3-blade).

Type 3 (blade) aedeagus appears very different from the other two types, lacking a well-defined beak (tip). Individuals that belonged to mtDNA genetic Clade 1 possessed type 1 (pointed) aedeagei, individuals of Clade 2 possessed type 2

(shouldered) and individuals of Clade 3 possessed type 3 aedeagei (blade). An exception to this was found in beetles from a single locality in Nelson (collected on apples) (n=2), which had type 1 (pointed) aedeagei but genetically belonged to Clade 3. Clades 1 and 2 were genetically most similar to one another, and their aedeagei appeared to be relatively similar (Figure 2.12).

2.3.5. Integration of morphometrics with genetic data and genitalia data

Four characters (PR-PUN, HD-PUN, AEL-PUN and EL-WID), out of total 10 measured characters, differed significantly among beetles belonging to different haplotype clades (stepwise discriminant analysis). Contrary to the genetic data where clades 1 and 2 were more similar (Tables 2.6 and 2.7), in morphometrics clades 2 and 3 were more similar (squared Mahalanobis distance = 5.4; p <.001) than clades 1 and 2 (squared Mahalanobis distance = 18.7; p <.001) (Figure 2.13). Can1 (X-axis in Figure 2.13) explains 86.4% variation among the clades, while Can2 (Y-axis in Figure 2.13) explains 13.6% variation. Pronotal punctures density (PR-PUN) contributed the highest variation between clades (F (2, 127) = 120.27; p <.001). Punctures density (PR-PUN, HD-PUN and AEL-PUN) is higher among indviduals on positive side of X-axis (mostly Clade 1) while elytral width (EL-WID) is higher among individuals on the negative side of X-axis (Clades 2 and 3) (Figure 2.13). On the Y-axis this is vice-versa.

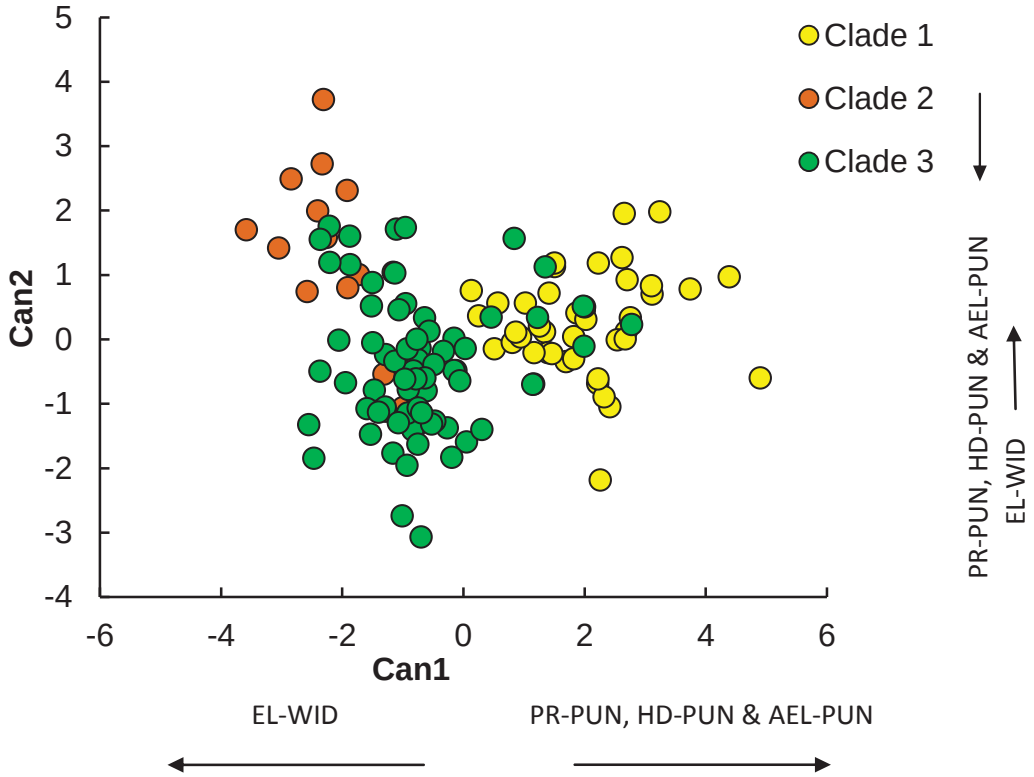


Figure 2.13 Morphometric relationships among the three mainland New Zealand genetic lineages of *Eucolaspis*. Can1 and Can2 denote the two canonical variables that best describe morphometric variation among the lineages. EL-WID = elytra width; PR-PUN = punctures density on the pronotum; HD-PUN = punctures density on the head; AEL-PUN = punctures density on the anterior elytral region. Direction of arrows indicates progression of values for these characters along the axes.

When morphometric data of only male *Eucolaspis* beetles were analysed by grouping individuals according to genitalic type, males clustered into three groups consistent with their aedeagus type, although there were a few outlier individuals (Figure 2.14). Elytra length (EL-WID), pronotum length (PR-LEN) and punctures density on the pronotum (PR-PUN) were the only significant characters, among the ten morphometric characters measured, that differed among the aedeagus types (stepwise discriminant analysis). Can1 (X-axis in Figure 2.14) explains 71% variation while Can2 (Y-axis in Figure 2.14) explains 29% variation. Males with aedeagus type 2 differed from the other two groups of males (squared Mahalanobis distance between 1 and 2 types = 27.12, p <.001; squared Mahalanobis distance between 2 and 3 types = 24.10, p <.001) especially in having longer elytra and pronotum and lesser density of punctures on pronotum. Males with aedeagei type 1 and 3 (squared Mahalanobis distance = 5.98, p <.001) mainly differed from one another in terms of puncture density on pronotum.

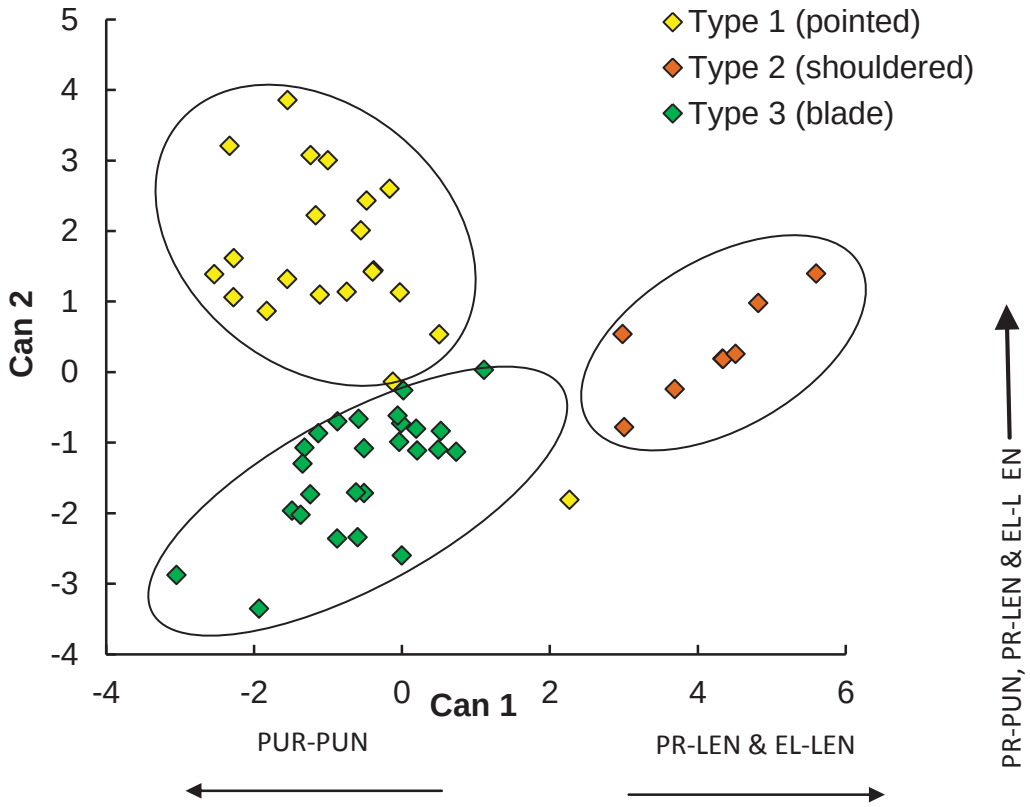


Figure 2.14 Morphometric relationships among male *Eucolaspis* beetles with different aedeagei types. Can1 and Can2 denote the two canonical variables that best describe morphometric variation among the three types. PR-PUN = punctures density on the pronotum; PR-LEN = pronotum length; EL-LEN = elytra length. Direction of arrows indicates progression of values for these characters along the axes.

2.3.6 Ecological and geographic associations of Eucolaspis lineages in New Zealand

Spatial distribution of the three *Eucolaspis* lineages showed a clear demarcation between East and West, with clade 1 (Yellow) occupying mostly Eastern areas (Figure 2.15). In some of the samples two clades were represented within the same location sample, e.g., Golden Bay, Waikato, Manaia (Clades 2 and 3), Rotoma (Clades 1 and 3) and Tuai (Clades 1 and 2), whereas at Torere - Bay Of Plenty, New Zealand all three clades were represented in a single location sample of 5 individuals. Clade 2, which has more within-clade genetic diversity than the other two clades, has diverse geographic distribution and appears to occur mostly in sympatry with either or both of Clade 1 and 2.

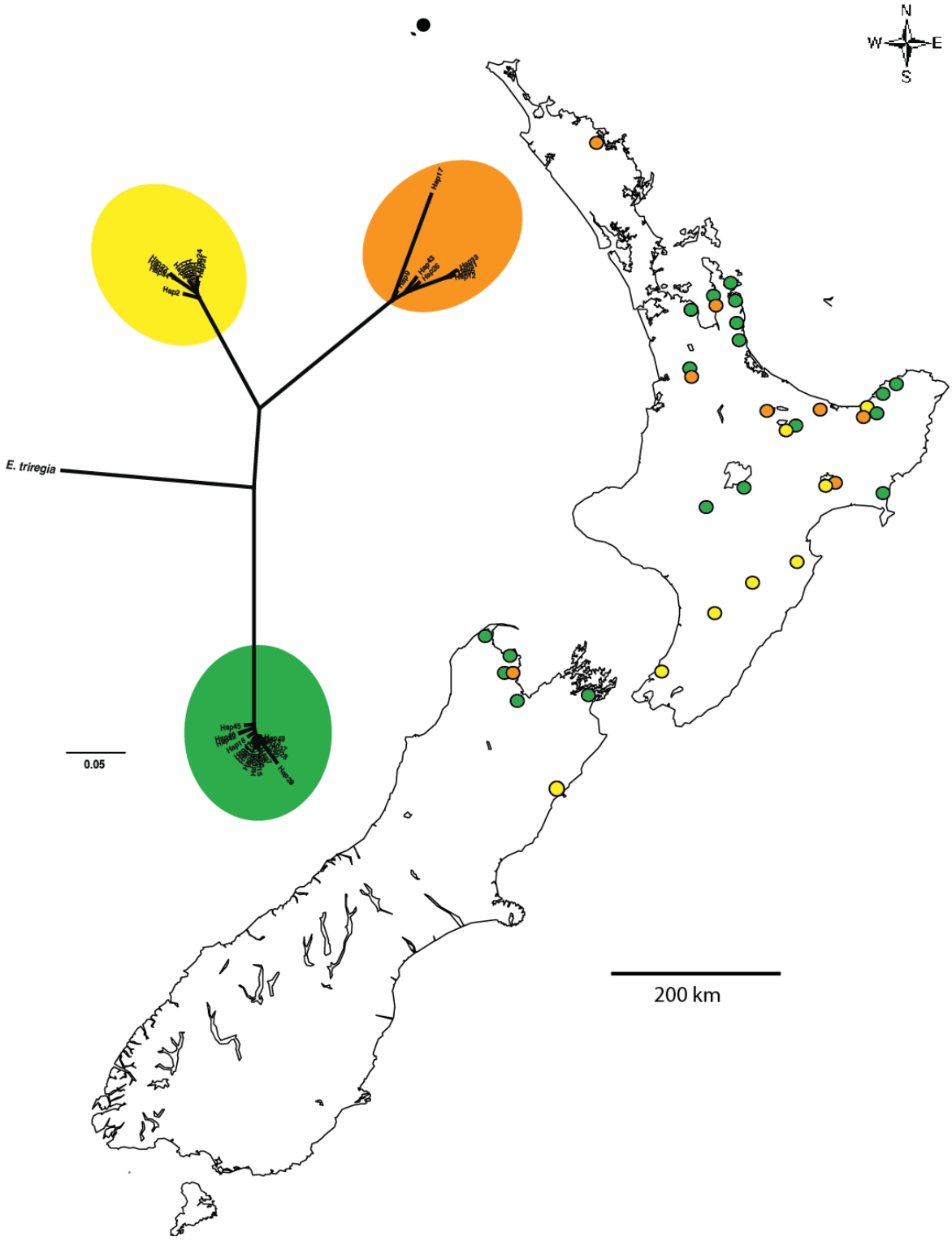


Figure 2.15 Geographical distribution of *Eucolaspis* lineages in New Zealand. Each location point on the map represent a sampling site and colour codes denote respective clades (Yellow: Clade1, Orange: Clade 2, Green: Clade 3 and Black: *E.* n. *triregia*).

Among the host plants sampled, blackberry and manuka are used by beetles of all the three mainland New Zealand *Eucolaspis* clades while apple is found to be used by only Clades 1 and 2 (Table 2.8). Kanuka and wineberry are used by only Clade 3.

Table 2.8 Host plant usage by *Eucolaspis* clades in mainland New Zealand

	Apple	Blackberry	Blueberry	Linden	Manuka	Kanuka	Wineberry
Clade 1	✓	✓		✓	✓		
Clade 2		✓	✓		✓		
Clade 3	✓	✓	✓		✓	✓	✓

2.3.7 Relationship with other New Zealand and international Eumolpinae genera

A total of 9 COI sequences corresponding to 9 different global genera were obtained from GenBank® (National Centre for Biotechnology Information – NCBI, USA) database. Along with these 9 genera, New Zealand's *Peniticus* sp., formed a sister group to a monophyletic lineage that included *Eucolaspis* (including *E*. sp. nov. *triregia*), *Atrichatus ochraceus* and *A. aeneicollis* clades, when Bayesian inference method was used to construct phylogeny (Figure 2.16). Other New Zealand genera in the subfamily Eumolpinae appear to be closely related to the *Eucolaspis* clades, however, *Peniticus* was clearly more distant to *Eucolaspis* than *Atrichatus*, and indeed the current data yield a polytomy comprising *Eucolaspis* and *Atrichatus.*

Phylogeny was also reconstructed for COI region of mtDNA using the Maximum Likelihood method after pruning out the most distant global Eumolpinae genera; retaining only *Chrysochus auratus* and *Tymnes tricolor* of USA along with New Zealand Eumolpinae taxa. All *Eucolaspis* taxa including *E*. sp. n. *triregia* formed a monophyletic clade separate from *Atrichatus ochraceus*, *A. aenicollis* and *Peniticus* sp. (Figure 2.17).

In addition to the mtDNA data, a total of 68 sequences for 18S rDNA gene were obtained from GenBank® (NCBI, USA) database (please see appendix for GenBank® accession numbers), corresponding to different Eumolpinae taxa worldwide, including two species of New Zealand *Eucolaspis* (*E. jucunda* and *E. picticornis*) that were sequenced by BMNH scientists (unpublished data). A phylogenetic tree constructed using the Maximum Likelihood method of these 68 18S sequences, placed *E. jucunda* and *E. picticornis* along with another unidentified Eumolpinae taxon from New Caledonia into a well supported separate clade (Figure 2.18). This lineage branching

highlights the theory that New Zealand *Eucolaspis* is loosely separated from other pacific Eumolpinae genera such as *Dematochroma* and *Colaspoides* (C. Reid, 2011, pers. comm.).

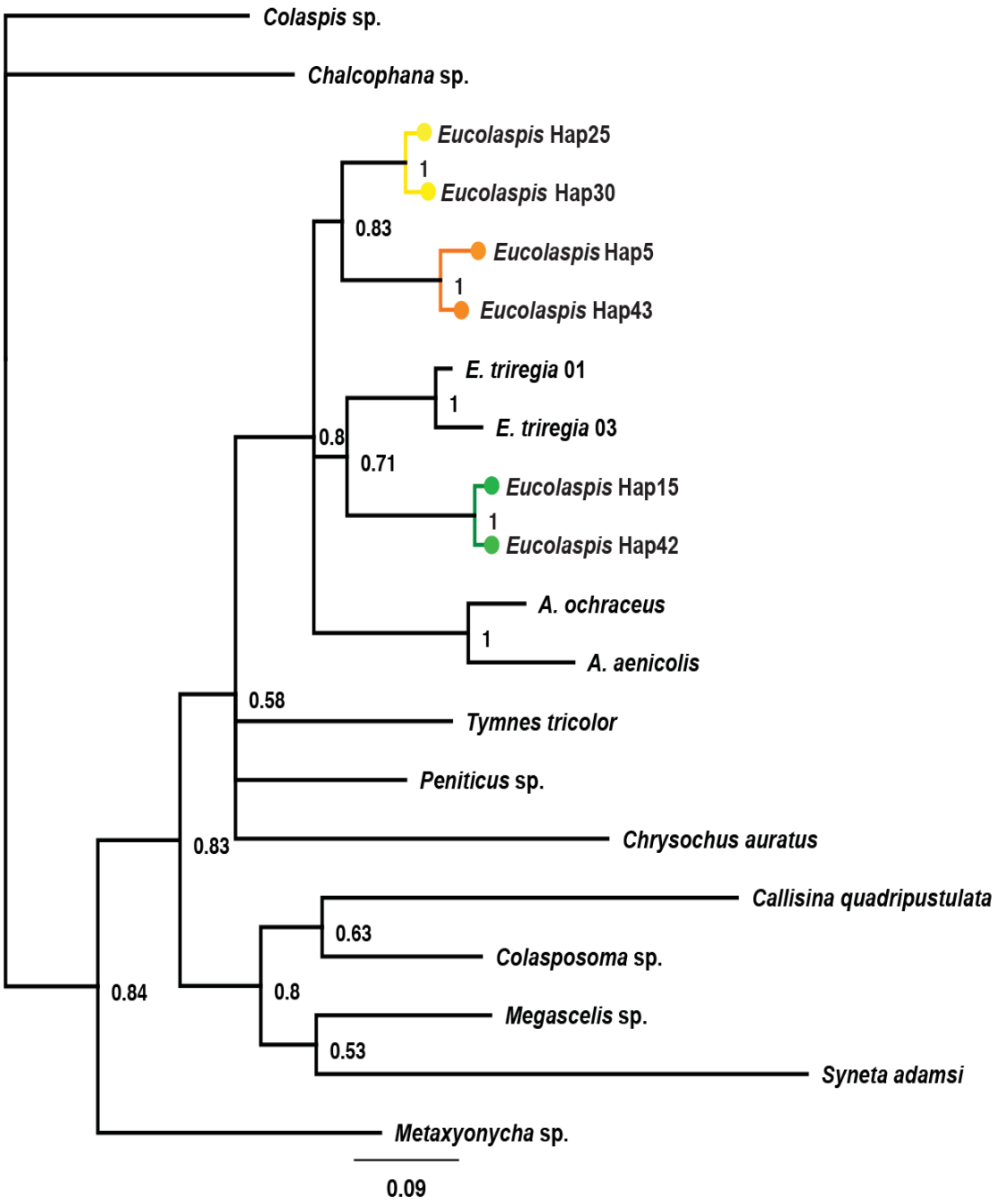


Figure 2.16 Phylogenetic tree (for COI region of mtDNA) of New Zealand and international Eumolpinae genera (Chrysomelidae) constructed by Bayesian inference method in Geneious with MrBayes plugin using GTR+G+I model. SBL= 3.617. Node labels indicate posterior probability support, and tip labels indicate corresponding taxon. Mainland New Zealand *Eucolaspis* clades are colour coded: Yellow- Clade 1; Orange- Clade 2; Green- Clade 3.

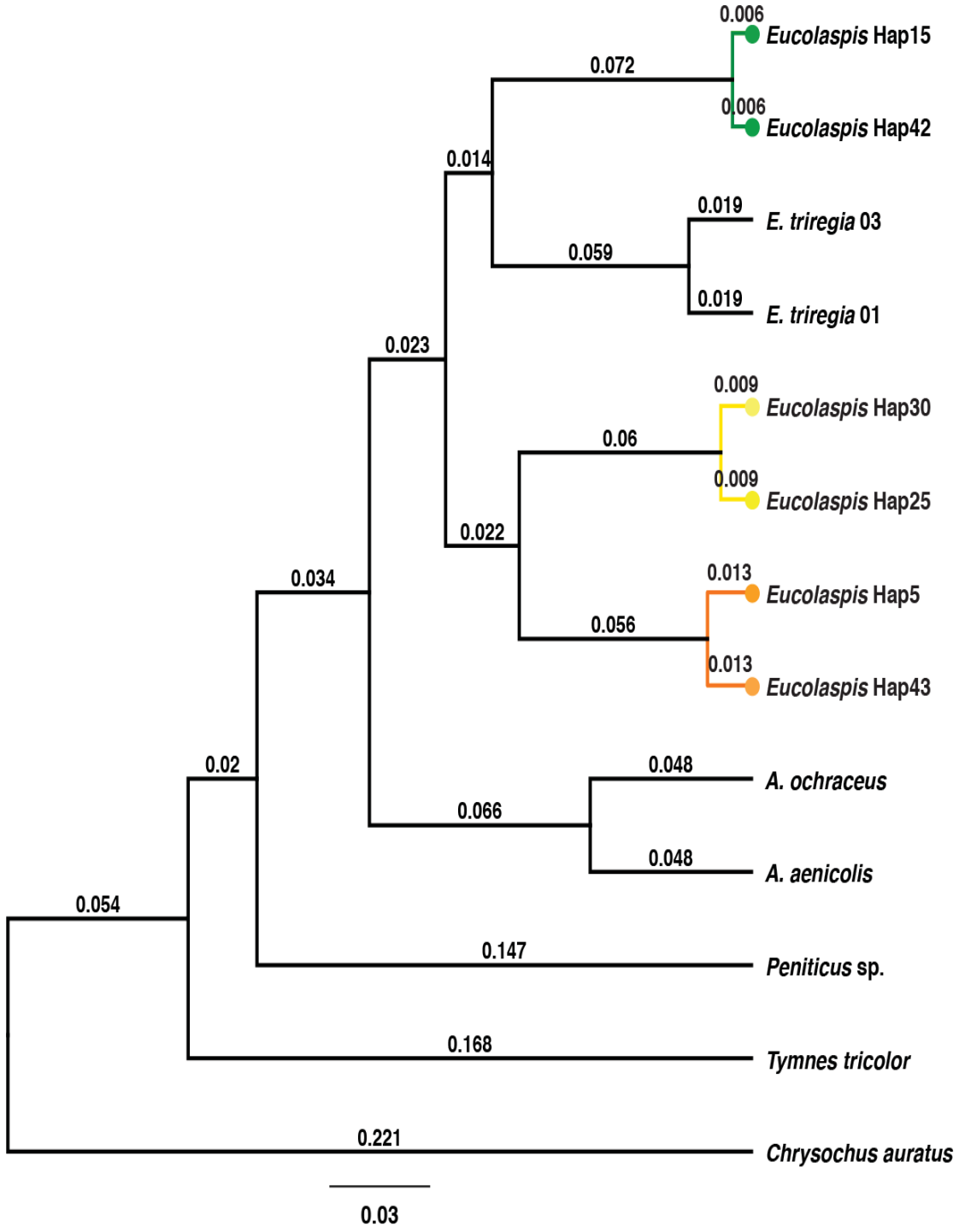


Figure 2.17 Maximum Likelihood tree, for COI sequences of three (*Eucolaspis*, *Atrichatus* and *Peniticus*) New Zealand Eumolpinae genera, and *Tymnes tricolor* and *Chrysochus auratus* (Chrysomelidae: Eumolpinae) of USA, found by heuristic search (score of the tree=2565.27171) in PAUP* using the GTR+G+I model. SBL = 1.2042 Branch labels indicate substitutions per site and tip labels indicate corresponding taxon. Mainland New Zealand *Eucolaspis* clades are colour coded: Yellow- Clade 1; Orange- Clade 2; Green- Clade 3.

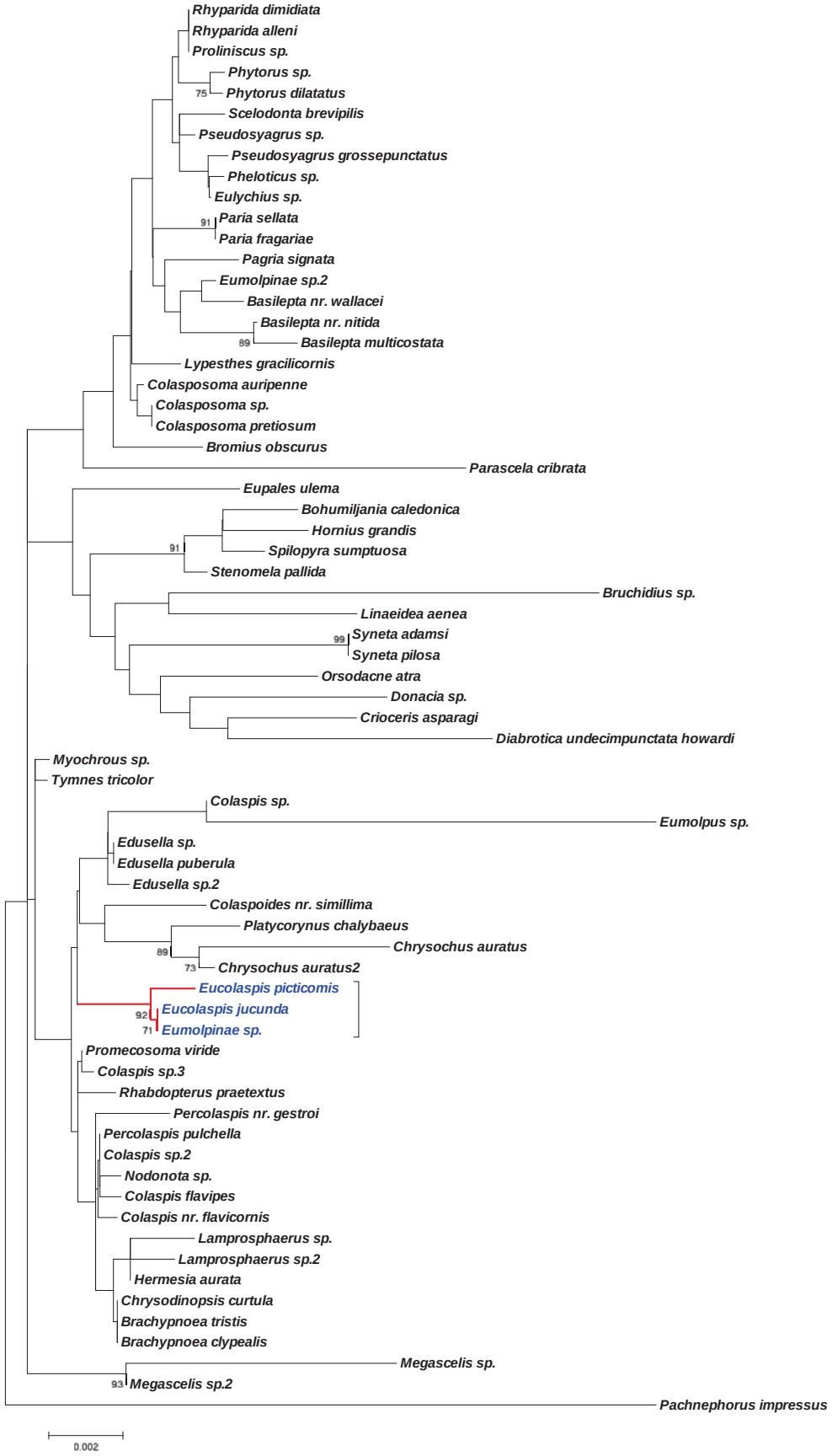


Figure 2.18 Bootstrap consensus tree of 18S rRNA sequence alignment of different genera in the subfamily Eumolpinae (Coleoptera: Chrysomelidae) constructed by Maximum Likelihood method using GTR+G+I model in MEGA5. Log Likelihood=-5119.42; SBL=0.17093617. Branch labels denote proportion (%) of tree occurrences in total of 500 replicates (consensus support values <75% are not shown). *Eucolaspis picticornis* and *E. jucunda* from New Zealand and one undescribed taxa (Eumolpinae sp.) from New Caledonia

2.4. Discussion

Mitochondrial DNA data, male genitalia data and morphometric data together provide strong evidence for just three mainland New Zealand species of *Eucolaspis* (Clades 1, 2 and 3) and possibly another on the Three Kings islands (*E.* sp. n. *triregia*) (Figures 2.9 and 2.10). Reconstructed phylogeny showed well-structured lineages, genetically different enough to be considered distinct species.

Genetic distances measured as sequence divergence (Table 2.6) reveal that the inter-clade distance at this locus is typical of inter-species genetic variation in Coleoptera when COI mtDNA sequences were used (Hebert et al., 2003b). A threshold of 3% divergence has been found to be generally applicable as per barcoding standards (Hebert et al., 2003a). There was a prominent barcode "gap" between the intraclade pairwise distances and those from interclade comparison (Figure 2.11). This indicates that barcode taxonomic division is not being arbitrarily imposed on a continuous distribution of diversity. A similar barcode gap was observed in other leaf beetles such as *Crioceris* (Coleoptera: Chrysomelidae), where the maximum intrascpecific genetic distance was about 2.5% whereas the interspecific genetic distances ranged from 16.9 to 20.3% (Kubisz et al., 2012). Likewise, the interspecific genetic distance varied from 8.1% to 14.4% while intraspecific genetic distance was between 0.3 to 0.6% in *Arsipoda* (Coleoptera: Chrysomelidae) (Gómez-Zurita et al., 2010). It has been suggested that using mean interspecific genetic distances might exaggarate the "barcode gap" and, instead, use of smallest interspecific genetic distance should be favoured (Meier et al., 2008). When mean genetic distance was used, it was found that there will be about 13% chance of misidentififcation in coleopterans (Meier et al., 2008). In the current study, the smallest interlineage genetic distance was about 7.9% whereas mean interlineage genetic distance was about 10% (Figure 2.11), both of which fall well beyond "congeneric barcode gap of 3%". More importantly, the inter-specific and intra-specific genetic distances did not overlap (Figure 2.11). Although on its own, this single locus evidence is insufficient for taxonomic distinction (Will et al., 2005; Cognato, 2006; Rubinoff et al., 2006; Trewick, 2008), it is notable that only three mainland lineages are indicated rather than 15 (Broun, 1893b; 1893a; 1909; Hudson, 1923) or 5 (Shaw, 1957).

Genitalia data and morphometric data corroborate with genetic data confirming the identitfy of three mainland New Zealand lineages (Figures 2.13 & 2.14). The data sets (mtDNA, genitalic and morphometric) utilized in the current study have shown to complement each other but not mutually exclusive, and therefore, integrative taxonomy is possible in this particular genus. Such an integration of different character sets provides infallible taxonomic decisions (Schlick-Steiner et al., 2010). Congruence of different types of data as observed in my study, has also been reported in many recent studies on other coleopterans (for e.g., Bell et al., 2004; Gatto et al., 2008; Ruiz et al., 2009; Mitchell & Maddox, 2010; Tosevski et al., 2011).

Analysis of morphological characters from specimens identified to species using existing taxonomy, does not support existing classification (Figure 2.8). Body size (length and width) of the beetles, the main characters that Broun (1880; 1893b; 1893a; 1909) used in addition to body colour to describe many of his 13 *Eucolaspis* species, did not differ significantly among the randomly selected groups of named voucher specimens from the NZAC collection. Instead, other characters such as the width of elytra and puncture density on head and posterior elytra partitioned the "species" into clusters. This provided a nice impartial test of the existing *Eucolaspis* taxonomy. Partial to complete overlapping of different "species" (such as *E. vittiger, E. colorata and E. brunnea*) supports in part the synonymy proposed by Shaw (1957), although he used a different set of characters for that inference. Close examination of beetles from different genetic lineages in the current study in regards to the shape of the punctures, the main character used to delineate species by Shaw (1957), suggested that this character is highly variable. The shape of the punctures varied among individuals within a population, and differences among individuals of different populations (and lineages) showed no consistency. Puncture density (on pronotum, head and elytra), a character also used by Shaw (Table 2.2) and Broun (Table 2.1) in species descriptions, displayed consistent differences among the genetic lineages (Figure 2.13).

Genitalic shape in males correlated well with the genetic pattern among mainland New Zealand *Eucolaspis.* This is consistent with shared descent of Clade 1 and Clade 2. Variation in the shape of male genitalia also indicates that these reproductive structures are under evolutionary selection and this selection pressure

may reflect reproductive isolation, especially in sympatric populations. Several reproductive isolation mechanisms such as variation in size and shape of cerci of male grasshoppers (*Parapodisma setouchiensis* and *P. subastris*) (Kawakami & Tatsuta, 2010) and difference in cuticular hydrocarbon profiles that act as sex pheromones in leaf beetles *Chrysochus auratus* and *C. cobaltinus* (Peterson et al., 2007) have been reported in sympatric populations. However, reproductive isolation and / or incompatibility between "species" in *Eucolaspis*, was not the focus of the current study.

The spatial structure of the lineages (Figure 2.14) reveals that only one lineage (putative species), i.e., Clade 1 of *Eucolaspis*, infests apple orchards in Hawke's Bay, while apples elsewhere (Nelson) are infested by beetles of a different lineage (Clade 3). Clade 1 (Yellow) also includes populations collected from other host plants in Hawke's Bay, and also populations from other locations such as Palmerston North, Kapiti Coast and Canterbury. Host plant use of the three mainland New Zealand lineages suggests that all three lineages can infest exotic fruit crops as well as native plants. The specific pattern of host plant use appears to depend on regional availability, implying that all three lineages are polyphagous in nature. The current sampling included only 7 host plants and, hence, inferred utilization of hosts by different genetic lineages could underestimate the real situation, as the members of the genus are reported to feed on many plant species in New Zealand (Chapter 1).

On a broad geographic scale, there was significant correspondence between ecological regions and external morphological characters, especially as evidenced in the beetles of the Leeward Districts ecological region of New Zealand, which is occupied by Clade1 (Yellow). This matches the pattern of East-West partitioning of genetic clades. Geographical distribution of the lineages (putative species) is quite interesting, since a clear demarcation between North-West and South-East populations was observed. Leeward districts, which are the driest among the sampled ecological regions, are separated geographically from the rest of the area by axial ranges (Ruahines, Tararuas) in North Island and the Alps in South Island. It could be that these ranges act as a physical barrier limiting mobility. However, Palmerston North (Windward districts) populations were genetically similar to Hawke's Bay (Leeward districts) populations, suggesting contact and dispersal between regional and local populations that is possible through discontinuity in the ranges. A similar

trend of species distribution was observed in some other New Zealand biota such as peripatus *Peripatoides* (Trewick, 2000), *Priasilpha* beetles (Leschen & Michaux, 2005), *Paryphanta* snails and corophiid amphipods (in Trewick et al., 2011).

Analyses of phylogenetic relationships (Figures 2.16 & 2.17) among New Zealand Eumolpinae genera using available data suggest that the genera *Eucolaspis* Sharp and *Atrichatus* Sharp are more closely related to each other, rather than to *Peniticus* Sharp; this confirms the previous suggestions by Broun (1893b) and Shaw (1957). A fourth genus, *Pilacolaspis* Sharp, could not be included in this study as the available specimens were old and did not yield amplifiable DNA.

In the current study, Nelson-Marlborough and Canterbury regions were covered but not the rest in South Island, while much of the North Island is covered. However, *Eucolaspis* beetles are not generally found south of Canterbury region of New Zealand (Chapter 1). As per genetic and genitalic data there are only three putative species in mainland New Zealand, unless the current sampling missed any rather cryptic species with limited and isolated distribution. However, this appears unlikely, as most of the earlier descriptions (Broun, 1880; 1893b; 1893a; 1909) were made from few isolated locations (Table 2.1) which are extensively sampled in my current study (e.g., Tairua).

I therefore propose three mainland *Eucolaspis* taxa, tentatively distinguished by haplotype clade, aedeagus shape, puncture density (on pronotum, head and anterior elytra) and elytra width. Beetles that belong to Clade 1 are distinguished morphologically by having denser puncturation (on pronotum, head and anterior elytra) and narrower elytra than the other two clades. Assignment of taxonomic names will depend primarily on chronological priority in nomenclature and would require DNA analysis of type material.

Taxonomic support for pest management has been achieved through the current study, as the Hawke's Bay bronze beetle populations were shown to comprise just one putative species. Species-specific pest control strategies would benefit from my findings and at the same time, wide applicability of such species-specific methods to apple orchards in other places warrants prior trials with local populations of interest. Infestation of fruit orchards in different localities appear to be by different species (Figure 2.15 and Table 2.8).

Closeness of evolutionary histories of New Zealand biota, in this case *Eucolaspis*, and those of other Pacific biota was bolstered by analysis of 18S rDNA data (Figure 2.18). However, these higher order evolutionary relationships (inter-generic and intra-subfamilial) within New Zealand and at international level need to be researched further for confirmation and might have implications for genus level taxonomy.

2.5. Conclusions

The spatial and putative taxonomic diversity of New Zealand *Eucolaspis* was encompassed in this study. The inclusion of identified voucher specimens from several major collections provided the basis of a test of the current classification. As has been indicated (Kuschel, 1990; Hutcheson, 1992), this classification is found wanting. Here, I show that a combination of morphological and genetic evidence supports the existence of just 3 mainland New Zealand species, whose differences are consistent with species status. At the same time, I demonstrate that most morphological information used previously to define New Zealand *Eucolaspis* species is inappropriate. Instead, these characters (colour, size and markings), which vary considerably within lineages and even within populations, are better suited for a generic treatment of "bronze beetles". It appears that there may be another closely related lineage (putative species) on Three Kings islands in addition the three mainland *Eucolaspis* lineages. The genetic structure among the lineages was well supported by genitalic data and also morphometric data to some extent. It was found that only a single species (lineage) infests apples in Hawke's Bay – *Eucolaspis* sp. "Hawke's Bay".

Chapter 3: Host location and feeding preference

Part of this chapter was presented at the Entomological Society of New Zealand's 59th annual conference (April 11-14th 2010, Wellington, New Zealand).

3.1. Introduction

Host specificity is common in the family Chrysomelidae (Insecta: Coleoptera), with the majority being either mono- or oligophagous; some species from subfamily Eumolpinae (Coleoptera: Chrysomelidae) are polyphagous (Fernandez & Hilker, 2007). *Eucolaspis*, one of the only four native Eumolpinae genera in New Zealand (Leschen et al., 2003), is a group of polyphagous species. Recent findings on taxonomy and distribution of *Eucolaspis* suggest there may be fewer species of the genus in New Zealand than formerly accepted and that it is a single "species" that infests apple orchards in Hawke's Bay – *Eucolaspis* sp. "Hawke's Bay" (see Chapter 2).

Eucolaspis was first reported on flowers of a New Zealand native bushy-shrub, manuka (*Leptospermum scoparium* – Myrtaceae) (Fabricius 1781 in White, 1846). The beetles of the genus have since been recorded as feeding on at least another 67 plant species in New Zealand (including 27 natives and 40 exotics), covering 33 families, although the most common are Myrtaceae and Rosaceae (see Table 3.1). In Rosaceae and Myrtaceae, 11 and 6 plant species respectively are used as host plants. The range of host plants extends from small pasture crops such as *Trifolium* to orchids such as *Cymbidium* and conifers like *Pinus*, and the habitat range extends from cultivated pastures to orchards, native scrub and forests (Table 3.1).

It appears that the beetles have a special preference for exotic fruit plants of the family Rosaceae as often huge populations are found on these (personal observation). Fruit crops (from families other than Rosaceae) such as avocado (Lauraceae), black currant and gooseberry (Grossulariaceae), blueberry (Ericaceae) and feijoa (Myrtaceae) are also damaged. There may be more host plants for the genus *Eucolaspis* in New Zealand than are listed here, as some of the specimens in NZAC and some publications such as Rogers et al. (2008) indicated "unidentified plants" as hosts.

Table 3.1 Host plants of adult *Eucolaspis* in New Zealand.

Common Name	**Scientific name**	**Family**	**Native / Introduced***	**Reference**
Pukanui	*Meryta sinclairii*	Araliaceae	Native	Coll. by R.E. Beever (NZAC specimen label)
Astelia	*Astelia* sp.	Asteliaceae	Native	NZAC specimen label
Boneseed	*Chrysanthemoides monilifera* ssp. *monilifera*	Asteraceae	Introduced	(Winks et al., 2004)
Californian thistle	*Cirsium arvense*	Asteraceae	Introduced	(Rogers et al., 2008)
Alder	*Alnus glutinosa*	Betulaceae	Introduced	NZAC specimen label
Pink bindweed	*Calystegia sepium* ssp. *roseata*	Convolvulaceae	Native	(Kuschel, 1990)
Wineberry	*Aristotelia serrata*	Elaeocarpaceae	Native	M.A.Minor 2009, personal obs.
Prickly mingimingi	*Leptecophylla juniperina* subsp. *juniperina*	Ericaceae	Native	Personal obs., 2011
Blueberry	*Vaccinium corymbosum*	Ericaceae	Introduced	L.Hawes 2009, personal obs.
Acacia	*Acacia* sp.	Fabaceae	Introduced	(Lysaght, 1930)
NZ Broom	*Carmichaelia* sp.	Fabaceae	Native	Coll. by C.F. Butcher (NZAC specimen label)
Clover	*Trifolium* sp.	Fabaceae	Introduced	(Lysaght, 1930)
Gorse	*Ulex europaeus*	Fabaceae	Introduced	Coll. by P. Maddison (NZAC specimen label)
Geranium	*Geranium* sp.	Geraniaceae	Introduced	(Lysaght, 1930)
Black currant	*Ribes nigrum*	Grossulariaceae	Introduced	(Miller, 1926)
Gooseberry	*Ribes uva-crispa*	Grossulariaceae	Introduced	(Miller, 1926)
Fire weed	*Haloragis* sp.	Haloragaceae	Native	(Kuschel, 1990)
Tawa	*Beilschmiedia tawa*	Lauraceae	Native	Coll. by C.F. Butcher (NZAC specimen label)
Bay tree	*Laurus* sp.	Lauraceae	Introduced	(Lysaght, 1930)
Avocado	*Persea Americana*	Lauraceae	Introduced	(New Zealand Avocado Growers' Association & Industry Council Ltd, 2011)
Hangehange	*Geniostoma* sp.	Loganiaceae	Native	Coll. by P. Maddison (NZAC specimen label)
Hollyhock	*Alcea* sp.	Malvaceae	Introduced	(Lysaght, 1930)
Whau	*Entelea arborescens*	Malvaceae	Native	Coll. by J.C. Watt (NZAC specimen label)

Poor Knights houhere	*Hoheria equitum*	Malvaceae	Native	Coll. by J.S. Dugdale (NZAC specimen label)
Mallow	*Malva sylvestris*	Malvaceae	Introduced	(Lysaght, 1930)
Linden	*Tilia* sp.	Malvaceae	Introduced	S.Trewick 2008, personal obs.
Feijoa	*Acca sellowiana*	Myrtaceae	Introduced	Personal obs.,2009
Eucalyptus	*Eucalyptus* sp.	Myrtaceae	Introduced	(Lysaght, 1930)
Lilly pilly	*Syzygium smithii*	Myrtaceae	Introduced	NZAC specimen label
Manuka	*Leptospermum scoparium*	Myrtaceae	Native	(White, 1846; Broun, 1893b)
Kanuka	*Kunzea ericoides*	Myrtaceae	Native	Personal obs., 2010
Pohutukawa	*Metrosideros excelsa*	Myrtaceae	Native	(Martin, 2010)
Silver Beech	*Nothofagus menziesii*	Nothofagaceae	Native	NZAC specimen label
Kotukutuku	*Fuchsia excorticata*	Onagraceae	Native	(Lysaght, 1930)
Cymbidium	*Cymbidium* sp.	Orchidaceae	Introduced	(Dymock & Holder, 1996)
Dendrobium	*Dendrobium* sp.	Orchidaceae	Introduced	(Dymock & Holder, 1996)
Pine	*Pinus* sp.	Pinaceae	Introduced	(Kay, 1980)
Pittosporum	*Pittosporum* sp.	Pittosporaceae	Native	(Lysaght, 1930)
Kahikatea	*Dacrycarpus dacrydioides*	Podocarpaceae	Native	NZAC specimen label
Podocarpus	*Podocarpus* sp.	Podocarpaceae	Native	(Lysaght, 1930)
Totara	*Podocarpus totara* var. *totara*	Podocarpaceae	Native	S.Trewick 2011, personal obs.
Pohuehue	*Muehlenbeckia* sp.	Polygonaceae	Native	NZAC specimen label
Broad-leaved dock	*Rumex obtusifolius*	Polygonaceae	Introduced	Personal obs., 2007
Scarlet pimpernel	*Anagallis arvensis* ssp. *arvensis*	Primulaceae	Introduced	(Auckland Botanic Gardens, 1999)
Weeping mapou	*Myrsine divaricata*	Primulaceae	Native	(Lysaght, 1930)
Red mapou	*Myrsine australis*	Primulaceae	Native	Coll. by J.C. Watt (NZAC specimen label)
NZ honeysuckle	*Knightia excelsa*	Proteaceae	Native	NZAC specimen label
Columbine	*Aquilegia* sp.	Ranunculaceae	Introduced	(Rogers et al., 2008)
Hawthorn	*Crataegus*	Rosaceae	Introduced	(Lysaght, 1930)

Strawberry	*Fragaria* sp.	Rosaceae	Introduced	NZAC specimen label
Apple	*Malus domestica*	Rosaceae	Introduced	(Huntley, 1867; Miller, 1926)
Peach & nectarines	*Prunus persica*	Rosaceae	Introduced	(Lysaght, 1930)
Plum	*Prunus* sp.	Rosaceae	Introduced	(Miller, 1926)
Cherry	*Prunus* sp.	Rosaceae	Introduced	(Lysaght, 1930)
Apricot	*Prunus armeniaca*	Rosaceae	Introduced	NZAC specimen label
Pear	*Pyrus communis*	Rosaceae	Introduced	(Miller, 1926)
Rose	*Rosa indica*	Rosaceae	Introduced	(Lysaght, 1930)
Blackberry	*Rubus fruticosus*	Rosaceae	Introduced	(Miller, 1926)
Raspberry	*Rubus idaeus*	Rosaceae	Introduced	(Miller, 1926)
Coprosma	*Coprosma* sp.	Rubiaceae	Native	(Lysaght, 1930)
Poplar	*Populus* sp.	Salicaceae	Introduced	(Lysaght 1930)
Titioki / NZ Ash	*Alectryon excelsum*	Sapindaceae	Native	(Lysaght, 1930)
Camellia	*Camellia* sp.	Theaceae	Introduced	(Royal New Zealand Institute of Horticulture, 2011)
Elm	*Umulus* sp.	Ulmaceae	Introduced	(Lysaght, 1930)
Mahoe	*Melicytus ramiflorus*	Violaceae	Native	Coll. by R.C. Craw (NZAC specimen label)
Violet	*Viola* sp.	Violaceae	Introduced	(Lysaght, 1930)
Grape	*Vitis* sp.	Vitaceae	Introduced	(Woodfin, 1927)

***Status verified from http://www.NZPCN.org.nz**

Infestation of exotic fruit plants or native plants by *Eucolaspis* depends on local and regional availability, as revealed from host plant use by different genetic lineages in New Zealand (Chapter 2). However, intra-specific variation in host plant use by bronze beetles cannot be ruled out as variation in host plant use between conspecific populations were reported in other phytophagous insects such as *Chrysomela lapponica* (Coleoptera: Chrysomelidae) (Zvereva et al., 2010), *Agelasa nigriceps* (Coleoptera: Chrysomelidae) (Kohyama et al., 2012) and *Apagomerella versicolor* (Coleoptera: Cerambycidae) (Logarzo et al., 2011). Long-term association with a host plant might lead to stronger preference through tolerance, detoxification, recognition and avoidance (Jermy, 1984; Mostafa et al., 2011).

