Fruit-tree borer (*Marogra melanostigma*):
Investigations on its biological control in prune trees

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DEDICATION

In memory of Maddy and those lost along the way…
ACKNOWLEDGEMENTS

There are many people I would like to thank and acknowledge for the help and support they have shown me throughout this process.

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STATEMENT OF AUTHENTICATION

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

_______________________________________________
(Signature)
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SUMMARY

Fruit-tree borer, *Maroga melanostigma* (Wallengren), is a native Australian pest in many species of trees. It is of particular economic importance in prune (*Prunus domestica*) trees because the presence of this wood boring insect can reduce productivity by an average of 5% per tree. Large areas of orchards can be affected. There are currently no chemicals registered for control of this pest. Young, New South Wales is the second largest prune-growing district in Australia and the area most seriously affected by *M. melanostigma*. Prune growers in the district utilise integrated pest management and were supportive of a project to investigate biological control options for this economically damaging pest.

The two main objectives of the project were 1) to understand the life cycle of *M. melanostigma*, so biological controls could be timed appropriately; and 2) to investigate biological control options for this pest.

Life cycle studies were commenced in the first season (2003/04) using field cages and light trapping. These investigations continued throughout the project. In Young, moths were found to emerge from wood over a two month period (December and January). Oviposition was assumed to be during this period however, even after extensive searches of trees, no eggs were observed. Historical data were collated to determine locations and timings of moth emergence elsewhere in Australia. The data
showed that *M. melanostigma* has been found in every state and territory Australia, with moths observed from October through to March.

The biological control options reviewed were egg parasitoids (*Trichogramma* species only), entomopathogenic nematodes and entomopathogenic fungi. *Trichogramma* were favoured because of previous research undertaken against the same pest in pecans in Moree, NSW. Entomopathogenic nematodes were also investigated due to research indicating their effectiveness in cryptic situations, such as borer tunnels in trees. Fungi were considered but dismissed due to lack of literature supporting their effectiveness in reducing lepidopteran pest damage in trees.

A major field trial was designed with the assistance of a biometrician and the trial blocks laid out based on this advice. There were three trial sites, each containing four blocks of approximately 200 trees (~800 trees/site). Two blocks were designated as release blocks and two as non-release blocks to correspond with the trial’s two treatments. In the first season (2003/04) an initial visual assessment of borer damage was undertaken after leaf fall on each of the trees in the trial. This information was used as baseline data, to compare against damage levels following biological control releases in the second and third years of the project. Natural parasitism in the field was assessed using cultured eggs of *Helicoverpa armigera* (Hübner) before and between *Trichogramma carverae* (Oatman & Pinto) releases. Temperature and relative humidity were recorded in each of the trial sites, for the duration of the trial, using commercially available data loggers.
In the second season (2004/05), *Trichogramma* releases were made during the period of moth activity and *H. armigera* eggs were used to monitor parasitism in the trial orchards. Parasitised eggs were reared through and all parasitoids were identified as *T. carverae*. Damage assessments were again carried out after leaf fall to compare release versus non-release blocks, as well as to determine if there was any change in borer activity. Early instar larvae were collected from non-trial blocks and exposed to the entomopathogenic nematode *Steinernema carpocapsae* in a laboratory investigation. Results from this limited bioassay were inconclusive.

In the third season (2005/06), *Trichogramma* releases were again made during the period of moth activity and *H. armigera* eggs were used to monitor parasitism in the trial orchards. Parasitised eggs were reared through to emergence. The emerged parasites were identified as *T. carverae*, *T. pretiosum* and *T. nr brassicae*. Damage assessments were again made of all the trees in the trial. Results were statistically analysed to detect any differences between treatments.

There was no statistically significant evidence that the releases of *T. carverae* reduced damage from *M. melanostigma* over the duration of the trial. Although damage increased across both release and non-release treatments in most blocks during the trial investigations, the increase was slightly lower in trees in which *Trichogramma* had been released. It should be noted that the experiments were affected by serious drought conditions which prevailed during the three seasons of the trial.
Chapter 1 Introduction

Prunes have been grown in the Young, NSW district since the 1800s. Not until after World War I, when returning soldiers were given settlement blocks in rural areas, were prunes planted in large quantities. Commercial production began after this time, with larger areas being planted not only in Young but also in the Murrumbidgee Irrigation Area around Griffith, NSW. Smaller areas are now also planted near Renmark, South Australia.

Prunes are a European plum. The main variety of prune grown in Australia is d’Agen. There are also a number of d’Agen clones which are suited to Australian growing conditions. The trees are generally planted as grafted trees, where the scion is grafted onto a rootstock. The most common rootstock used today in Australia is Myrobalan H29C. In mature trees, the fresh fruit is mechanically harvested from the tree and dehydrated. In the dehydrated form, the fruit is stored until needed. The dried prunes then undergo processing, or re-hydration, before being packaged.

One reason prunes are chosen as a crop to grow is because there are very few pests and diseases which attack prune trees in Australia. Thwaite (2004) completed a four-year project on integrated pest and disease management (IPDM) in prunes in 2003. One of the project recommendations was for the Australian prune industry to fund a separate project to investigate biological control options for the fruit-tree borer, *Maroega melanostigma* (Wallengren) (Lepidoptera: Oecophoridae).
The damage by this pest can be found in any part of the tree. Early instar larvae feed on the surface of the tree which can lead to ringbarking and death, especially in young trees. Later instars tunnel into the tree, which causes structural damage and decreased tree vigour. The Industry Development Manager estimates that the presence of this pest in a tree can cause a loss in fruit production by an average of 5% (J. Granger, Pers. comm.). This can amount to a significant loss in production and income in a commercial enterprise when large areas of orchard are affected.

There are no chemical controls currently registered for this pest. The general control recommendation is to clear away the frass (the material left behind by the pest as it feeds) and probe the tunnel beneath with a thin piece of wire, and pierce the larva to kill it. While effective, commercial growers find this method to be time consuming and impractical.

The industry’s uptake of Thwaite’s IPDM work, where he stressed the importance of the pest and beneficial species complex, meant that in the Young district growers were no longer using broad spectrum insecticides in their orchards. The industry accepted the recommendation to fund a separate project because they wanted to investigate possible biological control options for this economically damaging pest. The three-year project began in 2003 and was funded by the Australian Prune Industry Association, Horticulture Australia Limited and the NSW Department of Primary Industries.
Biological control of *M. melanostigma* was recommended to industry because of the success of work previously carried out on the same pest in pecans in Moree, NSW. The team in Moree achieved acceptable control of *M. melanostigma* using a native egg parasitoid wasp, *Trichogramma nr ivelae* (Pang & Chen) (Hymenoptera: Trichogrammatidae). Because of this research, the likely biocontrol option in prunes would focus on *Trichogramma*.

Although this parasitoid was effective in pecans in Moree, there was clearly a different set of variables to consider for attempting to implement a similar strategy in prune trees at Young. This project was designed with two main aims. The first was to look at the life cycle of *M. melanostigma* in the Young district. The data from Moree were not necessarily going to be accurate for the climate and other site conditions at Young.

An accurate assessment of the moth’s life cycle in Young was crucial in order to carry out the second aim, which was to investigate biological control options. While the egg parasitoid was a likely candidate, its success was highly dependent on wasp releases being made during the egg-laying phase of the pest’s life cycle. Because there was a potential to miss this critical timing with releases, alternative controls were also considered to target the larval stages of the pest’s life cycle. The use of entomopathogenic nematodes was considered due to their recorded success in controlling other lepidopteran larvae. Entomopathogenic fungi were also considered, however no experiments were conducted because nothing in the literature suggested effective control of lepidopteran larvae feeding in tree wood.
CHAPTER 2 LITERATURE REVIEW

2.1 The prune industry

The prune industry in Australia is relatively small on a global scale, producing approximately 2% of the world’s production. There are three main growing regions – Young and Griffith in New South Wales; and Renmark in South Australia (Figure 1). Amblard (2005) summarises that there are 2370 hectares planted to prune trees with a potential to produce 5000 dried tonnes per year. Griffith produces between 50-55% of the Australian total; Young between 35-40%; and South Australia contributes between 5-10%.

The industry contributes about $20 million per annum to the Australian economy. Australia under-produces for its domestic needs and is therefore reliant on imports. The main region Australia imports from is North America. The Californian industry can potentially produce 160,000 tonnes (55% of world production) of dried product per year. Other major areas of prune production are France (21%), Chile (12%),
Argentina (9%), South Africa (1%) and Italy (<1%) (Amblard, 2005). In recent years, the export of sugar plums (fresh prunes) to south-east Asia has decreased the dried tonnage of prunes in Australia but caused an increase in tree plantings to cater for this early fresh market (J. Granger, Pers. comm.).

Prunes are the dried product of European plum (*Prunus domestica*). In Australia, prune trees flower in September with fruit harvested, depending on the year, from mid-January to early March (Hetherington *et al*, 2004). Fresh fruit is referred to as prunes or sugar plums. The main variety is d’Agen, originally from France. Other varieties of prunes planted in Australia are generally clones of the d’Agen or d’Ente varieties, such as 698, 303 and 707. They are generally planted on the rootstock Myrobalan H29C. This stock is a *Prunus* species (*P. cerasifera*) and well-suited to Australian conditions (Menzies, 2001).

The prune industry in Young began to expand around 1920 with the Soldier Settlement blocks north and south of the township. At that time, the practice was for clean cultivation and the trees were grown under dry-land conditions. Because of the decline in tree and orchard health, sod culture was introduced in the 1970s, with drip irrigation becoming more common especially after the drought of 1982/83 (Menzies, 2001).

Prune trees in Australia are relatively free from pests and diseases. Thwaite (2004) found in his four-year prune integrated pest and disease management (IPDM) study that the main pests of prunes were plum rust mites (*Aculus fockeui* Nalepa and Trouessart), two spotted mite (*Tetranychus urticae* Koch), San José scale (*Quadraspidiotus perniciosus* Comstock) and fruit-tree borer (*Maroga melanostigma*)
Diseases included prune rust (*Tranzschelia discolor* Funkel, Tranzschel and Litv.), brown rot (*Monilinia fructicola* G. Wint. Honey) and shot hole (*Wilsonomyces carpophilus* Lév. Adaskaueg, Ogawa and Butler). The health and vigour of the tree is important in pest and disease control, as healthy trees are less prone to attack. Because of the growing conditions in Young (i.e. prone to drought, saline water, *et cetera*) trees are often not grown under optimal conditions and are therefore more likely to be attacked by pests such as *Maroga melanostigma*.

