Investigating organic control methods for bronze beetle (*Eucolaspis* sp.) in New Zealand organic apple production

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Abstract

Bronze beetle (*Eucolaspis* sp.), an insect native to New Zealand (NZ), is one of the most prevalent threats to the NZ organic apple industry. In organic orchards, bronze beetle can potentially damage or destroy 40-50% of the crop. At Bostock New Zealand, a large organic company that produces approximately 90% of NZ's organic apples, the beetle causes losses of approximately $6 million per year. The lack of effective control methods available for use in organic production systems exacerbates the population numbers and severity of this pest.

Two separate experiments were carried out to help alleviate this problematic pest. The first was a laboratory bioassay trial comparing the efficacy of three conventional insecticides with three organic insecticides containing the active ingredients spinosad (Entrust™ SC Naturalyte™ 240), pyrethrin (PYNZ28 EC), and azadirachtin (NeemAzal-T/S™ 40 EC). The objective of this experiment was to determine if any of the organic insecticides had the potential to provide an acceptable level of control in controlled conditions. If so, they should be investigated commercially. The organic insecticide Entrust SC Naturalyte (commonly called Entrust) provided over 90% control five days after application to leaves, a level of control similar to all three conventional insecticides trialled (Vayego® 200 SC, Calypso® 480 SC, and Avaunt® 300 WDG). NeemAzal-T/S, the organic insecticide containing azadirachtin, also showed some potential but at a lower level compared to Entrust, as the control achieved was over 80% seven days after direct application to leaves.

The second experiment investigated the host plant attraction of bronze beetle, as it is known that they use plant volatiles to locate host plants. Based on this, apple, plum, and blackberry leaves were compared (with a clean air control) to identify which of these crops was the most attractive to bronze beetle. The purpose of this was to investigate the possibility of using these crops as attractants in a potential organic control programme. However, there were few responses to treatments, and all four treatment options appeared equally attractive to bronze beetle during this experiment, with any differences attributed to random variability.

Keywords: Bronze beetle, organic insecticides, efficacy, New Zealand (NZ), apple production, laboratory, host plant(s).

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Chapter 1 Introduction

1.1 Overview

Organic horticulture is growing on a global scale because of an increasing consumer demand for sustainable food products free from synthetic chemicals (Granatstein & Kupferman, 2006; Rigby & Caceres, 2001). The industry aims to be economically successful, socially responsible, and safer for the environment (Sumner, 2006). Organic production focuses on increasing the organic matter content of soil and growing crops with better resistance to pests and diseases (Granatstein & Kupferman, 2006). One of the most important guiding principles of organic production is the use of natural inputs and materials instead of synthetic alternatives (Granatstein & Kupferman, 2006; Prange et al., 2006). Another key principle is working with natural systems and processes (Granatstein & Kupferman, 2006). In comparison to this, conventional systems rely on broad-spectrum synthetic pesticides and fertilisers to maximise production (Condron et al., 2000; Suckling et al., 1999), creating concerns related to environmental degradation and depletion of natural resources (Page, 2009). New Zealand (NZ) has also developed an Integrated Fruit Production (IFP) system aimed at increasing the use of a range of ecologically safe control methods and minimising the use and side effects of agrichemicals (Suckling et al., 1999; Wiltshire, 2003).

Organic certification programmes exist to ensure that organic products meet specific standards and rules related to the principles of organic horticulture (Rigby & Caceres, 2001; Sumner, 2006). These programmes vary between countries, but all guarantee that organic products, which tend to have higher prices, have been grown, prepared, and treated in the manner claimed (Granatstein & Kupferman, 2006; Rigby & Caceres, 2001). In NZ both BioGroNZ and AssureQuality provide organic certification, allowing produce to be marketed as organic in both domestic and international markets (Organics Aotearoa New Zealand, 2021; Wearing et al., 2011).

Compared with conventional horticulture, organic horticulture is generally perceived to be more beneficial to the environment. However, it is usually less productive and requires more frequent spray applications for pest and disease control (Granatstein & Kupferman, 2006;

Muller et al., 2015). The increased spray frequency is caused by organic pesticides being less effective and having shorter residues than conventional alternatives (Granatstein & Kupferman, 2006). Furthermore, produce grown in organic systems may be of lower quality. For example, the use of sulphur based organic fungicides can reduce the size of ‘Royal Gala’ apples by 9% and ‘Braeburn’ apples by 30% (McArtney & Walker, 2002).

In 2020, there were 85,850 hectares (ha) of land under organic certification in NZ, of which 18,890 ha were used for fruit and vegetable production. Organic fruit and vegetables made up 3.6% of NZ’s fresh fruit and vegetable exports, with a value of $135.9 million and $143.9 million in 2017 and 2020 respectively (New Zealand Institute for Plant and Food Research Limited, 2021; Organics Aotearoa New Zealand, 2021). Approximately 6% of all apples exported from NZ are organic. Bostock New Zealand, a large organic company based in Hawke’s Bay, grows approximately 90% of these (F. Gillies, personal communication, February 28, 2022; Organics Aotearoa New Zealand, 2021). Hawke’s Bay in NZ has one of the highest concentrations of organic growers in the world, with about 25% of apple growers being organic certified (Delate et al., 2008).

1.2 Bronze beetle (*Eucolaspis* sp.)

The bronze beetle is a small Chrysomelid insect native to NZ (Gómez-Zurita, 2019; Kay, 1980). However, there is taxonomic uncertainty over the species of bronze beetle (Doddala, 2012; Rogers et al., 2007). Prior to 2007 bronze beetle was most frequently referred to as *Eucolaspis brunnea* (Fabricius) in literature (Kay, 1980; Lysaght, 1930; Rogers et al., 2006). However, in December 2006, adult bronze beetle from a single organic apple orchard in Hawke’s Bay were tentatively identified as *Eucolaspis pallidipennis* (White), creating taxonomic uncertainty which is yet to be solved (Doddala, 2012; Rogers et al., 2007). Due to this, bronze beetle tends to be referred to as *Eucolaspis* sp. in more recent literature (Doddala et al., 2016; Gómez-Zurita, 2019; Malone et al., 2017; Rogers et al., 2007; Rogers et al., 2009; Sofo et al., 2020). Further confusion is added due to *E. brunnea* being called *Colaspis brunnea* in a publication by A. White in 1846, creating a homonymy with the grape colaspis (*C. brunnea*) in North America which is sometimes called bronzed beetle (Doddala et al., 2015). Based on this information, the bronze beetle studied in this research project will be referred to as *Eucolaspis* sp.

1.2.1 Description

Bronze beetle were first described by J. C. Fabricius in 1783. A range of revisions followed, culminating in a description by D. Miller in 1925 (Lysaght, 1930). Fully grown bronze beetle larvae are approximately 5 mm long and 2 mm wide with a curved shape. The head is light yellow tinged with brown, while the body is cream (Figure 1.1). In comparison, pupae have a creamy-white colour, but as they mature wing cases, eyes, and mandibles develop a brownish colour (Figure 1.2) (Kay, 1980; Lysaght, 1930). Adult bronze beetle are 3-5 mm long and 2-3 mm wide, and have an oval shape. They range in colour from yellowish-brown to black (Doddala, 2012; Lysaght, 1930). Convex elytra extend past the end of the abdomen and are a similar length as the thread-like antennae. Bronze beetle adults also have robust legs for jumping and burrowing (Figure 1.3) (Doddala, 2012; Kay, 1980). Finally, eggs are less than 1 mm long and turn from white to yellow during development. Eggs tend to be laid in clusters of 3-14 in earthen cells 10-30 mm below the soil surface (Kay, 1980).

Figure 1.1: *Bronze beetle larvae (dorsal view)*

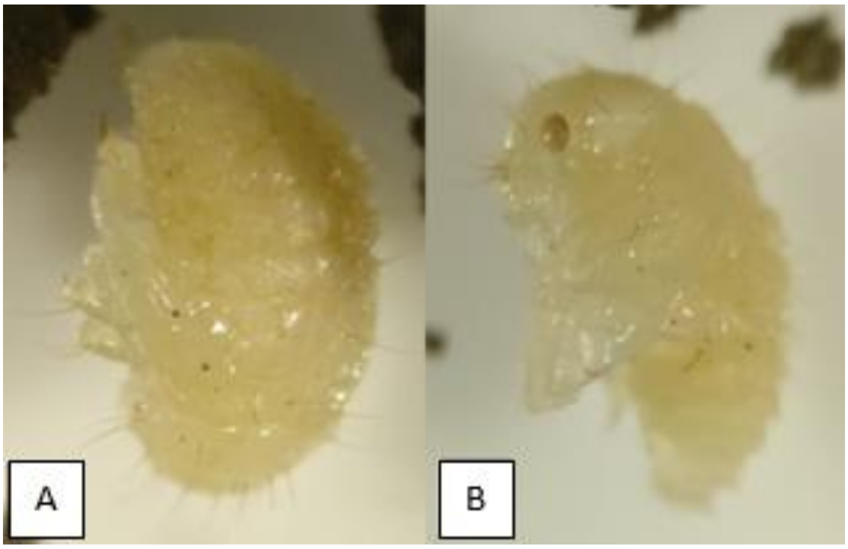

Figure 1.2: *Bronze beetle pupae - (A) dorsal view, and (B) lateral view*

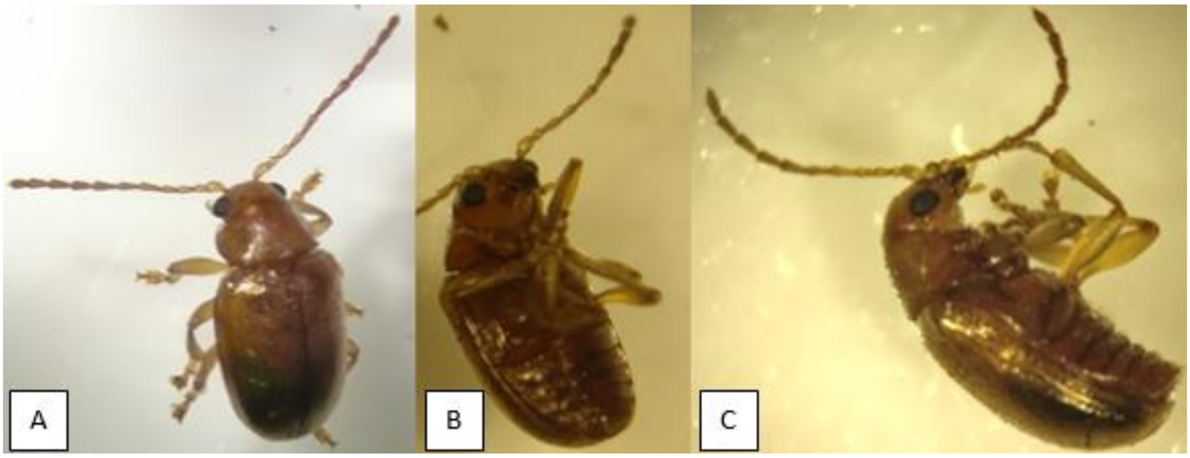

Figure 1.3: *Bronze beetle adults - (A) dorsal view, (B) ventral view, and (C) lateral view*

Bronze beetle are polyphagous, feeding on a varied range of host plants (Doddala et al., 2016; Gómez-Zurita, 2019). They feed on both native and introduced plants but occur in larger numbers on introduced species (Lysaght, 1930). In NZ, they were first reported on Manuka, a native shrub, in 1781. Since then, they have been recorded feeding on at least 67 other plant species including 27 native species and 40 introduced species. These species belong to 33 different plant families, of which Rosaceae (six species) and Myrtaceae (11 species) are the most common (Doddala et al., 2016).