Eucolaspis sp. "Hawke's Bay" was found to feed on apple, blackberry, manuka, linden (Chapter 2), raspberry, feijoa, strawberry, mallow, clover, broad-leaved dock (personal observations) and possibly many other plants. The instance of *Eucolaspis* beetles infesting exotic plants presents a contrast to the more common occurrence in New Zealand of exotic animal and plant pests targeting native flora and fauna. In fact, the situation of the *Eucolaspis* presents an apparent parallel to the classic cases of Colorado potato beetle and apple maggot in the USA. The Colorado potato beetle (*Leptinotarsa decemlineata*), a native North American leaf beetle that fed on native solanaceous plants (*Solanum rostratum*) became successfully established as a major pest on introduced potato (*Solanum tuberosum*) and the beetle subsequently spread to Europe along with potato plants (Schoonhoven et al., 2005). Apple maggot fly (*Rhagoletis pomonella*) that is native to North America originally fed on native wild hawthorn (*Crataegus* spp.), and later became a pest of cultivated apples upon their introduction to North America (Weems Jr., 2002).

However, in the case of both the Colorado potato beetle and the apple maggot fly, both the native (old) and exotic (new) host plant species belong to the same family and hence are more closely related than the various unrelated hosts of *Eucolaspis*. *Eucolaspis*'s infestation of plant family Rosaceae that was relatively underrepresented in New Zealand flora naturally until the introduction of fruit crops, suggests pre-adaptation to a plant family. Such pre-adaptation exists in other insects such as bruchids (Chrysomelidae: Bruchinae) that are able to sustain low humidity conditions that enables them to explore and infest dry seeds of new host plants (Shimada, 1990; Tuda, 2007), and rolled leaf beetles (Chrysomelidae: Cassidinae) that specialize in

tissue-feeding by rolling leaves (McKenna, 2006). Similar host-plant chemistries sometimes enable pre-adaptation in insects such as *Ophraella* leaf beetles (Futuyma, 1990) and Colorado potato beetle (Schoonhoven et al., 2005). This adaptive behaviour highlights the stimuli that are used in locating various hosts and specificity of the mechanism involved. Currently, there is no information available on how bronze beetles locate their host plants, the role of olfactory stimuli in host-finding or any evidence of preference between host plants for the beetles. To assess this, verification of whether bronze beetles use plant volatiles to locate host plants would be crucial. Preference between host plants, either due to evolutionary affiliation or due to ecological and nutritional incentives, would have important implications in understanding host-breadth expansion and behavioural management of the bronze beetles.

Host plant choice usually includes three steps: locating the host, recognizing the host, and, finally, either accepting or rejecting the host plant (Bernays & Chapman, 1994). Locating a suitable host plant would be a basic requirement for a herbivore to proceed further in its life cycle. Insects locate their hosts using a number of strategies, which may include a mere random searching and/or more selective searching (Bernays & Chapman, 1994; Jolivet et al., 2004; Schoonhoven et al., 2005). Selective searching in phytophagous insects is essentially driven by responses to stimuli associated with host plants; the stimuli involved are predominantly visual and olfactory (Visser, 1986).

Vision is a relatively short-range sense compared to olfaction (Schoonhoven et al., 2005). Visual cues could be insufficient alone, but in combination with specific plant odours may play a significant role in host location (Bernays & Graham, 1988). Olfactory signals are often taxon-specific, whereas visual signals such as plant shape and colour tend to be less specific and may vary even within a species (Jaenike, 1990).

Plant volatiles, a combination of ubiquitous and species-specific compounds (Visser, 1986; Dudareva et al., 2004), have been shown to shape insect-plant relations in many insects. In fact, the most prevalent mechanism of host plant location in phytophagous insects is by perception of the ratio and blend of ubiquitous compounds, rather than by perception of species-specific compounds (Bruce et al., 2005). Highly volatile blends of six carbon aldehydes and alcohols, collectively known as “green leaf volatiles”, are ubiquitous in the plant kingdom (Bruce et al., 2005). Certain plant groups such as Fabaceae, Rosaceae and Poaceae emit large quantities of alcohols and aldehydes

in their head space (Schoonhoven et al., 2005). These alcohols and aldehydes, along with esters and terpenes, are frequently used by chewing insects in locating their host plants (Szendrei & Rodriguez-Saona, 2010). Therefore it is possible that bronze beetles are attracted to Rosaceae plants owing to the volatiles emitted.

Plant volatiles have been shown to mediate host plant location in various coleopteran herbivores. For instance, adult plum curculio weevils *Conotrachelus nenuphar* were found attracted to apple and wild plum (both Rosaceae) (Leskey & Prokopy, 2000). Pollen beetle *Meligethes aceneus* was attracted to the fresh odour from buds and flowers of its host plant oilseed rape (Brassicaceae) (Jönsson, 2005), while adult agave weevils (*Scyphophorus acupunctatus*) were attracted to volatile compounds found in fresh leaves of cultivated agaves in Mexico (Altuzar et al., 2007). In a New Zealand study, Wee et al. (2008) found that Fuller's rose weevil adults were attracted to odours from fresh lemon and clover plants. Likewise, it is possible that bronze beetles may also use plant volatiles to locate suitable host plants.

In Chrysomelidae, cues that mediate attraction to undamaged host plants include plant size (Colorado potato beetle *Leptinotarsa decemlineata* – (Visser, 1976; Bolter et al., 1997; Hoy et al., 2000)), visual cues (*L. decemlineata* – (Zehnder & Speese, 1987; Jermy et al., 1988; Szentesi et al., 2002); *Phyllotreta striolata* – (Visser, 1976; Bolter et al., 1997; Hoy et al., 2000; Yang et al., 2003); *Oreina cacaliae* – (Kalberer et al., 2001)) and plant volatiles (Fernandez & Hilker, 2007). However, the most prevalent host-finding mechanism appears to be olfactory cues (Stenberg & Ericson, 2007). For example, Hibbard and Bjostad (1988) found that corn rootworm larvae (*Diabrotica virgifera*) (Chrysomelidae) are attracted to CO_2 and corn volatiles. Similarly, Park et al. (2004) found that the alder leaf beetle *Agelastica coerulea* females were attracted to fresh leaf odours from their host plants of the family Betulaceae. Other leaf beetle genera such as *Acalymma*, *Altica*, *Chrysolina*, *Entomolscelis*, *Galerucella*, *Leptinotarsa*, *Longitarsus*, *Oreina*, *Phratora*, *Phyllotreta*, *Trirhabda* and *Xanthogaleruca* were also found attracted to host-plant volatiles (see the following reviews: Mitchell, 1988; Fernandez & Hilker, 2007; Stenberg & Ericson, 2007; El-Sayed, 2011). None of these genera belongs to the subfamily Eumolpinae, which includes approximately 500 genera and 7000 species (Jolivet & Verma, 2008) and remains poorly studied in terms of host location.

It is important to know the host location strategies used by a pest such bronze beetle, in order to evaluate different ecological niches that are being invaded. It is also crucial to understand the herbivore's evolutionary and physiological adaptations (or lack of one) in doing so, as relative advantages and disadvantages exist between generalist and specialist dietary behaviours (Bernays & Chapman, 1994). Unravelling the means of insect-host plant interactions may provide tools for pest management, in addition to improving our ecological and evolutionary understanding of the responses of insect herbivores to plant volatiles (Agelopoulos et al., 1999; Rodriguez-Saona & Stelinski, 2009). In cultivated plant ecosystems, plant volatiles have been widely deployed in the behavioural manipulation of herbivores through applications such as lures to monitor (or mass trap) insect pests (Light et al., 2001), spraying plants to enhance attractiveness in trap cropping (Martel et al., 2005), or making certain crop plants unattractive by application of plant-derived repellents (Pickett et al., 2006).

3.1.1. Research objectives

I studied the ecological and behavioural associations of *Eucolaspis* sp. "Hawke's Bay" with host plants focusing on the following specific questions.

1. Does adult *Eucolaspis* sp. "Hawke's Bay" respond to host plant odours?
2. Do adult beetles discriminate between damaged and undamaged hosts?
3. Do adult beetles discriminate between odours of host plants from two different species? Is this discrimination consistent across different populations with different host plant and geographic association i.e., is there any evidence of evolutionary affiliation?
4. Do adult beetles demonstrate feeding preference when a choice of host plants is available?

3.2. Materials and methods

Host location and feeding preference were explored through Y-tube olfactometry experiments and feeding bioassays carried out in the laboratory during spring / summer of 2008-09, 2009-10 and 2010-11.

3.2.1. Insects

Normal healthy adult beetles of *Eucolaspis* sp. "Hawke's Bay" were used for all bioassays. Two different populations were used in the bioassays, one from an organic apple orchard ('Royal Gala') in Havelock North (Hawke's Bay, New Zealand) and another from wild blackberry on a private property in Waikanae (Kapiti, New Zealand) (see Figure 2.1, in Chapter 2 for map). Beetles from Havelock North were collected as pupae from the field during the spring from an organic apple orchard (Orchard A), and raised to adults in the laboratory. The pupae were collected in vented 1.5 ml micro-centrifuge vials (Eppendorf) along with a small amount of soil. The pupae were kept cool and transported to the laboratory, where they were maintained at controlled conditions (18 ^{0}C temperature, 0: 24 h Light: Dark photoperiod) until adults emerged. Care was taken that humidity did not fall below 80% by occasional water spraying and leaving water in open containers. Beetles from Waikanae were collected as adults in the field; all insects were collected in individual vials and kept cool during immediate transfer to the laboratory. Adult beetles were maintained in individual vials in controlled conditions (20 ^{0}C, 16 h light: 8 h dark). Fresh host plant material ('Royal Gala' apple leaves / fruitlets) was provided as food, and moisture provided by moist filter paper. Food and water were checked daily and replenished as necessary. Beetles used in the study were starved for at least 4 hours immediately before a bioassay. Starving is necessary to induce host finding or feeding, and is a widely used practice in similar experiments with herbivorous insects (Park et al., 2004; García-Robledo & Horvitz, 2009).

3.2.2. Plants

Host detection was tested with four plant species which are all known hosts of the adult beetles: apple (*Malus domesticus* CV 'Royal Gala') (Rosaceae), blackberry (*Rubus fruiticosus*) (Rosaceae), white clover (*Trifolium repens*) (Fabaceae) and broad-leaved dock (*Rumex obtusifolius*) (Polygonaceae). Apple and blackberry were used to test whether the attraction consistent among these two Rosaceae plants, whereas white clover and broad-leaved dock were used to test whether the degree of attraction to host plants is uniform across host plant families. White clover and broad-leaved dock are predominant under-storey plant species in organic apple orchards in Hawke's Bay region, and both are eaten by beetles in the field (personal observation). Fresh leaves / fruitlets / flower heads of 'Royal Gala' apple, white clover and broad-leaved dock were

collected from Massey University's organic apple orchard in Palmerston North; blackberry leaves were collected from a spray-free private property on Turitea road in Palmerston North. Fresh plant material was transferred immediately to the laboratory in plastic bags lined with moist paper towels.

3.2.3. Olfactometry

A glass Y-tube olfactometer was used to run the bioassays (Figure 3.1) to test to host plant attraction. The two arms of the Y-tube were connected to an airflow meter (Figure 3.1, d), which in turn was connected to a compressed air source via an activated charcoal filter (Figure 3.1, c). Air was passed through the two arms of the Y-tube simultaneously and into the main central arm at a rate of 400 ml/s. The activated charcoal filter was included in the circuit to absorb any potential contaminating volatiles in the airflow entering the system. All connections were secured with two layers of adhesive tape (Sellotape®) to prevent any air leakage.

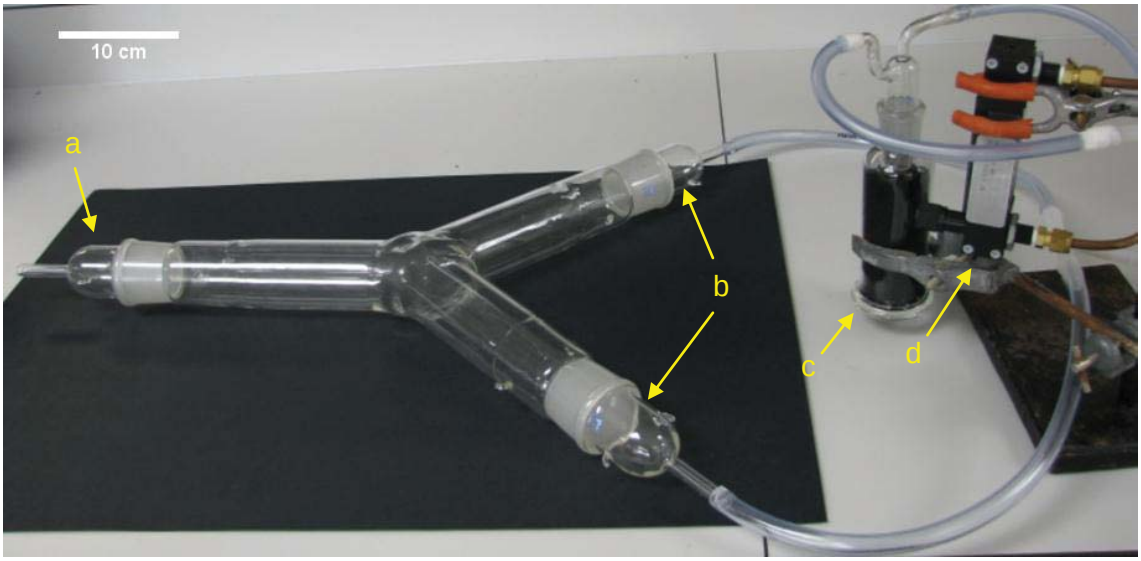


Figure 3.1 Y-tube olfactometer used for host location and preference bioassays of adult *Eucolaspis* sp. "Hawke's Bay" beetles, showing (a) the specimen chamber, (b) treatment chambers, (c) charcoal filter, and (d) the air flow meter.

Individual insects were released into the opening of the central arm of the Y tube through the specimen chamber (4 cm diameter) (Figure 3.1, a) and exposed to two different treatments placed in the treatment chambers of the two lateral arms. The two chambers containing treatment choices (4 cm diameter) (Figure 3.1, b) were separated from the Y-tube by black muslin cloth, which acted as a visual barrier and prevented the

beetles from entering the choice chambers. Insects were prevented from walking out of the specimen chamber by a muslin cloth that closed its opening.

The beetle responses were recorded based on the movement of insects towards a treatment. Beetles were allowed to spend a maximum of 20 minutes (experimental run) in the Y-tube. A positive response was scored when a beetle reached the end of an arm, at which point the run was deemed finished. Beetles that failed to reach a treatment in 20 minutes were recorded as non-responsive and eliminated from further analysis. The time spent by each beetle before choosing an option (response time) was recorded using a digital timer. Each beetle was used only once in the bioassays. After each run, the test beetle was removed, and the Y-tube was rinsed with n-hexane and air-dried before being used in the next bioassay. Treatment choices are detailed in Table 3.2.

Table 3.2 Treatment details of Y-tube olfactometer experiments, exploring host location by adult *Eucolaspis* sp. "Hawke's Bay" beetles.

Experiment No.	**Population Source**	**N**	**Option 1**	**Option 2**
1	Havelock North on apple	45	Apple leaves & fruitlets	Control*
2	Havelock North on apple	36	Blackberry leaves	Control*
3	Havelock North on apple	32	White clover leaves & flower heads	Control*
4	Havelock North on apple	33	Broad-leaved dock leaves	Control*
5	Havelock North on apple	21	Apple fruitlet	Apple fruitlet with 2 ♂ and 2 ♀ feeding beetles feeding
6	Havelock North on apple	18	Apple leaves	Blackberry leaves
7	Waikanae, on blackberry	25	Apple leaves	Blackberry leaves

N=number of beetles tested

*control was an empty chamber with clean air flowing through

Attraction of adult *Eucolaspis* sp. "Hawke's Bay" beetles to host plant odours was tested in bioassays 1-4 whereas preference in attraction to a colonized and un-colonized plant was tested in bioassay 5 (Table 3.2). Habituation or evolutionary affiliation to ancestral host plant was tested by offering a choice of two host plants in bioassays 6 and 7, and also in feeding bioassays (section 3.2.4.) using two different

populations with different geographical and host plant association. Both populations were found to be of the same "species" and genetically similar (see Chapter 2).

My laboratory colonies of the bronze beetles suffered heavy fungal infections limiting the number of available insects for each bioassay. However, similar number of replications (number of individuals) have also been used by other researchers in similar experiments (for e.g., Heisswolf et al., 2007; Wee et al., 2008).

3.2.4. Feeding bioassays

Feeding preference for host plants apple and blackberry was tested in the laboratory during summer 2010-2011 with two populations of *Eucolaspis* sp. "Hawke's Bay" ("apple population" from Havelock North and "blackberry population" from Waikanae). Two populations were used to test whether host preference was affected by ecological or evolutionary affiliation. Leaf discs of 2.5 mm diameter were cut from fresh leaves of host plants; one leaf disc of each apple and blackberry were presented to each test beetle in a Petri dish (5.5 cm diameter) lined with moist filter paper (Whatman® Cat No 1001-055). All the leaf discs provided were weighed prior to the bioassay. The Petri dish was then sealed with tape to retain moisture. The beetles were allowed to feed on the leaf discs for 24 h, after which they were removed. The leaf discs were photographed using a camera (Olympus Camedia C-5050) fitted to a dissecting microscope (Olympus SZX-ILLD2-200). These photographs were analysed using the leaf area analysis software, Compu-eye (Bakr, 2005; Wyman et al., 2010) to calculate the consumed area of all the leaf discs. Leaf mass consumed was calculated for all the insects by multiplying the leaf area consumed with the initial weight of the leaf disc.

3.2.5. Data analysis

The total number of beetles that responded in the olfactometry was compared to the non-responsive ones by logistic regression and non-responsive beetles were omitted from further statistical analysis. The number of beetles attracted to each of the two options in the olfactometer bioassays was compared by binary logistic regression (Proc Logistic) and the difference in response times between treatments was tested by ANOVA (Proc GLM). Difference between the two sexes in attraction or non-attraction in a bioassay was tested using binary logistic regression. The differences in response times between the two sexes were tested within each of the two categories (beetles that chose option1 and beetles that chose option2 in a bioassay) in all the bioassays by

ANOVA. Each olfactometer bioassay was analysed separately and the 95% level of confidence was used to interpret the significance of results. The proportion of apple and blackberry leaf (in area and in mass) consumed in the feeding bioassays were compared using a t-test. Normality of data on response times and leaf area was checked with Proc Univariate. SAS v.9.2 (SAS Inc., USA) was used to perform all the statistical analyses.

3.3. Results

3.3.1. Host location

The beetles showed strong positive anemotaxis (moving upwind) in all of the olfactometer bioassays (overall response was 87.4%; Wald χ^2 (1, N = 214) = 88.36, p <.0001). Males (88.3% of tested males responded) and females (86.4% of tested females responded) were equally responsive (Wald χ^2 (1, N = 214) = 0.17, p >.05).

When a host plant odour was tested against control (experiments 1-4), apple (Wald χ^2 (1, N = 36) = 8.15, p=.0043) and blackberry (Wald χ^2 (1, N = 36) = 3.84,p =.049) were attractive to *Eucolaspis* sp. beetles, whereas white clover (Wald χ^2 (1, N = 29) = 0.03, p =.85) and broad-leaved dock (Wald χ^2 (1, N = 30) = 0.55, p =.46) were not attractive (Figure 3.2). In fact, clean air was more attractive to beetles than fresh leaf odour from either white clover or broad-leaved dock. There was no effect of sex in the degree of attraction to a treatment / control in all the four bioassays (p > .05; see Table IV of Appendix).

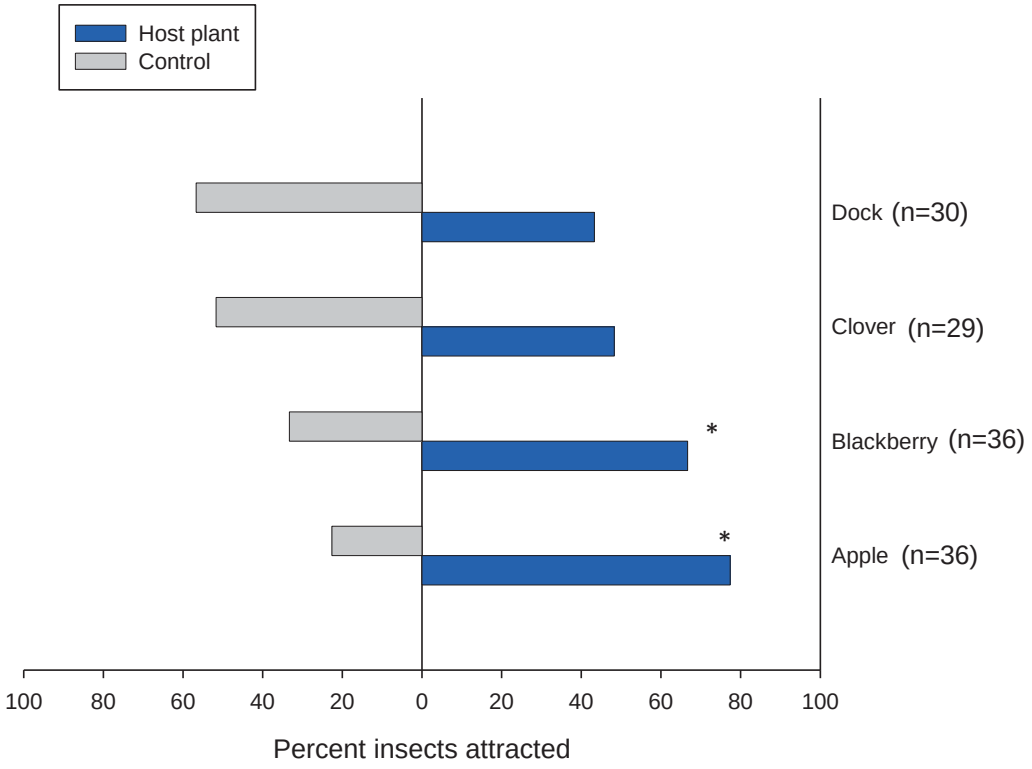


Figure 3.2 Attraction of adult *Eucolaspis* sp. "Hawke's Bay" beetles to host plant odour (apple / blackberry / white clover / broad-leaved dock) or clean air (control) in Y-tube olfactometer bioassays. * p <.05; n = number of beetles responded.

Beetles that chose control (clean air) took more time to respond than those that chose a host plant odour in bioassays 2 (with blackberry) and 3 (with clover), whereas it was the opposite in bioassays 1 (with apple) and 4 (with broad-leaved dock) (Figure 3.3). However, the differences in response times of the beetles for choosing between the two options (host plant / control) were not significant in any of the four bioassays (all p > .05; see Table IV of Appendix).

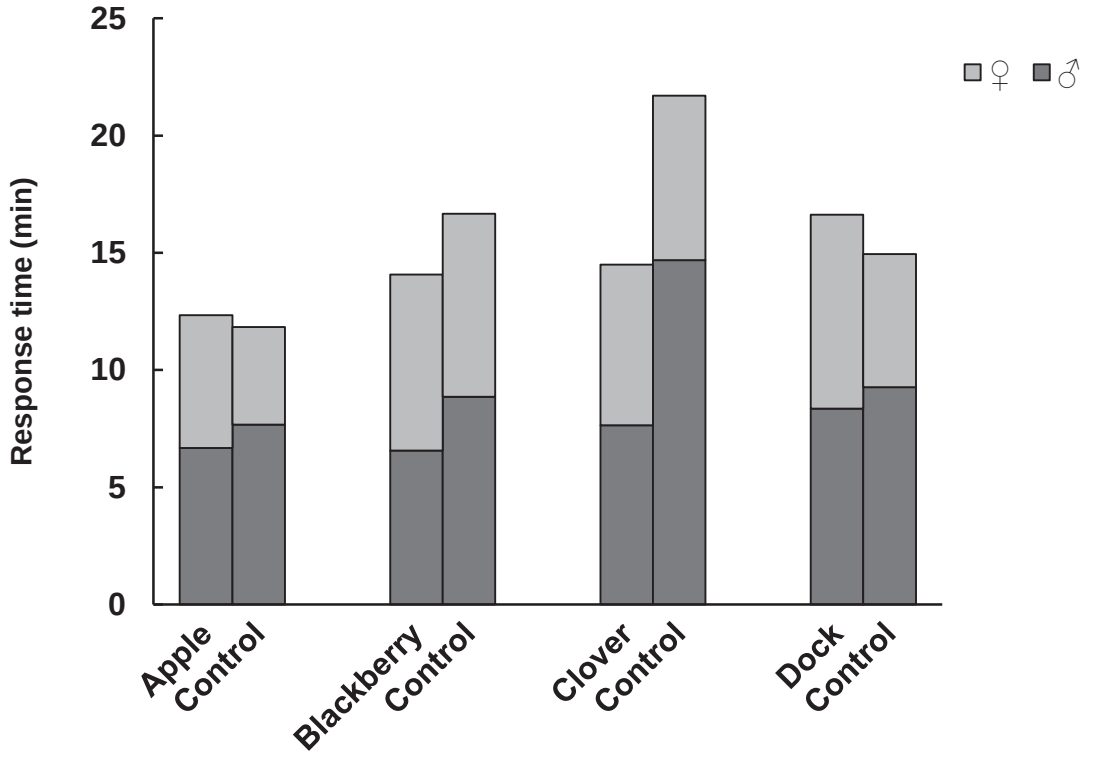


Figure 3.3 Mean response time (minutes) of adult *Eucolaspis* sp. "Hawke's Bay" beetles for choosing a host plant odour (apple / blackberry / white clover / broad-leaved dock) or clean air (control) in Y-tube olfactometer bioassays (no host/control differences significant at $\alpha = 0.05$).

Male and female beetle response times were similar to the treatments (Figure 3.3), although in the bioassay 1 (apple vs. control) there was a weakly significant difference (p =.052) between male and female beetles in response times.

In the dual treatment-choice olfactometer bioassays (experiments 5-7), neither treatment was more attractive than the other for the *Eucolaspis* sp. "Hawke's Bay" beetles. Beetles did not show any preference between odours from an apple fruitlet alone and odours from an apple fruitlet with other beetles feeding on it (41.18% chose apple, 58.82% chose apple w/ beetles; Wald χ^2 (1, N = 17) = 0.52,p =.47). When a choice of two host plant odours (apple and blackberry) was offered to beetles from two different host populations, there was a slight preference towards ancestral host plant, although the results were inconclusive (Figure 3.4).

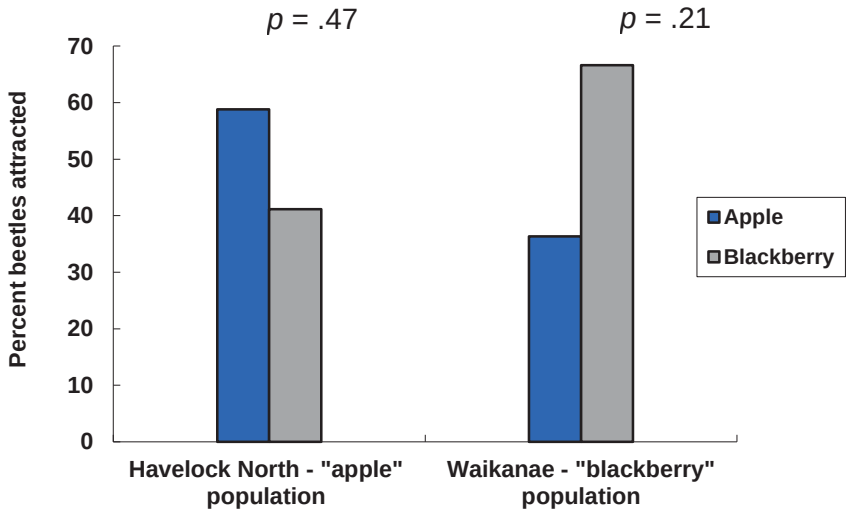


Figure 3.4 Response of adult *Eucolaspis* sp. "Hawke's Bay" beetles (% attracted) from two populations from different host plants (Havelock North - "apple population" and Waikanae - "blackberry population") to fresh leaf odour of apple and blackberry in dual choice Y-tube olfactometer bioassays in the laboratory (not significant at $\alpha = 0.05$).

There was no difference in response times between beetles that chose different treatments in the bioassays 5-7 (all $p > .05$; see Table IV of Appendix). There was no significant effect of sex in choosing a treatment and in response times (all $p > .05$; see Table IV of Appendix) (Table 3.3). The response times were compared between sexes within a choice in each of these bioassays i.e. differences between males and females that chose option1 were compared.

Table 3.3 Choice of treatment and mean response times (minutes) of male and female *Eucolaspis* sp. "Hawke's Bay" beetles in choosing either of the two treatments in Y-tube olfactometer bioassays. Response times between sexes within each set of beetles that chose a option were compared such that there were 6 possible comparisons from the data of bioassays 5-7 (not significant at $\alpha = 0.05$, also see Table IV of Appendix).

Bioassay	**Sex**	**N**	**n**	**Chose option 1 (mean response time in minutes)**	**Chose option 2 (mean response time in minutes)**
5. Apple vs. apple with beetles feeding	♂	14	11	7 (11.55)	4 (12.27)
	♀	7	6	3 (10.21)	3 (14.89)
6. Apple vs. blackberry, (Havelock North "apple" population)	♂	10	10	5 (4.88)	5 (7.92)
	♀	8	7	5 (6.03)	2 (3.3)
7. Apple vs. blackberry, (Waikanae "blackberry" population)	♂	12	11	3 (9.72)	8 (6.89)
	♀	13	11	5 (7.66)	6 (7.44)

N= Number of beetles tested; n=number of beetles responded

3.3.2. Host feeding preference

Beetles of both host plant populations ("apple population" from Havelock North and "blackberry population" from Waikanae) preferred to feed on blackberry leaves (Table 3.4). The proportion of leaf area and amount of leaf mass consumed was significantly different in apple-blackberry choice (Table 3.4). Male and female beetles from Waikanae population displayed similar food preferences whereas males from Havelock North were less choosy between the two leaf discs provided (Table 3.4). Although beetles from the Havelock North population fed on both apple and blackberry leaf discs provided, they consumed about twice the leaf area of blackberry (about 66% of the total meal consumed) comparing to that of apple (Figures 3.5 and 3.6).

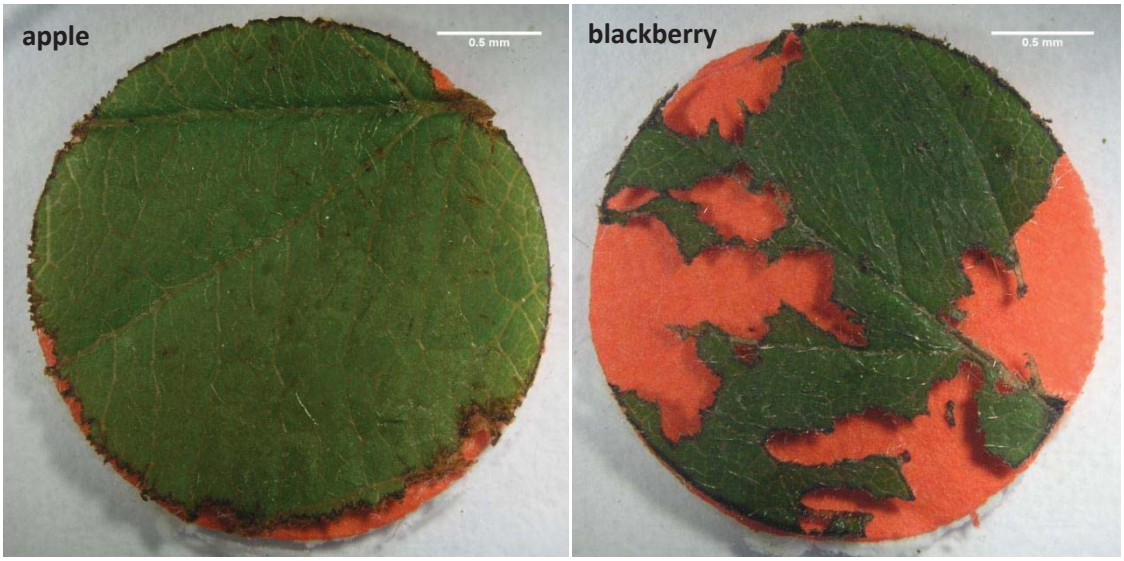


Figure 3.5 An example of leaf area of apple and blackberry consumed by *Eucolaspis* sp. "Hawke's Bay" beetles from the Havelock North "apple population" in the feeding preference bioassay (relative feeding by a single beetle; both leaf discs were offered simultaneously).

Very few of the 31 test insects from Waikanae "blackberry" population fed on apple, preferring instead to feed on blackberry exclusively (Table 3.4 and Figure 3.6). Beetles of the Waikanae population consumed less leaf irrespectively of the host plant, compared to beetles of Havelock North population (Figure 3.6). Females consumed more total leaf area than males irrespective of population source (Table 3.4).

Table 3.4 Leaf area of apple and blackberry consumed in feeding bioassays by the adult *Eucolaspis* sp. "Hawke's Bay" from two different host plant populations (apple population in Havelock North, blackberry population in Waikanae).

Population source	**Sex**	**N**	**Percent leaf area consumed (mean±SE)**		***t***	***p***
			apple	**blackberry**		
Havelock North	♂	29	1.5979 ± 0.258	2.3908 ± 0.464	-1.49	.141
	♀	19	1.4877 ± 0.326	3.9819 ± 0.549	-3.91	<.001
	Total	48	1.5543 ± 0.200	3.0206 ± 0.369	-3.49	<.001
Waikanae	♂	20	0.0018 ± 0.001	0.1018 ± 0.020	-4.87	<.001
	♀	11	0	0.2297 ± 0.079	-2.9	.009
	Total	31	0.0011 ± 0.001	0.1472 ± 0.032	-4.53	<.001

N = number of insects tested

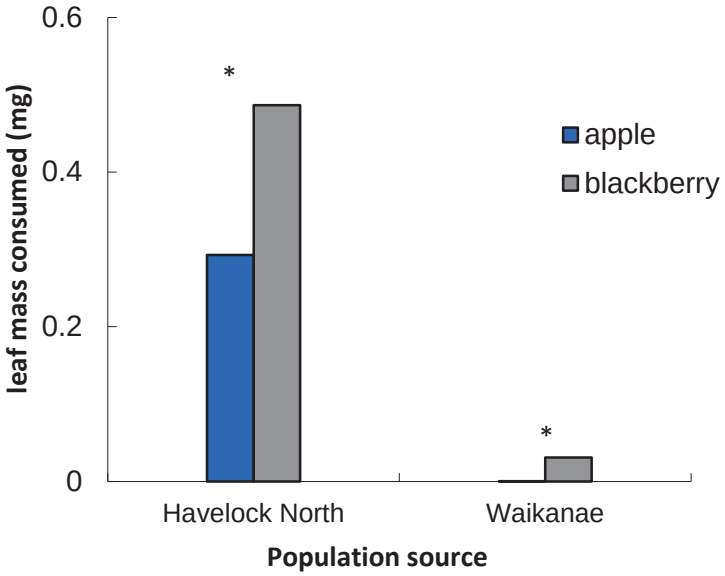


Figure 3.6 Apple and blackberry leaf mass consumed by adult *Eucolaspis* sp. "Hawke's Bay" from two different host plant populations (apple population in Havelock North, blackberry population in Waikanae) in feeding bioassays. (*p <.01)

3.4. Discussion

Adult *Eucolaspis* sp. "Hawke's Bay" beetles were attracted, to odours from both apple and blackberry when offered each alone. Field observations indicate these plant species are the most intensely used hosts. Insects move towards odour source in field anticipating to find a familiar host-plant (Visser, 1986). Strong anemotaxis shown by bronze beetle adults in the current study represents a likely odour-oriented host-finding behaviour in nature. The attraction of beetles to the odours of the tested host plant (apple and blackberry) indicates that the beetles use olfactory cues to invade suitable host plants in the habitat.

Attraction of pest insects to odours from apple plants is a widely established phenomenon. For example, odours from apple leaves and fruitlets attract pest insects such as codling moth *Cydia pomonella* (Reed & Landolt, 2002; Ansebo et al., 2004; Coracini et al., 2004; Hern & Dorn, 2004), oriental fruit moth *Cydia* (*Grapholita*) *molesta* (Piñero & Dorn, 2007), apple maggot fly *Rhagoletis pomonella* (Prokopy et al., 1973; Fein et al., 1982; Zhang et al., 1999) and apple fruit moth *Argyresthia conjugella* (Bengtsson et al., 2006).

Some of the plant odour components could be common between groups of plant taxa. Bengtsson et al. (2001) found that as many as 11 volatiles co-occur in rowan

berries and apples (both Rosaceae) in different proportions, and all these volatiles elicit antennal response in apple fruit moth *Argyresthia conjugella*. Hori et al. (2006) found that strawberry leaf beetle *Galerucella vittaticollis* (Chrysomelidae) is attracted to odours from a variety of host plants: strawberry (Rosaceae), buckwheat, dock, knot-weed, Japanese knot-weed (Polygonaceae), but not to odours from non-host plants such as radish (Brassicaceae), wolfberry (Solanaceae), Japanese mugwort (Asteraceae) and wheat (Graminae). Hori et al. (2006) also identified a common green leaf volatile, cis-3-hexnyl acetate, as the critical chemical used by the beetles to identify the host plants. Though this chemical was also found in non-host plants, its concentration varied. Similar behaviours have been demonstrated in rolled leaf beetles (García-Robledo & Horvitz, 2009), which were attracted to host plants belonging to families Marantaceae, Costaceae and Zingiberaceae. I suspect that both apple and blackberry have some key volatiles in common which attract bronze beetles to these plants. In contrast to our findings, the leaf beetle *Altica engstroemi* (Chrysomelidae: Alticinae) uses visual but not olfactory cues to locate its host plant, meadowsweet *Filipendula ulamaria* (Rosaceae) (Stenberg & Ericson, 2007).

My findings corroborate information for other chrysomelids, such as Colorado potato beetles (Thiery & Visser, 1986) and golden rod beetles *Trirhabda canadensis* (Puttick et al., 1988) which were attracted to their host plant odours and show positive anaemotaxis. Likewise, Pivnick (1992) found that mustard oils, characteristic to brassica plants, attract flea beetles *Phyllotreta cruciferae* and *Phyllotreta striolata*. In another similar study, Park et al. (2004) found that the alder leaf beetle *Agelastica coerulea* females were attracted to fresh leaf odours from their host plants of the family Betulaceae.

In the experimental setting, *Eucolaspis* sp. "Hawke's Bay" beetles were not attracted to either white clover or dock by olfaction, although in the field the beetles were observed to feed on both these plants. It is possible that the bronze beetles do not have olfactory abilities to recognize these two plants and use other cues to find them. Also, in this study the beetles did not distinguish between blackberry and apple odours when both were offered. Though not statistically significant, there was a slight preference for ancestral host plant in the two beetle populations tested. The two beetle populations tested (Havelock North and Waikanae) belong to the same mtDNA lineage, are genetically similar, and the average difference between the two populations is

limited to just two mutations at CO1 locus (Chapter 2). However, affiliation to specific host plants by geographically separated conspecific populations is not uncommon in Chrysomelidae. For example, host-plant preferences varied within populations of *Chrysomela lapponica* (Coleoptera: Chrysomelidae); some populations preferred *Salix mirsinifolia* while others preferred *S. caprea* (Zvereva et al., 2010). Similarly, different locality populations of *Agelasa nigriceps* (Coleoptera: Chrysomelidae) showed different levels of acceptance towards a recently established host plant, *Pterostyrax hispidus* (Kohyama et al., 2012). It is also possible that either both apple and blackberry are equally favoured as host plants, and hence the beetles could not choose one over the other, or beetles could not make a choice based on olfaction alone. In some cases olfactory cues alone could be insufficient for the insects to exactly decipher the host plant. In such cases, insects might often use other plant cues in combination with odours. For example, Bjorklund et al. (2005) tested the role of visual or odour plant cues in host-plant finding by pine weevil *Hylobius abietis* and found that both types of cues were crucial and had an additive effect in helping the beetle to find the conifer seedlings. Similarly, Heisswolf et al. (2007) identified that contact stimuli in addition to olfactory stimuli, were required to identify host plants by the tortoise beetle *Cassida canaliculata* (host plant meadow sage, *Salvia pratensis*, family Lamiaceae).

It was found that *Eucolaspis* sp. "Hawke's Bay" beetles did not differentiate between a colonised and non-colonised plant. This could be because no change was induced in either volatile quantity or quality by damage. This is in contrast to findings by Bolter et al. (1997) and Landolt et al. (1999) that adult Colorado potato beetles are attracted to damaged plants. Damaged and undamaged potato plants both released the same volatile chemicals, but the blend differs. Another chrysomelid, *Oreina cacaliae* was attracted to both its spring host, alpine butterbur and primary host, grauer alpendost (both Asteraceae) when damaged by conspecifics (Kalberer et al., 2001). This type of response may indicate the host damage offers the opportunity to locate conspecifics.

When feeding preferences were tested, beetles from both test populations preferred blackberry to apple (Figures 3.5 and 3.6). The beetles did not choose blackberry or apple over the other in olfactory bioassays (Figure 3.4), but when more cues were available in the feeding bioassays, the beetles preferred blackberry. This indicates that blackberry leaves are more palatable for the beetles than apple. This may be because of differences in leaf morphology and chemistry (including dietary values

and secondary metabolites) between apple and blackberry. In the case of polyphagous insects, the extent of feeding on a plant is largely governed by phagostimulants (carbohydrates, amino acids and other nutrients that stimulate feeding) and deterrents (plant secondary metabolites such as alkaloids that act as feeding deterrents) (Bernays & Chapman, 1994). This has been shown in many herbivorous chrysomelids: for instance, the feeding preference of willow- feeding leaf beetles (*Phratora vitellinae*, *Plagiodera versicolora*, *Lochmaea capreae* & *Galerucella lineola*) on different cultivated and wild willow species plants was influenced by leaf trichome density (Soetens et al., 1991) and composition of phenolic glycosides in the leaves (Tahvanainen et al., 1985). Similarly, Eben et al. (1997) found that chrysomelids *Acalymma* spp. and *Diabrotica* spp. preferred cucurbitacin-containing Cucurbitaceae plant (squash variety "Ambassador") over non-cucurbitaceous plants such as corn and soybean, or a squash variety "Early Summer crook-neck" that doesn't contain cucurbitacin.

Female beetles appear to consume more total leaf area than males which may be due to relative size and metabolic rate differences between sexes (females are generally larger – see Chapters 1 and 2 for sexual dimorphism details). Beetles of the Waikanae population consumed less total leaf area than the beetles of Havelock North population which may be due to difference in age (age of Waikanae population was unknown – as the beetles were collected as adults from the field) or other unknown physiological factors.

I believe that there may be chemical compounds common to both blackberry and apple that are being recognized by the beetles to use as host location stimuli. In their natural environment beetles may use visual cues in addition to volatiles; but this study has demonstrated the beetles could locate hosts even when only olfactory cues are available. The ability of the bronze beetles to locate host plants when only olfactory cues are available would perhaps enable them to locate and feed on host plants even during the night, as they do (personal observations). Having identified specific olfactory abilities of *Eucolaspis* sp. "Hawke's Bay" in host plant location, exploring the host range and associated behaviours of different species of *Eucolaspis* would be interesting. In addition to this, testing attraction of the beetles to native host plant volatiles would provide a verification of host-breadth dynamics of the beetles and contribute vastly to the knowledge on feeding behaviour of polyphagous herbivores.

Blackberry, a common weed plant with distribution all over New Zealand (Popay et al., 2010), could be providing a persistent source of bronze beetle populations which could potentially invade into economically important fruit crops such as organic apple orchards or strawberries. Numerous insect pests were shown to move from weeds to crop hosts (Norris & Kogan, 2000). For example, Ruthglen bug (*Nysius vinitor)* in Australia was found to migrate into field crops (sunflower, safflower, canola and sorghum) from a range of weed host plants (Kehat & Wyndham, 1973; Miles, 2010). It is not known how far the bronze beetles can disperse, to better estimate the importance of this reservoir and the possibility of crop invasion. Although adjacent cropping activity was found to have no influence on bronze beetle density in infested apple orchards (Rogers et al., 2006), I suspect fresh invasions are common owing to attraction of beetles to apple volatiles.

3.5. Conclusions

The current findings show that *Eucolaspis* sp. "Hawke's Bay" beetles use plant odours to detect and discriminate host and non-host plants, but the beetles were not able to distinguish between damaged and undamaged host plants and between closely related species of host plants just by olfaction. There are apparent differences in host plant preference by beetles of different populations, but the findings were not quite conclusive. Irrespectively of their geographical location and ancestral host plant, beetles obviously preferred to feed on blackberry over apple. My results provide a basis for further research into the role of volatiles in host plant location by *Eucolaspis* beetles. Plant volatile (head-space) and leaf chemistry analyses of apple, blackberry and few other Rosaceae plants would complement the outcome of our behavioural bioassays and provide a compelling verification of host selection by *Eucolaspis* sp. "Hawke's Bay" beetles. This could lead to development of a behavioural control or monitoring method using attractants or trap crops.

Chapter 4: Adult sex ratios, mating behaviour and mate recognition in *Eucolaspis* sp. “Hawke’s Bay”

4.1. Introduction

Most of the leaf beetles (Chrysomelidae) mate multiple times; and males search competitively for potential female mates (Dickinson, 1992; 1995; 1997). I suspect a similar mating system in *Eucolaspis,* but there is very little information available so far. Lysaght (1930) supposed that there may be equal number of male and female *Eucolaspis* beetles in nature; but no data was provided in support of this opinion. Multiple mating has been observed in both sexes of *Eucolaspis* (Lysaght, 1930; personal observations). Apart from these, our knowledge of mating behaviour and sex ratios in *Eucolaspis* is restricted at the moment. There is no information available on most aspects of mating behaviour, such as sexual maturity, mating sequence, mating duration, role of vision and close range or contact pheromones utilized in mate location.

Hanks et al. (1996) suggested that sexual dimorphism in longhorn beetles (Coleoptera: Cerambycidae), where males have longer antennae than females, is an evolutionary adaptation to perceive sex pheromones. *Eucolaspis* males also have longer and more robust antennae than the females (Shaw, 1957), suggesting a similar selection trait to that of male longhorn beetles. However, no evidence for a long-range sex pheromone for mate location has been observed in *Eucolaspis* sp. "Hawke's Bay" (Rogers, unpublished data). Close range or contact sex pheromones are a possibility which if found, would have important implications in devising environmentally safe control strategies.

From ecological and evolutionary perspectives, we need to understand the mating system in *Eucolaspis* including how sexual dynamics operate (i.e., when and which sex dominates emergence and the active population) and implications of these in terms of population growth. Studying the mate location process and sex ratios of *Eucolaspis* sp. "Hawke's Bay" would greatly enhance our understanding of the ecology and reproductive biology of *Eucolaspis* in particular and Chrysomelidae in general.

4.1.1. Sex ratios

Sex ratio, estimated as the proportion of males in the offspring produced, is an intrinsic attribute that defines dynamics of reproduction in species that have separate sexes (Hardy, 2002). Sex ratios can be measured as either the population sex ratio or operational sex ratio, each of which conveys different biological inferences. Population

sex ratio refers to the physical number of males and females present in a population at a given time. The operational sex ratio refers to the number of individuals of either sex (usually males) that are readily available to mate with each individual of the opposite sex (Emlen & Oring, 1977; Kvarnemo & Ahnesjo, 1996). The operational sex ratio may or may not be the same as the population sex ratio. Sex ratios essentially outline the mating system of a species, such as competition between individuals of the same sex, selection within a sex, inter-sex selection, relative roles of each sex, etc. (Kvarnemo & Ahnesjo, 2002).

