Biology and Integrated pest management of the elephant weevil borer, *Orthorhinus cylindrirostris* (EWB) (Fabricius) (Coleoptera: Curculionidae) in blueberries

Gregory Murdoch
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Biology and integrated pest management of the elephant weevil borer, *Orthorhinus cylindrirostris* (EWB) (Fabricius) (Coleoptera: Curculionidae) in blueberries

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A thesis submitted to the University of Sydney in fulfilment of the requirements for the degree of Doctor of Philosophy
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Preface

The work presented in this thesis, to the best of my knowledge and belief, is original and as a result of my work, except where due acknowledgement has been made. This work has not been submitted either whole or in part for the award of any other degree.

Greg Murdoch

[Signature]
Abstract

Orthorhinus cylindrirostris (Fabricius) (Coleoptera: Curculionidae), elephant weevil, is an Australian native insect and is a known pest of citrus, grapes, apples, peaches, apricots and recently blueberries. Orthorhinus cylindrirostris has steadily increased its population density on blueberry plants at Blueberry Farms of Australia (BFA) from early 1990. By 1999 it was clear there was a severe problem with this insect. The lack of knowledge and prior research regarding O. cylindrirostris was the primary reason for this project. The objective was to gain an improved understanding of O. cylindrirostris biology and potential management strategies for this important pest. Cultural and chemical control options were evaluated, including the use of myco-insecticides. Although attacks by EWB do not always cause tree mortality, tree growth and quality may be reduced significantly. Effective management of O. cylindrirostris requires integration of control tactics including cultural practices to avoid plant stress, crop sanitation to reduce the spread of adult weevils, and the use of commercially available insecticides. Results obtained from this research will increase the effectiveness and reduce the costs of control measures against O. cylindrirostris.

The biology of O. cylindrirostris was investigated over two years on four known host plants: Acacia falcata, Angophora floribunda, Citrus limon var. Eureka and Vaccinium corymbosum var. 390. Variables measured in this study included duration of all life stages and the entire life cycle, host plant and sex effects, and adult behaviour. Females spent up to five hours boring a hole for oviposition whilst males engaged in copulation and mate guarding. The larvae develop in the branches, crown and roots of healthy blueberry plants and Australian native trees. O. cylindrirostris body shape and size shows a large diversity both in males and females. Male adults are distinguishable from females by the antennal base lying closer to the tip of the rostrum and having large tarsal pads, which are used in male-male combat when guarding a female. Survival and development time differed among host tree species with V. corymbosum considered more suitable for O. cylindrirostris than other plant species because it had a higher emergence of adults. Host plant had no effect on insect sex, adult lifespan, insect weight and insect length.
The density of *O. cylindrirostris* was surveyed on the premises of BFA during 2007 and 2008 by counting adult emergence holes on blueberry crowns on a 5 x 5m grid (every fifth blueberry tree in every fifth row of each field). *Orthorhinus cylindrirostris* attacked all varieties but the attack and/or survival rate varied between varieties. Tolerance of *O. cylindrirostris* was limited across all blueberry varieties surveyed with all Rabbiteye (RE) and Southern high-bush (SHB) varieties showing at least some susceptibility to *O. cylindrirostris* damage, however modern RE varieties were attacked by fewer *O. cylindrirostris* relative to SHB varieties, as indicated by fewer adult emergence holes. The newer RE varieties Powderblue, Premier and Britwell exhibited more tolerance for *O. cylindrirostris* than all other blueberry varieties. Older RE varieties Becky blue and Bonito were very susceptible to *O. cylindrirostris* infestation. Bonito has been totally eaten out by *O. cylindrirostris* larvae and has been removed from planting at BFA. Becky blue is still utilised due to its high quality berries, although plants are infested by significantly higher densities of *O. cylindrirostris* than all other varieties and this variety may be serving as brood trees for further infestations. All SHB varieties showed moderately high susceptibility to *O. cylindrirostris* damage with SHB 115 the least susceptible to *O. cylindrirostris* attack.

The 5m x 5m grid survey of *O. cylindrirostris* distribution was compared with historical emergence data (obtained from BFA records) using geo-statistical techniques to characterise spatial and temporal variability of *O. cylindrirostris*. Regression kriging was used to produce 5-m resolution continuous maps for adult *O. cylindrirostris* populations (i.e. emergence holes and adult numbers) and blueberry variety location (historic and present). The two maps showed that *O. cylindrirostris* damage occurred most often in the southern area of the property. The area with the highest presence of *O. cylindrirostris* was also where the most susceptible varieties were found historically and the distribution of *O. cylindrirostris* populations is likely to correlate with variety susceptibility and selection.

While there are broad-spectrum and selective insecticides available for the control of coleopteran pests in fruit crops, no insecticides are currently registered for use against *O. cylindrirostris* in blueberries. The management of *O. cylindrirostris* is likely to focus on control of the free-living adult stage rather than the egg, larval and pupal stages, which are protected inside the host plant. Chemical residues on harvested blueberries and compatibility with
pollinators are key factors that affect the choice of chemical control options. The insecticides that caused the greatest mortality in *O. cylindrirostris* adults under laboratory and field conditions were applications of Indoxacarb (Dupont® Avatar®) Clothianidin (Sumotomo® Clothianidin 200SC®), imidacloprid (Bayer® Confidor 200SC®), imidacloprid (Bayer® Initiator®), Nutritech® Mycoforce® (which contains Beauveria bassiana, Verticillium leccinii and Metarhizium anisopliae) and an aqueous suspension of Beauveria bassiana var EWB (a locally derived isolate).

*Beauveria bassiana* showed potential for use against *O. cylindrirostris* and has been isolated from field-collected specimens. However some commercial pesticides registered for use in blueberries may be harmful to entomopathogenic fungi. In vitro toxicity of malathion (Hy-MAL Crop-care®), methomyl (Marlin®, Bayer®), indoxacarb (Avatar® Dupont®), Spinosad (Natralure®, Dow AgriScience™) propiconazole (Throttle, NuFarm™) and nucleopolyhedrovirus or NPV (Vivus Gold Ag Biotec Australia™) towards *B. bassiana* was evaluated at three concentrations for each pesticide (label rate, half the label rate and twice the label rate). Effects of these products on conidia germination, vegetative growth and sporulation were compared. All pesticides tested had varying degrees of toxicity to germination, vegetative growth and sporulation of *B. bassiana*, dependent on the chemical nature of the compounds and the application rate. Malathion, methomyl, indoxacarb, nucleopolyhedrovirus and spinosad had the least effect on the germination, vegetative growth and conidia production of *B. bassiana* and can be recommended as compatible for use with *B. bassiana*. In contrast, use of propiconazole should avoid overlap with *B. bassiana* applications to minimize harmful effects on conidia germination which could decrease greatly the effectiveness of the entomopathogenic fungi.

The project has established integrated pest management (IPM) recommendations for *O. cylindrirostris* by identifying 1) lifecycle and behaviour, 2) blueberry varieties’ susceptibility, 3) biological and chemical insecticides and 4) bio-insecticide compatibility with other pesticides. The IPM recommendations are for the use of *O. cylindrirostris* tolerant varieties, introduction of *O. cylindrirostris* screening into the existing blueberry plant breeding program, removal of *O. cylindrirostris* infested blueberry trees and the use of two commercially available products (Mycoforce® and indoxacarb) against *O. cylindrirostris* that should not disrupt bee pollination, harvesting or cause residue concerns when managed appropriately.
Chapter 1 Motive and background for the study

*Orthorhinus cylindrirostris*, elephant weevil or the elephant weevil borer (EWB), is a major pest of cultivated blueberry in New South Wales, Victoria and South Australia. *Orthorhinus cylindrirostris* is native to these local environments and over a period of 15 years an increase has occurred in the horticultural damage caused by the larval feeding.

*Orthorhinus cylindrirostris* is thought to be responsible for current losses of blueberry yields approaching 25% (figure 1-1), representing economic losses of an estimated Au $3 million per year. The increased damage has impacted heavily on the major cultivator of blueberries in Australia, Blueberry Farms of Australia (BFA). The species therefore represents a significant impediment to the long term profitability of the blueberry industry on the Corindi Plateau. This investigation was commissioned into potential biological and other control methods that may assist in the long-term control of *O. cylindrirostris*.

Adult *O. cylindrirostris* are long lived and emerge continually over a 3-6 month interval, resulting in adult and egg laying activity throughout the harvest time. The adults are very mobile, readily moving between the fields. An initial survey done during the summer of 1996/97 indicated that the Southern Highbush group of varieties was more heavily infested than Rabbiteye varieties.

The pesticide options are very limited because of the long time adults are present, the long interval over which the fruit is harvested, and the requirement for bees to pollinate the flowers for fruit set (figure 1-2). The control for *O. cylindrirostris* needs to satisfy the requirements and goals of the producer whilst being safe to use, cost effective and environmentally friendly.

The goal of this project was to get a comprehensive appreciation of the habits and life cycle of *O. cylindrirostris* in connection with blueberry cultivation within Australia, potential for biological control strategies using bio-pesticides and to quantify variety tolerance. This research provides insight into the many issues affecting the implementation of pest management strategies, identifies areas of knowledge currently lacking regarding *O. cylindrirostris* and potential solutions.
Chapter 2 From egg to adult: the story of *Orthorhinus cylindrirostris*

2.1 Introduction

The incidence of *Orthorhinus Cylindrirostris* (Coleoptera: Curculionidae), on BFA, Corindi, NSW has steadily increased each year from the late 1980s until by 1997 it was clear there was a severe problem (Clift, 2005). *Orthorhinus cylindrirostris* is a native Australian insect (Smith et al., 1997) and is a known pest of citrus (Olliff, 1890), grapes, apple, peach, apricot trees (Hely et al., 1982) and recently blueberries (Clift, 2005). The known native hosts of the weevil include *Eucalyptus* spp. (Hely et al., 1982), *Acacia decurrens* and a native chestnut, *Castanospermum australe* (Froggatt, 1900) (Table 2-1). *Orthorhinus cylindrirostris* is prominently distributed along the east coast of Australia through temperate to sub tropical forestes with apparent isolated patches in South Australia where occurrence is distributed through mediterian grasslands and forests.

Two types of damage result from *O. cylindrirostris* activity. The adult weevil feeds on the bark of the blueberry bush, causing a minor level of damage (figure 2-1) (Smith et al., 1997). While the damage caused by such grazing is minimal, marks left on the plant are an important clue to the orchardist of the presence of the weevil (Froggatt, 1900). The more important damage is economic loss due to the feeding habits of the larval stage of the weevil (figure 2-1). As described by Olliff (1890), the female, after boring a hole into the stem with her rostrum, oviposits an egg directly into the wood of the plant, approximately 10-15cm above the soil line (Froggatt, 1900). Upon hatching, the larva chews its way down into the root system of the plant where it pupates, after which it bores a tunnel approximately to where the original egg was oviposited, finally boring laterally and emerging from the stem of the plant (Froggatt, 1900). The time from egg hatch to adult emergence is thought to be between twelve months (Smith et al., 1997) and two years (Clift, 2005).
Table 2-1 *Orthorhinos cylindrirostris* reported hosts.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Author</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Angophora florabunda</em></td>
<td>Moore 1955</td>
<td>Lisarow</td>
</tr>
<tr>
<td><em>Cup sempervirens</em></td>
<td>Moore 1963</td>
<td>Jilliby</td>
</tr>
<tr>
<td><em>Angophora sp.</em></td>
<td>Tylor 1944</td>
<td>Rushcutters bay</td>
</tr>
<tr>
<td><em>Melaleuca maideni</em></td>
<td>Moore 1956</td>
<td>Woolgoolga</td>
</tr>
<tr>
<td><em>Angophora intermedia</em></td>
<td>Moore 1958</td>
<td>Lisarow</td>
</tr>
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<td>Moore 1964</td>
<td>Lisarow</td>
</tr>
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<td><em>Cup sempervirens</em></td>
<td>Moore 1964</td>
<td>Lisarow</td>
</tr>
<tr>
<td><em>Cedrela australis</em></td>
<td>Moore 1956</td>
<td>Jilliby</td>
</tr>
<tr>
<td><em>Acacia florabunda</em></td>
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<td>unknown</td>
</tr>
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<td><em>Eucalyptus florabunda</em></td>
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</tr>
<tr>
<td><em>Citrus limon</em></td>
<td>Olliff 1890</td>
<td>unknown</td>
</tr>
<tr>
<td><em>Citrus limon</em></td>
<td>Smith et al., 1980</td>
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</tr>
<tr>
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<td>Corindi</td>
</tr>
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<td>Hely et al., 1982</td>
<td>unknown</td>
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<td><em>Castanospermum australe</em></td>
<td>Froggatt 1900</td>
<td>unknown</td>
</tr>
<tr>
<td><em>Acacia falcata</em></td>
<td>Froggatt 1900</td>
<td>unknown</td>
</tr>
</tbody>
</table>
Chapter 2  From egg to adult the story of Orthorhinus cylindrirostris

The considerable extent of defoliation by *O. cylindrirostris* larvae is the major cause of yield losses to the blueberry plant (Cliff, 2003). In severe infestations yield loss up to 25% is attributed to *O. cylindrirostris* larvae (Williams et al., 2008). From the time the larvae hatch in late April through to late Orthorhinus cylindrirostris burrows into trees and feeds from inside the trees (Frognall, 1999). The larvae burrow deep into the tree trunk and damage the bark and lichen surrounding the larval burrow. Larvae also feed on the tree trunk, feeding damage is shown in Figure 2-1. 

![Figure 2-1](image)

**Figure 2-1** Orthorhinus cylindrirostris feeding damage (A) Adult feeding damage on Acacia decurrens (B) larval feeding damage in crown cross section of Vaccinium corymbosum

The larval stage has a body size of 6-10 mm long. The body is soft, fleshy, creamy yellow, and oval (Italy et al., 1982). The pupal cuticle (figure 2-2) is glabrous, soft, and white, and some are dark brown (Zimmerman, 1994). The adult is 10-20mm long. The body of the adult *O. cylindrirostris* is very robust with a dense internal fit body, this is very visible in mature (Zimmerman, 1994). Their exoskeletons are densely covered with scales, which
The considerable extent of tunnelling by *O. cylindrirostris* larvae is the major cause of yield losses to the blueberry plant (Clift, 2005). In severe infestations yield loss up to 25% is attributed to *O. cylindrirostris* larvae with weakened plants destroyed entirely by the mechanical harvester pushing over the plant, or the plant may simply die of starvation or water stress (Clift, 2005). From the time the eggs are laid until the time the adult emerges in early September through to late October, larvae inhabit a highly protected environment. The bark and timber surrounding the larva protects the insect from natural predators and parasites (Froggatt, 1900), as well as chemical controls (Olliff, 1890). Tunnels filled with tightly packed frass further protect the larvae from external attack.

### 2.1.1 Taxonomy of the *Orthorhinus cylindrirostris*

Curculionidae is the family of snouted beetles. With over 60,000 species described worldwide and 6000 described Australian species, it is the largest of the beetle families (Lawrence and Britton, 1991). The genus *Orthorhinus* exhibits a distinctly large rostrum, geniculate clubbed antennae, and excessively large forelegs. The elytra exhibit distinctive protuberances towards the wing tips (Hely et al., 1982).

### 2.1.2 *Orthorhinus cylindrirostris* description

*Orthorhinus cylindrirostris* ranges from 10 to 20mm in length; females are commonly larger than males; the dominant colour of the weevil is a dark brown, with mottles of grey-white (Olliff, 1890). The larva is a legless grub, light yellow in colour, which tunnels extensively within the plant stem, trunk and roots. The fully grown larvae (figure 2-2) then pupate in a cocoon of woody fibres within the tunnel near the crown of the plant (Goodwin and Pettit, 1990).

The larval stage has a maximum size of 16 x 7 mm with a head width of 2.5 - 4mm and is soft, fleshy, creamy yellow, and legless (Hely et al., 1982). The pupal cuticle (figure 2-2) is glabrous, soft and white and setae are dark brown (Zimmerman, 1994). The adult is 10-20mm long. The body of the adult *O. cylindrirostris* is very robust with a dense internal fat body, that is very wide at thorax (Zimmerman, 1994). Their exoskeletons are densely covered with scales, which
vary from grey to black. The adult (figure 2-2) has a thorax covered externally with irregular bosses, which form ridges on the elytra (Froggatt, 1900). The wing covers have four distinct prominences towards the tip and smaller ones near the base. Another pair on the thorax slightly overhangs the head. The prominences bear longer scales. It has a long prominent slender snout turned down in the front and very long forelegs terminating in large feathered tarsi (Froggatt, 1900; Hely et al., 1982). The long proboscis of 5-7mm is distinctive of the adults and is the basis of the frequently used common name of elephant weevil borer (Goodwin and Pettit, 1990) although the official common name is elephant weevil (Naumann 1993).
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2.1.3 Lifecycle of the Orthorhinus cylindrirostris

The duration and exact lifecycle of Orthorhinus cylindrirostris was unclear because the few published studies are inconsistent and based on field observations using Caras assay. For example, there are reports of Orthorhinus cylindrirostris having their first flight at 100 days. Smith et al. (1993) noted that emergence of Orthorhinus cylindrirostris occurs at the beginning of the first rain after the eggs were laid but Chiff (2005) detected emergence of Orthorhinus cylindrirostris at the beginning of the first rain after the eggs were laid in October. However, in Chiff (2005) there were no further records of emergence after the first rain. Is the duration of the lifecycle for Orthorhinus cylindrirostris known? It is not clear whether the lifecycle of Orthorhinus cylindrirostris is longer or shorter than that of other insects.

Figure 2-2 External morphology of three life stages of Orthorhinus cylindrirostris (larva (A), pupa (B) and adult (C))
2.1.3 Lifecycle of the *Orthorhinus cylindrirostris*

The duration and exact lifecycle of the *O. cylindrirostris* was unclear because the few published studies are inconsistent and based solely on opportunistic field observations using *Citrus limon*. For example, there are several observations regarding emergence of adult *O. cylindrirostris* from their host plants. The original studies by Olliff (1890) and Froggatt (1900) describe the emergence of *O. cylindrirostris* during September and October. Smith et al., (1997) noted the emergence of *O. cylindrirostris* during October to November. This time frame was extended to January in blueberry varieties (Clift, 2005).

Oviposition on *Citrus limon* was described by Froggatt (1900) as occurring shortly after adult emergence, holes are drilled with the proboscis using her mandibles (Zimmerman, 1994) in a trunk near the ground. The newly created hole is then used for egg laying. On hatching the weevil larva begins to feed on the wood, boring and eating downwards (Zimmerman, 1994). Froggatt (1900) reports the larvae tunnel for about ten months, when fully grown, the larva makes its way back up the tunnel where it will begin pupating. The fully fed larva pupates within the trunk in a short horizontal cell between a few centimetres and a metre above ground level. The pupal stage lasts up to three weeks and the adults emerge a year after the eggs were laid but Clift (2005) reported the larvae hatch in *Vaccinium corymbosum* and in the first 8 to 12 months tunnel within the canes of the plants down to the crown where they then spend an additional 6 months prior to pupating. When the weevil emerges from the pupal case it cuts a clean round hole to the surface (Hely et al., 1982). Reproductive behaviour and capacity are unknown according to Haywood (1996).

This chapter presents the first comparative study of *O. cylindrirostris* life history on different host plants and provides a basis for the development of an integrated pest management program. Specifically the aims of this chapter are to:

1. Measure the duration of the entire life cycle (oviposition to adult death) and the duration of individual stages (adult lifespan, larval development and pupation).
2. Determine if host plant or insect sex affects the duration of the life cycle or the adult lifespan, length and weight.
3. Determine if female *O. cylindrirostris* show oviposition preferences for different host plants or if host plant affects the sex ratio of emerged adults.
4. Observe and describe the behaviour of adult *O. cylindrirostris*, particularly in relation to feeding, mating and oviposition.

2.2 Materials & Methods

2.2.1 Orthorhinus cylindrirostris collection and colony maintenance

Adult *O. cylindrirostris* were field collected between November and January from BFA, Corindi, NSW. Specimens were obtained as part of normal fruit harvesting when operating mechanical harvesters (Korvet™ USA) (see appendix 1-1 for pictures) through blueberry fields. Adult weevils removed from the blueberry bushes and collected in fruit crates as part of normal berry harvesting operations were gathered into 20L plastic containers (500 mm x 300 mm x 300 mm) with 20 adults per container. The adults were fed on Southern high-bush blueberry and transported to the University of Sydney, Camperdown. The weevils were fed on fresh blueberry cuttings every 7 days and fed approximately 3 kg of blueberry stems per week until used for an experiment. Insect cages were cleaned weekly before feeding and any dead weevils were removed to reduce disease. Mating pairs were collected from the containers when needed to ensure an equal sex ratio for experiments. The first field collection of weevils was made in November 2006, followed by weekly collections until December 2006. Four adult *O. cylindrirostris* were sent to Rolf Oberprieler (Zimmerman Fellow, CSIRO Entomology, Canberra, ACT Australia) as voucher specimens.

2.2.2 Plant growth and maintenance

Plants for this experiment were sourced from Tuggerah, NSW. The four species (n = 12 for each species) used for *O. cylindrirostris* feeding and oviposition were:

i. *Acacia falcata* (Hickory wattle)
ii. *Angophora floribunda* (Rough-barked Apple)
iii. *Citrus limon* (lemon var. Eureka)
iv. *Vaccinium corymbosum* 390 (Northern Highbush blueberry)
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The four plant species were selected as the plant species were previously observed to harbour *O. cylindrirostris* adults, plants of an appropriate size were available from commercial suppliers and the chosen species included both native and exotic host plants. Plants were approximately 1.3m tall when purchased and replanted into 40L pots (Botany Bag, Botany Horticultural, Nerang Qld) in the open space at Darlington greenhouse at the University of Sydney. The plants were exposed to natural light and temperature conditions and only fertilized at the time of replanting with granular form fertilizer (Osmocote Native Fertilizer, Scotts Australia Pty Ltd, Bauklkham Hills NSW). The plants were drip irrigated (Drip Line, Netafim™, Mildura VIC Australia) daily with 50mL of water. The plants were kept for one month prior to the release of the weevils in case there were any unexpected insect pests that needed to be controlled before conducting experiments. Replicate plants of the four species were arranged in a Latin square design (3 plants of each species within a block $n = 12$ plants per block) to control for potential environmental gradients e.g. shade or wind, at the site.

2.2.3  **Field study of Orthorhinus cylindrirostris lifecycle**

Experimental cages were 1000 mm in diameter and 2000 mm high constructed from saran (mesh size 0.05mm x 0.05mm). Cages, open on the bottom with elastic strapping, were placed atop trees in 40L black pots and pulled tight around pot bases. Adult weevils (three males and three females per plant = 180 mating pairs in total) were released onto the plants in November 2006. Each plant was covered by a mesh cage (as described above) for four weeks while the adult weevils were on the plants. Scars made by females during oviposition were marked with white Dulux® weather shield™ paint (Dulux®, Australia) and recorded. Length and circumference of each branch / crown oviposition site was also measured in order to calculate branch / crown diameter.