1.2.2 Life cycle

Bronze beetle have an annual life cycle that coincides with apple fruit development and growth (Hurst et al., 2011; Sofo et al., 2020). Immature stages (eggs, larvae, and pupae) live in the soil, and larvae feed on the roots of grass and broad leaf weeds in apple orchards (Doddala et al., 2013; Hurst et al., 2011). Larvae go through winter diapause approximately 25-200 mm below the soil surface (Kay, 1980; Rogers et al., 2009). When soil temperatures increase in September and October. Larvae come out of diapause and move towards the topsoil, where they pupate in individual earthen cells in the top 70 mm of the soil. The pupal stage lasts approximately three weeks (Doddala et al., 2013; Kay, 1980). Adults emerge from the soil from October to January, with peak emergence occurring between November and December. They then either fly or climb into the apple trees, feeding on leaves and developing fruit (Hurst et al., 2011; Rogers et al., 2006). Adults live for up to one month during which time they lay eggs in the soil. Larvae hatch from these eggs after approximately three weeks (Doddala et al., 2010; Kay, 1980). As the timing of adult emergence coincides with apple fruit development, adults have the potential to cause significant damage to developing apple crops (Rogers et al., 2006).

1.2.3 Damage to apple crops

The main damage to apple crops caused by bronze beetle adults includes raised feeding scars on fruit and a distorted fruit shape (Delate et al., 2008; Rogers et al., 2006). This damage results from adult feeding at flowering and fruitlet stages of apple growth. Figure 1.4 shows symptoms on immature fruit. The scars caused during the immature fruit stage remain on mature fruit (Figure 1.5). The beetles also feed on the stalks of immature fruit (Figure 1.6), causing the fruit to drop from the tree prematurely (Rogers et al., 2006). Finally, leaves damaged by adults exhibit a shot-hole effect (Figure 1.7) (Delate et al., 2008; Doddala et al., 2010).

Figure 1.4: *Feeding scars on immature fruit*

Figure 1.5: *Feeding scars on a mature fruit that were caused by feeding on immature fruit*

Figure 1.6: *Feeding marks on stalks of immature fruit*

Figure 1.7: *Shot-hole effect in apple leaves*

1.2.4 Impact on organic apple production

Bronze beetle was a significant pest in apple orchards during the 1920s and 1930s, but later became rare due to applications of organochlorine insecticides. IFP was introduced using pilot programmes in 1996 and was widely adopted by 2001, after which time bronze beetle caused no yield or economic loss in both conventional and IFP apple orchards (Rogers et al., 2006; Wiltshire, 2003). However, in 2000, significant damage from bronze beetle was first reported in an organic orchard and the problem has escalated since then. It is now one of the most challenging and significant pests for organic apple production in NZ (Delate et al., 2008; Delate et al., 2010). Bronze beetle is such a danger to the viability of organic apple production that it has caused some organic orchards to revert to conventional production (Doddala, 2012). In an infested organic orchard, prior to the widespread use of cultivation, it caused a yield loss of up to 40-50% (Doddala et al., 2010; Rogers et al., 2009). For Bostock New Zealand the financial loss caused by bronze beetle is thought to be approximately $13,500/ha. This loss is caused by the cost of cultivation for control, the cost of searching for damaged fruit during thinning, and the value of damaged fruit found during picking and packing. This results in an estimated loss of over $6 million per year (Bostock New Zealand, 2021). Because bronze beetle is primarily an issue for organic apple production, the focus of my research was on investigating potential organic control methods.

1.2.5 Potential organic control methods for bronze beetle

There are a range of organic control methods that have been trialled or used for controlling bronze beetle. These include organic insecticides (Delate et al., 2008; Rogers et al., 2006) and soil cultivation (Doddala et al., 2010; Hurst et al., 2011). Trapping has also been used for monitoring purposes (Rogers et al., 2006) and could be adapted for control purposes (Doddala, 2012; Doddala et al., 2016).

No effective organic insecticides have been found for controlling bronze beetle despite several trials testing a range of organic insecticides (Delate et al., 2008; Doddala, 2012; Rogers et al., 2006). The main difficulty in the search for an effective organic insecticide is that bronze beetle has a long emergence window and multiple applications of a persistent insecticide would be required during the growing season (Rogers et al., 2006). The lack of a known effective organic insecticide confirms that further ongoing research is required in this area.

Traps have previously been used to monitor adult bronze beetle populations (Rogers et al., 2006). Because bronze beetle use plant volatiles to locate host plants, trapping has the potential to be used as a control method (Doddala, 2012; Doddala et al., 2016). Based on this, investigating the host plant preferences of bronze beetle could be valuable.

Soil cultivation to target pupae in the soil is the most common organic method for controlling bronze beetle (Rogers et al., 2009). The most effective practice includes two cultivations during the growing season, which has the potential to reduce crop damage by 46-61% (Rogers et al., 2009). The timing of cultivation is critical as the larvae tend to live deep in the soil. However, the pupae are close to the surface so it is essential to carry out the cultivation during the pupal stage (Doddala et al., 2010). Soil cultivation is effective in the short term as a control method but damages soil structure and plant roots (Doddala, 2012; Hurst et al., 2011). It is also a costly and time-consuming method. It costs Bostock New Zealand $3,500/ha per annum, and in the 2016/2017 season it required 16,604 man hours. However, even with these negative aspects, soil cultivation is the only effective control method currently available for organic growers (Bostock New Zealand, 2021; Doddala, 2012).

1.3 Aims and objectives

The aims of this thesis were to investigate potential organic control methods for bronze beetle through a comparison of the efficacy of conventional and organic insecticides, as well as an assessment of adult preferences for different host plant odours.

The objectives of this thesis were to:

1. Determine the control (combined percent dead and percent moribund) of bronze beetle provided by conventional (Vayego®, Calypso®, and Avaunt®) and organic (Entrust™ SC Naturalyte™, NeemAzal-T/S™, and PYNZ28) insecticides in the laboratory.
2. Determine if bronze beetle are most strongly attracted to apple, plum, or blackberry odours.

Chapter 2 Comparison of the efficacy of organic and conventional insecticides

2.1 Abstract

Presently, there are no suitable organic insecticides that are known to control bronze beetle. The aim of this experiment was to compare the efficacy of several organic and conventional insecticides against this pest in a laboratory setting. My results show that the three conventional insecticides (Vayego, Calypso, and Avaunt) and the organic insecticide Entrust provided the highest level of control. Entrust achieved over 90% control five days after application to leaves. NeemAzal-T/S also showed potential, as all trialled concentrations reached over 80% control when assessed seven days after application to leaves. However, these products were only trialled in a controlled laboratory environment. Therefore, field trials in organic apple orchards in NZ would be necessary to conclude if they have the potential to control bronze beetle to an acceptable level commercially.

2.2 Introduction

The three most widely used categories of organic insecticides are products with spinosad, pyrethrin, and azadirachtin as the active ingredients (Dively et al., 2020; Kamminga et al., 2009). There are few organic insecticides available compared to the number of conventional insecticides (Kamminga et al., 2009; Tofangsazi et al., 2018). This creates resistance management issues as there are a smaller number of organic insecticide groups to rotate (Sial et al., 2019). They are also relatively short-lived in the environment (DiGiacomo et al., 2021; Dively et al., 2020), having considerably shorter residual activity periods than conventional insecticides. This is positive because organic sprays can be applied closer to harvest, but it also creates the need for more frequent spray applications (Sial et al., 2019; Tofangsazi et al., 2018). Another consideration is that organic insecticides tend to have higher efficacy on immature life cycle stages of insects than on adults (Dively et al., 2020). This compounds the difficulty in controlling bronze beetle because immature stages dwell in the soil (Doddala et al., 2013; Hurst et al., 2011). Finally, organic insecticides have lower efficacy and higher costs than their conventional counterparts (DiGiacomo et al., 2021; Dively et al., 2020).

The aim of this experiment was to compare the efficacy of three organic insecticides with that of three conventional insecticides for activity against bronze beetle in a laboratory setting. The three organic insecticides have the active ingredients spinosad, pyrethrin, and azadirachtin. Some of the organic insecticides tested either have not been trialled on bronze beetle or formulations have changed since they were previously trialled. While the conventional insecticides are registered or commonly used for controlling bronze beetle. The objectives of the experiment were to:

(1) Investigate and compare the efficacy of the individual products.

(2) Evaluate the efficacy of the organic insecticides with regard to a range of concentrations.

(3) Assess the time taken for any efficacy results to be observed for all trialled insecticides.

2.2.1 Organic insecticides

Organic insecticides with spinosad as the active ingredient, such as Entrust, are among the most effective organic insecticides available (Sial et al., 2019). Entrust is registered for use against leafroller caterpillars in apple production in NZ and operates through ingestion and contact. Insects cease feeding after exposure but may take up to 3 days to die (Corteva Agriscience, 2021a, 2021b). It is also effective against other lepidopteran insects, thrips, and some beetles (Dively et al., 2020; Kamminga et al., 2009). Spinosad is a metabolite from the soil bacterium *Saccharopolyspora spinosa* and operates by binding to the nicotinic acetylcholine receptor in the central nervous system (Corteva Agriscience, 2021a, 2021b; Dively et al., 2020). This interferes with the transmission of synaptic signals by disrupting the binding of acetylcholine to the receptors, resulting in paralysis ("798 spinosad," 2012; Dively et al., 2020). Entrust is BioGroNZ certified #4560 (Dow AgroSciences, 2017) and contains 240 g/L spinosad in the form of a Suspension Concentrate (SC) (Corteva Agriscience, 2021a, 2021b). Persistence in the field is likely to be between 1 and 2 weeks with degradation caused by exposure to UV light. Creating the need for repeated applications during the growing season (Balusu & Fadamiro, 2012; Reddy & Antwi, 2016).

NeemAzal-T/S is a broad-spectrum organic insecticide. The leading active compound, azadirachtin, is derived from the seed kernels of the neem tree (*Azadirachta indica*) (Danelski et al., 2014; Saber et al., 2004). NeemAzal-T/S is an Emulsifiable Concentrate (EC) containing 40 g/L azadirachtin (EcoGrape Service, 2021b; Saber et al., 2004). It can be used to control pests such as aphids, thrips, whiteflies, leafroller caterpillars, scale insects, and mealybugs (EcoGrape Service, 2021b, n.d.). NeemAzal-T/S is BioGroNZ certified #433 (EcoGrape Service, 2021a; Trifolio-M, n.d.). Azadirachtin has several modes of action that affect the behaviour and physiology of insects. It acts as a growth regulator by inhibiting production of the hormone ecdysone, influencing chitin synthesis (Balayara et al., 2019; Kamminga et al., 2009). There are also potential anti-feedant, repellent, and oviposition and fecundity inhibition modes of action (Balayara et al., 2019; Dively et al., 2020). Azadirachtin is ingested through sucking or biting while feeding, and after ingestion insects stop feeding and damaging the plants. Following this, development and reproduction are inhibited and mortality occurs after several days (EcoGrape Service, n.d.; Trifolio-M, n.d.). Apart from azadirachtin, there are over 60 other active ingredients in NeemAzal-T/S. This high number improves the activity of the product and prevents resistance development (EcoGrape Service, 2021a, 2021b). NeemAzal-T/S is degraded after field application by high temperatures and exposure to UV light. Consequently, repeated applications are required (Kumar & Poehling, 2006; Thoeming et al., 2006). Foliar applications of commercial neem products may persist for 5-7 days in the orchard (Schmutterer, 1990). However, some trials have shown a half-life of 0.73 or 0.8 days (Caboni et al., 2002; Caboni et al., 2006), or a persistence of only 3 days (Kumar & Poehling, 2006).