In animals that are capable of polygamy, operational sex ratios are usually male-biased, as females need time out for brood-related activities. However, there are exceptions, where sex roles are reversed and males care for the brood (Hardy, 2002). Sexual selection leads to sexual dimorphism and other sex-specific adaptations (Stuart-Fox & Moussalli, 2007).

Studying sex ratios provides useful quantitative information, with many basic and applied implications in evolutionary biology, and the sex ratios are also the main inputs in population dynamic models (West et al., 2002). Seasonal changes in sex ratios show us sex-specific traits that maintain equilibrium in the population.

4.1.2. Mating behaviour

Insect mating is a complex process involving mediation of cues such as visual, acoustic, or chemical (Bailey, 1991). Chemical stimuli, collectively known as pheromones, play a crucial role in communication between individuals of the same species. Pheromones are often used in various levels of mate choice information such as species recognition, mate recognition, and mate assessment (Johansson & Jones, 2007). Pheromones are used to recognize nest-mate in social insects, in defense, and even in chemical mimicry (e.g., in blister beetle *Meloe franciscanus* larvae) (Howard & Blomquist, 2005). Pheromones can be either sequestered from chemicals derived from host plants or produced *de novo* (Tillman et al., 1999). The pheromones may be produced in specialized glands (such as abdominal glands in many beetles) or produced elsewhere and stored in specific glands (Jurenka, 2004).

Sex pheromones that typically facilitate mating between two individuals can be of different types: long-range, close-range or contact pheromones. The more volatile a

pheromone is (shorter carbon chain), the greater the distance over which it can be perceived by the target individuals. Long-range pheromones, such as those used by many moth species, have been shown to attract potential mates from great distances (e.g. Kochansky et al., 1975; Wall & Perry, 1987). In Coleoptera, such long-range sex pheromones are rather rare, although exceptions include, but are not limited to, the rove beetle *Aleochara curtula* (Peschke & Metzler, 1987; Bartelt, 2010) and the cowpea weevil *Callosobruchus maculates* (Phillips et al., 1996). However, close-range and contact sex pheromones have been reported for many beetles (Sugeno et al., 2006).

Hydrocarbons (n-alkanes, methyl-branched alkanes and alkenes), that render insect cuticle hydrophobic, have crucial roles in chemical communication in many insect species (Blomquist et al., 1987; Howard & Blomquist, 2005; Blomquist, 2010). These hydrocarbon pheromones are produced in subcuticular abdominal epidermal cells (oenocytes), deposited onto the cuticular surface (Blomquist, 2010) and are perceived by olfactory sensillae (on antennae) and/or tactile sensillae (on palpi and tarsi). Utilization of cuticular hydrocarbons as contact sex pheromones has been reported for many longhorn beetles (Coleoptera: Cerambycidae) (Hanks et al., 1996; Wang et al., 2002; Ginzel et al., 2003; Ginzel & Hanks, 2003; Zhang et al., 2003; Lu et al., 2007) and also for some weevil species (Coleoptera: Curculionidae) (e.g. Mutis et al., 2009). Among leaf beetles (Chrysomelidae), the existence of contact sex pheromones has so far been reported for Colorado potato beetle *Leptinotarsa decemlineata* (Jermy & Butt, 1991; Otto, 1997), green dock beetle *Gastrophysa atrocyanea* (Sugeno et al., 2006), dogbane beetle *Chrysochus auratus* (Peterson et al., 2007), mustard leaf beetle *Phaedon cochleariae* (Geiselhardt et al., 2009) and coconut leaf beetle *Brontispa longissima* (Kawazu et al., 2011).

Vision also has an important role in mate location, especially in diurnal species and those that exhibit luminescence (Bailey, 1991). Some beetles use vision alone (Wang & Chen, 2005), while others use a combination of visual and chemical cues for mate-finding (Fukaya et al., 2004; 2005). Szentesi et al. (2002) showed that Colorado potato beetles used reflectance pattern in their searching behaviour and were attracted to yellow and yellow-green boards and beads that matched the reflectance spectra of beetles' elytra. In most cases different stimuli act synergistically, and enable the insect to assess the bigger picture such as species, gender and fertility status of a potential

mate.

Investigating mate location processes helps us to understand reproductive strategies that govern insect mating systems. These reproductive strategies and insect sex signals, which are generally species-specific, are often the basis on which to formulate control strategies. Behavioural pest control methods, that utilize strategies such as mating disruption or mass capturing, are designed based on the specific sex pheromones employed in mate location by the insect of interest (Witzgall et al., 2010). For example, pests of stone fruit orchards (nitidulid beetles *Carpophilus* spp.) in Australia have been successfully managed by an attract-and-kill strategy with pheromone traps and insecticide-treated baits (Bartelt, 2010). In New Zealand apple orchards examples include codling moth (*Cydia pomonella*), light brown apple moth (*Epiphyas postvittana*) and apple leaf rollers (green headed leaf roller *Planotortix octo* and brown headed leaf roller *Ctenopseustis obliquana*) which can be managed by using pheromone traps and mating disruption (El-Sayed et al., 2006; Suckling et al., 2007; Suckling et al., 2011). Moreover, species-specificity of certain reproductive strategies (such as pheromones) assists differentiation of closely related and otherwise similar taxa (Smadja & Butlin, 2008). Chemical taxonomy is a fast advancing field used to delineate species boundaries (Bagneres & Wicker-Thomas, 2010). Peterson et al. (2007) were able to identify a group of contact sex pheromone compounds which maintain reproductive isolation between sympatric species of *Chrysochus* (Coleoptera: Chrysomelidae), *C. cobaltinus* and *C. auratus*.

4.1.3. Research objectives

This chapter outlines various experiments I conducted with *Eucolaspis* sp. "Hawke's Bay" beetles to study sex ratios and mate location, with the following specific questions.

1. What is the emergence pattern and adult sex ratio of *Eucolaspis* sp. "Hawke's Bay" in organic apple orchards in Hawke's Bay?
2. How does the emergence sex ratio vary over time?
3. How is short-range mate location in *Eucolaspis* sp. "Hawke's Bay" mediated?
4. Is there any evidence of contact sex pheromones that mediate mate recognition in *Eucolaspis* sp. "Hawke's Bay"?

4.2. Materials and methods

4.2.1. Sex ratios

Fifteen emergence traps (Figure 4.1) were installed per orchard in 8 organic apple ('Royal Gala') orchards (orchards H1, H2, H3, H4, L1, L2, L3 and L4) (see Ch. 5) in Hawke's Bay, New Zealand during spring 2007 to study emergence sex ratios. A bucket trap was an upturned black plastic bucket (9 L capacity and 270 mm diameter) with circular a hole (60 mm diameter) in the base. A Petri dish base that also has an equivalent hole was glued to the bucket base, and a Petri dish lid was then placed on top of the Petri dish base (Rogers et al., 2006). The Petri dish lid was smeared with Tanglefoot® insect glue to trap emerged adult beetles as they move upwards from the ground. The beetles are negatively geotropic and light passing through the clear Petri dish lures the beetles to it. The traps were installed near randomly selected trees, within 1m radius from the trunk and preferably under a branch (Rogers et al., 2006). Each bucket trap was held to the ground by two iron pegs. The traps were monitored weekly from 1st October 2007 and trapped beetles were collected into individual labelled vials, transferred to the laboratory and stored at -20 ^{0}C until processed. Adult *Eucolaspis* sp. "Hawke's Bay" caught in these emergence traps were used for determining emergence (population) sex ratio.

Figure 4.1 Bucket trap used to monitor *Eucolaspis* sp. "Hawke's Bay" adult emergence in 8 organic apple orchards in Hawke's Bay, New Zealand.

Adult *Eucolaspis* sp. "Hawke's Bay" for the study of the active population sex ratio in apple foliage were collected by beating branches of randomly selected trees in two other organic apple orchards ('Royal Gala', orchard A and orchard B) in Hawke's Bay, New Zealand during summer 2009, onto circular plastic plates (22.5 cm diameter) smeared with Tanglefoot® insect glue. The plates along with the beetles caught were then transferred to the laboratory and stored at -20 ^{0}C until further processing. Sampling of adult *Eucolaspis* from other locations and host plants was achieved by beating branches of a host plant over a beating sheet; collected beetles were then preserved in 95% alcohol in plastic vials, transferred to the laboratory, and stored until further processing. All the beetles collected from emergence traps and trees were dissected to determine the sex under a dissecting microscope (Olympus SZX-ILLD2-200).

4.2.2. Mating behaviour

Olfactometer bioassays and mating experiments were conducted in the laboratory at Massey University, Palmerston North, during summers of 2008-09, 2009-10 and 2010-11. Adult beetles raised from pupae collected from an organic apple orchard ('Royal Gala', orchard A) in Hawke's Bay, New Zealand were used in this study. Insect collection and culturing methods were as detailed in Chapter 3. Individual adult beetles were sexed immediately after emergence based on the morphology of last abdominal segment (Shaw, 1957) and maintained in individual Petri dishes (5.5 cm diameter) lined with moist filter paper (Whatman® Cat No 1001-055), with apple leaves and / or fruitlets (organic 'Royal Gala') provided as food. Three to five days old normal healthy-looking virgin insects were used in the bioassays.

4.2.2.1. Olfactometry

The existence of any female-specific close range sex pheromone was assessed by dual-choice olfactometer bioassays. These bioassays were carried out with a glass Y-tube (Figure 3.1 in Chapter 3) using virgin healthy male beetles as test insects, with the two choices being 4 virgin females vs. 4 virgin males. No food was provided in either of the choice chambers. The test male beetles (36 males) were individually released into the central arm of the Y-tube and the beetles were allowed to spend a maximum of 20 minutes to choose either of the two options. Males that did not choose any of the two choices after 20 minutes were deemed unresponsive and removed from further analysis. A black muslin cloth was used as a vision barrier to separate specimen (choice)

chambers from the rest of the Y-tube. The bioassay and data collection procedures were followed as described in Chapter 3.

4.2.2.2. Mating bioassays

The role of vision in mate location and existence of a female-specific contact sex pheromone was evaluated through a series of mating bioassays in the laboratory. Pairings were carried out in small Petri dishes (5.5 cm diameter) lined with moist filter paper (Whatman® Cat No 1001-055) under controlled conditions of 20 ± 1 ^{0}C temperature and artificial light. The mating bioassays were recorded by video camera (Sony® digital video recorder - model number DCR-SR35E) and the videos were later analysed. Each bioassay was continued for a maximum of 1 hour. Petri dishes were washed with acetone and air dried after each trial. All insects in the experiments were used only once.

1. Role of vision in mate location was tested through mating bioassays between blind (with compound eyes covered) virgin male and normal virgin female insects.

Covering compound eyes: Insects were anaesthetized by placing on ice for 5 minutes (Mpho & Seabrook, 2003). An eyelash glued to a needle tip was used for painting the insects' compound eyes under a dissecting microscope. Three types of paint (acrylic, water soluble, and face paint) and nail polish were used. The insects with covered compound eyes were allowed to recover for 24 hours before conducting the bioassays, by which time they had resumed normal feeding and walking activities. None of the beetles with paint covered eyes retained the paint after 24 hours: all of them had removed the paint by grooming activities. Nail polish was detrimental to the beetles and most of them died within 24 hours. Hence, the trial on vision (treatment 1 in the above list) had to be abandoned and no data was available for further analysis.

2. Role of antennae in mate location (assuming antennae alone are used to perceive pheromones) was tested through mating bioassays between virgin male whose antennae were ablated and normal virgin female insects.

Ablation of antennae: Antennae of virgin male beetles were ablated surgically using a pair of fine scissors under a dissecting microscope. The scissors were sterilized with 95% alcohol between ablations. Insects were anaesthetized by placing on ice for 5

minutes before ablation, and were allowed to recover for 24 hours before being presented each with a live healthy normal female.

3. Existence of female-specific contact sex pheromone was verified through bioassays involving normal virgin male and treated female (Lu et al., 2007; Peterson et al., 2007). Males were offered one of the following:

a) a freshly-freeze killed female cadaver (cuticular chemistry intact)
b) freshly freeze-killed and washed female cadaver (cuticular chemistry stripped)
c) a freshly freeze-killed, washed and reconstituted female cadaver (cuticular chemistry reconstituted)
d) a freshly freeze-killed male cadaver (negative control)
e) a normal live healthy virgin female (positive control)

From freeze-killing to reconstitution of cuticular hydrocarbon extracts (see treatment 3.c above), slightly modified methods of Peterson et al. (2007) were followed.

Freeze-killing: Live virgin beetles (male and female) were kept at -20 ^{0}C for 1 hour for freeze-killing; the cadavers were allowed to warm to room temperature by placing them at 25 ^{0}C for up to 1 hour before presenting to a virgin male.

Stripping cuticular chemistry: Freshly freeze-killed virgin females were each put through three 10-minute washes in 1 ml *n*-hexane each. The cadavers were then allowed to dry at room temperature before being presented to a virgin male.

Sourcing cuticular hydrocarbons (CHC): Freshly freeze-killed virgin females were rinsed individually for 10 seconds in 1 ml *n*-hexane; the rinsing was repeated with another 1 ml *n*-hexane. Both extracts (1+1 ml) were combined, then dried to a crust in a SpeedVac (Savant SpeedVac® - Thermo Fisher Scientific Inc.) and stored at -20 ^{0}C until further use.

Reconstituting CHC extracts and painting on female cadavers: 10 µL of *n*-hexane were added to each of the crusts of crude extract, centrifuged, and applied by a micropipette to the elytra and pronotum of a female cadaver that had its cuticular chemistry stripped.

The positive control bioassays (normal male and female pairs) were also used to observe general mating behaviour, in which the sequence of courtship behaviours,

copulation, and post-copulatory mate guarding were recorded. In addition to these mating bioassays, field and laboratory populations were observed for any contests between rival males over receptive females for mating.

4.2.3. Data analysis

Sex ratio data were analysed for deviation from normal 0.50 ratio using binomial tests. The effect of orchard and sampling time on the sex ratio were evaluated using logistic regression. Y-tube olfactometry data were analysed by binary logistic regression. All videos of mating bioassays were viewed and data for number of encounters, behavioural sequences and mating success were recorded; the data on mating success were analysed by binary logistic regression. Data on number of mating encounters and response times were analyzed by ANOVA for differences between control and different treatments, and also among treatments after confirming the data normality with univariate tests. All statistical analyses above were performed in SAS v.9.2 (SAS Inc.). Data on directions (angles) from which encounters were initiated by test males in treatment (please see treatment 3.a above) involving intact female cadavers were recorded (by superimposing freezed frames on to a protractor) and these data were analysed for directional bias using Rayleigh test and Watson's U^2 test in Oriana v.3.21 (Kovach Computing Services). Fungal infections in the laboratory to field-collected beetle pupae limited the number of available insects for some experiments. However, similar numbers of replications (number of individuals) have also been used by other researchers in similar experiments (Ginzel et al., 2003; Fukaya et al., 2004; Ibeas et al., 2008; Silk et al., 2011).

4.3. Results

4.3.1. Sex ratios

In all orchards the emergence (population) sex ratio was female-biased. No bronze beetles were caught in orchard L1 and only a few beetles were caught in orchards L3 and L4 over the entire season. These three orchards were therefore omitted from further analysis.

The overall mean emergence sex ratio (mean of all orchards and all sampling periods) was 0.35 (proportion of males), which was significantly different ($z = 9.73$, $p < .001$) from the 0.5 ratio. Though the sex ratio slightly varied among different orchards

tested, the differences were not statistically significant (Wald χ^2 (4, N=1035) = 12.05, p = .06). Among all sites, orchard H2 had the highest total of females emerge, whereas orchard H1 had the highest total of males emerge (Figure 4.2). The sex ratio varied over the season, with more males emerging early in the season (Figure 4.3). Differences in the sex ratio between sampling dates were significant (Wald χ^2 (8, N=1035) = 28.18, p <.001). Two peaks in emergence were observed for female beetles, separated by about 3 to 4 weeks. The number of males emerging continued to increase until the 23rd November 2007, while the number of females emerging continued to increase until the 30th November.

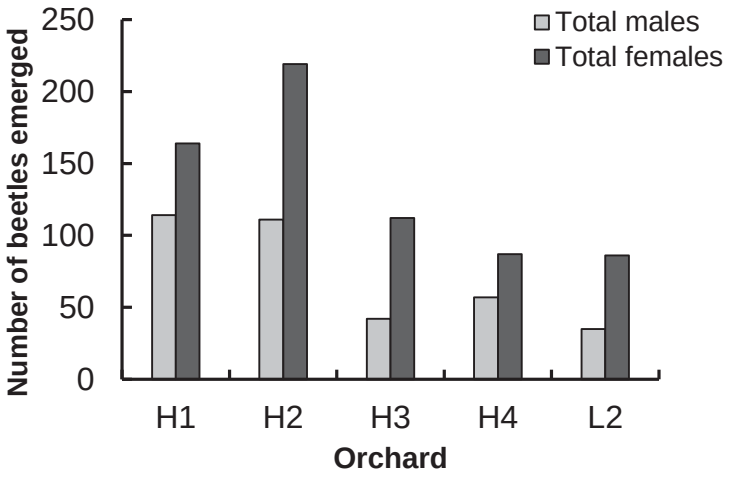


Figure 4.2 Total number of male and female *Eucolaspis* sp. "Hawke's Bay" beetles emerged from each of the various organic apple ('Royal Gala') orchards (orchards H1-H4 and L2) in Hawke's Bay, New Zealand, during 2007-08. Differences between male and female numbers were significant within each of all the 5 orchards (p <.05, binomial exact test)

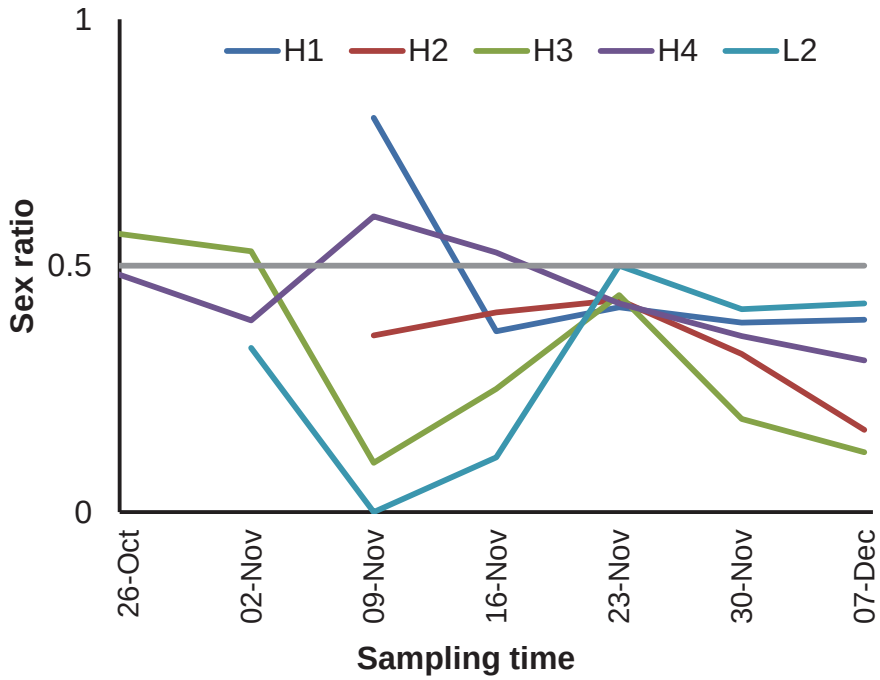


Figure 4.3 Seasonal variation of sex ratio (proportion of males) in adult bronze beetles emerging from different organic apple orchards ('Royal Gala') in Hawke's Bay, New Zealand during spring-summer 2007 (horizontal grey line indicates sex ratio 0.5).

Adult sex ratios in active populations on foliage were quite different from the emergence sex ratio. Adult sex ratio (proportion of males) assessed by beating foliage, on 25th November 2009 in orchard "A" (see Methods) was found to be 0.55 (z = -1.67, *p* = .107), whereas it was 0.56 (z = -2.52, *p* = .013) in orchard "B" on 10th December 2009.

Adult sex ratio of active population in different host plants' foliage varied from 0.39 to 0.69. However, data were obtained from a single host plant per location (and a single sample for some of the host plants) in most cases, and thus may not provide an actual estimation of variation; no statistical analysis was performed on these data.

4.3.2. Mating behaviour

Mating duration (from mounting to dismounting) varied from a few minutes to a few hours. While attempting to copulate and during copulation, males engaged in antennal vibration and licking behaviours (Figure 4.4). In all observed mating events, antennal contact preceded actual copulatory attempts. Copulatory attempts involved a sequence of behaviours including: male stopping after contact, male chasing female, male aligning with female, male grasping female with legs, mounting, bending abdomen, and protruding aedeagus (Figure 4.4).

Males vigorously vibrated their antennae in a characteristic manner, holding both antennae perpendicular to the body. Antennal vibration was intermittent and repeated several times. Males also tapped their antennae on female elytra and sometimes even climbed further towards female's elytra and pronotum, bit and/or licked the female, which I suspect was an effort to appease the female if it was non-receptive (Figure 4.5). Sperm transfer occurred between genitalia and whether males extract female genitalia was not evident.

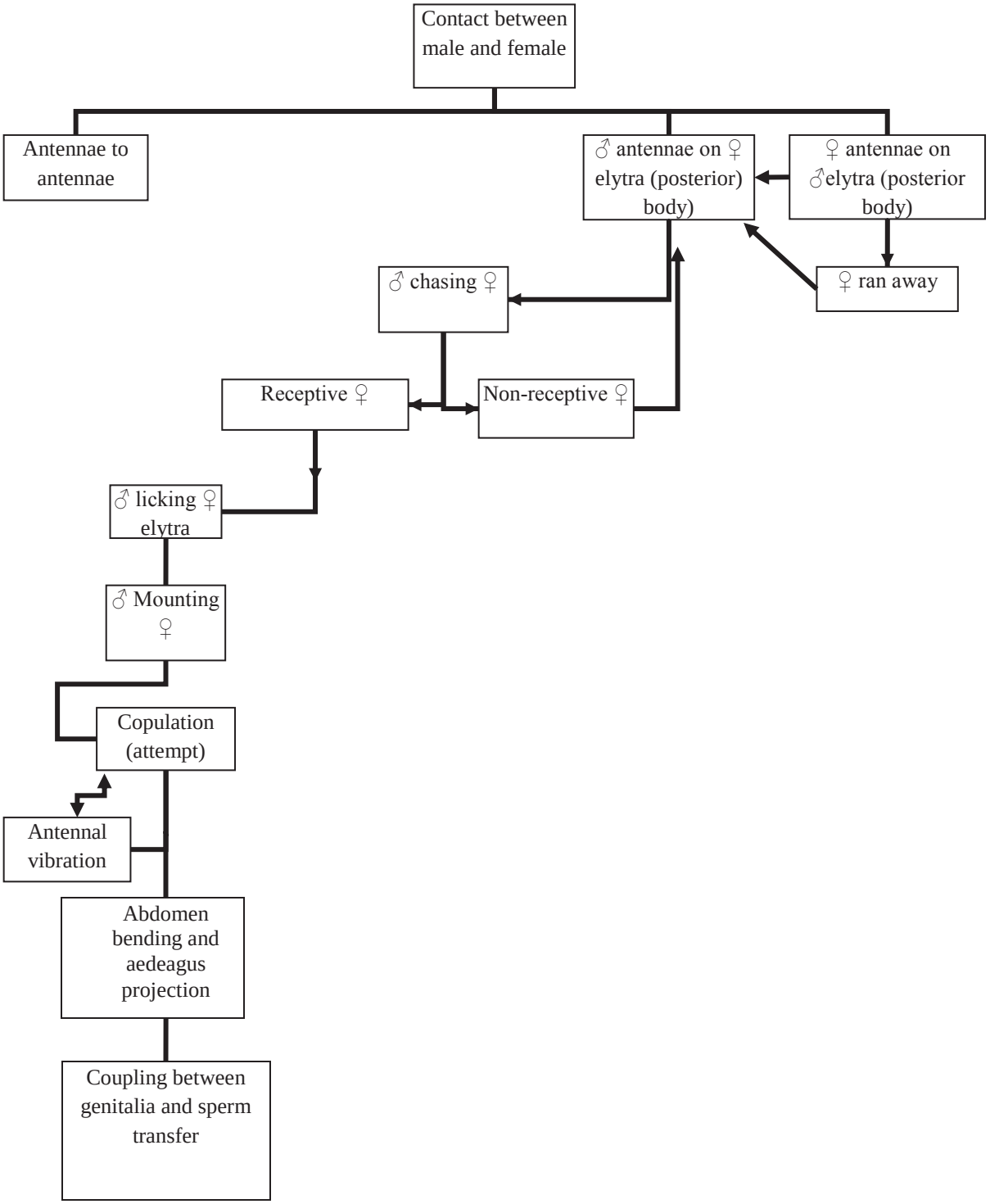


Figure 4.4 Mating sequence of *Eucolaspis* sp. "Hawke's Bay". Direction of arrows denotes chronological succession of events. Antennal vibration and licking were observed multiple times during the course of a successful mating.

Figure 4.5 A mating pair of *Eucolaspis* sp. "Hawke's Bay" in the laboratory arena; protruded aedeagus (a) and licking of female's elytra by male (b) can be seen.

Post-copulatory mate guarding was also observed in some but not all cases, wherein males continued to ride on females' backs or stayed in close contact. The duration of guarding varied from a few seconds to more than an hour. Contests between two males for a receptive female were also observed; most of these fights involved violent antennal strokes by rival males, with occasional biting.

Olfactometer bioassays

A total of 36 virgin males were tested for attraction towards either male or female beetles; 29 males responded, of which 17 were attracted to females, whereas the remaining 12 were attracted to males. The 7 males that were unresponsive did not choose either treatment within the stipulated 20-minute observation time. Though males showed a slight affinity towards females, the difference was not statistically significant (Wald χ^2 (1, N=29) = 0.85, p =.356). Males that chose females took an average of 8.23 minutes (S.D. 5.10), whereas males that chose males took an average of 8.68 minutes

(S.D. 5.35) to respond and the difference in time was not significant ($F(1, 27) = 0.05$, $p = .82$).

Role of chemical cues

All except one of the 15 normal virgin males mated with normal virgin females. Normal males took on average 2.5 minutes to initiate copulation with normal females, the minimum time was 45 seconds and maximum was 7.5 minutes.

Males whose antennae were ablated performed well in mating; no significant difference in the proportional mating success when compared to the positive control (Wald χ^2 (1, N=31) =1.69, p = .194), although treated males needed slightly more encounters per successful mating (2.25 encounters) than normal males (1.5 encounters) before successful mating ($F(1, 24) = 1.18$, $p = .288$) (Table 4.1). Only 4 out of 16 males without antennae failed to mate with normal females. Despite many encounters in these 4 pairs, mating did not proceed further. Males without antennae took significantly more time than normal males to initiate copulation ($F(1, 24) = 17.24$, $p = .0004$) (Table 4.2). The minimum time elapsed was 2.08 minutes and the maximum time was 25.13 minutes before copulation occurred between a male without antennae and a normal female. In all these matings, mating advanced only after male licked female's elytra.

About half of the males tested (13 out of 29) attempted to mate with freshly freeze-killed female cadavers (with cuticular chemistry intact), which is a significantly lower proportion when compared to the positive control (Wald χ^2 (1, N=44) =6.69, p = .0097) (Table 4.1). Males took from 1.75 minutes to 40 minutes to initiate a mating attempt with a freshly freeze-killed female cadaver and, on average, the response time was significantly greater than in the control (live female) bioassays ($F(1, 25) = 16.3$, p = .0004) (Table 4.2).

Table 4.1 Response of normal and treated virgin male *Eucolaspis* sp. "Hawke's Bay" beetles towards normal and treated female beetles in the laboratory bioassays.

Treatment	**N**[1]	**Mean number of encounters**[2] **per a successful attempt**	**Mean number of encounters per an unsuccessful attempt**	**Percent attempted to copulate (%)**[3]
Normal male and normal female	15	1.50	5.00	93.33 a
Normal male and freshly freeze killed female	29	1.62	1.38	44.83 b
Normal male and freshly freeze killed and washed female	17	1.17	1.00	35.29 b
Normal male and freshly freeze killed, washed and reconstituted female	12	1.00	1.82	8.33 b
Normal male and freshly freeze killed male	8	NA[+]	8.38	0.00 b
Male without antennae and normal female	16	2.25	26.00	75.00 a

[1] N= number of males tested; [2] Number of encounters initiated by both sexes where a live female was among the mating pair.

[3] Percentages with same letter suffixes are not significantly different at $\alpha = 0.05$ (binary logistic regression). [+] Not applicable as none of the males attempted to mate

Table 4.2 Elapsed time before a successful mating attempt in different treatments in the laboratory mating bioassays.

Treatment	**N**	**Mean time (minutes) taken to initiate mating**[+]
Normal male and normal female	14	2.50 (2.09) a
Normal male and freshly freeze killed female	13	15.84 (12.19) b
Normal male and freshly freeze killed and washed female	6	24.33 (11.67) b
Normal male and freshly freeze killed, washed and reconstituted female	1	10.45
Male without antennae and normal female	12	10.03 (6.41) c

N=Number of males succeeded in mating or attempting to mate; [+] Values within parentheses are standard deviations; values suffixed by the same letters are not significantly different ($\alpha = 0.05$, one-way ANOVA).

In order to further investigate the bioassays that involved intact female cadavers, circular statistical analysis was performed (Figure 4.6). The analysis suggests that there was no concentration of encounters near a mean direction and the angles at which encounters were initiated were uniformly distributed ($p = .371$, Rayleigh test) (Figure 4.6). There was no difference between two groups of males (successful and unsuccessful) in circular distribution of the angles from which encounters were initiated ($p > .05$, Watson's U^2 test). This indicates that there was no directional bias in approaching the female cadavers and that approach direction did not differ from random irrespective of whether males attempted to mate or not.

Some of the males tested (6 out of 17) attempted to mate with freshly freeze-killed and washed female cadavers (cuticular chemistry stripped). Mating success of these bioassays was significantly reduced (Wald χ^2 (1, N=32) = 7.92, $p = .0049$) and significantly delayed ($F(1, 18) = 48.81$, $p < .0001$) when compared to normal male and normal female bioassays (control). Males, which attempted to mate with washed female cadavers, took a minimum of 13.27 minutes and a maximum of 44.38 minutes to initiate a mating attempt.

There was no significant difference between the proportion of males successful in attempting to mate with freeze-killed intact or washed female cadavers (Wald χ^2 (1,

N=46) = 0.4, p = .527). On average, males took more time to initiate a mating attempt with a washed cadaver than with an intact female cadaver, however this difference was not statistically significant (F(1, 17) = 2.04, p = .171) (Table 4.2).

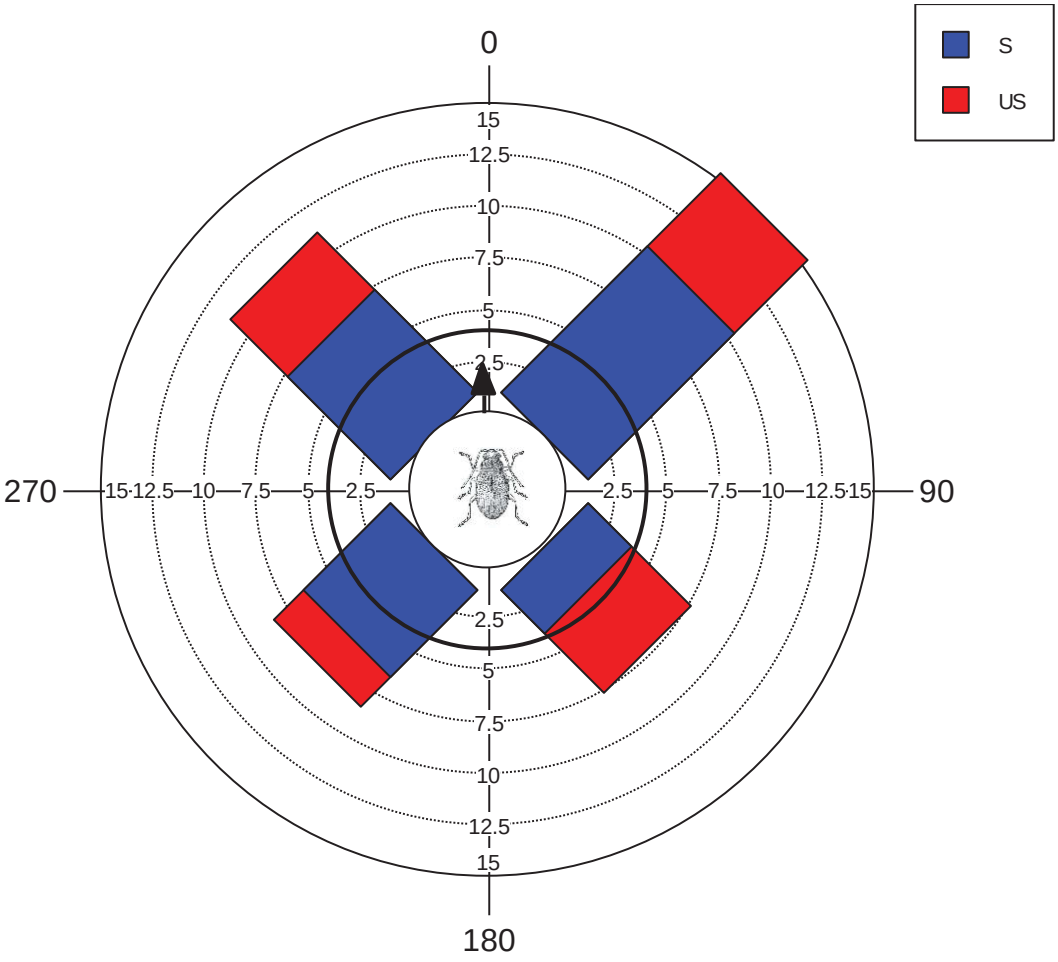


Figure 4.6 Histogram of the angles from which mating encounters were initiated by *Eucolaspis* sp. "Hawke's Bay" males ("S"=Successful; "US"=Unsuccessful) towards freshly-freeze killed female cadavers placed in the centre of a Petri dish in mating bioassays. The small arrow near the centre of the circle above denotes the length of the mean vector (mean direction of all encounters); each circle denotes 2.5 encounters; darker circle between 2.5 and 5 encounter circles denotes significance ($\alpha = 0.05$). As the vector length is much less than the significance level, we would infer that there is no directional bias and the encounters are randomly distributed over the 360-degree space.

Only one out of 12 tested males attempted to mate with reconstituted female cadavers, and none of the tested males attempted to mate with freshly freeze-killed male cadavers (Table 4.1). The only male that attempted to mate with a reconstituted female cadaver took 10.45 minutes to initiate the mating attempt (Table 4.2).

4.4. Discussion

The emergence sex ratio of *Eucolaspis* sp. "Hawke's Bay" in organic apple orchards in Hawke's Bay was clearly female-biased, whereas adult sex ratios in the active population on apple foliage were slightly male-biased.

Population (emergence) sex ratios were around 0.40 (proportion of males) for the first 5 weeks out of total 8 week emergence period (Figure 4.2). According to Lysaght (1930) males of *Eucolaspis* live longer than females and I suspect that this longer life period of males would compensate a female-biased emergence sex ratio. Seasonal variations in sex ratio (i.e. more distortion towards the end of the season) could be an adaptation resulting from selection pressure on adult longevity, so that there are sufficient males at all times to maximise the number of females to be fertilized. This strategy may serve to achieve exponential population growth, with a highly female-biased progeny production. Skewed sex ratios, especially female-biased sex ratios, have been reported for other chrysomelids such as *Calligrapha* spp. (Robertson, 1966), Western corn rootworm (*Diabrotica virgifera*) (Weiss et al., 1985; Bereś & Sionek, 2010), walnut leaf beetle (*Gastrolina depressa*) (Chang et al., 1991), thistle tortoise beetle (*Cassida rubiginosa*) (Koji & Nakamura, 2006) etc. Distortion in sex ratio may result from sex-specific population dynamics traits (such as sex-specific dispersion and mortality) or existence of sex ratio distorters (e.g., B chromosomes in haplodiploid insects, male-killing bacteria like *Wolbachia*, *Spiroplasma* and *Rickettsia*, parthenogenesis, cytoplasmic male-sterility etc.) (Werren & Beukeboom, 1998; Stouthamer et al., 2002). The mechanism of sex ratio distortion in bronze beetles is unknown; if identified, it could assist with better understanding of the rationale behind this distortion. Sex determination among invertebrates may be generally by either genetic sex determination (e.g., male heterogamety, female heterogamety, arrhenotoky, paternal genome loss etc.) or environmental sex determination (e.g., temperature, nutrition level, photoperiod, mate availability etc.) (Cook & Hardy, 2002).

Despite fewer males emerging at any point of time, adult sex ratios (proportion of males) in the active population on foliage were slightly male-biased. Two possible explanations for this are: (1) females are absent from the foliage while engaged in activities such as, finding a suitable oviposition site and ovipositing and (2) males may

pursue females vigorously, and occupy a major proportion of active population at the sampling sites (foliage) (so that male count is cumulative, but the female count is not).

Mating behaviour of *Eucolaspis* sp. "Hawke's Bay" involved a series of behaviours by males, including stopping walking, antennal vibration, licking of female's elytra and post-copulatory mate guarding (Figure 4.4.). Male beetles were equally attractive to virgin male and female insects in Y-tube olfactometer bioassays (that lasted for 20 minutes each) suggesting an absence of any short-range sex-specific pheromone.

Observations on normal male and female mating pairs suggest that antennal contact is necessary for male beetles before they mount female beetles, as a confirmation of the opposite sex. However, the lower number of cases (3 out of 15) in normal mating pairs in which males actually sensed the presence of females, which were out of visual range (e.g., behind), suggests that vision also aids males in identification of females.

Freshly freeze-killed females were, in about half of the cases, still attractive to male beetles. In most of these cases, male beetles stopped in action at a distance to the female, started walking towards female cadaver, touched the cadaver with either antennae or licked female elytra by palpi, and then proceeded to attempt to copulate. This suggests the existence of a contact sex pheromone in females that is perceived by male beetles in association with vision. However, only 44.8% of the males attempted to mate with female cadavers. This might be because freezing reduced the cuticular hydrocarbon profile and / or subtly altered some visual cues leading to reduced attraction (Ginzel, 2010). A similar effect has been observed in a study with longhorn beetle *Glenea cantor*, where only 42% of males attempted to mate with freshly freeze killed female cadavers (Lu et al., 2007). The washed female *Eucolaspis* sp. "Hawke's Bay" cadavers, which were expected to be devoid of their sex pheromones (through washing), remained as attractive as the cadavers with cuticular chemistry intact. Similarly, Mutis et al. (2009) found that 33% of males attempted to mate with washed female cadavers in raspberry weevil *Aegorhinus superciliosus.* Other researchers found that none of the tested males attempted to copulate with washed female cadavers in experiments with various beetle species (Ginzel et al., 2003; Ginzel & Hanks, 2003; Silk et al., 2011). It is possible that the cuticular hydrocarbons were not entirely

removed by the brisk washing protocol followed in the present study, and that vision is also important in identification of prospective mates by male beetles. However, no directional bias while initiating an encounter was found in test beetles in these bioassays. Orientation of males towards intact and washed female cadavers even before antennal contact indicates that volatile chemical and /or visual cues are used (Fukaya et al., 2004).

Freshly freeze-killed washed and reconstituted female cadavers were unattractive to all but one of the male beetles. This was unexpected, as visual cues should be the same as with intact cadavers. Moreover, the cuticular chemistry, if stripped, should have been reinstated by reapplication. However, the cadavers became almost repellent after the reapplication step, suggesting that during collection of total body extracts, certain compounds may have dissolved from internal body tissues. These compounds may be unattractive or even repel males, and when the extracts along with these compounds were reapplied, the reconstituted cadavers became unattractive. A similar effect was observed in longhorn beetles *Tetropium fuscum* and *T. cinnamopterum*, in which reconstituted female cadavers were attractive in only 10% of the cases, while up to 90% of males attempted to mate with intact cadavers (Silk et al., 2011).

Ablation of antennae did not reduce the ability of males to copulate with females, but more encounters were needed and the mating took longer than for normal males. Likewise, removal of antennae alone had no effect on sex recognition in mealworm beetles (Obata & Hidaka, 1982) and 47% of Colorado potato beetle males whose antennae were ablated successfully mated with normal females (Mpho & Seabrook, 2003). In contrast, Lu et al. (2007) found that only 3% of the male *Glenea cantor* beetles mated successfully with normal females. The current findings in *Eucolaspis* suggest that though antennae play a key role in mate location, contact sex pheromones may also be perceived by palpi so that males without antennae can still recognize potential mates. However, in most cases, female beetles touched males multiple times before males could recognize females and initiate mating attempts. In field situations, where females do not typically seek out males, but rather males chase females, antennae could be crucial to guide a chase and impress a female (Mpho & Seabrook, 2003). In some of the bioassays, males with ablated antennae might have

sensed a female, but failed to actually chase her down, which was not the case for normal males.

Freshly freeze-killed males were not expected to be attractive to males, and this was found to be true. Although males may rely on visual cues to recognize male and female beetles, in all cases males actually did touch the male cadavers for confirmation. This could indicate subtle differences in overall visual cues in addition to sex pheromones between live and dead males, which prevent any chance of homosexual mating. Same-sex (male-male) mating attempts were sometimes observed in the laboratory when a large number of insects were kept together.

It can be inferred that *Eucolaspis* sp. "Hawke's Bay" males use both visual and contact cues to locate a potential mate. Short distance or close-range mate location is generally mediated in beetles by visual, chemical and tactile stimuli (Petersson & Solem, 1987). Due to the problem with the vision treatment, this study could not determine the relative effect of these two types of cues. Use of both contact pheromones and vision has been found in other chrysomelids such as the Colorado potato beetle (Otto, 1997; Mpho & Seabrook, 2003). Current findings are also in agreement with those of Fukaya (2004; 2005) who showed that both visual and chemical cues were required for mate location in white-spotted longhorn beetle *A. malasiaca.* The role of vision in chrysomelid mate location is relatively unexplored, as most of the studies are focused exclusively on evaluation of chemical cues. Contact sex pheromones have been identified in relatively few chrysomelids, including coconut hispine beetle *Brontispa longissima* (Kawazu et al., 2011), green dock beetle *Gastrophysa atrocyanea* (Sugeno et al., 2006), compared to a higher frequency of cerambycids. This does not mean that Chrysomelidae do not use pheromones, but rather that they await thorough exploration.

Male-biased sex ratios in the active population (in the foliage), as assessed in this study, suggest that male *Eucolaspis* sp. "Hawke's Bay" males may compete with each other for access to potential mates, which is typical in Chrysomelidae (Dickinson, 1992; 1995; 1997; Nahrung & Allen, 2004). This further highlights the possibility of pre-copulatory sexual selection and evolution of mate attraction mechanisms (such as producing pheromones) in females. In light of the current findings, future research should aim to find out the sex determination mechanism, which would help explain the

selection pressures operating in *Eucolaspis*, and the means of sex dynamics. In addition, chemical analyses of virgin male and female cuticular hydrocarbon profiles would provide further evidence of sex-specific pheromones in *Eucolaspis*. The current study on mating behaviour was conducted exclusively with insects raised from a single population (from one organic apple orchard in Hawke's Bay); similar behavioural bioassays with beetles from other locations and other genetic lineages (see Chapter 2 for details of genetic diversity within the genus in New Zealand) would be interesting to study from a taxonomic perspective. The distinct differences in genitalia structures between the three genetic lineages of *Eucolaspis* (Chapter 2) suggest the possibility of different mating behaviours among the lineages.

4.5. Conclusions

Emergence sex ratio in *Eucolaspis* sp. "Hawke's Bay" in organic apple orchards in Hawke's Bay was female-biased (0.35), and progressively more females emerged during the season, whereas adult sex ratio in the active population on foliage was slightly male-biased (0.55). The adult sex ratios in the genus were variable among various locations and host plants. No evidence for a short-range sex pheromone was found through olfactometer bioassays. All the mating attempts in mating bioassays proceeded only after either antennal contact or licking of female's elytra by the males. Ablating antennae didn't impair mating, but a significant delay was observed in locating the mate. A total of 45% and 35% of the tested males attempted to mate with intact and washed female cadavers, respectively, whereas no mating attempts were initiated towards male cadavers. Only one of the test males attempted to mate with reconstituted female cadavers. Males of *Eucolaspis* sp. appear to utilize both contact sex pheromones and vision in locating potential female mates. However, the current study could not tease apart the relative contribution of vision and pheromones to mate location.

Chapter 5: Bronze beetle and other soil macro-invertebrate assemblage in organic apple orchards

A version of this chapter was published as:

P.R.C.Doddala, Minor M.A. & Rogers D.J. 2010. Population dynamics of bronze beetle, *Eucolaspis* spp (Coleoptera: Chrysomelidae) in relation to other soil macro-invertebrates in organic apple orchards in Hawke's Bay, New Zealand. New Zealand Plant Protection 63: 195-200

5.1. Introduction

Eucolaspis sp. "Hawke's Bay" is currently a significant threat to the viability of organic apple orchards in Hawke's Bay, with some of the orchards on the verge of converting back to conventional production, after failing to cope with huge losses incurred from beetle damage (D. Rogers, pers. comm.). As mentioned in Chapter 1 (Introduction), adult *Eucolaspis* are the life stage causing economically important damage. Larvae live in the soil, where they feed on small grass roots (Kay, 1980). Emergence of adult beetles in Hawke's Bay organic apple orchards generally starts in October and continues till the end of December, with most emergence during November (Rogers et al., 2006; Rogers et al., 2007). Beetles are generally rare in orchards after December, although occasionally few beetles can be seen till February (Rogers et al., 2006). There are no known parasitoids or specific predators of the bronze beetle. Generalist arthropod predators such as larval Carabidae (ground beetles) and Asilidae (robber flies) have been reported to predate on bronze beetle (Lysaght, 1930), but it is not clear from the literature which life stage of the beetle was predated upon.

Incidence of *Eucolaspis* sp. "Hawke's Bay" has been shown to vary greatly between and within organic apple orchards in Hawke's Bay, where some orchards and blocks suffer much greater damage than the others. Rogers et al. (2006) found no correlation between bronze beetle densities in various orchards, and orchard characteristics such as under storey composition, surrounding habitat, and site physical characteristics. Although the variation in beetle densities could not be attributed to any of the tested management practices or site characteristics, the population variations remained fairly consistent over 2 years of observation (Rogers et al., 2006; Rogers et al., 2007). This suggests that some other ecological factor(s) may be influencing the beetle populations in different orchards. For example, generalist arthropod predators such as centipedes, ground beetles, rove beetles, spiders, etc., all have a potential to exert suppression pressure on bronze beetle while in or on the soil.

Trophic interactions with either bottom-up or top-down effect, or horizontal displacement (competition within a trophic level) can potentially regulate population densities of soil herbivores (Wardle, 2006). Trophic cascades (top-down control of herbivores by predators), which were long thought to be a feature of aquatic ecosystems, have also been found in many terrestrial ecosystems (Schmitz et al., 2000; Halaj & Wise, 2001). Generalist arthropod predators such as spiders, ground beetles and

centipedes have been proved to regulate herbivore populations in agricultural / horticultural systems (Allen & Hagley, 1990; Brust & House, 1990; Merfield et al., 2004; Monzó et al., 2011) and are able feed on a variety of pest taxa (Symondson et al., 2002; Maloney et al., 2003). There is no information available on abundance of these generalist predators, which have potential to regulate the bronze beetle, in organic apple orchards in New Zealand,

There were very few New Zealand studies involving edaphic arthropod predators in organic orchards, with none in organic apple orchards. However, studies in other crops such as dairy pastures (Fukuda et al., 2011), vegetables (Berry et al., 1996), and kiwifruit (Maher & Logan, 2007; Todd et al., 2011) provide a hint of rich predator biodiversity and abundance in New Zealand organic systems.