Although pest and disease management in prunes is important, the sprays needed for control are considerably less than for other stone fruit crops (Menzies, 2001). Often there is a copper spray program to control fungal diseases as well as a program of HMOs (Horticultural Mineral Oil) to control scale insects (Hetherington *et al.*, 2004). Insecticides are not recommended unless a pest population approaches an economic threshold. This makes prunes an encouraging candidate for IPDM programs which use beneficial insects for biological control.

When the organochlorine pesticide DDT (dichlorodiphenyltrichloroethane) was available, it was widely used in the Young district for insect control by the fruit industry. Two to three years after its withdrawal in 1973, an increase in fruit-tree borer damage was noticed (J. Granger, Pers. comm.). Since then, there have been no effective chemicals registered for use in controlling this pest. Growers wanting control penetrate the borer tunnels with thin wire to kill the larvae. Control during the moth stage of the life cycle is not undertaken as this pest is nocturnal and difficult to locate (G. Thwaite, Pers. comm.). Control of eggs is by whatever natural enemies are present during that time.
2.2 Fruit-tree borer (*Maroga melanostigma*)

This native Australian species has gone through numerous name changes since its first recorded discovery in the 1850s. The earliest reference, in about 1861, is for *Cryptophasa unipunctata* Don (French, 1891). It changed to *Maroga unipunctata* Don by 1917. At some points it was also known as *C. gigantella* Walker, and *M. gigantella* Walker. In the 1970s the nomenclature became *C. melanostigma* Wallengren and in the 1990s the name was changed to what the pest is known as today – *Maroga melanostigma* (Wallengren).

**Life cycle**

The literature differs on the length of the life cycle of *M. melanostigma*. Nicholls (1933) stated that the larval stage probably lasted over a year. An unpublished report (J. Friend) found that the life cycle was definitely a year and a Tasmanian Garden Guide (1981) indicated that the life cycle started in summer, with eggs being laid on trees. The larvae burrow into the wood of the tree and emerge as adults the next summer, completing the life cycle.

In the Young district, the life cycle is thought to be at least two years (Jones, 1978). Collecting specimens in autumn will find larvae in different instars (Lloyd, 1972). In Moree, NSW, research into *M. melanostigma* was undertaken in a pecan orchard and the life cycle there was confirmed to be one year (R. Llewellyn, Pers. comm.). A difficulty with investigating wood boring insects is that they may remain in their
enclosed habitat for a number of years before conditions become favourable for pupation and emergence (P. Gillespie & M. Fletcher, Pers. comm.).

Time of moth emergence is variable. In Moree, NSW, moths were trapped using light traps during October and November. In Young, moth emergence is generally in December and January. As emergence is degree-day dependent (P. Gillespie, Pers. comm.), timings will change from year to year, and vary between locations. Moths in collections have been caught as early as 3 October and as late as 18 March. They have been found in every state and territory in Australia.

French (1933) noted that as many as 40 eggs could be laid by one female. The eggs are laid on the surface of the bark. When the small larva hatches, it feeds on the bark and bores downward into the tree. As the larva goes through its five instars, determined by the width of the head capsule, the size of the tunnel increases in length to reach approximately 75 - 100 mm before the larva pupates (Anon., 1948).

The adult moth is satiny-white and has a 30 - 50 mm wingspan. The upper surface of the abdomen is black with an orange-coloured fringe of hairs. There is a small black spot located near the centre of each forewing (Anon., 1950).

**Damage**

Damage by this pest was first recorded in Australia in the late 1800s, near Melbourne, Victoria. Damage was initially seen on ornamental and native trees but by 1920 *M. unipunctata* was noted as a pest of cherry trees in NSW (Allen and Hogg, 1920).
By 1922 damage from *M. unipunctata* was noted as occurring on the stems and branches of wattle trees, as well as fruit trees, in Brisbane, Queensland (Illidge, 1922; Jarvis, 1922). Its presence was noticed in South Australia between 1928 and 1930 when *C. unipunctata* was noted as causing damage to swamp oak (*Casuarina* sp.) (Davidson, 1931).

Pescott (1932) reported that the cherry borer moth was a serious pest in street trees while French (1933) noted that not only was *M. melanostigma* a problem in the native acacias, banksias and casuarinas, but was also a pest in cherry, quince, peach, plum, pear and apple. The first mention of attack to prunes was in 1948 (Anon., 1948). By 1959 citrus susceptibility was also observed (Anon., 1958).

In native trees, *M. melanostigma* attacks the forks between the branches but in fruit trees, attack can occur anywhere (Ward, 1922). Larvae tunnel into bark, usually in a downward direction (Nicholls, 1933).

Unhealthy or weakened trees are more susceptible to attack by *M. melanostigma*. Tree health is important in not only preventing attack, but also in the tree’s response to damage caused by borers (Anon., 1948). Clear or yellowish exudate from the bark may be an early indication there is a borer infestation (Anon., 1958). It was noted that the almond varieties grown in South Australia tend to exude a gum which can encourage oviposition by this pest (Quinn, 1934).
**Control measures**

Control of *M. melanostigma* has always been difficult. This is in part due to the nature of borers. Because they are protected by frass, and sheltered in the tree in most cases, it is difficult to achieve adequate pesticide coverage for control. Another reason is the cost and difficulty in procuring a registered product which would control *M. melanostigma* or damage caused by the pest.

In the past, many products have been investigated to control *M. melanostigma*. Recommendations have been made since the first appearance of the pest, some of which have worked adequately. The limiting factor in control is usually time. Finding borer sites and then inserting wire into the tunnels to kill the larvae is not a practical method of control for most commercial enterprises. Another problem is that by the time damage is noticed, the pest is already established in the tree.

The first advice recorded in 1899 recommended that copper wire was effective if inserted down borer tunnels. Only slight scratching of the cuticle of the larva causes death. Kerosene or soap applied over the borer holes worked effectively and also deterred egg-laying on the bark (Froggatt, 1899).

Recommendations through the decades have not changed considerably. The standard recommendation is to clear frass away from borer workings. A knife or scraping tool is effective in removing the early stages of larvae from the tunnels on fruit trees. Other than using a wire to penetrate the tunnels, most of the advice involved application of a residual substance which was lethal to the pest, soaked into a rag or wood, and plugged into the borer tunnel. Apart from kerosene, different oils were
recommended as was Stockholm tar (Assistant Entomologist, 1909) and petrol (Quinn, 1934). Pruning off affected limbs and subsequently burning them was also considered an effective control (Evans, 1939). Timing of tree pruning may also be critical as in summer, pruning can leave wounds which are more susceptible to pest attack (Lloyd, 1972).

Chemicals used for injecting into tunnels included carbolic acid (French, 1917), carbon bisulphite (Ward, 1922) and carbon tetrachloride (Nicholls, 1933). By 1950, a few drops of a DDT emulsion, at a concentration of 0.1%, was reported to be effective in killing the larvae if it was injected into the borer tunnels (Anon., 1950). Painting the affected area with dieldrin or another suitable insecticide was recommended after the larvae were removed (Anon., 1958).

For control of larvae feeding close to the surface of the tree, as well as for eggs laid on the bark, recommendations included spraying the tree with coal-tar water during dormancy (French, 1917), or destroying the eggs with a winter-strength lime sulphur spray (Ward, 1922). A spray of lead arsenate and flour paste was also reported to be effective in killing larvae feeding on the tree bark and buds (Nicholls, 1933).

**Research undertaken**

In a survey conducted in 1973/74 of eleven orchards in the Young area, it was found that 58% of prune trees had been attacked by *M. melanostigma* larvae (Jones, 1978). Attack was primarily through pruning cuts and grafting sites. Drought and defoliation appeared to exacerbate the problem.
Insecticide treatments were undertaken in the field for *M. melanostigma* control during March and April 1974 in Young. Rows 300 m long were sprayed using high volume orchard spraying equipment. Infested limbs were tagged and the frass brushed away. Sites were re-examined a week later. It was assumed that if the frass had been replaced then the larvae were still alive. The most effective chemicals for control were 0.1% DDT, 0.025% methomyl and 0.025% chlorpyrifos. The mortality increased from 60-70% to >90% for both DDT and methomyl when 0.013% wetter was added (Jones, 1978).

An insecticide paste developed in Brazil was successfully used for borer control in that country. An attempt in 1985 to introduce it into Australia was unsuccessful. The application of the product was labour intensive and this would have hindered uptake by industry (Thwaite, 1985).

Esfenvalerate (*Hallmark®*), a synthetic pyrethroid insecticide, was trialled in 1989 to control *M. melanostigma*. It was sprayed throughout a pecan orchard in Moree, NSW four times between October and December. It was an expensive trial which appeared to have little or no effect on the pest (Seymour and Crouch, 1993). However, based on an egg exposure program in the pecan orchard in 1991/92, a native *Trichogramma* wasp was found which had an effect on the pest in this situation. Although natural parasitism of *M. melanostigma* eggs by *Trichogramma* in the field did occur, the percentage was low (~3%). Seymour and Crouch (1993) thought that inundative releases of *Trichogramma* might increase parasitism rates and, while not eradicating

1 Registered trade name
*M. melanostigma* from the orchard, believed it could be suitable as a biological control.

Survey work undertaken by Thwaite (2004) and his team during the 1999 – 2002 period of his IPDM project, found for each year of the trial borer damage increased across the trial blocks and was by far the most serious pest problem. During the period of this trial, Thwaite discussed biocontrol options with the researchers in Moree, NSW. It was thought that the native *Trichogramma* they were using could be of benefit as a biological control for prune growers in Australia against the same pest. Thwaite did not pursue a control as part of the IPDM project but recommended to industry that they consider a separate project to assess the feasibility of biological control. That recommendation forms the basis of this current research project.
2.3 Biological control

Classical biological control involves introducing natural enemies into an area where a pest is causing economic damage (Waterhouse and Sands, 2001). The aim of a biological control program, according to Common (1993) is to “discover the most effective control organisms in the pest’s country of origin, carry out exhaustive tests to ascertain that, if introduced, those organisms would not themselves prove to be pests, and if safe, to introduce and establish them to aid in the pest’s control”. Insect parasitoids are one consideration for biological control, as are entomopathogenic nematodes, viruses and fungi.