PYNZ28 contains 28 g/L pyrethrin in the form of an oil in water Emulsifiable Concentrate (EC) (PyrethrumNZ, 2019b). Pyrethrin is found in the flowers of *Chrysanthemum cinerariaefolium*, which are commonly known as pyrethrum daises (Figure 2.1) (Dively et al., 2020; Valentine, 1990). Pyrethrin is an effective biopesticide because it contains a mixture of six different compounds with strong synergistic activity (pyrethrin I and II, cinerin I and II, and jasmolin I and II) (Jeran et al., 2021). PYNZ28 can be used to control a wide range of insect pests (Murray, 2020; PyrethrumNZ, 2019b). However, this formulation is not BioGroNZ certified and cannot currently be used in registered organic production systems (PyrethrumNZ, 2019a). Pyrethrum products have a sodium channel modulation mode of action (Pavoni et al., 2019; Valent®, n.d.). They bind to sodium channels, extending their opening and causing repetitive discharges within nerve cells. This results in rapid pest knockdown and paralysis, with death occurring at a later stage ("748 pyrethrins (pyrethrum)," 2012; Pavoni et al., 2019). Pyrethrum insecticides are known for being non-persistent due to rapid degradation after exposure to UV light (Antonious, 2004; Pan et al., 2017). The half-life of pyrethrum products after foliar field application varies between 2 hours and 2 days (Antonious, 2004; Lybrand et al., 2020). This is positive from an environmental viewpoint, but confirms the need for regular applications (Antonious, 2004; Pan et al., 2017). Another consideration is that pyrethrum products are not selective, creating potential for non-target effects (Pavoni et al., 2019).

Figure 2.1: *Pyrethrum daisies (Chrysanthemum cinerariaefolium) grown near Havelock North, Hawke's Bay*

2.2.2 Conventional insecticides

Conventional insecticides used to control bronze beetle include Vayego, Calypso, and Avaunt. These products were included in my trials as positive controls.

Vayego contains 200 g/L tetraniliprole in the form of a Suspension Concentrate (SC). It acts mainly through ingestion and also has some contact activity (Bayer Crop Science, 2020, n.d.-b). It targets the ryanodine receptors in insects, keeping them open and causing uncontrolled calcium release, resulting in cessation of feeding followed by paralysis. This product has systemic and translaminar activity, and is used to control codling moth, bronze beetle, and leafroller caterpillars in pipfruit (Bayer Crop Science, 2020, n.d.-b).

Calypso contains 480 g/L thiacloprid, also in the form of a Suspension Concentrate (SC). It is systemic and translaminar, and acts through both ingestion and contact. Calypso acts on the nervous system of insects as an agonist of the nicotinic acetylcholine receptor in the central nervous system, disturbing synaptic signal transmissions ("853 thiacloprid," 2012; Bayer Crop Science, 2007). It is used to control pests such as armoured scales, bronze beetle, mealy bugs, and codling moth in apples along with thrips in avocados, nectarines, and peaches; plus armoured scales in kiwifruit (Bayer Crop Science, 2007, n.d.-a).

Avaunt belongs to the oxadiazines class of insecticide. It contains 300 g/kg indoxacarb as an active ingredient and is in the form of Water Dispersible Granules (WDG) (FMC New Zealand Limited, 2021a; Sandeep et al., 2016). It is a broad-spectrum insecticide registered for use on apples and pears against codling moth and leafroller caterpillars, and for use on grapes against leafroller caterpillars (FMC New Zealand Limited, 2021a; Liu et al., 2002). Avaunt is also commonly used to control bronze beetle (D. Rogers, personal communication, October 10, 2021). Indoxacarb operates by blocking the sodium channels in insect nervous systems. This causes feeding to cease, resulting in paralysis, regulated growth, and eventual death (Liu et al., 2003; Sandeep et al., 2016). Feeding ceases within 24 hours, but death may take 2-3 days to occur (FMC New Zealand Limited, 2021a, 2021b). Avaunt is mainly taken up by ingestion, but also has some contact activity (FMC New Zealand Limited, 2021b).

2.3 Materials and Methods

2.3.1 Beetle collection

Beetles were collected from an organic apple orchard owned by Bostock New Zealand at 81 Raukawa Road, Bridge Pa, Hawke’s Bay (Figure 2.2). Collections occurred on the same day each bioassay trial was set up.

Figure 2.2: *Apple trees in the organic orchard where bronze beetle were collected*

Beetles were collected by shaking fruit and leaf clusters, causing beetles to drop onto a plastic plate (30 cm diameter). From this plate beetles were transferred into specimen vials using an insect aspirator (Figure 2.3). This aspirator was made using a specimen vial (5.6 cm height and 4.4 cm diameter), with two holes drilled in the lid. Two lengths of clear PVC (polyvinyl chloride) tubing (49 cm and 32 cm) were fitted into the holes. The shorter tube was sucked on to create a suction force, with the end inserted into the lid covered in gauze to prevent beetles from entering the tube. The longer length was used as a hose to transfer the beetles from the plastic plate into the vial. This was the same collection method used by Rogers et al. (2006) for collecting bronze beetle in a manner which would reduce damage and injury to the beetles.

Figure 2.3: *Plastic plate and insect aspirator used for collecting bronze beetle*

2.3.2 Apple crop and leaves used

The apple leaves were collected from 'Scifresh' apple trees located at Plant and Food Research Hawke's Bay, 30 Crosses Road, Parkvale, Havelock North 4172. The trees from which leaves were collected had not been sprayed with any insecticides. The leaves collected were all mature, relatively flat, and of a similar size. The petiole of each leaf was embedded in water saturated oasis floral foam in a Petri dish (90 x 25 mm Labserv© LB560015TS) to ensure they stayed fresh for the duration of the experiment (seven days, see below).

2.3.3 Products and treatments

The properties and batch numbers of the six insecticides used in the experiment are shown in Table 2.1. Table 2.2 presents application rates, treatment concentrations used in the experiments, and product costs.

Table 2.1: Product properties and batch numbers

Product name	Active ingredient	Product formulation	Batch number
Vayego®	tetraniliprole at 200 g/L (Bayer Crop Science, n.d.-b)	Suspension Concentrate (SC) (Bayer Crop Science, n.d.-b)	PQ19050001
Calypso®	thiacloprid at 480 g/L (Bayer Crop Science, n.d.-a)	Suspension Concentrate (SC) (Bayer Crop Science, n.d.-a)	Unknown
Avaunt®	indoxacarb at 300 g/kg (FMC New Zealand Limited, 2018)	Water Dispersible Granule (WDG) (FMC New Zealand Limited, 2018)	APR19AC028
Entrust™ SC Naturalyte™	spinosad at 240 g/L (Corteva Agriscience, 2021a, 2021b)	Suspension Concentrate (SC) (Corteva Agriscience, 2021a, 2021b)	0074J9C152
NeemAzal-T/S™	azadirachtin at 40 g/L (EcoGrape Service, 2021b)	Emulsifiable Concentrate (EC) (EcoGrape Service, 2021b)	190221AB
PYNZ28	pyrethrin at 28 g/L (PyrethrumNZ, 2019b)	Emulsifiable Concentrate (EC) (PyrethrumNZ, 2019b)	Unknown

Table 2.2: Application rates, treatment concentrations, and product costs

Product name	Labelled application rate	Treatment concentrations used in trials	Cost of product (excluding GST) Prices are for original concentrate products
Vayego®	15 ml/100 L water (Bayer Crop Science, n.d.-b)	15 ml/100 L water	1 L: $502.61 (J.Payne, personal communication, February 16, 2022)
Calypso®	30 ml/100 L water (Bayer Crop Science, n.d.-a)	30 ml/100 L water	1 L: $203.04 5 L: $1015.50 (J.Payne, personal communication, February 16, 2022)
Avaunt®	20 g/100 L water (FMC New Zealand Limited, 2018)	20 g/100 L water	400 g: $150.87 (J.Payne, personal communication, February 16, 2022)
Entrust™ SC Naturalyte™	20 ml/100 L water (Dow AgroSciences, 2017)	20 ml/100 L water 40 ml/100 L water 80 ml/100 L water	1 L: $477.39 (J.Payne, personal communication, February 16, 2022)
NeemAzal-T/S™	300-500 ml/100 L water (EcoGrape Service, 2021b)	500 ml/100 L water 1000 ml/100 L water 2000 ml/100 L water	1 L: $101.73 5 L: $479.96 20 L: $1920.06 (J.Payne, personal communication, February 16, 2022)
PYNZ28	1000 ml/100 L water (PyrethrumNZ, 2019b)	1000 ml/100 L water 2000 ml/100 L water 4000 ml/100 L water	1 L: $120.00 (PyrethrumNZ, 2019b)

2.3.4 Experimental design

The experiments were carried out in November and December to coincide with bronze beetle adult emergence from the soil (Rogers et al., 2006). Beetles were exposed to residues of Vayego, Calyspso, Avaunt, Entrust, NeemAzal-T/S, PYNZ28, or reverse osmosis water (negative Control) in the laboratory to determine efficacy. Application was carried out using a Potter Spray Tower (Figure 2.4). The method used was based on Rogers et al. (2006). Because the insecticides used here have the potential to operate through either contact or ingestion, or a combination of both, they were applied in two different ways:

(1) Sprayed leaves and then released beetles onto them to test potential contact and ingestion modes of action.

(2) Sprayed beetles directly to determine if there was activity by contact only.

Thirteen treatments were set up for both application methods. The treatments were one concentration each of three conventional insecticides, three concentrations each of three organic insecticides, and Control. All experiments were carried out at 20°C.