The current study considered macro-invertebrates to study trophic interactions in orchard soil. While less numerous than the smaller soil biota, macro-invertebrates (> 2 mm diameter) (Bardgett, 2005) are a highly diverse and integral component of many ecosystem services. Soil macro-invertebrates mediate important ecosystem processes such as nutrient recycling, litter decomposition, improving soil porosity, and pest control (Lavelle et al., 2006; Smith et al., 2008).

5.1.1. Research objectives

Keeping in view the potential of generalist predators to suppress herbivore population in organic orchards, the current research hypothesized that there is significant difference in soil macro-invertebrate (both hypogeic & epigeic) community structure between orchards with varying history of bronze beetle incidence. To test this, soil macro-invertebrate community structure and dynamics were studied in organic orchards (‘Royal Gala’ apples) and the specific objectives were

1) How does *Eucolaspis* sp. “Hawke’s Bay” abundance vary spatially (among orchards) and temporally?
2) How do sub-surface macro-invertebrate community dynamics vary spatially and temporally?
3) How do surface-dwelling macro-invertebrate community dynamics vary spatially and temporally?
4) Do trophic interactions influence variations in the abundance of *Eucolaspis* sp. “Hawke’s Bay”?

5.2. Materials and methods

5.2.1. Study sites

Eight certified organic orchards containing blocks of 'Royal Gala' apples in Hawke's Bay region of New Zealand were selected (Tables 5.1 & 5.2) as study sites. Among these, four orchards had a history of high bronze beetle damage (will be referred to as "High BB orchards" from here on) and the other four orchards had a history of low damage (will be referred to as "Low BB orchards"). The beetle damage histories were established through previous studies (Rogers et al., 2006; Rogers et al., 2007) and by talking to orchard managers. The orchard owners and / or managers have requested to keep the orchard identities anonymous and hence, any names or coordinates that identify the orchards were deliberately omitted.

Table 5.1 Organic apple orchards ('Royal Gala') used as study sites for examining soil macro-invertebrate community, Hawke's Bay, New Zealand, spring-summer 2007-2008.

Site #	**Location**	**Degree of Bronze beetle damage**	**Orchard Code (as used in this chapter)**
1	St Georges Rd., Havelock North	High	H1
2	Irongate Rd, Hastings	High	H3
3	Omahu Rd., Hastings	High	H3
4	Evenden Rd., Hastings	High	H4
5	St Georges Rd., Havelock North	Low	L1
6	Irongate Rd., Hastings	Low	L2
7	Evans Rd., Hastings	Low	L3
8	Napier Rd., Havelock North	Low	L4

Orchards H1 & L1 belonged to the same owner and are within 500m distance to each other; so are orchards H1 & L1 (Table 5.2). Even though in proximity to each other, the two orchards in each of these orchard pairs had varying degree of bronze beetle infestation historically.

Table 5.2 Inter-orchard distances (in km) among the 8 organic orchards ('Royal Gala' apples) in Hawke's Bay, New Zealand that were used as study sites.

orchard	H1	H2	H3	H4	L1	L2	L3	L4
H1								
H2	10.5							
H3	9.1	5.3						
H4	10.2	7.3	3.2					
L1	0.5	10	8.6	9.7				
L2	10	0.5	4.8	6.8	9.5			
L3	14.9	10.8	5.8	7.4	14.4	10.3		
L4	7	15.2	13.3	10.5	6.5	14.7	17.9	

5.2.2. Soil sampling

Five soil samples per orchard were collected once a month for 4 months, starting from October 2007 till January 2008. Soil samples (18 × 18 cm, 14 cm deep) were obtained by digging with a spade beneath a branch within a 1 m radius from five randomly selected apple tree trunks (Rogers et al., 2007). Five trees in each orchard were randomly selected during each of the 4 sampling times, and the trees once sampled were avoided subsequently. The samples were transferred to the lab in labelled clear plastic bags and stored at 4°C until processed for macro-invertebrates.

All soil samples were hand-sorted and macro-invertebrates found were counted, identified to appropriate taxonomic level by observing under a dissecting microscope (Olympus SZX-ILLD2-200), and stored in 70% ethanol.

5.2.3. Pitfall trap sampling

Five pitfall traps per orchard were set up in October 2007 beneath the tree line within a 1 m radius from the trunk of five randomly selected trees. Each pitfall trap comprised a small plastic cup (250 ml capacity) inserted into a PVC pipe (8.0 cm diameter) sunken into the soil flush with the soil surface level (Figure 5.1). A corrugated iron lid was placed on top to protect the trap from rainwater flooding, allowing a gap for crawling invertebrates. The trap was half-filled with Polyethylene Glycol (PGPLUS Concentrate – Fleetguard, Australia) to preserve invertebrates (Minor

& Robertson, 2006). The traps were permanently positioned for the entire sampling period.

Figure 5.1 A pitfall trap with lid (left) and another pitfall trap (right) with lid removed to retrieve macro-invertebrates.

Captured invertebrates were retrieved from all traps once a month for 4 months from November 2007 to February 2008. The preservative liquid from each trap was emptied into a spare cup through a cloth filter; invertebrates caught on the cloth filter were then transferred to a separate labelled vial. The trap was then refilled using the preservative liquid from the spare cup; the preservative was topped up if below half mark. A small quantity of 70% ethanol was added to each vial once brought to the lab. The samples were stored at room temperature until processed. All macro-invertebrates collected were counted and identified to appropriate taxonomic level by observing under a dissecting microscope (Olympus SZX-ILLD2-200).

5.2.4. Adult Eucolaspis sp. "Hawke's Bay" emergence

Emergence bucket traps were used to monitor adult bronze beetle emergence from the soil in the orchards (see Chapter 4). The traps were checked weekly from the 1st week of October 2007 till the end of 2nd week of January 2008. The beetles caught in each trap were counted, removed into separate labelled 1.5 ml micro-centrifuge tubes (Eppendorf), transferred to the lab at Massey University and frozen.

5.2.5. Data analysis

Due to the different sampling methods, data for sub-soil (soil cores) and surface-dwelling (pitfall traps) macro-invertebrates were analysed separately. All macro-

invertebrates were grouped into three main trophic groups (herbivores, detritivores & predators) based on their feeding habits according to following references (Petersen & Luxton, 1982; Dindal, 1990; Bejakovich et al., 1998; Minor & Robertson, 2006). Using trophic groupings allows a synthetic functional approach to assess the structure and dynamics of soil assemblages, and may provide information on ecosystem functioning (Clough et al., 2007). Herbivores were further divided into bronze beetles and other herbivores. The predators from pitfall traps were further split into spiders, predatory beetles (Carabidae and Staphylinidae), centipedes and other predators (ants, flatworms and earwigs) for additional analysis. Earwigs were considered as predators following Suckling et al. (2006). Very few unidentified insects (including some Diptera and Hymenoptera) which could not be allotted to a particular functional group were removed from statistical analyses.

Abundance of functional (trophic) groups of surface and sub-soil macro-invertebrate between two groups of orchards (with history of low and high bronze beetle incidence), among all orchards, among orchards within each group, and among sampling dates was compared. Data on *Eucolaspis* sp. "Hawke's Bay" adult emergence was analysed for differences between orchards and sampling dates. Following advice by a Massey University statistician, Poisson regression (Proc Genmod, SAS 9.2) was used to analyse all datasets; χ^2 and P-values from type3 likelihood ratio analysis were used to compare the effects. Correlation among densities of different groups of macro-invertebrates were analysed in each orchard separately, using Pearson correlation analysis (Proc CORR, SAS 9.2) (data were log transformed for these analyses: log(1+n) where n is the density of particular invertebrate). Relationships of sub-soil and surface dwelling predators' abundance with other invertebrates' abundance were tested using Poisson regression and these analyses were conducted separately for each orchard. To show arrangement of orchards based on macro-invertebrate abundance, ordination was done using the nonmetric multidimensional scaling (NMS) technique. Sorensen (Bray-Curtis) distance was used to represent the distance between orchards in n-dimensional ordination space. Pearson and Kendall correlations were used to infer the correlation of invertebrate abundance with ordination axes. Effect of orchard and date were tested using multi-response permutation procedures (MRBP). NMS and MRBP analyses were conducted in PC-ORD v.5.0 (MjM Software Design Ltd., USA). Surface-dwelling

invertebrate abundance data were transformed by the power of 0.25 to reduce stress in the scaling.

5.3. Results

Both the sub-soil invertebrates obtained by soil sampling and surface-dwelling macro-invertebrates obtained by pitfall trapping were numerically dominated by detritivores (54.18% and 69.25%, respectively). Herbivores (bronze beetle and other herbivores) were the second largest group found in the sub-soil (34.6%), whereas predators were the second largest group (28.82%) in the surface-dwelling macro-invertebrate community. Table 5.3 lists the taxa found in the orchards, their associated abundances and trophic functional groups (see Appendix for raw data).

Table 5.3 Macro-invertebrate taxa in the soil ("sub-soil") and pitfall trap ("orchard floor") samples collected during spring to summer 2007-08 in eight organic apple orchards in Hawke's Bay, New Zealand. Values are total counts.

Taxon	**Sub-soil**	**Orchard floor**	**Trophic group**
Oligochaeta - earthworms	2337	401	Detritivores
Amphipoda – land hoppers	99	1601	Detritivores
Isopoda – slaters	247	15672	Detritivores
Diplopoda – millipedes	71	71	Detritivores
Gastropoda - snails and slugs	408	228	Herbivores
Chrysomelidae – bronze beetle (*Eucolaspis* sp. "Hawke's Bay")	649	24	Herbivores
Curculionidae – clover root weevil (*Sitona lepidus*)	3	3	Herbivores
Elateridae – click beetles	6	31	Herbivores
Scarabeidae – grass grub (*Costelytra zealandica*) and other unidentified	123	8	Herbivores
Soldier flies (Stratiomyidae) and other Dipteran larvae	766	0	Herbivores
Unidentified Diptera	0	169	NA*
Cicadidae - cicadas	26	2	Herbivores
unidentified Coleoptera	156	138	Herbivores
unidentified Lepidoptera	0	12	Herbivores
other Hymenoptera	7	59	NA*
Chilopoda – centipedes	463	138	Predators
Opiliones - harvestmen	0	52	Predators
Araneae – spiders	21	6136	Predators
Staphylinidae – rove beetles	20	96 (28^{+})	Predators
Carabidae – ground beetles	13	301	Predators
Formicidae – ants	81	526	Predators
Dermaptera – earwigs	2	13	Predators
Turbellaria – flatworms	23	83	Predators

*Trophic functional group not known; $^{+}$ larvae of Staphylinidae and Carabidae together

5.3.1. Eucolaspis sp. "Hawke's Bay" – density in soil and adult emergence

As expected, significantly higher numbers of *Eucolaspis* sp. "Hawke's Bay" in the soil (cumulative total of larvae, pupae and adults) were found in High BB orchards during all four sampling months ($p < .0001$, Poisson regression type3 analysis of likelihood ratio) (Table 5.4). During October, orchard H3 had the highest bronze beetle density, whereas L4 orchard had the lowest (nil) (Figure 5.2). *Eucolaspis* sp. "Hawke's Bay" density in the soil (cumulative total of all life stages) varied between orchards in three out of four sampling periods; during January 2008 there was no difference between orchards in beetle densities.

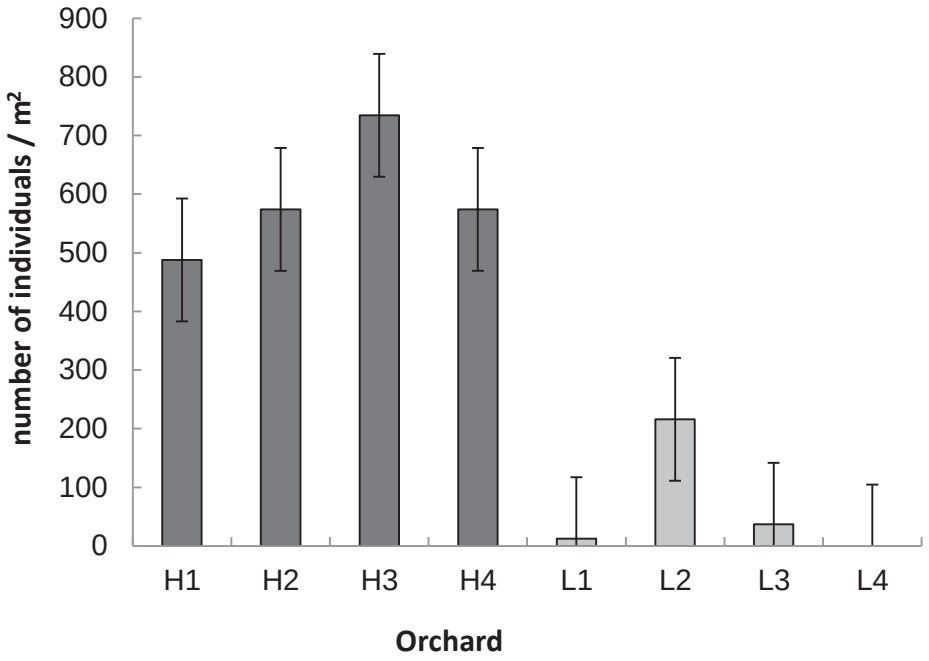


Figure 5.2 Total number of bronze beetles (larvae + pupae + adults)/m^2 (mean ± SE) found in the soil. Data are for October 2007 when beetle density in the soil was at its highest. H1-H4 – orchards with high bronze beetle damage; L1-L4 – orchards with low bronze beetle damage.

Larvae were the most abundant life stage of bronze beetle in the soil during October (56.21%), whereas adults were more abundant from November onwards (71.28%, 96% and 100% of total beetle population during November, December and January respectively) (Figure 5.3). Across all orchards the density of bronze beetle (cumulative for all life stages) in the soil decreased from spring to summer as the adults emerged and left the soil (Figure 5.3). *Eucolaspis* sp. "Hawke's Bay" density varied

significantly between sampling periods in all but L1 and L4 orchards, which had very few and nil beetles, respectively. Very few bronze beetle adults were collected in pitfall traps, with none in the November and February samples.

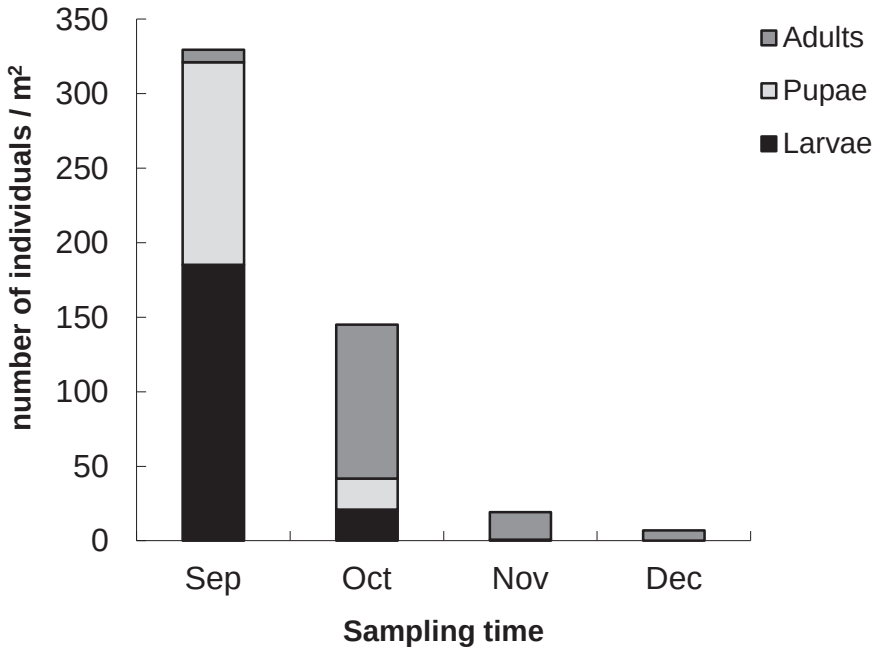


Figure 5.3 Relative abundance (mean number of individuals/m^2 cumulative of all the orchards) of different life stages (larvae, pupae & adults) of bronze beetle in soil over the sampling period.

Adult beetles started emerging after first week of October and continued emerging until the second week of January (data from emergence traps). Highest emergence among all the orchards was observed in orchard H2 in one of November samplings (Figure 5.4). No beetles were caught in orchard L1, and very few were caught in orchards L3 (total: 2) and L4 (total: 9). These three orchards were removed from statistical analysis while comparing effect of site (orchard) and sampling dates in order to minimize bias.

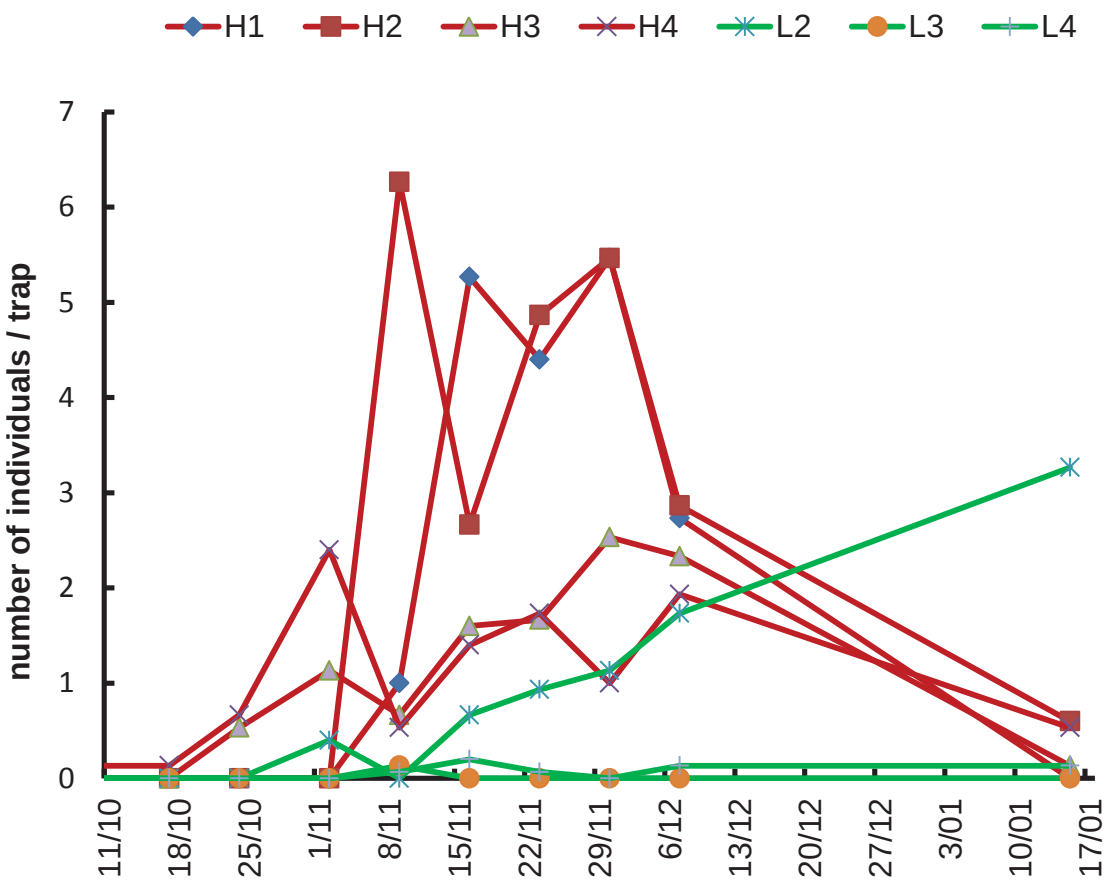


Figure 5.4 Weekly emergence (mean number of beetles per trap) of adult bronze beetles (*Eucolaspis* sp. "Hawke's Bay") in organic apple orchards in Hawke's Bay, New Zealand during 2007-08. H1-H4 – orchards with high bronze beetle damage; L1-L4 – orchards with low bronze beetle damage (data collected from emergence traps; L1 had no beetles).

Beetle emergence significantly varied among orchards ($\chi^2(6, N=1050) = 787.42$, $p < .001$) and between sampling dates ($\chi^2(1, N=1050) = 135.39$, $p < .001$) and there was a significant interaction effect of orchard and sampling date ($\chi^2(6, N=1050) = 70.95$, $p < .001$). Peak emergence of beetles was observed during November in all orchards except L2 (Figure 5.4). About 80% of beetles emerged during November in all orchards except orchards L2 (39%) and L4 (56%).

Table 5.4 Sub-soil macro-invertebrates (mean number/m^2) in orchards with a history of high (High BB) and low (Low BB) bronze beetle damage, spring-summer 2007-08, Hawke's Bay, New Zealand.

Previous history	Bronze beetle				Other herbivores				Detritivores				Predators			
	Oct	**Nov**	**Dec**	**Jan**	**Oct**	**Nov**	**Dec**	**Jan**	**Oct**	**Nov**	**Dec**	**Jan**	**Oct**	**Nov**	**Dec**	**Jan**
High BB	592.6	265.4	34.0	12.3	561.7	402.8	188.3	216.0	899.7	409.0	696.0	478.4	274.7	106.5	185.2	120.4
Low BB	66.4	24.7	4.6	1.5	242.3	189.8	104.9	57.1	935.2	429.0	521.6	273.1	142.0	66.4	29.3	37.0
χ^2 value	313.0	151.18	16.31	6.2	84.56	50.72	15.56	63.88	0.44	0.31	16.24	36.79	27.88	6.09	81.8	30.1
p	**<.001**	**<.001**	**<.001**	**.013**	**<.001**	**<.001**	**<.001**	**<.001**	.505	.577	**<.001**	**<.001**	**<.001**	**.014**	**<.001**	**<.001**

5.3.2. Other herbivores

Other herbivores (other than bronze beetles) found in soil samples were mainly immature stages of Coleoptera and Diptera. The abundance of the other herbivores declined over the sampling period. High BB orchards had significantly more other herbivores than Low BB orchards in all sampling months (Table 5.4). The density of other herbivores varied significantly between sampling dates in all orchards, and between orchards.

Adult Coleoptera (Scarabeidae and Elateridae) comprised the majority of the very few surface-dwelling herbivores found in pitfall traps. High BB orchards had slightly fewer other herbivores on the orchard floor than Low BB orchards, although the differences were not significant (Table 5.5).

5.3.3. Detritivores

Earthworms were the most abundant detritivores found in the soil samples, followed by slaters (Isopoda). Epigeic earthworms (*Lumbricus rubellus*) and endogeic earthworms (*Aporrectodea calignosa, A. rosea,* and *Octalasion cyaneum*) were present in all the orchards though some species such as *A. rosea* and *O. cyaneum* were very rare. Anecic earthworms (*Aporrectodea longa*) were found only in one orchard (L4). Earthworms in pitfall traps could not be identified to species level due to deterioration of morphological characters.

Detritivores were slightly more abundant in Low BB orchards during October and November, although the differences were not significant. In contrast, in January and February samples detritivores were significantly more abundant in High BB orchards (Table 5.4).

Surface-dwelling detritivores caught in pitfall traps were mostly Isopoda and Amphipoda; their abundance varied greatly over time. January samples had the highest density of detritivores (178.28 individuals per trap on average) between the four sampling periods. The differences in abundance of detritivores between High BB and Low BB orchards were significant throughout the sampling period (Table 5.5). Millipedes (Diplopoda) were found only in orchards H1, H2, L1 and L2 whereas land hoppers (Amphipoda) were found only in orchards H1, H2, and L2.

5.3.4. Predators

Generalist sub-soil predators were mostly Chilopoda (centipedes) along with few Insecta (Carabidae – ground beetles, Staphylinidae – rove beetles, Formicidae - ants) and Turbelleria (terrestrial flatworms) (Table 5.3). The density of soil predators was higher in High BB orchards than in Low BB orchards. Although this difference was significant during all the sampling periods, it became even more obvious during December and January (Table 5.4).

Surface-dwelling predator community structure was relatively similar across all the orchards. Spiders outnumbered all others, representing about 80% of the predators caught in all pitfall traps. The surface predators were significantly more abundant in Low BB orchards than in High BB orchards throughout the sampling period (Table 5.5). Spiders were more abundant in Low BB orchards than in High BB orchards on all sampling dates (Table 5.6.). Predatory beetles and other predators such as ants were also significantly more abundant in Low BB orchards on some sampling dates, although the numerical difference was small (Table 5.6). Centipedes were more abundant in High BB orchards in January and February. Earwigs (Dermaptera) were only found in four (orchards H2, H3, L1 & L2) of the eight orchards whereas flatworms (Turbellaria) were found in six (orchards H2, H3, H4, L1, L3 & L4) of the eight orchards. Harvestmen (Opiliones) were not found in orchards H4, L3 & L4.

Table 5.5 Surface-dwelling macro-invertebrates (mean number/trap) caught over the preceding month in pitfall traps in orchards with a history of high (High BB) and low (Low BB) bronze beetle damage, spring-summer 2007-08, Hawke's Bay, New Zealand.

Previous history	**Bronze beetle**				**Other herbivores**				**Detritivores**				**Predators**			
	Nov	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**
High BB	0	0.95	0.1	0	4.1	3.15	2.55	1.85	83.79	56.6	137.35	71.15	23.79	35.35	66.60	35.45
Low BB	0	0.15	0	0	4.6	3.25	2.84	1.80	114.75	97.85	221.37	125.4	40.75	44.85	84.68	45.55
χ^2 value		12.97	-	-	0.51	0.03	0.31	0.01	94.34	223.04	387.85	303.4	87.69	22.56	42.27	25.25
p		**.0003**	-	-	.46	.86	.58	.91	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**

Table 5.6 Surface-dwelling generalist predators (mean number / pit-fall trap) in orchards with a history of high and low bronze beetle damage. [1]Predatory beetles = Carabidae and Staphylinidae. [2]Other predators = ants+flatworms+earwigs.

Previous history	**Spiders**				**Predatory beetles[1]**				**Centipedes**				**Other predators[2]**			
	Nov	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**	**Nov**	**Dec**	**Jan**	**Feb**
High BB	19.05	30.30	55.15	29.75	1.42	1.60	3.10	1.35	0.26	0.40	2.70	1.20	3.05	3.05	5.65	3.15
Low BB	31.80	35.45	71.58	38.25	2.00	2.95	5.53	3.65	0.50	0.25	1.26	0.40	6.45	6.20	6.31	3.25
χ^2	62.8	8.08	41.67	21.31	1.92	8.13	13.51	21.98	1.45	0.7	10.35	8.37	24.13	21.89	0.72	0.03
p	**<.0001**	**.005**	**<.0001**	**<.0001**	0.166	**.004**	**.0002**	**<.0001**	.228	.403	**.0013**	**.004**	**<.0001**	**<.0001**	.395	.86

5.3.5. Community composition

Sub-soil macro-invertebrate community composition varied among different orchards and sampling dates. Detritivores dominated the community in orchards L1-L4 in all four sampling periods, whereas herbivores (bronze beetle and other herbivores together) dominated the community in orchards H1-H4 in October and November samplings (Figure 5.5). Orchard H3 had highest proportion of sub-soil predators in October (20%) and December (30%) samplings, whereas orchard H4 had highest proportion of predators in November 2007 (22%) and January 2008 (28%).

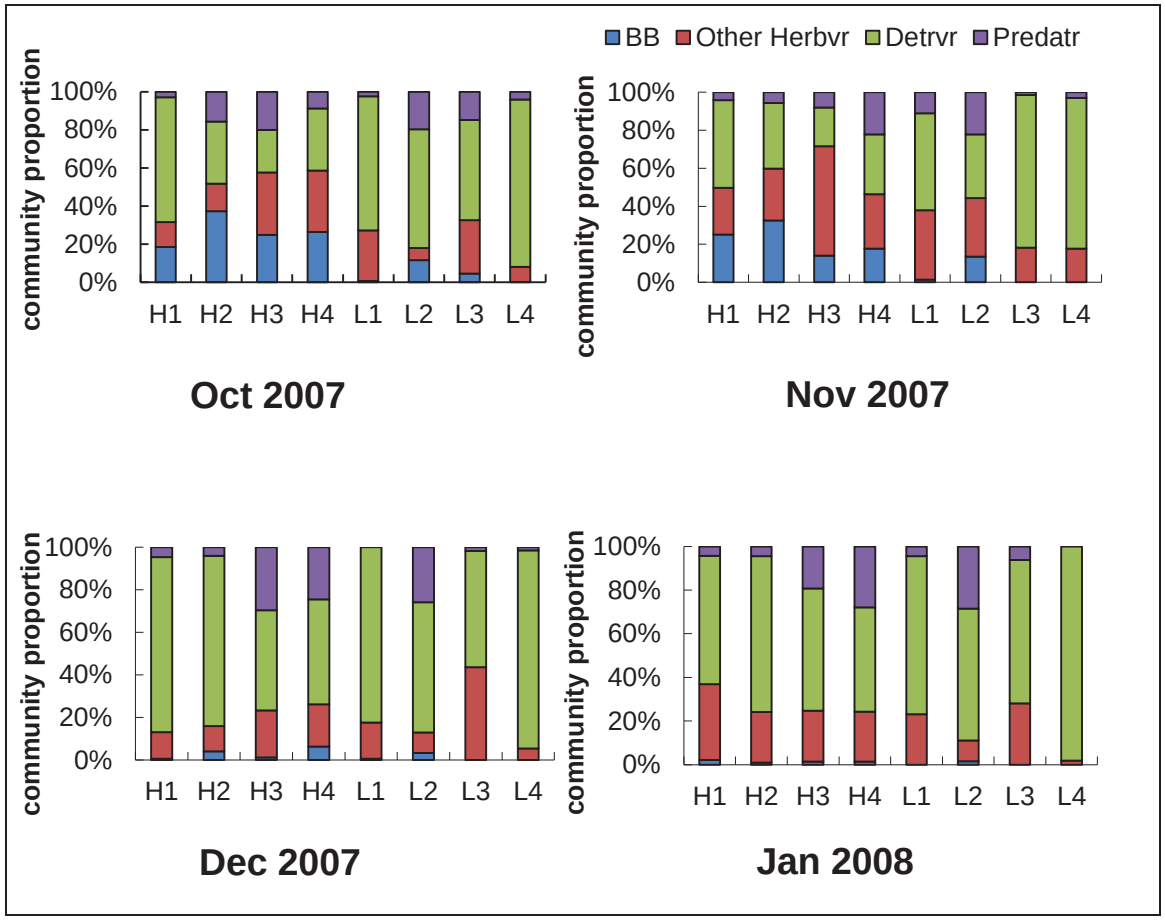


Figure 5.5 Sub-soil macro-invertebrate community composition in organic apple orchards with high (H1-H4) and low (L1-L4) bronze beetle (BB) damage, Hawke's Bay, New Zealand, October 2007-January 2008. BB = Bronze beetle; Other Herbvr = Other herbivores; Detrvr = Detritivores; Predatr = Predators.

In the sub-soil macro-invertebrate community, significant correlations were found between bronze beetles and other herbivores in orchards H1 (r=0.51, *p*=.02) and L2 (r=0.48, *p*=.03). Significant correlations were found between density of other herbivores and detrititvores in L1 (r=0.46, *p*=.04) and L3 (r=0.57, *p*=.008), and between densities of predators and detritivores in L2 (r=0.69, *p*<.01). Density of predators in the soil appears to be influenced by the density of bronze beetles, other herbivores and detritivores some orchards (Table 5.7).

Table 5.7 Relationships between sub-soil predators' density in soil and densities of other sub-soil invertebrates in eight organic apple orchards in Hawke's Bay, New Zealand during spring-summer 2007-08 (significant regressions at $\alpha = 0.05$ are shown, Poisson regresson).

Orchard	**Predators**		
H1	$\chi^2(1, N=20) = 3.88$, $p=.049$ ↑		
H2	$\chi^2(1, N=20) = 6.12$, $p=.013$ ↑		
H3	$\chi^2(1, N=20) = 5.21$, $p=.022$ ↑		$\chi^2(1, N=20) = 39.28$, $p=.0001$ ↑
H4	$\chi^2(1, N=20) = 4.92$, $p=.027$ ↑	$\chi^2(1, N=20) = 8.93$, $p=.003$ ↑	$\chi^2(1, N=20) = 6.08$, $p=.014$ ↑
L1		$\chi^2(1, N=20) = 10.38$, $p=.001$ ↓	$\chi^2(1, N=20) = 8.77$, $p=.003$ ↓
L2			$\chi^2(1, N=20) = 43.59$, $p=.0001$ ↑
L3			
L4			
	Bronze beetles	**Other herbivores**	**Detritvores**

↑ positive regression, ↓ negative regression

Surface-dwelling macro-invertebrate community composition also varied among different orchards and different sampling dates. Orchards H1, L1 and L2 consistently had more detritivores, whereas in other orchards the surface-dwelling invertebrate community was dominated by predators (Figure 5.6).

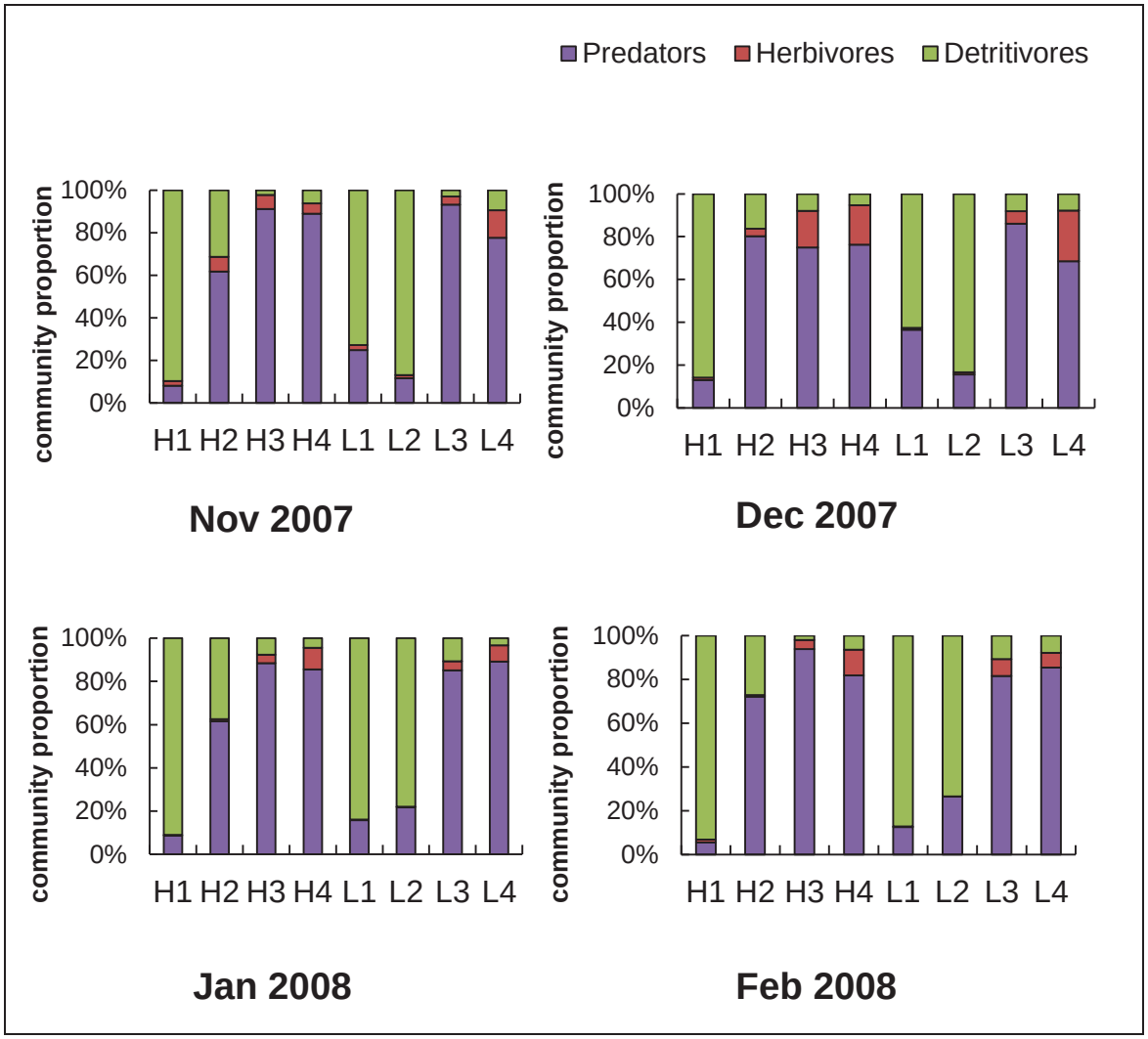


Figure 5.6 Epigeic macro-invertebrate community composition in organic apple orchards with high (H1-H4) and low (L1-L4) bronze beetle damage, Hawke's Bay, New Zealand, November 2007-February 2008.

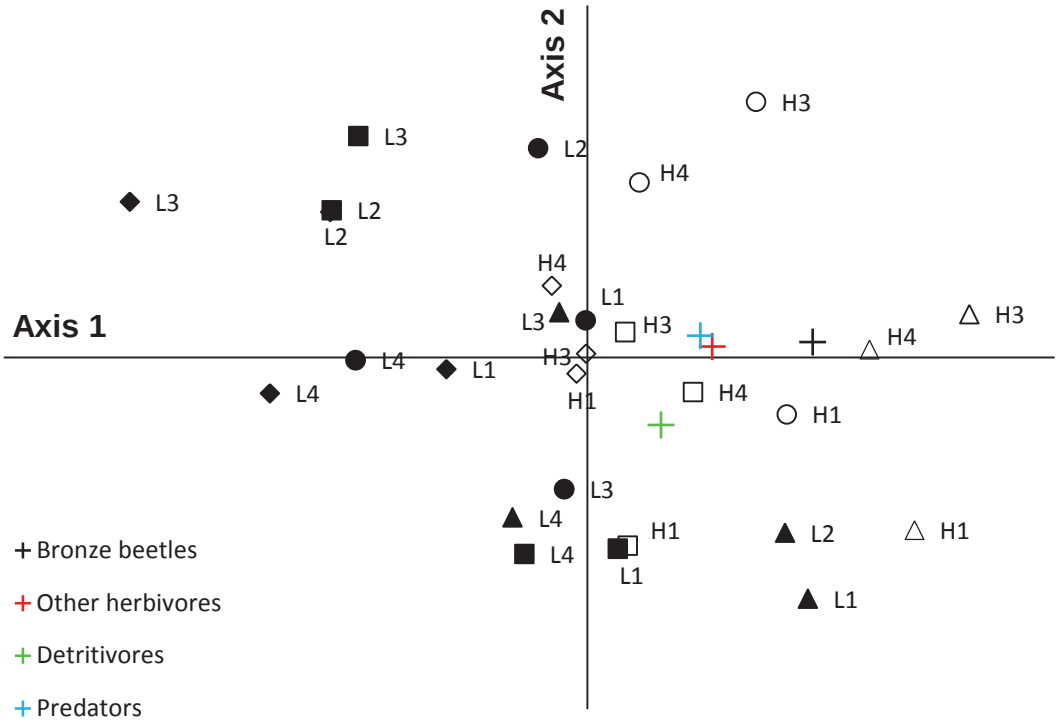
In the surface-dwelling macro-invertebrate community, significant correlation between herbivores and detritivores was found only in orchard L2 (r=-0.67, *p*=.001) whereas correlation between detritivores and predators was found to be significant only in orchard H4 (r=0.46, *p*=.04). Density of surface-dwelling predators varied with density of detritivores in most orchards (6 out of 8) whereas it varied with density of herbivores in only three orchards (H2, L2 and L4) (Table 5.8).

Table 5.8 Relationships between densities of epigeic predators and other epigeic macro-invertebrates in eight organic apple orchards in Hawke's Bay, New Zealand during spring-summer 2007-08 (significant regressions at $\alpha = 0.05$ are shown, Poisson regresson).

Orchard	Predators	
H1		$\chi^2(1, N=20) = 38.56$, $p=.0001$ ↑
H2	$\chi^2(1, N=19) = 91.20$, $p=.0001$ ↑	$\chi^2(1, N=19) = 31.89$, $p=.0001$ ↑
H3		$\chi^2(1, N=20) = 3.91$, $p=.048$ ↑
H4		$\chi^2(1, N=20) = 45.90$, $p=.0001$ ↑
L1		
L2	$\chi^2(1, N=20) = 225.27$, $p=.0001$ ↑	$\chi^2(1, N=20) = 38.59$, $p=.0001$ ↑
L3		
L4	$\chi^2(1, N=19) = 13.46$, $p=.0002$ ↑	$\chi^2(1, N=19) = 6.11$, $p=.013$ ↑
	Herbivores	**Detritvores**

↑positive regression

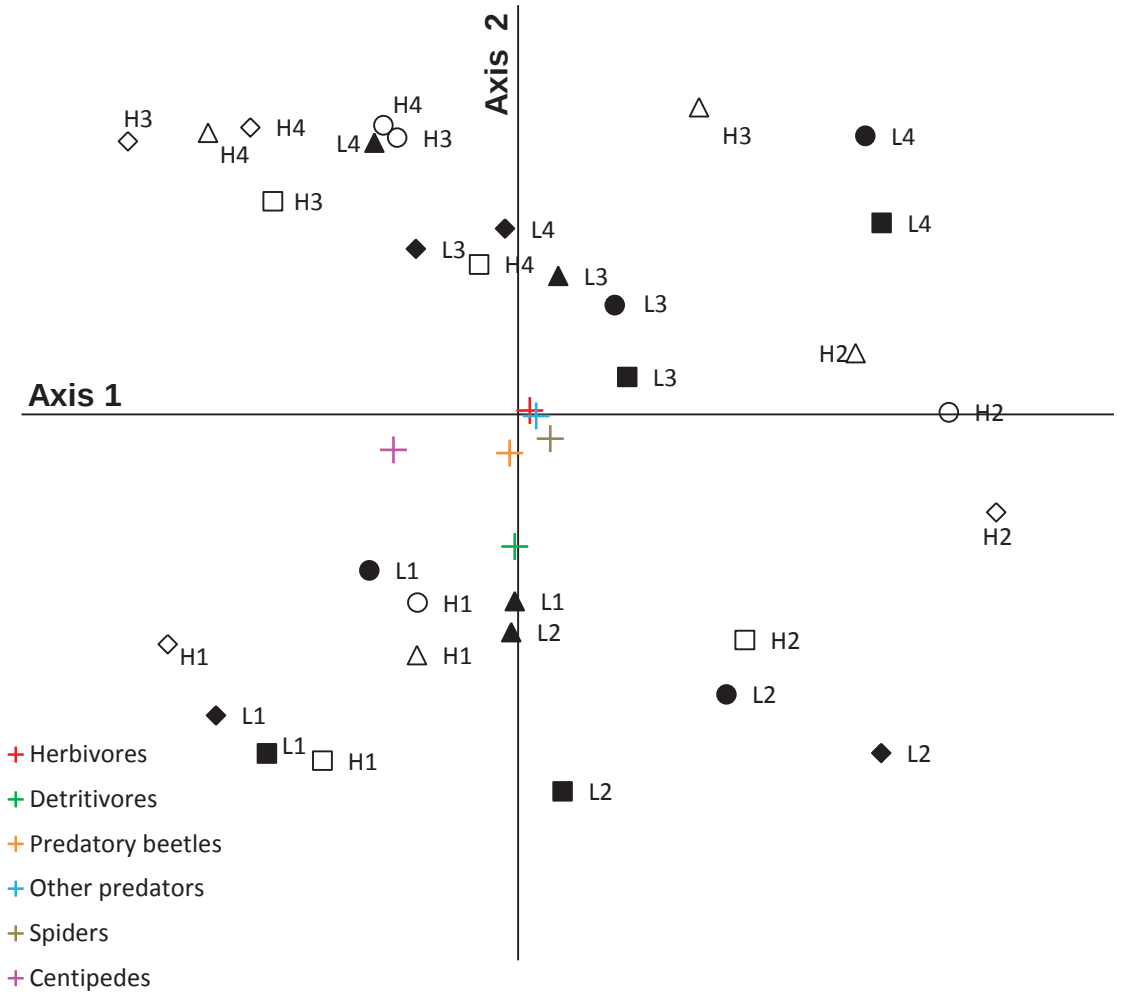
Similarities among orchards in assemblage (abundance) of different functional groups of sub-soil macro-invertebrates, analysed by non-metric multidimensional scaling (NMS), revealed that high bronze beetle damage history orchards (H1-H4) differ from low bronze beetle damage history orchards in having higher numbers of sub-soil macro-invertebrates (Figure 5.7). Abundance of invertebrates differed among different sampling dates and the abundance decreased as the New Zealand summer progressed (from October to January) in all the orchards. Axis1 (r^2=0.665) in the ordination plot (Figure 5.7) explains the distance between orchards in n-dimensional space better than Axis2 (r^2=0.263). Abundance of other herbivores was highly correlated with ordination Axis1 (r^2=0.581) whereas abundance of detritivores was highly correlated with ordination Axis2 (r^2=0.66). Multi-response permutation procedures (MRBP) analysis suggested that different orchards were significantly different from one another ($p < .001$). Likewise, bronze beetle damage histories (high BB and low BB) were different from each other ($p < .001$) and different dates were also significantly different from each other ($p < .001$) in terms of soil macro-invertebrate assemblages.



	Oct-07	Nov-07	Dec-07	Jan-08
High bronze beetle incidence:	△	○	□	◇
Low bronze beetle incidence:	▲	●	■	◆

Figure 5.7 Non-metric multidimensional scaling plot of similarities in sub-soil macro-invertebrate abundance among orchards with different bronze beetle damage histories. Major invertebrate groups (viz., bronze beetle, other herbivores, detritivores and predators) are represented by "+" signs; each other labelled data point on the plot represent an orchard (mean for 5 samples per date).

Ordination plot (NMS) of surface-dwelling macro-invertebrate abundance in orchards reveals essentially 3 clusters (Figure 5.8). Orchards H1, L1 and L2 were similar to one another than others so were orchards L3 and L4. The third cluster contained orchards H3 and H4 that were different from the rest. The similarities were generally constant during different sampling dates except in orchard H2 which had significant differences in invertebrate abundance among different sampling dates. Axis2 (r^2=0.772) in the ordination plot explained the distance in n-dimensional space better than Axis1 (r^2=0.107) (Figure 5.8). Abundance of surface-dwelling detritivores was highly correlated with ordination Axis2 (r^2=0.977) whereas abundance of centipedes was highly correlated with ordination Axis1 (r^2=0.636).



	Nov-07	Dec-07	Jan-08	Feb-08
High bronze beetle incidence:	△	○	□	◇
Low bronze beetle incidence:	▲	●	■	◆

Figure 5.8 Non-metric multidimensional scaling plot of similarities in surface-dwelling macro-invertebrate abundance among orchards with different bronze beetle damage histories. Invertebrate groups are represented by "+" signs (Other predators includes ants, flatworms and earwigs); each other labelled data point on the plot represent an orchard (mean for 5 samples per date).

Distance between data points in the ordination plot (Figure 5.8) is positively correlated with distance in n-dimensional space i.e. the orchard points that are nearer have similar invertebrate abundance. Multi-response permutation procedures (MRBP) analysis suggested that different orchards were significantly different from one another ($p < .001$) but not bronze beetle damage histories ($p > .05$) while different dates were

significantly different from each other ($p < .001$) in the abundance of surface-dwelling macro-invertebrates.

5.4. Discussion

The variation in bronze beetle populations found between the two groups of orchards corroborates with previous history (Rogers et al., 2006; Rogers et al., 2007) and growers' observations. The observed emergence pattern of beetles in organic apple orchards in Hawke's Bay was consistent with previous findings (Rogers et al., 2006; Rogers et al., 2007), with peak emergence during November. There were obvious differences between orchards in terms of beetle abundance (Figure 5.4), but not in the temporal pattern. In all but one of the observed orchards, the beetle abundance decreased after November and no beetles emerged after mid-January. These observations corroborate findings by previous studies on seasonal incidence and adult activity in the field (Miller, 1926; Lysaght, 1930; Clearwater & Richards, 1984; Rogers et al., 2006). The beetles appear to be able to synchronise their life cycle with resource availability, i.e., young foliage and fruit-lets of apple.