Insect parasitoids

A parasitoid is defined as an insect which lives off its host, eventually killing it (Common, 1993). There are many advantages to using natural parasitoids for pest management, especially when compared with using pesticides. Advantages include: no toxic residues left on plants or soil; resistance problems are less likely to occur; equipment needs are less complicated; and non-target pests and predators are not adversely affected (Hassan, 1993). A benefit of using an egg parasitoid is that by laying its eggs inside a host egg, it kills the pest before it hatches, thereby eliminating any damage the host can cause in other stages of its life cycle (Lundgren et al, 2002).
**Trichogramma as a biological control agent**

*Trichogramma* species are used worldwide as egg parasitoids against a range of agricultural pests. *Trichogramma* are minute wasps which measure less than 0.5 mm long. The female lays her eggs into host eggs, where the wasp eggs hatch and the developing larvae feed on the developing host. The *Trichogramma* larvae pupate inside the host egg and when fully formed emerge from the host egg by chewing a hole through it. The host egg turns black as this parasitisation takes place, with the whole process taking between 7-20 days, depending on temperature (Hassan, 1993; Llewellyn, 2002). Males emerge first and wait for females so mating can take place immediately (Seymour *et al*, 1994). During her 5-14 day life, a female wasp can parasitise over 50 moth eggs. While mated females can produce both male and female offspring, fertilised eggs will generally become female offspring while unfertilised eggs will become male offspring (Martel and Boivin, 2004). A *Trichogramma* female will usually stay near a preferred host until all or most of the eggs are parasitised (Hassan, 1993) and better parasitism tends to occur with pests that lay their eggs in clusters rather than singly (Smith, 1996).

Controlling lepidopteran pests with *Trichogramma* has been considered for over 100 years (Smith, 1996). The first account of *Trichogramma* release in Australia was in Queensland for control of codling moth (*Cydia pomonella* Linnaeus) in 1927 (Seymour *et al*, 1994). Worldwide, much of the research and consistent success into using *Trichogramma* as a biocontrol against lepidopteran pests, has been in corn. Cotton, sugarbeet, vegetables, vineyards, rice and fruit trees (including plum) have also been targeted in the past (Smith, 1996). Using *Trichogramma* instead of insecticides helps to sustain the natural complex of pests and predators already
present, and therefore helps to reduce the need to control secondary pests which can occur with pesticide use. Biocontrol using *Trichogramma* also reduces human and environmental health risks which are associated with pesticides (Lundgren *et al*, 2002).

It is difficult to assess the effectiveness of *Trichogramma* releases because there are many factors involved and the results are often variable. Level of control varies depending on crop, pest pressure and other beneficial insects present (Llewellyn, 2002). There is a relationship between egg parasitism, larval populations and damage but conclusive studies have not been undertaken. Although the effectiveness of using *Trichogramma* for biocontrol is being investigated in many countries, only a small number of pests and their relationship to *Trichogramma* species have been studied in detail. Most studies assess parasitism rates of host eggs, but this may underestimate actual control rates because of the difficulty in recovering, and therefore assessing, all parasitised eggs in the field (Smith, 1996). Parasitism is a useful indicator of *Trichogramma* activity but to obtain an indication of pest control, monitoring pest activity and damage is more reliable.

It is important to have knowledge of both the target pest life cycle and the biological control agent’s life cycle and preferences. Literature from around the world indicates that one aspect hampering the use of *Trichogramma* as a biological control is lack of taxonomic information regarding the species (Seymour *et al*, 1994). Understanding the taxonomy of *Trichogramma* species is essential in trying to evaluate the results, and distinguishing between native and released wasps helps to understand the effects of inundative releases (Seymour *et al*, 1994).
Approaches for biological control

There are generally three biological control approaches used with releasing *Trichogramma*: introduction, inoculation and inundation. Introduction involves introducing a species to an area and/or crop. Inoculation involves releases where the progeny of the parasitoids are able to build up over a growing season so their effect is over a longer period (Smith, 1996). Inundative release, which is the most widely used for *Trichogramma*, relies on releases being made which have immediate effects on reducing host populations but not on long-term establishment of the *Trichogramma* species. Inundative release involves large numbers of the parasitoid being released and timed to coincide with the oviposition period of the pest (Smith, 1996). Rates of release depend on the target pest and crop situation. Generally, after the first release a second release is made five to six days later (Crouch, 1999). Selection of an appropriate strain of *Trichogramma* for inundative release to control a particular pest should be based on laboratory, semi-field and field experiments. The most important selection criteria when choosing a suitable species are host preference, searching behaviour and tolerance to environmental conditions (Hassan, 1993).

*Trichogramma* are more habitat-specific than host-specific and therefore different levels of egg parasitism will be found in different crops. It is thought that *Trichogramma* search randomly throughout a plant for host eggs. If this is true then factors such as size of plant, the plant’s surface area and the complexity of plant structure will all affect parasitism levels. This would also indicate that there could be different levels of parasitism of the same pest in different crops. Plants emit volatile chemicals which stimulate *Trichogramma* searching and parasitism abilities. All these
factors relate to *Trichogramma’s* ability to be successful as a parasitoid (Smith, 1996).

Another important trait when selecting *Trichogramma* species for biological control is host acceptance. It is important to survey a potential release site to know what, if any, *Trichogramma* species are currently present. *Trichogramma* species are host-specific and depending on the species, strain and rearing conditions of the *Trichogramma*, the acceptance of hosts can vary greatly (Hassan, 1993; Seymour *et al*, 1994; Cerutti and Bigler, 1995; Steidle *et al*, 2001).

The effects of both contact and residual chemicals on beneficial organisms have often been associated with field failure of biocontrol (Hewa-Kapuge *et al*, 2003). There may be a level of control occurring naturally but the balance is often displaced due to the use of pesticides or poor management (Common, 1993). It has been demonstrated that some pesticides are less toxic to beneficials and these may be able to be used in integrated pest and disease management programs (Hewa-Kapuge *et al*, 2003). According to a study by Voegelé, *Trichogramma* sensitivity to certain pesticides can occur up to one kilometre away from a spray site (Smith, 1996). Mass releases are therefore best suited to situations using organic or IPM practices (Llewellyn, 2002).

One of the target pests for *Trichogramma* release is *M. melanostigma* (Llewellyn, 2002). On a pecan orchard in Moree, NSW Australia, Seymour found that eggs of *M. melanostigma* were being parasitised by a native wasp in the genus *Trichogramma* (Creagh, 1992). Seymour and Crouch (1993) reported that in Moree the moth emergence of *M. melanostigma* occurred over approximately 76 days, with the peak
moth activity in mid-November. They also found that natural parasitism levels of moth eggs in the field were very low, at < 3%. Inundative releases of the parasitoid (release rate unpublished), weekly during the period moths were active and laying eggs, were shown to achieve a “reasonable” level of control of this pest in the orchard. Pest damage in the pecan trees was reduced following *Trichogramma* releases, although there was some seasonal variation (Llewellyn, 2001).

*T. nr brassicae* Bezdenko sensu Pintureau, formerly known as *T. nr ivelae*, had been shown to be the effective *Trichogramma* species against *M. melanostigma* in pecans in Moree. In that same orchard, *T. pretiosum* Riley and *T. carverae* Oatman & Pinto were later used (Llewellyn, 2001), however with less successful outcomes (G. Bowman, Pers. comm.).

**Commercial use and release of Trichogramma**

Smith (1996) stated that to successfully reach the requirements for commercial application, all *Trichogramma* programs must take into account four points: an appropriate population for release needs to be selected; a system needs to be in place for mass rearing; effective distribution of the parasitoid needs to be in place; and there needs to be a field release strategy.

A critical aspect of any control program using *Trichogramma* is the method of release. Apart from aerial releases, there are two generally accepted ground release methods which are both used depending on circumstances. ‘Broadcast releases’ involve distributing *Trichogramma* evenly throughout a release area. ‘Point source’ releases use parasitised hosts or recently emerged adults placed in one or more fixed
sites in an agricultural setting (Lundgren et al, 2002). Although a broadcast release may give a more even distribution, a point source release offers protection for the released material in the form of shelter from other predators or parasites. A point source release relies on the wasps dispersing through the area and evenly parasitising host eggs. Because of many variables, this is not always achievable (Lundgren et al, 2002). One method of point source release is to use capsules. They usually contain a minimum of 1000 parasitised moth eggs, are stapled or placed in a crop and have exit holes for the *Trichogramma* to escape from when they hatch. The capsule also offers some protection for the *Trichogramma* from predation in the field (Llewellyn, 2004).

In Australia, *Trichogramma* are normally shipped in the form of parasitised Angoumois grain moth, *Sitotroga cerealella* (Olivier) eggs and attached to cards. Cards can be placed in a container until wasps emerge, then the container is opened and the wasps can move about in an area. Alternatively, cards with parasitised eggs can be placed in the area to be serviced. Releasing fully emerged wasps may reduce their time of efficacy in the field for parasitisation, while putting egg cards out increases chances of hyperparasitism. In large areas, releases can be made by small aircraft (Seymour et al, 1994).

Proper delivery of material is important for a release to be successful. Hot conditions and long distance of delivery can jeopardise a successful release. These factors can impact on the survival of the released *Trichogramma* species (Smith, 1996).

Of the many factors affecting the release and disappearance rate of *Trichogramma* in the field, weather is critical. Temperature and humidity are the most influential
components because an extreme in either will adversely affect results. Other factors are the host pest, predation, pesticide use, quality of the parasitoid and the crop being targeted (Seymour et al., 1994; Smith, 1996). Other conditions which can adversely affect Trichogramma include dew, high light intensity, heavy rain and wind greater than 1.1 km/h (Smith, 1996). Rate of predation can increase if there is a delay in parasite emergence in the field. Another component affecting releases is parasitoid quality. By providing a food source such as honey or molasses to adult wasps, quality of the parasitoids may increase from two to ten times in efficacy above those parasitoids without a food source (Smith, 1996).