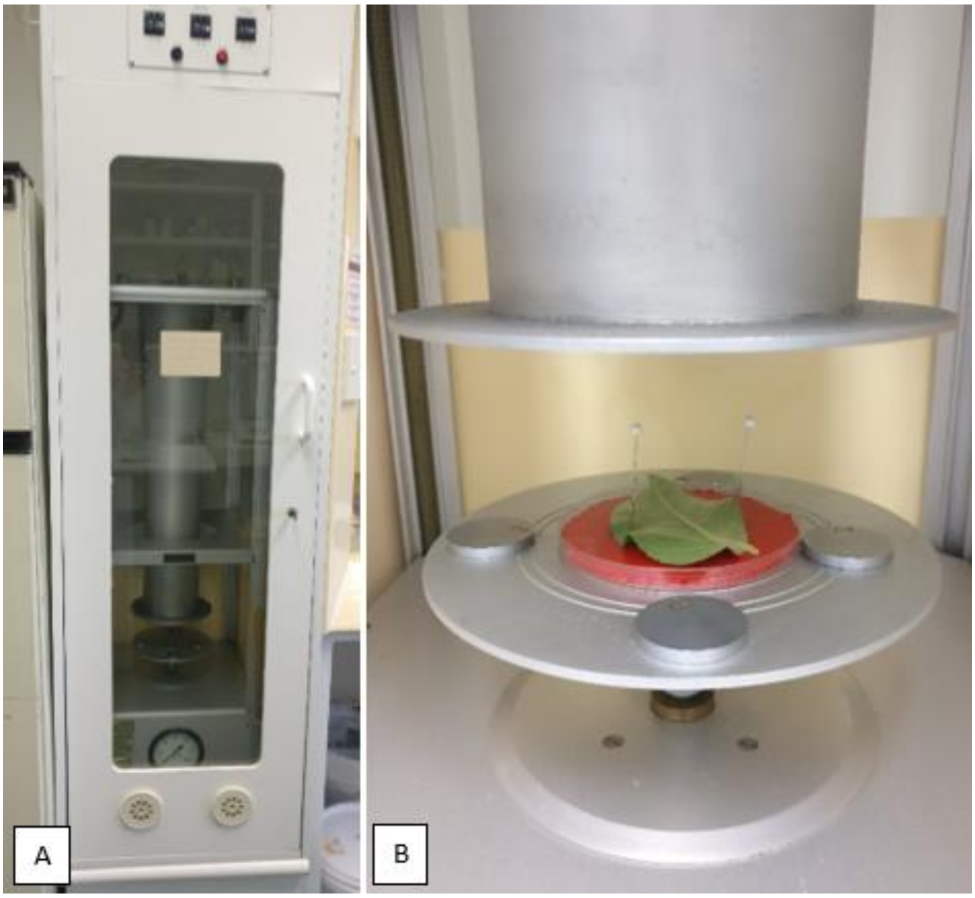

Figure 2.4: *(A) Potter Spray Tower in a cabinet with extraction system, and (B) a leaf positioned to be sprayed in the Potter Spray Tower*

Application to leaves (ingestion and contact)

Each treatment was sprayed with 2ml of a diluted chemical or water (Control) on each side of a leaf. The leaf was dried by being placed into a running fume hood for 15 minutes and was then placed into a Petri dish. Twenty unsprayed beetles were released onto the leaf and allowed to feed (20 beetles in one Petri dish as a replicate). There were five replicate Petri dishes (5 x 20 beetles) for each treatment. Figure 2.5 shows the Petri dishes with the Control and three treatments for Entrust after it was applied to leaves and the beetles were added. Assessments occurred 2, 5, and 7 days after application of the treatments. Figure 2.6 shows the first replicate of the three concentrations of PYNZ28 treatments applied to leaves, as well as the Control treatment, on the three assessment days. A beetle was classified as dead if no movement was observed when touched, and moribund if it was twitching, unable to walk, or its wings were out. The number of dead and moribund beetles were combined to determine the percent control provided by each treatment. It was suitable to combine these two parameters to indicate that the pest is under control because if the beetles are dead or moribund, they are no longer feeding and damaging the crop.

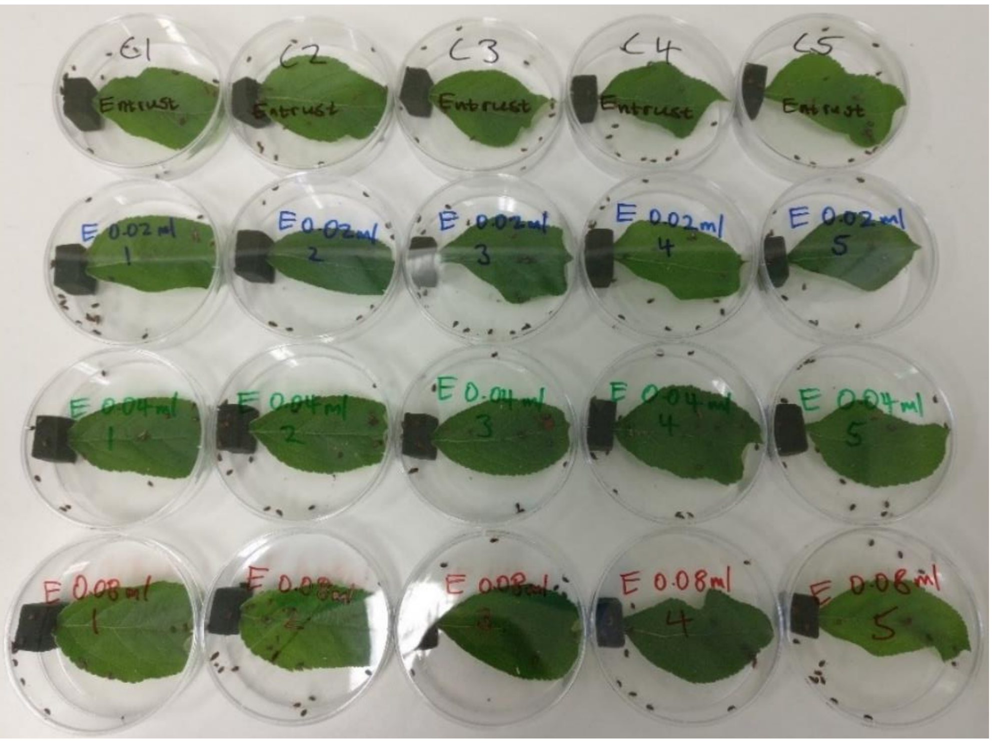

Figure 2.5: *Treatments and replicates for Entrust™ SC Naturalyte™ when applied to leaves. C, Control; E0.02ml, 20 ml/100 L water; E0.04ml, 40 ml/100 L water; E0.08ml, 80 ml/100 L water*

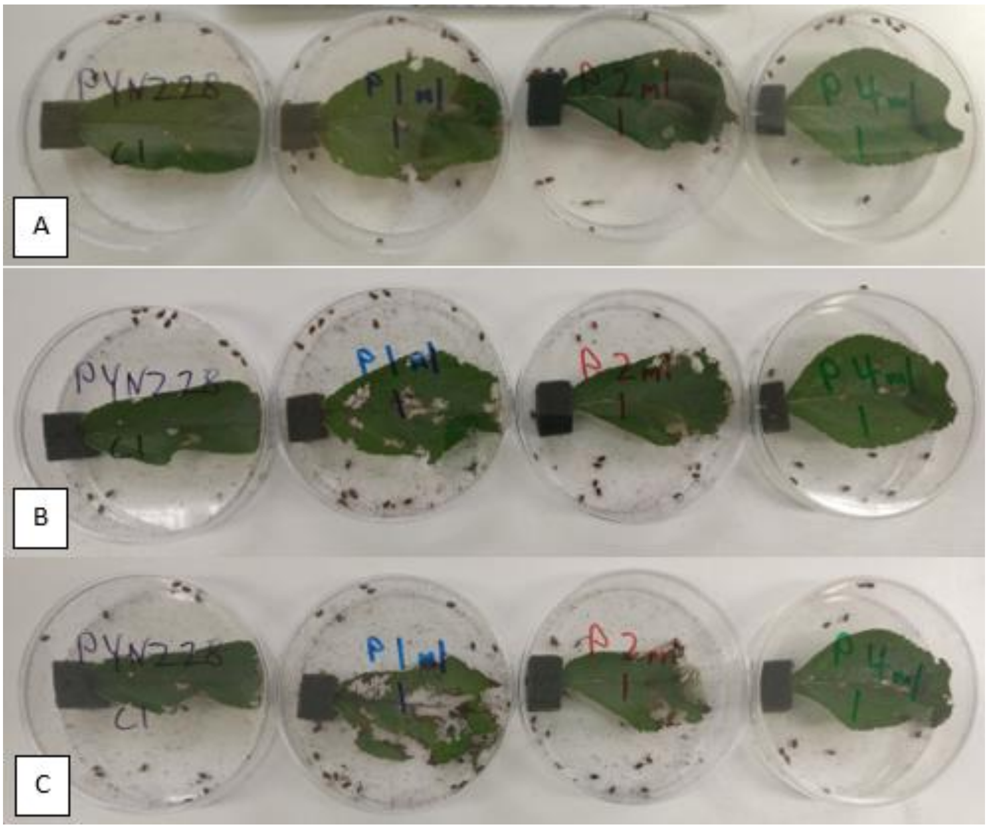

Figure 2.6: *The first replicate of the PYNZ28 treatments applied to leaves - (A) 2 days after application, (B) 5 days after application, and (C) 7 days after application. C, Control; P1ml, 1000 ml/100 L water; P2ml, 2000 ml/100 L water; P4ml, 4000 ml/100 L water*

Application to beetles (direct contact)

Beetles were anaesthetised using carbon dioxide to prevent them from escaping during application (Figure 2.7). Each treatment was sprayed with 2ml of a diluted chemical or water (Control) on each group of 20 beetles. After application, the beetles were transferred into a Petri dish with an unsprayed apple leaf and allowed to feed. Each Petri dish with 20 beetles constituted a replicate. Death and moribundity of the beetles were assessed 2, 5, and 7 days after transfer to determine the percent control achieved by each treatment. I defined dead, moribund, and percent control as above. There were five replicate Petri dishes (5 x 20 beetles) for each treatment.

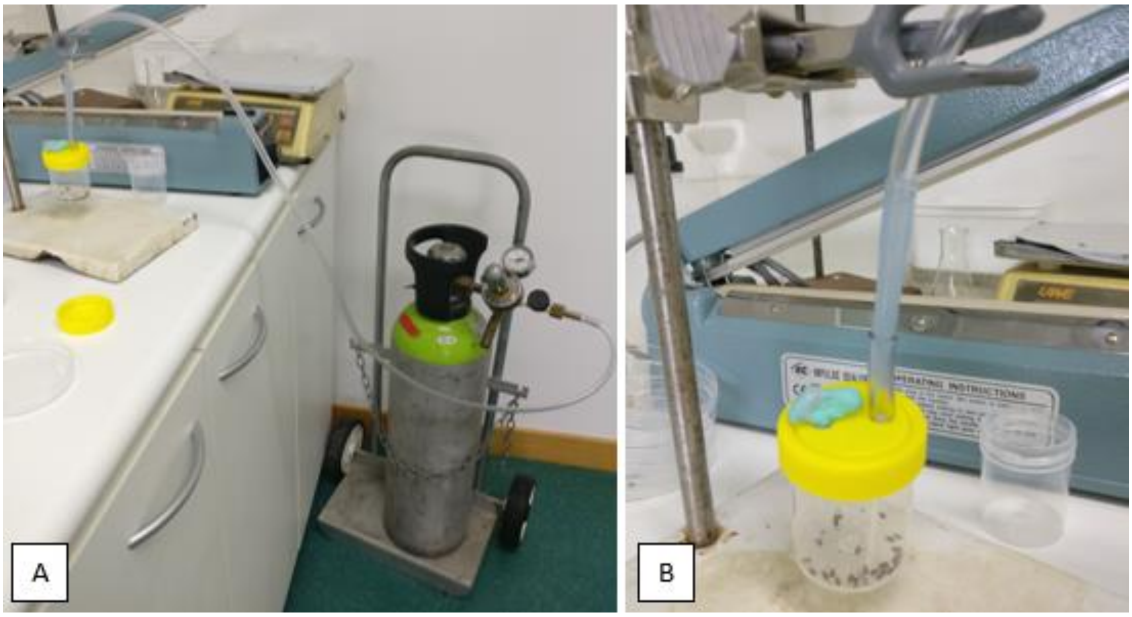

Figure 2.7: *(A) The set up used to anaesthetise beetles, and (B) anaesthetised beetles in a modified specimen vial connected to the carbon dioxide bottle*

2.3.5 Data analysis

Statistical analysis was carried out using R version 4.0.5. Generalized linear models with a binomial distribution were fitted to the data. This choice of model was suitable as the data included many units (bronze beetle) and a binary outcome (healthy or not) for each unit. ANOVA (F-test) and pairwise t-tests were carried out from these models to allow comparison of the efficacy of different treatments. The rejection level was set at $P > 0.05$.