Although high BB orchards had more sub-soil generalist predators (mostly centipedes), their soil also supported higher bronze beetle population and much higher density of other herbivores (Table 5.4) than low BB orchards. There may be some other yet unidentified factor responsible for the consistent differences in bronze beetle population densities between orchards. Abundance of bronze beetles and other herbivores appear to be positively correlated in two orchards (H1 and L2), suggesting that a common factor, such as availability of food, could support these invertebrates. Sub-soil predators that were more abundant in high incidence orchards (Table 5.4) seem to be influenced by abundance of bronze beetles (Table 5.7).

Orchards with historically low bronze beetle damage had significantly more surface-dwelling predators than high bronze beetle orchards. Further analysis showed this was due to spider numbers. In a similar study conducted in Europe, it was found that spiders (Araneae) were abundant and species-rich generalist predators in apple orchards (Bogya et al., 1999).

Orchards H1, L1 and L2 had extremely high densities of surface-dwelling detritivores, mostly slaters (Isopoda), during the entire sampling period (Figure 5.7). In

these three orchards and also to some extent in orchard H2, thinned fruit from previous season was left on the orchard floor, which would have provided abundant food resources for detritivores. Surge of detritivores (in density and diversity) in these orchards later in the sampling period (January) could be linked to the ongoing fruit thinning of the current crop. Litter-feeding invertebrates (e.g. slaters, millipedes and epigeic and anecic earthworms) respond to the quantity and quality of litter inputs (Hopkin & Read, 1992; Curry & Schmidt, 2006). It is possible that high density of detritivores in orchards H2, L1 and L2 resulted in recruitment of more predators (spiders). However, other low incidence orchards, such as L3 and L4, also had high density of surface-dwelling spiders throughout the sampling period, despite there being very few detritivores. In fact, in most orchards, the abundance of surface-dwelling predators appears to depend on the abundance of prey resources (herbivores and detritivores) (Table 5.8).

The abundant surface-dwelling generalist predators (especially spiders) have potential to contain bronze beetles. Many researchers have previously reported reductions in pest populations due to successful predation pressure by surface-dwelling generalist predators. For example, when the surface-dwelling generalist predator population was enhanced by habitat manipulation, it resulted in successful control of *Cydia pomonella* (Lepidoptera) in apple orchards (Mathews et al., 2004). Spiders have been reported to limit pest populations in apple orchards (Marc & Canard, 1997) and in citrus groves (Mansour & Whitecomb, 1986). Spiders have been shown to feed on many insect pest taxa including species of Coleoptera, Lepidoptera, Hemiptera and Diptera (Symondson et al., 2002; Maloney et al., 2003) and were capable of controlling key orchard pests such as apple blossom weevil *Anthonomus pomorum* (Coleoptera: Curculionidae) and codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) (Marc & Canard, 1997). Spiders have been shown to significantly decrease insect damage to harvest in apple orchards in Israel, Europe, Australia, and Canada (Maloney et al., 2003). Studies in Europe found that ground beetles (Carabidae) were the dominant predatory group in apple orchards (Epstein et al., 2000; Miñarro et al., 2009) and the ground beetles have been found to regulate Mediterranean fruit fly populations in citrus orchards in Spain (Monzó et al., 2011). Generalist epigeal predators such as the spiders, the ground beetles, the rove beetles and the ants were found to predate on apple maggot flies in the USA (Allen & Hagley, 1990).

Large populations of generalist predators supported by a range of prey species are capable of providing efficient biocontrol for a pest species, switching to the pest prey during its availability (e.g. during a pest outbreak), and returning to alternative prey species when the pest population declines (Symondson et al., 2002). It may be possible, therefore, that the surface-dwelling spiders, which must feed on other prey throughout the year, may be able to utilize the soft and vulnerable emerging adult bronze beetle, as they emerge from the soil during early summer, as a facultative prey. Following emergence bronze beetles tend to feed, mate and live within the foliage, and are less vulnerable to ground- dwelling predators. Whether ground-dwelling spiders, whose population was highest during the month of January, are responsible for containing the population growth of bronze beetle in low BB orchards should be an objective of further research. For example, observations of specific predation by spiders on bronze beetles emerging from the ground are needed to add support to my hypothesis that persistent high abundance of surface-dwelling spiders may be containing bronze beetle establishment in low incidence orchards.

The current findings could not explain conclusively why some orchards have more spiders than others. There is conflicting evidence in literature on what influences spider abundance in orchards. Some studies suggest that spider diversity and density in orchards (and other agricultural fields) depends on surrounding habitat factors such as distance from nearest non-crop habitat, habitat similarity between surroundings and the orchard (Bogya et al., 2000; Schmidt et al., 2005; Isaia et al., 2006). Other studies imply that the habitats surrounding orchards and other agricultural fields often have different spider assemblages, and the colonisation of orchards by spiders may be due to long range dispersal (Bishop & Riechert, 1990; Topping & Lovei, 1997; Samu et al., 1999; Samu & Szinetar, 2002). The current study didn't look into surrounding habitat factors, such as identifying nearest non-crop habitat, landscape similarities, and hence spider abundance and diversity could not be linked to any of these factors. Predation on bronze beetle by different generalist arthropod predators that occur in organic orchards could be verified qualitatively by techniques such as molecular analysis of gut contents of the predators (Chen et al., 2000; Greenstone & Shufran, 2003). This would conclusively elucidate the potential of these generalist predators in controlling the beetle.

5.5. Conclusions

Endogeic macro-invertebrates were more abundant in orchards that historically had high bronze beetle incidence (2569.75 per m^2), whereas epigeic macro-invertebrates were more abundant in orchards that had historically low bronze beetle incidence (6588.58 per m^2). Orchards with high bronze beetle damage history had significantly higher number of bronze beetles and sub-soil predators than orchards with low bronze beetle damage history. About eighty percent of adult bronze beetles emerged during November in most orchards. Abundance of sub-soil macro-invertebrates decreased in all the orchards as the New Zealand summer progressed. Significant effect of orchard and date was detected in variation of sub-soil and surface-dwelling macro-invertebrates assemblage (Figures 5.8 and 5.9). Low bronze beetle damage history orchards had significantly higher number of surface-dwelling predators (53.96 per trap) than high bronze beetle damage history orchards (40.30 per trap). It may be that abundant surface-dwelling generalist predators (especially spiders) in these orchards control bronze beetle from establishing. Future research should aim at qualitative evaluation of bronze beetle predation by different generalist predators, which would have important implications in beetle biocontrol.

Chapter 6: Phenology: temperature-dependent development of pupae and modelling adult emergence

Results from this chapter were presented at the New Zealand Plant Protection Society's 64th annual conference (9-11th August 2011, Rotorua, New Zealand).

6.1. Introduction

Phenology is an integrated environmental science that deals with timing of recurring biological events in relation to key environmental factors (Tauber & Tauber, 1976). Knowledge of insect phenology is crucial for the timing of pest management practices to target the most susceptible life stage of an insect pest (Delahaut, 2003). Temperature-based insect phenology models have long been used in decision support systems to predict the timing of crop protection activities (Nietschke et al., 2007). To effectively use phenology, the pest's life cycle must be understood, including what are the most vulnerable stages and when these vulnerable stages occur within the season (Speight et al., 1999; Delahaut, 2003).

Adult *Eucolaspis* sp. "Hawke's Bay" beetles, which cause economically significant damage in organic apple orchards in Hawke's Bay, New Zealand, emerge during spring / summer (October to December) (Chapter 5). It is difficult to control adults with insecticides permitted for use in organic orchards, whereas larvae live too deep in the soil to control (except during spring) (Rogers et al., 2006). All immature stages in *Eucolaspis* life cycle live exclusively underground, whereas adults emerging from pupation live above ground, except when females are ovipositing (Miller, 1926; Doddala et al., 2010). It is believed that larvae undergo winter diapause in deep soil layers (Kay, 1980; Rogers et al., 2008). During late New Zealand autumn (May), larvae were found exclusively between 450-600 mm below the soil surface where there are few plant roots available as food, suggesting that the larvae were starting to overwinter (Rogers et al., 2008). Once soil temperatures are sufficiently high during late winter (August), larvae conclude their diapause and begin moving upwards, seeking food and shelter to pupate (Kay, 1980). As an evidence for this, during spring (September and October) most larvae were found less than 70 mm deep in the ground (Rogers et al., 2007; Rogers et al., 2009). Pupation occurs in small earthen cells in the upper soil layers during spring (Lysaght, 1930; Rogers et al., 2006). Pupal duration varies and lasts about 3½ weeks (Lysaght, 1930). Thus, spring provides a small window of opportunity for controlling bronze beetle immature stages as they occur close to ground surface.

An effective control method aimed at the immature stages (especially pupae) of *Eucolaspis* sp. "Hawke's Bay" has been trialled successfully; it is a mechanical control

by the way of tilling soil between and within orchard rows near tree roots (Rogers et al., 2007). However, timing is critical for this control method, and it has been observed that cultivating too late in the season gave limited control of *Eucolaspis* sp. "Hawke's Bay" (Rogers et al., 2009). A more recent control method aimed at adults, application of a bacterial formulation (*Yersinia entomophaga*), appears promising, with up to 40 percent mortality of adult beetles (Hurst et al., 2011).

Any of bronze beetle's control methods (either bacterial insecticide, or soil cultivation, or any other developed in future) would be deficient unless the seasonal occurrence of the beetle's immature stages and adults could be predicted precisely. Currently, there is very little information available on many aspects of the phenology of *Eucolaspis* sp. "Hawke's Bay". Seasonal development and occurrence of the bronze beetle, when modelled accurately, would help us to know when adults would be active in the field, when is the good time for planning control of larvae / pupae / adults in the field. This would also help us to understand ecological plasticity of the beetle.

The development of insects may depend on many environmental factors (temperature, photoperiod, humidity, etc.); of these, temperature is considered as the most important factor (Howe, 1967; Trudgill et al., 2005). Photoperiod may be crucial to certain biological events, such as onset and termination of diapause, whereas humidity may limit distribution range (Regniere & Logan, 2003). Temperature, on the other hand, appears to have a more direct effect on development of insects; being ectothermic animals, they depend on external (environmental) temperature for their thermal requirements (Delahaut, 2003). Rates of development of ectotherms are slower at low temperatures than at high temperatures, and accelerate within specific range of tolerable temperatures (Jarosik et al., 2002; Jarosik et al., 2004; Trudgill et al., 2005). This range of temperatures is often referred to as optimum temperature range for development.

The base temperatures above and below which the development of an organism ceases are called, respectively, the upper and lower threshold temperatures (Sharpe & DeMichele, 1977; Honek, 1996; Jarosik et al., 2002). These thermal values (upper and lower temperature thresholds) are usually species-specific, easy to measure under controlled conditions in the laboratory, and can be used effectively to model phenology of the insect of interest (Delahaut, 2003; Jarosik et al., 2004; Trudgill et al., 2005).

Another crucial parameter in phenology models, thermal constant, or the sum of effective temperatures (usually expressed in degree-days), is the total amount of heat required for an organism to develop from one stage to another in its life cycle (Honek & Kocourek, 1990; Honek, 1996). A degree-day is defined as one degree temperature accumulated above the lower threshold temperature over a 24 hour period (Wilson & Barnett, 1983; Garcia & Morrell, 2009). Degree-days have been used to predict phenology of insects, plants and other ectothermic life forms (Schwartz, 2003).

When rate of development (which is the inverse of development duration at a given temperature) is plotted against temperature, the relationship appears to be linear for most parts of the temperature range, with non-linear regions towards the upper and lower thresholds (Gilbert & Raworth, 1996; Honek, 1996). Researchers use various linear or non-linear models to describe insect development (Herrera et al., 2005). Non-linear models enable description of insect development over a wide range of temperatures, but these models may not be practically relevant, as most of these models do not allow for calculation of threshold temperatures and, moreover, nonlinearity reflects biases in the data (Jarosik et al., 2002). Thus, if an insect rarely experiences the temperature extremes that fall in the non-linear regions of the temperature-development rate curve, a non-linear model has no ecological meaning. Linear models usually give a good fit for the rate of development over a range of ecologically relevant temperatures, and are simple to calculate and use (Honek, 1996; Jarosik et al., 2002; Trudgill et al., 2005; Garcia & Morrell, 2009). Linear models are usually sufficient to predict insect development in the field (Campbell et al., 1974; Jarosik et al., 2002). Jarosik et al. (2002) and Trudgill et al. (2005) advocate that linear models are practical in terms of providing opportunity to calculate temperature thresholds, and provide true representation of actual field conditions.

In milder climate regions, such as in New Zealand, the temperatures extremes near lower and upper developmental thresholds are unlikely to be experienced during the active growth period of *Eucolaspis* sp. “Hawke’s Bay”. Pupation of the beetles occurs once environmental conditions are favourable, and pupal stage is preceded by actively feeding and developing post-diapause larval stage(s) (Kay, 1980). I believe that a linear model is adequate to describe pupal development in bronze beetle, to calculate threshold temperature and to model adult emergence.

Phenology models that predict various aspects of population dynamics, including seasonal occurrence, are available for many phytophagous insects (Nietschke et al., 2007). Phenology models are used in applications such as prediction of potential geographical distribution and establishment, or prediction of seasonal occurrence of a particular growth stage for a range of crop pests and biocontrol agents. For example, Honek et al. (2003) calculated threshold temperatures and degree-days requirements for different life stages of green dock beetle (biocontrol agent of dock) and estimated that the beetle can only complete 2.4 generations per year in central Europe, significantly reducing biocontrol efficacy of newly established dock seedlings. Phenology models and thermal requirements have been used to predict geographical distribution and potential success of other weed biocontrol agents such as Mediterranean tamarisk beetle (biocontrol agent of saltcedar) (Herrera et al., 2005), TSA Tortoise beetle (biocontrol agent of tropical soda apple –TSA) (Diaz et al., 2008), the Mexican beetle (bioconrol agent of congress weed) (Omkar et al., 2008), etc.

Phenology models have been effectively used in the mountain pine beetle (Coleoptera: Curculionidae) management in North America (Logan & Powell, 2003). Walgama and Zalucki (2007) calculated thermal requirements of the shot-hole borer of tea (Coleoptera: Scolytidae) and found that altitudinal distribution of the pest across tea-growing areas in Sri Lanka is governed by temperature. Phenology models have been constructed for many other coleopteran pests, such as the Western corn rootworm (Jackson & Elliott, 1988; Stevenson et al., 2008), the spruce bark beetle (Wermelinger & Seifert, 1998), the juniper bark borer (Ma et al., 2008), pine sawyer (Naves & de Sousa, 2009), powderpost beetle (Garcia & Morrell, 2009), brassica leaf beetle (Wang et al., 2009), etc.

In New Zealand, phenology models are available for an apple pest, the codling moth *Cydia pomonella* (Walker, 2008). Models are also available for other species of biosecurity risk to New Zealand. For example, Hartley and Lester (2003) developed a cumulative degree-day model for development of different life stages of Argentine ant *Linepithema humile*, and produced a map of potential invasion range, taking into account soil and air temperatures in different regions of New Zealand.

6.1.1. Research objectives

In order to provide phenology support for *Eucolaspis* sp. "Hawke's Bay" control programs, development of beetle pupae was studied in the laboratory. Specific research aims were:

1) To estimate the lower threshold temperature and the degree days required for development of *Eucolaspis* sp. "Hawke's Bay" pupae into adults under controlled conditions
2) To propose and test a phenology model for adult emergence in the field.

6.2. Materials and methods

Development of pupae of *Eucolaspis* sp. "Hawke's Bay" was observed at three different constant temperature regimes in the laboratory; threshold temperature and degree-days were calculated from the data obtained. A phenology model was devised and validated using data on adult emergence in the field. The details are described below.

6.2.1. Insects and incubators

Fully grown *Eucolaspis* sp. "Hawke's Bay" larvae were collected during the last weeks of September 2009 and September 2010 from an organic apple orchard near Havelock North, Hawke's Bay (Orchard A, in Chapter 4). Soil samples were taken below tree branches and hand-sorted (Rogers et al., 2006; Doddala et al., 2010). The larvae were collected in vented 1.5ml micro-centrifuge vials (Eppendorf) along with a small amount of soil. The larvae were kept cool and transported to the laboratory, where they were maintained at controlled conditions (18 ^{0}C temperature, 0: 24 h Light: Dark photoperiod) until pupated. Care was taken that humidity did not fall below 80% by occasional water spraying and leaving water in open containers. The larvae were observed every morning for pupation. Once a larva has pupated, it was allocated randomly to one of three constant temperature treatments and transferred immediately to its respective incubator.

Three different incubators were used for the experiment, each one set at a constant temperature of 12 ^{0}C, 15 ^{0}C or 18 ^{0}C. These three temperatures were chosen as approximate representation of soil temperatures in the field (in orchards) during pupal

stage (September to October) (see Table 6.1). No light was provided in the incubators to simulate dark conditions in the soil where pupation occurs in nature. Humidity was not controlled but monitored with digital hygrometers. The vials were sprayed with distilled water if humidity fell below 85%. Temperatures were monitored using data loggers. As there were only three incubators available at any point of time, the experiment was replicated in time (once each in the years 2009 and 2010) with same incubators each year for the same temperature. Care was taken to have equal number of adults emerged from each temperature regime by adding more pupae depending on mortality in individual incubators (see table 6.3 for details). The pupae were observed daily at noon for adult emergence. Adult status was accepted when the beetles achieved darker pigmentation (Lysaght, 1930). Pupal duration was calculated as the number of days between the date of pupation and the date of adult emergence.

6.2.2. Calculation of thermal requirements

Lower threshold temperature for development and the thermal constant (degree days) required for *Eucolaspis* sp. "Hawke's Bay" pupa to develop into adult were calculated based on two different methods as described below.

In **method 1,** rate of pupal development (y) which is the inverse of pupal duration (N) at a particular constant temperature was regressed against temperature (T) as $y=a+bT$, where "y" is rate of development, "T" is temperature in ^{0}C, "a" is the intercept and "b" is the slope of the regression line. Proc REG in SAS v.9.2 (SAS Inc., USA) was used for the regression analysis.

Threshold temperature (C_t) was calculated according to the formula $C_t = -a/b$ (the temperature at which the regression line intercepts X-axis). Thermal constant (degree-days required) (K) was calculated as the inverse of the slope of the regression line ($K=1/b$) (Campbell et al., 1974; Naves & de Sousa, 2009). Standard errors for threshold temperature ($S.E._{Ct}$) and degree-days ($S.E._K$) were calculated from the following formulae (Campbell et al., 1974; Walgama & Zalucki, 2007):

$$S.E._{Ct} = \frac{y}{b}\sqrt{\frac{s^2}{n * y^2} + \left(\frac{S.E._b}{b}\right)^2}$$

$$S.E._K = \frac{S.E._b}{b}$$

where *y* is mean development rate;

n is number of temperature regimes;

s^2 is the residual mean square of *y*;

$S.E._b$ is the standard error of b.

For **method 2,** I followed Zong et al. (2004) and Ma et al. (2008). Thermal constant (*K*), threshold temperature (C_t) and their standard errors ($S.E._K$ and $S.E._{Ct}$ respectively) were calculated based on the following formulae:

$$K = \frac{n\sum VT - \sum V \sum T}{n\sum V^2 - (\sum V)^2}$$

$$S.E._K = \sqrt{\frac{\sum (T - T`)^2}{(n-2)\sum (V - V`)^2}}$$

$$C_t = \frac{\sum V^2 \sum T - \sum V \sum VT}{n\sum V^2 - (\sum V)^2}$$

$$S.E._{Ct} = \sqrt{\frac{\sum (T - T`)^2 \left[\frac{1}{n} + \frac{V`^2}{\sum (V - V`)^2}\right]}{n-2}}$$

where *n* is the number of temperature regimes in the experiment;

T is the experimental temperature;

N is the average number of development days (i.e. pupal duration);

V is the average development rate and equals to 1/N;

$V` = \frac{\Sigma V}{n}$ where n is the number of temperature regimes in the experiment;

T` is the calculated value of temperature (or effective temperature) and equals to $C_t + KV$.

6.2.3. Weather data

Weather data on daily mean soil temperature (at 10 cm depth) in Hawke's Bay, New Zealand (Table 6.1) was retrieved for 2006-2010 from an online database (Pipfruit NZ, 2012) and used in model testing.

Table 6.1 Soil temperatures (monthly mean) at 10 cm depth during spring in years 2006-2008 in Hawke's Bay, New Zealand (Pipfruit NZ, 2012).

Month	**Monthly mean soil temperature (^{0}C) at 10 cm depth**			
	2006	**2007**	**2008**	**Mean**
September	13.56	12.60	11.53	12.56
October	13.60	15.09	13.37	14.02
November	19.08	19.78	16.86	18.57

6.2.4. Development and evaluation of phenology model

Accumulated degree days were calculated using the horizontal calculation method (Wilson & Barnett, 1983) for years 2005, 2006, 2007 and 2009 using several different biofix dates:

$$\text{Date of emergence} = \text{Biofix date} + N$$

where N is the pupal duration which is equal to n when $\sum_1^n (T_n - C_t) \geq K$;

T_n is the mean soil temperature at 10 cm depth (^{0}C) on n^{th} day;

C_t is the lower threshold temperature (^{0}C);

K is the degree-days required (^{0}C days).

A biofix date is an arbitrary date of the start of a developmental stage (in this case, day 1 of pupation). As it was not possible to estimate accurately the start date of pupation in the field, I have tested four dates (September 1st, 6th, 11th and 16th) as possible biofix dates. Testing different biofix dates in the absence of an empirical date is well supported by published literature (Umble & Fisher, 2000; Naves & de Sousa, 2009). Predicted emergence dates (date of first beetle emergence) were generated for years 2005, 2006, 2007 and 2009, and validated by comparing predicted emergence dates with actual emergence dates observed in those years.

Actual emergence dates were obtained from emergence traps data either collected by other researchers (Rogers et al., 2006; Rogers et al., 2007; Rogers, unpublished data) or generated in the current research project (see Chapter 5). The emergence traps (see Chapter 4. for detailed description) were monitored weekly and hence the actual emergence dates were calculated as -4 days of the first beetle catch date in emergence traps in the field.

6.3. Results

The rate of development of pupae increased as the temperature increased from 12 ^{0}C to 18 ^{0}C in the laboratory bioassays, with a strong linear relationship ($p < .0001$) (Figure 6.1). It took on average 33.2, 22.8 and 18.4 days for pupae of *Eucolaspis* sp. "Hawke's Bay" to develop into adults at constant temperatures 12 ^{0}C, 15 ^{0}C and 18 ^{0}C, respectively (Table 6.2). The sex of emerging adults was not recorded.

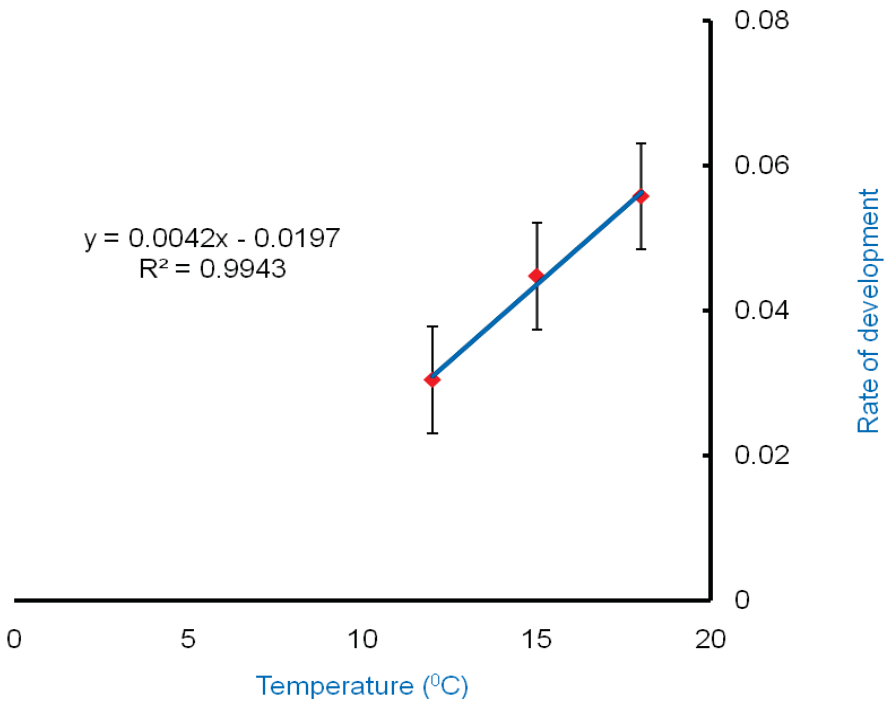


Figure 6.1 Relationship between temperature and the rate of development in *Eucolaspis* sp. "Hawke's Bay" pupae in the laboratory; rate of development is equal to inverse of pupal duration at a given temperature (error bars show S.E.s).

6.3.1. Threshold temperature and degree-days required

Lower threshold temperature for development of *Eucolaspis* sp. pupae was 4.69 ± 0.89 ^{0}C, if calculated using method 1 as (x; 0) intercept of the regression line in Figure

6.1. The threshold temperature was 4.47 ± 0.84 ^{0}C when calculated using method 2 (using parameters in Table 6.2).

Degree-days required for completion of pupal development, which were equal to the inverse of the slope of the regression line (Figure 6.1), were found to be 236.98 ± 21.67 (^{0}C days) according to method 1. Degree-days required were 246.18 ± 19.06 (^{0}C days) according to method 2 (using parameters in Table 6.2).

Table 6.2 Parameters for calculation of thermal requirements for the emergence of *Eucolaspis* sp. "Hawke's Bay" adults from pupae, at three constant temperature regimes in the laboratory

T (^{0}C)	**n_p**	**n_a**	**N**	**V**	**VT**	**V^2**	**T`**	**$(T-T`)^2$**	**$(V-V`)^2$**
12	54	15	33.2	0.03	0.361	0.0009	11.88	0.01334	0.00015824
15	50	16	22.8	0.044	0.658	0.00192	15.27	0.07117	0.00000169
18	61	16	18.4	0.054	0.978	0.00295	17.85	0.02289	0.00013567
Σ			74.4	0.128	1.998	0.00578	45.00	0.10739	0.00029561

T is the experimental temperature,

n_p is number of pupae monitored,

n_a is number of adults emerged,

N is the mean pupal duration;

V is the mean development rate and equals to 1/N;

VT, V^2, T`, $(T-T`)^2$ and $(V-V`)^2$ are the parameters that were used in calculation of threshold temperature and degree-days using method 2 following Zong et al. (2004) and Ma et al. (2008): V` = $\frac{\Sigma V}{n}$ = 0.0428; n=3, is the number of temperature regimes in the experiment (12, 15 and 18 ^{0}C); T` is the calculated value of temperature (or effective temperature) and equals to $C_t + KV$

6.3.2. Phenology model

Thermal requirements (lower threshold temperature and degree-days) calculated using the two methods did not differ greatly; values obtained from the more widely referenced method 1 (linear regression model) were used to predict adult beetle emergence.

Accumulated degree days were calculated for each of the four test years (2005-2007 and 2009) by the horizontal method starting from 4 different biofix dates. A degree-day is a period of 24 hours with temperature 1 ^{0}C above the lower threshold temperature (Garcia & Morrell, 2009). The value from linear regression method (4.69 ^{0}C) was used as the lower threshold temperature for bronze beetle pupae. For each of the four years, the predicted first beetle emergence dates were generated when the accumulated degree-days reached the required 237 degree days; these dates were compared with actual emergence dates (Table 6.3).

Different biofix dates compared for the accuracy of prediction revealed that the best biofix date could be either 6th or 11th of September (Table 6.3); September 11th that has least absolute deviation from actual emergence dates was chosen as the biofix date.

When September 11th was used as the biofix date, the 237 degree-days (^{0}C) required for pupal development and emergence of bronze beetle adults appeared to have accumulated almost at the same time (within few days) in the years 2005, 2006 and 2007 (Figure 6.2). Although there were differences in soil temperatures between these three years in any particular week during the period considered, the inequalities were compensated overall by the end of the four weeks period between September 11th and October 8th.

Table 6.3 Predicted dates of first adult emergence using four different biofix dates, and the absolute deviation (in days) from the actual emergence dates of adult *Eucolaspis* sp. "Hawke's Bay" beetles in organic apple orchards in Hawke's Bay, New Zealand, in different years.

Year	**Predicted emergence date (absolute deviation from actual emergence date in days)**			
	Biofix date			
	1st Sept	**6th Sept**	**11th Sept**	**16th Sept**
2005	1st Oct (20)	4th Oct (17)	9th Oct (12)	15th Oct (12)
2006	28th Sept (11)	2nd Oct (7)	8th Oct (1)	12th Oct (3)
2007	30th Sept (5)	5th Oct (0)	8th Oct (3)	11th Oct (6)
2009	12th Oct (1)	15th Oct (2)	17th Oct (4)	22nd Oct (9)
Average deviation (days)	9.25	6.5	5	7.5

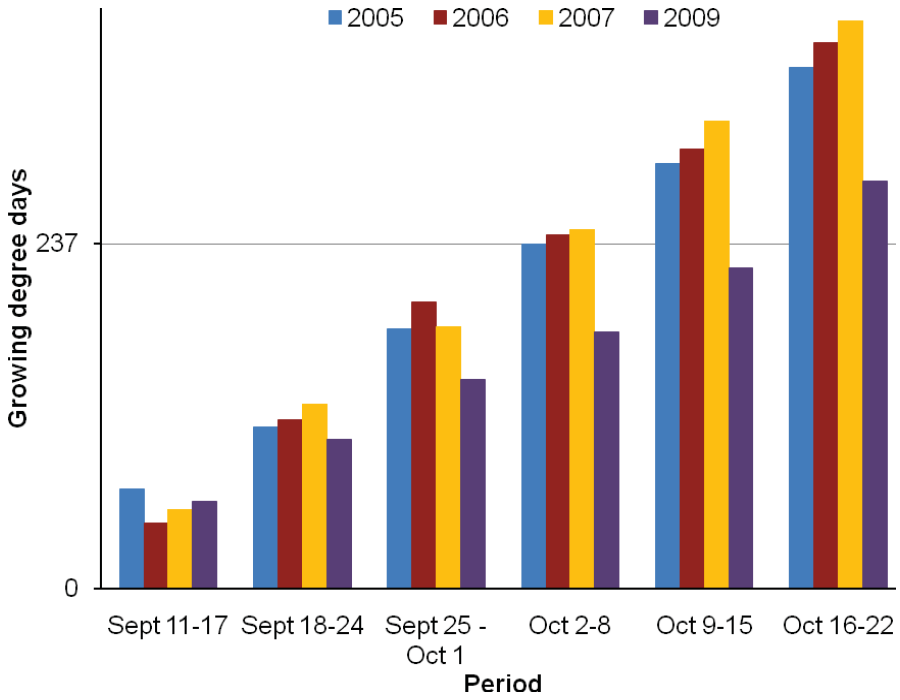


Figure 6.2 Accumulation of degree-days (^{0}C days) for pupal development of bronze beetles, in Hawke's Bay, New Zealand during different years. Biofix date was September 11th; 237 degree-days (shown by a horizontal line) were required for emergence of adults from pupae. Values on X-axis represent 7-day periods.

The predicted and actual dates of first adult beetle emergence, when September 11th was used as the biofix date, were within ± 4 days for all the years except for 2005 where the difference between the two dates was more than 10 days (Figure 6.3). Among the investigated years, 2006 and 2007 had the earliest predicted emergence date due to higher soil temperatures, whereas 2009 had the latest predicted emergence date due to the lower soil temperatures in that year.

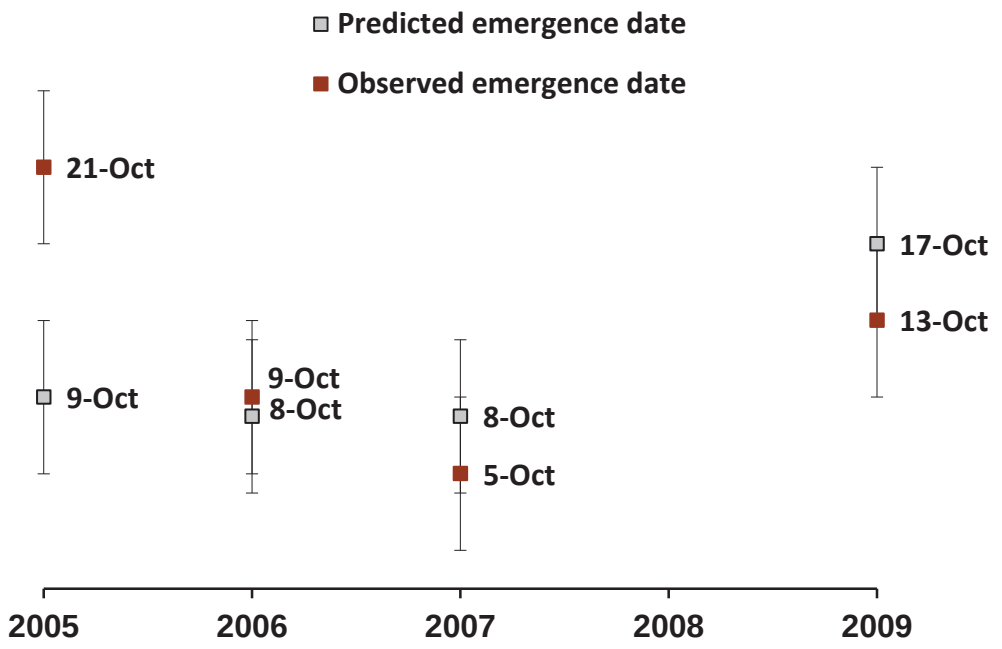


Figure 6.3 Predicted (±4 days) and actual dates (±4 days) of first emergence of adult *Eucolaspis* sp. "Hawke's Bay" beetle in organic apple orchards in Hawke's Bay, New Zealand, in different years.

6.4. Discussion

Pupae required 237 degree days above a threshold temperature of 4.69 ^{0}C to develop as adults. A phenology model based on these values accurately predicted adult emergence in the field for 3 of the 4 years tested when September 11th was used as the biofix date (but see further discussion regarding 2005 emergence data).

Pupae of *Eucolaspis* sp. "Hawke's Bay" developed faster as the temperature progressed in the laboratory studies. Pupae took almost half of the time to develop into adults at the constant temperature of 18 ^{0}C than they did at 12 ^{0}C. Greater reduction in duration of pupal development occurred with the increase in temperature from 12 to 15 ^{0}C, but the overall trend was linear (Fig 6.1). The increased rate of development at higher temperatures was also observed in another life stage (eggs) of the bronze beetle in a study by Lysaght (1930), who observed that bronze beetle eggs hatched in 21-22 days during early in the season (late spring), whereas they hatched in only 15 days later in the season (summer) when temperatures were high. Humidity was not controlled in this study; the humidity is not expected to affect the rate of development, but would rather affect the survival (Lapointe & Shapiro, 1999).

Developmental thermal values (threshold temperatures and degree-days) are usually taxon-specific, and represent the evolutionary adaptations of an organism to its thermal environment (Trudgill et al., 2005). I believe that the 4.69 ^{0}C threshold temperature calculated for *Eucolaspis* sp. "Hawke's Bay", which appears to be much lower than the observed average for Coleoptera (13.6 ^{0}C) (Nietschke et al., 2007), is an ecological strategy deployed by the beetles to adapt to cooler New Zealand spring temperatures. I have also observed that pupae kept at 6 ^{0}C developed into adults after a long time (>4 months), whereas no development was observed in pupae kept at 4 ^{0}C (unpublished data). Similar lower threshold temperature for pupal development (<5^{0}C) was also observed in other temperate zone coleopterans such as strawberry root weevil *Otiorhynchus ovatus* (Coleoptera: Curculionidae) (4.3^{0}C) (Umble & Fisher, 2000) and powderpost beetle *Dinoderus minutus* (Coleoptera: Bostrichidae) (4.5^{0}C) (Garcia & Morrell, 2009).

The accuracy of thermal constants calculated from linear models (as used in the current Chapter) depends on the ecological relevance of the experimental temperatures, i.e., the experimental temperatures should be representative of the environmental temperatures during the organism's active developmental period (Bergant & Trdan, 2006). The temperatures used in my experiment (12, 15 and 18 ^{0}C) were within ±1 ^{0}C from mean monthly soil temperatures in the region during September, October and November in the three years (2006 to 2008) immediately preceding the year of experiment (i.e., 2009) (Table 6.1). These three months (September-November) is the period during which active pupal growth occurs in the field (Lysaght, 1930; Rogers et al., 2008). Thus, the experimental temperatures represent the most probable range of temperatures in the field during active pupal growth. In this study, the linear relationship between temperature and pupal development within the optimum developmental range was of interest, so the temperatures which would represent non-linear regions of temperature-development relationship towards the upper and lower thresholds were not included in the experimental setup.

A particular strength of the proposed phenology model for *Eucolaspis* sp. "Hawke's Bay" is that is has been validated with the field data. The model was efficient in estimating the emergence of adult beetles in the field. Among the four different biofix dates tested, September 11th (11th day after the start of New Zealand meteorological

spring in a year - 254th day in a 365 day year) was more accurate in prediction the emergence date (Figure 6.3). The first pupal appearance in the field was not usually observed before the first week of September each year (Rogers, unpublished data), which gives further support for the chosen biofix date. When September 11th was used as the biofix date, all predicted emergence dates (with the exception of the year 2005) of the bronze beetle adults fell within ±4 days of the observed emergence dates in organic apple orchards of Hawke's Bay. Usually, the predictions that are within 10-15% deviation from the actual dates are considered adequate (Higley et al., 1986; Naves & de Sousa, 2009). The large discrepancy between predicted and observed date of beetle emergence in 2005 is probably an artifact, because it has been suggested by Rogers et al. (2006) that field monitoring of adult bronze beetles may have started rather late in 2005, and the beetles that emerged prior to October 21st may have been missed. As a result, it is possible that in 2005 the beetles started emerging earlier than recorded, which would explain the larger discrepancy between observed and predicted emergence dates for that year.

The emergence of bronze beetle adults in the field typically continues for approximately 8 weeks, and about 80% of the beetles emerge during November (Rogers et al., 2007). More than 50% of the adults had emerged by the 2nd week of November each year (except in 2007 - 36% emergence) and 90% of the adults had emerged by the 1st week of December, as inferred from emergence traps data in Chapter 5, from published literature (Rogers et al., 2006; Rogers et al., 2007), and from unpublished data. By using the phenology model proposed here and weather data, corresponding pupation dates were calculated for the years 2005-2006 and 2009 (Table 6.4).

Table 6.4 Emergence and corresponding pupation dates of *Eucolaspis* sp. "Hawke's Bay" populations during different years in organic apple orchards in Hawke's Bay, New Zealand

Year	50% Pupation	90% Pupation	50% Emergence	90% Emergence
2005	24 October	14 November	12 November	3 December
2006	15 October	12 November	6 November	27 November
2007	6 November	24 November	23 November	7 December
2009	17 October	15 November	13 November	4 December

These pupation dates (Table 6.4) suggest that control methods (like cultivation) aimed at immature stages of bronze beetle should be executed before 3rd week of October to achieve better success. For example, 90% pupation of the bronze beetle would be reached by 2nd week of November (except in 2007) (Table 6.4). Other applications for the suggested phenology model would be predicting emergence of beetles in the orchards by using historic weather data. When biofix date of September 11th and historic soil temperature (mean temperatures for 30 years period 1971-2000) (NIWA, 2011b) were used, the predicted appearance of bronze beetles in Hawke's Bay apple orchards would be 15th October. If one can monitor larvae and pupae in the orchard soil for a couple of weeks, starting from the last week of August and note down the date of first appearance of pupae, the phenology model can be used to accurately predict the start of beetle emergence in that particular orchard, by replacing the biofix date with first pupal appearance date and using weather forecast for accumulating degree-days. However the biofix date recommended here (September 11th) gave accurate predictions for 3 out of the 4 years considered, and so the model can be used successfully as it is.

Studying thermal regulation of diapause and thermal requirements of post-diapause pre-pupal larval instar(s) would provide more robustness to the phenology model. For example, Naves and de Sousa (2009) calculated thermal requirements of post-diapuse larvae-to-adult development for pine sawyer *Monochamus galloprovincialis* (Coleoptera: Cerambycidae) and successfully tested the model for accuracy with field emergence data. In their case, wood-boring pine sawyer larvae (last instar larvae which were assumed to be still in diapause) were collected during late winter and used for the study. There is no information available so far on which larval stage(s) would undergo diapause in *Eucolaspis*. However, if it is found that only one larval instar undergoes the diapause, and also if proper diapause termination data are available, thermal requirements of the diapause-undergoing instar could be estimated. This would give us more accuracy on when the pupation occurs and thus will fine-tune the proposed model. Further, studying thermal biology of all other immature stages of *Eucolaspis* sp. "Hawke's Bay" would also enable us to predict outbreaks, expansion to new geographic areas and future challenges under the influence of changing climate. Currently the distribution of *Eucolaspis* appears to be limited by temperature, as the beetle is not found south of Canterbury in New Zealand (personal communications with

various researchers and entomologists). By 2040 annual temperatures across all regions of New Zealand are predicted to increase by up to 1.3 ^{0}C (NIWA, 2011a). This might have important implications for Central Otago apple orchards, where bronze beetle is not yet established.

6.5. Conclusions

Estimation of thermal requirements for the bronze beetle pupae reported here is the first of its kind for any of the beetle's life cycle stages. Adults emerged from pupae in 33.2, 22.8 and 18.4 days at constant temperatures of 12, 15 and 18 ^{0}C, respectively. The lower threshold temperature was found to be 4.69 ± 0.89 ^{0}C, whereas degree-days required were found to be 237 ± 22 (^{0}C days) for development of *Eucolaspis* sp. "Hawke's Bay" pupae into adults. Phenology model based on these values gave best fit when September 11th was used as the biofix date. The model predicted adult emergence with a precision of ±4 days.

Chapter 7: Synthesis

An estimated NZ$485 million was contributed to the New Zealand economy during 2009 by organic horticulture (Frost, 2011). The organic exports from New Zealand are dominated by fresh fruits and vegetables (Organics Aotearoa New Zealand, 2011). Organic apples, which are worth about NZ$35 million annually (Plant and Food Research Institute of New Zealand Ltd., 2008), are threatened by a native leaf beetle, *Eucolaspis* sp. (Chrysomelidae: Eumolpinae), commonly called the bronze beetle. Damage due to bronze beetles could result in the loss of up to 40% fruit in some of the Hawke's Bay orchards (Rogers et al., 2006). There is little available information on the biology and ecology of these beetles and this has impaired the invention of effective pest control strategies. This thesis set out to fill gaps in research, targeting the most important, practically feasible and readily applicable aspects of the beetle's ecology.

The species infesting apple in Hawke's Bay was initially thought to be *Eucolaspis brunnea*, but this was later disputed and identified as *E. pallidipennis* (Rogers et al., 2007). This reflects the underlying taxonomic instability of the genus (Kuschel, 1990; Dugdale & Hutcheson, 1997), but also raises the important question of whether it is a single species or a complex of species that is infesting organic apples in Hawke's Bay. Without taxonomic certainty any research on biology and ecology could be misleading.

Accordingly, the first objective of this thesis was to resolve the taxonomic complexity and verify the species status of the populations that infest organic apples in Hawke's Bay. This was achieved by extensive sampling throughout New Zealand, including beetles from many host plants. Extensive museum collections from NZAC and LUNZ were also examined to complement the fresh samples. Phylogenetic analysis based on a fragment of the mitochondrial Cytochrome oxidase subunit1 locus, revealed only one genetic lineage in Hawke's Bay and only 3 putative species in mainland New Zealand, and a fourth on Three Kings Islands. A clear gap in inter and intra-clade genetic distances was observed among the CO1 haplotypes of mainland New Zealand *Eucolaspis* and this corroborates with allospecific information for other Coleoptera. The beetles infesting apples in Hawke's Bay region of New Zealand i.e. Clade 1 will be referred as *Eucolaspis* sp. "Hawke's Bay" until type material is examined and precedence in nomenclature is established. Phylogenetic relationships found through molecular data were consistent with variation in male genitalia that I identified. Analysis of some morphometric characters such as elytral width and punctures density

on pronotum, head and anterior elytra (but not others) also supported the phylogeny to some extent. Integration of all three data sets gave consensus support for three species in mainland New Zealand. Other species that are rare and localised might exist, but this appears unlikely given the geographic distribution of the lineages and clear demarcation of genitalia shapes between lineages. Examination of early species descriptions reveals that many of the proposed taxa were abundant and sympatric, and my analysis suggests that these are likely to have been only colour and size polymorphisms.

Use of host plants by different lineages appears to depend on local / regional availability. However, the current sampling included only a few host plants and hence the host plant usage could be an underestimate of the actual situation. Perhaps, the range of host plants of the genus, as compiled in Chapter 3, shows a more realistic estimate and highlights the vast diet breadth of the *Eucolaspis* beetles. Genetic diversity at the genus and species levels suggests multiple and isolated invasions of the bronze beetles into crop plants. Preference for Rosacease plants is evident across all the species, a unique fondness towards a naturally underrepresented plant family (New Zealand Rosaceae were represented by very few taxa until the introduction of fruit crops such as apple (NZPCN, 2012)). A preadaptation towards Rosaceae may exist in *Eucolaspis.* Such preadaptation could stem from similar plant chemistry, but no information is available so far on host selection in *Eucolaspis.* My experiments show that *Eucolaspis* sp. “Hawke’s Bay” beetles use plant odours to detect and discriminate among potential host plants, but the beetles were not able to distinguish between damaged and undamaged host plants and between closely related species of host plants by olfaction alone. Bronze beetles of different populations appear to show different host plant preferences, but this may be due to habituation. Irrespective of their geographical location and ancestral host plant, beetles preferred to feed on blackberry over apple (both exotic plant species). As both blackberry and apple belong to plant family Rosaceae, they possibly emit some common volatiles to which the beetles are attracted. Plant volatile (head-space) and leaf chemistry analyses of apple, blackberry and other Rosaceae plants would complement the outcome of my behavioural bioassays and provide a compelling verification of host selection by *Eucolaspis* sp. “Hawke’s Bay” beetles. Chemical signals play important role in communication between different species in the insect-plant world (Du, 2001). Often plant volatiles that have a different aim attract unwanted herbivorous guests that feed on the eimitting individual.

Sometimes these volatiles also attract predators which act for the benefit of the releasing plant species. Any of such plant volatile attractant, if chemically identified, would be useful to devise pest control and monitoring methods. More importantly, host-range expansion in *Eucolaspis* could be better understood from any future research on identification of specific plant volatiles that attract beetles.

Chemical signals also play a crucial role in communication between sexes in insects (Johansson & Jones, 2007). For example, sex pheromones emitted by female moths attract males from long distances (Kochansky et al., 1975). Aggregation pheromones in insects such as mountain pine beetles attract individuals of both sexes to feeding sites to conduct a mass attack on pine trees (Miller & Lafontaine, 1991; DNRC, 2012). Olfactory bioassays that involved live beetles feeding on apple fruit suggested an absence of aggregation pheromones in *Eucolaspis*. However, in the orchards, the adult beetles often seem to congregate in some apple branches. This may be the result of plant physical or nutritional characters. As an evidence for this, beetles prefer apple varieties such as Royal Gala that produce clusters of fruits and thus provide a hiding place with plenty of food (Rogers et al., 2006). Aggregation has advantages to individuals by way of advertising an abundant qualitiy resource available, and increasing chance of finding potential mating partners. However, increased chance of predation may negate the advantages of aggregation to some extent. Existence of a long range sex pheromone that could contribute to bronze beetle aggregation was ruled out in previous studies (Rogers, unpublished data).