Cages and weevils were then removed at the same time to prevent continued damage to the plants from the weevils or lack of sunlight. Each plant was searched carefully and all oviposition holes counted and the level of adult feeding damage noted. Plants were checked visually for any external changes every week for two years. During the summer periods of 2007 and 2008 when *O. cylindrirostris* is recorded to emerge (Clift, 2005), the plants were caged again and emerged.
adults collected into 20L plastic containers (500 mm x 300 mm x 300 mm) with 20 adults per container and moved into the laboratory for daily observation. The numbers of eggs, larvae, pupae, adults, and the size, weight and sex ratio of emerged adults were recorded for each individual plant. The larval and pupal development of Orthorhinus cylindrirostris inside the plants could not be monitored directly because this would require destructive sampling of the plant and would prevent subsequent emergence of adult weevils (an essential part of the study). However after a maximum of two years observation the plants were cut into 10mm disc sections to search for eggs, larvae or pupae that failed to complete development. Plants that died part way through the two year experiment were held for six months to determine if Orthorhinus cylindrirostris emerged and destructively sampled in the same method as all other trees. Temperature, humidity and rainfall were recorded throughout the observation period using an on-site meteorological station.

2.2.4 Laboratory study of Orthorhinus cylindrirostris lifecycle

The size, weight and sex of all emerged adults were recorded and individuals were monitored daily until death. All weevils reared from the lifecycle study were used to assess weekly fecundity, viability, and adult (male and female) lifespan. The adults were given the same food and treatment as field collected specimens – but kept separate so that their progress could be tracked after emergence from the life cycle plants. Stems with oviposition holes were kept under ambient conditions in the laboratory for observation. General observations were taken whenever possible from laboratory and field weevil populations to understand behavioural characteristics and to complement the experimental data. Images were taken with a Canon EOS 1000D camera (Canon® Sydney NSW Australia ) with 10x magnification. Sexual dimorphism was described using an Olympus SZ 61 stereomicroscope (Olympus Corporation, Tokyo, Japan).

2.3 Statistical analysis

The time taken from oviposition to adult emergence and the adult lifespan for individual Orthorhinus cylindrirostris were subjected to survival analysis using a Kaplan-Meier estimator and curves fitted to the data using the Weibull distribution. Host plant and sex effects on these distribution curves were determined by log rank tests for significance. The effect of host plant on oviposition and adult emergence was investigated through separate analyses of variance (ANOVA) because
the sample sizes of these two variables differed substantially i.e. not all oviposition holes produced an adult weevil. The effects of host plant and sex on adult weight and length were investigated using multivariate analysis of variance (MANOVA, test statistic was Pillai's Trace, $v$). A chi-square goodness of fit test was used to check for any sex bias in adult emergence. All statistics were calculated using JMP 8 for windows (SAS).

2.4 Results

2.4.1 Survival and duration of different life stages

The time needed to complete development from oviposition to adult emergence for *O. cylindrirostris* differed between the four host plant species (log rank test $\chi^2_3 = 17.537, P < 0.001$, figure 2-3, see appendix 1-2, for Kaplan-Meier graphs). More adults emerged from the plant species *V. corymbosum* than from *An. floribunda, C. limon* and *Ac. falcata*. No adults emerged from any plants before 180 days. Emergence patterns from the plant species *An. floribunda, V. corymbosum* and *C. limon* were similar with emergence events occurring from 190 days up to 680 days after oviposition. Adult weevils emerged faster from *Ac. falcata* than the other plants with adult emergence occurring from 180 days up to 400 days after oviposition.

Host plant did not appear to affect the adult lifespan of *O. cylindrirostris* (log rank test $\chi^2_3 = 0.098, P = 0.992$, figure 2-4, see appendix 1-3 for Kaplan-Meier graphs). The life span of male and female *O. cylindrirostris* was similar (log rank test $\chi^2_1 = 0.999, P = 0.317$ figure 2-5, see appendix 1-4 for Kaplan-Meier graphs) with an observed minimum lifespan of 50 days and a maximum lifespan of 380 days.
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Figure 2-3 Survival analysis of juvenile development (from oviposition to adult emergence) by host plant, the graph shows time of occurrence (emergence of Orthorhinus cylindrirostris adult) accumulated to 100 day intervals, the line is Orthorhinus cylindrirostris emergence events
Figure 2-4 Survival and adult lifespan of *Orthorhinus cylindrostris* reared from different host plants. The graph shows time of occurrence (adult lifespan) accumulated to 50 day intervals, the line is *Orthorhinus cylindrostris* death events.
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Female Orthorhinus cylindrirostris emerged more often on *A. flaccida*, *P. euryphloea* and *C. brunia* than on *A. indica* \( F_{3,96} = 0.379, P = 0.794 \), Figure 2-6). More adults emerged from the plant species *A. flaccida* in the first week. Figure 2-6 shows that 13% of *C. brunia* seeds produced 2% of the Orthorhinus cylindrirostris. There were no significant environmental gradients affecting either of these variables (i.e. block, weight and length, Figure 2-7). However, it was observed that larger adults tended to emerge from larger seeds and that larger seeds had higher survival rates. Figure 2-5 shows a male and female Orthorhinus cylindrirostris.

Figure 2-5 Survival and adult lifespan of male and female Orthorhinus cylindrirostris
Females oviposited more often on *An. floribunda*, *V. corymbosum* and *C. limon* than on *Ac. falcata* ($F_{3, 57} = 0.379, P = 0.014$, Figure 2-6). More adults emerged from the plant species *An. floribunda* and *V. corymbosum* than *C. limon* and *Ac. falcata* ($F_{3, 57} = 0.596, P = 0.001$, figure 2-7). All plants were oviposited on by females, but of the plants used in the lifecycle experiment no adults emerged (i.e. no exit holes were found) from 40% of *Ac. falcata*, 25% of *An. floribunda* and 15% of *C. limon* compared with 2% of *V. corymbosum* plants. There was no evidence for any external environmental gradients affecting either of these variables (i.e. block was not a significant factor for oviposition ($F_{3, 57} = 0.8593, P = 0.4675$) or adult emergence ($F_{3, 57} = 0.2618, P = 0.8526$).

Host plant $V_{6, 72} = 1.834$, $P = 0.104$, and sex ($F_{2, 35} = 0.575, P = 0.570$) did not significantly affect adult weight and length (figure 2-8). There was no evidence for any external environmental gradients affecting either of these variables (i.e. block was not a significant factor $V_{2, 35} = 1.013, P = 0.373$). However it was observed that larger adults tended to emerge from larger diameter logs and from softer heartwood plant species such as *V. corymbosum* (mean weight 23 mg ± 3 mg). There was a 1:1 ratio of female to male emergence ($\chi^2_{1} = 0.70, p = 0.873$) across all host plants.

The results of 10 mm plant sections to determine larvae hatching success found only one egg failed to hatch (did not have a tunnel from egg, see appendix 1-5 for picture of egg). In other cases of failed insect development it was impossible to determine at what point of its life stage the insect died. There was no evidence of larval or pupal remains (due to decomposition) at the end of the tunnel.
Figure 2-6 Oviposition (mean ± se) by female *Orthorhinus cylindrirostris* on the four host plant species, results followed by the same letter are not significantly different.
Sexual dimorphism was apparent in the external features of adult *Ortorhinus cylindrostris* and the differences between males and females were noted from direct observations. Six structural differences were visible with the naked eye or stereomicroscope (Figure 2-8).

Males have legs that are long and slender; females have shorter legs with thicker front femurs.

The males have mandibles that are 3.5 ± 0.5mm wide and have hairs on the lateral edges of the mandible, whereas the mandibles of the female's antennae are 1.0 ± 0.1mm wide. The tip of the rostrum length is 2.2mm longer in males than the females, and the males are significantly larger in size.

The females have broader pectoral prothoracic claspers and thicker, more substantial ovipositors.

The males have longer and thinner mandibles relative to the females, which have smaller and thicker mandibles.

The males have shorter but thicker front femurs, with the females having longer but thinner front femurs.

The males have longer and thinner hind femurs, with the females having shorter but thicker hind femurs.

The males have longer and thinner front tibiae, with the females having shorter but thicker front tibiae.

The males have longer and thinner hind tibiae, with the females having shorter but thicker hind tibiae.

The males have longer and thicker front tarsi, with the females having shorter but thicker front tarsi.

The males have longer and thicker hind tarsi, with the females having shorter but thicker hind tarsi.

The males have longer and thicker claws, with the females having shorter but thicker claws.

The males have longer and thicker ovipositors, with the females having shorter but thicker ovipositors.

The males have longer and thicker claspers, with the females having shorter but thicker claspers.

Figure 2-7 Mean emergence (mean ± se) of *Ortorhinus cylindrostris* adults on the host plant species, results followed by the same letter are not significantly different.
Sexual dimorphism was apparent in the external features of adult *Orthorhinus cylindrirostris* and the differences between males and females were noted from direct observation. Six structural differences were visible with the naked eye or stereomicroscope (figure 2-9):

1. Males have legs that are long and slender; females have shorter legs with thicker front femora.
2. Males have tarsal pads that are $3.5 \pm 0.5$ mm wide and have hairs on the lateral edges of the tarsal pad.
3. The base of the female’s antennae is situated $1.5 \pm 0.3$ mm from the tip of the rostrum whereas the base of the male’s antennae is $1.0 \pm 0.1$ mm from the tip of the rostrum.
4. Rostrum length is $3 \pm 2$ mm longer in the female than the male.
5. The females have a wider pronotum.
6. Females have bigger eyes relative to their head size than males.
Figure 2-8 Sexual dimorphism of adult *Orthorhinus cylindriostris*, distinguishing features are circled.
2.4.2 Behavioural and other observations

Under laboratory and field conditions adult weevils were most often observed resting in the fork of branches \((n = 200)\) (figure 2-10); adult feeding, walking, flight and mating activity was greatest after 10:30 am when the temperature was above 10°C. Adult female *O. cylindrirostris* were selective in their choice of oviposition sites, preferring crown / branches / canes that were > 10mm in diameter (figure 2-11); the number of oviposition pits and exit holes increased with log diameter up to 100 mm. Males and females met at potential oviposition sites i.e. plant parts with a diameter > 10mm \((n = 200)\), apparently by chance. Aggregations of adult weevils were not observed nor did females show any behaviour suggestive of pheromone ‘calling’ to attract males \((n = 200)\). Males approached females head on, then they touched rostrums and antennae before the males climbed onto females followed by a delayed period before copulation \((n = 80)\) (figure 2-12). Females spent up to 4 ± 1 hours \((\text{mean} \pm \text{sd})\) boring a hole for oviposition \((n = 200)\). Males engaged in copulation and mate guarding while the female prepared an oviposition hole. A male guarded a female by standing on her abdomen or at the rear of the female. Copulation lasted about 1 min ± 30 sec with up to 10 events observed with a single female and the male continued to guard the female between copulation events \((n = 200)\) (figure 2-12).

Male competition and fighting was observed in both laboratory and field life cycle experiments when there were as little as two males to one female \((n = 50)\). Fighting occurred when a male was mate guarding and was confronted by another male \((n = 50)\). Multiple fights did occur with the same or multiple male contenders during the several hours of guarding while the female bored an oviposition hole \((n = 50)\). Fights between males may have been more frequent due to high density conditions in captivity compared with the natural situation. *O. cylindrirostris* body size varies substantially in both males and females (figure 2-9). There was no indication of different behavioural responses such as holding and mounting by males of different sizes but females mated more frequently with larger males because larger males were victorious in combat for females \((n = 200)\).
Weekly and lifetime oviposition rates from lifecycle experiments show *O. cylindrirostris* females lay $2 \pm 1$ egg / week and $86 \pm 40$ eggs / lifetime. 99% of these oviposition sites contained an egg that hatched successfully, as shown by the larvae which bored into the centre of the branches or crown. Eggs hatched in $30 \pm 8$ days when laid into *V. corymbosum*. Larvae at 1 to 5 days old are $1 \times 0.85$ mm and weigh $6.0 \pm 1.0$ mg. Larvae at 300 days old are $10 \times 5$ mm and weigh $108.0 \pm 50.0$ mg. The female with the highest fecundity (109 eggs) lived 356 days.
Figure 2-9 Adult Orthorhinus cylindrirostris resting in the fork of branches (A) lateral view of resting (B) anterior view of resting.
Figure 2-10 (A) Oviposition pit bored by female *Orthorhinus cylindrirostris* with egg inside, (B) larval *Orthorhinus cylindrirostris* boring through blueberry stem. (C) Oviposition pits on *Angophora floribunda* (circled).
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Figure 2-11 (A) Male and female *Orthorhinus cylindrirostris* meeting at a potential oviposition site on a blueberry stem in the laboratory. (B) Male *Orthorhinus cylindrirostris* on top of female mate guarding (C) Male *Orthorhinus cylindrirostris* copulating with female.
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Figure 2-12 Orthorhinus cylindrirostris lifecycle in Vaccinium corymbosum.
Figure 2-13 Small *Orthorhinus cylindrirostris* emerged from cut *Vaccinium corymbosum* branches.
Figure 2-14 *Orthorhinus cylindrirostris* emergence numbers over the year
Figure 2-15 *Orthorhinus cylindriostris* oviposition numbers on different size stem hosts.
2.5 Discussion

This study has demonstrated that the life history of *Orthorhinus cylindricus* is more complex than previously thought. *Orthorhinus cylindricus* develops in the crown and roots of healthy blueberry and Australian native trees. Eggs are laid in small matches on the bark of trees, the crown or branches, excavated by the adult weevils in late spring and the larvae hatch and tunnel throughout the year before emergence (Figure 2-11, see appendix 1-6 for a picture of a weevil oviposition). The number of larvae production cannot be determined because of the concealed nature of the host plant. Adults oviposit in the oviposition sites where females spend up to 12 hours ovipositing. Females engaged in oviposition are not able to lay eggs in the field. The number of larvae emerging is significantly reduced and emergence can be postponed until the following year. An adult emergence from paperest peaks in December of the year after their first overwintering. The larva spends two years in the soil in a depth of approximately 4 cm (Figure 2-11). The oviposition period lasts from September to March of the following year, with oviposition numbers peaking in March. After oviposition, females overwinter in the soil and develop into adults the following year. The larva remains in the soil for multiple years as long as they do not die from *Orthorhinus cylindricus* attack or other causes. As the temperature decreases (below 10°C minimum temperature), usually March in Northern NSW, the adult weevils rest during the cooler periods of the day, often being found in the fork of blueberry branches or in grass near forest stands.

The red palm weevil *Rhynchophorus ferrugineus* (Oli-Sy) (Coleoptera: Rhynchophoridae) shares wide-ranging similarities in its biology and behavior with *Orthorhinus cylindricus*; the identification includes that the females use the rear leg to bore into the tissue to form a hole in the crown for oviposition.

Figure 2-16 *Orthorhinus cylindricus* oviposition numbers over the year.
2.5 Discussion

This study has demonstrated that the life history of *O. cylindrirostris* is more complex than previously thought. *O. cylindrirostris* develops in the crown and roots of healthy blueberry and Australian native trees. Eggs are laid in small notches on the bark of roots, the crown or branches, excavated by the adult weevils in late spring and the larvae hatch and tunnel throughout trees before emergence (figure 2-11, see appendix 1-6 for a picture of a weevil emerging). The number of larval instars could not be determined because of their concealed habitat inside the host plant. Adults meet at the site of oviposition where females spend up to five hours boring a hole for oviposition whilst males engaged in copulation and mate guarding. Females start to lay eggs in the first week of emergence suggesting that the pre-oviposition period is short relative to the adult lifespan. Female weevils were observed to oviposit continually after their first mating experience without the need for subsequent mating events. Pupation and emergence can take place within one year of oviposition or can be postponed until late summer of the following year (figure 2-12). Adults emerged from pupation peaks in September to December (figure 2-14), but late emergence in January was not uncommon.

The adult weevils feed on the bark of woody plants throughout the year, ovipositing in the spring (figure 2-14). After oviposition, the adult weevils remain on the plant for the rest of the summer. The adults can live for over one year and the sex ratio is approximately 50:50. After mating, females oviposit in stems or regions of the crown that are greater than 10 mm in diameter (figure 2-15 & 2-16). One egg is laid in each hole and the holes are gnawed in the bark to a depth of approximately 4 mm (figure 2-11). The oviposition period lasts from September to February, but peaks in October (figure 2-16). Plants can remain suitable as oviposition sites for multiple years as long as they do not die from *O. cylindrirostris* attack or other causes. As the temperature decreases (below 10°C minimum temperature), usually March in northern NSW, the adult weevils rest during the cooler periods of the day, often being found in the fork of blueberry bushes or in mature forest stands.

The red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Rhynchophoridae) shows wide-ranging similarities in its biology and behavior with *O. cylindrirostris*; the similarities include that the females use the rostrum to bore into the tissue to form a hole in
which to lay their eggs. Oviposition occurs most frequently in crowns and then the hole is sealed to protect the eggs (Bokhari and Abuzuhairah, 1992). When hatched, larvae feed on the surrounding tissue. As the larvae feed, they produce frass which fills the tunnels made by the larvae. In severely infested palms, cavities are formed by the feeding larvae which weaken the crown of the tree. Once mature, the larvae form an oval cocoon and pupate. Just like the *O. cylindrirostris* larvae the red palm weevil is concealed and symptoms of attack at an early stage of infestation are difficult to detect (Bokhari and Abuzuhairah, 1992). Infestations of red palm weevil are also detected through the occurrence of emergence holes and in severe infestations by stem or crown breakage (Abraham et al., 1998). Similarities are also evident in other life history parameters, with a lot of temporal variation, and no clear pattern related to climatic conditions. However unlike *O. cylindrirostris*, larval development times and the total life cycle period reported is much shorter with larval development time of two months on average and a pupal period of three weeks (Wattanapongsiri, 1966).

Survival analysis from this study showed that tree species, which are commonly found in the blueberry field landscape, differed in their attractiveness and effects on the performance of *O. cylindrirostris*, with the greatest oviposition by females occurring on blueberry bushes. Larval development of *O. cylindrirostris* differed between host tree species with a shorter development time on *Ac. falcata* than other plant species and higher emergence of adults from *V. corymbosum*. Host plants used in this study showed no influence on adult lifespan, weight and length of *O. cylindrirostris*.

Investigations into weevil response to plants are not new (Prokopy et al., 1995). However this was the first controlled experiment using multiple families of host plants to determine the lifecycle of adult *O. cylindrirostris*. Previous authors used observations to describe *O. cylindrirostris* taxonomically (Hely et al., 1982; Zimmerman, 1994) and to determine the feeding habits of the larval stage through field collected crowns and branches from lemon trees (Froggatt, 1900; Olliff, 1890), grape vines (Goodwin and Pettit, 1990) and blueberries (Clift, 2005). The larval development time and larval tunnelling pattern was the only finding to contradict previous studies. Previous studies described the larval period being 1 year and the
direction of larval boring as downwards from point of oviposition (Froggatt, 1900) however my results suggested no specific directionality and a larval period up to 24 months.

In *O. cylindrirostris* the flexibility of the larval-pupal development and interaction with the host plant show broad diversity. Individuals varied significantly in the time taken to reach adult maturity. The full range of previous estimates of lifecycle length from 365 days (Froggatt, 1900; Smith et al., 1997) up to 730 days (Clift, 2005) were found in this study, demonstrating that *O. cylindrirostris* has a flexible lifecycle with juvenile development time of 200 to 680 days and adult lifespan of 50 to 400 days. This variation in the life cycle duration could be attributed to differences in host plant nutritional quality as seen in the large pine weevil *Hylobius abietis* Linnaeus (Coleoptera: Curculionidae) in pine plantations (Leather et al., 1999). In contrast to their findings however, *O. cylindrirostris* larval development in our study was longer on the more favoured plant species, with adult *O. cylindrirostris* emergence greatest from blueberry. Alternatively, these host plant effects may be an experimental artefact if one of the plant species, particularly *A. falcata*, did not respond well to being grown in a pot for two years.

*Orthorhinus cylindrirostris* larval development shows host plant interaction and flexibility, possibly because *O. cylindrirostris* colonizes and feeds only on living trees. When a host plant dies containing developing larvae, *O. cylindrirostris* larvae respond to food deprivation with a reduction in the length of the larval period and premature pupation, leading to the early eclosion of a small adult, as small as 2 mm (figure 2-14). It appears that larvae initiate pupation once food availability has ceased, as it allows an individual to reach adulthood and hence maintains the possibility of reproduction. This phenomenon also occurs if plant parts containing *O. cylindrirostris* larvae are removed by pruning. The forced pupation of underdeveloped larvae is a likely behaviour to escape desiccation as the host plant dries up after death.

Similar flexibility in development time has been seen in the larvae of the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae) (Shafiei et al., 2001) and yellow-spotted longicorn beetle, *Psacothea hilaris* (Coleoptera: Cerambycidae) (Munyiri et al., 2003) Larvae of *O. taurus* responded to food deprivation by initiating pupation. This has striking similarities to *O. cylindrirostris* with the degree of flexibility in the dynamics and timing of
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larval development. Perhaps premature exhaustion of food supplies can serve as a cue for the initiation of metamorphosis. Premature metamorphosis in response to food deprivation has been documented extensively in amphibians, but to the best of my knowledge, this is only the second time (O. taurus the first) such behaviour has been documented for a holometabolous insect (Nijhout, 1999).

For many insect species if larvae are deprived of food or inadequate nutrition, the majority of insects will not pupate (Bradshaw and Johnson, 1995), or will respond through delaying progressive development until sufficient resources have been secured (Nijhout, 1999). Many borer beetle larvae however are largely immobile and develop in chambers that contain a finite amount of resource provided by their parents. Once this resource is used up, locating additional food sources is impossible. In such instances, completion of larval development would require either correct assessment of the initial resource size and quality by the parents (Moczek, 1998) or the ability to terminate larval development prematurely and undergo pupation and metamorphosis at a smaller body size once the food supply is exhausted (Nijhout, 1999) as seen in O. cylindriostris.

The choice of oviposition hosts by adult female O. cylindriostris is critical, as weevil larvae are apodous and thus incapable of moving between hosts, similar to many other coleopteran wood borers (Gardiner, 1961). Therefore females influence the fitness of their progeny according to where they lay their eggs (Mousseau! and Charles 1998). The adult O. cylindriostris may locate suitable larval hosts by olfaction in the way other coleopteran insects do (Linsley, 1961). For example, females may use ethanol volatiles and semiochemicals (Ch’enier and Philog’ene, 1989) from other feeding O. cylindriostris to locate host plants. An aggregation pheromone and plant kairomones (ethyl acetate, ethyl propionate or ethyl butyrate) are known to attract red palm weevils to potential hosts (Bokhari and Abuzuhairah, 1992). The role of volatiles in host selection by O. cylindriostris was not addressed in this study as weevils were caged on single plants with no choice between plants for oviposition.