Normality of the models was determined by examining a Normal Q-Q (quantile-quantile) plot for each assessment day to check if the residuals are normally distributed. The closer to the diagonal line in the centre of the plot, the more normal the residuals are. However, as the data were not normally distributed, the Q-Q plots contain a transformed residual (Standardised Pearson residual). For all three plots, the transformed residuals were close enough to the diagonal line that the models were acceptable to use.

2.4 Results

2.4.1 Assessment at 2 days after application

The percent control provided by all treatments, including the Control treatment, at 2 days after application (DAA) is shown in Figure 2.8. There were significant differences in the percent control provided between treatments ($F_{12,145} = 84.2$, $p < 0.001$). There was also a significant interaction between these 13 products and application rates as well as between application methods ($F_{13,145} = 4.6$, $p < 0.001$). The results indicate that the conventional insecticides Calypso and Avaunt provided a significantly higher level of control than did Vayego and the organic insecticides Entrust, NeemAzal-T/S, and PYNZ28. For the organic insecticides, Entrust, at the concentration of 80 ml/100 L water, provided about 50% control regardless of application method. This was a significantly higher level of control than was provided by lower concentrations of Entrust and all the other organic insecticides. At lower concentrations of Entrust and all concentrations of NeemAzal-T/S, application to leaves gave significantly better control than direct application to beetles. PYNZ28 at all tested concentrations did not appear effective in control of bronze beetle.

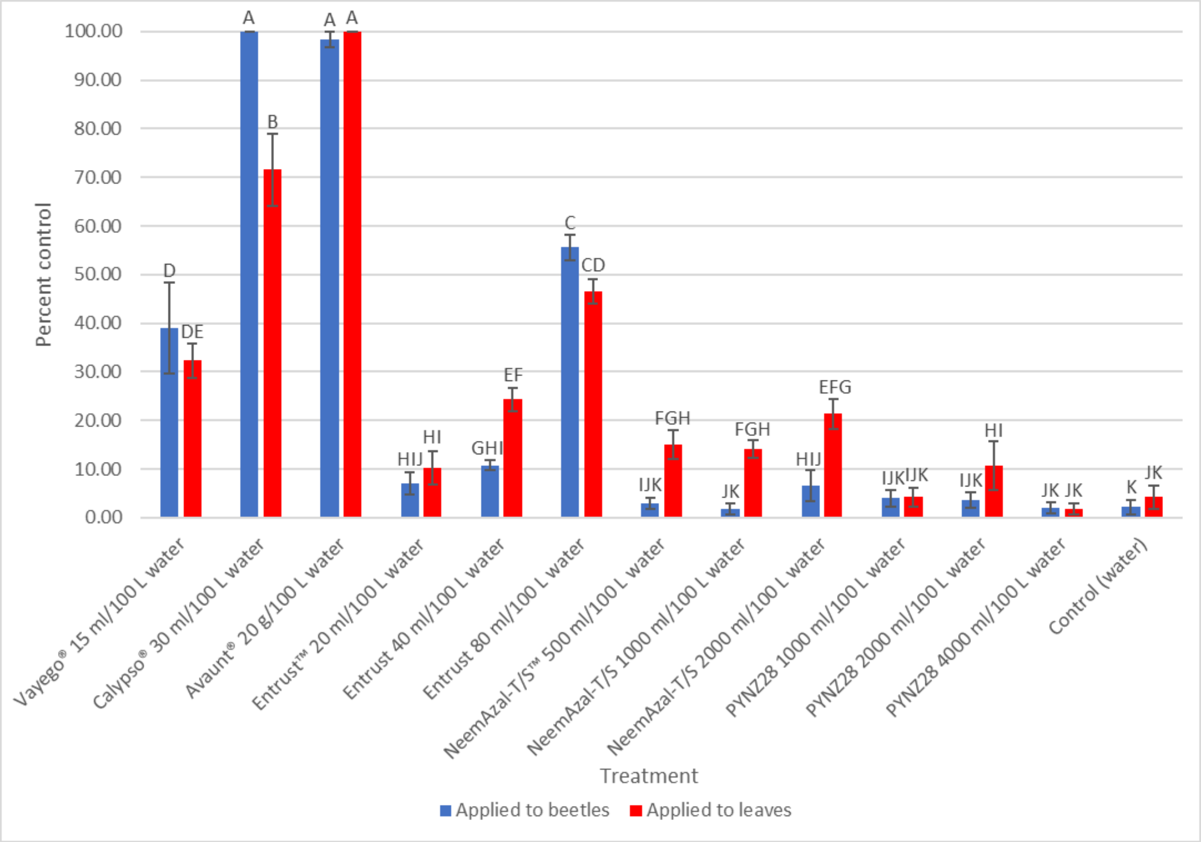

Figure 2.8: *Mean (± SE) percentage of dead and moribund bronze beetle (percent control) at 2 days after application. Columns with the same letters are not significantly different (P > 0.05)*

2.4.2 Assessment at 5 days after application

The level of control that was provided by all treatments at 5 DAA can be seen in Figure 2.9. There were significant differences between the percent control provided by the different treatments ($F_{12,145}$ = 80.2, p < 0.001), as well as a significant interaction between the 13 treatments and the application methods (F =$_{13,145}$ 1.9, p < 0.05). The results indicate that Entrust, at concentrations of 40 ml/100 L water and 80 ml/100 L water, reached over 90% control. This level of control was comparable to that reached by all three conventional insecticides and significantly higher than all other organic insecticide treatments. All concentrations of NeemAzal-T/S had 43-55% control. However, the control achieved when applied to leaves was significantly better than when applied to beetles.

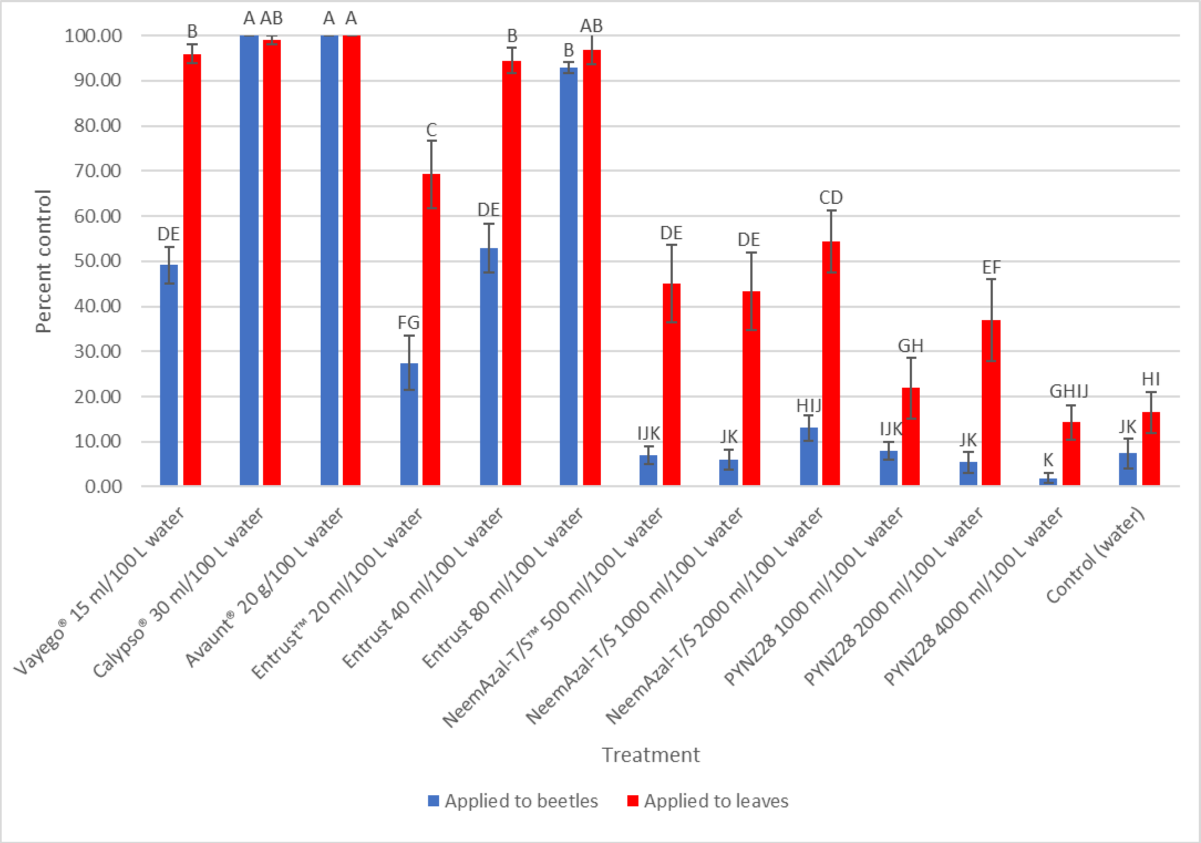

Figure 2.9: *Mean (± SE) percentage of dead and moribund bronze beetle (percent control) at 5 days after application. Columns with the same letters are not significantly different ($P > 0.05$)*

2.4.3 Assessment at 7 days after application

At 7 DAA, the percent control provided by all treatments can be seen in Figure 2.10. There were significant differences between the percent control provided by the 13 different treatments ($F_{12,145} = 33.1$, $p < 0.001$). There was also a significant interaction between these 13 products and application rates and the application method ($F_{13,145} = 2.2$, $p < 0.05$). Findings show that all three trialled concentrations of Entrust when applied to leaves achieved over 90% control. This also pertained to Entrust applied to beetles at a concentration of 80 ml/100 L water. In addition, all concentrations of NeemAzal-T/S when applied to leaves attained over 80% control. The control provided by these Entrust and NeemAzal-T/S treatments was comparable to all three conventional insecticides and significantly better than the control provided by the remaining organic treatments and Control.