The dispersal pattern of Trichogramma is uncertain. Some studies have shown they do not travel far once released. Other studies have recaptured them 400 m downwind of a release site, while another study has found marked females 700 m from the release site (Kuske et al., 2003). Wasps gradually move downwind in a release site so it is recommended to place extra capsules along the windward boundary (Llewellyn, 2004).

Release of Trichogramma should coincide with moth activity and egg laying as inundative releases at the beginning of a pest infestation are the most effective for biological control (Hassan, 1993). Timing of releases can be hard to calculate so techniques to monitor the pest life cycle are important (Seymour et al., 1994; Smith, 1996). Light trapping or pheromone traps are the best predictive tool because they indicate when moths are present. It has been shown that Trichogramma releases prior to oviposition have achieved better results than releasing as egg laying commences (Smith, 1996).
There are general recommendations for release rates, but some variation is allowed due to pest pressure or plant physiology. Smith (1996) estimates that in arboreal situations, such as with fruit trees, several million wasps per hectare should be released. Llewellyn, a consultant with an Australian insectary raising *Trichogramma*, recommends 60,000-120,000 wasps per hectare in a similar situation (Llewellyn, 2002).

Smith (1996) discussed studies undertaken on timing and application rate models. The three predictors Smith mentions are that to reduce pest populations: more than 80% parasitism needs to occur on freshly laid host eggs; the rate of release needs to increase proportionally with parasitoid disappearance rate and the leaf surface area; and if the emergence of parasitoids is staggered then the release rate can be reduced by half. Smith further comments that while field trials testing parasitoid longevity have not been formally carried out, it is commonly accepted that releases at 5-7 day intervals should ensure a sustained parasitoid population.

The study by Kuske *et al* (2003) concluded that inundative releases of *Trichogramma* species were unlikely to have a major impact on *Trichogramma* species already in the area. For ecological reasons, releases of *Trichogramma* are usually done with a local species. This is because they are thought to be better adapted to the habitat, climate and host conditions in the area. It is advisable that before a release is commenced, a survey of the local pest/beneficial species population is undertaken. Natural levels of parasitism can be as high as 40-100% in some eco-systems (Smith, 1996). Inundative releases can thus be used to complement the species complex already present.
**Nematodes**

Nematodes are known to infect hundreds of different insects in a number of ways. While some nematodes show no effect on their hosts others can cause decreased fecundity, sterility, delayed development or a reduction in flight activity and longevity. Entomopathogenic nematodes (ENs) can rapidly kill their insect host, mainly due to the mutualistic bacteria they are associated with. Whereas the insect-parasitic nematodes are classed as classical biological control agents, ENs are used primarily as biological insecticides (Kaya *et al.*, 1993).

Entomopathogenic nematodes are considered to be a valuable biocontrol option as an alternative to conventional pesticide use (Bélair *et al.*, 1998). This interest in insect control has increased the commercial production and development of formulations over the past twenty years (Arthurs *et al.*, 2004). Acceptance of ENs has varied because efficacy results have not been consistent. They have potential as an integrated approach in control of soil insects and also in some field conditions (Grant and Villani, 2003). Although ENs may provide a complete solution to a pest problem, they probably work best as one of a number of biocontrol options within an IPM program (Bedding *et al.*, 1993).

The two main entomopathogenic nematode families are Steinernematidae and Heterorhabditidae. In nature, they are obligate pathogens (Kaya *et al.*, 1993) found in soil as insect parasites. The infective juveniles (IJs) have a symbiotic association with bacteria and have been shown to kill host pests in 24-48 h (Bélair *et al.*, 1998). The Steinernematids are symbiotically associated with the *Xenorhabdus* bacterium and the Heterorhabditids with the *Photorhabdus* bacterium (Simões and Rosa, 1996). The
relationship between ENs and bacteria is very specific. Each Steinernematid species has only one *Xenorhabdus* species associated with it, but a *Xenorhabdus* species may be associated with more than one Steinernematid species (Akhurst, 1993). Laboratory assays and field work have shown that ENs are able to invade and kill a number of pest species, including lepidoptera (Simões and Rosa, 1996).

Akhurst (1993) explained that it is the symbiotic relationship that the Steinernematids and Heterorhabditids have with the bacteria which make these ENs successful in biological control. The bacteria need the nematodes to survive the soil environment and the nematodes need the bacteria to provide and protect nutrients needed for reproduction. The nematodes penetrate the host and the bacteria then enter and release toxins which eventually kill the host pest. The IJs enter through their host’s mouth, anus or spiracles, with Heterorhabditid IJs also able to enter through the interskeletal membranes. The IJs release the symbiotic bacteria into the insect’s haemolymph after they penetrate to the haemocoel. This causes septicaemia which kills the host insect. The nematodes then reproduce in the insect’s body and within two weeks thousands of new IJs are formed, ready to infect other insects (Bedding *et al.*, 1983).

The ability to penetrate a host may be a vital key in the pathogenic process. Specific cues produced by a host aid in the infection process. If the insect is not seen as a potential host to the nematode then penetration may be limited (Simões and Rosa, 1996). Heterorhabditids seek prey in an active way by following trails of CO₂ and other cues given by host insects, whereas Steinernematids are ambush parasites, adopting the ‘sit and wait’ approach towards parasitism (Booth *et al.*, 2002).
As there are a number of ways a nematode can enter a host, success with this as a biocontrol hinges on the nematode being able to get through the insect’s natural defences and establishing itself in its body cavity. The ability of the nematode to invade and establish is dependent on the host’s defence mechanisms. For symbiotic bacteria to establish in the host, the antibacterial factors produced by an insect need to be destroyed and it is the nematode which does this. Once the nematode penetrates its host, it needs to establish itself. This is vital for the nematode’s development, maturation and reproduction (Simões and Rosa, 1996).

Although applications of the ENs of the Steinernematid and Heterorhabditid families have traditionally been used for biocontrol in soil dwelling pests, research is now showing they may also have a place in controlling above-ground pests (Arthurs et al, 2004). Past research has shown that ENs are able to find and infect insects in cryptic environments, such as beneath tree bark and in tunnels created by larvae. This is very important in management of those insects which are difficult to control with traditional insecticides (Fallon et al, 2004). Most of the insects which Simões and Rosa (1996) tested showed that a particular pest tended to have a higher susceptibility towards infection with a specific nematode. Since the host range of pests susceptible to infection by nematodes appears to be small this helps to ensure that non-target organisms are not affected by large-scale biocontrol releases (Simões and Rosa, 1996).
**Parameters of infectivity**

Parasitism of a host is influenced by the nematode species and there can be some variability between strains of the same species (Simões and Rosa, 1996) so it is important to select the most appropriate species for the target host (Gaugler, 1993). Factors affecting nematode performance in soil include light, temperature, moisture, texture and bulk density of soil. Nematodes need a humid environment to survive and moisture is probably the greatest factor in determining their survival in soil (Grant and Villani, 2003).

Temperature plays an important part in the infectivity and effectiveness of ENs. In laboratory studies, 25°C was the most effective temperature for parasitisation by entomopathogenic nematodes, while temperatures above 30°C prevented infection from occurring. In the field, temperatures which showed the best results were between 16.8–27.3°C, with lower temperatures resulting in a longer time for infection and mortality to occur. Higher temperatures were responsible for decreased infectivity percentage (Huaiwen *et al.*, 1993). Temperature also affects development, respiration, dispersal and survival of IJs, and nematode use is restricted both in temperate areas where soil temperatures can be too low, as well as tropical areas where ambient temperatures can be too high (Griffin, 1993).

Selecting the appropriate species for a pest control situation is crucial and the nematodes need to be applied correctly to be effective. Susceptibility of an insect to nematode parasitism can be subject to the developmental stage of the insect so this must be considered (Simões and Rosa, 1996). Other factors to consider include providing adequate moisture and correct temperature to achieve positive results. Time
of application, as well as formulation and distribution of the species, also needs consideration (Bedding et al, 1993).

One factor affecting the infectivity of ENs is their short-term persistence in ultraviolet light after application. In laboratory tests, infectivity has been shown to be greatly reduced with exposure to ultraviolet light. This may explain the reduction in efficacy often found in field applications in environments other than soil (Wilson and Gaugler, 2004).

Although a commonly recommended field rate of application is ~2.4 billion IJs/ha of soil surface area (Grant and Villani, 2003), Arthurs et al (2004) found that an increase in dosage application rates of IJs did not necessarily increase infectivity rates. Under ideal application conditions, low dosage IJ application can have very positive effects.

**Field investigations**

Early studies using nematodes out of their natural soil environment proved relatively unsuccessful. It has only been in recent years they have been considered again for cryptic situations. In some areas, such as larval tunnels or in leaf canopy, the nematodes have some protection (Bedding et al, 1993; Arthurs et al, 2004). Huaiwen et al (1993) state that ENs are an effective alternative to chemical insecticides in controlling lepidopteran borers, with *Steinernema carpocapsae* Weiser being the favoured entomopathogenic nematode to use as a biocontrol in above-ground pests (Arthurs et al, 2004). Arthurs et al (2004) found that among lepidopteran pests, those whose larvae bore into the bark and cambium were best controlled with ENs, probably due to the
protection of the tunnels. The tunnels may provide a moist environment with increased humidity and a reduction in UV light. For larvae which bore into bark, bark treatments using orchard sprayers were effective. Hand spraying the borer galleries was also effective, but very time consuming. Huaiwen et al (1993) experimented with nematode application techniques. Of four methods tried, the two which showed most favourable results were achieved with injection into borer tunnels and sponge blocking of borer holes. They found the injection method the best overall but because of the labour involved, it became quite costly. The sponge blocking was less labour intensive and favourable in areas with low water availability.

In studies conducted by Huaiwen et al (1993) in China against the wood boring lepidopteran pest *Holocerus insularis* Staudinger, the highest mortality rate was achieved with *S. carpocapsae* (100%) followed by *H. bacteriophora* Poiner (97.8%). This pest is similar to *M. melanostigma* in that it lays its eggs in scarred areas near borer tunnel entrances. Larvae feed under the bark until the third instar, then bore into the xylem to complete their two year life cycle.