Previous work hypothesised that stressed host plants were targeted for attack by O. cylindriostris (Goodwin and Pettit, 1990; Hely et al., 1982). This is a characteristic particular to
bark beetles (Coulson et al., 1976) and weevils (Harari and Landolt, 1997; Leskey et al., 1998; Loughrin et al., 1995; van Tol and Visser, 2002; van Tol et al., 2002), including the black vine weevil (Hamilton-Kemp et al., 1988; van Tol and Visser, 2002; van Tol et al., 2002). This hypothesis was not supported by this study because *O. cylindrostris* oviposit into living, healthy trees and escape dying plants by premature metamorphosis. The hosts may survive for years despite harbouring borer larvae, something which is evident in blueberry plantations as multiple generations can be produced from one blueberry tree. Larval feeding does contribute to decline of hosts, particularly when they are repeatedly attacked by multiple generations. The larval boring in the heartwood and sapwood of healthy plants may circumvent host plant resistance by preventing the flow of sap and may elevate nitrogen content in the damaged portion owing to pooling of amino acids (Orcella, 1982).

*Orthorhinus cylindrostris* performed equally well on a non-native host plant as on its natural, co-evolved host plants. Since oviposition preference and larval performance are both favoured on blueberry, this host is likely to act as a population source for *O. cylindrostris*, not just as a sink for *O. cylindrostris* insects coming from the surrounding native host community (Clift, 2005). It may be that the native host plants exhibit some degree of biotic resistance due to their shared evolutionary history with *O. cylindrostris* (Maron and Vila, 2001; Tallamy, 2004). Or the novel host plant (blueberry) may be selectively attacked in its new geographic range because it is defensively naive against native consumers (an idea first proposed by Darwin, 1859). Another hypothesis is that the nutritional quality of blueberries may be superior to the native host plants. Very few studies have looked at differences in insect herbivory between a non-native species and its native congener.

The potential defences of native plants which could be acting on *O. cylindrostris* populations include cuticular waxes, secondary metabolites, inhibitory proteins, enzymes, relatively low protein concentrations, relatively low lipid concentrations and toxic compounds (Maron 2001). Little is known about the defensive value of volatiles against herbivorous insects for the four host plants used in this study (Law and Regnier, 1971). This does not imply that the compounds and defence barriers of the native plants are themselves more toxic than those of horticultural plants but it would appear they are merely more effective in their ability to defend large
infestation of *O. cylindrirostris* as seen in blueberry fields. Alternatively the production of the novel secondary compounds may be increased in native plants, thereby making them even more tolerant to attack by *O. cylindrirostris*. Further research needs to be conducted to determine the mechanisms of tolerance to *O. cylindrirostris* in plants and the effects of plant defences on the biology of this species.

Until the present study little attention had been given to sexual dimorphism of *O. cylindrirostris*. There is a large diversity in *O. cylindrirostris* body size both in males and females. Body shape and size are not distinctly different between the sexes, something which is also observed in *Sternochetus frigidus* (Coleoptera: Curculionidae) (Fabricius) (Rowena et al., 2002). Male *O. cylindrirostris* however are distinguishable by large front tarsal pads and the antennal base close to the rostrum tip; it is not uncommon to find external morphological differences in Curculionidae (Rowena et al., 2002). These results should be added to the taxonomic literature of Zimmerman (1994) to reduce confusion with species identification.

Small adult body size in *O. cylindrirostris* relative to conspecifics is likely to be due to reduced larval food availability and may reduce fecundity in females (Hunt and Simmons, 1997; Hunt and Simmons, 2000). The fitness costs for small-sized males are more difficult to predict (Pare and Tumlinson, 1999) although direct observation suggested a link between male body size and success in mate competition. An explicit test of this hypothesis was beyond the scope of this thesis.

Oviposition rates and fecundity in insects are affected by a number of factors, e.g. size, longevity, adult and larval nutrition and plant stimulants (Leather, 1987; Leather, 1988; Leather et al., 1995). The effect that adult nutrition has on the fecundity and longevity of *O. cylindrirostris* is unknown and was not addressed in this study but the plant species tested did not affect adult lifespan. Larval nutrition may be important in determining the adult size attained, but as *O. cylindrirostris* is a relatively long lived insect, it is likely that adult nutrition and the factors affecting this will have the most marked effect on fecundity (Leather et al., 1999). Oviposition occurs 20 mm (Olliff, 1890) to 1000 mm (Clift, 2005) from the soil surface. It is possible that the regions of the stump closer to the soil surface are warmer than those
further above ground, aiding egg and larval survival and development (Leather et al., 1999). This is a direct consequence of the oviposition location and subsequent larval boring behaviour.

For most Coleoptera, adult males usually play the active role in mate location. It has long been held that many beetles, like most other insects, depend on pheromones that act over long distances for mate location (Linsley, 1961), however convincing evidence of long-range pheromones in *O. cylindrostris* was not evident in this study. Conspecific aggregation was not observed in laboratory or field experimentation. However this aspect of mating behaviour was not tested specifically and therefore olfactometer experiments and chemical analysis are needed to eliminate the possibility of pheromone use in this species. One hypothesis is that mate location is a behaviour associated with males seeking females at the adult feeding site, which is also the larval host. Mate location appears to depend on males encountering females by chance, through mutual attraction to the larval host where males rely on antennal contact to recognize females, possibly by very short-range pheromones operating over distances of a few centimetres, or visually (Kim et al., 1992). This behaviour is seen in Japanese pine sawyer beetle, *Monochamus alternatus* Hope and white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) (Fukaya, 2004). However the probability of encountering a mate by chance on adult host plants, and hence mating opportunity, would seem to be very low, particularly on large host trees (Harman and Harman, 1987; Leather et al., 1999).

Whilst it is common for female beetles of some species to select ideal males for copulation e.g. yellow-spotted longicorn beetle *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae) (Fukaya, 2004; Fukaya et al., 2004), females were not observed to refuse any males of a particular size. Although the effect of male size on mating success was not specifically tested, observations suggested that small males were less successful. Once a male has found a female, *O. cylindrostris* males do not show any form of pre-copulatory courtship behaviour; males typically approach females directly and attempt to mount and copulate immediately. Although copulation is very brief, the male remains with the female as she chews the oviposition site, repeating copulation and fending off rival males. Repeated mating may be necessary for complete fertilization in *O. cylindrostris* however this was untested in this study.
Orthorhinus cylindrirostris is not nocturnal like many weevil pests such as black vine weevil Otiorhynchus sulcatus Fabricius (Coleoptera: Curculionidae) and garden weevil Phlyctinus callosus Boheman (Coleoptera: Curculionidae), where the adults only feed at night. Therefore monitoring of *O. cylindrirostris* and population assessments can be conducted during daylight hours. Biological knowledge has been used to develop insect scouting techniques in commercial blueberry fields in Massachusetts, New Jersey, Wisconsin, and Michigan for the monitoring of Cranberry weevil Anthonomus musculus Say (Coleoptera: Curculionidae) (Szendrei et al., 2009). Growers use insect activity during the day and the emergence periods to determine the best times for insect scouting (Szendrei et al., 2009). However these techniques are labour intensive and may be inaccurate for *O. cylindrirostris* as the adult distribution can be patchy. Furthermore, weevils tend to drop to the ground when scouts move plants while searching.

These observations and descriptions of biology should help with management of *O. cylindrirostris*. Counting of adult emergence holes might be one of the easiest methods to apply in practice. There are indeed no strict and reliable relationships between *O. cylindrirostris* adult numbers and damage to blueberry plants (see chapter 3). However, it is important to know the beginning of adult activity in blueberry fields each spring because early control of the more vulnerable adult weevils is essential for breaking their life-cycle (Clift, 2005). The life history of *O. cylindrirostris* appears to be more K- than r-selected although K-strategists are less common in agro-ecosystems (Southwood and Comins, 1976). The extended duration of the protected juvenile life stages inside the host plant, combined with the fact that *O. cylindrirostris* appears to be forming a long-term stable population within blueberries, suggests that it will take longer to see the effects of any control strategies on abundance of this pest.

2.6 Conclusion

Relatively little work has been aimed at *O. cylindrirostris* biology in the past so this study has contributed to the basic understanding of *O. cylindrirostris* biology including: clarifying lifecycle length and longevity, displaying the flexibility of larvae by escaping desiccation in dying hosts, describing the lifecycle / biology, describing the sexual dimorphism in adult *O. cylindrirostris* and showing that *O. cylindrirostris* performs equally as well on *V. corbybassum*
as other horticultural and native plants. Because *O. cylindrirostris* are a flexible insect pest with a protected larval stage, it is a challenging weevil to study and for which to develop monitoring and control methods. Much more needs to be learnt of the biology and behaviour of *O. cylindrirostris* to provide insight into its reproductive strategies and many of the observations need to be confirmed through experiments, e.g olfactometer testing, Y maze testing, behavioural studies using different insect densities and testing to determine the overall host range of adult and larval *O. cylindrirostris*. The life history information presented here provides the basis for an integrated pest management program against *O. cylindrirostris*.

2.7 References


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3.1 Introduction

Orthorhinus cylindrirostris population dynamics have not been a focal point for producers dealing with *O. cylindrirostris* populations. The level of accuracy and detail in previous research and field surveys is also an issue. Blueberry susceptibility to attack and larval feeding by *O. cylindrirostris* is poorly described.

A pilot survey conducted in 2003 indicated that varieties of the Southern Highbush (SHB) were heavily infested when compared to Rabbiteye (RE) varieties and more susceptible to damage, however the mechanism of resistance was not apparent (Clift, 2005). Infestation of blueberries planted on the Corindi plateau is presumed to be a result of the recent conversion of native vegetation to blueberry production, which has forced *O. cylindrirostris* adults to move into the crop in search of alternative food sources (Froggatt, 1900). However colonization is believed to be non-random as the weevil demonstrates preferences for certain blueberry varieties based on as yet unknown plant characteristics (Clift, 2005).

Due to the lack of research about blueberry susceptibility to insect borers and the population dynamics of *O. cylindrirostris*, this review will focus on plant-insect interactions, plant tolerance and/or resistance to insect borers, resistant varieties used against insect pests in agriculture generally and examples of resistant blueberry varieties used against other insect pests.

3.1.1 Plant resistance to herbivory

Herbivory by an insect often induces morphological, phenological, and chemical changes in a host plant (Harrison and Karban, 1986; Moran and Whitham, 1990; Strauss, 1991). Herbivore-induced changes affect plant quality, such as decreases in nutritional status and biomass, and/or increases in secondary substances and the density of thorns/spines (Tindall and Stout, 2001; Wise and Weinberg, 2002). Herbivore-induced change may either decrease or improve plant
quality for subsequent herbivores (Damman, 1989; Martinsen et al., 1998). Insect herbivory that stimulates re-growth in host plants may increase their susceptibility to insect herbivores that emerge at a later time (Denno et al., 1995; Kaplan and Denno 2007; Ohgushi 2005).

Plant resistance can be defined as "including those characteristics which enable a plant to avoid, tolerate, or recover from the attacks of insects under conditions that would cause greater injury to other plants of the same species" (Beck, 1960). Plants have evolved a variety of strategies for defending themselves against herbivores including escape in time or space, tolerance or replacement or repair of damaged parts, confrontation or active defence using physical or chemical barriers, and specialized biological associations (Harris and Frederiksen, 1984). Entomologists however, usually deal with the mechanisms of non-preference (also known as antixenosis), tolerance, and antibiosis (Painter, 1958).

- Antixenosis occurs when the plant lacks certain qualities necessary for recognition by the insect as a potential host (Beck, 1957; Beck, 1960).
- Tolerance is present when a plant is capable of supporting an insect population without loss of vigour and without reduction of crop yield (Beck, 1957; Beck, 1960; Thorsteinson, 1960).
- Antibiosis encompasses more direct physical and chemical defences, which are either constitutive, i.e. structures and compounds that confer resistance are present at all times, or induced, i.e. structures or compounds that confer resistance are produced only in response to attack (Berryman, 1988).

Plant resistance has been studied almost exclusively in terms of its agricultural implications for plant breeding programs. Emphasis is thus placed on economic damage by the insects and agronomic properties of the crop plants (Beck, 1960). Insect-resistant varieties have been a good alternative to insecticides to keep herbivore populations below their economic injury level.
3.1.2 Insect Resistant Varieties

Numerous borers prefer to attack stressed trees and therefore host location is usually the primary focus of tree susceptibility to borer pests such as the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera; Buprestidae), which is a threat to street trees in Minnesota (McCullough and Siegert, 2007). The most common method used to assess borer susceptibility of plant varieties is to count emergence holes (Reagan, 2001). The presence of adult emergence holes represents a seasonal record of adult production (Bessin et al., 1991) and can be used to determine the relative survival of larvae (Reagan, 2001). The impact of variety resistance can be estimated from the rate of increase of borer populations (Bessin et al., 1991).

Variety resistance allows for a more permanent control of pest populations (Bessin et al., 1991; Smith, 1989). Resistant varieties have been used against insects in sugar cane, blueberries and eucalyptus. For example, Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), was first reported as a major pest of commercial sugarcane, *Saccharum* spp., in 1924 (Van Zwaluwenburg, 1926). Sugarcane damage can average 20-30% yield loss with an estimated cost of between $US$575 to $US$1,181/ha (Legaspi et al., 1997; Meagher et al., 1993). Insecticides have had such poor success in controlling Mexican rice borer that their use has been abandoned by most sugarcane growers (Legaspi et al., 1997). There was great variation in sugarcane variety susceptibility to Mexican rice borer. A recently released variety, which represented 85% of the production area in Louisiana, was significantly more susceptible than other commonly planted varieties and now different varieties that are resistant to Mexican rice borer are recommended (Calvo and Molina, 2004).

3.1.3 Herbivory

It is widely accepted that reallocation of resources within individual plants following herbivory can result in compensatory regrowth, depending on the relationship between plant parts that consume energy (e.g., apical meristems, flowers, and fruits) and photosynthetic organs or storage tissues for growth (Larsson, 2002). Herbivore damage often removes apical meristems, flowers, fruits or storage tissues, thereby modifying the pattern of resource allocation (Dyer et
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Leaf herbivory has been considered as a significant factor in the initiation of plant responses because leaf-eating herbivores often induce the production of defensive chemical substances. Consequently, most studies on plant regrowth have also focused on responses to leaf-eating herbivores (Karban and Strauss, 1993; Karlsson and Weih, 2003; Paige and Whitham, 1987; Peinetti et al., 2001; Utsumi and Ohgushi, 2007) with fewer studies looking at plant responses to wood boring insects (Rosenthal and Welter, 1995; Utsumi and Ohgushi, 2007).

3.1.4 Insect resistant blueberry varieties

There are very few examples of insect-resistant blueberry varieties. The only known example involves the lappet moth, Streblote panda (Lepidoptera; Lasiocampidae) (Hübner), which is a common species found on blueberry in Western Andalusia (Calvo and Molina, 2004). The biology of this species is poorly known and it appears that the pest switched from unknown native host plants to blueberry. Larval mortality and development time varied between varieties and particular varieties were identified as most suitable for planting where the pest occurred. Investigation of plant resistance provided useful information for planning and managing blueberry orchards in the presence of S. panda populations and an interesting similarity to the pest status of O. cylindrirostris, another species that has shifted from native host plants onto blueberry.

From even a limited review of existing literature, the complexity of plant resistance to insects is apparent. It is doubtful that any example of resistance can be explained on the basis of a single simple biological characteristic of the plant. Suppression of pest populations through the use of resistant plants has long been considered to be the ideal control method (Bergman and Tingey, 1979; Berryman, 1988; Gould, 1978; Gould, 1986; Gould et al., 1991; Obrycki et al., 1983; Price, 1984; Price et al., 1980).
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In a few instances, outstanding success has been attained e.g. rice, potatoes (Obrycki et al., 1983) and many other staples (Gould et al., 1991). Many other cultivars have been developed that display some degree of insect resistance, which complements other control methods. Much of this agronomic development has depended on a good knowledge of plant genetics, the development of reliable criteria by which to measure resistance, and knowledge of the general biology of the insect species.

3.1.5 Objectives

The first stage for host plant resistance is to demonstrate variation in susceptibility between existing varieties. Then if that variation exists, the next stage is to determine the mechanism(s) and to select for those characteristics as part of a planned breeding program. As greater understanding of *O. cylindrirostris* ecology in blueberries and plant chemistry is attained, it will be possible to approach the goal of developing agronomic plants that are deliberately designed to be insect resistant. The ultimate goal of this research was to improve management decisions for control of *O. cylindrirostris*.

The objectives of the proposed work was to

1. Measure susceptibility of commercial blueberry varieties to attack by *O. cylindrirostris*.
2. Determine the economic injury level for *O. cylindrirostris* in commonly grown blueberry varieties.
3. Map spatial and temporal trends in *O. cylindrirostris* abundance at Blueberry Farms Australia.

3.2 Materials & Methods

3.2.1 Site location

The location of the study area is in the north coast region, New South Wales (NSW), Australia. Blueberry Farms of Australia (BFA) is located about 42 km south of Grafton at Corindi (Figure 3-1) (see appendix 2-9 for farm map). The north coast region is primarily used for grazing and tropical fruit horticulture. The bioregion is characterised by four main soil types: red-brown
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earths, grey, brown and red clays, and red earths. The size of the study area is 650 ha and is a mixed horticultural farming business with its primary source of income coming from blueberry production and smaller holdings of macadamia and raspberry plantations. The north coast region is dominated by a subtropical climate. The hottest month is January and the coldest month is July. The daily mean temperature in January range from 22°C - 28°C and for July it is 12°C - 22°C. The rainfall is summer dominant but there are high intensity thunderstorms in winter.
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2.2.2 Data collection

In 2007 – 2008, 18 blueberry varieties andage groups were surveyed in different fields over geographically important areas in the Bega Valley, NSW. There were three replicates for each variety and each field was under the same fertilizer and pest control regime.

As part of normal harvesting practices, mechanical harvesting was used to collect blueberry rows that were approximately 200 m in length with a total of 230 trees sampled per field. Fruit losses were counted on each tree and the area sampled by the harvester was sampled using a global positioning system (GPS) (Garmin 60CSx). The following were collected on a per-tree basis:

- Fruit set
- Harvested fruit

Analysis was carried out using analysis of variance to calculate differences between varieties and a one-way ANOVA for variety effects, using a statistical software.

Figure 3-1 Blueberry Farms of Australia location map relative to Australia, location within NSW and aerial photo of the farm.

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3.2.2 Data collection

In 2007 - 2008, 18 blueberry varieties in five age groups were surveyed in different fields over geographically separated paddocks on BFA, Corindi NSW (figure 3-1). There were three replicate fields for each variety-age combination. All fields were under the same fertilizer and insect control regime.

As part of normal harvesting practices mechanical harvesters (Korvet™, USA) worked through single blueberry rows that were approximately 200 m in length with a total of 250 trees sampled per field. Exit holes were counted on each tree and the adults collected by the harvest machines from each row were counted. Tree location was recorded for all trees sampled using a global positioning system (GPS) (Garmin®, GPS72, Olathe, Kansas, USA).

3.2.3 Statistical analysis

3.2.3.1 Economic injury level (EIL)

At least two blueberry varieties are grown in alternate rows, to increase hybrid vigour by cross pollination (see appendix 2.1 for a picture of blueberry fields). Yield data was collected for each variety per field from the harvesters. To determine per tree yields, tree numbers for the varieties were divided into the total yield of a single variety per field. Emergence numbers were collected on a per tree basis and summed together to determine field emergence of *O. cylindrirostris* for each of the varieties. The relationship between exit holes and yield was analysed using analysis of covariance (ANCOVA), with blueberry varieties and plant age as the covariates and a one-way ANOVA on emergence holes and adult numbers per field to test for variety effects, using a Tukey’s HSD to determine varietal differences. All statistics were caculated using JMP 8 for windows (SAS).
3.2.3.2 Mapping of *Orthorhinus cylindrirostris* distribution at Blueberry Farms of Australia

To analyse the spatial distribution of *O. cylindrirostris*, two separate steps were required: fitting of the variogram for the whole area (using actual data points) followed by kriging estimates to allow interpolation across points that were not sampled.

Regression kriging was applied to the variables, *O. cylindrirostris* adult counts and *O. cylindrirostris* emergence holes to produce 5 m resolution continuous maps. The two maps were visually compared to assess the relationship between *O. cylindrirostris* distribution and replanting time (as a measure of crop damage) and relationship with varieties.

Kriging estimates the value of an unknown real-valued function, at a point, given the values of the function at some other points. A kriging estimator is said to be linear because the predicted value is a linear combination of the variables. Kriging interpolation takes into account the distance and the spatial autocorrelation. The program used was Variogram Estimation and Spatial Prediction with Error (VESPER) (Australian Centre for Precision Agriculture, The University of Sydney).