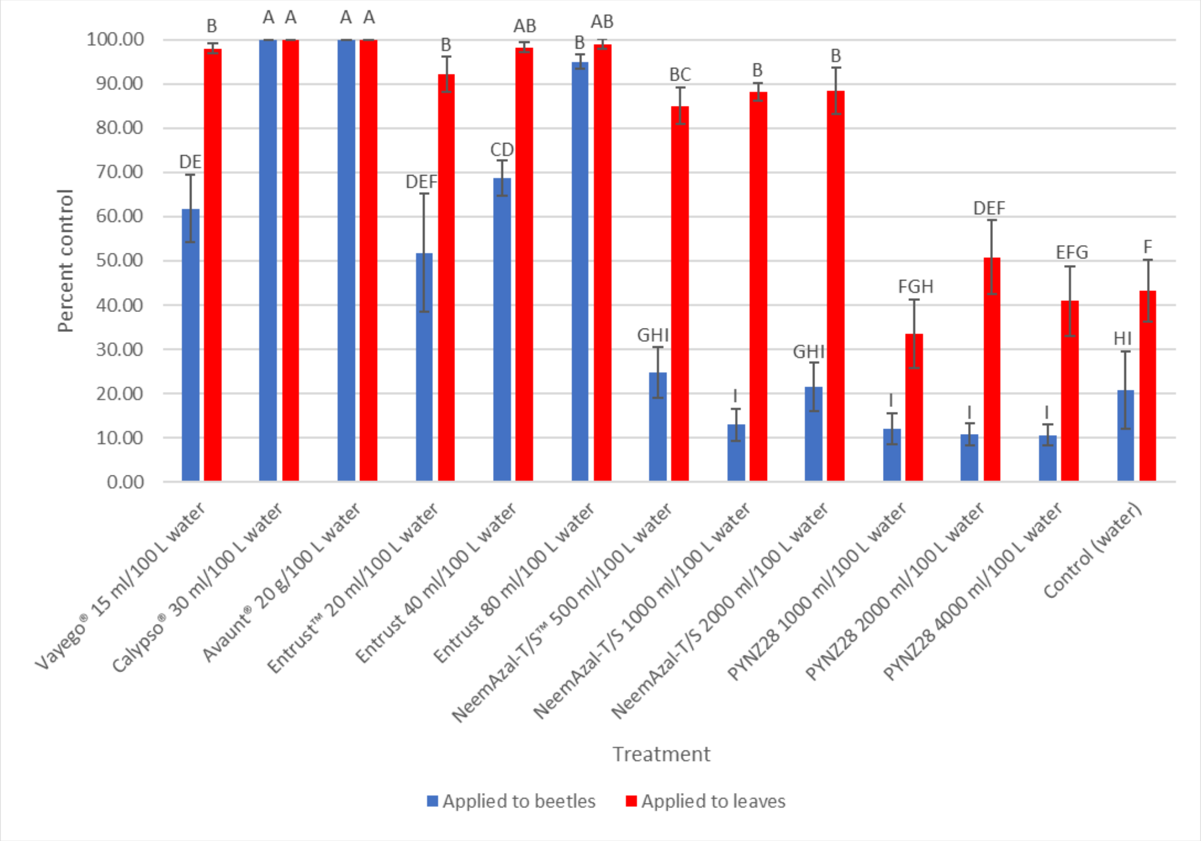

Figure 2.10: *Mean (± SE) percentage of dead and moribund bronze beetle (percent control) at 7 days after application. Columns with the same letters are not significantly different (P > 0.05)*

2.5 Discussion

My results indicate that the organic insecticide Entrust has the highest potential for controlling bronze beetle, and NeemAzal-T/S has the potential to be used in conjunction with Entrust. At 2 DAA, Entrust at the concentration of 80 ml/100 L water provided around 50% control regardless of the application method. In comparison, at 5 DAA, Entrust, at the concentrations of 40 ml/100 L water applied to leaves and 80 ml/100 L water applied to either beetles or leaves, provided over 90% control. At all concentrations, NeemAzal-T/S applied to leaves achieved 43-55% control at 5 DAA. At 7 DAA, all three concentrations of Entrust applied to leaves and 80 ml/100 L water applied to beetles provided over 90% control. Comparatively, all concentrations of NeemAzal-T/S applied to leaves provided over 80% control. At 7 DAA, the percent control was not significantly different between the Control (water) and PYNZ28 treatments (10.7-50.8%). This suggests that PYNZ28 is not an effective insecticide for controlling bronze beetle and the only control observed may be caused by unfavourable conditions in the Petri dishes for both treatments.

It is generally accepted that a reduction of a pest population over 75% (compared to an untreated Control) indicates that an organic insecticide provides a sufficient level of control (Caldwell et al., 2012; Dively et al., 2020). However, it is also considered that reductions of 50-74% provide a fair level of control (Caldwell et al., 2012). Based on the assessment at 2 DAA, the only organic insecticide that marginally achieved a fair level of control was Entrust at the concentration of 80 ml/100 L water when applied directly to beetles. Nevertheless, at 5 DAA, Entrust provided sufficient control at the concentrations of 80 ml/100 L water (applied to both beetles and leaves), and 40 ml/100 L water (applied to leaves), while Entrust at the concentration of 20 ml/100 L water (applied to leaves) achieved a fair level of control. However, at 7 DAA, there was no sufficient control provided by either Entrust or NeemAzal-T/S, with only fair control provided by Entrust 40 ml/100 L water applied to leaves and both 80 ml/100 L water treatments. This is due to the high beetle mortality in the Control (water only) treatments. At 7 DAA, the percent control caused by the organic treatments appeared low when compared to untreated Control, even though the percent control reached up to 98.98% for Entrust and 88.35% for NeemAzal-T/S.

Many factors need to be considered before an insecticide is adopted for the control of bronze beetle in organic orchards. These include the cost, the number of applications required per season, resistance development, and adverse impacts on beneficial insects. For example, Entrust costs $477.39 for 1 L and NeemAzal-T/S costs $101.73 for 1 L (Table 2.2), but the former can achieve good control at a much lower concentration than NeemAzal-T/S (Figures 2.8-2.10). Because of short residue periods, most organic insecticides need repeated applications during the season. However, the reported persistence of Entrust in the field is about twice as long as NeemAzal-T/S, thus providing an advantage (Balusu & Fadamiro, 2012; Kumar & Poehling, 2006; Schmutterer, 1990). NeemAzal-T/S additionally has over 60 active ingredients which help prevent resistance development (EcoGrape Service, 2021a, 2021b). In contrast, resistance against Entrust may develop if it is used excessively (Dow AgroSciences, 2017). Another factor to consider is that Entrust has adverse effects if directly applied to honeybees, but no impact after the spray has dried (Dow AgroSciences, 2017). There is also the potential for adverse effects for *Aphelinus mali* (parasitoid of woolly apple aphid). To preserve *A. mali*, application should occur either late in the season or when the insects activity is low (Dow AgroSciences, 2017; Rogers et al., 2011). In direct comparison to this, NeemAzal-T/S has no or insignificant toxicity effects on honeybees, predators, and beneficial insects (EcoGrape Service, n.d.; Ogburn & Walgenbach, 2019).

The differences between trials carried out in a laboratory and field trials are also important to consider. This is because organic insecticides are prone to environmental degradation when applied in the orchard, which means that they may be effective for a shorter time in the field than in the laboratory (DiGiacomo et al., 2021; Dively et al., 2020). Due to this difference, laboratory trials only indicate if there is a possibility for sufficient control of bronze beetle. Therefore, field trials are required to truly understand if a spray programme of Entrust and NeemAzal-T/S might provide sufficient control of bronze beetle.

The results of this experiment are supported by several studies on other pests (Evans & Hallett, 2016; Shrestha et al., 2020; Sial et al., 2019). My experiment, like these studies, trialled insecticides containing spinosad, pyrethrin, and azadirachtin, which are the three most common active ingredients for organic insecticides (Dively et al., 2020; Kamminga et al., 2009). Evans and Hallett (2016) trialled these products on swede midge (*Contarinia nasturtii* Kieffer), Shrestha et al. (2020) on pea leaf weevil (*Sitona lineatus* L), and Sial et al. (2019) on spotted-wing drosophila (*Drosophila suzukii* Matsumura). All three studies used Entrust and found this product was the most effective organic insecticide trialled, consistent with my experiment. The products used in these studies containing azadirachtin also provided moderate mortality, which is also consistent with my experiment. However, in these studies, pyrethrin caused moderate mortality (Evans & Hallett, 2016; Shrestha et al., 2020; Sial et al., 2019), which did not occur in my experiment. This difference could be because of the different concentrations and formulations of the products, as well as the target pests that the products were trialled on.

Chapter 3 Host plant attraction

3.1 Abstract

Bronze beetle use plant volatiles to locate host plants. This has the potential to be used to develop a control programme based on the use of attractants. This experiment attempted to determine the attractiveness of apple, plum, and blackberry leaves to bronze beetle using an olfactometer. However, my results were inconclusive, and any differences observed were likely due to random variability. This was possibly caused by the methodologies that were used. Future work could focus on the improvement of the experimental criteria used to determine beetle responses.

3.2 Introduction

Many insects, including bronze beetle, locate their host plants using plant volatiles (Doddala, 2012; Wang et al., 2020). This response to olfactory cues from plants has potential to be useful for pest control in organic apple production. If the host plant(s) to which bronze beetle are most strongly attracted can be identified, then further research could allow the use of these volatiles for pest monitoring, mass trapping, and lure and kill operations (Addesso et al., 2011; Davidson et al., 2008). This knowledge could have value for the organic apple industry in terms of increased control of bronze beetle and a decreased requirement for the use of other control methods. Research on this concept has been widely carried out on other beetles, such as the oak ambrosia beetle (*Platypus quercivorus*) (Pham et al., 2020), western tarnished plant bug (*Lygus hesperus*) (Blackmer et al., 2004), and oriental fruit moth (*Cydia molesta*) (Natale et al., 2004). For these pests, a laboratory olfactometer bioassay method was used to investigate the host plants that the pests were attracted to. Seedlings or shoots of the plants being trialled were used as odour sources to determine the attractiveness of the volatiles emitted from the plant samples (Blackmer et al., 2004; Natale et al., 2004; Pham et al., 2020).

If the preferred host plant of bronze beetle can be identified, then there is opportunity to identify the specific volatiles or compounds from the plant which are attractive, and then extract them. This knowledge could then be applied in pest control programmes in NZ organic apple orchards. Research related to this concept has been carried out for the pollen beetle (*Meligethes aeneus*) (Mauchline et al., 2005) and the spotted wing drosophila (*Drosophila suzukii*) (Revadi et al., 2015). For the pollen beetle, a range of essential oils from several plants were trialled with the purpose of determining how the use of these semiochemicals could manipulate the behaviour of the beetle if used in a push-pull control strategy. This strategy uses attractants to 'pull' natural enemy populations into the crop, and repellents to 'push' pest populations out (Mauchline et al., 2005). For the spotted wing drosophila, extracts from five fruit species known to be attractive revealed 91 different compounds, 29 of which produced reliable antennal responses from female insects. The compounds which produced responses tended to be esters and alcohols, which could be used as attractants for the pest (Revadi et al., 2015).

Bronze beetle are thought to be most highly attracted to plants from the Rosaceae and Myrtaceae families (Doddala et al., 2016). Rosacea is one of the most predominant families of fruit crops in the temperate zones, where NZ is located (Bennett, 2010). Because of this, three crops from the Rosaceae family were chosen for the olfactometer experiment. These crops were apple, plum, and blackberry, which all have economic importance as fresh and processed fruit. Apple is the most economically important Rosaceae crop, while plum is also popular, and blackberry is growing in popularity (Hummer & Janick, 2009). The aim of this study was to determine whether there were any differences between the attractiveness of apple, plum, and blackberry leaves to bronze beetle.

3.3 Materials and Methods

3.3.1 Experimental device

A four-choice olfactometer was used to establish bronze beetle host plant attraction (Figures 3.1 and 3.2). The olfactometer used was 100 cm across, while the central arena had four points, and was 26 cm across with a depth of 4 cm. Treatment areas of 2.5 cm were marked on the olfactometer. These marked treatment areas were located at the ends of each of the four points of the central arena, adjacent to where air entered into the arena. The base of the olfactometer (30 cm across) had a plexiglass covering held in place with butterfly screws.

There were four globular glass treatment chambers attached to each side of the base of the olfactometer. Fitted into the ends of these chambers were tubular connectors into which air flowed from Tygon® tubing.