If success using ENs to control wood boring pests is to be practical there are a number of management issues which need to be considered: the ecology of the target pest has to be matched with the IJs activity; applications need to be made at the best (i.e. most vulnerable) stage in the pest’s life cycle; application methods need to be appropriate; environmental factors need to be optimal; and nematodes need to be able to search and locate the pest quickly (Arthurs et al, 2004). Due to environmental conditions, control of above-ground pests using ENs should coincide with favourable conditions. Studies using foliage and bark surfaces have shown that an elevated
relative humidity (>90%) for a minimum 8-24 h after application increases infection rates (Arthurs et al, 2004).

Arthurs et al (2004) showed that efficacy in one of their experiments depended on the target pest’s habitat. The model they used found greater efficacy in borer holes followed by cryptic foliage, and then exposed foliage. Their experiments also showed higher efficacy in green house studies than in the field. There also seemed to be a significant correlation between temperature and humidity during and up to 8 h post-application and the rates of infection by nematodes.

In the study done in China, Huaiwen et al (1993) found that the cost of using nematodes for control of certain tree boring insects was cheaper than the cost of insecticides. But Bedding et al (1993) found that the most negative aspect against using ENs in controlling borers was cost of application. The environmental benefits of using entomopathogenic nematodes as a biological control are worth investigation and should be considered in the cost equation.
CHAPTER 3 MATERIALS & METHODS

3.1 The trial sites

The field investigations for this research were conducted at three orchard trial sites located in traditional prune growing areas on the southwest slopes and plains around Young, NSW (Figure 2). The randomised complete block trials were designed by Remy van de Ven, a NSW DPI biometrician, located at the Orange Agricultural Institute. In each of the three orchard sites there were four blocks (A, B, C & D). Two blocks were used for biological control releases and two were non-release blocks. There were approximately 200 trees in each block and therefore ~800 trees per trial site. The growers agreed to not use broad spectrum insecticides in the orchard nor attempt to control borers in the trial blocks during the trial period.

Figure 2. Location of trial sites
Orchard 1

Orchard 1 was located at Kingsvale, 20 km south of the Young township. This orchard had 20 ha planted to prunes. The variety planted was d’Agen, grafted onto Myrobalan H29C rootstock. The trees in Blocks A & B were 13 years old when the trial began, and the trees in Blocks C & D were 5 years old. These blocks were chosen as trial sites due to the uniformity in age of the trees within each block. Tree spacings in the blocks were 4 m (between trees) × 6 m (between rows). Blocks A & B had 220 trees each (10 rows × 22 trees); Block C had 206 trees (14 rows of various lengths); and Block D had 92 trees (11 rows of various lengths). Blocks A & B were biological control release blocks and Blocks C & D were non-release blocks. Figure 3 shows a map of the trial area with each square representing one tree. The trial blocks were managed in the same way as the rest of the orchard for pruning, harvesting, irrigation, chemical and fertiliser applications. Table 1 shows the spray program over the trial period.

Table 1. Orchard 1 spray program by season

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F - fungicide, H - herbicide, I - insecticide
Figure 3. Orchard 1 trial blocks. These blocks are within an orchard area and this map is not to scale. Not all trees in the orchard are represented. Randomisation of block treatments is shown in Figures 14 – 16.
**Orchard 2**

Orchard 2 was located at Wirrimah, NSW, 40 km northeast of the Young township. This orchard had 20 ha of prunes, comprising varieties d’Agen and the varietal clone 698. Block A had 5 year old d’Agen trees on Myrobalan H29C rootstock at the commencement of the trial. Block B had 5 year old 698s on Myrobalan H29C rootstock and Blocks C & D had 2 year old 698s, grafted onto Myrobalan H29C rootstock. The trees within each block were of uniform age and variety. Tree spacings were 5 m between trees and 6 m between rows.

Blocks A, C & D had 200 trees each (10 rows \( \times \) 20 trees); and Block B had 208 trees (16 rows \( \times \) 13 trees). Blocks A & B were non-release blocks and Blocks C & D were used as biological control release blocks. **Figure 4** shows a map of the trial block, with each square representing one tree.

The blocks were managed in the same manner as the rest of the orchard for pruning, irrigation, chemical and fertiliser application. However, there was a difference in harvest technique. In 2004 and 2005, Blocks C & D were hand-harvested due to their young age and in 2006 they were mechanically harvested for the first time. Blocks A & B were mechanically harvested throughout the trial. **Table 2** shows the spray schedule over the trial period.

After the 2005 season, the grower carried out limited non-chemical control on larvae that were found while pruning. This resulted in only one larva being killed in Block D, one of the release blocks. The tree where this occurred was noted and disregarded in subsequent statistical analysis.
Figure 4. Orchard 2 trial blocks. These blocks are within an orchard area and this map is not to scale. Not all trees in the orchard are represented. Randomisation of block treatments is shown in Figures 14 – 16.
### Table 2. Orchard 2 spray program by season

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F - fungicide  I - insecticide

**Orchard 3**

Orchard 3 was also located in Wirrimah, approximately 6 km from Orchard 2. This orchard was planted with 66 ha of d’Agen prunes. Tree spacings were 4 m between trees and 6 m between rows. All trees were 12 years old at the beginning of the trial and were on Myrobalan H29C rootstock. Blocks A & D had 200 trees each (10 rows × 20 trees); Block B had 192 trees (6 rows × 23 trees and 2 rows × 27 trees); and Block C had 209 trees (11 rows × 19 trees). Blocks B & D were biocontrol release blocks and Blocks A & C were non-release blocks. Figure 5 shows a map of the trial block, with each square representing one tree.

The trial blocks were managed in the same way as the rest of the orchard for pruning, harvesting, irrigation, chemical and fertiliser applications. Table 3 shows the spray program for only one season of the trial period.
There were some management issues which impacted the investigations on this orchard, which was for sale when the trial began. The owner was agreeable to having the orchard used in the trial and said she would indicate this to any potential buyer.

Due to severe drought in the district since 2001, the availability of quality water was limited. Available bore water was saline (3.2 dS m\(^{-1}\)). Because of the quality of the water, and the costs associated with pumping from the bores, the owner ceased irrigating the orchard in November 2004. No sprays of any kind were applied to the orchard from mid-2004 to August 2005. Only larger fruit were harvested that year and smaller fruit were left on trees. The owner then leased the property for the following season (2005/06). The new manager agreed to the terms of the trial so research continued at that site, but again due to water and other management issues, this site was effectively abandoned as a commercial orchard in April 2006.

**Table 3** Orchard 3 spray program by season

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F - fungicide  H - herbicide

* - There are no records for 2003/04 and 2004/05 seasons because of management changes.
Figure 5. Orchard 3 trial blocks. These blocks are within an orchard area and this map is not to scale. Not all trees in the orchard are represented. Randomisation of block treatments is shown in Figures 14 – 16.

Logging of temperature & humidity data

Gemini Tinytag Ultra data loggers (www.geminidataloggers.com, distributed in Australia by Hastings Data Loggers, Port Macquarie NSW 2444) were placed in one trial block at each site. At the beginning of the trial they measured temperature (°C) and relative humidity at 15 minute increments (September 2003 – May 2004). From the end of May 2004 until the end of the trial period (February 2006) they measured temperature and relative humidity in one hour increments. Data were downloaded
from the loggers into the Gemini computer package (GLM Version 2) at three monthly intervals throughout the trial period. The daily maximum and minimum for temperature and relative humidity for the duration of the trial were imported into Microsoft Office Excel (2003) spreadsheets and the data graphed using the Excel program (Appendices 3 & 4).
3.2 Monitoring for Maroga melanostigma moths and borer damage in trees

Moths

From information obtained through previous trial work in Moree, NSW, it was shown that *M. melanostigma* followed a typical lepidopteran life cycle, completed in one year, with moth emergence from October to mid-December (Seymour and Crouch, 1993). However, Hely *et al* (1982) stated that the life cycle took at least two years to complete (location not stated), so a more precise understanding of the *M. melanostigma* life cycle in Young was required so that any potential biological control releases could be timed appropriately.

In the first investigative season (2003/04) borer-infested limbs of cherry and prune trees were placed under three 1 m³ field cages (*Figure 6*) constructed of green shade cloth (50% shade) attached to a wooden baton frame. The base/bottom was left open to fit over a pile of limbs. The limbs were placed on the ground, on top of black plastic porous weed mat. Cages were placed at the trial site in Kingsvale and one of the sites in Wirrimah, as well as a site in Monteagle (*Figure 2*). These three locations were used because they were geographically different and would therefore be able to help predict timings of likely moth emergence in these prune growing areas.
When the first emergence of moths was observed from the wood in the cages, light trapping at night began. This nocturnal moth had been observed to readily come to light traps in the Moree pecan orchards (Seymour and Crouch, 1993). Using this information, two light traps were set up at the Kingsvale site. The traps were constructed from galvanised sheet iron, mounted on a solid base with a protective cap over the electrical connections and collecting funnel. The overall trap height was 500 mm. The funnel directed insects into the collecting box (Figure 7). Different light globes were used in each trap. One globe was a Philips 160 watt blended light lamp and the other was an “Eye” 100 watt blended mercury lamp (Iwasaki Electrical Co. Ltd.). The traps were placed in the same orchard and were approximately 300 m apart. Light from one trap was not visible at the other trap site. Each trap was run by a petrol-fuelled generator. The traps were set up on reflective grey plastic tarpaulins (3 m × 4 m), at dusk (between 2030 and 2100 h Eastern Australia Daylight Savings Time). In the first season (2003/04) light trapping occurred from early January through to late February. Traps were set up two to three times per week, depending on weather conditions. In 2004/05, trapping occurred from early December through to mid-February. Trapping was again weather dependent. The generators connected to the light traps ran for approximately 8.5 hours overnight. Traps were checked before dawn the following morning for presence of moths and other insects. The tarpaulin and adjoining orchard area were also scouted for moths.