The local variogram model used a nonlinear least-squares method. Interpolation with block kriging was used giving a value that represents a statistically weighted average for an area centred on the grid point. The size of the area for the block size was specified to 5 m² due to the sampling design. Neighbourhood Interpolation used a minimum of 40 known points and maximum of 300 known points to calculate the unknown areas, allowing the formation of maps for unsampled areas. Images were used in ArcGIS® (ESRI™, Redlands, USA) to overlay data to the land cover image and map the data on a continuous scale.
3.3 Results

3.3.1 Economic injury level (EIL)

There were varying rates of *O. cylindrostris* emergence from different blueberry varieties but no consistent relationship between yield and emergence once the effects of variety and plant age were accounted for ($F_{60, 124} = 0.94, P = 0.615$). Yield differed between varieties ($F_{1, 124} = 8.85, P = 0.004$) but not between age groups ($F_{1, 124} = 0.25, P = 0.621$) even though emergence holes tend to accumulate with increasing plant age because the holes persist long after the weevils have left the plant. It proved impossible to determine a consistent EIL from the emergence-yield relationship because there was too much variation both between and within varieties. Visual comparison of emergence-yield plots for the different varieties showed three different types of response (figure 3-2, see appendix 2-2 to 2-8 for graphs of all varieties): decreasing yield with increased *O. cylindrostris* emergence, no change in yield with increased emergence, and (apparently) increasing yield with increased emergence. This latter trend is thought to reflect plant growth over time (bigger plants produce more berries) and accumulation of emergence holes over time rather than a direct positive effect of *O. cylindrostris* damage on yield. The increasing yields suggest those varieties are compensating for larvae damage and illustrate tolerance.
Figure 3-2 *Orthorhinus cylindrirostris* emergence and *Vaccinium corymbosum* yield relationships for the varieties (A) emergence showed no effect on yield (B) increasing emergence reduced yield and (C) yield increased with accumulated emergence.
Emergence rates and adult numbers on a per field basis for different varieties did indicate considerable variation in susceptibility to *O. cylindrirostris* attack when using adult emergence ($F_{15,269} = 11.607, P = 0.001$) and adult presence ($F_{15,269} = 32.322, P = 0.001$) (table 3-1). Older style RE c.v. Becky blue, Bonito and SHB cv Misty had the highest abundance of *O. cylindrirostris* in general. The newer RE c.v. Powderblue, Premier, Britwell exhibited the lowest abundance of *O. cylindrirostris* compared with all other RE and SHB blueberry varieties. SHB c.v. 42 had the lowest *O. cylindrirostris* emergence of all SHB varieties with moderate *O. cylindrirostris* abundance found in the remaining SHB c.v. 209A, 115, Sharps, 41 and 390. Visual comparison of yield and emergence per tree from the different varieties revealed RE c.v. Powderblue, Britwell and SHB c.v. 115 are the highest yielding varieties (2.5 – 3kg per tree) with relatively low *O. cylindrirostris* emergence per tree compared with other varieties (Figure 3-3). RE c.v. Premier and Climax are the highest yielding varieties able to support high rates of *O. cylindrirostris* emergence. In contrast RE c.v. Becky blue, Bonito and Misty are the most affected by *O. cylindrirostris* damage with yield of only 1 kg per tree when median emergence exceeds three *O. cylindrirostris* per tree.
Table 3-1 *Orthorhinus cylindrirostris* emergence holes and adults (mean ± se) collected from *Vaccinium corymbosum* varieties. Classic varieties were created ≥20 years ago, new varieties have only been in production since 2000. Means followed by different letters within each column are significantly different (P ≤ 0.05)

<table>
<thead>
<tr>
<th>Level</th>
<th>Emergence holes per field</th>
<th>Adults collected per field</th>
<th>Variety type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becky blue (RE)</td>
<td>188.3 ± (50.1) (a)</td>
<td>43.4 ± (15.5) (a)</td>
<td>Classic</td>
</tr>
<tr>
<td>Bonito (RE)</td>
<td>143.0 ± (48.5) (ab)</td>
<td>47.7 ± (6.6) (a)</td>
<td>Classic</td>
</tr>
<tr>
<td>Misti (SHB)</td>
<td>115.1 ± (45.0) (b)</td>
<td>43.2 ± (6.4) (ab)</td>
<td>Classic</td>
</tr>
<tr>
<td>Climax (RE)</td>
<td>76.4 ± (24.8) (c)</td>
<td>46.6 ± (15.5) (a)</td>
<td>Classic</td>
</tr>
<tr>
<td>Sharps (SHB)</td>
<td>71.9 ± (29.4) (c)</td>
<td>48.8 ± (5.2) (a)</td>
<td>Classic</td>
</tr>
<tr>
<td>Windy (RE)</td>
<td>67.5 ± (35.2) (c)</td>
<td>38.7 ± (14.5) (ab)</td>
<td>Classic</td>
</tr>
<tr>
<td>Tiffanies (RE)</td>
<td>61.6 ± (22.5) (c)</td>
<td>46.0 ± (19.3) (a)</td>
<td>Classic</td>
</tr>
<tr>
<td>Crunchy (SHB)</td>
<td>55.6 ± (18.4) (c)</td>
<td>25.8 ± (3.5) (c)</td>
<td>Classic</td>
</tr>
<tr>
<td>Star (SHB)</td>
<td>51.3 ± (24.7) (d)</td>
<td>22.7 ± (5.0) (c)</td>
<td>Classic</td>
</tr>
<tr>
<td>42 (SHB)</td>
<td>51.0 ± (30.8) (d)</td>
<td>8.8 ± (1.3) (d)</td>
<td>New</td>
</tr>
<tr>
<td>115 (SHB)</td>
<td>48.4 ± (14.2) (d)</td>
<td>30.5 ± (4.8) (bc)</td>
<td>New</td>
</tr>
<tr>
<td>41(SHB)</td>
<td>45.1 ± (14.0) (d)</td>
<td>34.3 ± (10.0) (bc)</td>
<td>New</td>
</tr>
<tr>
<td>390 (SHB)</td>
<td>44.9 ± (15.6) (d)</td>
<td>23.5 ± (7.1) (c)</td>
<td>New</td>
</tr>
<tr>
<td>209A (SHB)</td>
<td>44.1 ± (15.5) (d)</td>
<td>24.4 ± (6.3) (c)</td>
<td>New</td>
</tr>
<tr>
<td>Britwell (RE)</td>
<td>43.2 ± (11.03) (d)</td>
<td>33.2 ± (18.7) (bc)</td>
<td>New</td>
</tr>
<tr>
<td>Powderblue (RE)</td>
<td>35.0 ± (22.8) (d)</td>
<td>3.8 ± (1.0) (d)</td>
<td>New</td>
</tr>
<tr>
<td>Premier (RE)</td>
<td>27.3 ± (18.4) (d)</td>
<td>2.9 ± (1.2) (d)</td>
<td>New</td>
</tr>
</tbody>
</table>

(SHB) = Southern highbush. (RE) = Rabbiteye
# Chapter 3 Blueberry variety susceptibility & Orthorhinus cylindrostris population dynamics

## 3.5 Blueberry variety susceptibility

<table>
<thead>
<tr>
<th>Variety</th>
<th>Yield (blueberry Kg/Tree)</th>
<th>Median EWB emergence (Median EWB emergence / tree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder Blue</td>
<td>3.5</td>
<td>115</td>
</tr>
<tr>
<td>Britwell</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Premier</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Climax</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bonito</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Sharp</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tiffanies</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Star</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95 x crunchy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>209A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Becky Blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Windy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3-3 Blueberry varieties yield (mean ± se) relative to median *Orthorhinus cylindrostris* emergence holes/plant. Median emergence was used to minimise the effect of outliers.
3.3.2 Mapping of *Orthorhinus cylindrostris* population on Blueberry Farms of Australia

Figures 3-4 to 3-7 map the abundance of *O. cylindrostris* adult and larval distributions in 2007/08 and the spatial arrangement of current (last 2 years) and historic (pre 2001) blueberry varieties grown in the different fields. Woody field borders are denoted by the dark grey areas surrounding fields. The spread of adults show there are clear areas of low and high density on the map. However there is no evidence of edge effects on adult abundance. Figure 3-4 and 3-5 shows *O. cylindrostris* adult and emergence distribution is in medium to high density (yellow to red) over 30% of the blueberries under cultivation, with the greatest infestation over the southern areas of BFA.

In the past (figure 3-7) blueberry varieties were planted in large expanses e.g. 15 ha intervals. The current practice (figure 3-6) of blueberry cultivation is to remove and plant smaller areas, e.g. 5 ha. The smaller land holdings are planted with different varieties to the adjacent paddocks to increase hybrid vigour. However there are large expanses of unknown varieties in the historic data (figure 3-7). The observed distribution of *O. cylindrostris* from the 2007/2008 survey relates to the spatial arrangement of different varieties at BFA and to the evidence for differences in susceptibility to attack between varieties.

The varieties which are currently planted in the high infestation areas predominantly SHB var Sharp, 390, 209A Climax, Crunchy, Becky blue and 115. The varieties which are currently found in low infestation areas are RE var Powderblue, Britwell, and Premier and newly planted blocks of SHB var Sharp and 390. When the abundance data is compared with the historic spatial arrangement of varieties (figure 3-7), weevils were found in the areas where the highly susceptible varieties Bonito, Misty and Becky blue were originally planted.
Figure 3-4 2008 *Orthorhinus cylindrostris* adult distribution map over BFA
Figure 3-5 2008 *Orthorhinus cylindrostris* emergence distribution map over BFA
Chapter 3 Blueberry variety susceptibility & Orthorhinus cylindrostris population dynamics

Legend

- Major roads
- Sharp & 209A
- Powder blue & Britvell
- 390 & Sharp
- 115 & 390
- Climax & Becky blue
- Crunchy & 42
- Star & Crunchy
- 115 & Crunchy
- 41 & 390
- 209A & 115
- Windy & Tiffteries & Climax
- 42 & Sharp
- Dam
- Grass

Figure 3-6 2008 Recent Vaccinium corybosum varieties on BFA
Figure 3-7 Historic *Vaccinium corybosum* varieties over BFA
3.4 Discussion

All blueberry varieties commonly grown in Australia were attacked successfully by *O. cylindrirostris* but susceptibility to attack differed between varieties. Modern RE varieties (Powderblue, Britwell, and Premier) were attacked by fewer *O. cylindrirostris* relative to all SHB varieties. The varieties which have the highest *O. cylindrirostris* populations (RE c.v. Bonito and Misty) have now sustained so much damage that all plants have been eaten out and removed from the fields. Approximately 30% of the land under blueberry cultivation is heavily infested with *O. cylindrirostris*. The populations thus far are concentrated throughout the southern extent of the blueberry farm and infestation appears to be linked to susceptible varieties. The results indicate that no blueberry variety is resistant to attack but an element of tolerance is present in some varieties.

The varieties which looked best to use as tolerant varieties with no consideration of other pests, diseases or consumer preferences were Powderblue, Britwell and Premier. These varieties gave high yields even when subject to some degree of attack and did not support large numbers of adults. These varieties would be acceptable for management of *O. cylindrirostris* although other factors also determine which is favoured by producers. Powderblue and Premier yield increased with age and had low *O. cylindrirostris* emergence. Britwell yield was moderately affected by *O. cylindrirostris* emergence. The varieties, SHB var 42, 115, 41, 390 and 209A were moderate to high yielding varieties when subject to attack by *O. cylindrirostris* but supported higher numbers of adults than Powderblue, Britwell and Premier. Any of these varieties would be a better choice when compared with the varieties Climax, Sharp, Windy, Tiffanies, Crunchy and Star which are highly damaged by *O. cylindrirostris* emergence, and harbour high numbers of adults and emergence and are currently planted in large areas throughout BFA.

The variety Becky blue needs to be removed from the property. The yield is heavily reduced in the presence of weevils and the variety is heavily infested with large adult population and emergence. The variety is still retained due to its berry quality; however it may be serving as brood trees for further infestations in other areas of BFA due to the dispersal capability of adult *O. cylindrirostris* and their ability to invade new areas of the farm (Froggatt, 1900).
Chapter 3 Blueberry variety susceptibility & Orthorhinus cylindrirostris population dynamics

The inconsistent spread of *O. cylindrirostris* suggests that adults likely colonized the plots by successive flights from infested trees within the crop. The generally low aggregation levels and inconsistent spatial structures (figure 3-4 and 3-5) indicate distribution is determined by the plantings of different varieties. The presence of brood trees on BFA may be an explanation for the clumped rather than regular or random spatial abundance of *O. cylindrirostris* (figure 3-6 and 3-7). The variability makes it difficult to predict damage based on recording numbers of emergence in a particular area as fields closely located to heavily infested areas will have a spill over effect (Marshall and Moonen 2002), an effect that was seen when trying to determine potato variety susceptibility to potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) on alternate planting of varieties in Israel (Coll et al., 2000). This alone is unlikely to be the only factor. *O. cylindrirostris* damage is likely to also include factors such as farming practices, variety layout, plant health, general climatic conditions, farm hygiene (Wright, 2008) and the fluctuation of *O. cylindrirostris* numbers from one year to another (Clift, 2005). However growers should still be encouraged to continue monitoring for better assessment of the general trends in the pest populations (Pedigo et al., 1986).

Host plant defensive strategies such as antibiosis are likely to be an effective control option for *O. cylindrirostris*. Escape in time or space could not be used in Australian blueberry production as it is only effective when close synchronization of host and pest phenology occurs, or for annual plants that can be grown in different locations each year (Hanks, 1999; Hanks et al., 1991; Mattson and Haack 1987; Mattson et al., 1988). The variety survey suggests that there no varieties with resistance to *O. cylindrirostris* attack but there is evidence of some tolerant varieties. These varieties show tolerance by continuing to give high yields even in the presence of *O. cylindrirostris* attack. In particular, the RE c.v. Powderblue, Premier, Britwell exhibited the greatest tolerance of the variety tested. Rabbiteye varieties are considered to be tough and fast growing (Wright, 2008) and their tolerance of *O. cylindrirostris* may be attributed to physical plant characteristics (Harris and Frederiksen, 1984). Tolerance of attack by the squash vine borer *Melitta cucurbitae* (Harris) (Lepidoptera; Sesiidae) in cucurbits is associated with hard woody stems with closely packed, tough vascular bundles. These make larval entry and feeding difficult (Painter, 1958). There was not time to investigate the actual mechanism
producing tolerance in this study given the long life cycle of the pest (chapter 2) but it is an important topic for future research.

Maps of insect abundance are useful and often the only practical means available to estimate insect populations because of the time and expense involved in more precise measures (Fierke et al., 2005). The spatial patterns can be used as a tool to categorize *O. cylindrirostris* severity and will help growers to determine spatial and temporal changes in abundance of this long-lived pest. The maps can be used to supplement control for the standard blueberry varieties or screen for blueberry progeny with particularly good or poor insect resistance. The current spatial distribution of *O. cylindrirostris* corroborates the initial observations of Clift (2005). *O. cylindrirostris* was observed to heavily infest Bonito and Misty varieties. Bonito were planted in the southern extent of BFA and Misty to the north (figure 3-7). From the maps it can be seen that the very susceptible varieties, Bonito and Misty, have now been removed, an appropriate strategy for heavily infested trees (Dubbert et al., 1998; Milinski and Parker, 1991). This should have an impact on the *O. cylindrirostris* pressure in the existing plantations however because movement patterns and residence times have not been well documented in the past it is difficult to speculate about future trends (Clift, 2005). Indeed the levels of *O. cylindrirostris* infestation are still extremely high although these areas are now planted under different blueberry varieties. Continued removal of susceptible varieties and replanting with tolerant varieties identified in this study or through future breeding programs would be advisable as part of integrated pest management for *O. cylindrirostris*.

### 3.5 Conclusions

Variety susceptibility of blueberry varieties to *O. cylindrirostris* attack illustrated varying tolerance and as a result no single EIL is applicable for all varieties. Although this study was unable to determine an EIL, tolerant varieties are currently grown on BFA and host tree tolerance represents a promising strategy that offers the potential for reducing the impacts of *O. cylindrirostris*. Trees which harbour lower *O. cylindrirostris* populations and are high yielding varieties include RE c.v. Powderblue, Britwell and Premier. These varieties could be used to restock areas where infested trees have been removed, as well as to minimize the probability of
establishment of *O. cylindrirostris* in areas with a high risk of infestation that were identified through spatial maps. With the removal of heavily infested trees, *O. cylindrirostris* populations should be reduced in the long term.

Unquestionably, current knowledge of the mechanisms underlying blueberry plant tolerance to *O. cylindrirostris* is inadequate. However since tolerant varieties were identified, a study using these varieties to determine the mechanisms of tolerance should be conducted. These varieties should be used to develop a blueberry breeding program that targets tolerance of *O. cylindrirostris* and perhaps to incorporate the desirable berry characteristics found in some susceptible varieties into new tolerant variety. The programme will need to operate over a three to six year time scale due to the long lifecycle of *O. cylindrirostris* and the production life of *Vaccinium* trees.

### 3.6 References


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Chapter 3 Blueberry variety susceptibility & Orthorhinus cylindriostris population dynamics


Chapter 4 - Potential control options for Orthorhinus cylindrirostris

Chapter 4 Potential control options for *Orthorhinus cylindrirostris*

4.1 Introduction

*Orthorhinus cylindrirostris* is an emerging pest in cultivated blueberries. Control measures need to be identified to determine an integrated pest management program. Given consumer demand and current Australian Pesticides & Veterinary Medicines Authority (APVMA) reviews of broad spectrum products such as organophosphates and the popular view against broad-spectrum and persistent pesticides, the time is right to further develop, register, and utilize reduced-risk insecticides. Here within is discussed newer insecticidal technologies based on biological components and reduced risk chemicals to provide a tandem approach to *O. cylindrirostris* control. This literature examines the current knowledge and needs in relation to development of integrated pest management for *O. cylindrirostris*.

Integrated pest management (IPM) combines chemical, biological, and cultural control to provide targeted and efficient pest management solutions that can be tailored to specific climates and habitats in order to maximize efficacy. Integrated pest management in the context of agricultural entomology is a pest control strategy using complementary methods: mechanical, physical, genetic, biological, cultural, and chemical management. These methods are utilized in three stages of prevention, observation and intervention. It is an ecological approach whose main goal is to reduce the use of pesticides for pest suppression whilst maintaining beneficial insects such as predators, parasitoids and pollinators (Howarth, 1991; Simberloff and Stiling, 1996). IPM is an ideal fit for an environmentally friendly approach to farming. IPM can reduce human and environmental exposure to hazardous chemicals, potentially lower overall costs once initiated and is an alternative to calendar-based insecticide programs (Bostanian et al., 2005).

The basic components that need to be attained to achieve a successful IPM program include: proper identification of the pest or pest complex, knowledge of the pest’s life cycle and biology, monitoring pest abundance and damage levels and the effect of control measures, record keeping (reproductive cycles, population dynamics), establish an action threshold (economic, health or aesthetic), preventive cultural practices (maintaining healthy crops and mechanical
removal of insects), biological controls (natural biological processes), chemical controls and evaluation of the results (Apple et al., 1976). All of these aspects are considered in this thesis.

4.1.1 Current control measures for Orthorhinus cylindrirostris

Control of *O. cylindrirostris* was introduced in vineyards using fipronil (Regent 200SC NUFARM 200 g/L) at 20mL/100L in conjunction with azinphos methyl (Gusathion 200 SC Farmoz 200 g/L) at 245mL/100L as foliar applications to provide consistent control of elephant weevil adults. Broad spectrum insecticide use is discouraged in blueberries due to consumer pressure for minimal residues and the high toxicity to social hymenopteran pollinators (particularly when insecticides are carried back to the nest by foraging workers) that are required for successful fruit set (Macdonald, 1996).

4.1.1.1 Potential chemicals for Orthorhinus cylindrirostris

For development of new insecticides or to register existing insecticides for use against off label insect pests, laboratory efficacy tests are performed before semi-field or field application to ensure that the candidate insecticide will be effective on the target insect populations under typical environmental conditions. Over the past 40–50 years, the choice of insecticide compounds available in horticulture has moved away from the organochlorines to include organophosphate, carbamate and pyrethroid compounds (Hagstrum and Flinn, 1996; Schöller et al., 1997). Depending on their type and application rate, insecticides can ensure protection from noxious insects (Sinha, 1995). Indoxacarb and imidacloprid are two insecticides which may provide chemical tools for integrated pest management of *O. cylindrirostris*.

Indoxacarb is produced by DuPont™ and marketed as a reduced-risk pesticide worldwide for use on apples, pears, brassicas, sweet corn, lettuce and fruiting vegetables. Indoxacarb is classified as an oxidiazine, which is a new class of pyrazoline-type insecticides (McCann et al., 2001). Activity has been shown against several lepidopteran pests as well as numerous coleopteran pests (Pluschkell et al., 1998; Tong-Xian et al., 2002). Indoxacarb's key feature is its novel bioactivation. Insect voltage-gated sodium channels are blocked off by N-decarbomethoxylated
metabolite interference. The ion channels are inhibited and the flow of sodium into nerve cells is stopped, causing paralysis and death of pests (Wing et al., 2000).

Organophosphate resistant and susceptible populations of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, (Parimi et al., 2003) Egyptian alfalfa weevil (EAW), *Hypera brunneipennis* and alfalfa weevil, *Hypera postica*. (Godfrey and Pickel, 1998) southern corn rootworm, *Diabrotica undecimpunctata*, Colorado potato beetle *Leptinotarsa decemlineata*, and boll weevil *Anthonomus grandis* (Greene et al., 2001; Wing et al., 2000) have all shown susceptibility to indoxacarb by causing mortality to larvae and adults via contact exposure from indoxacarb based pesticides.

*Imidacloprid* has proved to be a very effective insecticide by blocking elements of the insect nervous system (Suchail et al., 2001) and acting as a nicotinic acetylcholine receptor antagonist. Previous research has shown differences in the nicotinic acetylcholine receptor to be responsible for the specificity of the insecticide; the mammalian receptor contains an anionic binding subsite, whereas the insect receptor contains a cationic sub-site (Tomizawa and Casida, 1999). It is considered to be among the best insecticide candidates because of this specificity for the insect nervous system and its low mammalian toxicity. Currently, imidacloprid is registered for a wide range of uses including as a soil, seed and foliar insecticide to control coleopteran pests on crops including rice, cotton, cereals, maize, sugar beet, potatoes, vegetables, citrus and stone fruit (Liu and Casida, 1993; Yamamoto et al., 1998).

Chloronicotinyl compounds are distributed throughout plant tissues, providing cultivated crops with protection from both root and foliar pests. Clothianidin and Imidacloprid, the newest member of the chloronicotinyl insecticide family, has recently been registered for foliar spray and seed treatment applications. Clothianidin has a high activity against a broad range of insects, including sucking insects and chewing insects (Liu and Casida, 1993; Yamamoto et al., 1998).

Chloronicotinyl compounds have proved to be a very effective insecticide by blocking elements of the insect nervous system (Suchail et al., 2001) and acting as a nicotinic acetylcholine receptor antagonist. Previous research has shown differences in the nicotinic acetylcholine.
receptor to be responsible for the specificity of the insecticide; the mammalian receptor contains an anionic binding subsite, whereas the insect receptor contains a cationic sub-site (Tomizawa and Casida, 1999). It is considered to be among the best insecticide candidates because of this specificity for the insect nervous system and its low mammalian toxicity. Currently, imidacloprid and clothianidin is registered for a wide range of uses including as a soil, seed and foliar insecticide to control coleopteran pests on crops including rice, cotton, cereals, maize, sugar beet, potatoes, vegetables, citrus and stone fruit (Liu and Casida, 1993; Yamamoto et al., 1998).

The toxicity of chloronicotinyl compounds to a number of coleopteran pests throughout the world has been measured under laboratory and field conditions as part of a program to develop and integrate a wider spectrum of controls. Results have been exceptional with up to 90% mortality. Targeted coleopteran pests include the cereal leaf beetle, *Oulema melanopus* (L.), (Tharp et al., 2000), Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Therese et al., 2006), Melolonthine scarab beetles (Hurley et al., 2002) and Childers cane grubs, *Antitrogus parvulus* Britton. chloronicotinyl compounds are now used to control existing infestations of the above examples and as a rotation with the organophosphate-based cadusafos and chlorpyrifos (suSCon® Blue) to minimise the development of insecticide resistance (McGill et al., 2003).

### 4.1.2 Potential biological pesticides for Orthorhinus cylindrirostris

**4.1.2.1 Entomopathogenic Fungi**

Entomopathogenic fungi are increasingly recognized as important regulatory factors in insect populations (Zimmerman and Cranshaw, 1991) and numerous species are employed for biological control of insect pests or as a supplement to broad-spectrum chemical insecticides (Burges, 1980; Devi et al., 2003; Lacey et al., 2001; Meyling and Eilenberg, 2007). The following sections discuss entomopathogenic fungi as well a range of chemical insecticides that show potential for control of *O. cylindrirostris*.

Although more than 700 species of entomopathogenic fungi have been reported (Hajek et al., 2003; Samson et al., 1988; St. Leger et al., 1991), the most studied are *Beauveria bassiana*
Chapter 4 - Potential control options for Orthorhinus cylindrirostris

(Balsamo) Vuillemin (Deuteromycota: Hyphomycetes), Metarhizium anisopliae (Metschnikoff) Sorokin (Deuteromycota: Hyphomycetes), Verticillium lecanii (Zimmermann) Viegas (Deuteromycota: Hyphomycetes), and Paecilomyces fumosoroseus (Wize) (Deuteromycota: Hyphomycetes), which have been used for the control of a range of insect pests (Goettel and Hajek, 2001; Keller and Zimmermann, 1989; Zimmerman, 1992).