Larvae are abundant in the soil directly under apple tree branches, an indication of poor dispersal as larvae, rather than congregation near future feeding site (for new adults that emerge after completion of metomorphosis). But also it is more likely a result from selection of oviposition by parents, given the comparative mobility and selection abilities of adult and larval stages. If the site selection by ovipositing felames is responsible for larval densities in the soil, the larvae developing in close proximity are likely to be siblings, which can be tested using DNA analysis. It would be also interesting to test the local emerging beetles' sex ratio. Sex ratios are crucial in understanding the mating system of the beetles. Contrary to previous assumptions (Lysaght, 1930), emergence sex ratio in *Eucolaspis* sp. "Hawke's Bay" was found to be female-biased (65% of emerging population) and progressively more females emerged during the season, whereas the adult sex ratio in the active population on foliage was

slightly male-biased (55%). Fewer male beetles emerging at any point of time does not seem to have an influence on active population sex ratio; this manifests behavioural differences but could also represent differences in longevity between sexes (Lysaght, 1930). Adult sex ratios varied among samples from various locations and host plants but these data are unreliable due to low numbers of samples from some host plants.

No evidence of a short range sex pheromone was found through my olfactometer bioassays. Cuticular hydrocarbons that render insect cuticle hydrophobic in nature are used as contact sex pheromones in many cerambycid beetles (Ginzel, 2010). Contact sex pheromones have also been reported to mediate mate choice in some chrysomelids (El-Sayed, 2011). I hypothesised that the bronze beetles may use female-specific contact sex pheromones in mate recognition. A series of mating bioassays were conducted as detailed in Chapter 4, to test this hypothesis. All the mating attempts in mating bioassays proceeded only after either antennal contact or licking of the female's elytra by male. Ablating antennae did not impair mating, but significantly delayed location of the mate. Forty five percent and thirty five percent of the tested males attempted to mate with intact and washed female cadavers, respectively, whereas no mating attempts were initiated towards male cadavers. Only one of the test males attempted to mate with reconstituted female cadavers. It can be concluded that male *Eucolaspis* sp. "Hawke's Bay" utilize both contact sex pheromones and vision in locating potential female mates. However, further research is needed to tease apart the relative effect of vision and pheromones in mate location. A species-specific contact sex pheromone could reinforce sexual isolation in congeneric sympatric populations of the beetles described in Chapter 2. Distinctive differences male genitalia between different lineages could also contribute to sexual isolation if incompatible with allospecific female genitalia.

Bronze beetle infestation levels vary among regions. For example, Massey University's organic block of apples is completely devoid of any bronze beetles while these beetles persist on other home gardens in Palmerston North and more widely distributed in orchards in Hawke's Bay. The beetle densities vary between orchards and sometimes among blocks in an orchard. The reason for this patchy distribution was not evident from previous studies. The beetle lineage (Clade 1) that infests apples in Hawke's Bay also includes beetles from Palmerston North, Waikanae and Canterbury. The intra-clade genetic differences among *Eucolaspis* haplotypes are typical of intra-

specific differences in Coleoptera (Hebert et al., 2003b), confirming "single species" status for the haplotypes under each clade (Clades 1, 2 and 3). Thus, the patchy distributions are not due to allospecific differences. Experiments detailed in Chapter 5 were designed to investigate whether population dynamics of bronze beetles is related to population dynamics of other soil macro-invertebrates in organic orchards. Soil predators such as spiders, ground beetles, rove beetles and centipedes play an important role in suppressing herbivore population in orchards and arable crops (Symondson et al., 2002). I found that endogeic macro-invertebrates (e.g., earthworms, centipedes, etc.) were more abundant in orchards that historically had high bronze beetle density, whereas epigeic macro-invertebrates (such as spiders) were more abundant in orchards that had historically low bronze beetle incidence. Abundance of all sub-soil macro-invertebrates in the high BB orchards could be due to related factors such as soil fertility and having greater resources to support abundant invertebrate community. Sub-soil predators, such as centipedes, appeared to have benefited from abundant prey (bronze beetle larvae and pupae) in high BB orchards, but could not suppress the beetle populations, perhaps as they were an overwhelming prey resource (Table 5.7). I suspect that surface-dwelling generalist predators, such as spiders and ground beetles, which have access to diverse prey resources (herbivores and detritivores) to support high predator density, may limit the establishment of bronze beetles. This inference could be further assessed by direct observation of predation behaviour of spiders and other generalist predators.

Though it was found by previous studies that most adult emergence occurred during November (Rogers et al., 2006; Rogers et al., 2007; Rogers et al., 2008), seasonal variations in adult emergence resulted in reduced efficiency of population control during some years. This prompts for adequate scientific knowledge on beetle phenology to be part of control strategies. Understanding phenology is crucial for planning pest management practices to target the most susceptible life stage of the pest (Delahaut, 2003). Such prediction models have long been used in decision support systems (Nietschke et al., 2007). I designed an adult emergence prediction model (chapter 6) that performed well when tested with field data. Estimated thermal requirements of bronze beetle pupae reported here are the first of their kind for any life cycle stage of this beetle. Adults emerged from pupae in 33.2, 22.8 and 18.4 days at constant temperatures of 12, 15 and 18 ^{0}C respectively. The lower threshold temperature

was found to be 4.69 ± 0.89 ^{0}C, whereas degree-days required were found to be 237 ± 22 (^{0}C days) for development of *Eucolaspis* sp. "Hawke's Bay" pupae into adults. Phenology model based on these values gave best fit when September 11th was used as the biofix date. The model predicted adult emergence with a precision of ±4 days. At the moment, bronze beetle is economically important only in organic apple orchards. But conventionl apple production systems that would soon adopt nill pesticide residue system under "Apple Futures" programme (Pipfruit NZ, 2011) could become vulnerable to bronze beetle, as application of presistant insecticides will be reduced. Different aspects found by my research would help mitigate such future challenges. For example, my phenology model could be used accurately to predict adult emergence in any orchard system and, instead of multiple applications, a single application of less-persistant yet effective insecticide could be used to control beetles.

Contribution to the body of knowledge

Most of the findings from my research are new to science and hence make substantial contribution to the body of knowledge. My findings highlighted taxonomic inflation in the genus, *Eucolaspis* Sharp 1886 confining the species compendium to just three (putative species) in mainland New Zealand, that are well supported by genetic, aedeagal and morphometric data sets (Chapter 2). Key findings on host location behaviour include attraction of beetles to fresh leaf/fruit odour from host plants and preference of blackberry over apple to feed on by the adult beetles (Chapter 3). Plant volatiles and feeding preference results found would aid in managing beetle infestation and in understanding evolutionary development of host-range expansion. Adult sex ratios in the emerging and active population of the beetles revealed a contrast situation while mating behaviour bioassays established that the male beetles use both vision and contact sex pheromones in locating and identifying a potential mate (Chapter 4). A large data set of population density and diversity of bronze beetle and other soil macro-invertebrates in organic apple orchards in Hawke's Bay, New Zealand was generated through my field study (Chapter 5). The data revealed that surface-dwelling generalist predators were abundant in the same orchards that also contained low density of bronze beetles, suggesting a top-down effect. I have proposed a phenology model to predict adult emergence in the field based on threshold temperature and degree-days required for pupal development (Chapter 6). This has a wide applicability in prediction of adult

emergence, pest monitoring, planning pest management practices and expansion to new areas at the effect of global climate change.

A significant advance has been made through the current research in our knowledge on *Eucolaspis* in particular but also on Chrysomelidae in general. The diversity of topics allowed use of a variety of advanced techniques. Inclusion of field and laboratory experiments helped to assess lab-to-land technology transfer feasibility. Already established scientific theory (such as reliability of mitochondrial genomic data to species phylogeny and temperature-dependant development of ectotherms) has been put to practical use, especially to test the applicability. Given the lack of any biology/ecology studies on *Eucolaspis* since 1930, the current research had a huge gap to fill in. Nevertheless, many useful inferences made here give rise to several further questions that warrant urgent attention by future researchers.

Application to management of beetles

It has been found through my research that a single putative species infests apple orchards in Hawke's Bay and these findings give taxonomic certainity to species-specific pest control methods. I also found that the beetle lineage infesting apples in Hawke's Bay is different from the one that infests apples in Nelson. Likewise, different lineages infest blueberries in Hamilton and Kerikeri. This has to be taken into consideration when developing and adminstering species-specific control measures such as biopesticides (bacteria, nematodes etc.) or pheromones.

Hawke's Bay lineage beetles were shown to utilize plant volatiles to locate host plants of the family Rosaceae (Chapter 3). Similar olfactory abilities can be expected in other lineages to locate their respective host plants, as the three lineages were shown to associate with similar wild / crop plants (Chapter 2). Bronze beetles preferred to feed on blackberry (Chapter 3), which I believe has important impliactions for orchard management. Blackberry, a common weed widely distributed within New Zealand, as well as being commercially grown, has a great potential to attract and harbour bronze beetles, which could find their way into any apple orchards (either organic or conventional) in the vicinity. Although the beetles can be controlled easily in conventionally managed orchards, risk of resurgence always exists, as phytophagous insects in agricultural and horticultural systems are known to develop resistance to pesticides sooner or later (Georghiou, 1972). Native host plants that are abundant

through out New Zealand are another source which could harbour persistent populations of bronze beetles. Having no blackberry near an apple orchard would be a precaution to prevent any potential invasion by bronze beetles.

Host breadth expansion is an exiting area that has potential to offer insights into causes of such occurrences in the future. For this type of study, *Eucolaspis* provides a great opportunity. As reviewed in Chapter 3, these Myrtaceae-feeding natives now use >67 plant species covering 33 families. It is one of the few polyphagous insects that use plant volatiles in host location, similar to other polyphagous chrysomelids, *Diabrotica balteata*, *D. undecimpunctata* (Jackson et al., 2005), *D. speciosa* (Ventura et al., 2005) and *Galerucella vittaticollis* (Hori et al., 2006). Larval host plants are not yet known for any of the species of *Eucolaspis*, though often speculated to be some grass species. Another aspect of larval biology that remains unsolved is the initiation and termination of diapause. These types of studies would offer venues for management of this economically important insect. For example, if monophagy of larval *Eucolaspis* is established, as it has been in the case of the Western corn rootworm *Diabrotica virgifera virgifera* (Chyb et al., 1995), a simple alternate under-storey planting with non-host plants (larval) would offer an exellant control for beetles in apple orchards.

I have shown, for the first time that *Eucolaspis* utilizes contact sex pheromones in mate location (Chapter 4). Although sex pheromones tend to be highly species-specific, I suspect that all three lineages use sex pheromones which would serve as sexual isolation mechanism in congeneric sympatric populations, as it is observed in other chrysomelids. For example, both *Chrysochus auratus* and *C. cobaltinus* use contact sex pheromones, but a clear difference between species exists in composition of the pheromone (Peterson et al., 2007). Pheromones have long been widely used in pest management in many crop / forest systems (Witzgall et al., 2010).

It appears that surface-dwelling generalist predators, especially spiders, may effectively suppress herbivore population, and the predator community seem to have benefited from abudant alternate prey such as detritivores (Chapter 5). As most bronze beetles appear to emerge during mid October to November, conservation of predator activity during these months would be crucial in maintaining a top-down pressure on emerging beetles. As such, one can not suggest abandoning taking up any agronomic practices during this time that can potentially displace ground-dwelling predators.

However, minimum disturbance of surface-dwelling predator community is advised. For example, with historic weather data (30 years average), I predict that bronze beetles would start emerging from 15th October each year (Chapter 6) and would continue to emerge for up to 8 weeks, although most beetles would emerge within first 4-6 weeks. So any efforts to conserve and improve predator activity during these periods would be highly beneficial for pest control.

Future research

Use of data on multiple loci is preferred to that of a single locus, in order to infer phylogeny. However, in the current study, the phylogeny inferred using just COI locus was well supported through other data sets, i.e., genitalic and morphometric. A quick comparison with other genera in the subfamily Eumolpinae highlighted the often well-supported biogeographic link, i.e., close evolutionary histories of Austro-Papuan-Pacific biota. Future research should be focused on exploring relationships among New Zealand Eumolpinae in the light of Pacific-Gondwanan-origin. At a genus level, autecology and population ecology studies on other "species" of New Zealand *Eucolaspis* would provide insights into species interactions, more importantly host-switch or host-breadth-expansion of this economically important native beetle. Keeping in view of these, this thesis identified some of the future research areas related to *Eucolaspis*, as listed below.

- Evolution of different genera of New Zealand Eumolpinae and their relationship with other Pacific and Austro-Papuan eumolpine genera: Phylogenetic relationships among New Zealand and global Eumolpinae suggested paraphyly of the New Zealand taxa with other global taxa. This higlights isolated invasion / colonisation in this island country by different genera. Seperation of *Eucolaspis* from some pacific genera such as *Colaspoides* and *Dematochroma* has been questioned by some researchers. However this theory needs proper evaluation by inclusion of a wide range of samples.
- Biogeographic study of *Eucolaspis* in New Zealand: The geographic distribution identified in this study, indicates an interesting demarkation between East and West parts of New Zealand. Testing the geographic distribution in the light of existing geological and biotic evidence would be crucial to understand the evolution of species ranges.

- Larval diapause (which instar initiates it, and what are the biochemical changes that lead to diapause initiation and termination), and thermal biology of pre-pupal larval instar: So far, there is no data available regarding diapause in brozne beetles, which is crucial to estimate any future challenges under the influence of climate change. Increased winter temperatures may have an effect on number of generations that the beetles could complete in a year. Overlapping generations are a possibility if diapause is not necessary to survive through winter with increase in environmental temperatures.
- Larval feeding, using stable isotopes and DNA analyses to identify the host plants: Stable isotopes such as Carbon (^{13}C) and Nitrogen (^{15}N) are good tools assess the ecological nitche the larvae occupy (Hood-Nowotny & Knols, 2007). For example, whether the larval food includes grass or broad-leaved species could be found out with ease using stable isotopes. Molecular analysis of gut contents would be crucial identify the specific host plant species that are fed by the larvae.
- Attraction of bronze beetle adults to fresh leaf volatiles from native host plants such as manuka, and also preference between different native and introduced host plants for attraction and feeding: Bronze beetles exihbit an interesting situation in terms of diet-breadth. All the three lineages were found to use both native and exotic host plants. An effect of habituation was shown in apple and blackberry populations. Assessing the beetles' choice between native and exotic host plants would offer incites into host-switch or diet-breadth-expansion and evolutionary lability of such behaviour.
- Chemical analysis of cuticular hydrocarbon profiles from male and female bronze beetle adults and testing their role in mate location.
- Female's choice in selecting mates, its implications for fitness gain and population growth.
- Predator gut-analysis to investigate predation of bronze beetles by generalist arthropod predators in apple orchards: A qualitative confirmation of generalist predators' prey would help to evaluate the ecosystem services provided by predators in the light of bronze beetle and other herbivores suppression.

Bibliography

Agelopoulos, N., Birkett, M. A., Hick, A. J., Hooper, A. M., Pickett, J. A., Pow, E. M., et al. (1999). Exploiting semiochemicals in insect control. *Pesticide Science, 55*(3), 225-235.

Allen, W. R. & Hagley, E. A. C. (1990). Epigeal arthropods as predators of mature larvae and pupae of the apple maggot (Diptera: Tephritidae). *Environmental Entomology, 19*(2), 309-312.

Altuzar, A., Malo, E. A., Gonzalez-Hernandez, H. & Rojas, J. C. (2007). Electrophysiological and behavioural responses of *Scyphophorus acupunctatus* (Col., Curculionidae) to *Agave tequilana* volatiles. *Journal of Applied Entomology, 131*(2), 121-127.

Ansebo, L., Coracini, M. D. A., Bengtsson, M., Liblikas, I., Ramírez, M., Borg-Karlson, A. K., et al. (2004). Antennal and behavioural response of codling moth *Cydia pomonella* to plant volatiles. *Journal of Applied Entomology, 128*(7), 488-493.

Auckland Botanic Gardens. (1999). *Pohutakawas and related species for Auckland gardens*.

Bagneres, A.-G. & Wicker-Thomas, C. (2010). Chemical taxonomy with hydrocarbons. In G. J. Blomquist & A.-G. Bagneres (Eds.), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology* (pp. 121-162). Cambridge, the UK: Cambridge University Press.

Bailey, W. J. (1991). Mate finding: selection on sensory cues. In W. J. Bailey & R.-S. J. (Eds.), *Reproductive behaviour of insects: individuals and populations* (pp. 42-74). London: Chapman & Hall.

Bakr, E. M. (2005). A new software for measuring leaf area, and area damaged by *Tetranychus urticae* Koch. *Journal of Applied Entomology, 129*(3), 173-175.

Bardgett, R. D. (2005). *The biology of soil : a community and ecosystem approach* Oxford: Oxford University Press, 2005.

Bartelt, R. J. (2010). Volatile hydrocarbon pheromones from beetles. In G. J. Blomquist & A.-G. Bagneres (Eds.), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology* (pp. 448-476). Cambridge, the UK: Cambridge University Press.

Bejakovich, D., Pearson, W. D. & O'Donnell, M. R. (1998). *Nationwide survey of pests and diseases of cereal and grass seed crops in New Zealand. 1. Arthropods and Molluscs.* Paper presented at the 51st New Zealand Plant Protection Conference.

Bell, K. L., Yeates, D. K., Moritz, C. & Monteith, G. B. (2004). Molecular phylogeny and biogeography of the dung beetle genus *Temnoplectron* Westwood (Scarabaeidae: Scarabaeinae) from Australia's wet tropics. [doi: DOI: 10.1016/j.ympev.2003.09.010]. *Molecular Phylogenetics and Evolution, 31*(2), 741-753.

Bengtsson, M., Bäckman, A.-C., Liblikas, I., Ramirez, M. I., Borg-Karlson, A.-K., Ansebo, L., et al. (2001). Plant odor analysis of apple: antennal response of codling moth females to apple volatiles during phenological development. *Journal of Agricultural and Food Chemistry, 49*(8), 3736-3741.

Bengtsson, M., Jaastad, G., Knudsen, G., Kobro, S., Bäckman, A.-C., Pettersson, E., et al. (2006). Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella*. *Entomologia Experimentalis et Applicata, 118*(1), 77-85.

Bereś, P. & Sionek, R. (2010). The occurrence and sex ratio of *Diabrotica virgifera* Le Conte beetles on sweet maize (*Zea mays* var. Saccharata) in South-Eastern Poland near Rzeszów in 2007-2009. *Vegetable Crops Research Bulletin, 73*(-1), 87-97.

Bergant, K. & Trdan, S. (2006). How reliable are thermal constants for insect development when estimated from laboratory experiments? *Entomologia Experimentalis et Applicata, 120*(3), 251-256.

Bernays, E. A. & Chapman, R. F. (1994). *Host-plant selection by phytophagous insects*. New York, USA: Chapman & Hall Inc.

Bernays, E. A. & Graham, M. (1988). On the evolution of host specificity in phytophagous Arthropods. *Ecology, 69*(4), 886-892.

Berry, N. A., Wratten, S. D., McErlich, A. & Frampton, C. (1996). Abundance and diversity of beneficial arthropods in conventional and "organic" carrot crops in New Zealand. *New Zealand Journal of Crop and Horticultural Science, 24*(4), 307-313.

Bishop, L. & Riechert, S. E. (1990). Spider colonization of agroecosystems: Mode and source. *Environmental Entomology, 19*(6), 1738-1745.

Björklund, N., Nordlander, G. & Bylund, H. (2005). Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis*. *Physiological Entomology, 30*(3), 225-231.

Blomquist, G. J. (2010). Structure and analysis of insect hydrocarbons. In G. J. Blomquist & A.-G. Bagneres (Eds.), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology* (pp. 19-34). Cambridge, the UK: Cambridge University Press.

Blomquist, G. J., Nelson, D. R. & De Renobales, M. (1987). Chemistry, biochemistry, and physiology of insect cuticular lipids. *Archives of Insect Biochemistry and Physiology, 6*(4), 227-265.

Bogya, S., Marko, V. & Szinetar, C. S. (2000). Effect of pest management systems on foliage- and grass-dwelling spider communities in an apple orchard in Hungary. *International Journal of Pest Management, 46*(4), 241-250.

Bogya, S., Szinetar, C. S. & Marko, V. (1999). Species composition of spider (Araneae) assemblages in apple and pear orchards in the Carpathian Basin. *Acta Phytopathologica et Entomologica Hungarica, 34*(1-2), 99-122.

Bolter, C. J., Dicke, M., Van Loon, J. J. A., Visser, J. H. & Posthumus, M. A. (1997). Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology, 23*(4), 1003-1023.

Broun, T. (1880). *Manual of the New Zealand Coleoptera* (Vol. 1): The New Zealand Institute, Wellington.

Broun, T. (1893a). LVI.—Descriptions of new Coleoptera from New Zealand. *Journal of Natural History Series 6, 12*(71), 374-392.

Broun, T. (1893b). *Manual of the New Zealand Coleoptera* (Vol. 5): The New Zealand Institute, Wellington.

Broun, T. (1909). XXXII.—Descriptions of new genera and species of New-Zealand Coleoptera. *Journal of Natural History Series 8, 4*(21), 275-291.

Bruce, T. J. A., Wadhams, L. J. & Woodcock, C. M. (2005). Insect host location: a volatile situation. *Trends in Plant Science, 10*(6), 269-274.

Brust, G. E. & House, G. J. (1990). Effects of soil moisture, no-tillage and predators on southern corn rootworm (Diabrotica undecimpunctata howardi) survival in corn agroecosystems. *Agriculture, Ecosystems & Environment, 31*(3), 199-215.

Bryant, G. E. & Gressitt, J. L. (1957). Chrysomelidae of Fiji (Coleoptera). *Pacific Science, 11*(1), 2-91.

Campbell, A., Frazer, B. D., Gilbert, N., Gutierrez, A. P. & Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology, 11*(2), 431-438.

Chang, K. S., Shiraishi, T., Nakasuji, F. & Morimoto, N. (1991). Abnormal sex-ratio condition in the walnut leaf beetle, *Gastrolina depressa* (Coleoptera: Chrysomelidae). *Applied Entomology and Zoology, 26*(3), 299-306.

Chen, Y., Giles, K. L., Payton, M. E. & Greenstone, M. H. (2000). Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology, 9*(11), 1887-1898.

Chyb, S., Eichenseer, H., Hollister, B., Mullin, C. A. & Frazier, J. L. (1995). Identification of sensilla involved in taste mediation in adult western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Journal of Chemical Ecology, 21*(3), 313-329.

Clark, A. F. (1938). A survey of insect pests of eucalyptus in New Zealand. *New Zealand Journal of Science and Technology, 19*(12), 750-761.

Clearwater, J. R. & Richards, M. N. (1984). *Bronze beetle control in Auckland apple orchards.* Paper presented at the 37th New Zealand Weed and Pest Control Conference.

Clough, Y., Kruess, A. & Tscharntke, T. (2007). Organic versus conventional arable farming systems: Functional grouping helps understand staphylinid response. *Agriculture Ecosystems & Environment, 118*(1-4), 285-290.

Cognato, A. I. (2006). Standard Percent DNA Sequence Difference for Insects Does Not Predict Species Boundaries. *Journal of Economic Entomology, 99*(4), 1037-1045.

Cook, J. M. & Hardy, I. C. W. (2002). Sex determination in invertebrates *Sex Ratios: Concepts and Research Methods*: Cambridge University Press.

Coracini, M., Bengtsson, M., Liblikas, I. & Witzgall, P. (2004). Attraction of codling moth males to apple volatiles. *Entomologia Experimentalis et Applicata, 110*(1), 1-10.

Cottier, W. (1935). Experiments on the control of the bronze beetle (*Eucolaspis brunnea*). *New Zealand Journal of Science and Technology, 17*(2), 433-453.

Curry, J. P. & Schmidt, O. (2006). The feeding ecology of earthworms - A review. *Pedobiologia, 50*(6), 463-477.

Delahaut, K. (2003). Insects. In M. D. Schwartz (Ed.), *Phenology: An Integrative Environmental Science* (pp. 405-419): Kluwer Academic Publishers, Dordrecht, The Netherlands.

Diaz, R., Overholt, W. A., Samayoa, A., Sosa, F., Cordeau, D. & Medal, J. (2008). Temperature-dependent development, cold tolerance, and potential distribution of *Gratiana boliviana* (Coleoptera: Chrysomelidae), a biological control agent of tropical soda apple, *Solanum viarum* (Solanaceae). *Biocontrol Science and Technology, 18*(2), 193 - 207.

Dickinson, J. L. (1992). Scramble competition polygyny in the milkweed leaf beetle: combat, mobility, and the importance of being there. *Behavioral Ecology, 3*(1), 32-41.

Dickinson, J. L. (1995). Trade-offs between postcopulatory riding and mate location in the blue milkweed beetle. *Behavioral Ecology, 6*(3), 280-286.

Dickinson, J. L. (1997). Multiple mating, sperm competition, and cryptic female choice in the leaf beetles (Coleoptera: Chrysomelidae). In J. C. Choe & B. J. Crespi

(Eds.), *The evolution of mating systems in insects and arachnids* (pp. 164-183). Cambridge, UK: Cambridge University Press.
Dindal, D. L. (1990). *Soil Biology Guide*: Wiley - Interscience.
DNRC. (2012). Mountain Pine Beetle. Retrieved 29/06/2012, from http://dnrc.mt.gov/forestry/Assistance/Pests/mtnpinebeetle.asp#
Doddala, P. R. C., Minor, M. A. & Rogers, D. J. (2010). Population dynamics of bronze beetle, *Eucolaspis* spp. (Coleoptera: Chrysomelidae) in relation to other soil macro-invertebrates in organic apple orchards in Hawke's Bay. *New Zealand Plant Protection, 63*, 195-200.
Du, J. W. (2001). Plant-insect chemical communication and Its behavior control. *Acta Phytophysiologica Sinica, 27*(3), 193-200.
Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of Plant Volatiles. *Plant Physiology, 135*(4), 1893-1902.
Dugdale, J. & Hutcheson, J. (1997). *Invertebrate values of kanuka (Kunzea ericoides) stands, Gisborne region*. Wellington: Department of Conservation.
Dymock, J. J. & Holder, P. W. (1996). Nationwide survey of arthropods and molluscs on cut flowers in New Zealand. *New Zealand Journal of Crop and Horticultural Science, 24*(3), 249-257.
Eben, A., Barbercheck, M. E. & Aluja, M. S. (1997). Mexican diabroticite beetles: I. Laboratory test on host breadth of *Acalymma* and *Diabrotica* spp. *Entomologia Experimentalis et Applicata, 82*(1), 53-62.
Edkins, J. (2009). Imperial Measures of Length. Retrieved 17/02/2012, from http://gwydir.demon.co.uk/jo/units/length.htm
El-Sayed, A. M. (2011). The Pherobase: Database of Insect Pheromones and Semiochemicals. Retrieved 05-09-2011, from http://pherobase.com
El-Sayed, A. M., Suckling, D. M., Wearing, C. H. & Byers, J. A. (2006). Potential of mass trapping for long-term pest management and eradication of invasive species. *Journal of Economic Entomology, 99*(5), 1550-1564.
Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*(4300), 215-223.
Epstein, D. L., Zack, R. S., Brunner, J. F., Gut, L. & Brown, J. J. (2000). Effects of broad-spectrum insecticides on epigeal arthropod biodiversity in Pacific Northwest apple orchards. *Environmental Entomology, 29*(2), 340-348.
Fabricius, J. C. (1781). *Species Insectorum* (Vol. 1): Hamburgi et Kilonii.
Fein, B. L., Reissig, W. H. & Roelofs, W. L. (1982). Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *Journal of Chemical Ecology, 8*(12), 1473-1487.
Fernandez, P. & Hilker, M. (2007). Host plant location by Chrysomelidae. *Basic and Applied Ecology, 8*(2), 97-116.
Ferro, D. N., Lowe, A. D., Ordish, R. G., Somerfield, K. G. & Watt, J. C. (1977). Standard names for common insects. *Bulletin of the Entomological Society of New Zealand 4*, 1-42.
Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.
Frost, C. (2011). New Zealand Organic Report 2010. Retrieved 28/11/2011, from http://www.organic-market.info/web/Continents/Australia-Japan-Pacific/New_Zealand/201/204/0/7914.html

Fukaya, M., Akino, T., Yasuda, T., Yasui, H. & Wakamura, S. (2004). Visual and olfactory cues for mate orientation behaviour in male white-spotted longicorn beetle, *Anoplophora malasiaca*. *Entomologia Experimentalis et Applicata, 111*(2), 111-115.

Fukaya, M., Yasui, H., Yasuda, T., Akino, T. & Wakamura, S. (2005). Female orientation to the male in the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) by visual and olfactory cues. *Applied Entomology and Zoology, 40*(1), 63-68.

Fukuda, Y., Moller, H. & Burns, B. (2011). Effects of organic farming, fencing and vegetation origin on spiders and beetles within shelterbelts on dairy farms. *New Zealand Journal of Agricultural Research, 54*(3), 155-176.

Futuyma, D. J. (1990). Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, Chrysomelidae). *Evolution, 44*(8), 1885-1913.

García-Robledo, C. & Horvitz, C. C. (2009). Host plant scents attract rolled-leaf beetles to Neotropical gingers in a Central American tropical rain forest. *Entomologia Experimentalis et Applicata, 131*(2), 115-120.

Garcia, C. M. & Morrell, J. J. (2009). Development of the powderpost beetle (Coleoptera: Bostrichidae) at constant temperatures. *Environmental Entomology, 38*(2), 478-483.

Gatto, L., Mardulyn, P. & Pasteels, J. M. (2008). Morphological and mitochondrial DNA analyses indicate the presence of a hybrid zone between two species of leaf beetle (Coleoptera; Chrysomelidae) in Southern Spain. *Biological Journal of the Linnean Society, 94*(1), 105-114.

Geiselhardt, S., Otte, T. & Hilker, M. (2009). The role of cuticular hydrocarbons in male mating behavior of the mustard leaf beetle, *Phaedon cochleariae* (F.). *Journal of Chemical Ecology, 35*(10), 1162-1171.

Georghiou, G. P. (1972). The Evolution of Resistance to Pesticides. *Annual Review of Ecology and Systematics, 3*(ArticleType: research-article / Full publication date: 1972 / Copyright © 1972 Annual Reviews), 133-168.

Gilbert, N. & Raworth, D. A. (1996). Insects and temperature : A general theory. *The Canadian Entomologist, 128*(1), 1-13.

Ginzel, M. D. (2010). Hydrocarbons as contact pheromones of longhorned beetles (Coleoptera: Cerambycidae). In G. J. Blomquist & A.-G. Bagneres (Eds.), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology* (pp. 375-389). Cambridge, the UK: Cambridge University Press.

Ginzel, M. D., Blomquist, G. J., Millar, J. G. & Hanks, L. M. (2003). Role of contact pheromones in mate recognition in *Xylotrechus colonus*. *Journal of Chemical Ecology, 29*(3), 533-545.

Ginzel, M. D. & Hanks, L. M. (2003). Contact pheromones as mate recognition cues of four species of longhorned beetles (Coleoptera: Cerambycidae). *Journal of Insect Behavior, 16*(2), 181-187.

Gómez-Zurita, J., Cardoso, A., Jurado-Rivera, J. A., Jolivet, P., Cazères, S. & Mille, C. (2010). Discovery of new species of New Caledonian Arsipoda Erichson, 1842 (Coleoptera: Chrysomelidae) and insights on their ecology and evolution using DNA markers. *Journal of Natural History, 44*(41-42), 2557-2579.

Greenstone, M. H. & Shufran, K. A. (2003). Spider predation: Species-specific identification of gut contents by polymerase chain reaction. *Journal of Arachnology, 31*(1), 131-134.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate Maximum-Likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology, 59*(3), 307-321.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. *Systematic Biology, 52*(5), 696-704.

Gurney, A. B., Kramer, J. P. & Steyskal, G. C. (1964). Some Techniques for the Preparation, Study, and Storage in Microvials of Insect Genitalia. *Annals of the Entomological Society of America, 57*(2), 240-242.

Halaj, J. & Wise, D. H. (2001). Terrestrial trophic cascades: how much do they trickle? *The American Naturalist, 157*(3), 262-281.

Hanks, L., Millar, J. & Paine, T. (1996). Mating behavior of the eucalyptus longhorned borer (Coleoptera: Cerambycidae) and the adaptive significance of long "horns". *Journal of Insect Behavior, 9*(3), 383-393.

Hardy, I. C. W. (2002). *Sex Ratios: Concepts and Research Methods*. Cambridge, UK: Cambridge University Press.

Hartley, S. & Lester, P. J. (2003). Temperature-dependent development of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae): a degree-day model with implications for range limits in New Zealand. *New Zealand Entomologist, 26*, 91-100.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321.

Hebert, P. D. N., Ratnasingham, S. & de Waard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(Suppl 1), S96-S99.

Heisswolf, A., Gabler, D., Obermaier, E. & Müller, C. (2007). Olfactory *versus* contact cues in host plant recognition of a monophagous chrysomelid beetle. *Journal of Insect Behavior, 20*(2), 247-266.

Hern, A. & Dorn, S. (2004). A female-specific attractant for the codling moth, *Cydia pomonella* from apple fruit volatiles. *Naturwissenschaften, 91*(2), 77-80.

Herrera, A. M., Dahlsten, D. D., Tomic-Carruthers, N. & Carruthers, R. I. (2005). Estimating temperature-dependent developmental rates of *Diorhabda elongata* (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (*Tamarix* spp.). *Environmental Entomology, 34*, 775-784.

Hibbard, B. E. & Bjostad, L. B. (1988). Behavioral responses of Western corn rootworm larvae to volatile semiochemicals from corn seedlings. *Journal of Chemical Ecology, 14*(6), 1523-1539.

Higley, L. G., Pedigo, L. P. & Ostlie, K. R. (1986). DEGDAY: A program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology, 15*, 999-1016.

Honek, A. (1996). Geographical variation in thermal requirements for insect development. *European Journal of Entomology, 93*, 303-312.

Honek, A., Jarosik, V. & Martinkova, Z. (2003). Effect of temperature on development and reproduction in *Gastrophysa viridula* (Coleoptera: Chrysomelidae). *European Journal of Entomology, 100*, 295-300.

Honek, A. & Kocourek, F. (1990). Temperature and development time in insects: a general relationship between thermal constants. *Zoologische Jahrbuecher Systematik 117*, 401-439.

Hood-Nowotny, R. & Knols, B. G. J. (2007). Stable isotope methods in biological and ecological studies of arthropods. *Entomologia Experimentalis et Applicata, 124*(1), 3-16.

Hopkin, S. P. & Read, H. J. (1992). *The biology of millipedes / Stephen P. Hopkin and Helen J. Read*: Oxford [England] ; New York : Oxford University Press, 1992.

Hori, M., Ohuchi, K. & Matsuda, K. (2006). Role of host plant volatile in the host-finding behavior of the strawberry leaf beetle, *Galerucella vittaticollis* Baly (Coleoptera: Chrysomelidae). *Applied Entomology and Zoology, 41*(2), 357-363.

Howard, R. W. & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology, 50*(1), 371-393.

Howe, R. W. (1967). Temperature effects on embryonic development in insects. *Annual Review of Entomology, 12*, 15-42.

Hoy, C. W., Vaughn, T. T. & East, D. A. (2000). Increasing the effectiveness of spring trap crops for *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata, 96*(3), 193-204.

Hudson, G. V. (1923). An index of New Zealand beetles. *Transactions and Proceedings of the Royal Society of New Zealand, 54*, 353-399.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England), 17*(8), 754-755.

Huffaker, C. (1974). Some ecological roots of pest control. *BioControl, 19*(4), 371-389.

Huntley, R. H. (1867). *Remarks on some of the coleopterous insects which injure fruit and other trees in the neighbourhood of Wellington.* Paper presented at the Wellington Philosophical Society, Wellington, New Zealand.

Hurst, M. R. H., Rogers, D. J., Wright, D. A., Townsend, R. J., Bruening, R., Cole, L. M., et al. (2011). Effect of the bacterium *Yersinia entomophaga* on adult bronze beetle. *New Zealand Plant Protection, 64*, 209-214.

Hutcheson, J. (1992). Observations on the effects of volcanic activity on insects and their habitat on White Island. *New Zealand Entomologist, 15*, 72-76.

Hutton, F. W. (Ed.). (1904). *Index Faunæ Novæ Zealandiæ*. London: Published for the Philosophical Institute of Canterbury, New Zealand.

Ibeas, F., Díez, J. & Pajares, J. (2008). Olfactory sex attraction and mating behaviour in the pine sawyer *Monochamus galloprovincialis* (Coleoptera: Cerambycidae). *Journal of Insect Behavior, 21*(3), 101-110.

Isaia, M., Bona, F. & Badino, G. (2006). Influence of landscape diversity and agricultural practices on spider assemblage in Italian vineyards of Langa Astigiana (Northwest Italy). *Environmental Entomology, 35*(2), 297-307.

Jackson, D. M., Sorensen, K. A., Sorenson, C. E. & Story, R. N. (2005). Monitoring cucumber beetles in sweetpotato and cucurbits with kairomone-baited traps. *Journal of Economic Entomology, 98*(1), 159-170.

Jackson, J. J. & Elliott, N. C. (1988). Temperature-dependent development of immature stages of the Western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Environmental Entomology, 17*, 166-171.

Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics, 21*(1), 243-273.

Jarosik, V., Honek, A. & Dixon, A. F. (2002). Developmental rate isomorphy in insects and mites. *The American Naturalist, 160*(4), 497-510.

Jarosik, V., Kratochvíl, L., Honék, A. & Dixon, A. F. G. (2004). A general rule for the dependence of developmental rate on temperature in ectothermic animals. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(Suppl 4), S219-S221.

Jermy, T. (1984). Evolution of insect / host plant relationships. *The American Naturalist, 124*(5), 609-630.
Jermy, T. & Butt, B. A. (1991). Method for screening female sex pheromone extracts of the Colorado potato beetle. *Entomologia Experimentalis et Applicata, 59*, 75-78.
Jermy, T., Szentesi, Á. & Horváth, J. (1988). Host plant finding in phytophagous insects: the case of the Colorado potato beetle. *Entomologia Experimentalis et Applicata, 49*(1), 83-98.
Johansson, B. G. & Jones, T. M. (2007). The role of chemical communication in mate choice. *Biological Reviews, 82*(2), 265-289.
Jolivet, P., Cox, M. L. & Petitpierre, E. (1994). *Novel aspects of the biology of chrysomelidae / edited by P.H. Jolivet, M.L. Cox, and E. Petitpierre*: Dordrecht ; Boston : Kluwer Academic Publishers, c1994.
Jolivet, P., Santiago-Blay, J. A. & Schmitt, M. (Eds.). (2004). *New developments in the biology of Chrysomelidae*. The Hague, The Netherlands: SPB Academic Publishing bv.
Jolivet, P. & Verma, K. K. (2008). Eumolpinae a widely distributed and much diversified subfamily of leaf beetles (Coleoptera, Chrysomelidae). *Terrestrial Arthropod Reviews, 1*(1), 3-37.
Jönsson, M. (2005). *Responses to oilseed rape and cotton volatiles in insect herbivores and parasitoids.* Swedish University of Agricultural Sciences.
Jurenka, R. (2004). Insect pheromone biosynthesis. *Topics in Current Chemistry, 239*, 97-132.
Kalberer, N. M., Turlings, T. C. J. & Rahier, M. (2001). Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *Journal of Chemical Ecology, 27*(4), 647-661.
Kawakami, Y. & Tatsuta, H. (2010). Variation in the shape of genital appendages along a transect through sympatric and allopatric areas of two brachypterous grasshoppers, *Parapodisma setouchiensis* and *Parapodisma subastris* (Orthoptera: Podisminae). *Annals of the Entomological Society of America, 103*(3), 327-331.
Kawazu, K., Ichiki, R. T., Dang, D. T. & Nakamura, S. (2011). Mating sequence and evidence for the existence of a female contact sex pheromone in *Brontispa longissima* (Coleoptera: Chrysomelidae). *Japan Agricultural Research Quarterly, 45*(1), 99-106.
Kay, M. K. (1980). *Bronze beetle*. Rotorua: New Zealand Forest Service, Rotorua, New Zealand.
Kehat, M. & Wyndham, M. (1973). The relation between food, age and flight in the Rutherglen bug, *Nysiusm vinitor* (Hemiptera : Lygaeidae). *Australian Journal of Zoology, 21*(3), 427-434.
Kochansky, J., Tette, J., Taschenberg, E. F., Cardé, R. T., Kaissling, K. E. & Roelofs, W. L. (1975). Sex pheromone of the moth, *Antheraea polyphemus*. *Journal of Insect Physiology, 21*(12), 1977-1983.
Kohyama, T. I., Matsumoto, K. & Katakura, H. (2012). Geographic variation of host use in the leaf beetle *Agelasa nigriceps* suggests host range expansion. *Entomologia Experimentalis et Applicata, 142*(2), 165-174.
Koji, S. & Nakamura, K. (2006). Seasonal fluctuation, age structure, and annual changes in a population of *Cassida rubiginosa* (Coleoptera: Chrysomelidae) in a natural habitat. *Annals of the Entomological Society of America, 99*(2), 292-299.

Kubisz, D., Kajtoch, Ł., Mazur, M. & Rizun, V. (2012). Molecular barcoding for central-eastern European *Crioceris* leaf-beetles (Coleoptera: Chrysomelidae). *Central European Journal of Biology, 7*(1), 69-76.

Kuschel, G. (1990). *Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989)*. Auckland: DSIR Plant Protection Report 3.

Kvarnemo, C. & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution, 11*(10), 404-408.

Kvarnemo, C. & Ahnesjo, I. (2002). Operational sex ratios and mating competition. In I. C. W. Hardy (Ed.), *Sex Ratios: Concepts and Research Methods* (pp. 424): Cambridge University Press.

Landolt, P. J., Tumlinson, J. H. & Alborn, D. H. (1999). Attraction of Colorado potato beetle (Coleoptera: Chrysomelidae) to damaged and chemically induced potato plants. *Environmental Entomology, 28*, 973-978.

Lapointe, S. L. & Shapiro, J. P. (1999). Effect of soil moisture on development of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *The Florida Entomologist, 82*(2), 291-299.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., et al. (2006). Soil invertebrates and ecosystem services. *European Journal of Soil Biology, 42, Supplement 1*(0), S3-S15.

Lea, A. M. (1915). Notes on Australian Eumolpides (Coleoptera: Chrysomeldiae), wtih descriptions of new species. *Transactions of the Royal Soceity of South Australia, 39*, 102-339.

Lea, A. M. (1916). Notes on some miscellaneous Coleoptera, with descriptions of new species. Part II. *Transactions of the Royal Soceity of South Australia, 40*, 272-436.

Leschen, R. A. B., Lawrence, J. F., Kuschel, G., Thorpe, S. & Wang, Q. (2003). Coleoptera genera of New Zealand. *New Zealand Entomologist, 26*, 15-28.

Leschen, R. A. B. & Michaux, B. (2005). Biogeography and Evolution of New Zealand Priasilphidae. *New Zealand Entomologist, 28*, 55-64.

Leschen, R. A. B. & Reid, C. A. M. (2004). New Zealand Chrysomelidae. *Chrysomela News Letter, 43*, 7-8.

Leskey, T. C. & Prokopy, R. J. (2000). Sources of apple odor attractive to adult plum curculios. *Journal of Chemical Ecology, 26*(3), 639-653.

Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 25*(11), 1451-1452.

Light, D., Knight, A., Henrick, C., Rajapaska, D., Lingren, B., Dickens, J., et al. (2001). A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). *Naturwissenschaften, 88*(8), 333-338.

Logan, J. A. & Powell, J. A. (2003). *Modelling mountain pine beetle phenological response to temperature.* Paper presented at the Mountain Pine Beetle Symposium: Challenges and Solutions, Kelowna, British Colombia, Canada.

Logarzo, G., Casalinuovo, M., Piccinali, R., Braun, K. & Hasson, E. (2011). Geographic host use variability and host range evolutionary dynamics in the phytophagous insect *Apagomerella versicolor* (Cerambycidae). *Oecologia, 165*(2), 387-402.

Lopatin, I. K. (1984). Leaf Beetles (Chrysomelidae) of Central Asia and Kazakhstan (pp. 416). New Delhi: Oxonian Press.

Lu, W., Wang, Q., Tian, M. Y., He, X. Z., Zeng, X. L. & Zhong, Y. X. (2007). Mate location and recognition in *Glenea cantor* (Fabr.) (Coleoptera: Cerambycidae: Lamiinae): roles of host plant health, female sex pheromone, and vision. *Environmental Entomology, 36*(4), 864-870.

Lysaght, A. M. (1930). Bronze beetle research. *New Zealand Department of Scientific and Industrial Research, Wellington, New Zealand., Bulletin 25*, 32 pp.

Ma, L.-q., Gao, S.-j., Wen, J.-b., Zong, S.-x. & Xu, Z.-c. (2008). Effective accumulated temperature and developmental threshold temperature for *Semanotus bifasciatus* (Motschulsky) in Beijing. *Forestry Studies in China, 10*(2), 125-129.

Maher, B. J. & Logan, D. P. (2007). European earwigs, *Forficula auricularia*, and predation of scale insects in organic and conventionally managed kiwifruit. *New Zealand Plant Protection, 60*, 249-253.

Maloney, D., Drummond, F. A. & Alford, R. (2003). *Spider Predation in Agroecosystems: Can Spiders Effectively Control Pest Populations?* : Maine Agricultural and Forest Experiment Station.

Mannering, R. (2006). Beetle threatens organic pipfruit viability. *The Orchadist, December 2006*, 20-21.

Mansour, F. & Whitecomb, W. (1986). The spiders of a citrus grove in Israel and their role as biocontrol agents of *Ceroplastes floridensis* [Homoptera: Coccidae]. *BioControl, 31*(3), 269-276.

Marc, P. & Canard, A. (1997). Maintaining spider biodiversity in agroecosystems as a tool in pest control. *Agriculture, Ecosystems & Environment, 62*(2-3), 229-235.

Martel, J. W., Alford, A. R. & Dickens, J. C. (2005). Synthetic host volatiles increase efficacy of trap cropping for management of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *Agricultural and Forest Entomology, 7*(1), 79-86.

Martin, N. A. (2010, 29/04/2010). Pohutakawa leaf miner - *Neomycta rubida*. *New Zealand Arthropod Collection Factsheet Series* Retrieved 08/02/2012, from http://nzacfactsheets.landcareresearch.co.nz/factsheet/OrganismProfile/Pohutukawa_leaf_miner_-_Neomycta_rubida.html

Masters, B. C., Fan, V. & Ross, H. A. (2011). Species delimitation – a Geneious plugin for the exploration of species boundaries. *Molecular Ecology Resources, 11*(1), 154-157.

Mathews, C. R., Bottrell, D. G. & Brown, M. W. (2004). Habitat manipulation of the apple orchard floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Biological Control, 30*, 265-273.

McGlone, M. (2011). Ecoregions - Te Ara Encyclopedia of New Zealand, updated 25-Sep-11. Retrieved 5/01/2012, from http://www.teara.govt.nz/en/ecoregions/1/1

McKenna, D. D. (2006). *Ecological and evolutionary radiation of Cephaloleia beetles (Coleoptera: Chrysomelidae).* Harvard University.

Meier, R., Zhang, G. & Ali, F. (2008). The use of mean instead of smallest interspecific distances exaggerates the size of the "Barcoding Gap" and leads to misidentification. *Systematic Biology, 57*(5), 809-813.

Merfield, C. N., Wratten, S. D. & Navntoft, S. (2004). Video analysis of predation by polyphagous invertebrate predators in the laboratory and field. *Biological Control, 29*(1), 5-13.