At the end of the first season the difficulties in having cages for monitoring moth emergence 60 km apart became apparent. It was impractical to check these traps on a daily basis. To overcome this, ten orchard-grown prune trees (2 year old 698s) were infested with early instar *M. melanostigma* larvae (Jones, 1978). These trees were
then dug out of the field and placed in round plastic pots (400 mm × 400 mm) with soil from the field. The potted trees were then transferred to an enclosed shade (50% shade) house (dimensions 2.4 m × 3.7 m) (Adloheat, Pakenham, Victoria 3810 Australia) in the researcher’s back yard in the Young township and watered by hand twice-weekly. The shade house was monitored daily for any moth emergence. If the *M. melanostigma* life cycle was at least two years in Young (Lloyd, 1972), that meant there would be no moth emergence the next season from the infested trees in the shade house. To compensate for this, borer-infested limbs cut from local prune and cherry trees were also added to the shade house. As well as assessing moth emergence, the shade structures were also used to collect moth specimens. The intention was to collect moths, monitor oviposition and use the eggs to test potential egg parasitoids.

Small borer-infested limbs from cherry and prune trees were kept in a clear plastic container (380 mm × 250 mm × 150 mm) covered with fine muslin gauze, in the researcher’s office. Temperature in the office ranged from 16° – 23°C. It was envisaged that emerged moths would be used for further study into the life cycle of this pest and any eggs laid by the moths could be used for preference testing with commercially available *Trichogramma* species.

Since there was difficulty in capturing sufficient moths to monitor their oviposition, in the final season of the project (2005/06) enclosures were put over borer damage on trees *in situ*. Chicken wire (20 mm × 25 mm mesh) was loosely fitted over the damage site, to give shape to the enclosure, and then green 50% shade cloth was fitted, and stapled, over the top of the structure. The top and bottom of the enclosure
was cinched with thin wire. The shape of the enclosure ensured adequate space for movement of any moths that emerged. Emerged moths would be monitored for oviposition and any eggs produced used for parasitism studies.

Emerged moths were collected and kept in a glass container (200 mm × 200 mm × 250 mm) in the researcher’s office, and held at ambient temperature. Thin twigs were added to the container for moth oviposition. Water was provided in a small plastic lid (30 mm × 10 mm) (R. Llewellyn, Pers. comm.). The container top was covered with fine muslin to prevent the moths from escaping.

**Damage**

Borer damage is very noticeable in trees. There is a characteristic frass which is left behind by the insect as it feeds (Jones, 1978). Damage can be located anywhere in the tree (Figure 8).

While laboratory studies to determine biological control efficacy can indicate whether a control works against a pest, judging its effectiveness in the field is more difficult. Although levels of parasitism can be monitored, the effect that a control strategy is having on damage levels is more difficult to ascertain. Few studies actually focus on the effects that biological control is having on pest damage levels in the field.

For this project, gauging damage levels before and after biocontrol releases was important. If the control strategy was unable to reduce damage levels, the adoption of this research by industry would be marginal.
Figure 6. Field cage for gauging moth emergence

Figure 7. Light trapping at night
Figure 8. Typical frass damage on a prune tree

Figure 9. Locations of damage assessment in tree
In order to compare damage levels between years, baseline data on damage already present in the field was collected before biological control releases began. This was done by examining every tree within the trial blocks at all three trial sites. Damage assessments were again carried out after the first and second year of *Trichogramma* releases. These assessments took place each winter in July, after the trees had defoliated. Assessments recorded the presence and position of borer damage (crotch, limb or trunk – **Figure 9**). Multiple borer sites within a tree were also recorded. The extent, or area, of damage at each site was not assessed.

At each damage site the activity of the frass was also recorded as either old (inactive) or new (active). The feeding habit of *M. melanostigma* larvae allows the frass to indicate recent pest activity (Jones, 1978). Active frass is normally soft or spongy and is a reddish brown colour. Inactive frass tends to be hard or crumbly, and is a sandy grey colour. Early instar larvae feed on the bark of the tree and the frass tends to be finer than frass left behind by later instar larvae which have started tunnelling into the tree (Lloyd, 1972). Once the larvae enter into the tree, they remain cryptic during the day and come out to feed during the night. It is this habit which makes it easy to determine whether there are active larvae under the frass. Although the trees were scouted for moths each night while light trapping, no larvae were ever observed during these times.

In the first year (2004) of collecting damage data, the recording of old versus new damage was inconsistent even though there was a standard assessment technique. The inconsistency occurred because there were two observers assessing damage. In
the second and third years, only one observer was used and therefore all damage sites were marked consistently.

Also in the first year of damage assessment, the quadrant (N, S, E or W) in which the damage occurred in the tree was recorded. This was not continued in the second and third seasons because the data from the first year indicated there was no directional correlation with damage (Appendix 1). The results from each year’s assessment were entered into Microsoft Excel spreadsheets and marked on all the trial block maps (Figures 14 – 16).

Analysis of data
The hypothesis tested in this trial was that the introduction of a biological control, in this case Trichogramma, within a block would reduce the incidence of borer damage, as indicated by presence of frass. Statistical analysis considered the proportion of trees within each trial block with frass (i.e. borer damage) and the proportion of trees within each block with new frass (i.e. active larvae). In each case the proportion, or the log$_e$ proportion for new frass, was analysed using the same linear mixed model. This model had, as fixed effects, a regression on time (Year) which was allowed to differ across the two treatments of release (Wasp) and non-release (Control), but with the constraint that in year 2004 the mean response was identical for the two treatments. The random effects in the model were effects for Orchard; Orchard × Year; Treatment × Orchard; Treatment × Orchard × Year; Orchard × Block; Orchard × Block × Year; and deviations from linearity across years (overall, across Orchards and across combinations of Treatment × Orchard). Also included was a random error term. This model was fitted using ASREML (Gilmour et al, 2002). To test for a
significant difference in mean regression over time for the two treatments, a scaled Wald test statistic was used. The level of significance of this test statistic was based on an F-test approximate with denominator degrees of freedom determined using a Kenward adjustment for small sample inference (Kenward and Roger, 1997). This same analysis was used for testing the significance of proportion of trees with ‘new’ frass sites.

The same linear mixed model analysis was performed to determine mean number of damage sites per tree to ascertain if there was a difference between treatments. The mean number of new (active) frass sites per tree was also considered. The data for number of new frass sites per tree were log$_e$ transformed prior to analysis.
3.3 Biological controls

*Trichogramma*

*Trichogramma* spp. were considered to be the most feasible biological control agents for investigation in this project, based on previous research (Seymour and Crouch, 1993). *Trichogramma* are known parasitoids of lepidopteran eggs (Smith, 1996) and are commercially available in many countries including Australia. The species of *Trichogramma* for these investigations was obtained from the commercial insectary Bugs for Bugs Pty Ltd (Munduberra, Queensland, 4626). The *Trichogramma* were reared on eggs of *Sitotroga cerealella* and the parasitised eggs were enclosed in cardboard capsules, which had small exit holes to allow for *Trichogramma* emergence. These capsules also provided some protection from predation for the *Trichogramma* prior to their emergence. Of the two species commercially available, namely *T. carverae* and *T. pretiosum*, the former species was chosen because of its reputation as the more arboreal of the two species (R. Llewellyn, Pers. comm.). This is consistent with the finding by Smith (1996) that *Trichogramma* spp. are more habitat–specific than host–specific.

The recommended release rate of *Trichogramma* in tree crops is between 60,000 – 120,000 wasps per hectare (Llewellyn, 2004). The higher release rate of 120,000 wasps per hectare was chosen for two reasons: firstly, because there was a lot of damage present and larger numbers of wasps were needed for greater dispersal within the area; and secondly, because the primary objective of this investigation was
to determine whether *Trichogramma* at any release rate were able to provide any control of the pest. If *Trichogramma* were shown to be effective then the optimal release rate could be assessed in further research.

*Trichogramma* were sent overnight from Bugs for Bugs by Express Post. Along with the sheets of capsules, most shipments also included a vial of parasitised eggs (Figure 10). This vial was used as an indicator for wasp emergence, since they emerged 24 h before the rest of the shipment. This information was important for the timing of releases. A couple of the capsules from each shipment were kept in a Glad® Snap Lock™ Resealable mini plastic bag (150 mm × 90 mm), at ambient temperatures in the researcher’s office, to check that there was a reasonable level of *Trichogramma* emergence.

![Figure 10. Trichogramma capsules and indicator vial](image)

The sheets and vial were kept at ambient temperature in the researcher’s office until the wasps in the vial started to emerge. At that point, field releases began. There were two releases in the 2004/05 season and six in the 2005/06 season (Figures 11-
13). These were timed to coincide with moth emergence in the district (Table 4a & 4b).

Releases were made by stapling the cardboard capsules onto leaves in the tree canopy, approximately 1.6 m above ground level. Because of the high temperatures experienced during some of the releases, the capsules were positioned in the trees so as to provide some protection from direct sunlight.

The number of trees per hectare was calculated to ensure the recommended release rate of 120,000 wasps/ha. It was recommended by the suppliers that releases be spread out across the orchard area to encourage dispersal of the parasitoids throughout the block. In Orchard 1, in both release blocks A & B, 63 capsules were used at each release date in both years. In Orchard 2, 80 capsules were used in each release block C & D at each release date in both years. In Orchard 3, 55 capsules were used in release block B and 57 capsules were used in release block D, at each release date in both years. The only exception to this was in one consignment during the second release season. The level of parasitism from the material in the capsules was estimated by the supplier to be about half of what was guaranteed and to compensate for this, the company sent double the number of capsules. In that week, two capsules were put in every designated release tree.

There were 417 trees/ha planted in Orchards 1 and 3. To release 120,000 wasps/ha, capsules were required on every 3.5 trees. To ensure this release rate, capsules were stapled in every third, then every fourth, tree. This pattern was continued throughout the block. There were 333 trees/ha planted in Orchard 2, so capsules were required
on every 2.8 trees. In these blocks, capsules were placed every second, then every third tree and this pattern continued throughout the block. In the first release season, the first capsule in the first release was placed in the first tree in every block and the numbering carried on from there to obtain the recommended release rate. The second release started in the second tree in every block, and continued from there. In the second release season, this same practice was continued. The only difference was that there were more releases made so the first capsule in each release moved on accordingly within the block (i.e. the third release commenced in the third tree). After the third release, the first capsule in each block restarted at the first tree to maintain the optimal dispersal pattern. The exception was Orchard 2 where the third release moved back to the first tree due to the release pattern of every second then third tree.