Among the entomopathogenic fungi, B. bassiana is a popular registered myco-insecticide (Glare and Milner, 1991; Goettel et al., 2000; Humber, 1991; Li et al., 2005). It is ubiquitous in distribution and is pathogenic to a wide spectrum of arthropods including most insect orders (Butt and Goettel, 2000; Lacey et al., 2001; Zimmermann, 2007).

4.1.2.2 Entomopathogenic fungi as a biological control

There are problems with the practical use of entomopathogenic fungi such as market awareness and quality control, particularly variability in virulence of the products and observed mortality rates (Bateman and Chapple, 2001; Butt et al., 2001). The successful integration of entomopathogenic fungi into a blueberry production system for use against O. cylindrirostris will rely on survival of the fungus in the environment prior to infection of the target host, mortality rate and compatibility for integration with current control measures against other pests. Infection of B. bassiana conidia occurs through an initial deposition onto the integument of the target insect. When contact with the insect cuticle occurs, the conidia germinate, producing a germ tube which, through a combination of mechanical pressure and enzymatic action, pushes through the integument and into the hemocoel (Inglis et al., 1997; Samson et al., 1988). Once inside the body, mitosis rapidly takes place, and is obvious visually 5-7 days after insect death, eventually leading to mummification of the insect (Bartlett and Jaronski, 1988). Mycelia appear first at the joints of the insect femur, tibia and cranial sutures (Boucias et al., 2000). Attachment of conidia to the insect cuticle is assisted by forces enacting highly hydrophobic responses at the conidia wall and the insect epicuticle (Boucias et al., 2000). B. bassiana produces an appresorium at the contact point of the conidia and insect integument prior to mechanical impaction upon the cuticle; this site is where penetration of the cuticle takes place (Samson et al., 1988). The mechanical force exerted also needs the help of secreted enzymes as cuticular
lipids have been shown to have antifungal properties. These enzymes (lipases, chitinases, proteases and elastases) aid penetration of the germ tube (Samson et al., 1988).

Effects of physical conditions like temperature and moisture on the virulence of isolates have been well documented (Ferron et al., 1991; Glare and Milner, 1991; Vandenberg et al., 1998). For example, significant differences in virulence were observed with *Beauveria brongniartii* isolates tested against two different populations of the same insect species, the European cockchafer *Melolontha melolontha*, under varying climatic conditions (Keller et al., 1999).

### 4.1.2.3 Survival and application of entomopathogenic fungi in the field

The host range of *B. bassiana* in the laboratory is much larger than that observed in the field (Hajek et al., 2001). This is due to the environmental conditions of the laboratory, which provide optimal temperatures, water availability and limit UVB exposure. The temporal and spatial separation of pathogen and host in the natural environment also explains the lack of natural epizootics (Hajek et al., 2001).

Persistence of *B. bassiana* in the field will be an important determinant of the level of mortality that the fungi enact upon the target host (Inglis et al., 1997). Persistence can be defined by survival of either conidia or mycelia and active capacity to infect. Abiotic factors such as water availability, microclimate, UVB exposure, canopy type and temperature all have an effect on fungal biological controls (Ekesi et al., 1999). Endophytic persistence of *B. bassiana* has been demonstrated via stem injections into maize plants, which provided systemic protection against stem borers on a year round basis (Cherry et al., 1999). *B. bassiana* can be used to control a wide variety of pests. Nevertheless the focus of this review is application of the fungus to weevil or borer-like pests. There are several application methods for insect control including: dry conidia seed dressings, placing conidia suspensions onto leaf axils, and direct injection of conidia suspensions into plants stems (Cherry et al., 1999). Commercial *B. bassiana* products are applied via micro-granules, wettable powders and as suspension concentrates in high volume applicators (Bateman and Chapple, 2001). Oils such as Tween-80® may be used to suspend spores in solution (Godonou et al., 2000). Currently concentrations of spores for use against weevils range from $10^6$ to $10^9$ conidia mL$^{-1}$ (Godonou et al., 2000). For example the application
of *B. bassiana* in Ugandan banana plantations reduced banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), populations by 63 - 72% over an 8 week period (Nankinga and Moore, 2000). Similarly the transmission of *B. bassiana* infection to pecan weevils *Curculio caryae* (Horn) (Coleoptera: Curculionidae) using soil based inoculum resulted in high larval mortality (Gottwald and Tedders, 1984).

4.1.3 Objectives

Biological and chemical insecticide applications have proved a useful tool in the control of many coleopteran pests. A rigorously followed IPM program requires a number of options for success; these options are intended to manage pest populations rather than eliminate them altogether.

The aim of the following experiments was to examine chemical and biological control methods for use in an integrated pest management program against *O. cylindrirostris*. Examples of insecticides that have potential to manage *O. cylindrirostris* populations in cultivated blueberries are presented and the development of non-chemical pest control was a key focus.

4.2 Materials & methods

4.2.1 Insect collection and rearing

Scouting and mechanical removal from September to December 2006 (see chapter 2) was used to obtain adult *O. cylindrirostris* from Corindi, NSW. Insects were taken back to the University of Sydney and maintained in rearing cages (20L plastic containers, 385 x 270 x 250mm, Starmaid, Officeworks, Australia), on fresh blueberry stems, which were replaced weekly. Blueberry stems and foliage (*Vaccinium corymbosum var. 390*) were maintained in a chemical free environment at Darlington glass house (the University of Sydney) in 40L pots (Botany bag planter bag™) and drip irrigated (Netafim Drip Line™). Insects could not be kept in continuous culture under laboratory conditions (22-25 °C 70% relative humidity (RH) and natural light) due
to their long life cycle and the need to rear larvae on living plants (Chapter 2). All insects were monitored daily for food consumption and mortality.

### 4.2.2 Isolation and culture of entomopathogenic fungi

Insects were monitored daily and any cadavers were removed from the rearing containers. The cadavers were transferred intact to sterile Petri dishes containing moistened sterile filter paper, sealed with Parafilm and kept at 22 °C temperature, 60% RH, and 12L:12D (ProSciTech E14 Incubator, Thuringowa Qld Australia) for 14 days (under these conditions *B. bassiana* hyphae rapidly emerged from experimentally infected *O. cylindrirostris* adults).

The cadavers were monitored for fungal growth (hyphal emergence) and external growth of secondary microbes. When fungal hyphae emerged mycelium from 20 randomly selected cadavers was sampled and cultured on PDA plates for identification using Humber (1991). In addition, two insects were killed and surface-sterilized using serial immersion in sterile water and 70% ethanol. Cadavers were blotted dry with sterile Kimwipes (Kimberley-Clark™ Pty Ltd Australia NSW Australia) then broken open with sterile tweezers. A sample of the internal contents was spread on PDA plates. Culture plates were incubated aerobically in 25°C temperature, 60% RH, and 12L:12D for 14 days.

Koch Postulate testing identified *B. bassiana* and *A. parasiticus* fungal cultures as potential entomopathogenic fungi candidates with concentrations of $1 \times 10^6$ spores mL$^{-1}$ the most efficacious from a pilot study.

Koch Postulate (Knight and Burgess, 2008):
1. Observe a consistent association between the disease condition and the presence of a specific microbe.
2. Isolate the microbe and grow it in pure culture outside of the original host.
3. Inoculate a healthy, susceptible host with the pure culture and observe disease symptoms that are the same as those in the original host.
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4. Isolate the microbe from the inoculated host and demonstrate that it is the same as the microbe from the original diseased organism.

*B. bassiana* conidia were harvested into water by rubbing the surface of a culture with a sterile bent glass rod to dislodge the spores. For long-term storage, spores were kept in 50% glycerol at -20 °C, or in 15 - 20% glycerol at -80 °C.

Cultures of *B. bassiana* and *A. parasiticus* were maintained on potato dextrose agar at 24 ± 4°C. For bioassays, hyphae and spores were spread on PDA plates and cultured aerobically at 25 ± 4°C. Following sporulation, conidio-spores were harvested by scraping plates flooded with sterile water, then suspended in distilled water and diluted to a final concentration of 1 x 10^6 spores mL⁻¹, using a counting chamber (hemocytometer) (Double Neubauer Ruled Metallized Counting Chamber, ProSciTech, Australia) to determine the spore concentration.

4.2.3 Dose response tests of entomopathogenic fungi and chemical insecticides

A series of dose-response trials were conducted using laboratory bioassays to determine the efficacy of several insecticides and three bio-insecticides against *O. cylindrirostris* adults (table 4-1). *O. cylindrirostris* larvae were tested only against systemic insecticides (table 4-1) because of their cryptic wood-boring habit (Chapter 2).

To measure mortality rates in *O. cylindrirostris* adults a logarithmic series of four application rates, including an untreated control, was tested in the dose response assays (the number of application rates for each insecticide was restricted because all insects had to be collected from the field). All treatments (except crown drenching) were applied by topical application of 10mL using a 100mL hand spray bottle (Taizhou Huangyan Dinghao Plastic Factory, China). The solution was sprayed onto 10 adults (50: 50 sex ratio) then insects were released into cages (10 L Starmaid plastic containers, 285 x 170 x 200 mm, Starmaid, Officeworks, Australia) and fed cut blueberry stems (approximately 0.3m in length) (for blueberry maintenance see insect collecting and rearing). Each treatment was replicated five times (a total of 150 insects per insecticide treatment, n = 50 per application rate). Before spray treatment, enough individual
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branch segments (300mm long) for five replicates for four bioassays were cut, i.e. approximately 5000 g of stem material.

The assessment of systemic chemicals was done for liquid formulations using a crown drench method applied by a knap sack sprayer (Silo 435, Silo™, Germany) while tablet formulations were applied in the root zone as specified by the manufacturer. The systemic applications were made 14 days prior the removal of blueberry stems for bioassays to ensure the chemical had translocated through the phloem and xylem. The blueberry bushes used for the systemic applications were 2 years old and 1 meter tall in 40L pots.

Cages were checked daily for a maximum of 24 days and the number of dead weevils recorded and removed each day. Plastic rearing containers were kept in 22 ± 4 °C temperature, 65% ± 5% RH, and ambient light conditions.

To measure mortality rates in *O. cylindrirostris* larvae, ten weevils (50: 50 sex ratio) were caged on potted blueberry bushes at Darlington glass house for ten days to allow mated females to oviposit. Weevils were then removed and systemic insecticides were either crown drenched or applied in tablet form; applications were made as recommended by the chemical manufacturer using three application rates (table 4-1). Larvae were allowed to feed for three months. Trees were then dissected into 1cm segments (sampling without replacement) to determine efficacy. Trees were housed outdoors in 28 ± 12°C temperatures, 62% ± 23% RH, and ambient light conditions.

Probit analysis was conducted on all dose response tests to determine the relative toxicity of the different insecticides (Probits5® for Windows 1991, P. Gillespie, Orange, N.S.W., Australia). Probit analysis is a measure of mortality rates of organisms when subject to the effect of a mortality factor. Usually a control trial is tested against a range of trials, each of a different concentration of the pesticide. Important details given from the analysis are: the difference between the actual and the expected counts and the chi-square statistic for testing the significance of these differences; the total of the Chi-square values which is used to test the overall significance of the differences from the model; degrees of freedom for the chi-square
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test; the fiducial limits - the boundaries within which a parameter is considered to be located; and the heterogeneity factor – indicating the "soundness" of the data. Heterogeneity factors of $< 1$ indicate homogeneous data; factors $> 1$ indicate heterogeneous data. Results with heterogeneous data should be treated with some caution.
## Chapter 4 - Potential control options for Orthorhinus cylindrirostris

Table 4-1 Synthetic chemicals and biopesticides used in dose response tests against *Orthorhinus cylindrirostris* adults

<table>
<thead>
<tr>
<th>Chemical trade name</th>
<th>Active Constituent</th>
<th>1/2 Rate tested</th>
<th>Rec Rate* tested</th>
<th>2 x Rate tested</th>
<th>Chemical Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marlin® (CropCare™)</td>
<td>Methomyl</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>Carbamate</td>
</tr>
<tr>
<td>Avatar® (Dupont™)</td>
<td>Indoxacarb</td>
<td>6.25</td>
<td>12.5</td>
<td>25</td>
<td>Oxidiazine</td>
</tr>
<tr>
<td>Clothianidin 200SC® (Sumotomo™)</td>
<td>Clothianidin</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
<td>Neonicitinoid</td>
</tr>
<tr>
<td>Clothianidin 200SC® butt drench (Sumotomo™)</td>
<td>Clothianidin*</td>
<td>1.25</td>
<td>3.5</td>
<td>7</td>
<td>Neonicitinoid</td>
</tr>
<tr>
<td>Confidor 200SC® (Bayer™)</td>
<td>Imidacloprid</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
<td>Neonicitinoid</td>
</tr>
<tr>
<td>Confidor 200SC® butt drench (Bayer™)</td>
<td>Imidacloprid*</td>
<td>1.25</td>
<td>3.5</td>
<td>7</td>
<td>Neonicitinoid</td>
</tr>
<tr>
<td>Initiator® tablets (Bayer™)</td>
<td>Encapsulated</td>
<td>½ tablet (1.25g)</td>
<td>1 tablet (2.5g)</td>
<td>2 tablet (5g)</td>
<td>Neonicitinoid</td>
</tr>
<tr>
<td>Mycoforce® (Nutri-Tech©)</td>
<td>Mixed conidia</td>
<td>1x 10⁴/mL</td>
<td>1x 10⁶/mL</td>
<td>1x 10⁸/mL</td>
<td>Fungus</td>
</tr>
<tr>
<td><em>Aspergillus parasiticus EWB</em></td>
<td>conidia</td>
<td>1x 10⁴/mL</td>
<td>1x 10⁶/mL</td>
<td>1x 10⁸/mL</td>
<td>Fungus</td>
</tr>
<tr>
<td><em>Beauveria bassiana EWB</em></td>
<td>conidia</td>
<td>1x 10⁴/mL</td>
<td>1x 10⁶/mL</td>
<td>1x 10⁸/mL</td>
<td>Fungus</td>
</tr>
</tbody>
</table>

*In addition to adult bioassays, these chemicals were tested against larvae. *Fungal species are *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae*. *Rec Rate was the recommended rate on the label for control of coleopteran pests in edible horticulture production.*
4.2.4 Insecticide and entomopathogenic fungi efficacy under field conditions

A subset of chemicals and entomopathogenic fungi were tested under either field or semi-field conditions (table 4-2). The observed mortality in the dose response assays was considered when selecting chemicals for field tests.

Field plot experiments for entomopathogenic fungi were conducted at BFA from September 2006 to September 2008 to assess the efficacy of the entomopathogenic fungi against *O. cylindrirostris* adults. Two blueberry varieties *V. corymbosum* var. 390 and *V. corymbosum* var. sharp were selected for the study. Control plots treated with water and wetting agent (Tween 80) Emergence holes from previous seasons were marked with paint so that new emergence holes could be identified. The myco-pesticide was prepared and applied in a Latin square design with two varieties per treatment and ten blueberry bushes per variety (10 vav. 390 and 10 var. sharp); each treatment was replicated ten times. A hand sprayer (Solo 480 Knap sack sprayer) was used to apply the treatment solutions. All insecticides were applied at 100 L/ha total volume; changes in application rate were achieved by a dilution series if needed. Treatments were reapplied every 100 days (due to the long lifecycle, chapter 2). Efficacy was measured by counting new emergence holes at the end of the experiment, September 2008. The level of insect infestation was compared between sprayed and unsprayed plots using Chi square ($\chi^2$) analysis (JMP© version 8, SAS™ 2007).

Field plot experiments for synthetic chemicals were conducted in potted blueberry bushes at the University of Sydney because APVMA regulations do not permit use of unregistered chemicals in commercial crops. Trees 1m tall were maintained in 40L pots (Botany bag planter bag™) and drip irrigated daily (Netafim Drip Line™). Trees were sprayed with indoxacarb and imidacloprid, crown drenched with imidacloprid or treated with an encapsulated tablet imidacloprid.

The insecticide was applied in a Latin square design with one *V. corymbosum* c.v. 390 per treatment, each treatment replicated five times (30 trees total including an untreated control). A hand sprayer (Solo 480 Knap sack sprayer) was used to apply the treatment solutions.
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Insecticides were applied at the rates specified in table 4-2. After spray applications were completed ten adults per treatment (50: 50 sex ratios) were caged on the plants with the same methods used in the lifecycle methods chapter 2.2.3. Trees were assessed weekly for dead insects from September 2007 to December 2007. The level of insect infestation was compared in both sprayed and unsprayed plots. No oviposition occurred on any chemical treated plants in the field cages so larval mortality was not compared. Chi square ($\chi^2$) analysis was used to determine mortality of adults due to entomopathogenic fungi and chemicals all statistics were calculated using JMP 8 for windows (SAS).

Table 4-2 Synthetic chemicals and entomopathogenic fungi tested under field conditions

<table>
<thead>
<tr>
<th>Chemical trade name</th>
<th>Active Constituent</th>
<th>Active Conc.</th>
<th>Rate applied$^\wedge$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avatar® (Dupont™)</td>
<td>Indoxacarb</td>
<td>400g/L</td>
<td>12.5g/100l</td>
</tr>
<tr>
<td>Confidor 200SC®(Bayer™)</td>
<td>Imidacloprid</td>
<td>200g/L</td>
<td>25mL/100l</td>
</tr>
<tr>
<td>Initiator® tablets (Bayer™)</td>
<td>Encapsulated</td>
<td></td>
<td>2.5g /tree</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid*</td>
<td>200g/L</td>
<td>$1 \times 10^6$ conidia</td>
</tr>
<tr>
<td>Mycoforce® (Nutri-Tech©)</td>
<td>Mixed conidia #</td>
<td>/mL</td>
<td>$1 \times 10^8$ /mL</td>
</tr>
<tr>
<td>Beauveria bassiana EWB</td>
<td>conidia</td>
<td>N/A</td>
<td>$1 \times 10^8$ /mL</td>
</tr>
</tbody>
</table>

$^\wedge$Fungal species are Beauveria bassiana, Verticillium lecanii and Metarhizium anisopliae. $^\wedge$Rate Applied was the most efficacious rate found from the laboratory bioassays.
4.3 Results

4.3.1 Dose response bioassays

All 50 insects in the control group showed no change in their behavior or activity, with low mortality (< 4%) in the control colony. The mortality of *O. cylindrirostris* adults caused by different dosages of entomopathogenic fungi (figure 4-1) and chemical insecticides showed a linear relationship between dose and probit mortality with non-significant chi square values (indicating a consistent homogenous population, table 4-3, see appendix 3 for dose response graphs). No mortality was recorded after treatment with imidacloprid or clothianidin as crown drenches so these insecticides were not included in the probit analysis (table 4-3). Insect numbers were limited for the bioassay experiments hence only a few application rates for each insecticide or entomopathogenic fungi could be tested. Therefore the LD<sub>50</sub> values reported here should be interpreted with some caution (table 4-3).

Mortality of adults due to entomopathogenic fungi was first recorded 18 days after treatment at concentrations from 1 x 10<sup>4</sup> conidia per mL, and 14 days at the rate of 1 x 10<sup>8</sup> conidia per mL regardless of fungal species. No entomopathogenic fungi were able to produce 100% mortality in these bioassay experiments. However all adults were considered to be moribund (very slow movement and disorientation compared with normal untreated adults) after treatment. All of the entomopathogenic fungi caused a similar degree of mortality in *O. cylindrirostris* adults with >50% mortality for the lowest application rate of 10<sup>4</sup> conidia per mL (half rate). *B. bassiana* var EWB achieved 50%, 65% and 75% mortality for the three application rates 1 x 10<sup>4</sup>, 1 x 10<sup>6</sup> and 1 x 10<sup>8</sup> conidia per mL respectfully. These results are similar to *A. parasiticus* var EWB (55%, 63% and 82%) and Mycoforce® (60%, 66% and 70%) (see appendix 4). All the mortality from Mycoforce in this study was due to *B. bassiana* alone. *V. lecanii* and *M. anisopliae* were not isolated from dead cadavers, indicating that the strains of the *V. lecanii* and *M. anisopliae* in Mycoforce® did not show any infectivity to *O. cylindrirostris* adults.

There were significant differences in the mortality of adults and larvae from the various chemical insecticide treatments. The application of indoxacarb, clothianidin, imidacloprid and encapsulated Imidacloprid exhibited the greatest toxicity to *O. cylindrirostris* adults of all foliar
applied chemical insecticide tested (see table 4-3 and appendix 3). indoxacarb exhibited the
greatest level of toxicity to *O. cylindrirostris* adults for foliar applied insecticides with 80% to
100% mortality achieved in five days after application. Foliar applications of nicotinamides
clothianidin and imidacloprid performed equally well with mortality ranging from 70% for half
rate application to 100% mortality for double recommended rate. 100% mortality occurred in
seven days after application of nicotinamides. methomyl showed the lowest level of toxicity of
all foliar applied insecticides to adult with mortality of 48% 55% 58% for half, recommended
and double recommended rate (see appendix 3 and table 4-3). In contrast applications of
Clothianidin and imidacloprid as a crown drenches were not lethal to either adult or larval *O.
cylindrirostris*. Applications of the encapsulated imidacloprid was the only chemical to show
toxicity towards *O. cylindrirostris* larvae with mortality of 50%, 65% and 75% for half,
recommended and double recommended rate respectively. Mortality of adult *O. cylindrirostris*
ranged from 64% to 77% and occurred 14 days after treatment but active feeding by adults
stopped five days after application.
# Chapter 4 - Potential control options for *Orthorhinus cylindrirostris*

## Table 4-3: Log dose response (Probit analysis) of insecticides and entomopathogenic fungi tested against *Orthorhinus cylindrirostris*

<table>
<thead>
<tr>
<th>Control type</th>
<th>Insect life stage</th>
<th>N</th>
<th>$LD_{50}$</th>
<th>95% Fiducial limit $LD_{50}$</th>
<th>Slope</th>
<th>Chi square</th>
<th>P Value</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutri-Tech</td>
<td>Adult</td>
<td>150</td>
<td>11633 Conidia/mL</td>
<td>1989 - 2700000 Conidia/mL</td>
<td>0.6</td>
<td>2.8</td>
<td>P=0.09</td>
<td>1</td>
</tr>
<tr>
<td>Mycoforce®</td>
<td>Adult</td>
<td>150</td>
<td>123935 Conidia/mL</td>
<td>2894 - 53603 Conidia/mL</td>
<td>1.3</td>
<td>7.8</td>
<td>P=0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>Aspergillus parasiticus</em> var EWB</td>
<td>Adult</td>
<td>150</td>
<td>1219246 Conidia/mL</td>
<td>21417 - 69408720 Conidia/mL</td>
<td>0.8</td>
<td>0.9</td>
<td>P=0.32</td>
<td>1</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em> var EWB</td>
<td>Adult</td>
<td>150</td>
<td>2.3 g/100L</td>
<td>0.1 - 5.1227 g/100L</td>
<td>3.1</td>
<td>1.2</td>
<td>P=0.30</td>
<td>1</td>
</tr>
<tr>
<td>Indoxacarb Avatar®</td>
<td>Adult</td>
<td>150</td>
<td>254.4 mL/100L</td>
<td>4.7 - 13642 mL/100L</td>
<td>1.5</td>
<td>1.2</td>
<td>P=0.27</td>
<td>1</td>
</tr>
<tr>
<td>Methomyl Marlin®</td>
<td>Adult</td>
<td>150</td>
<td>7 mL/100L</td>
<td>0.2 - 235 mL/100L</td>
<td>3</td>
<td>0.5</td>
<td>P=0.46</td>
<td>1</td>
</tr>
<tr>
<td>imidacloprid Confidor 200SC</td>
<td>Adult</td>
<td>150</td>
<td>1.5 g/Tree</td>
<td>0.18 - 130 g/Tree</td>
<td>0.96</td>
<td>2.175</td>
<td>P=0.13</td>
<td>1</td>
</tr>
<tr>
<td>imidacloprid Initiator® tablets</td>
<td>Adult</td>
<td>150</td>
<td>0.9 g/Tree</td>
<td>0.1 - 75 g/Tree</td>
<td>1.4</td>
<td>1.8</td>
<td>P=0.17</td>
<td>1</td>
</tr>
<tr>
<td>Clothianidin Clothianidin 200SC®</td>
<td>Adult</td>
<td>150</td>
<td>6 mL/100L</td>
<td>0.1 - 75 mL/100L</td>
<td>1.6</td>
<td>0.3</td>
<td>P=0.55</td>
<td>1</td>
</tr>
</tbody>
</table>

**N = total number of insects tested**
Figure 4-1 Dead Orthorhinus cylindrirostris (A) infected with Beauveria bassiana holding onto Vaccinium corymbosum (B) infected with Aspergillus parasiticus
4.3.2 Field evaluation results

From the dose response tests the entomopathogenic fungi in Mycoforce®, and the local isolate of *B. bassiana* showed the greatest promise for field testing. Due to the myco-toxins produced by *A. parasiticus* further testing was not conducted with the local isolate of this species (see discussion). The chemicals indoxacarb, imidicloprid and encapsulated imidacloprid showed the greatest promise for field testing due to their toxicity in laboratory bioassays.