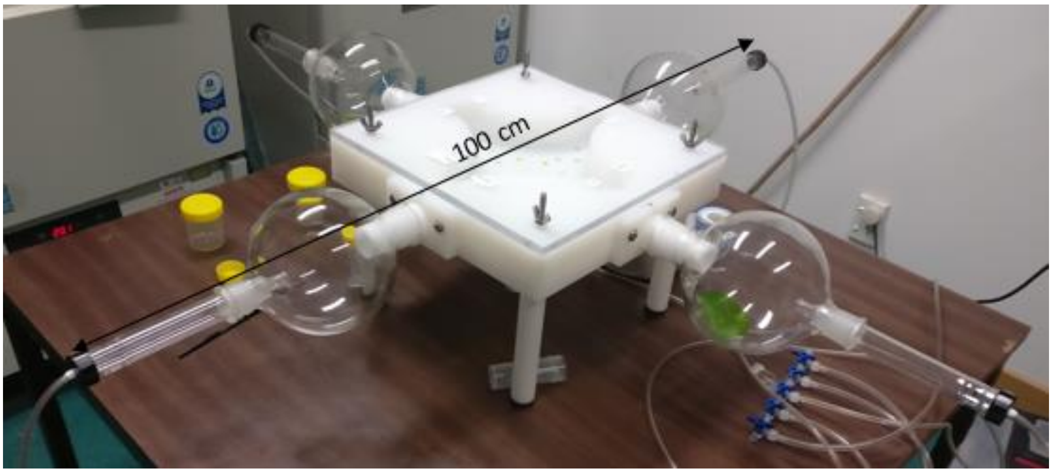

Figure 3.1: *Four-choice olfactometer used for olfactometer experiment*

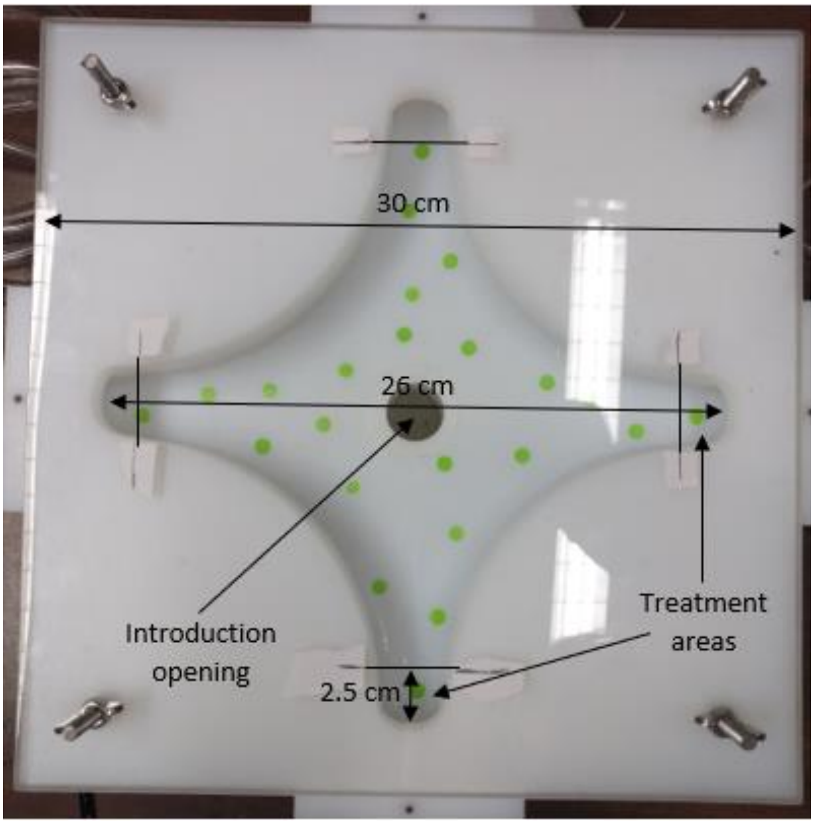

Figure 3.2: *Central arena of four-choice olfactometer used for olfactometer experiment*

3.3.2 Bioassay procedures

Bronze beetle were collected from wild roses growing on Kopanga Road, Havelock North, to prevent host bias. They were collected using the method previously discussed (section 2.3.1). Then held for starvation in specimen vials (5.6 cm height and 4.4 cm diameter) with 10 beetles per vial. They were starved for 24 hours at 20°C before the bioassay occurred to induce host finding (Doddala, 2012).

A Reciprotor electromagnetic piston pump was used to create airflow through the olfactometer. An airflow meter was used to determine the flow rate, a needle valve regulator used to control the flow, and an activated charcoal filter absorbed contaminants from the air (Figure 3.3) (Addesso et al., 2011; Doddala, 2012). All connecting tubing used was Tygon tubing, due to its ability to resist chemicals and prevent contamination (Saint-Gobain, 2021). Air was flowing concurrently through all four treatment chambers of the olfactometer into the central arena and out through the central introduction opening. The air flow rate at the introduction opening was 280 ml/minute to ensure sufficient air flow to transfer the plant volatiles to the beetles in the central arena. The airflow through each of the four treatment chambers was equal.

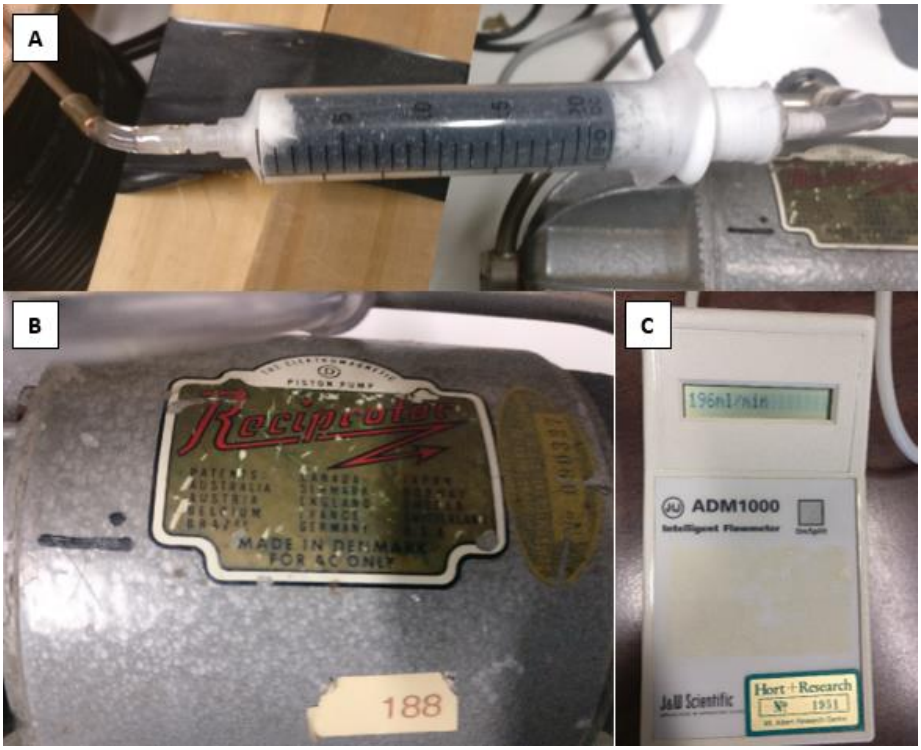

Figure 3.3: *(A) activated charcoal filter, (B) Reciprotor electromagnetic piston pump, and (C) airflow meter*

Each experimental run consisted of one starved beetle, randomly selected regardless of sex, being released into the introduction opening at the centre of the arena with the aid of a paint brush and left for 5 minutes (Lacey et al., 2008; Riddick et al., 2000). The beetle was recorded as making a choice either if it spent at least 3 minutes, or the last minute of the experimental run, within any marked treatment area (Addesso et al., 2011; Ginzel & Hanks, 2005). If the beetle did not make a choice within 5 minutes, then 'no response' was recorded. Each beetle was only used once and 100 beetles were used to complete 100 experimental runs (Blackmer et al., 2004; Doddala, 2012). The four treatment chambers, adjacent to the four marked treatment areas, contained leaves from three different host crops (apple, plum, and blackberry). With one chamber remaining empty as a control.

After each experimental run, the treatment chambers were alternated by rotating in a clockwise direction to control for positional bias (Natale et al., 2004; Riddick et al., 2000). The leaves in the treatment chambers were also changed every 15 trial runs to ensure freshness of samples (Natale et al., 2004). Finally, the olfactometer was cleaned with n-hexane and air dried at the end of each day of use, after approximately 25 runs (Doddala, 2012; Lacey et al., 2008).

3.3.3 Data analysis

Statistical analysis was carried out using R version 4.0.5. Pearsons's chi-squared test was applied using the counts of outcomes to establish if the beetles were likely to pick any of the four treatment choices or to not respond. This test was suitable to use because it determined if the different categorical variables or response options (apple, plum, blackberry, control, and 'no response') had a significant correlation between them.

A second chi-squared test was used to determine if there was a significant difference between the treatment options (apple, plum, blackberry, and control). The 'no response' option was excluded from this second test to allow comparison of only the experimental runs that resulted in a response to a treatment.

3.4 Results

My results show that there was a significant difference between the number of beetles which chose an option and those which made 'no response'. With a significantly higher number of beetles not responding to any treatment option ($X^2 = 240.9$, df = 4, P = < 0.0001) (Figure 3.4). When the 'no response' data were excluded, there was no significant difference between the number of beetles which responded to each treatment ($X^2 = 2.89$, df = 3, P = 0.409) (Figure 3.5).

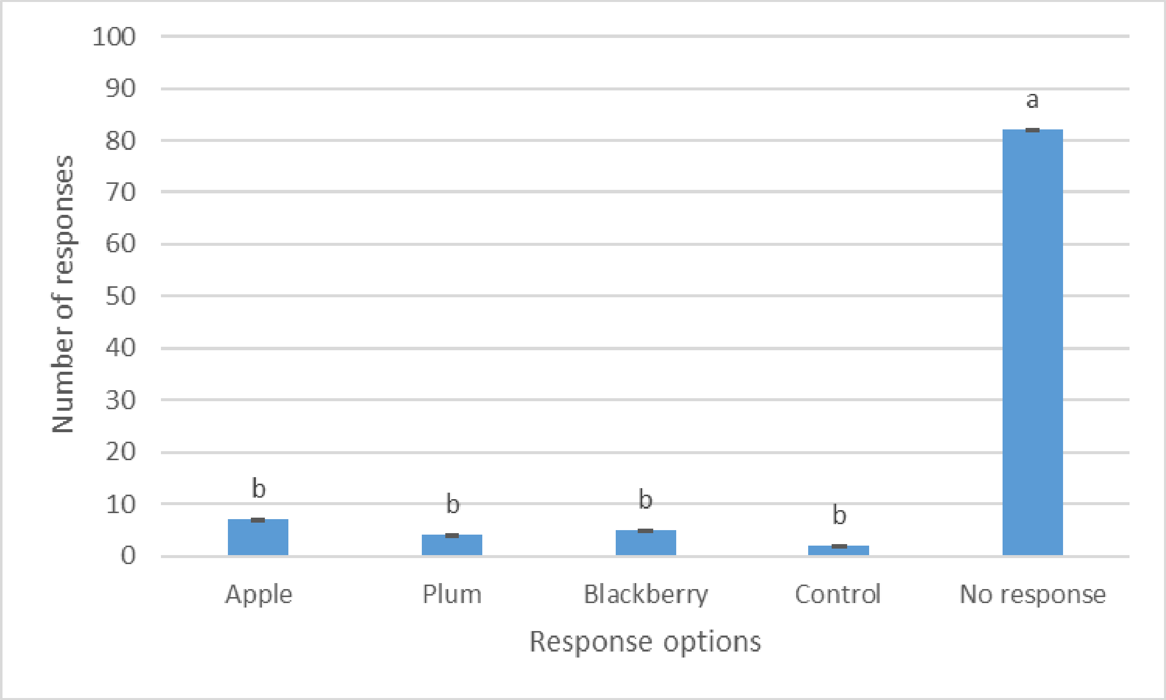

Figure 3.4: *Observed number (± SE) of responses to each of the five response options. Columns with the same letters are not significantly different (P > 0.05)*