**Entomopathogenic nematodes**

Considering *M. melanostigma* spends the majority of its life cycle in larval stages, an investigation into a biological control for this stage was undertaken. Previous research has shown entomopathogenic nematodes (ENs) to be effective in infecting lepidopteran larvae in the field, thereby terminating the completion of their life cycle (Huaiwen et al, 1993).

In order to test the effectiveness of ENs, as many *M. melanostigma* larvae as possible were collected from the field in the autumn (March) of 2005. Young trees in the district were scouted for early instar larvae, as they tend to feed on the outside of the tree and are easier to collect than later instar larvae which have tunneled into the tree (Lloyd, 1972). Trees in trial blocks were excluded from this search. When larvae
were found, they were carefully removed, so as not to damage them, and were placed in plastic vials (100 mm × 25 mm). When a number of larvae were collected, they were transferred to a plastic container (170 mm × 120 mm × 40 mm) filled with moist paper towel, small prune tree twigs (1-2 mm diameter) and recycled Oregon pine sawdust. The towel was kept moist with tap water. The container was covered with fine muslin which was held in place with the lid of the container, the majority of which had been removed. At the end of the collection period, only two of the original ten larvae collected had survived. The two larvae were taken to Ecogrow Australia Pty Ltd in Canberra, ACT.

Ecogrow is an Australian company specialising in production and supply of ENs (www.ecogrow.com.au). After consulting with Craig Wilson, Technical Advisor/Facility Manager, it was determined there were two possible ENs to use with *M. melanostigma*: *Steinernema carpocapsae* and *S. feltiae*. Both nematodes infect through orifices in the body cavity. Since the limited number of larvae meant it was impractical to test both nematodes *S. carpocapsae* was selected because it was the more ‘robust’ of the two species (C. Wilson, Pers. comm.).

The ENs are harvested from a slurry, dehydrated, mixed with cellulose (50/50) and stored as a dry product. They are shipped in this dry formulation and reconstituted before use. Before they are shipped, Ecogrow completes a ‘Harvesting & Formulating Record’ to assess the average number of nematodes per gram. This information is passed on to the recipient so the target number of nematodes for application can be met. The Standard Operating Procedure (SOP) which Ecogrow uses for infectivity testing is Commercial in Confidence and therefore is not reported
here. Permission to report on the procedure for counting nematodes, though, was
given (Appendix 2) so that the steps to reconstitute and count \textit{S. carpocapsae} could
be outlined.

After confirming under magnification (20\times - 25\times) that the nematodes were in an
acceptable concentration (100/mL\pm 5\%) the larvae were prepared for infection. Each
larva was placed in the bottom of a 30 mL plastic container. These were then lightly
filled with sand having a 7\% m/w content (7 mL water:100 g sand). An indentation
was made in the centre on top of the sand to hold 1 mL of the nematode solution.
After the nematodes were added, the containers were covered and left in a 23\(^{\circ}\)C
controlled temperature room. One larva was assessed after seven days and the other
after 14 days.
3.4 Monitoring Trichogramma releases

Eggs of *Helicoverpa armigera* were used to assess the presence of any egg predators and parasites in the trial orchards, as well as to gauge movement and activity of the *Trichogramma carverae* after inundative releases.

In the first season of the trial (2003/04) there was one *Helicoverpa* egg application made on 21 January 2004 to begin the process of assessing the presence of lepidopterous egg predators and parasitoids. The *Helicoverpa* were obtained from Australian Produced Biologicals Pty Ltd in Richmond NSW, 2753. The eggs, having been laid the day before and then shipped by overnight courier, arrived on one piece of cotton muslin. The muslin sheet was then cut into 80 squares (approximately 40 mm × 30 mm), with each square containing ~50 eggs. These squares were randomly distributed throughout two of the trial sites and at the site in Monteagle. There were 28 squares at Monteagle, 35 squares at Kingsvale (Orchard 1) and 17 squares at Wirrimah (Orchard 2). They were pinned into trees, left for 24 h, removed and placed into Glad® Snap Lock™ Resealable mini plastic bags (150 mm × 90 mm) and held inside the researcher’s office at ambient temperature. The eggs were checked daily for signs of parasitism.

In the first season of *Trichogramma* releases (2004/05) two *Helicoverpa* egg applications were made. The first application was on 7 December 2004. The second application was on 11 January 2005, between that season’s two *Trichogramma*
releases (Figures 11a - 13a). Eggs were again obtained from Australian Produced Biologicals. The eggs arrived laid on one piece of cotton muslin. For the application in December the muslin sheet was cut into 136 squares with each square containing ~30 eggs. The squares were randomly distributed throughout the blocks at the three trial sites. There were 41 squares used at Orchard 1; 51 at Orchard 2; and 44 at Orchard 3. The squares were pinned on the trees (limb or trunk), left for 24 h, collected and placed in Mini plastic bags. The bags were held inside the researcher’s office at ambient temperature and checked daily for parasitism. For the application in January, the muslin was cut into 75 squares, with each square containing ~50 eggs. Twenty-five squares were pinned into trees at each of the trial sites. Five were pinned in consecutive trees in each of the trial blocks, with a further five pinned in a block of five random trees near the trial blocks (see legend in Figures 14 – 16). Again, the squares were removed after 24 h, placed in Mini plastic bags, held inside at ambient temperature and assessed daily for parasitism.

In the second Trichogramma release season (2005/06), Helicoverpa eggs were obtained from the Queensland Department of Primary Industries in Toowoomba. These eggs, having been laid the day before and then shipped by overnight courier, came laid on brown paper towel. As in the last application of the previous season, the paper towel was cut into 75 squares with each square containing ~50 eggs. These squares were placed in the same trees as the last application of the previous season. After 24 h the squares were removed from the trees, placed in Mini plastic bags and held inside at ambient temperature and assessed daily for parasitism. This procedure was followed for the three Helicoverpa applications during that season. These releases occurred on 15 December 2005; 23 December 2005; and 6 January 2006 (Figures 11b - 13b).
CHAPTER 4 RESULTS

4.1 The trial sites

The data loggers in each of the sites were downloaded at three-monthly intervals throughout the three years of the trial. Daily maximum and minimum temperatures for the trial period (September 2003 to February 2006) are given in Appendix 3. The maximum and minimum temperatures for December and January for each site over the *Trichogramma* release years are shown in Figures 11 – 13. The release dates of *Trichogramma* and application dates of *Helicoverpa* egg cards are indicated on these graphs. In the first release season (2004/05) there were two *Trichogramma* releases in each treatment block and two assessments of parasitism, using *Helicoverpa* eggs. In the second release season (2005/06) there were six *Trichogramma* releases and three *Helicoverpa* applications. While relative humidity (RH %) was also downloaded, these graphs are not included in this results section but are presented in Appendix 4.
Figure 11a. Orchard 1 temperature, Trichogramma release and Helicoverpa egg application data for 2004/05

Figure 11b. Orchard 1 temperature, Trichogramma release and Helicoverpa egg application data for 2005/06

Figure 12a. Orchard 2 temperature, Trichogramma release and Helicoverpa egg application data for 2004/05
Figure 12b. Orchard 2 temperature, Trichogramma release and Helicoverpa egg application data for 2005/06

Figure 13a. Orchard 3 temperature, Trichogramma release and Helicoverpa egg application data for 2004/05

Figure 13b. Orchard 3 temperature, Trichogramma release and Helicoverpa egg application data for 2005/06
4.2 *Maroga melanostigma* moths & damage

In the first season (2003/04) of the trial, moths began emerging in the field cages in early December (**Table 4a**). They stopped emerging in early February. There was always a number of earwigs (O. Dermaptera) in the enclosures, in particular located amongst the frass. Considering that earwigs can be both pest and predator (Nicholas, 1996), they may have been responsible for reducing larvae numbers in the limbs. In the second season (2004/05) moths began emerging in the shade house in late November, with the last moth emerging mid-January (**Table 4b**). Both these seasons indicated that moth emergence occurred over approximately 55 days, during the months of December and January, with the peak emergence during late December/early January. This information was used to time the *Trichogramma* releases. In the third season (2005/06), no moths emerged in the shade house. *In situ* traps were used to cover borer frass on individual prune trees in two orchards (one trial orchard but not in trial blocks; and one orchard on the edge of town, within easy monitoring distance). No moths were ever observed in these traps, although earwig activity was noted.

**Table 4. Number and timing of M. melanostigma moth emergence**

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<th>b. Emerged moths for the 2004/05 season</th>
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<td>Date</td>
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Once moths began emerging in the field cages, light trapping began at night using two traps at different locations within the same orchard. In the first season (2003/04) light trapping was undertaken on five occasions between 12 Jan 04 –16 Feb 04. The researcher being overseas prevented light trapping from occurring any earlier in that season. In the 2004/05 season, light trapping occurred over 12 nights between 22 Nov 04 – 11 Jan 05. Issues which hindered light trapping included inclement weather (e.g. rain, lightning, etc) and cold nights (lack of moth activity). No light trapping was attempted during the 2005/06 season.

Over the two seasons, light trapping occurred on 17 occasions. The collection box of each of the two light traps was examined after each night. There were very few insects observed in the traps. Most of the insects were beetles (O. Coleoptera). Only one *M. melanostigma* moth was recovered in that time. The night when the moth was recovered, the temperature remained above 22°C. It was also the only night when the traps were full of small flying insects (unidentified). The moth was in the collection box and appeared partially consumed by the time it was recovered at dawn.