Field tests of Mycoforce® and *B. bassiana* var EWB in a commercial blueberry orchard found more trees without new emergence holes due to *O. cylindrirostris* after treatment compared with untreated trees ($\chi^2 = 5.84$, $P = 0.049$, figure 4-2). This trend was most noticeable in the Mycoforce® treatment.

Mortality of *O. cylindrirostris* adults increased following insecticide applications on potted plants compared with the control ($\chi^2 = 87.32$, $P < 0.001$, figure 4-3). Foliar applied Imidicoprid and Indoxacarb showed highest toxicity to *O. cylindrirostris* adults with 100% mortality on treated plants encapsulated imidacloprid systemic tablets did increase mortality of *O. cylindrirostris* adults when compared with the control but not as much as the foliar applications.
Figure 4-2 Emergence of *Orthorhinus cylindriostris* from total number blueberry trees after field application of *Beauveria bassiana* var *EWB* (1x 10^8 conidia/mL) and Mycoforce (1x 10^8 conidia/mL).
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4.5 Discussion

All pesticides tested had varying degrees of toxicity to *O*. *cylindrirostris* dependent on the chemical nature of the compounds, the application rate and application method. Of the insecticidal formulations and concentrations tested in laboratory bioassay experiments, foliar applications of indoxacarb, imidacloprid and thiacloprid showed the greatest toxicity on *O*. *cylindrirostris* adults. Indoxacarb had the lowest application rate of 2.5g per 100L to achieve 90% mortality which is well below the 7.5g per 100L recommended for application against *O*. *cylindrirostris* larvae. Clothianidin and thiacloprid foliar applications also achieved 50% mortality in 25mL per tree applications. Imidacloprid was the only insect formulation to show toxicity to *O*. *cylindrirostris* larvae, while still showing moderate toxicity to *O*. *cylindrirostris* adults. Imidacloprid was effective against adults in suggested laboratory bioassays, but was not effective against larvae when applied to plants. Imidacloprid as foliar applications was ineffective. Systemic treatment with imidacloprid (Initiator, 2.5g/tree) was not effective at the dosage tested. The results of the bioassay experiments for these pesticides to *O*. *cylindrirostris* adults corresponded provided 100% control of larvae on hardwood and hardwood susceptible *O*. *cylindrirostris* larvae from field bioassays. Other pest genera included potato leafhopper, *Empoasca fabae* (Hemiptera: Cicadellidae), potato scab mite, *Tetranychus urticae* (Acari: Tetranychidae) and the union mite, *Dela anthogramma* (Mesostigmata: Caparidae) and *O*. *cylindrirostris* larvae from field bioassays. Imidacloprid foliar applications have shown field efficacy of 85% mortality against the hop blight brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae) the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) and the potato leafhopper, *Empoasca fabae* (Hemiptera: Cicadellidae) in the field. However, these tests were conducted in the laboratory and do not represent field conditions for these products to *O*. *cylindrirostris* adults.

![Graph showing total mortality of adult Orthorhinus cylindrirostris on potted blueberry plants after foliar application of indoxacarb (Avatar®, 12.5g/100L) and imidacloprid (Confidor 200SC®, 25mL/100L) and systemic treatment with imidacloprid (Initiator®, 2.5g / tree)](image)

Figure 4-3 Total mortality of adult *Orthorhinus cylindrirostris* on potted blueberry plants after foliar application of indoxacarb (Avatar®, 12.5g/100L) and imidacloprid (Confidor 200SC®, 25mL/100L) and systemic treatment with imidacloprid (Initiator®, 2.5g / tree)
4.5 Discussion

All pesticides tested had varying degrees of toxicity to *O. cylindrirostris* dependent on the chemical nature of the compounds, the application rate and application method. Of the insecticide formulations and concentrations tested in laboratory bioassay experiments, foliar applications of indoxacarb, imidacloprid and clothianidin showed the greatest toxicity on *O. cylindrirostris* adults. Indoxacarb had the lowest application rate of 2.3g per 100L to achieve 50% mortality which is well below the 12.5g per 100L recommended for application against horticulture pests. Clothianidin and imidacloprid foliar application also achieved 50% mortality with application rates well below the recommended rates for horticulture pests of 25mL per 100L. Encapsulated Imidacloprid tablets were the only insect formulation to show toxicity to *O. cylindrirostris* larvae, whilst still showing moderate toxicity to *O. cylindrirostris* adults. Clothianidin was effective against adults in targeted laboratory bioassays, but was not effective against adults or larvae when applied as a crown drench. Use of methomyl as a foliar application was also ineffective. Susceptibility to these compounds clearly depended on the exposed life stage and application method.

The field experiments of entomopathogenic fungi Mycoforce®, *B. bassiana* var EWB and chemical insecticides, indoxacarb and imdicloprid showed high insect control rates under laboratory and field conditions suggesting commercial efficacy for these products to *O. cylindrirostris* adults.

In previous research with chemical insecticides, both neonicotinoid (imidacloprid and clothianidin) and oxidiazine indoxacarb foliar applications have shown insecticidal activity against insect herbivores (Wise et al., 2007). For example, treatment with imidacloprid compounds provided 100% control of larvae on herbivorous insects; the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae) (Wu et al., 2006) potato leafhopper, *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae) and the onion maggot, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), (Nault, 2006) and flea beetle larvae *Phyllotreta cruciferae* Spinola (Coleoptera: Chrysomelidae) (Dosdall et al., 1999). Indoxacarb foliar applications have shown field efficacy of >85% mortality against light brown apple moth *Epiphyas postvittana* Walker, (Lepidoptera; Tortricidae) the diamondback moth *Plutella sp.* (Lepidoptera;
Plutellidae) and the Colorado potato beetle *Leptinotarsa decemlineata* Say (coleoptera; Chrysomelidae) (Wing et al., 2000)

Borers are a problematic pest to control because the larvae are internal crown feeders. Larvae of the cabbage seedpod weevil *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) and diaprepes root weevil *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae) (Quintela and McCoy, 1997) still emerged from imidacloprid and clothianidin treated plants (Nault et al., 2004). This indicates that treatments with either clothianidin or imidacloprid do not cause systemic insecticidal activity on larval cabbage seedpod weevil life stages, which is similar to the finding from *O. cylindrirostris* experiments. However although clothianidin results suggest that it is less effective than imidacloprid as a systemic insecticide on *O. cylindrirostris*, efficacy against black vine weevil *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) showed high mortality of more than 80% (Pattena and Metzger, 2009). *Orthorhinus cylindrirostris* oviposition was reduced significantly on potted plants subjected to the systemic treatments, indicating that the imidacloprid formulation compounds provide a repellent effect on female oviposition.

The previous research of entomopathogenic fungi show that although several entomopathogenic fungi, including several *Beauveria* spp, have been reported for use on an extensive number of insect coleopteran pests (Reeks and Smith, 1956; Wagner and Leonard, 1980), this is the first study when *B. bassiana, A. parasiticus* and a commercial formulation Mycoforce® (*B. bassiana, M. anisopliae, V. lecanii*) were used against *O. cylindrirostris. Orthorhinus cylindrirostris* field mortality from infection with *B. bassiana var Mycoforce* and *B. bassiana var EWB* was considerably lower than the mortality rates of > 50% exhibited following field application against red imported fire ant *Solenopsis invicta* Buren, (Hymenoptera; Formicidae) (Siebeneicher et al., 1992), rice hispa *Dicladispa armigera* (Olivier) (Coleoptera; Hispidae) (HazariKa and Puzari, 1990), eastern tent caterpillar *Malacosoma americanum* Fabricius (Lepidoptera; Lasiocampidae) (Leathers and Gupta, 1993), convergent lady beetle *Hippodamia convergens* Guérin-Méneville, (Coleoptera; Coccinellidae) (James and Lighthart, 1994), western corn rootworms *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) (Parimi et al., 2003)
and coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) (De la Rosa et al., 1997).

As stated earlier (in field application methods) the myco-toxins produced by *A. parasiticus* excluded the entomopathogenic fungi from further testing. *A. parasiticus* commonly grows on carbon-rich substrates. *Aspergillus* species contaminate agricultural commodities (Leontopoulos et al., 2003; Weidenborner et al., 2000) that may result in the production of aflatoxins B1 (AFB1) and allergenic spores (D'Mello et al., 1998). Aflatoxins are naturally occurring myco-toxins that are produced by many species of *Aspergillus*, most notably *A. flavus* and *A. parasiticus*. Aflatoxins are secondary metabolites toxic and carcinogenic to animals, including humans and as a result exclude the use of this entomopathogenic fungi in insect control (D'Mello et al., 1998).

### 4.1.4 Compound effectiveness

Non-systemic pesticides have little effect on insect borer larvae once they have established colonies within their hosts, since the chemical does not reach its target; and in the case of *O. cylindrirostris*, the systemic pesticides imidacloprid and clothianidin applied as crown drenches were not successful. However encapsulated imidacloprid was moderately successful in bioassays analysis on *O. cylindrirostris* larvae, despite sharing the same target site and mode of action (Nauen et al., 2003). The variation in action among the tested neonicotinoids is striking. Possible explanations include a difference in formulation, so that the poison is too dilute by the time it reaches the larvae, or the chemical may not actually reach the larval feeding zone (Nauen, 1995).

A potential reason for dilution of imidicloprid and clothianidin when applied as a crown drench has been shown in imidacloprid runoff tests, where high levels of runoff (up to 90% chemical loss) in formulations of wettable powder were reported (Armbrust and Peeler, 2002). Tablet formulated insecticides exhibit excellent efficacy when compared with crown drench insecticide application because tablets exhibit longer lasting activity (Takahashi et al., 2001). Chemical exposure to roots may be far greater with tablets than suspended concentrate formulations which
have the potential for the chemical to bypass the root system without absorption, hence reducing the dosage available to insects (Jawara et al., 2001; Oliver et al., 2009). This is shown in incorporation of imidacloprid into the upper 40 mm of soil as optimal for absorption and translocation by the roots of lettuce (*Lactuca sativa*) to control white fly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Palumbo et al., 1996).

The mortality and feeding deterrence of *O. cylindrirostris* following systemic applications of encapsulated imidacloprid is a response seen in other horticultural pests (Kaakeh et al., 1997; Quintela and McCoy, 1997). The antifeedant effect of imidacloprid has been reported for green peach aphid *Myzus persicae* (Sulzer) (Hemiptera; Aphididae), leading to weight loss and subsequent death from starvation (Nauen, 1995). This suggests that the antifeedant effect is more pronounced when imidacloprid was applied by the systemic method. Such antifeedant properties were only observed for systemic application of imidacloprid in this study. However when aphid mortality and deterrence is compared with *O. cylindrirostris* mortality, it becomes obvious that an acceptable level of mortality or plant protection was not reached in this study.

Foliar applied treatments provided increased *O. cylindrirostris* control compared with the systemic treatment. Therefore *O. cylindrirostris* adult control will need to be the focal point of insecticide control, as conventional insecticides (i.e. foliar applications of indoxacarb imidacloprid and clothianidin) encompass a suite of lethal and sub lethal mechanisms that work together to achieve toxicity (Wing et al., 2000).

The entomopathogenic fungi strains *B. bassiana* var EWB and Mycoforce® effectively colonize and kill adult *O. cylindrirostris* in laboratory bioassays. Field tests show that *B. bassiana* formulations can suppress *O. cylindrirostris* emergence however the commercially formulated Mycoforce® was effective at a lower concentration of conidia than the local isolate *B. bassiana* var EWB. The efficacy of Mycoforce® may be a result of common and complex interactions between mixed infections involving two or more species, increasing lethality. Asymptomatic infections of other pathogens stress the immune system of the insect (Tounoua et al., 2008), such that the burden of virulent *M. anisopliae* and *V. lecanii* agents increased the infection of *B. bassiana* to *O. cylindrirostris* adults. Mixed infection with a largely avirulent fungal pathogen in
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the desert locust have shown to increase the virulence and reproduction of a second, highly virulent pathogen (Chandler et al., 1993) with mixed strains giving 46% greater infection and subsequent mortality than the single strain formulation (Cox, 2001). However differences in infection as large as this were not observed in *O. cylindrirostris* bioassays and differences were smaller again in field applications.

The activity of entomopathogenic fungi can vary according to the host plant species to which it is applied and environmental conditions (Farrar et al., 1996). There are several reports regarding the influence of climatic factors on fungal colonization (Bustillo et al., 1999; Lighthart et al., 1988; Mathieu et al., 1997; Steinhaus, 1958; van Frankenhuyzen and Nystrom, 1987). Fungal success is very much dependent upon weather conditions, particularly on high relative humidity (RH). High RH is an essential factor in the development of fungal propagules in the field (Murphy and Moore, 1990; Romoska, 1984). Ultimately, success of *B. bassiana* as a microbial control agent will depend on the ability to maintain efficacy under fluctuating environmental conditions. The subtropical location of blueberry production on the east coast of NSW is likely to satisfy the humidity requirements for sporulation of entomopathogenic fungi.

While there are broad-spectrum and selective insecticides available for the control of coleopteran pests in fruit crops, no insecticides are currently registered for use against *O. cylindrirostris* in blueberries. The long interval over which the fruit is harvested, the requirement for bees to pollinate the flowers for fruit set and the cryptic nature of *O. cylindrirostris* larvae may limit the acceptability of systemic and foliar insecticides with residues as a desirable option for blueberries. Conversely the neonicotinyl and indoxacarb based insecticides provide control of many indirect pests, notably aphids, leafhoppers and other hemipteran pests (Beers et al., 1993). Applications intended for *O. cylindrirostris* control may have the added benefit of suppressing some of these pests in blueberry crops.

These experiments should be viewed as a screening for potential control candidates given the limited dose range tested for each chemical or entomopathogenic fungi. The long lifecycle of *O. cylindrirostris* prohibited the use of laboratory reared insects for these experiments (see chapter 2). Efficacious insecticides from this study will need further investigation to meet the
requirements of the APVMA and to determine maximum residue limit (MRLs) for registration of imidacloprid and indoxacarb based insecticides in blueberry production for *O. cylindrirostris* control (Australian Pesticides & Veterinary Medicines Authority, 2006).

### 4.6 Conclusions

The objectives of this study were to determine insecticides that have physiological effects on *O. cylindrirostris* adults and/or larvae. The results revealed one or more positive examples from each of the following groups: oxadiazines, neonicotinoids and, entomopathogenic fungi. (Mycoforce®), Indoxacab, imidacloprid, foliar applications on adults and imidacloprid, systemic tablets towards larvae exhibited a reduction in insect survival following exposure to treated substrate expanding the potential life stage targets for blueberry IPM. Comprehensive control of *O. cylindrirostris* will likely require a suite of tactics and life-stage targets. Although adults will be the likely life stage targeted for control during the growing season, investigation of alternative avenues are needed to completely understand the impact of field treatments on *O. cylindrirostris* populations. However, in commercial orchards, where *O. cylindrirostris* pressure is not severe, it is likely that *B. bassiana* formulated as Mycoforce® could provide levels of *O. cylindrirostris* control. The neonicotinyl and indoxacarb insecticides have been shown to provide control of many indirect pests of horticultural fruits, notably aphids, leafhoppers and some hemipteran pests. Therefore, applications timed for *O. cylindrirostris* control would likely have the added benefit of suppressing some of these pests. None of the chemical and entomopathogenic fungi insecticides are likely to be used as a stand-alone method for season-long control of *O. cylindrirostris*.

### 4.7 References


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Chapter 5 Compatibility of Beauveria bassiana with insecticides registered for use with blueberries

5.1 Introduction

Beauveria bassiana (Balsamo Crivelli) Vuillemin is an entomopathogenic fungus which is formulated into several myco-insecticide based formulations adopted for IPM (Burges, 1980; Devi et al., 2003; Oliveira et al., 2003). When used in IPM, these novel compounds may increase efficacy and provide an alternative to synthetic chemical insecticide applications (Neves et al., 2001).

Although the use of chemical insecticides and biological insecticide agents together has been framed as a contentious and complicated issue (Devine and Furlong, 2007), the two are being used increasingly in tandem (Carruthers and Hural, 1990; Dent, 2000; Oi et al., 2008). The utilization of compatible insecticides in association with entomopathogenic fungi may increase the efficiency of pest control, reduce the amount of insecticides applied against the target insect and minimize environmental contamination through a reduction in chemical insecticide applications (Klingen and Haukeland, 2006; Neves et al., 2001). Control accomplished by entomopathogenic fungi is dependent on survival of the organism after application (Anderson and Roberts, 1983), hence it is critically important to determine the interaction between the myco-insecticide and conventional chemical pesticides to establish an IPM program (Neves et al., 2001).

5.1.1 Concept of compatibility

While use of biological and chemical control though insecticidal sprays is designed to be extremely effective at killing the targeted insect pests, they also have the potential to adversely interact when used in combination (Ishaaya et al., 2007). However quantifying the impact of pesticides on biological organisms can be difficult (Hossain and Poehling, 2006; Stark and Banks, 2003; Stark et al., 2004a; Stark et al., 2004b; Stark et al., 2007). Myco-pesticides are living organisms so it is important to quantify the impact of chemical insecticides on different
stages of fungal development. Germination toxicity may be considered the most important measure of toxicity because entomopathogenic fungi infect insects through conidial germination on the insect cuticle and the fungi survive in the field as conidia (Oliveira et al., 2003). Potential mechanisms of incompatibility arise due to a number of causes, with fungicides likely to have the most serious impact on entomopathogenic fungi (Majchrowicz and Poprawski, 1993) due to their mode of action and activity against fungal pathogens generally (Majchrowicz and Poprawski, 1993; Olmert and Kenneth, 1974; Poprawski and Majchrowicz, 1995; Todorova et al., 1998).

Many fungicides are reported to be non-specific to pathogenic, saprophytic or harmless species of fungi, so will affect all types of fungi present in a treated area. Fungicides are often created with multiple modes of action in one compound so it can be difficult to understand which mode of action causes incompatibility or other effects on non-target fungi (Clark et al., 1982). Fungicides may interfere with the infection process of entomopathogenic fungi by a) inhibiting ribosome RNA synthesis (Griffith et al., 1992), b) preventing adhesion (conidial hydrophobicity or electrostaticity) of the conidia to the host surface in the case of copper oxide or mancozeb, or c) affecting conidia viability, germination, differentiation and penetration process or act during cell division by inhibiting the synthesis of ergosterol (Chitarra, 2003).

Insecticide effects on entomopathogenic fungi are likely to be a result of either additives in spray formulations (e.g. antifungal compounds in bacterial toxin insecticides such as Bacillus thuringiensis) or an effect of the active ingredient itself. The mechanisms of additives can lead to the blocking of conidia metabolic functions and thus drastically affect germination. For example, metabolic ion accumulation on the surface of the cellular membrane (Moore-Landecker, 1982) interferes with cell wall formation due to inhibition of the enzyme that converts phosphatidylethanolamine into chitin, which drastically reduces conidia germination, vegetative growth and sporulation (Oliveira et al., 2003).
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindrirostris with chemicals used in blueberry production

5.1.2 Measurement of Compatibility

In the field, vegetative growth of entomopathogenic fungi will only occur inside the body of the insect host as *B. bassiana* is rarely found in the environment in the saprophytic form (Neves et al., 2001). Therefore testing of vegetative growth and sporulation alone for compatibility is misleading, particularly if the entomopathogenic fungi are being applied as a bio-pesticide in the form of conidia. Therefore germination must be included in tests for chemical compatibility with entomopathogenic fungi (Batista Filho et al., 2001; Feng et al., 1994). Inhibition of conidial germination by chemical insecticides will influence the time required to kill the target pest (Pedro et al., 2001) as the conidia are responsible for initiating infection by either ingestion or contact (Gottel and Hajek, 2001; Hajek and St. Leger, 1994). Entomopathogenic fungi infection is a result of two factors: survival of the fungal inoculum (conidia) in the field, which are responsible for the disease, and infection of insects after conidial germination (Oliveira et al., 2003).

Laboratory tests of compatibility are considered a worst case scenario that are likely to overestimate potential impacts to entomopathogenic fungi (Hassan et al., 1991) because organism exposure is significantly higher than that experienced in field applications (Latteur and Jansen, 2002). Therefore use of laboratory results will produce conservative compatibility guidelines that minimize impacts in the field.

The International Organization for Biological Control (IOBC) played an early role and continues to develop compatibility tests through a joint testing program (Louda et al., 2003; Louda et al., 1997). The “Commission on IP Guidelines” on “Pesticides and Beneficial Organisms” uses established IOBC criteria for classification of pesticide side-effects. The method involves initial pesticide screening under laboratory conditions, and, depending on the results obtained, either semi-field or field tests are also conducted. This method has been designed to evaluate the acute residual toxicity as well as sub lethal effects of pesticides on reproductive performance of non-target organisms (Vogt et al., 1992). In the case of entomopathogenic fungi, the treated fungi are tested against its target pest for control to determine if there has been a mycotoxic effect which has led to decreased viability of the fungi
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindriostris with chemicals used in blueberry production

(Overmeer and Van Zon, 1982; Neves et al., 2001; Olmert and Kenneth, 1974). This is then measured after adjustment using Abbott's formula (Abbott, 1925).

\[
\text{Adjusted } \% \text{ mortality} = \frac{\% \text{ alive in control} - \% \text{ alive in treatment}}{\% \text{ alive in control}} \times 100\%
\]

(Abbott, 1925)

Where the values are obtained relative to the control (100%), the value of T defines toxicity within the following intervals. The percent reduction is relative to control that is reported for each class: Class 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%), and Class 4: harmful (>75%). These ratings are assigned to chemicals after calculating results using Abbott’s formula. Compatible insecticides are those which cause less than 25% reduction in sporulation, conidia germination and vegetative growth.