Miles, M. (2010). Rutherglen bug, grey cluster bug. Retrieved 21/06/2012, from http://www.daff.qld.gov.au/26_5084.htm

Miller, D. (1926). The Bronze beetle, its habits and control as an orchard pest. *New Zealand Journal of Agriculture, 32*(1), 9-14.
Miller, D. (1931). Report on the control experiments using chemical spray. *New Zealand Journal of Science and Technology, 13*, 34-36.
Miller, D. (1971). *Common insects in New Zealand*. Wellington: AH & AW Reed Ltd., Wellington, New Zealand.
Miller, D. R. & Lafontaine, J. P. (1991). cis-Verbenol: An aggregation pheromone for the mountain pine beetle, *Dendroctonics ponderosae* Hopkins (Coloeoptera: Scolytidae). *Journal of the Entomological Society of British Columbia, 88*, 34-38.
Miñarro, M., Espadaler, X., Melero, V. X. & Suárez-Álvarez, V. (2009). Organic versus conventional management in an apple orchard: effects of fertilization and tree-row management on ground-dwelling predaceous arthropods. *Agricultural and Forest Entomology, 11*(2), 133-142.
Minor, M. A. & Robertson, A. W. (2006). Soil Bugs - An Illustrated Guide to New Zealand Soil Invertebrates (updated 12-Jan-2011). Retrieved 06-10-2011, from http://soilbugs.massey.ac.nz
Mitchell, A. & Maddox, C. (2010). Bark beetles (Coleoptera: Curculionidae: Scolytinae) of importance to the Australian macadamia industry: an integrative taxonomic approach to species diagnostics. *Australian Journal of Entomology, 49*(2), 104-113.
Mitchell, B. K. (1988). Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. *Journal of Insect Physiology, 34*(3), 213-225.
Monzó, C., Sabater-Muñoz, B., Urbaneja, A. & Castañera, P. (2011). The ground beetle *Pseudophonus rufipes* revealed as predator of *Ceratitis capitata* in citrus orchards. *Biological Control, 56*(1), 17-21.
Mostafa, A. M., Lowery, D. T., Jensen, L. B. M. & Deglow, E. K. (2011). Host plant suitability and feeding preferences of the grapevine pest *Abagrotis orbis* (Lepidoptera: Noctuidae). *Environmental Entomology, 40*(6), 1458-1464.
Mpho, M. & Seabrook, W. D. (2003). Functions of antennae and palpi in the mating behaviour of the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Bulletin of Entomological Research, 93*(01), 91-95.
Mutis, A., Parra, L., Palma, R., Pardo, F., Perich, F. & Quiroz, A. (2009). Evidence of contact pheromone use in mating behavior of the raspberry weevil (Coleoptera: Curculionidae). *Environmental Entomology, 38*(1), 192-197.
Nahrung, H. & Allen, G. (2004). Sexual selection under scramble competition: mate location and mate choice in the eucalyptus leaf beetle *Chrysophtharta agricola* (Chapuis) in the field. *Journal of Insect Behavior, 17*(3), 353-366.
National Research Council. (1996). *Ecologically based pest management: new solutions for a new century*: National Academy Press.
Naves, P. & de Sousa, E. (2009). Threshold temperatures and degree-day estimates for development of post-dormancy larvae of *Monochamus galloprovincialis* (Coleoptera: Cerambycidae). *Journal of Pest Science, 82*(1), 1-6.
New Zealand Avocado Growers' Association & Industry Council Ltd. (2011). Avocado: Avogreen Insect Biology. Retrieved 08/02/2012, from http://www.nzavocado.co.nz/index.php/pi_pageid/86
Nietschke, B. S., Magarey, R. D., Borchert, D. M., Calvin, D. D. & Jones, E. (2007). A developmental database to support insect phenology models. *Crop Protection, 26*(9), 1444-1448.

NIWA. (2011a). Climate change scenarios for New Zealand. Retrieved 08/02/2012, from http://www.niwa.co.nz/our-science/climate/information-and-resources/clivar/scenarios?SQ_BACKEND_PAGE=main&safe_edit=yes&page=30638#downscaling

NIWA. (2011b). Mean 10cm Earth Temperature (^{0}C). Retrieved 01-05-2011, from http://www.niwa.co.nz/education-and-training/schools/resources/climate/earthtemp

Norris, R. F. & Kogan, M. (2000). Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Science, 48*(1), 94-158.

NZPCN. (2012). New Zealand Plant Conservation Network. Retrieved 29/06/2012, from http://www.nzpcn.org.nz/flora_search.asp?scfSubmit=1

Obata, S. & Hidaka, T. (1982). Experimental analysis of mating behavior in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). *Applied Entomology and Zoology, 17*(1), 60-66.

Omkar, Rastogi, S. & Pandey, P. (2008). Effect of temperature on development and immature survival of *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) under laboratory conditions. *International Journal of Tropical Insect Science, 28*(03), 130-135.

OrganicDirect NZ Ltd. (2011). OANZ News 11-28th May 2010. Retrieved 08/02/2012, from http://www.organicdirect.co.nz/organics-resource-details.php?name=OANZ%20News%2011%20-%A0%2028th%20May%202010%20&resourceID=387&page=15

Organics Aotearoa New Zealand. (2011). New Zealand Organic Report 2010.

Otto, D. (1997). Some properties of the female sex pheromone of the Colorado potato beetle *Leptinotarsa decemlineata* (Col. Chrysomelidae). *Anzeiger für Schädlingskunde, 70*(2), 30-33.

Park, I.-K., Lee, S.-G., Shin, S.-C., Kim, C.-S. & Ahn, Y.-J. (2004). Feeding and attraction of *Agelastica coerulea* (Coleoptera: Chrysomelidae) to Betulaceae plants. *Journal of Economic Entomology, 97*, 1978-1982.

Peschke, K. & Metzler, M. (1987). Cuticular hydrocarbons and female sex pheromones of the rove beetle, *Aleochara curtula* (Goeze) (Coleoptera:Staphylinidae). *Insect Biochemistry, 17*(1), 167-178.

Petersen, H. & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos, 39*(3), 288-388.

Peterson, M. A., Dobler, S., Larson, E. L., Juarez, D., Schlarbaum, T., Monsen, K. J., et al. (2007). Profiles of cuticular hydrocarbons mediate male mate choice and sexual isolation between hybridising *Chrysochus* (Coleoptera : Chrysomelidae). *Chemoecology, 17*(2), 87-96.

Petersson, E. & Solem, O. J. (1987). *Male mate recognition in Leptoceridae.* Paper presented at the 5th International Symposium on Trichoptera, The Hague.

Phillips, T. W., Phillips, J. K., Webster, F. X., Tang, R. & Burkholder, W. E. (1996). Identification of sex pheromones from cowpea weevil, *Callosobruchus maculatus*, and related studies with *C. analis* (coleoptera: Bruchidae). *Journal of Chemical Ecology, 22*(12), 2233-2249.

Pickett, J. A., Bruce, T. J. A., Chamberlain, K., Hassanali, A. & Khan., Z. R. (2006). Plant volatiles yielding new ways to exploit plant defence. In M. Dicke & W. Takken (Eds.), *Chemical Ecology: From Gene to Ecosystem* (pp. 161-174). Dordrecht, The Netherlands: Springer.

Piñero, J. C. & Dorn, S. (2007). Synergism between aromatic compounds and green leaf volatiles derived from the host plant underlies female attraction in the oriental fruit moth. *Entomologia Experimentalis et Applicata, 125*(2), 185-194.

Pipfruit NZ. (2011). Apple Futures. Retrieved 25/11/2011, from http://www.pipfruitnz.co.nz/Library/Apple_Futures.aspx

Pipfruit NZ. (2012). MetWatch daily climate info. http://www.pipfruitnz.co.nz/Tools/Weather_History/MetWatch_Daily_Climate_Info.aspx

Pivnick, K. A., Lamb, R. J. & Reed, D. (1992). Response of flea beetles, *Phyllotreta* spp., to mustard oils and nitriles in field trapping experiments. *Journal of Chemical Ecology, 18*(6), 863-873.

Plant and Food Research Institute of New Zealand Ltd. (2008). *Fresh Facts*.

Popay, I., Champion, P. D. & James, T. (2010). *An illustrated guide to common weeds of New Zealand / Ian Popay, Paul Champion, Trevor James* (3rd ed.). Christchurch, N.Z.: New Zealand Plant Protection Society.

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution, 25*(7), 1253-1256.

Prokopy, R. J., Moericke, V. & Bush, G. L. (1973). Attraction of apple maggot flies to odor of apples. *Environmental Entomology, 2*, 743-750.

Puttick, G. M., Morrow, P. A. & Lequesne, P. W. (1988). *Trirhabda canadensis* (Coleoptera: Chrysomelidae) responses to plant odors. *Journal of Chemical Ecology, 14*(8), 1671-1686.

Reed, H. C. & Landolt, P. J. (2002). Attraction of mated female codling moths (Lepidoptera: Tortricidae) to apples and apple odor in a flight tunnel. *Florida Entomologist, 85*(2), 324-329.

Regniere, J. & Logan, J. A. (2003). Animal life cycle models. In M. D. Schwartz (Ed.), *Phenology: An Integrated Environmental Science* (pp. 237-254): Kluwer Academic Publishers.

Reid, C. A. M. (1995). A cladistic analysis of subfamilial relationships in the Chrysomelidae sensu lato (Chrysomeloidea), *Biology, Phylogeny and classification of Coleoptera: papers celebrating the 80th birthday of Roy A. Crowson* (pp. 559-631). Warszawa: Muzeum i Instytut Zoologii PAN.

Robertson, J. G. (1966). The chromosomes of bisexual and parthenogenetic species of *Calligrapha* (Coleoptera: Chrysomelidae) with notes on sex ratio, abundance and egg number. *Canadian Journal of Genetics and Cytology, 8*(4), 695-732.

Rodriguez-Saona, C. R. & Stelinski, L. L. (2009). Behavior-Modifying Strategies in IPM: Theory and Practice. In R. Peshin & A. K. Dhawan (Eds.), *Integrated Pest Management: Innovation-Development Process* (pp. 263-315): Springer Netherlands.

Rogers, D. J., Cole, L. M., Delate, K. M. & Walker, J. T. S. (2006). Managing bronze beetle, *Eucolaspis brunnea*, in organic apple orchards. *New Zealand Plant Protection, 59*, 57-62.

Rogers, D. J., Cole, L. M., Fraser, T. M. & Walker, J. T. S. (2009). Managing bronze beetle (*Eucolaspis* sp.) in Hawke's Bay organic apple orchards using soil cultivation. *New Zealand Plant Protection, 62*, 285-290.

Rogers, D. J., Cole, L. M., Fraser, T. M., Walker, J. T. S. & Brownbridge, M. (2008). *Management of bronze beetle in organic production - 2008*: The Horticulture and Food Research Institute of New Zealand.

Rogers, D. J., Cole, L. M., Fraser, T. M. a. & Walker, J. T. S. (2007). Potential strategies to manage bronze beetle (*Eucolaspis* sp.) in organic apple orchards. *New Zealand Plant Protection, 60*, 1-6.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*(12), 1572-1574.

Royal New Zealand Institute of Horticulture. (2011). Bug detection. Retrieved 17/08/2011, from http://www.rnzih.org.nz/pages/Bug_Detection.htm

Rubinoff, D., Cameron, S. & Will, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *Journal of Heredity, 97*(6), 581-594.

Ruiz, E. A., Víctor, J., Hayes, J. L. & Zújñiga, G. (2009). Molecular and morphological analysis of *Dendroctonus pseudotsugae* (Coleoptera: Curculionidae: Scolytinae) : an assessment of the taxonomic status of subspecies. *Annals of the Entomological Society of America, 102*(6), 982-997.

Samu, F., Sunderland, K. D. & Szinetár, C. (1999). Scale-dependent dispersal and distribution patterns of spiders in agricultural aystems: A review. *Journal of Arachnology, 27*(1), 325-332.

Samu, F. & Szinetar, C. (2002). *On the nature of agrobiont spiders*.

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R. H. (2010). Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology, 55*(1), 421-438.

Schmidt, M. H., Roschewitz, I., Thies, C. & Tscharntke, T. (2005). Differential effects of landscape and management on diversity and density of ground-dwelling farmland spiders. *Journal of Applied Ecology, 42*(2), 281-287.

Schmitz, O. J., Hamback, P. A. & Beckerman, A. P. (2000). Trophic cascades in terrestrial systems: A review of the effects of carnivore removals on plants. *The American Naturalist, 155*(2), 141-153.

Schoonhoven, L. M., van Loon, J. J. A. & Dicke, M. (2005). *Insect-Plant biology* (2 ed.). New York, USA: Oxford University Press Inc.

Schwartz, M. D. (Ed.). (2003). *Phenology: An Integrative Environmental Science*: Kluwer Academic Publishers, Dordrecht, The Netherlands.

Seeno, T. N. & Wilcox, J. A. (1982). Leaf beetle genera, (Coleoptera: Chrysomelidae) (Vol. 1, pp. 221).

Sharp, D. (1886). On New Zealand Coleoptera with descriptions of new genera and species *The Scientific Transactions of the Royal Dublin Society* (Vol. 3, pp. 445-446). Dublin: The University Press, The Royal Dublin Society.

Sharpe, P. J. H. & DeMichele, D. W. (1977). Reaction kinetics of poikilotherm development. *Journal of Theoretical Biology, 64*(4), 649-670.

Shaw, S. (1957). A revision of the New Zealand genera *Eucolaspis* Sharp and *Atrichatus* Sharp (Coleoptera: Chrysomelidae) with descriptions of two new species. *Annals and magazine of natural history, 12*, 641-655.

Shennan, C. (2008). Biotic interactions, ecological knowledge and agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1492), 717-739.

Shimada, M. (1990). Preadaptation of a wild bean weevil, *Kytorhinus sharpianus* Bridwell (Coleoptera, Bruchidae), for infesting the storage green gram, *Vigna radiata* (L) Wilczek (Leguminosae) *Applied Entomology and Zoology, 25*(1), 132-134.

Silk, P. J., Sweeney, J., Wu, J., Sopow, S., Mayo, P. D. & Magee, D. (2011). Contact sex pheromones identified for two species of longhorned beetles (Coleoptera:

Cerambycidae) *Tetropium fuscum* and *T. cinnamopterum* in the subfamily Spondylidinae. *Environmental Entomology, 40*(3), 714-726.

Smadja, C. & Butlin, R. K. (2008). On the scent of speciation: the chemosensory system and its role in premating isolation. *Heredity, 102*(1), 77-97.

Smith, J., Potts, S. G., Woodcock, B. A. & Eggleton, P. (2008). Can arable field margins be managed to enhance their biodiversity, conservation and functional value for soil macrofauna? *Journal of Applied Ecology, 45*(1), 269-278.

Soetens, P., Rowell-Rahier, M. & Pasteels, J. M. (1991). Influence of phenolglucosides and trichome density on the distribution of insects herbivores on willows. *Entomologia Experimentalis et Applicata, 59*(2), 175-187.

Speight, M. R., Hunter, M. D. & Watt, A. D. (1999). *Ecology of Insects: Concepts and Applications*: Blacwell Science Ltd.

Stenberg, J. A. & Ericson, L. (2007). Visual cues override olfactory cues in the host-finding process of the monophagous leaf beetle *Altica engstroemi*. *Entomologia Experimentalis et Applicata, 125*(1), 81-88.

Stevenson, D. E., Michels, G. J., Bible, J. B., Jackman, J. A. & Harris, M. K. (2008). Physiological time model for predicting adult emergence of Western corn rootworm (Coleoptera: Chrysomelidae) in the Texas high plains. *Journal of Economic Entomology, 101*(5), 1584-1593.

Stouthamer, R., Hurst, G. & Breeuwer, J. (2002). Sex ratio distorters and their detection, *Sex ratios B2 - Sex ratios* (pp. 185-215). Cambridge, UK: Cambridge University Press.

Stuart-Fox, D. & Moussalli, A. (2007). Sex-specific ecomorphological variation and the evolution of sexual dimorphism in dwarf chameleons (*Bradypodion* spp.). *Journal of Evolutionary Biology, 20*(3), 1073-1081.

Suckling, D. M., Burnip, G. M., Hackett, J. & Daly, J. C. (2006). Frass sampling and baiting indicate European earwig (*Forficula auricularia*) foraging in orchards. *Journal of Applied Entomology, 130*(5), 263-267.

Suckling, D. M., McLaren, G. F., Manning, L.-A. M., Mitchell, V. J., Attfield, B., Colhoun, K., et al. (2011). Development of single dispenser pheromone suppression of *Epiphyas postvittana*, *Planotortrix octo* and *Ctenopseustis obliquana* in New Zealand stonefruit orchards. *Pest Management Science*, (accepted for publication).

Suckling, D. M., Walker, J. T. S., Lo, P. L., Shaw, P. W., McLaren, G. F., Clare, G., et al. (2007). Progress with pheromones and other semiochemicals for fruit pest management in New Zealand. In M. R. Butcher, J. T. S. Walker & S. M. Zydenbos (Eds.), *Future Challenges in Crop Protection: Repositioning New Zealand's Primary Industries for the Future* (pp. 99-107): Manaki Whenua Press.

Sugeno, W., Hori, M. & Matsuda, K. (2006). Identification of the contact sex pheromone of *Gastrophysa atrocyanea* (Coleoptera: Chrysomelidae). *Applied Entomology and Zoology, 41*(2), 269-276.

Sunnucks, P. & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution, 13*(3), 510-524.

Swofford, D. L. (2003). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.04beta*: Sinauer Associates, Sunderland, Massachusetts.

Symondson, W. O. C., Sunderland, K. D. & Greenstone, M. H. (2002). Can generalist predators be effective biocontrol agents? *Annual Review of Entomology, 47*(1), 561-594.

Szendrei, Z. & Rodriguez-Saona, C. (2010). A meta-analysis of insect pest behavioral manipulation with plant volatiles. *Entomologia Experimentalis et Applicata, 134*(3), 201-210.

Szentesi, Á., Weber, D. C. & Jermy, T. (2002). Role of visual stimuli in host and mate location of the Colorado potato beetle. *Entomologia Experimentalis et Applicata, 105*(2), 141-152.

Tahvanainen, J., Julkunen-Tiitto, R. & Kettunen, J. (1985). Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. *Oecologia, 67*(1), 52-56.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. *Molecular Biology and Evolution, 28*(10), 2731-2739.

Tauber, M. J. & Tauber, C. A. (1976). Insect seasonality: Diapause maintenance, termination, and postdiapause development. *Annual Review of Entomology, 21*(1), 81-107.

Thiery, D. & Visser, J. H. (1986). Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. *Entomologia Experimentalis et Applicata, 41*(2), 165-172.

Tillman, J. A., Seybold, S. J., Jurenka, R. A. & Blomquist, G. J. (1999). Insect pheromones — an overview of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology, 29*(6), 481-514.

Tillyard, R. J. (1926). *The Insects of Australia and New Zealand*. Sydney: Angus & Robertson Ltd.

Todd, J. H., Malone, L. A., McArdle, B. H., Benge, J., Poulton, J., Thorpe, S., et al. (2011). Invertebrate community richness in New Zealand kiwifruit orchards under organic or integrated pest management. *Agriculture, Ecosystems & Environment, 141*(1-2), 32-38.

Topping, C. J. & Lovei, G. (1997). Spider density and diversity in relation to disturbance in agroecosystems in New Zealand, with a comparison to England. *New Zealand Journal of Ecology, 21*(2), 121-128.

Tosevski, I. V. O., Caldara, R., JoviĆ, J., HernÁNdez-Vera, G., Baviera, C., Gassmann, A., et al. (2011). Morphological, molecular and biological evidence reveal two cryptic species in *Mecinus janthinus* Germar (Coleoptera, Curculionidae), a successful biological control agent of Dalmatian toadflax, Linaria dalmatica (Lamiales, Plantaginaceae). *Systematic Entomology, 36*(4), 741-753.

Trewick, S. A. (2000). Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand Peripatoides (Onychophora). *Molecular Ecology, 9*(3), 269-281.

Trewick, S. A. (2008). DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). *Cladistics, 24*(2), 240-254.

Trewick, S. A., Wallis, G. P. & Morgan-Richards, M. (2011). The invertebrate life of New Zealand: A phylogeographic approach. *Insects, 2*(3), 297-325.

Trudgill, D. L., Honek, A., Li, D. & Van Straalen, N. M. (2005). Thermal time – concepts and utility. *Annals of Applied Biology, 146*(1), 1-14.

Tuda, M. (2007). Applied evolutionary ecology of insects of the subfamily Bruchinae (Coleoptera: Chrysomelidae). *Applied Entomology and Zoology, 42*(3), 337-346.

Umble, J. R. & Fisher, J. R. (2000). Temperature-dependent development of *Otiorhynchus ovatus* (Coleoptera: Curculionidae) pupae. *Environmental Entomology, 29*(4), 758-765.

Ventura, M. U., Resta, C. C. M., Nunes, D. H. & Fujimoto, F. (2005). Trap attributes influencing capture of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) on common bean fields. *Scientia Agricola, 62*, 351-356.

Visser, J. H. (1976). The design of a low-speed wind tunnel as an instrument for the study of olfactory orientation in the Colorado beetle (*Leptinotarsa decemlineata*). *Entomologia Experimentalis et Applicata, 20*(3), 275-288.

Visser, J. H. (1986). Host odor perception in phytophagous insects. *Annual Review of Entomology, 31*(1), 121-144.

Walgama, R. S. & Zalucki, M. P. (2007). Temperature-dependent development of *Xyleborus fornicatus* (Coleoptera: Scolytidae), the shot-hole borer of tea in Sri Lanka: Implications for distribution and abundance. *Insect Science, 14*, 301-308.

Walker, J. T. S. (2008). General principles of codling moth control. 3. Retrieved from http://www.hortplus2.com/mwol/model/pipfruit/codling_info.pdf

Wall, C. & Perry, J. N. (1987). Range of action of moth sex-attractant sources. *Entomologia Experimentalis et Applicata, 44*(1), 5-14.

Wang, Q. & Chen, L.-Y. (2005). Mating behavior of a flower-visiting longhorn beetle *Zorion guttigerum* (Westwood) (Coleoptera: Cerambycidae: Cerambycinae). *Naturwissenschaften, 92*(5), 237-241.

Wang, Q., Zeng, W., Chen, L., Li, J. & Yin, X. (2002). Circadian reproductive rhythms, pair-bonding, and evidence for sex-specific pheromones in *Nadezhdiella cantori* (Coleoptera: Cerambycidae). *Journal of Insect Behavior, 15*(4), 527-539.

Wang, X., Zhou, X. & Lei, C. (2009). Development, survival and reproduction of the brassica leaf beetle, *Phaedon brassicae* Baly (Coleoptera: Chrysomelidae) under different thermal conditions. *Pan-Pacific Entomologist, 83*(2), 143-151.

Wardle, D. A. (2006). The influence of biotic interactions on soil biodiversity. *Ecology Letters, 9*(7), 870-886.

Wee, S.-L., El-Sayed, A. M., Gibb, A. R., Mitchell, V. & Suckling, D. M. (2008). Behavioural and electrophysiological responses of *Pantomorus cervinus* (Boheman) (Coleoptera: Curculionidae) to host plant volatiles. *Australian Journal of Entomology, 47*(1), 24-31.

Weems Jr., H. V. (2002, January 2012). Apple maggot - *Rhagoletis pomonella* (Walsh). Retrieved 08/02/2012, from http://entomology.ifas.ufl.edu/creatures/fruit/tropical/apple_maggot_fly.htm

Weiner, J. (2003). Ecology - the science of agriculture in the 21st century. *Journal of Agricultural Science, 141*, 371-377.

Weiss, M. J., Seevers, K. P. & Mayo, Z. B. (1985). Influence of Western corn rootworm larval densities and damage on corn rootworm survival, developmental time, size and sex ratio (Coleoptera: Chrysomelidae). *Journal of the Kansas Entomological Society, 58*(3), 397-402.

Wermelinger, B. & Seifert, M. (1998). Analysis of the temperature dependent development of the spruce bark beetle *Ips typographus* (L) (Col., Scolytidae). *Journal of Applied Entomology, 122*(1-5), 185-191.

Werren, J. H. & Beukeboom, L. W. (1998). Sex determination, sex ratios, and genetic conflict. *Annual Review of Ecology and Systematics, 29*(ArticleType: research-article / Full publication date: 1998 / Copyright © 1998 Annual Reviews), 233-261.

West, S. A., Reece, S. E. & Sheldon, B. C. (2002). Sex ratios. *Heredity, 88*(2), 117-124.

White, A. (1846). Insects. In J. Richardson & J. E. Gray (Eds.), *The Zoology of the Voyage of H.M.S. Erebus &Terror* (Vol. 2, pp. 23). London.

Will, K. W., Mishler, B. D. & Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology, 54*(5), 844-851.

Wilson, L. & Barnett, W. (1983). Degree-days: An aid in crop and pest management. *California Agriculture, 37*(1), 4-7.

Winks, C. J., Fowler, S. V. & Smith, L. A. (2004). Invertebrate fauna of boneseed, *Chrysanthemoides monilifera* ssp. *monilifera* (L.) T. Norl. (Asteraceae: Calenduleae), an invasive weed in New Zealand. *New Zealand Entomologist, 27*, 61-72.

Witzgall, P., Kirsch, P. & Cork, A. (2010). Sex pheromones and their impact on pest management. *Journal of Chemical Ecology, 36*(1), 80-100.

Woodfin, J. C. (1927). Control of vine diseases and pests occurring in New Zealand. *New Zealand Journal of Agriculture, 35*, 298-309.

Wyman, T. E., Trewick, S. A., Morgan-Richards, M. & Noble, A. D. L. (2010). Mutualism or opportunism? tree fuchsia (*Fuchsia excorticata*) and tree weta (*Hemideina*) interactions. *Austral Ecology*, 1-8.

Yang, E. C., Lee, D. W. & Wu, W. Y. (2003). Action spectra of phototactic responses of the flea beetle, *Phyllotreta striolata*. *Physiological Entomology, 28*(4), 362-368.

Zehnder, G., Gurr, G. M., Kühne, S., Wade, M. R., Wratten, S. D. & Wyss, E. (2007). Arthropod pest management in organic crops. *Annual Review of Entomology, 52*(1), 57-80.

Zehnder, G. & Speese, J. (1987). Assessment of color response and flight activity of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) using window flight traps. *Environmental Entomology, 16*, 1199-1202.

Zhang, A., Linn, C., Wright, S., Prokopy, R., Reissig, W. & Roelofs, W. (1999). Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. *Journal of Chemical Ecology, 25*(6), 1221-1232.

Zhang, A., Oliver, J., Chauhan, K., Zhao, B., Xia, L. & Xu, Z. (2003). Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis*; (Coleoptera: Cerambycidae). *Naturwissenschaften, 90*(9), 410-413.

Zong, S., Luo, Y., Wang, T., Xu, Z. & Lu, C. (2004). Developmental threshold temperature and effective accumulative temperature of pupae and eggs of *Holcocerus hippophaecolus*. *Forestry Studies in China, 6*(4), 34-38.

Zvereva, E., Kozlov, M. & Hilker, M. (2010). Evolutionary variations on a theme: host plant specialization in five geographical populations of the leaf beetle *Chrysomela lapponica*. *Population Ecology, 52*(3), 389-396.

Appendices

Appendix 1. Entomopathogenic nematodes in soils of organic apple orchards in Hawke's Bay, New Zealand

Introduction

Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) are usually soil-borne and found in many ecologically diverse soil habitats (Gaugler et al., 1997; Gaugler, 2010). These nematodes parasitise many insect pests and can offer an effective control measure against bronze beetle as the larvae and pupae of this beetle live in the soil. In order to test for the presence of any naturally occurring entomopathogenic nematodes in the orchard soil, a pilot study was conducted.

Materials and methods

Live insect bait (wax moth, *Galleria mellonella* larva) was used for detecting nematodes (Bedding & Akhurst, 1975; Barker and Barker, 1998). In this study, 4 organic orchards of Royal Gala apples in Hawke's Bay were surveyed for nematodes. Five soil samples were collected from each of these orchards during October and November 2007, using a soil corer (5 cm diameter X 10 cm deep). Each soil core was cut into two equal halves (top 5 cm and bottom 5 cm), stored separately in labelled polythene bags and transported to the lab. The soil was hand crumbled before use and any grass / weeds were removed. Soil from all 40 subsamples was placed into individual plastic specimen containers up to about $3/4^{th}$ full. Five live wax moth larvae were released into each container onto the soil surface. Then the containers were carefully inverted, leaving the larvae buried in soil and with the clear base of the container uppermost. The containers with larvae were kept at 25^0C with 24h light for a week, after which the wax moth larvae were recovered. The larvae that were alive were reused to run the test with the same soil and container. The dead larvae were observed for any indices of nematode infestation like colour and shape changes and any smell (N.L. Bell personal communication; Gaugler, 2010).

Results and Discussion

Entomopathogenic nematodes were not found in any of the surveyed orchards (Table 1). All wax moth larvae used for bait were either still alive after two consecutive runs after which they were discarded, or dead due to fungal / bacterial infections. This

seems to indicate that there were no resident populations of entomopathogenic nematodes in any of the surveyed orchards. Some nematodes were found attacking bronze beetle larvae / pupae that were collected in field and stored for a different study. These nematodes were isolated and identified as *Diplogaster* spp. These free living saprophages are facultative parasites of insects and generally are not considered as entomopathogenic (Barker & Barker, 1998).

Table1. Soil entomopathogenic nematodes in four organic apple orchards (H1, H2, L1, L2) in Hawke's Bay, New Zealand, presence tested using live insect bait.

Orchard	Number of samples tested	Number of samples tested positive for nematodes
H1	10	0
H2	10	0
L1	10	0
L2	10	0

Soil moisture can be crucial for successful establishment of nematodes and their detection through insect baiting (Grant & Villani, 2003), as the nematodes need a free-flowing film of water for movement. In the current study soil samples were collected during late spring when the soil was considerably moist, thus I believe lack of enough soil moisture was not a limitation here.

Absence of entomopathogenic nematodes in certain soils is not uncommon. For instance, Stock (1995) found no nematodes in any of the clay loam soil samples tested whereas, Bell et al (2005) did not detect any stenernematid or heterorhabditid nematodes in soils of indigenous tussock grasslands. However, it is possible that the sample volume in our study is too low to detect any entomopathogenic nematodes if they were very sparse and patchy. The huge population sizes of bronze beetles in some orchards (up to 1697 individuals / m2) (Doddala et al., 2010) suggest that the larvae and pupae of the beetle in the soil are not controlled by nematodes.

Entomopathogenic nematodes are used successfully to control many soil insect pests in orchards, for instance, in Florida, USA, 60000 acres of citrus are treated annually with *Steinernema riobravis* to control citrus root weevil (Gaugler, 2010). A

research in future in New Zealand towards evaluating entomopathogenic nematodes to control bronze beetle would be very useful in organic control perspective.

References

Barker C W and Barker G M 1998. General entomopathogens as biological indicators of deforestation and agricultural land use impacts on Waikato soils. *New Zealand Journal of Ecology*, 22(2), 189-196.

Bedding, R. A. & Akhurst, R. J. (1975). A simple technique for the detection o f insect parasitic rhabditid nematodes in soil. *Nematologica, 21*, 109-110.

Bell N L, Davis L T, Sarathchandra S U, Barratt B I P, Ferguson C M and Townsend R J. 2005. Biodiversity of indigenous tussock grassland sites in Otago, Canterbury and the central North Island of New Zealand II. Nematodes. *Journal of the Royal Society of New Zealand*, 35(3), 303-319.

Doddala P R C, Minor M A and Rogers D J 2010. Population dynamics of bronze beetle, *Eucolaspis* spp. (Coleoptera: Chrysomelidae) in relation to other soil macro-invertebrates in organic apple orchards in Hawke's Bay. *New Zealand Plant Protection*, 63, 195-200.

Gaugler R, Lewis E and Stuart RJ 1997. Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia*, 109, 483-489.

Gaugler R 2010. Nematodes (Rhabditida: Steinernema & Heterorhabditidae). Retrieved on 18.08.2010 from, http://www.nysaes.cornell.edu/ent/biocontrol/pathogens/nematodes.html

Grant JA, Villani MG 2003. Soil moisture effects on entomopathogenic nematodes. *Environmental Entomology*, 32(1), 80-87.

Organics Aoteaora 2010. Retrieved on 18.08.2010, from http://www.oanz.org.nz/aboutorganics

Plant and Food Research Institute of New Zealand Limited. 2009. *Fresh Facts*. Plant & Food Research, Auckland, New Zealand. 33 pp.

Rogers DJ, Cole LM, Delate KM, Walker JTS 2006. Managing bronze beetle, *Eucolaspis brunnea*, in organic apple orchards. *New Zealand Plant Protection*, 59, 57-62.

Stock SP 1995. Natural populations of entomopathogenic nematodes in the Pampean region of Argentina. *Nematropica*, 25(2), 143-148.

Appendix 2. Evaluation of mark-release-recapture methods to study dispersal behaviour of *Eucolaspis* sp.

Introduction

Spatial distribution of bronze beetle, *Eucolaspis* sp. "Hawke's Bay" population in organic apple orchards in Hawke's Bay, New Zealand appears very irregular with some orchards and some blocks / rows in some orchards having more beetles than others. Rogers et al (2006) did not find any correlation between population (damage) densities and orchard characteristics such as under cover, adjacent orchards, physical barriers (such as canals) or soil type. Similarly, though there was high predators' density in some orchards which had low bronze beetle densities, no significant correlation was found between beetle population densities and density of other invertebrates (Doddala et al., 2010). This suggests that beetle populations are generally localized and disperse little (Rogers et al., 2006). It is not known whether the beetles would generally disperse to wide areas after emergence every year or rather feed and spend at the tree below which they emerged. It is very unlikely that the larvae disperse and spread around a wide area, as they are root feeders and are most abundantly found below apple tree branches in the orchards (Rogers et al., 2006). The adult beetles are seen flying around trees some times (personal observation) and flight interception traps caught some beetles in the orchards (Rogers, unpublished data).

To test the general dispersal behaviour in terms of distance, direction, orientation, mark-release-recapture experiments were conducted with adult bronze beetles.

Materials and methods

Mark-release-recapture experiments were conducted in two organic apple orchards during 2009-10 and in one organic apple orchard during 2010-11 (table 1).

Table 1. Details of dispersal trials conducted with adult *Eucolaspis* sp. beetles in three organic apple orchards in Hawke's Bay, New Zealand

Orchard	**Management method**	**Number of beetles released**	**Date marked beetles released**	**Date beetles recaptured**
A	Organic – cultivated	1000	25-11-2009	26-11-2009
B	Organic – unmanaged (abandoned)	1000	10-12-2009	11-12-2009
C	Organic - cultivated	500	21-11-2010	Continuous monitoring through flight interception traps: monitored weekly

Adult beetles were collected by beating apple tree branches and aspiration at the rate of 50 per vial. During the two dispersal trials in 2009-10, the beetles were marked with 5 types of fluorescent colour powders: radiant green, sunset orange, fluorescent yellow, fluorescent blue, fluorescent pink. The beetles collected by beating trees were transferred into polythene bags at the rate of 50 beetles into each bag and a random colour powder (very little quantity) was introduced into the bag and the bag is remained closed with sufficient air and the beetles are allowed to walk around so that all the beetles are marked with sufficient amount of the fluorescent colour. The marked beetles were kept cool until released. 200 beetles per each colour were marked. All the marked beetles were released from a central point in the orchard between two tree rows: the bags with beetles were opened and the beetles were allowed move out of the bag and disperse into the orchard. Most of the beetles slowly walked into the grass and then some flown away whereas other disappeared into grass. The beetles were recaptured the following day by beating 50 random tree branches walking across a transect in different directions from the release point. The beetles were beaten onto circular plates (22.5 cm diameter) smeared with Tanglefoot® insect glue and the plates were labelled and sealed

with food wrap (GLAD® wrap) and transported to the laboratory. GPS coordinates were collected for the all the recapture points. The plates were observed under a UV lamp for any marked beetles: number and colour of marked beetles was noted for all the plates separately.

For dispersal trial in 2010-11 the beetles were collected in the same method as explained before, transported to the laboratory. The beetles were then individually marked with a small droplet of acrylic paint using an eyelash glued to a needle under a dissecting microscope. The beetles were anaesthetised before marking by placing on ice for 5 minutes. The marked beetles were then allowed to recover overnight: apple leaves were provided as food and the beetles were maintained in groups of 20 in small polythene bags with air vents. The beetles were observed next day morning, and all live marked beetles were released in orchard "C" (Table 1) between blocks of apple and alleyway at the poplar tree fence row. The beetles were observed, counted and activity noted after 3, 6 and 24 hours after release. There were flight interception traps set up in the apple block at the rate of 4 traps per row (in 4 rows starting from the row adjacent to alleyway), the traps were monitored once a week, the beetles were removed and transported to the laboratory and observed under dissection microscope for any of the marked beetles.

Results

2009-10 dispersal trials

Marked beetles were only recaptured from two samples at orchard "A" and one sample at orchard "B" and all three locations were not different from the release point i.e. all these three samples were collected from the trees adjacent at which the marked beetles were initially released.

2010-11 dispersal trials

Many beetles were observed walking and flying onto the fence row of trees (poplar) where some were just walking around whereas others were mating. No marked beetles were found on the nearest apple trees either at 3, 6 or 24 hours. No marked beetles were collected in any of the flight interception traps.

Discussion

As very few marked beetles were recaptured in 2009 trials and no marked beetles were recaptured in 2010 trial, it appears that the dispersal behaviour of the beetles is hard to study due to small size of the beetles and numerous population densities in the orchards. I estimated that there are at least 1 million adult beetles at one of the released orchard on the day of releasing beetles. Thus the probability of finding 1000 marked beetles in a total of 1 million beetles appears very rare. Moreover it could be that the recapturing was attempted too early i.e. before the marked beetles were back on trees and resumed normal activities. This should have been rectified by the constant recapturing system of flight interception traps; however these flight interception traps themselves appear to be very ineffective in catching beetles evident by low number caught compared actual population present (Rogers, unpublished data). Fluorescent powders were used successfully in mark-release-recapture experiments with a wide range of insects such as Western corn rootworm (Coleoptera: Chrysomelidae) (Toepfer & Kuhlmann, 2006), glassy-winged sharp shooters (Hemiptera: Cicadellidae) (Coviella et al., 2006) and painted apple moth (Lepidoptera: Lymantriidae) (Stephens et al., 2008); however these proved to be ineffective to study bronze beetle's dispersal. Future research should aim at using techniques such as stable isotopes (Hood-Nowotny & Knols, 2007) which appear promising to study insect dispersal.

References

Coviella CE, Garcia JF, Jeske DR, Redak RA, Luck RF 2006. Feasibility of Tracking Within-Field Movements of *Homalodisca coagulata* (Hemiptera: Cicadellidae) and Estimating Its Densities Using Fluorescent Dusts in Mark–Release–Recapture Experiments. *Journal of Economic Entomology*, 99, 1051-1057.

Doddala PRC, Minor MA, Rogers DJ 2010. Population dynamics of bronze beetle, *Eucolaspis* spp. (Coleoptera: Chrysomelidae) in relation to other soil macro-invertebrates in organic apple orchards in Hawke's Bay. *New Zealand Plant Protection*, 63, 195-200.

Hood-Nowotny R, Knols BGJ 2007. Stable isotope methods in biological and ecological studies of arthropods. *Entomologia Experimentalis et Applicata*, 124, 3-16.

Rogers DJ, Cole LM, Delate KM, Walker JTS 2006. Managing bronze beetle, *Eucolaspis brunnea*, in organic apple orchards. *New Zealand Plant Protection*, 59, 57-62.

Stephens AEA, Barrington AM, Bush VA, Fletcher NM, Mitchell VJ, Suckling DM 2008. Evaluation of dyes for marking painted apple moths (*Teia anartoides*

Walker, Lep. Lymantriidae) used in a sterile insect release program. *Australian Journal of Entomology*, 47, 131-136.

Toepfer S, Kuhlmann U 2006. Constructing life-tables for the invasive maize pest *Diabrotica virgifera virgifera* (Col.; Chrysomelidae) in Europe. *Journal of Applied Entomology*, 130, 193-205.

Table I. List of variable positions at COI region of mtDNA among NZ *Eucolaspis* haplotype sequences (Chapter 2)

```
Hap40 ACATCTTAATCATAAAATTATCATTACAATCCATTTACTGTAATTGATTTATTCAAACATCATCTCCATTCA
Hap42 .T.......................C.....................................
Hap46 .TC.....................................................G.....
Hap14 .TC.....................................................G.....
Hap39 .TC.....................................T.....G.....
Hap15 .TC.....................................................G.....
Hap6 .TC.....................................................G.....
Hap8 .TC.....................................T.G.....
Hap7 .TC.....................................G.....T.G.....
Hap28 GTCC.....................................T.G.....
Hap37 .TC.....................................T.G.....
Hap13 .TC.....................................T.G.....
Hap48 .TC.....T.....T.G.....
Hap29 -GC...T.....T.....A..C.....A.G.T.G.....
Hap32 .TC.....T.G.....
Hap41 ..C.....T.G.....
Hap47 ..C.....T.G.....
Hap1 ..C.....T.G.....
Hap16 .TC.....T.....
Hap45 .T.....G.....T.....T
Hap10 .ATC...G..CT.G.C...TTC....C.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap24 --------G..CT.G.CC..TTC....C.TAC...C..A.GG.CAG.....ATC....ATC.T..TCACTT
Hap11 .ATC...G..CT.G.C...TTC....C.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap25 .ATC...G..CT.G.C...TTC....C.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap21 -------..G..CT.G.C...TTC....C.TAC...C..A.GG..AG.....ATT....ATC.T..TCACTT
Hap22 ---C....G..CT.G.C...TTC....C.TAC...C..A.GG..AG.....ATC....ATC.T..TTACTT
Hap3 .ATC...G..CT...C...TT.....C.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap27 .ATC...G..CT...CC..TTC..T.T.TAC...C..A.GG..AG...G.ATC....ATC.T..TCACTT
Hap30 .ATC...G..CT...CC..TTC..T.T.TAC...C..A.GG..AG...G.ATC....ATC.T..TCACTT
Hap34 .ATCT...G..CT...CC..TTC..T.T.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap2 .ATC...G..CT.G.C...TTC....T.TAC...C..A.GG..AG.....ATC....ATC.T..TCACTT
Hap12 .ATAT.C..A.TCC.....G.TT.CGT.TCTAC...CT.A.GG..AG..C..AA..TT.AT..AATTTA.TT
Hap5 .ATAT.C..A.TCC.....G.TT.CGT.TCTAC...CT.A.GG..AGC....AA..TT.AT..AATTTA.TT
Hap31 .ATAT.C..A.TCC.....G.TT.CGT.TCTAC...CT.A.GG..AG.....AA..TT.AT..AATTTA.TT
Hap33 .ATAT.C..A.TCC.G...G.TT.C.T.TCTAC...CT.A.GG..AG.....AA..TT.AT..AATTTA.TT
Hap26 .ATAT.C..A.T.C.....G.TT.CGT.TCTAC...CT.A.GG..AG.....AA..TTTAT..A.TTTA.TT
Hap43 .ATAT.C..A.T.C.....G.TT.C.T.T.TAC...CT.A.GG...G.....AA..TT.AT..A.TTTA.TT
Hap9 -ATAT.C..A.T.C.....G.TT.CGT.TCTAC...CT.A.GG...G.....AA..TT.AT..A.T.TA.TT
Hap17 -----AC..ATT.C.....G.TT.C.T.T.TAC.C.CT.A.GG..AG.....AA..TT.AT..AC.TT..TT
E.triregia -ATAT.....T...T.T..GC.....TTCC.ATC.CT.C...G..A..CC...TT..T.AT.GT.ATTA.TT
```

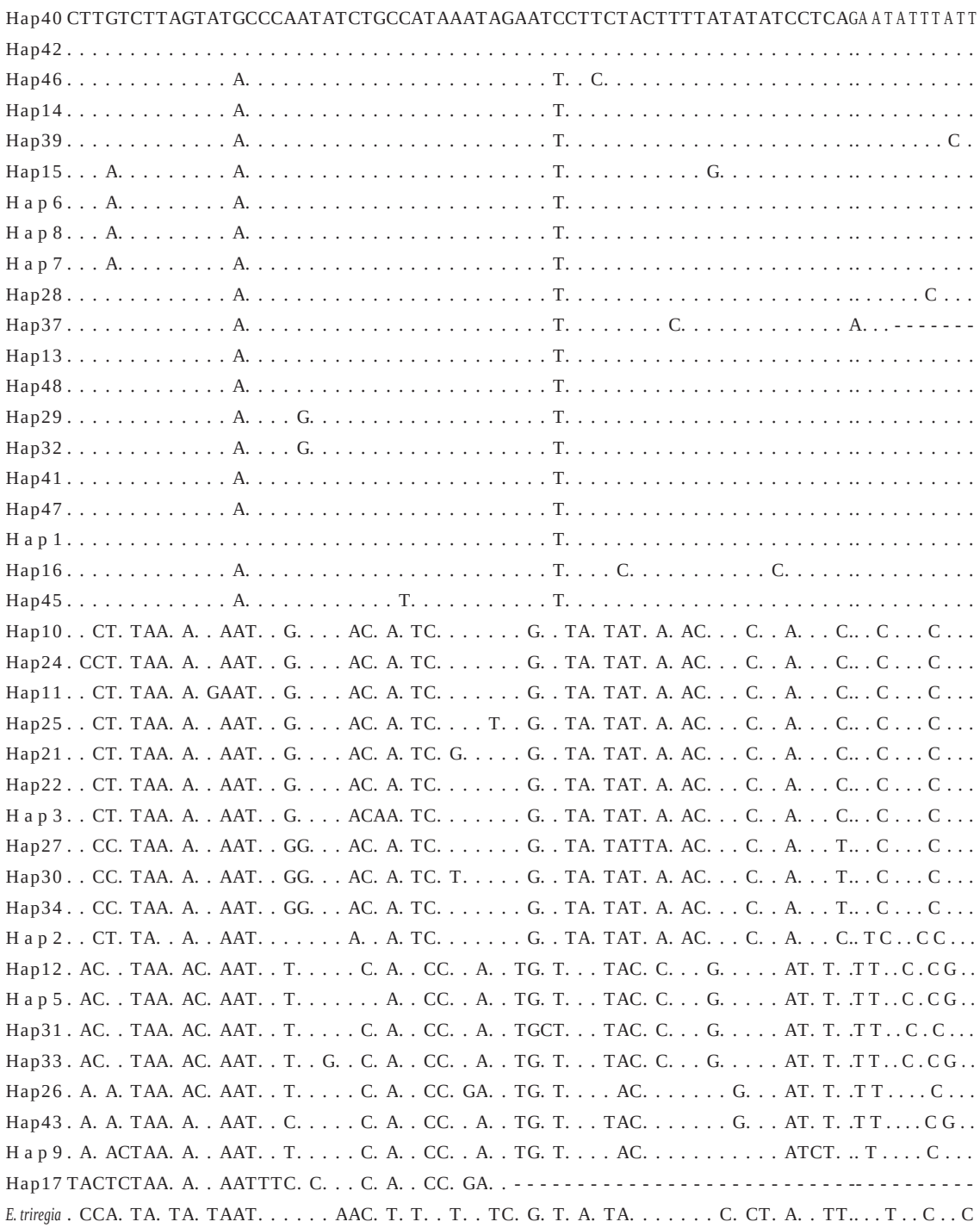

Hap40 CTTGTCTTAGTATGCCCAATATCTGCCATAAATAGAATCCTTCTACTTTTATATATCCTCAGAATATTTATT
Hap42
Hap46 A. T. . C.
Hap14 A. T.
Hap39 A. T. C .
Hap15 . . . A. A. T. G.
H a p 6 . . . A. A. T.
H a p 8 . . . A. A. T.
H a p 7 . . . A. A. T.
Hap28 A. T. C . . .
Hap37 A. T. C. A. - - - - - - - -
Hap13 A. T.
Hap48 A. T.
Hap29 A. . . . G. T.
Hap32 A. . . . G. T.
Hap41 A. T.
Hap47 A. T.
H a p 1 T.
Hap16 A. T. . . . C. C.
Hap45 A. T. T.
Hap10 . . CT. TAA. A. . AAT. . G. . . . AC. A. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap24 . CCT. TAA. A. . AAT. . G. . . . AC. A. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap11 . . CT. TAA. A. GAAT. . G. . . . AC. A. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap25 . . CT. TAA. A. . AAT. . G. . . . AC. A. TC. . . . T. . G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap21 . . CT. TAA. A. . AAT. . G. . . . AC. A. TC. G. . . . . G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap22 . . CT. TAA. A. . AAT. . G. . . . AC. A. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
H a p 3 . . CT. TAA. A. . AAT. . G. . . . ACAA. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. . C . . . C . . .
Hap27 . . CC. TAA. A. . AAT. . GG. . . AC. A. TC. G. . TA. TATTA. AC. . . C. . A. . . T.. . C . . . C . . .
Hap30 . . CC. TAA. A. . AAT. . GG. . . AC. A. TC. T. . . . . G. . TA. TAT. A. AC. . . C. . A. . . T.. . C . . . C . . .
Hap34 . . CC. TAA. A. . AAT. . GG. . . AC. A. TC. G. . TA. TAT. A. AC. . . C. . A. . . T.. . C . . . C . . .
H a p 2 . . CT. TA. . A. . AAT. A. . A. TC. G. . TA. TAT. A. AC. . . C. . A. . . C.. T C . . C C . . .
Hap12 . AC. . TAA. AC. AAT. . T. . . . . C. A. . CC. . A. . TG. T. . . TAC. C. . . G. . . . . AT. T. .T T . . C . C G . .
H a p 5 . AC. . TAA. AC. AAT. . T. . . . . A. . CC. . A. . TG. T. . . TAC. C. . . G. . . . . AT. T. .T T . . C . C G . .
Hap31 . AC. . TAA. AC. AAT. . T. . . . . C. A. . CC. . A. . TGCT. . . TAC. C. . . G. . . . . AT. T. .T T . . C . C . . .
Hap33 . AC. . TAA. AC. AAT. . T. . G. . C. A. . CC. . A. . TG. T. . . TAC. C. . . G. . . . . AT. T. .T T . . C . C G . .
Hap26 . A. A. TAA. AC. AAT. . T. . . . . C. A. . CC. GA. . TG. T. . . . AC. . . . . G. . . AT. T. .T T . . . . C . . .
Hap43 . A. A. TAA. A. . AAT. . C. . . . . C. A. . CC. . A. . TG. T. . . TAC. . . . . G. . . AT. T. .T T . . . . C G . .
H a p 9 . A. ACTAA. A. . AAT. . T. . . . . C. A. . CC. . A. . TG. T. . . . AC. . . . . ATCT. .. T . . . . C . . .
Hap17 TACTCTAA. A. . AATTTC. C. . . C. A. . CC. GA. . -
E. triregia . CCA. TA. TA. TAAT. . . . . AAC. T. T. . T. . TC. G. T. A. TA. . . . . C. CT. A. . TT.. . . T . . C . . C