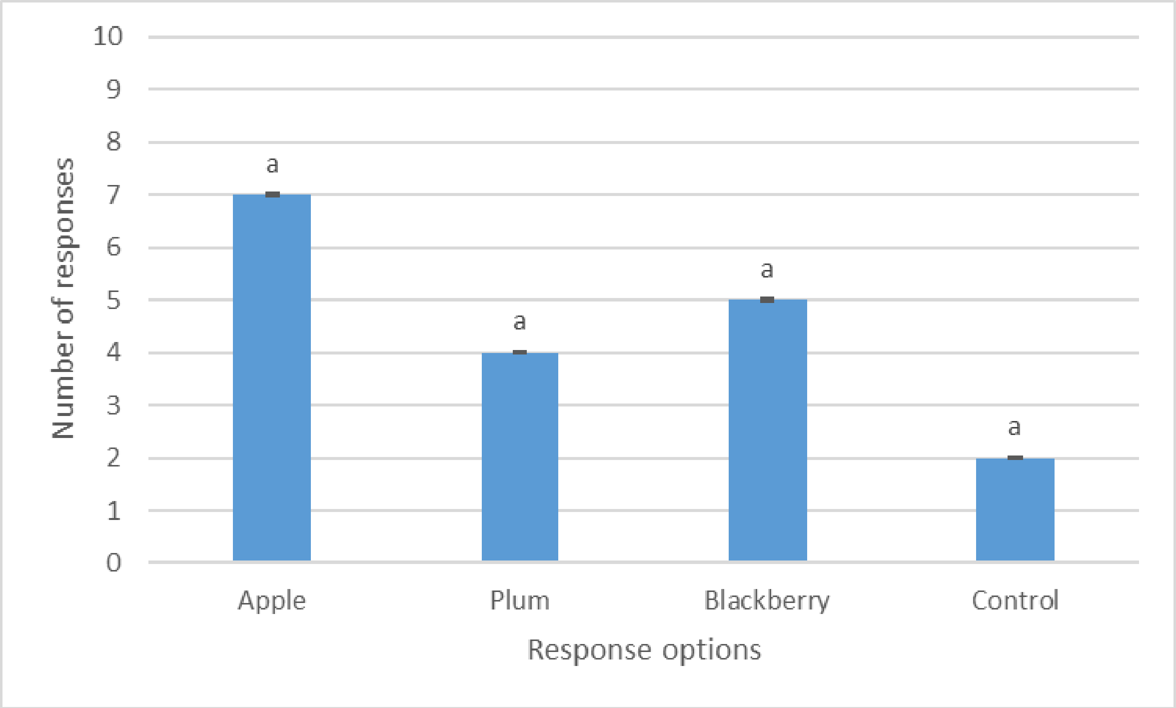

Figure 3.5: *Observed number (± SE) of responses to the four treatment response options (excluding 'no response'). Columns with the same letters are not significantly different (P > 0.05)*

3.5 Discussion

The results of the olfactometer experiment were unexpected, with only 18 out of 100 beetles responding to any of the treatments. Of the 18 beetles that responded, there were no significant differences between those that responded to each of the four treatment options. Any differences between the number of beetles which chose each option appears to be due to random variability. There are a range of possible reasons for this. For example, the treatment areas may have been too small. If the treatment areas were made larger (Figure 3.6), then a higher number of beetles may have made a choice, as there would be a larger treatment area associated with each treatment (Scholz et al., 1997; Verheggen et al., 2007). Another possible option is to increase the trial run time to give each beetle more time to make a choice. For instance, the trial run time could be increased from 5 minutes to 10 minutes (Lacey et al., 2008; Riddick et al., 2000), 15 minutes (Barrett et al., 2018; Ginzel & Hanks, 2005), or even 20 minutes (Doddala, 2012). All host plants tested belong to the Rosaceae family (Doddala et al., 2016; Hummer & Janick, 2009). This could have caused most beetles to be unable to respond in the allowed time because they were attracted to all three options and became disorientated. One way to overcome this potential drawback would be to include host plants from different plant families as the treatment options.

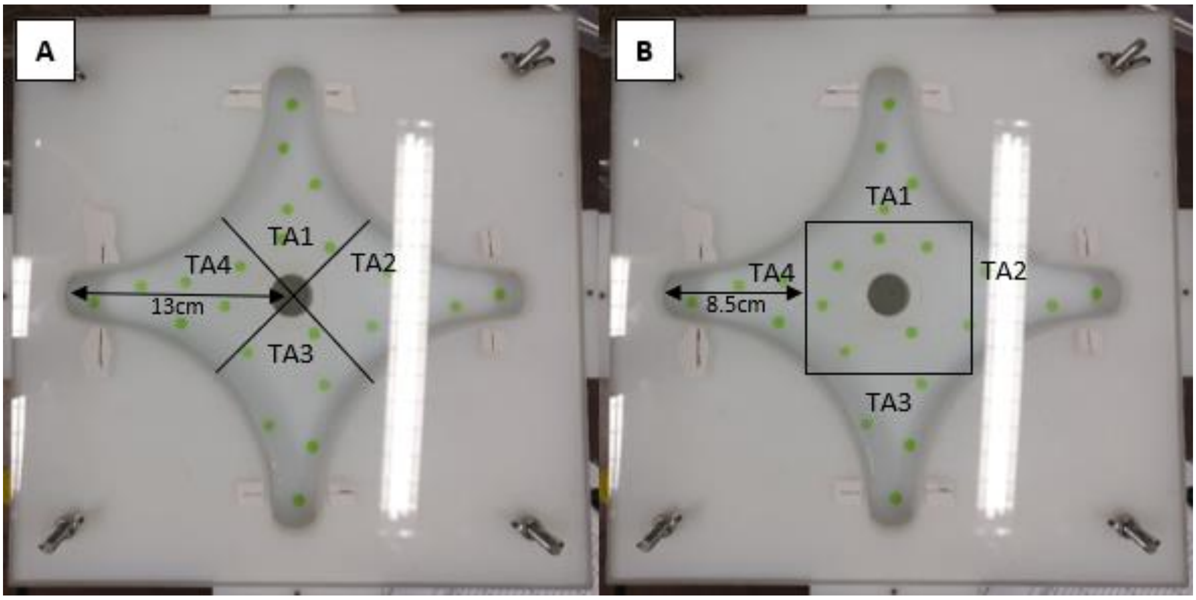

Figure 3.6: Alternative treatment areas (TA) - (A) 13 cm across based on Scholz et al. (1997), and (B) 8.5 cm across based on Verheggen et al. (2007)

Chapter 4 Conclusion

The aims of this thesis were focused on studying the potential of several organic methods for controlling bronze beetle, one of the most prevalent pests for organic apple production in NZ. This was carried out using an evaluation comparing the efficacy of several conventional and organic insecticides. In addition, a preliminary investigation into the host plant attraction of bronze beetle was carried out.

4.1 Objectives and outcomes

4.1.1 Objective one

Determination of the control (combined percent dead and percent moribund) of bronze beetle provided by conventional (Vayego, Calypso, and Avaunt) and organic (Entrust, NeemAzal-T/S, and PYNZ28) insecticides in the laboratory.

Overall, the conventional insecticides provided a higher level of control of bronze beetle than the organic insecticides when tested in the laboratory (Figures 2.8-2.10). This was expected because conventional insecticides are known to have higher efficacy than their organic counterparts (DiGiacomo et al., 2021; Dively et al., 2020). However, the organic insecticide Entrust provided sufficient control and NeemAzal-T/S showed potential for being used in a spray programme in conjunction with Entrust. Previous studies on other pests by Evans and Hallett (2016), Shrestha et al. (2020), and Sial et al. (2019) support these results. They state that out of the organic insecticides with the three most common active ingredients, insecticides based on spinosad, such as Entrust, are the most effective. Additionally, they state that insecticides with azadirachtin as the active ingredient, such as NeemAzal-T/S, also have potential.

In general, the efficacy of the organic insecticides improved as the concentration applied increased. This trend was apparent for both Entrust and NeemAzal-T/S, but not for PYNZ28. However, the differences were not always significant and were less apparent at 7 DAA. In terms of time taken for efficacious results to occur, it appears that both Calypso and Avaunt are fast acting (2 DAA), while Vayego (5 DAA), Entrust (5 DAA), and NeemAzal-T/S (7 DAA) are slower acting insecticides. PYNZ28 did not appear to have any efficacy at any of the three assessment days.

4.1.2 Objective two

Determination of bronze beetle preference between apple, plum, and blackberry odours.

In the olfactometer experiment the results were inconclusive. This means that it could not be determined if bronze beetle are most strongly attracted to apple, plum, or blackberry volatiles and odours. Very few beetles (18 out of 100) made a choice and any differences in the number which chose each of the three crop options was likely due to random variability. Therefore, this experiment could not determine which of these three plants is most attractive to bronze beetle.

4.2 Future research

Future research related to the insecticide trials could be to trial Entrust and NeemAzal-T/S in the field. Although the laboratory results were promising, efficacy in the field is often lower than in the laboratory due to environmental conditions causing degradation. Therefore, it is important to investigate residue toxicity under field conditions (Balusu & Fadamiro, 2012; Leach et al., 2017). Also, in the laboratory, direct toxicity was the only aspect of control monitored, while in the field, anti-feedant and repellence effects may also occur. Trialling these two insecticides in the field could provide insight into the behavioural impacts of the products as well as their potential for commercial use (Morehead & Kuhar, 2017).

For the olfactometer experiment, future research could be to improve the experimental device and procedures as discussed in Chapter 3. For example, trials could be conducted using the alternative treatment areas shown in Figure 3.6 or the trial run time could be increased (Lacey et al., 2008; Verheggen et al., 2007). Furthermore, the crops tested could also include fruit crops from a variety of plant families (Doddala et al., 2016).

4.3 Final summary

Based on the results of these experiments, Entrust (spinosad) and NeemAzal-T/S (azadirachtin) are the organic insecticides with the most potential for controlling bronze beetle. When Entrust was tested in the laboratory, it had comparable efficacy to the conventional insecticides trialled. NeemAzal-T/S also appeared to have potential for use in a spray programme to control bronze beetle, but at a lower level than Entrust. These findings are novel as according to previous research there are no organic insecticides with the potential to provide control of bronze beetle (Delate et al., 2008; Doddala, 2012; Rogers et al., 2006). This research also provides direction for possible further research on these two insecticides for controlling bronze beetle in NZ organic apple orchards.

Based on my results from the olfactometer experiment the attractiveness of apple, plum, and blackberry to bronze beetle could not be determined. This is due to most beetles not responding to any of the treatments, resulting in only a small amount of data to analyse. This is possibly caused by faults in the methodology used for this experiment. However, further studies using the modifications suggested previously may have value for the organic apple industry in NZ.

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