5.1.3 Compatibility currently known for Beauveria bassiana

Of the ten pesticides used in Australian blueberry production (table 5-1) toxicity testing has been conducted already with four chemicals on three different isolates of B. bassiana. Consequently these chemicals will not be re-tested on the new isolate of Beauveria bassiana var Mycoforce. From these previous findings, the pesticides registered for use in blueberry production which are toxic to B. bassiana are: dimethoate, (Oliveira and Neves, 2004), captan (Hassan et al., 1991; Hassan et al., 1994) mancozeb, (Kouassi et al., 2003) and chlorpyrifos, (Oliveira et al., 2003). However imidacloprid (Neves et al., 2001) and Bacillus thuringiensis (Bt) (Hassan et al., 1994) are compatible. Propiconazole, malathion, methomyl, indoxacarb, and nucleopolyhedrovirus (NPV) all need to be reviewed for compatibility with B. bassiana.
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindrirostris with chemicals used in blueberry production

Table 5-1 Insecticides registered for use in Australian blueberry production

<table>
<thead>
<tr>
<th>Chemical trade name</th>
<th>Active Constituent</th>
<th>Substance Chemical Family</th>
<th>Chemical target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penncozeb Dr (NuFarm)</td>
<td>Mancozeb</td>
<td>Carbamate</td>
<td>fungicide</td>
</tr>
<tr>
<td>Throttle (NuFarm)</td>
<td>Propiconazole</td>
<td>Triazole</td>
<td>fungicide</td>
</tr>
<tr>
<td>Captan WG (Crop-care)</td>
<td>Captan</td>
<td>Phthalimide</td>
<td>fungicide</td>
</tr>
<tr>
<td>Hymal (Crop-care)</td>
<td>Malathion</td>
<td>Organophosphate</td>
<td>insecticide</td>
</tr>
<tr>
<td>Dimethoate (4Farmers)</td>
<td>Dimethoate</td>
<td>Organophosphate</td>
<td>insecticide</td>
</tr>
<tr>
<td>Marlin (Bayer)</td>
<td>Methomyl</td>
<td>Carbamate</td>
<td>insecticide</td>
</tr>
<tr>
<td>Avatar (Dupont)</td>
<td>Indoxacarb</td>
<td>Oxadiazines</td>
<td>insecticide</td>
</tr>
<tr>
<td>Vivus Gold (Ag BIOTEC Australia)</td>
<td>Nucleopolyhedrovirus</td>
<td>Virus</td>
<td>insecticide</td>
</tr>
<tr>
<td>Natralure (Dow AgriScience)</td>
<td>Spinosad</td>
<td>Spinosyn</td>
<td>insecticide</td>
</tr>
<tr>
<td>Dipel DF (NuFarm)</td>
<td><em>Bacillus thuringiensis</em> kurstaki (Btk)</td>
<td>Bacterial toxin</td>
<td>insecticide</td>
</tr>
<tr>
<td>Confidor (Bayer)</td>
<td>Imidacloprid</td>
<td>Chloronicotinyl</td>
<td>insecticide</td>
</tr>
<tr>
<td>Lorsban 500EC (Dow AgriScience)</td>
<td>Chlorpyrifos</td>
<td>Organophosphate</td>
<td>insecticide</td>
</tr>
</tbody>
</table>
5.1.4 Objectives

The aim of this experiment is to 1) examine the case for using insecticides compatible with biological control in blueberry cultivation and 2) study mycotoxic effects on *Beauveria bassiana* var *Mycoforce* (i.e. selectivity and/or compatibility) of insecticides and fungicides not previously examined for entomopathogenic fungi compatibility. The commercially available *B. bassiana* isolate was used due to the availability of large quantities of the entomopathogenic fungi and to make the results useful for commercial farms which would need a commercial supply available. Presented are examples of pesticides that have the potential to be used successfully with myco-pesticides to manage insect pest populations in cultivated blueberries with recommendations for the use of chemicals on blueberry farms for the conservation of entomopathogenic fungi.

5.2 Materials & Methods

5.2.1 Chemicals used and application methods

Information on active ingredient, trade name, formulation, chemical group and recommended doses of the pesticides as well as the doses used (mL/grams per L/ha) in the experiments are shown in Table 5-2. For compatibility tests, the pesticides were used in three different concentrations: recommended application concentration (RC), half recommended application concentration rate ($\frac{1}{2}$RC) and twice the recommended application concentration rate (2RC). These rates were calculated using the recommended application rate given by the manufacturers for field application on blueberry crops when diluted in 100L of water for field practice.
Table 5-2 Chemicals tested for compatibility and application methods

<table>
<thead>
<tr>
<th>Chemical trade name</th>
<th>Active ingredient</th>
<th>Chemical target</th>
<th>Active Concentration on label</th>
<th>½RC /100L</th>
<th>RC /100L</th>
<th>2RC /100L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throttle®</td>
<td>Propiconazole</td>
<td>Fungicide</td>
<td>250 g/L</td>
<td>16mL</td>
<td>32mL</td>
<td>64mL</td>
</tr>
<tr>
<td>Hymal®</td>
<td>Malathion</td>
<td>Insecticide</td>
<td>1150 g/L</td>
<td>45mL</td>
<td>90mL</td>
<td>180mL</td>
</tr>
<tr>
<td>Marlin®</td>
<td>Methomyl</td>
<td>Insecticide</td>
<td>225g/L</td>
<td>100mL</td>
<td>200mL</td>
<td>400mL</td>
</tr>
<tr>
<td>Avatar®</td>
<td>Indoxacarb</td>
<td>Insecticide</td>
<td>700g/kg</td>
<td>10g</td>
<td>20g</td>
<td>40g</td>
</tr>
<tr>
<td>Vivus Gold®</td>
<td>Nucleopolyhedro virus</td>
<td>Insecticide</td>
<td>$2 \times 10^9$ Polyhedral inclusion bodies/mL</td>
<td>275mL</td>
<td>550mL</td>
<td>1.1L</td>
</tr>
<tr>
<td>Natralure®</td>
<td>Spinosad</td>
<td>Insecticide</td>
<td>0.24g/L</td>
<td>500mL</td>
<td>1L</td>
<td>2L</td>
</tr>
</tbody>
</table>
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindrostris with chemicals used in blueberry production

5.2.2 Fungal strain and culture methods

Conidia of B. bassiana var. Mycoforce (chapter 4) was isolated from the commercial formulation using single spore isolation. Three entomopathogenic fungi were present in the myco-insecticide and they may grow into the medium with the principal pathogen. To obtain a pure culture of the pathogen, a small sample of the growing edge of a colony with a flamed loop was taken and streaked over the surface of a pre-poured water agar (WA) plate. As the streak progresses over the agar, fungal spores are separated until single spores are obtained from which separate colonies will grow. A sample was then taken and placed in a tube containing 10mL of sterile water; this was then poured over a thin WA plate and incubated at 22 °C. After 24 hrs incubation, germinated spores were identified using a stereomicroscope (Olympus SZ61 stereomicroscope, Olympus Australia Pty Ltd, Australia) and transferred one spore at a time to a Potato-Dextrose-Agar (PDA) plate. Once a pure B. bassiana culture was obtained it was subsequently grown and multiplied on PDA (25°± 1°C; 12L:12D). Conidia produced on PDA were used for germination, vegetative growth and sporulation studies. Conidia from 14-day old B. bassiana colonies were used for comparisons of toxicity between different pesticide concentrations. All procedures were conducted in the plant pathology laboratory, Faculty of Agriculture, Food and Natural Resources, the University of Sydney. There were ten replications for each treatment for all three variables measured (germination, growth, sporulation) and a control (water and wetting agent Tween 20) for the three variables.

5.2.3 Conidia germination

The pesticides were diluted in sterile distilled water and made up to the concentrations given in table 5-2. 1mL of B. bassiana conidial suspension, standardized to 1x10⁴ conidia/ mL was then added to the pesticide suspension. Ten minutes after hand agitation of the mixture, 0.5 mL aliquots of each suspension were transferred to 90mm culture plates (Livingstone Labserv™, Australia) containing solidified WA medium. The control consisted of sterile distilled water, Tween 20 and the standardized spore suspension (0.5mL) without any pesticide. There were ten replications for each treatment. Culture plates were then transferred to an incubator (22 ± 1°C; 12L:12D) for 24h. After incubation, lines were drawn on the external surface of the lid of each
of the plate, dividing them into eight quadrants, in which approximately 100 conidia were counted per plate under 100x magnification using a stereomicroscope (Olympus SZ61 stereomicroscope, Olympus Australia Pty Ltd, Australia). Conidia were classified as germinated or not and percentage germination calculated for each plate.

5.2.4 Vegetative growth

The pesticides were diluted in sterile distilled water and made up to the concentrations given in table 5-2. PDA was made and poured into ten 90 mm culture plates (Livingstone Labserv™, Australia) per concentration per treatment. After media solidification, each plate was inoculated with B. bassiana conidia at the centre point of the plate. Treatments were applied by spraying 10 mL using a 50 mL hand spray bottle (Taizhou Huangyan Dinghao Plastic Factory, China) of chemical solution over the solidified PDA, 24 hours after inoculation of the fungi. The plates were then randomly transferred to an incubator (22 ± 2°C; 12L:12D) for 14 days. After incubation, the diameter of colonies in each culture plate was determined using calipers. Mean diameter of each colony was calculated after measuring the diameter three times in different directions; these measurements were taken perpendicular to one another.

5.2.5 Sporulation

Following the evaluation of vegetative growth, an area measuring 10 mm² was removed with a circular hole punch from the centre of each replicate colony for each treatment concentration. The 10 mm² fungal sample was transferred to glass test tubes (100 mm tall x 20 mm diameter) (Livingstone Labserv™, Australia) containing 10 mL sterilized distilled water. The colonies were hand agitated for two minutes. Conidia suspensions were subsequently diluted ten-fold and quantified by making ten spore counts from the diluted suspensions with a haemacytometer (Neubauer Chamber, Jessen double net ruling) and taking the average number of spores per mL.
5.2.6 Statistical Analysis

The effects of different chemicals and application rates were determined by a two way analysis of variance (ANOVA) with insecticide application and rate as the factors and if significant effects were detected treatment means were compared using Tukey’s HSD (JMP© version 8). Prior to analysis, sporulation data (section 5.2.5) were subjected to a log₁₀ transformation whilst percentage germination (section 5.2.3) data were subjected to \( \sin^{-1} \) (square root) transformation to achieve normality and fulfil the assumptions necessary for ANOVA.

Abbott’s formula was used to calculate compatibility and each pesticide classified for compatibility as follows: (Abbott, 1925)

Compatibility index:

1. **Class 1**: harmless (<25%)
2. **Class 2**: slightly harmful (25%-50%)
3. **Class 3**: moderately harmful (51%-75%)
4. **Class 4**: harmful (>75%)

Abbott’s formula results were calculated for each of the variables (germination, sporulation and vegetative growth). The median compatibility index class from these three variables was used as an overall rating of compatibility.

5.3 Results

5.3.1 Conidia germination

Conidia germination was significantly reduced by all pesticides used when compared with the water control (20% to 60% c.f. 90% germination, \( F_{7, 7} = 35.92, P < 0.001 \)). The fungicide propiconazole reduced conidia germination more than the other insecticide treatments.
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindrirostris with chemicals used in blueberry production

The effect of application rate on B. bassiana germination was not consistent across all chemicals tested (application effect, $F_{2, 2} = 3.14$, $P = 0.05$, interaction term, $F_{14, 14} = 3.19$, $P < 0.001$). For four of the six chemicals (propiconazole, NPV, malathion and methomyl) percentage germination declined further for the 2RC application rate compared with the lower application rates. In contrast, the decline in germination was similar across all application rates for indoxacarb and spinosad. Treatment with spinosad and the lower application rates of methomyl and malathion had the lowest impact on germination of B. bassiana (figure 5-1).

5.3.2 Sporulation

Sporulation of B. bassiana was significantly reduced by all pesticides used when compared with the water control (10,000 spores to 620,000 spores c.f. 680,000 spores, $F_{7, 7} = 6.68$, $P < 0.001$). The insecticide NPV at the 2RC application rate had the greatest impact of all treatments (95% decline in sporulation) and malathion reduced fungal sporulation (65% reduction) more than other insecticides for the lower application rates ($\frac{1}{2}$RC and RC).

Although not significant the effect of application rate on B. bassiana sporulation was not consistent across all chemicals tested (application effect $F_{2, 2} = 3.15$, $P = 0.06$, interaction term, $F_{14,14} = 2.74$, $P < 0.001$). For four of the six chemicals (NPV, methomyl, indoxacarb and spinosad) sporulation declined further for the 2RC application rate compared with the lower application rates. In contrast, the decline in sporulation was similar across all application rates for propiconazole and malathion. Lower application rates ($\frac{1}{2}$RC and RC) of spinosad and methomyl had the least impact on sporulation of B. bassiana (figure 5-2).

5.3.3 Vegetative growth

Vegetative growth of B. bassiana was significantly reduced by all pesticides used when compared with the water control (0.10 mm growth to 42 mm c.f. 45 mm, $F_{7, 7} = 18.48$, $P < 0.001$). The insecticides NPV and propiconazole at the 2RC application rate had the greatest impact of all treatments and had more effect than other insecticides at lower application rates ($\frac{1}{2}$RC and RC).
Although not significant the effect of application rate on *B. bassiana* sporulation was not consistent across all chemicals tested (application effect $F_{2,2} = 4.78$, $P = 0.06$, interaction term, $F_{14,14} = 35.96$, $P < 0.001$). With four of the six chemicals (propiconazole, NPV, malathion and indoxocarb) vegetative growth declined further for the 2RC application rate compared with the lower application rates. In contrast, the decline in vegetative growth was similar across all application rates for methomyl and spinosad. Lower application rates ($\frac{1}{2}$RC and RC) of spinosad, indoxacarb and malathion had the least impact on vegetative growth of *B. bassiana* (figure 5-3).
Figure 5-1 Effect of pesticides on *Beauveria bassiana* conidia germination, means followed by different letters within each column are significantly different (P ≤ 0.05) from the control.
Figure 5.2 Effect of pesticides on *Beauveria bassiana* conidia sporulation, means followed by different letters within each column are significantly different ($P \leq 0.05$) from the control.

Figure 5.3 Effect of pesticides on *Beauveria bassiana* vegetative growth, means followed by different letters within each column are significantly different ($P \leq 0.05$) from the control.
Figure 5-3 Effect of pesticides on *Beauveria bassiana* vegetative growth, means followed by different letters within each column are significantly different (P ≤ 0.05) from the control.
Chapter 5 - Compatibility of Bio-insecticides used for Orthorhinus cylindrirostris with chemicals used in blueberry production

Table 5-3 Compatibility of pesticides with *Beauveria bassiana*.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Chemical</th>
<th>Germination</th>
<th>Vegetative growth</th>
<th>Sporulation</th>
<th>Median Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throttle®</td>
<td>Propiconazole</td>
<td>3 (59%)</td>
<td>3 (58%)</td>
<td>2 (45%)</td>
<td>3</td>
</tr>
<tr>
<td>Vivus Gold®</td>
<td>Nucleopolyhedrovirus</td>
<td>2 (34%)</td>
<td>3 (53%)</td>
<td>2 (40%)</td>
<td>2</td>
</tr>
<tr>
<td>Hymal®</td>
<td>Malathion</td>
<td>2 (27%)</td>
<td>1 (21%)</td>
<td>3 (62%)</td>
<td>2</td>
</tr>
<tr>
<td>Marlin®</td>
<td>Methomyl</td>
<td>2 (32%)</td>
<td>2 (36%)</td>
<td>2 (30%)</td>
<td>2</td>
</tr>
<tr>
<td>Avatar®</td>
<td>Indoxacarb</td>
<td>2 (34%)</td>
<td>2 (25%)</td>
<td>3 (51%)</td>
<td>2</td>
</tr>
<tr>
<td>Natralure®</td>
<td>Spinosad</td>
<td>2 (27%)</td>
<td>1 (14%)</td>
<td>2 (27%)</td>
<td>2</td>
</tr>
</tbody>
</table>

Class 1: harmless (<25%), Class 2: slightly harmful (25%-50%), Class 3: moderately harmful (51%-75%), and Class 4: harmful (>75%)
Chapter 5 Compatibility of Beauveria bassiana var. EWB with insecticides registered for use with blueberries

The pesticides from table 5-3 caused B. bassiana to respond differently at different life stages. When using the median class to determine entomopathogenic fungi compatibility, propiconazole was the least compatible pesticide tested, being moderately harmful to B. bassiana germination, sporulation and vegetative growth. The pesticides nucleopolyhedrovirus, malathion, methomyl, indoxacarb and spinosad were similar in their compatibility with B. bassiana and classified as slightly harmful. Although toxicity can occur at sporulation to B. bassiana with pesticides, germination of the spores may not be affected e.g. indoxacarb and malathion. The converse can also be suggested for the effect of spinosad and malathion on vegetative growth, which was less affected by these insecticides than either sporulation or germination.

5.5 Discussion

5.5.1 Overview of results

All pesticides tested had varying degrees of toxicity to germination, vegetative growth and sporulation of B. bassiana, dependent on the chemical nature of the compounds and the application rate. Conidia germination, sporulation and vegetative growth were significantly reduced by all pesticides tested and the 2RC application rate had the greatest impact, as might be expected. Conidia germination was reduced by propiconazole more than the other insecticide treatments. Fungal sporulation and vegetative growth were reduced by NPV, malathion and propiconazole more than the other insecticides for lower application rates. Of the six chemicals propiconazole and nucleopolyhedrovirus were more toxic to all life stages (germination, sporulation and vegetative growth) of B. bassiana (55-58% effect for B. bassiana life stages) than any other chemical. In contrast treatment with spinosad and the lower application rates of methomyl, malathion, and indoxacarb had little negative effect on the germination, vegetative growth and conidia production of B. bassiana.

5.5.2 The observed toxicity of the chemicals tested and those tested previously

The pesticides chosen for compatibility testing against B. bassiana have not been tested before however the results are consistent with findings for other pesticides tested against B. bassiana.
Chapter 5 Compatibility of Beauveria bassiana var. EW B with insecticides registered for use with blueberries

(Neves et al., 2001). Previous authors have shown that B. bassiana shows high variability in compatibility with different pesticides and concentrations, (table 5-4) (O’Callaghan and Brownbridge, 2009; Anderson and Roberts, 1983).

There is a need for compatibility testing to make informed decisions about selection of bioinsecticides for use in IPM (Stark and Banks, 2003; Stark et al., 2004b; Stark et al., 2007). It can be seen from the literature that the organophosphates (OPs) dimethoate and chlorpyrifos are more toxic than the fungicides. In these experiments the fungicide had the greatest overall toxicity of all pesticides tested, probably because the insecticides tested here, e.g. spinosad and indoxacarb, do not have the broad spectrum of OPs and are considered reduced risk insecticides (Hassan et al., 1991; Hassan et al., 1994).
Chapter 5 Compatibility of Beauveria bassiana var. EWB with insecticides registered for use with blueberries

Table 5-4 Compatibility of pesticides with Beauveria bassiana

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Target</th>
<th>Compatibility Index</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captan</td>
<td>Fungicide</td>
<td>Slightly harmful</td>
<td>Hassan et al., 2001</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>Fungicide</td>
<td>Slightly harmful</td>
<td>Kouassi et al., 2003</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>Insecticide</td>
<td>Harmful</td>
<td>Oliveiro et al., 2004</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>Insecticide</td>
<td>Harmless</td>
<td>Hassan et al., 2001</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Insecticide</td>
<td>Harmful</td>
<td>Oliveiro et al., 2001</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Insecticide</td>
<td>Harmless</td>
<td>Neves et al., 2001</td>
</tr>
</tbody>
</table>

These experiments did not investigate the mechanisms of pesticide incompatibility with B. bassiana specifically so it is not clear how each chemical caused its effects. The additives in the Vivus gold™ formulation of NPV are a likely reason for causing deleterious effects on entomopathogenic fungi rather than the virus (Nucleopolyhedrovirus) causing direct harm to fungal growth and maintenance. Limited studies have focused on the mechanisms of toxicity to entomopathogenic or beneficial fungi however, in general, it has become widely accepted that the active ingredients do not affect the entomopathogenic fungi greatly and that formulations have more responsibility for toxicity (Anderson and Roberts, 1983; Moore-Landecker, 1982; Neves et al., 2001). Yet the only example found to support this hypothesis is a study on the aromatic petroleum distillates present in a commercial preparation of malathion insecticide. The study showed the distillates had a greater effect on viability of cultures of soil micro-organisms than did the malathion (Stanlake and Clark, 1975). Of the limited studies on toxicity of active ingredients to beneficial fungi, malathion was also studied. The results showed that malathion
and the related compounds have three pathways of toxicity through the process of membrane peroxidation: 1) a direct initiation by free radicals produced by metabolism of the chemical, initiating a chain of peroxidation by abstracting a hydrogen from other molecules; 2) indirect initiation by the production of reactive forms of oxygen during their metabolism, e.g. activating oxygen to reduce to the superoxide anion; 3) inhibition of enzymatic systems of defence (Yarsan et al., 1999).

In the case of non-target effects of fungicides on entomopathogenic fungi, it is expected that inhibition of germination by \textit{B. bassiana} conidia is a more significant effect than insecticide damage. In the case of propiconazole, the mode of action by demethylation of C-14 during ergosterol biosynthesis leads to accumulation of C-14 methyl sterols. The biosynthesis of these ergo-sterols is critical to the formation of cell walls of fungi. This lack of normal sterol production slows or stops the growth of the fungus, effectively preventing further infection and/or invasion of host tissues (germination) (Uesugi, 1998). This has been demonstrated with the effects of propiconazole on extra-cellular enzyme levels in Turkey Tail \textit{Trametes versicolor} Qué. (Poriceae: Trametes) during colonization and degradation. The metabolic pathways were altered, which led to an alteration of extra-cellular enzyme production (Lekounougou et al., 2008).

5.5.2.1 The recommendations for use in blueberry crops

Using a compatibility analysis approach, incompatible pesticides can be managed to minimize exposure of \textit{B. bassiana}. These factors are becoming increasingly important as the agricultural sector moves away from highly persistent broad-spectrum sprays to more selective, less persistent products (Neves et al., 2001).

Malathion, methomyl, indoxacarb NPV, and spinosad have slightly harmful effect on the germination, vegetative growth and conidia production of \textit{B. bassiana}, and as a result can be recommended for use with \textit{B. bassiana} var Mycoforce. In contrast, use of the 'moderately harmful' propiconazole should avoid overlap with \textit{B. bassiana} application periods to minimize toxicity. From previous work (table 5-4) \textit{B. thuringiensis} and imidacloprid can be used with
Chapter 5 Compatibility of *Beauveria bassiana* var. EWB with insecticides registered for use with blueberries

minimal impact on *B. bassiana* isolates but the fungicides captan and mancozeb would need to be managed diligently to reduce direct contact with fungi. Chlorpyrifos and dimethoate would not be recommended for use in combination with *B. bassiana* due to their high toxicity.