Table II. Pairwise p-distances (lower) and S.E.(upper) among NZ Eucolaspis haplotypes; measured as number of base differences per site for COI region of mtDNA (Chapter 2)

	Hap40	Hap42	Hap46	Hap14	Hap39	Hap15	Hap6	Hap8	Hap7	Hap28	Hap37	Hap13	Hap48	Hap29	Hap32	Hap41	Hap47	Hap1	Hap16	Hap45	Hap10	Hap24	Hap11	Hap25	Hap21	Hap22	Hap3	Hap27	Hap30	Hap34	Hap2	Hap12	Hap5	Hap31	Hap33	Hap26	Hap43	Hap9	Hap17	*E. trirejia*
Hap40		0.002	0.003	0.003	0.004	0.004	0.003	0.004	0.004	0.003	0.004	0.003	0.004	0.005	0.003	0.003	0.003	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.013	0.013	0.012	0.012	0.012	0.012	0.014	0.012
Hap42	0.002		0.004	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.006	0.004	0.004	0.004	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.013	0.013	0.013	0.012	0.012	0.012	0.014	0.012
Hap46	0.007	0.008		0.002	0.003	0.003	0.002	0.003	0.003	0.003	0.003	0.002	0.003	0.005	0.003	0.002	0.002	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.012	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap14	0.005	0.007	0.002		0.002	0.002	0.002	0.002	0.003	0.002	0.003	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap39	0.008	0.010	0.005	0.003		0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.012	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap15	0.008	0.010	0.005	0.003	0.007		0.002	0.002	0.003	0.003	0.004	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.012	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap6	0.007	0.008	0.003	0.002	0.005	0.002		0.002	0.002	0.003	0.003	0.002	0.003	0.005	0.003	0.002	0.002	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap8	0.008	0.010	0.005	0.003	0.007	0.003	0.002		0.002	0.002	0.003	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.011	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap7	0.010	0.012	0.007	0.005	0.007	0.005	0.003	0.002		0.003	0.003	0.002	0.003	0.005	0.003	0.002	0.002	0.003	0.004	0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap28	0.008	0.010	0.005	0.003	0.007	0.007	0.005	0.003	0.005		0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.011	0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap37	0.010	0.012	0.007	0.005	0.007	0.009	0.007	0.005	0.007	0.003		0.002	0.003	0.005	0.003	0.002	0.002	0.003	0.004	0.004	0.012	0.013	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.013	0.013	0.013	0.013	0.013	0.012	0.012	0.014	0.012
Hap13	0.007	0.008	0.003	0.002	0.005	0.005	0.003	0.002	0.003	0.002	0.003		0.002	0.004	0.002	0.000	0.000	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap48	0.008	0.010	0.005	0.003	0.007	0.007	0.005	0.003	0.005	0.003	0.005	0.002		0.005	0.002	0.002	0.002	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap29	0.018	0.020	0.015	0.013	0.017	0.017	0.015	0.013	0.015	0.013	0.015	0.012	0.013		0.004	0.004	0.004	0.005	0.005	0.005	0.012	0.012	0.012	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.011	0.012	0.013	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap32	0.008	0.010	0.005	0.003	0.007	0.007	0.005	0.003	0.005	0.003	0.005	0.002	0.003	0.010		0.002	0.002	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap41	0.007	0.008	0.003	0.002	0.005	0.005	0.003	0.002	0.003	0.002	0.003	0.000	0.002	0.012	0.002		0.000	0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap47	0.007	0.008	0.003	0.002	0.005	0.005	0.003	0.002	0.003	0.002	0.003	0.000	0.002	0.012	0.002	0.000		0.002	0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap1	0.005	0.007	0.005	0.003	0.007	0.007	0.005	0.003	0.005	0.003	0.005	0.002	0.003	0.013	0.003	0.002	0.002		0.003	0.003	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap16	0.008	0.010	0.008	0.007	0.010	0.010	0.008	0.007	0.008	0.007	0.009	0.005	0.007	0.017	0.007	0.005	0.005	0.007		0.004	0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.011	0.011	0.014	0.012
Hap45	0.010	0.012	0.010	0.008	0.012	0.012	0.010	0.008	0.010	0.008	0.010	0.007	0.008	0.018	0.008	0.007	0.007	0.008	0.008		0.011	0.012	0.011	0.011	0.012	0.011	0.011	0.012	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.012	0.012	0.011	0.014	0.012
Hap10	0.099	0.101	0.103	0.101	0.103	0.103	0.101	0.099	0.098	0.098	0.105	0.099	0.101	0.109	0.101	0.099	0.099	0.101	0.099	0.099		0.003	0.002	0.002	0.002	0.001	0.003	0.005	0.004	0.004	0.004	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap24	0.107	0.108	0.110	0.108	0.110	0.110	0.108	0.107	0.105	0.105	0.113	0.107	0.108	0.115	0.108	0.107	0.107	0.108	0.107	0.107	0.005		0.003	0.003	0.004	0.003	0.004	0.005	0.005	0.004	0.005	0.012	0.012	0.012	0.011	0.012	0.011	0.012	0.012	0.012
Hap11	0.101	0.103	0.104	0.103	0.104	0.104	0.103	0.101	0.099	0.099	0.107	0.101	0.103	0.111	0.103	0.101	0.101	0.103	0.101	0.101	0.002	0.007		0.002	0.003	0.002	0.003	0.005	0.005	0.004	0.004	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap25	0.101	0.103	0.104	0.103	0.104	0.104	0.103	0.101	0.099	0.099	0.107	0.101	0.103	0.111	0.103	0.101	0.101	0.103	0.101	0.101	0.002	0.007	0.003		0.003	0.002	0.003	0.005	0.005	0.004	0.004	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap21	0.102	0.104	0.105	0.104	0.105	0.105	0.104	0.102	0.100	0.100	0.107	0.102	0.104	0.112	0.104	0.102	0.102	0.104	0.102	0.102	0.003	0.008	0.005	0.005		0.003	0.004	0.005	0.005	0.005	0.005	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap22	0.099	0.101	0.103	0.101	0.103	0.103	0.101	0.099	0.098	0.098	0.105	0.099	0.101	0.109	0.101	0.099	0.099	0.101	0.099	0.099	0.002	0.007	0.003	0.003	0.005		0.003	0.005	0.005	0.004	0.004	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap3	0.098	0.099	0.101	0.099	0.101	0.101	0.099	0.098	0.096	0.096	0.103	0.098	0.099	0.109	0.099	0.098	0.098	0.099	0.098	0.098	0.005	0.010	0.007	0.007	0.008	0.007		0.005	0.005	0.004	0.005	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap27	0.106	0.108	0.109	0.108	0.109	0.109	0.108	0.106	0.104	0.104	0.112	0.106	0.104	0.114	0.104	0.106	0.106	0.108	0.106	0.106	0.015	0.017	0.017	0.017	0.018	0.017	0.017		0.002	0.002	0.006	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap30	0.106	0.108	0.109	0.108	0.109	0.109	0.108	0.106	0.104	0.104	0.112	0.106	0.104	0.114	0.104	0.106	0.106	0.108	0.106	0.106	0.015	0.017	0.017	0.017	0.017	0.017	0.017	0.003		0.002	0.006	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap34	0.103	0.104	0.106	0.104	0.106	0.106	0.104	0.103	0.101	0.101	0.108	0.103	0.101	0.111	0.101	0.103	0.103	0.104	0.103	0.103	0.012	0.014	0.013	0.013	0.015	0.013	0.013	0.003	0.003		0.005	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012

Table II. Pairwise p-distances (lower) and S.E.(upper) among NZ Eucolaspis haplotypes; measured as number of base differences per site for COI region of mtDNA (Chapter 2)

	Hap40	Hap42	Hap46	Hap14	Hap39	Hap15	Hap6	Hap8	Hap7	Hap28	Hap37	Hap13	Hap48	Hap29	Hap32	Hap41	Hap47	Hap1	Hap16	Hap45	Hap10	Hap24	Hap11	Hap25	Hap21	Hap22	Hap3	Hap27	Hap30	Hap34	Hap2	Hap12	Hap5	Hap31	Hap33	Hap26	Hap43	Hap9	Hap17	*E. triregia*
Hap2	0.098	0.099	0.101	0.099	0.101	0.101	0.099	0.098	0.096	0.096	0.101	0.098	0.099	0.108	0.099	0.098	0.098	0.099	0.098	0.098	0.010	0.015	0.012	0.012	0.013	0.012	0.015	0.022	0.022	0.018		0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.012	0.012
Hap12	0.116	0.118	0.116	0.114	0.116	0.114	0.116	0.116	0.114	0.113	0.117	0.114	0.113	0.124	0.116	0.114	0.114	0.116	0.114	0.111	0.086	0.090	0.088	0.088	0.088	0.084	0.084	0.088	0.088	0.084	0.088		0.003	0.003	0.003	0.006	0.006	0.006	0.009	0.012
Hap5	0.114	0.116	0.114	0.113	0.114	0.113	0.114	0.114	0.113	0.111	0.115	0.113	0.111	0.124	0.114	0.113	0.113	0.114	0.113	0.109	0.088	0.092	0.089	0.089	0.090	0.086	0.086	0.089	0.089	0.086	0.086	0.005		0.003	0.004	0.006	0.006	0.006	0.009	0.012
Hap31	0.114	0.116	0.114	0.113	0.114	0.113	0.114	0.114	0.113	0.111	0.117	0.113	0.111	0.124	0.114	0.113	0.113	0.114	0.113	0.109	0.084	0.088	0.086	0.086	0.087	0.083	0.083	0.086	0.086	0.083	0.086	0.005	0.007		0.003	0.006	0.006	0.006	0.009	0.012
Hap33	0.116	0.118	0.116	0.114	0.116	0.114	0.116	0.116	0.114	0.113	0.117	0.114	0.113	0.124	0.116	0.114	0.114	0.116	0.114	0.114	0.083	0.086	0.084	0.084	0.085	0.081	0.084	0.088	0.088	0.084	0.084	0.007	0.008	0.008		0.006	0.006	0.007	0.009	0.012
Hap26	0.108	0.109	0.108	0.106	0.108	0.106	0.104	0.104	0.103	0.104	0.112	0.106	0.104	0.118	0.108	0.106	0.106	0.108	0.106	0.103	0.088	0.092	0.089	0.089	0.090	0.086	0.086	0.089	0.089	0.086	0.089	0.022	0.023	0.020	0.025		0.005	0.005	0.009	0.012
Hap43	0.101	0.103	0.101	0.099	0.101	0.099	0.098	0.098	0.096	0.098	0.103	0.099	0.098	0.111	0.101	0.099	0.099	0.101	0.099	0.099	0.081	0.085	0.083	0.083	0.083	0.079	0.079	0.083	0.083	0.079	0.083	0.023	0.025	0.025	0.023	0.015		0.005	0.008	0.012
Hap9	0.099	0.101	0.099	0.098	0.099	0.098	0.096	0.096	0.094	0.096	0.103	0.098	0.096	0.109	0.099	0.098	0.098	0.099	0.098	0.094	0.086	0.090	0.088	0.088	0.088	0.084	0.084	0.088	0.088	0.084	0.088	0.026	0.028	0.025	0.030	0.015	0.017		0.009	0.012
Hap17	0.122	0.124	0.122	0.122	0.122	0.122	0.122	0.122	0.120	0.122	0.122	0.122	0.120	0.137	0.124	0.122	0.122	0.124	0.120	0.122	0.084	0.086	0.086	0.086	0.087	0.082	0.082	0.086	0.088	0.084	0.086	0.041	0.043	0.039	0.041	0.039	0.032	0.039		0.014
E. triregia	0.108	0.106	0.108	0.106	0.109	0.106	0.104	0.103	0.104	0.103	0.107	0.104	0.103	0.114	0.106	0.104	0.104	0.106	0.104	0.104	0.099	0.103	0.099	0.098	0.098	0.098	0.098	0.103	0.099	0.099	0.103	0.111	0.116	0.113	0.114	0.109	0.106	0.108	0.124	

Table III. GenBank® (NCBI, USA) accession numbers (GI and Version) for global Eumolpinae taxa 18S rDNA sequences used in the current study (Chapter 2)

	Taxon	GI	VERSION		Taxon	GI	VERSION
1	*Eucolaspis picticornis*	85013420	DQ337133.1	35	*Promecosoma viride*	58577593	AJ781601.1
2	*Eucolaspis jucunda*	85013407	DQ337120.1	36	*Percolaspis pulchella*	58577592	AJ781600.1
3	*Syneta adamsi*	53689710	AY676694.1	37	*Percolaspis* nr. *gestroi*	58577591	AJ781599.1
4	*Syneta pilosa*	10946199	AF267464.1	38	*Nodonota* sp.	58577590	AJ781598.1
5	*Megascelis* sp.	10946198	AF267463.1	39	*Lamprosphaerus* sp.2	58577589	AJ781597.1
6	*Chrysochus auratus*	10946197	AF267462.1	40	*Hermesis aurata*	58577587	AJ781595.1
7	*Colaspis* sp.	10946196	AF267461.1	41	*Colaspis* sp.2	58577586	AJ781594.1
8	*Eumolpus* sp.	10946195	AF267460.1	42	*Colaspis* sp.3	58577585	AJ781593.1
9	*Myochrous* sp.	58577607	AJ781615.1	43	*Colaspis flavipes*	58577584	AJ781592.1
10	*Edusella* sp.	58577598	AJ781606.1	44	*Chrysodinopsis curtula*	58577582	AJ781590.1
11	*Edusella* sp.2	58577597	AJ781605.1	45	*Brachypnoea tristis*	58577581	AJ781589.1
12	*Phytorus* sp.	58577573	AJ781581.1	46	*Brachypnoea clypealis*	58577580	AJ781588.1
13	*Megascelis* sp.2	58577559	AJ781567.1	47	*Colasposoma pretiosum*	58577578	AJ781586.1
14	*Lamprosphaerus* sp.	58577588	AJ781596.1	48	*Colasposoma auripenne*	58577577	AJ781585.1
15	*Colaspis* nr. *flavicornis*	58577583	AJ781591.1	49	*Proliniscus* sp.	58577576	AJ781584.1
16	*Colasposoma* sp.	58577579	AJ781587.1	50	*Pseudosyagrus* sp.	58577575	AJ781583.1
17	*Orsodacne atra*	58577615	AJ781623.1	51	*Pseudosyagrus grossepunctatus*	58577574	AJ781582.1
18	*Donacia* sp.	58577614	AJ781622.1	52	*Phytorus dilatatus*	58577572	AJ781580.1
19	*Crioceris asparagi*	58577613	AJ781621.1	53	*Pheloticus* sp.	58577571	AJ781579.1
20	*Bruchidius* sp.	58577612	AJ781620.1	54	*Paria sellata*	58577570	AJ781578.1
21	*Linaeidea aenea*	58577611	AJ781619.1	55	*Paria fragariae*	58577569	AJ781577.1
22	*Diabrotica undecimpunctata howardi*	58577610	AJ781618.1	56	*Eulychius* sp.	58577567	AJ781575.1
23	*Parascela cribrata*	58577609	AJ781617.1	57	*Rhyparida dimidiata*	58577566	AJ781574.1
24	*Pachnephorus impressus*	58577608	AJ781616.1	58	*Rhyparida alleni*	58577565	AJ781573.1

	Taxon	GI	VERSION		Taxon	GI	VERSION
25	*Bromius obscurus*	58577606	AJ781614.1	59	*Pagria signata*	58577564	AJ781572.1
26	*Lypesthes gracilicornis*	58577605	AJ781613.1	60	*Eumolpinae* sp.2	58577563	AJ781571.1
27	*Scelodonta brevipilis*	58577604	AJ781612.1	61	*Basilepta* nr. *wallacei*	58577562	AJ781570.1
28	*Colaspoides* nr. *simillima*	58577602	AJ781610.1	62	*Basilepta* nr. *nitida*	58577561	AJ781569.1
29	*Platycorynus chalybaeus*	58577601	AJ781609.1	63	*Basilepta multicostata*	58577560	AJ781568.1
30	*Chrysochus auratus* 2	58577600	AJ781608.1	64	*Eupales ulema*	58577557	AJ781565.1
31	*Tymnes tricolor*	58577599	AJ781607.1	65	*Spilopyra sumptuosa*	58577556	AJ781564.1
32	*Edusella puberula*	58577596	AJ781604.1	66	*Bohumiljania caledonica*	58577555	AJ781563.1
33	*Eumolpinae* sp.	58577595	AJ781603.1	67	*Stenomela pallida*	58577554	AJ781562.1
34	*Rhabdopterus praetextus*	58577594	AJ781602.1	68	*Hornius grandis*	58577553	AJ781561.1

Table IV Effect of sex on attraction and effect of sex and option chose on response time in olfactory bioassays with *Eucolaspis* sp. "Hawke's Bay" (Chapter 3)

Bioassay number	**Bioassay details**	**Effect of sex on attraction to hosplants**		**Effect of sex and option chose on response time**	
		Wald χ^2	***p***	**Sex**	**Option chose**
1	Apple vs. Control	0.34	0.56	F(1, 33) = 2.81, p = .103	F(1,33)=0.32, p=.578
2	Blackberry vs. Control	0	1	F(1,33) = .04, p=.834	F(1,33)=1.22, p=.278
3	Clover vs. Control	2.68	0.101	F (1, 26)=2.87, p=.102	F(1,26)=1.94, p=.175
4	Dock vs. Control	0.023	0.881	F(1,27) = 1.29, p=.266	F(1,27)=0.07, p=.797
5	Apple vs. Apple w/ beetles	0.29	0.587	F(1,14) = 0.01, p=0.908	F(1,14)=0.24, p=.635
6	Apple vs. Blackberry (apple population)	0.76	0.38	F(1,14) = 0.16, p=.695	F(1,14)=0.10, p=.753
7	Apple vs. Blackberry (blackberry population)	0.77	0.38	F(1,19) = 0.00, p=.959	F(1,19) =0.30, p=.591

Table V. Different macro-invertebrates found in soil samples in eight different organic apple orchards (Royal Gala) in Hawke's Bay during spring / summer 2007-08 (values are number of individuals retrieved per sample). BB is bronze beetle – *Eucolaspis* sp. "Hawke's Bay" (Chapter 5)

Orchard	Date	Sample #	BB Larvae	BB Pupae	BB Adults	*A. calignosa*	*A. rosea*	*L. rubellus*	*A. longa*	*O. cyaneum*	scarab larvae	Coleo larvae	Diptera	Cicadas	insect pupae	Adult beetls	Ground Beetls	Rove Beetls	Ants	Hymeno other	slater	Land hoper	Centiped	Milliped	Slugs	Snails	Spiders	Earwigs	Flat worm
H1	Oct-07	1	32	2		7	2	3				1	2			1					1	36	5	1	2	6			
H1	Oct-07	2	4	2	1	23		5				5	7									4		3	4				
H1	Oct-07	3	14	4		19	9	6				3	2		2	1	2				1	4	2		8	8			
H1	Oct-07	4	13	1		32	1	7					1			1						11	1		5	3			
H1	Oct-07	5	6			28		13				5	4			1						38	2	1	1	8			
H2	Oct-07	1	3			9	3	3				2	8							1						1	1		
H2	Oct-07	2	2	1		8		6			2		1		2				18						1	1	2		
H2	Oct-07	3	12	16	1	7		8				3	2									2	1		2				
H2	Oct-07	4	29	26		7		6				4							8						6		2		
H2	Oct-07	5	2	1		11	3	6				2				1			5				2						
H3	Oct-07	1	9	9		15	2	3				1											21		1	2			
H3	Oct-07	2		1		12	2	9					60	1	1	1			1				14		1	3	1		
H3	Oct-07	3	10	8		14		4				2	3										14		5		1		
H3	Oct-07	4	17	33	1	25		5				3	13		1			1					25				2		1
H3	Oct-07	5	11	19	1	8		3				2	63										15						
H4	Oct-07	1	11	2		28		3				3	2	1											3	4			
H4	Oct-07	2	6	4		11		5				1											3		1	3			
H4	Oct-07	3	1	2	7	15		5				2	41		3	1							16		5	2			
H4	Oct-07	4	26	25		17		2			5	6	17		1					1			3			9	1		1
H4	Oct-07	5	2	7		5		2				1	20										7		1	4			
L1	Oct-07	1				40	2	12		1			29		3						15			1	4	6	1		
L1	Oct-07	2	1									4	6		1						19				1	1	1		
L1	Oct-07	3	1			19	3	8		1		1	20			3				2	49		2		5		2		
L1	Oct-07	4				12		1				1									1		1		3				
L1	Oct-07	5				21		6			1	1	4			1					17	2	1		2				
L2	Oct-07	1	6	1		20						4			1				1		8		5	1		1			
L2	Oct-07	2	14	4		23		2				1	1			1					1		5			1			
L2	Oct-07	3	6	2		15		2					2						10	2	9		9	14					
L2	Oct-07	4	1			18						1	1			1					12		9	7					
L2	Oct-07	5	1			10							3			1				1	37		20	6	2	1			
L3	Oct-07	1		1		4		3				4				1			3						1	8			
L3	Oct-07	2				1						5	1						10							12			
L3	Oct-07	3		3		2		2				3	1		3				1							6			1
L3	Oct-07	4		2		17							1			1							1		11	6	1		
L3	Oct-07	5				6		2				2	4						2							2	1		
L4	Oct-07	1				14	2	5	2							1										14			
L4	Oct-07	2				6		8	2			1											1			14			
L4	Oct-07	3						2				1	1										3			8	1		
L4	Oct-07	4				3		6				2	1		1										2	8			
L4	Oct-07	5				5		5	2																	4			
H1	Nov-07	1	1	4	9	11		4					10										2	8	4	7			6
H1	Nov-07	2	1	1	24	6		1					5			3					1	2			5	9			
H1	Nov-07	3	8	3	7	18	6	3					8	1	1				1						3	8			

Orchard	Date	Sample #	BB Larvae	BB Pupae	BB Adults	*A. calignosa*	*A. rosea*	*L. rubellus*	*A. longa*	*O. cyaneum*	scarab larvae	Coleo larvae	Diptera	Cicadas	insect pupae	Adult beetls	Ground Beetls	Rove Beetls	Ants	Hymeno other	slater	Land hoper	Centiped	Milliped	Slugs	Snails	Spiders	Earwigs	Flat worm
H1	Nov-07	4			2	25		2		1		6	4			2									3	1			
H1	Nov-07	5		1	7	3		6		1		8	1			1					1		2	1	1				
H2	Nov-07	1	1	1	5	4						4	2	1		4		1	6		1					4			
H2	Nov-07	2	1		19	23		11					3										1		2				
H2	Nov-07	3	4	3	3	5		3				1	1												1				1
H2	Nov-07	4	1		1							1	8	1		5									1				
H2	Nov-07	5	5	4	3	2		1				3	1			3									1				
H3	Nov-07	1				2		4					10			2							5		1				
H3	Nov-07	2			1	6		7				1	52										1						
H3	Nov-07	3			3	6							14	1		1							2						
H3	Nov-07	4			8	3		7				2	3			3							5		1				
H3	Nov-07	5	1	1	12	1		2					14		2	1							2						
H4	Nov-07	1				4		3					13	1	1								6			2			
H4	Nov-07	2	2	3	6								14			2							5			3			
H4	Nov-07	3	1		8	4		8								1		1					3		1	1			
H4	Nov-07	4			4	3		12					2										7			2			
H4	Nov-07	5			3	2		3				1	4			4							12			1			
L1	Nov-07	1										2	2			4	4		9		2					2			
L1	Nov-07	2				6		3					17		3	2					9				2	2		2	
L1	Nov-07	3				8		3				1	1			3					3				1		1		
L1	Nov-07	4				6		1				1	3		2	3					2				4	4			
L1	Nov-07	5		2		19		2				1				1					2								
L2	Nov-07	1			1								2	1		1					5		4	1	3				
L2	Nov-07	2				1							6			1					3		7						
L2	Nov-07	3		1	1								1		1				2		3		4	1					
L2	Nov-07	4	1		3	4						1	1			8							2			2			
L2	Nov-07	5		3	4	1						2		2	1				2		13		2		1	1			
L3	Nov-07	1											1			1										2			
L3	Nov-07	2				33		3				1	5													2			
L3	Nov-07	3				31		17				1				2									1	5			
L3	Nov-07	4				1		1					1			2										3			
L3	Nov-07	5				5		3					7		1	3		1					1			9			
L4	Nov-07	1						4										1					1			3			
L4	Nov-07	2				8		3					1			1										1			
L4	Nov-07	3				3		1					2													2			
L4	Nov-07	4				5		5								2										3			
L4	Nov-07	5				10		5			1		3	1											1	1			
H1	Dec-07	1				13						2	3											1	3				3
H1	Dec-07	2			1	12		1					4			1					1		1		2	2	2		
H1	Dec-07	3				34	6	12					2								1			1		2			1
H1	Dec-07	4				25		21				1	3								1		1			1			
H1	Dec-07	5				18		6				1	1			1							1						
H2	Dec-07	1				1		4					1																
H2	Dec-07	2			2	5		5								1							1		2		1		
H2	Dec-07	3			2	8		17				1											2	1					

Orchard	Date	Sample #	BB Larvae	BB Pupae	BB Adults	*A. calignosa*	*A. rosea*	*L. rubellus*	*A. longa*	*O. cyaneum*	scarab larvae	Coleo larvae	Diptera	Cicadas	insect pupae	Adult beetls	Ground Beetls	Rove Beetls	Ants	Hymeno other	slater	Land hoper	Centiped	Milliped	Slugs	Snails	Spiders	Earwigs	Flat worm
H2	Dec-07	4	1			7		29		1		2													3				
H2	Dec-07	5				13		4					2		1	1							1		1	1			
H3	Dec-07	1				21							1					1					27		1				
H3	Dec-07	2				14		1					6										8						
H3	Dec-07	3			1	12		2					9			1							8		1				
H3	Dec-07	4			1	8		5					15	1									7						
H3	Dec-07	5				7		9				1	2																
H4	Dec-07	1			7	31		4			5		8										8						
H4	Dec-07	2			1	11		3			3			1									7		2	3			
H4	Dec-07	3			3	12	1	8					13										13		1				
H4	Dec-07	4			2						1		2			1		1					12		2				2
H4	Dec-07	5			1	22		13					4			1							12		1				
L1	Dec-07	1				22							2			1									1	1			
L1	Dec-07	2				17						1	2																
L1	Dec-07	3				48							19																
L1	Dec-07	4				12		8					4																
L1	Dec-07	5			1	36		6					1																
L2	Dec-07	1																			1		4						
L2	Dec-07	2				3															1		1	1					
L2	Dec-07	3				2													1		17		3	1					
L2	Dec-07	4			1	1						1		1	3			4			1		1						
L2	Dec-07	5			1	10										1							2						
L3	Dec-07	1				3		1					8	1		1										1			
L3	Dec-07	2				1		1					4																
L3	Dec-07	3				3																				1			
L3	Dec-07	4				3		3					7			1													
L3	Dec-07	5				11		4					2										1						
L4	Dec-07	1				25		18	1	1			1										1			3			
L4	Dec-07	2				16		3	5				3			1			1							1			
L4	Dec-07	3				10		2																					
L4	Dec-07	4				24		5	4	1		1														3			
L4	Dec-07	5				4			1				1													1			
H1	Jan-07	1			1	6							26											13	3	1			1
H1	Jan-07	2				6										2								1	1	1			
H1	Jan-07	3				15		6					1			2									2				
H1	Jan-07	4				11		2				2	1	1		1		1					1		3				
H1	Jan-07	5			2	14		3			1		1		1		1	1			2		1				1		
H2	Jan-07	1				6		5			1	1					1												
H2	Jan-07	2				12		12					8			1	1												
H2	Jan-07	3						4								1													
H2	Jan-07	4			1	13		1			1	2	2					1											
H2	Jan-07	5				21		4			7	1	1					2			1			1					
H3	Jan-07	1				11							5					1					9		2				1
H3	Jan-07	2				18		6			3	1	9										15						
H3	Jan-07	3				9		3					4												1				

Orchard	Date	Sample #	BB Larvae	BB Pupae	BB Adults	*A. caliginosa*	*A. rosea*	*L. rubellus*	*A. longa*	*O. cyaneum*	scarab larvae	Coleo larvae	Diptera	Cicadas	insect pupae	Adult beetls	Ground Beetls	Rove Beetls	Ants	Hymeno other	slater	Land hoper	Centiped	Milliped	Slugs	Snails	Spiders	Earwigs	Flat worm
H3	Jan-07	4			2	17		2					8	1															
H3	Jan-07	5				14		4					1										3						
H4	Jan-07	1				7		10				1	5	4									1						
H4	Jan-07	2												2									4		1				1
H4	Jan-07	3			1	16					5		2				1						8		2				
H4	Jan-07	4				28		3		1			3	1		1							2		1				
H4	Jan-07	5			1								2			1							17						4
L1	Jan-07	1				12		1					2																
L1	Jan-07	2				8		4					5			1		1			4				1				
L1	Jan-07	3				13										1	1								2		1		
L1	Jan-07	4				11		3			4		3				1				1				1		1		
L1	Jan-07	5				7		1					1					1			1						1		
L2	Jan-07	1				4											1						3	1					
L2	Jan-07	2				11		1			2	1											6						
L2	Jan-07	3				8		1															5	2					
L2	Jan-07	4			1						1																		
L2	Jan-07	5				7							1	1									3	3					
L3	Jan-07	1																1								1			
L3	Jan-07	2				9		1			4				2														
L3	Jan-07	3				1							2														1		
L3	Jan-07	4				1		2															1						
L3	Jan-07	5				4		1						1												1			
L4	Jan-07	1				5		4	1																	1			
L4	Jan-07	2				6			2							1										1			
L4	Jan-07	3				8			1																	2			
L4	Jan-07	4				3		3																					
L4	Jan-07	5				11		3	1																				

Table VI. Different macro-invertebrates found in pitfall traps in eight different organic apple orchards (Royal Gala) in Hawke's Bay during spring / summer 2007-08 (values are number of individuals caught per trap). BB is bronze beetle – *Eucolaspis* sp. "Hawke's Bay" (Chapter 5)

Orchard	Trap #	Date	BB	E.worms	Ground Beetle -	Rove Beetle Adults	Predatory beetle	Spiders	Opiliones	Ants	Wasps	Hymenoptera others	Centipeds	Millepeds	CRW	Click Beetles	Grassgrub adult	Coleoptera-others	Other Coleo larvae	Slaters	Land hoppers	Slugs	Flatworms	Mosquitoes	Blow flies	Diptera Others	Dipteran Larvae	Lepidoptera	Dermaptera	Cicada	Snails
H1	1	06.11.07	0	3	2			27		2			0	4				2	1	261	124	2		1				1			
H1	2	06.11.07	0	4		2		25	1	2			0	1						143	85	1		1							
H1	3	06.11.07	0	3	2			20	1				0	1				1		112	83	5									
H1	4	06.11.07	0	21	3			3	1				1	2				4		81	90	9		1	1			1			
H1	5	06.11.07	0	12	3			34	1				0	1						313	117	7		1	1						
H2	1	06.11.07	0	10	1			19		6			0							3	3	3									
H2	2	06.11.07	0	5				14		5			0								20	2	1	1		1					
H2	3	06.11.07	0	16	3			36		8			0								22	5		1		1					
H2	4	06.11.07	0	12	1	2		25	1	3			0				1		2	4	7	1		1					1		
H3	1	06.11.07	0	3	1			27		4			0									4	2	1							
H3	2	06.11.07	0	5	3			10		4			0					1											1		
H3	3	06.11.07	0	2				8					0									1									
H3	4	06.11.07	0	10	1			20		1			0									1		2							
H3	5	06.11.07	0	2	1			39		2			0					1				1									
H4	1	06.11.07	0	0				8		2			0									1		3							
H4	2	06.11.07	0	1				14		2			2									1				1					
H4	3	06.11.07	0	1		2		10		2			0									1		1							
H4	4	06.11.07	0	1				17		1			0									1									
H4	5	06.11.07	0	2				6		4			2																		
L1	1	06.11.07	0	11	2			33					1					4		57		1				2					
L1	2	06.11.07	0	10	2			52		2			1					2		272		5		1							
L1	3	06.11.07	0	4	1			62		3			0					1		107		2		1							
L1	4	06.11.07	0	3	2			45					2					5		202		2	1								
L1	5	06.11.07	0	11	5	2		59					1					1		175		2	4					1			
L2	1	06.11.07	0	3		2		34		5			1	3				2		223		2		1		2					
L2	2	06.11.07	0	3				25		8			0	5				2		213		1									
L2	3	06.11.07	0	10		1		36		6			0	8				2	2	428		2		1							
L2	4	06.11.07	0	7		1		24		3			0	13				2	1	214	1	1		1				1			
L2	5	06.11.07	0	2	4	1		31		2			0	14			1	2		243	4	2		1							
L3	1	06.11.07	0	6				21		21		1	2					1				3	1								
L3	2	06.11.07	0	2		2		19		29			0										1								
L3	3	06.11.07	0			1		70		11			0											2							
L3	4	06.11.07	0	7	2	2		45		18			1					2		1		2	1	2		1					
L3	5	06.11.07	0	3	1	5		29					0					1				2	2	2		1					
L4	1	06.11.07	0	2		1		6					0		1			1				2	3	1		1					
L4	2	06.11.07	0	10		1		18		2			0									1	1								
L4	3	06.11.07	0	11		1		12		1			0		1							1	2	1							
L4	4	06.11.07	0	2				7					1		1			1		5		1	1								
L4	5	06.11.07	0	0	1			8					0									1									
H1	1	10.12.07	3	0	1	1		28		2	3		1			2				195	13	1									
H1	2	10.12.07	0	0		2	1	18	2				1							209	20										
H1	3	10.12.07	2	0	3	1		35		2			1			1		2		179	6				2						
H1	4	10.12.07	0	0		1		19	3	2	1		3							258	9				2						

Orchard	Trap #	Date	BB	E.worms	Ground Beetle -	Rove Beetle Adults	Predatory beetle	Spiders	Opiliones	Ants	Wasps	Hymenoptera others	Centipeds	Millepeds	CRW	Click Beetles	Grassgrub adult	Coleoptera-others	Other Coleo larvae	Slaters	Land hoppers	Slugs	Flatworms	Mosquitoes	Blow flies	Diptera Others	Dipteran Larvae	Lepidoptera	Dermaptera	Cicada	Snails
H1	5	10.12.07	0	0		1		23	1	1			0					2		134	4	2			3						
H2	1	10.12.07	0	0		1		146					0				1			3	3	1				3					
H2	2	10.12.07	4	4	4			40					0								31					3					
H2	3	10.12.07	2	5	1		2	49		2	1		0			1	1				14					1		3			
H2	4	10.12.07	1	0	1		1	72	9	5			0			1			1	3	2	1				1					
H2	5	10.12.07	0	5	3	1	1	70	1				0			1				19											
H3	1	10.12.07	0	3				17					0					1	1			1									
H3	2	10.12.07	2	1		1		3	1	4			0									2							1		
H3	3	10.12.07	2	1				3				3	0									1				1					
H3	4	10.12.07	1	1		1		8		1			0			1											4				
H3	5	10.12.07	0	2	1			18					1										1			2			5		
H4	1	10.12.07	0	0	1	2		13		2			0						1			2									
H4	2	10.12.07	0	0				12		1			0									2					1				
H4	3	10.12.07	0	0				8		1			0					2				1				1					
H4	4	10.12.07	1	1				5		4			1					4				2	1								
H4	5	10.12.07	1	0				19		1			0						1							3					
L1	1	10.12.07	0	0	3		4	28		2			0				1			63		1				1					
L1	2	10.12.07	0	0	10	1		63		2			1	1						104						1					
L1	3	10.12.07	0	0	1		3	26		1			0					1		29											
L1	4	10.12.07	0	0	15			33					3					2		145			1			1					
L1	5	10.12.07	0	1	6			52					0					1		95		1	3			2					
L2	1	10.12.07	1	0				38		12			0	1				1	1	190		1									
L2	2	10.12.07	0	0	1			53		5	3		0	2				4		324											
L2	3	10.12.07	0	1	1			47					0	4				1	1	348						1					
L2	4	10.12.07	0	0				45		7			0	1			1			148		2							1		
L2	5	10.12.07	2	0		4		51	1	2			0	6				4		421		1									
L3	1	10.12.07	0	1				17		33			0							18		1									
L3	2	10.12.07	0	2		1	1	15		3			1									1									
L3	3	10.12.07	0	0				107		7			0							1								1			
L3	4	10.12.07	0	1		1	3	26		13			0						1	4		10	4								
L3	5	10.12.07	0	1	2	1		6		4			0									3	1								
L4	1	10.12.07	0	0				11		1			0					1	1	1		2	6								4
L4	2	10.12.07	0	1			1	20		1			0				1	2	1	1											15
L4	3	10.12.07	0	2				10		4			0						2			1									7
L4	4	10.12.07	0	3				21		2			0			2		1		10			3			2					
L4	5	10.12.07	0	1				40		1			0					1	1				1								
H1	1	11.02.08	0	0				3		2			2					1		108	19	4			2						
H1	2	11.02.08	0	0	1			2	2	3			6					2		288	7	2			2						
H1	3	11.02.08	0	0	1			6		4	1		2							112	3				3						
H1	4	11.02.08	0	1	3			6	1	2	1		0					4		301	27				3						
H1	5	11.02.08	0	0	1			9	10				3					2		316	5	2			1						
H2	1	11.02.08	0	2	2			121		6			0								21				2						
H2	2	11.02.08	0	3	2	1		31		2			0								28	2			1						
H2	3	11.02.08	0	10	6		1	107					0					1		7	96	1									

Orchard	Trap #	Date	BB	E.worms	Ground Beetle -	Rove Beetle Adults	Predatory beetle	Spiders	Opiliones	Ants	Wasps	Hymenoptera others	Centipeds	Millepeds	CRW	Click Beetles	Grassgrub adult	Coleoptera-others	Other Coleo larvae	Slaters	Land hoppers	Slugs	Flatworms	Mosquitoes	Blow flies	Diptera Others	Dipteran Larvae	Lepidoptera	Dermaptera	Cicada	Snails
H2	4	11.02.08	0	1	3			89		7			0						1	3	12				1		1				
H2	5	11.02.08	0	2				101		6			0							2	9										
H3	1	11.02.08	0	3				29					0									1									
H3	2	11.02.08	0	2	1	1		7		1			2										1								
H3	3	11.02.08	0	2		1		9		1	1		2			1															
H3	4	11.02.08	0	0				4			1		1									1			1						
H3	5	11.02.08	0	2				20		1	2		3									1	2		1						
H4	1	11.02.08	0	1		1		4		1			1									1		1	1						
H4	2	11.02.08	0	2		1		10		1	1		0												3			1			
H4	3	11.02.08	0	3				17		1			0									1									
H4	4	11.02.08	0	2				7					1					2													
H4	5	11.02.08	0	2		1		13		1			1					2				1	1					1			
L1	1	11.02.08	0	0	25			20					0							320		2	1								
L1	2	11.02.08	0	1	12			19					3							409											
L1	3	11.02.08	0	0	6			14		1			0							160		2									
L1	4	11.02.08	0	0	14			43					0							243		1									
L1	5	11.02.08	0	0	9			13		2			1							144						1					
L2	1	11.02.08	0	0				62					0							256											
L2	2	11.02.08	0	1		1		79		2			0							208		1			1						
L2	3	11.02.08	0	0				118					0							309		1			2						
L2	4	11.02.08	0	1				67		4	1		0							63					1						
L2	5	11.02.08	0	0		1		86	2	3	1		0							345					1				1		
L3	1	11.02.08	0	1				16		14			0			1				12					2						
L3	2	11.02.08	0	0				14		11			0									2	1								
L3	3	11.02.08	0	0				27		6			0									4						1			
L3	4	11.02.08	0	1	1	1		12		2			2							4		4									
L3	5	11.02.08	0	1				28		1			1									1									
L4	1	11.02.08	0	3				27					0										1		1						
L4	2	11.02.08	0	3				63			3		0			1		1	2	4		1	2		2						
L4	3	11.02.08	0	2				15					0							2		2	2		2						
L4	4	11.02.08	0	1	2			24		1	2		1					3		1											
L4	5	11.02.08	0	0		1		18		1			0			1		2		1						2					
H1	1	14.01.08	0	1	1			31		3			7							200	113				2		1				
H1	2	14.01.08	0	2	4	5	2	36		7	3		9			2		1		453	240	3			6						
H1	3	14.01.08	0	0	5	1		31		2			9					3		309	16				3						
H1	4	14.01.08	0	0	3			3					5							387	111				5						
H1	5	14.01.08	0	1	4	2		21	2	4			8							293	39				5						
H2	1	14.01.08	0	2	2			271		5	2		0								9	1									
H2	2	14.01.08	0	3		3		109		4	2		0						1		61	3			1	2					
H2	3	14.01.08	0	7	4	5	2	178	1	3	3		0						1	2	68										
H2	4	14.01.08	1	2	1			169		30	5		0	1		2				11	7	2							1	1	
H2	5	14.01.08	0	1	4	2		22	2	2	2		7							296	52	1				2					
H3	1	14.01.08	0	4		1	2	14				1	1			1							1								
H3	2	14.01.08	0	1		1		11		3	2		1										2								

Orchard	Trap #	Date	BB	E.worms	Ground Beetle -	Rove Beetle Adults	Predatory beetle	Spiders	Opiliones	Ants	Wasps	Hymenoptera others	Centipeds	Millepeds	CRW	Click Beetles	Grassgrub adult	Coleoptera-others	Other Coleo larvae	Slaters	Land hoppers	Slugs	Flatworms	Mosquitoes	Blow flies	Diptera Others	Dipteran Larvae	Lepidoptera	Dermaptera	Cicada	Snails
H3	3	14.01.08	1	0			1	21	1	1	1		1										1		5						
H3	4	14.01.08	0	4		1		5			2		1												2		1				
H3	5	14.01.08	0	2				14			2		1															1			
H4	1	14.01.08	0	3		2		32		1			2					2	4	3		2			1						
H4	2	14.01.08	0	2		3		41		1	4		0						1			1			5						
H4	3	14.01.08	0	2			1	50		3			1						1			1								1	
H4	4	14.01.08	0	3				31		1			0			1		3							1						
H4	5	14.01.08	0	0				13		2			1			2			1			2	1								
L1	1	14.01.08	0	0	9			20	1				1					1		266		2	2								
L1	2	14.01.08	0	0	26			36					4							654		1							1		
L1	3	14.01.08	0	0	16			53		2	1		2							133											
L1	4	14.01.08	0	2	30			41					4					1		282					1						
L1	5	14.01.08	0	1	7			31					3					1		198						1					
L2	1	14.01.08	0	0				116	1	7			1							608											
L2	2	14.01.08	0	0				180		2			2	1						285											
L2	3	14.01.08	0	1		1		83	2	4	1		1	1						821		2									
L2	4	14.01.08	0	0		1		219	1				0	1		1				378					2				1		
L2	5	14.01.08	0	2	1			94	3				1			1				472		3			1						
L3	1	14.01.08	0	2		1		17		19			1					3		32		1		1	3						
L3	2	14.01.08	0	6		3		63		21	2		0			2		1		1		1	1		1						
L3	3	14.01.08	0	1		1		85		5			2			2				1		2		1							
L3	4	14.01.08	0	9		3	1	54		15			2			1				3		1	8		1						
L3	5	14.01.08	0	0		1		37		1			0			1		2					7								
L4	1	14.01.08	0	3		1		46					0					2		1		7	4		1						
L4	2	14.01.08	0	15		1		53		1			0			1	1			3		3			1						
L4	3	14.01.08	0	3			1	35					0							2		4	2								
L4	5	14.01.08	0	6		1		97		3			0			1				1		2	2								

List of acronyms

Abbreviation	Expansion and /or Meaning
$	Dollar(s)
^{0}C	Degree Centigrade
AMNH	Auckland Museum
BMNH	British Museum of Natural History
bp	Basepair(s)
Buffer A.E.	a proprietery elution buffer of QIAZEN
ca.	Circa (approximately)
cm	Centimetre(s)
COI	Cytochrome Oxidase subunit 1
dH_20	De-ionised water
DNA	Deoxyribo Nucleic Acid
EtOH	Ethyl Alcohol / Ethanol
GPS	Global Positioning System
h	Hour(s)
H_20_2	Hydrogen peroxide
H_2O	Water
ha	Hectare(s)
IFP	Integrated Fruit Production
kb	Kilobasepair(s) (1 kb = 1000 basepairs)
km	Kilometre(s)
KOH	Potassium hydroxide
L	Litre(s)
LUNZ	Entomology Research Museum at Lincoln University, New Zealand
m	Mitre(s)
m^2	Square metre
$MgCl_2$	Magnesium Chloride
min	Minute(s)
mm	Millimetre(s)
mM	Milli molar(s)
mm^2	Square millimetre
mt	Mitochondrial
mtDNA	Mitochondrial DNA
NaCl	Sodium Chloride
ng	Nanogram(s)
nov.	Novus (new)
NZ	New Zealand
NZAC	New Zealand Arthropod Collection at Landcare Research Ltd., Auckland
PCR	Polymerase Chain Reaction

Abbreviation	Expansion and /or Meaning
Pers. comm.	Personal communication
Pers. obs.	Personal observations
RNA	Ribo Nucleic Acid
rpm	revolutions per minute
s	Second(s)
s.l.	*Sensu lato* (in broad sense)
µL	Micro Litre(s)
µM	Micro molar(s)