5.6 Conclusion

The action of pesticides on the vegetative and conidial structures of entomopathogenic fungi vary as a function of the chemical compound and pesticide formulation. Pesticides may inhibit fungal development through a number of undetermined metabolic functions. Of the pesticides used or being considered for use in blueberry production, imidacloprid *B. thuringiensis* poses a lower risk. Malathion, methomyl, indoxacarb, nucleopolyhedrovirus and spinosad pose slightly harmful risks to *B. bassiana*, whereas dimethoate, chlorpyrifos, captan, mancozeb, propiconazole and all need to be managed carefully to reduce contact with *B. bassiana*.

5.7 Reference


Chapter 5 Compatibility of Beauveria bassiana var. EWB with insecticides registered for use with blueberries


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Chapter 6 General Discussion

The aim of the research presented in this thesis was to understand *Orthorhinus cylindrirostris* and develop tools for integrated pest management. *Orthorhinus cylindrirostris* has become a pest of economic importance over the last 10 years. Limited published knowledge was available on all aspects of *Orthorhinus cylindrirostris* biology, behaviour and control. *Orthorhinus cylindrirostris* is responsible for current yield losses in blueberry crops through tree damage by larval feeding. *Orthorhinus cylindrirostris* therefore represents a significant impediment to the long term profitability of the blueberry industry. *Orthorhinus cylindrirostris* larvae are extremely difficult to manage, especially if an edible crop is involved due to the low acceptance of residue levels in fruit (Clift, 2005). *Orthorhinus cylindrirostris* is the only blueberry pest in Australia for which prophylactic treatment is not routinely applied.

Although most insect borers are attracted to weakened, damaged, dying or dead trees (secondary invaders) *Orthorhinus cylindrirostris* will attack healthy or apparently healthy trees (primary invaders), and may eventually kill them. *Orthorhinus cylindrirostris* infestations often go unnoticed until plants or parts of the plants begin to show symptoms. Although attacks by *Orthorhinus cylindrirostris* do not always result in tree mortality (chapter 1 and 2), tree growth and quality may be seriously reduced (chapter 2). *Orthorhinus cylindrirostris* damage has been shown to affect yield through the disruption of vascular tissue, preventing nutrients taken up by the roots being dispersed throughout the plant. In severe infestations, the plant may be so weakened that a strong wind or harvester may push over and destroy the plant entirely, or the plant may simply die of starvation or water stress (Wright, 2008).

Commercial blueberry growers will gain directly from the results of this project through development of effective control strategies (Chapter 3 and 4). The expected replacement cost for blueberry bushes is Aus$40,000/ha on a 20 year cycle (Wright, 2008). Currently replacement occurs on a five to six year cycle because of damage from *Orthorhinus cylindrirostris*. 
There is potential for expansion of any control strategies developed from this project into other crops attacked by *O. cylindrirostris* such as grapevines.

This is the first document to describe the biology of *O. cylindrirostris* in depth. The lifecycle of *O. cylindrirostris* shows extraordinary flexibility and endurance. Adult *O. cylindrirostris* are both long lived, up to 700 days (chapter 2), and continually emerge over a three to six month interval, resulting in adult and egg laying activity throughout the harvest time in blueberry crops. The adult longevity means there is potential for large numbers of eggs to be laid (up to 120) in the lifetime of a single female weevil. The adults are very mobile, readily walking or flying between fields of susceptible and favourable varieties (chapter 3). The duration of the larval-pupal stage in *O. cylindrirostris* can range from six months in the case of high larvae stress (pupating and emerging as an adult to escape desiccation) to two years in favourable conditions (Chapter 2). It must be recognized that the confines of the cages used in this study may have influenced the results, particularly with respect to the apparent preferences of adults for oviposition when offered no choice of host plant. Nevertheless, even if confinement within a cage caused *O. cylindrirostris* to attack trees that they would normally ignore in the field, such results would contribute to a conservative approach in surveying for signs of infestation and in selecting trees for future planting. Furthermore, all host species tested in this study are known hosts under natural conditions (Clift, 2005).

Aggregation of *O. cylindrirostris* was not observed however the possibility of pheromone communication between individuals of this species can not be excluded. Observations of *O. cylindrirostris* behaviour saw *O. cylindrirostris* adults meet at the site of oviposition for mating, females spent up to five hours boring a hole for oviposition whilst males engaged in copulation and mate guarding. However investigation using olfactorial techniques to determine if semiochemicals are present or the mechanisms of adult exploration may provide a valuable tool for IPM and should be conducted now the basic biology of *O. cylindrirostris* is addressed.
The larvae were observed to develop in the branches (Clift, 2005) and crown (Froggatt, 1900) of healthy blueberry and Australian native trees (chapter 2). The performance among tree species commonly found in the blueberry field landscape differed in their attractiveness and suitability for *O. cylindrostris* (chapter 2). Host plant preference should be investigated further by choice tests to determine if fecundity or other parameters are affected by host plant. Diversity in adult body size, both in males and females, was observed for all host plants tested in this study. Sexual dimorphism was identified with males distinguishable by large front tarsal pads and the antennae base close to the tip of the rostrum. Male *O. cylindrostris* use their enlarged fore legs and tarsal pads in male-male combat for hitting rival males (chapter 2).

The biology of *O. cylindrostris* described (chapter 2) express the extreme difficulties which are present in management of *O. cylindrostris*. Increases in crop tolerance using tolerant genotypes has been a successful option for boring insects (Kennedy et al., 1987; Strauss and Agrawal, 1999) and as a result was investigated for potential (chapter 3). Previous attempts to establish direct relationships between adult densities and yield losses have failed as a result of difficulties in manipulating densities of adults in the field (Clift, 2005). Therefore the survey conducted in this research used multiple generations of varieties with different densities of *O. cylindrostris* to determine the economic injury level and estimates of yield loss for *O. cylindrostris* larval feeding (*O. cylindrostris* emergence) on blueberry varieties. A marked difference between variety in terms of susceptibility to both attack by *O. cylindrostris* and the plant response to the attack was noted.

The bulk of the adult *O. cylindrostris* in the past were found in the varieties Bonito, Misty and Becky blue. The removal of large infested areas of these varieties during 2005 would be expected to reduce the numbers of *O. cylindrostris* that were collected in subsequent years however a lack of background information about the numbers of weevils infesting the total area of BFA made it difficult to judge the actual effects. The current blueberry varieties grown on BFA that were least affected by larval damage were RE c.v. Powderblue, Britwell, Premier, and SHB c.v. 115, 41, 42 and 209A. These
varieties should be recommended for future planting and further investigations into the mechanisms of tolerance are needed. These varieties could be used to cross breed with susceptible varieties to produce plant characteristics which satisfy the needs of producers whilst reducing the amount of *O. cylindriostris* damage to the orchard.

Efforts to quantify the relationship between damage and *O. cylindriostris* will need to be determined for future blueberry breeding programs. Varietal differences between Southern Highbush and Rabbiteye varieties were identified (chapter 3). Assessments of successful adult emergence relative to yield did not allow development of an EIL for all varieties. This may be attributed to the weak association between larval populations and intensity of damage over time and could be overcome in future by sampling the same trees for yield and *O. cylindriostris* over consecutive years so that adult emergence can be correlated with yield of individual trees.

An alternative theory is that the numbers of previous generations infesting a tree could be associated with rapid increases in weevil populations and current levels of infestation i.e. increased oviposition on natal trees from which beetles are emerging. Increased oviposition on natal trees by wood-boring insects has been documented e.g. female carpenter worm, *Prionoxystus robiniae* (Peck) (Lepidoptera: Cossidae) (Munro, 1925; Munro, 1928; Nilssen, 1984) and chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae) (Dunn et al., 1986) which could lead to epidemic population levels within tree varieties relatively rapidly. However little is known of *O. cylindriostris* movement patterns within a particular location once they have emerged from their host tree or migration into a particular area (Clift, 2005; Froggatt, 1900; Olliff, 1890). Regression models to determine best predictors for adult *O. cylindriostris* presence and larvae damage could not be developed from the data in this study, due to the long lifecycle of *O. cylindriostris* (chapter 2) relative to the limited time (three years) that was available for analysis, which would represent only about one generation of the pest.

Surprisingly little research has been conducted on the presence of parasitoids and predators of *O. cylindriostris* and the little information known is very incomplete. There
have been no observations of a parasitoid emerging directly from *O. cylindrirostris*. A wasp (Hymenoptera: Braconidae) that was identified only to family has been found at *O. cylindrirostris* infested vineyards (Tummel, 2003), but confirmation of a parasitoid-host relationship was not made from actual rearing. It is unlikely that this parasitoid would be able to parasitize *O. cylindrirostris* larvae in the thicker barked part of the stump. Predators capable of attacking a coleopteran insect as large as *O. cylindrirostris* are likely to be true predators such as ground beetles (Carabidae). However, candidate species were not observed to attack *O. cylindrirostris* at any stage during this study. Vertebrate predation is considered to be unimportant (Munro, 1928) although no quantitative studies have been undertaken; torresian Crows *Corvus orru* (Passeriformes: Corvidae) have been seen during this study taking *O. cylindrirostris* in Corindi blueberry fields.

The control of *O. cylindrirostris* using broad spectrum insecticides was the primary focus of past investigators. However the recent deregistration of several broad spectrum insecticides (Australian Pesticides & Veterinary Medicines Authority, 2006) has severely limited the use of residual pesticides in preventing borer attacks. Because of environmental concerns and public pressures, it is likely that this trend will continue into the future. Environmental considerations, worker health, high development of new pesticide formulations costs and the possibility of pesticide resistance development makes it challenging to control *O. cylindrirostris* effectively using repeated applications of insecticides through the season. A previous HAL project, FR00027, defined the problem and made recommendations for insect pathogenic fungi as a potential control method for *O. cylindrirostris*. It was established that bee toxicity was the major impediment to using conventional insecticides as bees are required for pollination to ensure fruit set in blueberries (Clift, 2005).

Taken into consideration these recommendations, chemical and biological insecticide control measures were identified for use in commercial blueberry production. The insecticides that caused the greatest mortality in *O. cylindrirostris* adults under laboratory conditions were foliar applications of indoxacarb, clothianidin, imidacloprid, *B. bassiana*
var EWB, *A. parasiticus* var EWB and commercially available Mycoforce® containing *B. bassiana*, *M. anisopliae*, and *V. lecanii* (chapter 3).

Mycoforce® is available for use on blueberry bushes as a “tonic” for plant health and can be used against *O. cylindrirostris* without disrupting bee pollination, harvesting or causing residue concerns. The use of biological pesticides for *O. cylindrirostris* control will be of benefit to domestic honeybees, the natural environment, agricultural workers and reduced exposure to chemical residues, which is important for continued availability and further development of these products for use in agriculture.

Formulations of clothianidin and imidacloprid are not currently labelled for use in Australian blueberry orchards. Indoxacarb is already registered for use on several types of fruit orchards against light brown apple moth and several weevil species. Indoxacarb is currently being considered for use against light brown apple moth in Australian blueberry orchards. Given the excellent activity against adult *O. cylindrirostris*, an extension to the permit needs to be sought. Once the permit for indoxacarb has been issued and a withholding period established, a detailed *O. cylindrirostris* management program can be developed in consultation with blueberry managers for the use of Mycoforce® and Indoxacarb. While considerably more environmentally friendly than broad spectrum insecticides, the use of oxidiazine and nicotinamide based insecticides are not without their problems. *O. cylindrirostris* itself has no substantial insect predators or parasitoids currently known but non-target effects from these insecticides may cause problems for management of other insect pests if they are disruptive of natural enemies.

Compatibility between biological control agents and indoxacarb has not been as well documented although indoxacarb ranked very highly overall for safety to beneficial insects, largely because of its low dermal toxicity (Michaud and Grant, 2003). and its suitability for inclusion in pest management through conservation of natural enemies has been demonstrated over other insecticides available for pest management (Hewa-Kapuge et al., 2003). The currently identified examples of beneficial insects affected by indoxacarb which may themselves be present in blueberry fields or have related families
species present are: a parasitoid of light brown apple moth (major blueberry pest) *Dolichogenidea tasmanica* (Hymenoptera: Braconidae), a parasitoid of whitefly *Encarsia formosa* (Hymenoptera: Aphelinidae), and the diamond back moth parasitoid *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) (Boucias et al., 2000; Haseeb et al., 2004; Newman et al., 2004; Studebaker and Kring, 2003).

The use of imidacloprid has raised concerns due to the impacts documented on beneficial insects. The systemic nature of the insecticides can potentially contaminate nectar and pollen when distributed throughout the plant (James and Price, 2002), leave residues in fruit and has been identified as a contributor to colony collapse disorder (Chauzat et al., 2006; Cutler and Scott-Dupree, 2007). Residues of imidacloprid have been detected in samples taken from nectar, pollen, plant tissues and soils (Bonmatin et al., 2005; Colin et al., 2004). This poses a threat to the use of imidacloprid in blueberry production which needs insect pollination for fruit set (Chauzat et al., 2006; Cutler and Scott-Dupree, 2007). Inadequate bee pollination limits blueberry production. Honey bees, *Apis mellifera* L., (Hymenoptera: Apidae) are currently the only manageable pollinators available for pollinating *Vaccinium* varieties. Honey bees are efficient pollinators of blueberry varieties (Sampson and Cane, 2000) and translocated chemicals increase the chances of contact toxicity from the insecticide to natural enemies and to nectar feeding pollinators (Lord et al., 1968). Ecotoxicology studies with imidacloprid showed that direct death of the bees is only part of the colony collapse problem, which includes behavioural changes such as disorientation (Bonmatin et al., 2005; Bortolotti et al., 2003), feeding problems (Suchail et al., 2001), and communication disturbance (Deglise et al., 2002; Yang et al., 2008). Although the systemic application of imidacloprid provides reduced direct exposure compared to foliar spray (Grafton-Cardwell et al., 2008; Smith and Krischik, 1999) there is still a risk that many non-target beneficial species can be indirectly exposed to systemic insecticides (Stapel et al., 2000). Imidacloprid should be used with caution by growers desiring long-term control of pests through IPM and the preservation of natural enemies (Grafton-Cardwell et al., 2008). As a result of the potential risks under the current climate, the recommendation for the registration and use of imidacloprid based insecticides in blueberry IPM is not currently warranted, particularly since two
alternatives with lower impacts on beneficial species have been identified i.e. entomopathogenic fungi and indoxacarb.

The risks associated with the use of *B. bassiana* formulations are the effect of conidia on non-target insects and the ecological consequences of application (Butt et al., 2001). Entomopathogenic fungi vary in how they impact natural enemies depending on whether natural enemies consume spores or they are directly affected by sprays (Cloyd and Sadof, 2000). Natural enemies may ingest fungal spores when either grooming or when feeding on a contaminated host or food source. The severity of the effect is very much dependent on the concentration of spores applied (James and Lighthart, 1994). The fungi *B. bassiana* has been observed to infect and harm ladybird beetles, causing as much as 95% mortality of adult ladybird beetles (Cloyd and Sadof, 2000). Pollinating bee populations when exposed to high concentrations of *B. bassiana* in the laboratory were found to have reduced individual longevity (Vandenberg et al., 1998). In contrast applications of *B. bassiana* caused low levels of mortality amongst hive bees, with no noticeable effect on bee behaviour and as a result poses little risk to natural hives (Neves et al., 2001). The lack of non-target outbreaks in nature may be the result of both virulence within a narrow host range and abiotic factors that affect the survival of the fungus in the field (Hajek and St. Leger, 1994).

The efficacy of Mycoforce® against *O. cylindrirostris* and its low environmental impact warranted identification of incompatibilities with current blueberry pest management. Therefore the effect of phytosanitary products was assessed on conidia germination, vegetative growth and sporulation. Previous authors have shown that *B. bassiana* appears to be compatible with a large number of insecticides and concentrations, which can make this fungus important when insecticides fail to control target pests (O’Callaghan and Brownbridge, 2009). All pesticides tested had varying degrees of mycotoxicity. Malathion, methomyl, indoxacarb, spinosad, *B. thuringiensis* and imidacloprid have little negative effect on germination, vegetative growth and conidia production so can be recommended for use with *B. bassiana* based myco-insecticides. In contrast captan, mancozeb, propiconazole, NPV, chlorpyrifos and dimethoate should avoid overlap with
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*B. bassiana* application periods to minimize toxicity and would need to be managed diligently to reduce direct contact with fungi which could render the myco-insecticide ineffective.

Cultural control techniques are limited for *O. cylindrirostris*. *O. cylindrirostris* attacks healthy trees, resulting in a decline in tree health that may render affected trees more susceptible to other harmful factors (chapter 2). Fertilization and irrigation practices that reduce stress in periods of drought are therefore essential in preventing stress to trees subject to attack by *O. cylindrirostris*. Monitoring for symptoms of borer attack, rapid removal and destruction of all infested trees and material thoroughly to prevent premature metamorphosis and emergence of any weevils in the trees plus the removal of *O. cylindrirostris* adults as they are collected by the mechanical harvesters (Clift, 2005), may help break population cycles. Leaving a fallow period has been shown to be an effective tool against the large pine weevil *Hylobius abietis* Linnaeus (Coleoptera: Curculionidae) (Munro, 1928). While research into fallow periods may provide considerable advantages for IPM (Nordlander, 1987; Nordlander et al., 2003) restocking blueberry sites at intervals greater than five years is unlikely to remedy *O. cylindrirostris* infestation because there is no shortage of breeding material available from nearby blueberry fields and the surrounding native vegetation (chapters 1 and 2). Cultural practices alone seldom provide the level of control needed in this situation so susceptible or high value trees may need to be protected from borer attack by pesticide applications once planted. A more rational pest management program for the weevil can be achieved by integrating the use of insecticides and plant resistance.

6.1 Conclusion

Integration of control tactics is essential to achieve the IPM objectives of preventing economic damage from *O. cylindrirostris* populations and reducing the risks of sole reliance on insecticides (Kogan, 1988). Effective management of *O. cylindrirostris* requires an integration of control tactics that satisfy the requirements of adequate levels
of efficacy, safety of beneficial insects and workers, the absence of residue problems and compatibility with existing technologies.

The current integrated pest management recommendations are: 1) Exercise good cultural practices to avoid plant stress to reduce yield loss in trees harbouring *O. cylindrirostris*. 2) Good crop sanitation practices to reduce spreading populations (removal of brood trees such as Becky blue). 3) Use of tolerant blueberry varieties in breeding and planting (e.g. RE var Powderblue, Britwell, Premier and SHB var 41, 390 and 209A). 4) The use of commercially available insecticides (particularly indoxacarb and Mycoforce®) optimising insecticide application times to make contact with the pest and not interfere with other agronomic practices. Using indoxacarb after harvest in peak weevil emergence and Mycoforce® during harvest period when weevils are emerging and limited contact with toxic phytosanitary products will occur are potential options which need investigation.

Future research topics discovered during this project’s investigations include: determine how adult *O. cylindrirostris* locate the opposite sex; determine the lifecycle of *O. cylindrirostris* in grapevines (*Vitis* sp); examine the effect of adult nutrition on the fecundity and longevity of *O. cylindrirostris* and the fitness costs for small-sized males which have had to emerge prematurely; explore the effect of mating competition and success; determine the mechanisms of plant tolerance to *O. cylindrirostris*; develop a blueberry breeding program for the expansion of tolerant blueberry varieties to *O. cylindrirostris* attack and registration, residue testing and large scale field tests using commercial spray equipment of the potencial control options (indoxacarb and Mycoforce®).

6.2 References

Australian Pesticides & Veterinary Medicines Authority, 2006. *The reconsideration of the active constituent azinphos-methyl, registrations of products containing azinphos-methyl and approvals of their associated labels*. Review Summary, APVMA, Canberra, Australia


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Appendix 1.

Appendix 1-1 Machine harvester, harvesting *Vaccinium corymbosum*
Appendix 1-2 Kaplan-Meier survival graphs for *Orthorhinus cylindrirostris* egg oviposition to adult emergence by host plant
Appendix 1-3 Kaplan-Meier survival graphs for Orthorhinus cylindrirostris adult emergence to adult death by host plant
Appendix 1-4 Kaplan-Meier survival graphs for Orthorhinus cylindrirostris adult emergence to adult death by insect sex
Appendix 1-5 *Orthrhinus cylindrirostris* egg removed from *Vaccinium corymbosum* and separated
Appendix 1-6 *Orthorhinus cylindriostris* emerging from *Vaccinium corymbosum*
Appendix 2.

Appendix 2-1 *Vaccinium corymbosum* growing on blueberry farms of Australia (A) under netting (B) young trees surrounded by native vegetation.
Appendix 2-1 Yield versus emergence of *Orthorhinus cylindrirostris* for Star and Sharp
Appendix 2-2 Yield versus emergence of *Orthorhinus cylindrirostris* for Powderblue and Premier
Appendix 2-3 Yield versus emergence of *Orthorhinus cylindrostris* for 42 and 41
Appendix 2-4 Yield versus emergence of *Orthorhinus cylindrirostris* for Britwell and Crunchy

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Appendix 2-5 Yield versus emergence of Orthorhinus cylindrirostris for Windy and Tiffanies
Appendix 2-6 Yield versus emergence of *Orthorhinus cylindrirostris* for 390 and Climax
Appendix 2-7 Yield versus emergence of *Orthorhinus cylindrirostris* for 209A and 115
Appendix 2-8 Blueberry Farms of Australia (BFA) farm map.
Appendix 3.

Appendix 3-1 Probit analysis for *Beauveria bassiana* Mycoforce formulation tested on adult *Orthorhinus cylindrirostris*
Appendix 3-2 Probit analysis for *Beauveria bassiana* var EWB formulation tested on adult *Orthorhinus cylindriostris*
Appendix 3-3 Probit analysis for *Aspergillus parasiticus* var EWB formulation tested on adult *Orthorhinus cylindrirostris*
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Appendix 3-4 Probit analysis for indoxacarb formulation tested on adult *Orthorhinus cylindrirostris*

\[ y = 2.6227x + 4.4302 \]

\[ R^2 = 0.7737 \]
Appendix 3-5 Probit analysis for methomyl formulation tested on adult *Orthorhinus cylindrirostris*

\[
y = 1.6277x + 1.0612 \\
R^2 = 0.9142
\]
Appendix 3-6 Probit analysis for clothianidin formulation tested on adult Orthorhinus cylindrirostris
Appendix 3-7 Probit analysis for imidacloprid formulation tested on adult Orthorhinus cylindriostris
Appendix 3-8 Probit analysis for imidacloprid tablets formulation tested on adult Orthorhinus cylindrirostris

\[ y = 1.5583x + 4.9581 \]

\[ R^2 = 0.8988 \]
Appendix 3-9 Probit analysis for imidacloprid tablets formulation tested on larve Orthorhinus cylindrirostris

\[ y = 3.7097x + 3.1263 \]

\[ R^2 = 0.9845 \]