Moths that were collected from the field cages, and those emerging from borer infested wood kept in containers in the researcher’s office, were introduced into a separate container to encourage oviposition (R. Llewellyn, Pers. comm.). There were no more than two moths in the container at the same time. The moths were not sexed upon collection and no eggs were obtained using this method. Therefore, it was not possible to subject *M. melanostigma* eggs to egg parasitoids in a controlled experiment during this trial.
4.3 Damage assessments & analysis

The damage assessments of all trees in the trial blocks were recorded on data sheets and marked on the Excel spreadsheet block maps (Figures 3 – 5). In the base year (2004), if there was damage anywhere in the tree, an ‘a’ was entered in the square which represented that tree (Figures 14a – 16a). These maps became the base year data. Damage assessments were again made after the first year of Trichogramma releases (2005) and if there was damage present it was marked as ‘b’ (Figures 14b – 16b). If damage was present in both years (2004 & 2005), the square was marked ‘ab’. Damage assessments were again carried out after the second year of Trichogramma releases (2006). If damage was present, then it was marked on the maps as ‘c’ (Figures 14c – 16c). If damage was present in all three years, the square was marked ‘abc’. If there was only damage in 2005 and 2006, the map square was marked ‘bc’. If there was damage in 2004 and 2006, it was marked as ‘ac’.

This information was then statistically analysed using a linear mixed model regression analysis. Analysis took place between the treatments, across years. Analysis took into account the presence of frass (i.e. damage) and whether there was an increase, decrease or no change in number of damage sites on trees, and within and between treatment blocks. The change in active (new) frass sites was also analysed.
Figure 14a. Orchard 1 borer damage - 2004
<table>
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<tr>
<th>Tree</th>
<th>a = damage in base year</th>
<th>b = damage in 1st year</th>
<th>B = buffer tree</th>
<th>ab = damage in base &amp; 1st year</th>
<th>D = dead tree</th>
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**Helicoverpa application**

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**Figure 14b. Orchard 1 borer damage – 2004 & 2005**
Figure 14c. Orchard 1 borer damage – 2004, 2005 & 2006
Figure 15a. Orchard 2 borer damage - 2004
Helicoverpa application

- = damage in base year
b = damage in 1st year
ab = damage in base and 1st year

Figure 15b. Orchard 2 borer damage – 2004 & 2005
Figure 15c. Orchard 2 borer damage – 2004, 2005 & 2006
Figure 16a. Orchard 3 borer damage - 2004
Figure 16b. Overhead 3 borer damage – 2004 & 2005

- N - North
- R - Row
- B - Block
- V - Vacant
- D - Dead
- A - Aged
- T - Tree

Legend:
- Tree
- Buffer tree
- Damage in base year
- Damage in 1st year
- Damage in base & 1st year
- Release blocks
- Helicoverpa application
Figure 16c. Orchard 3 borer damage – 2004, 2005 & 2006
The information from the data sheets was used to graph damage levels as a percentage of total trees between the years of the trial (Figure 17). These percentages are based on number of trees in each block which had at least one damage site. The graph shows that between 2004 and 2005, there was an increase in borer damage in all blocks at all sites, except one (Orchard 1; Block B). Damage levels remained constant in this block. Between 2005 and 2006, there was again an increase in borer damage in all blocks except two (Orchard 2; Blocks B & C). Damage levels decreased in those blocks.

Figure 17. Differences in damage levels (% of total trees) between years

It should be noted that in the 2006 data for Orchard 3, 11% of the trees in Block A had died. The tree deaths were associated with wind damage and overall poor tree health, which was attributed to extreme weather conditions. While borer damage was unlikely the cause of these deaths, it most likely exacerbated the already marginal conditions in that orchard. In all the other trial blocks, tree death ranged from 0 – 5%. The graph in Figure 17 does not take into account dead trees and is based on the total number of trees in the original trial design.
The graphs in Figure 18 present the proportion of trees in each block with damage (frass) over the trial years, as well as the proportion of those trees assessed as having active (new) frass, indicating current borer activity. Not all frass sites were identified as either ‘new’ or ‘old’ in the 2004 assessment, hence the missing data in those graphs. The proportion of trees in each block with frass increased, or remained constant, in all blocks except two (Orchard 2; Blocks B & C) where there was a slight decrease. The proportion of trees with new frass increased in most blocks but decreased in the non-release (control) blocks of Orchard 2 (Blocks A & B), and one of the release (wasp) blocks (Block A) in Orchard 1. The linear mixed model (LMM) analysis of the data showed there was no significant difference ($F_{1,8.4} = 0.16, p = 0.696$) (Table 5a) between treatments (Crt & Wasp) for proportion of trees with frass and no significant difference ($F_{1,7.5} = 0.14, p = 0.723$) (Table 5b) between treatments for proportion of trees with new frass.

**Table 5a. LMM analysis of variance for proportion of trees with frass**

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<th>Denominator DF</th>
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<th>Probability</th>
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<tr>
<td>Mean ($\mu$)</td>
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<td>25.65</td>
<td>0.037</td>
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<tr>
<td>Treatment.Year</td>
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<td>8.4</td>
<td>0.16</td>
<td>0.696</td>
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**Table 5b. LMM analysis of variance for proportion of trees with new frass**

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<tr>
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<th>Numerator DF</th>
<th>Denominator DF</th>
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<td>Mean ($\mu$)</td>
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<td>4.7</td>
<td>25.26</td>
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<tr>
<td>Year</td>
<td>1</td>
<td>3.7</td>
<td>2.71</td>
<td>0.175</td>
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<tr>
<td>Treatment.Year</td>
<td>1</td>
<td>7.5</td>
<td>0.14</td>
<td>0.723</td>
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The graphs in Figure 19 show that again there was no statistically significant treatment effect identified for either the mean number of frass sites per tree within each block, or mean number of new frass sites per tree within each block. While not statistically significant the analysis did show that the non-release blocks (control) had a slower increase of borer damage than the release blocks (wasp) for mean number of frass sites per tree within each block ($F_{1,8.4} = 0.08$, $p = 0.78$), but not for mean number of new frass sites per tree within each block ($F_{1,7.3} = 0.05$, $p = 0.83$).
Based on the linear mixed model fitted to the data, the estimated mean proportion of trees with frass for each treatment for the three years ($2004 - 2006$) is given in **Figure 20**. Here the means are across all orchards and blocks. While there was an increase in the mean proportion of trees with frass for both treatments, the increase was less in the release (wasp) trees. This difference was not statistically significant.
Figure 20. Mean proportion of total trees with borer damage

The mean proportion of trees with new frass sites over the trial years was also examined (Figure 21). As with the previous analysis, there was a smaller increase in borer damage, assessed by new frass, in the release (wasp) trees than the non-release (control) trees but this difference was not significant. The level of new frass sites is an important parameter in determining if there are longer term, or cumulative, effects of *Trichogramma* releases.

Figure 21. Mean proportion of total trees with active (new) frass sites
4.4 *Trichogramma* and parasitism

From the monitoring investigations using *Helicoverpa* eggs to generate baseline data in the first season (2003/04), there were no parasitised eggs recovered from the field. Low numbers of green lacewing (Neuroptera: Chrysopidae) larvae were however present at two orchard sites, indicating some beneficial organism activity in at least those trial orchards.

In the second season (2004/05), there were two *Helicoverpa* egg applications made. The one in early December did not yield any parasitised eggs. In the second application, between the two *Trichogramma* releases that season, parasitised eggs (~70) were recovered from two of the trial sites. The emerged insects were identified as *T. carverae*. Morphological identification was done by Peter Gillespie at the Agricultural Scientific Collections Unit, NSW Department of Primary Industries, Orange NSW. Of the 75 egg cards applied to the trees, 20 were returned with no eggs at all, almost certainly due to predation, and the other cards had varying levels of predation from 30% - 95%.

In the third season (2005/06), there were three *Helicoverpa* egg applications, all occurring between *Trichogramma* releases. From the first application, parasitised eggs (~180) were recovered from two of the trial orchards. Of the 75 egg cards applied to the trees, 19 were returned with no eggs at all and the other cards had varying levels of predation. The emerged insects from parasitised eggs from Orchard 1 were all identified as *T. carverae*, while those from parasitised eggs
recovered from Orchard 2 were identified as *T. carverae, T. pretiosum* (the other commercially available *Trichogramma* species) and *T. nr brassicae* (the species used with success in Moree, NSW (Seymour and Crouch, 1993)). The two non-released species of *Trichogramma* were recovered from the random *Helicoverpa* egg application block in the trial orchard (see legend Figure 15a). After the second *Helicoverpa* egg application, only one parasitised egg was recovered in Orchard 2. Emerged insects were identified as *T. carverae*. Of the 75 egg cards used in this application, 34 returned with no eggs, with other cards showing varying levels of predation. No parasitised eggs were recovered from the third application.
4.5 Entomopathogenic nematodes

The two *M. melanostigma* larvae which were exposed to *S. carpocapsae*, were left at Ecogrow in a controlled temperature (23°C) room for two weeks. After 7 days, one larva was checked for signs of nematode infection. The larva pulled apart (i.e. “snotting”), indicating it had been infected, but only one infective juvenile (IJ) was found in the body cavity. The second larva was left for a further week, to examine whether signs of infection would increase. However when the larva was examined there was no indication of IJs, either living or dead. These results were therefore inconclusive and did not confirm nematode efficacy.
CHAPTER 5 DISCUSSION

5.1 Damage assessments

Overall, the damage levels found in this investigation (Figure 17) were consistent with those that Jones (1978) found in his survey of prune orchards in 1973 and 1974. He reported 58% of prune trees in the Young district had *M. melanostigma* damage. He also noted that drought appeared to exacerbate the problem.

While most studies assess efficacy of an egg parasitoid by measuring egg parasitism, this investigation aimed to measure efficacy of the released *Trichogramma* by assessing pest damage levels in the field. The relationship between egg parasitism, pest larval populations and damage exists yet it is rarely examined. In a study using field corn, it was reported that comparing larval damage on treated versus untreated plots underestimated *Trichogramma* efficacy (Smith, 1996). Knutson (1998) noted that when evaluating the effectiveness of *Trichogramma* releases it is important to assess the density of target pest larvae already present in the crop. He stated that an increase in egg parasitism may not be synonymous with a reduction in damage by larvae. However, when Smith (1996) considered larval reduction and egg parasitism together, there appeared to be a positive relationship in that an increase in parasitism generally resulted in a reduction in larvae over time.
Between the base assessment year (2004) and the first year after *Trichogramma* releases (2005), incidence of borer damage increased in each of the trial blocks, except one release block (Orchard 1, Block B) where damage levels remained the same (Figure 17). It was interesting that one of the release blocks did not record an increase in damage levels. One possibility for this could have been that the *Trichogramma* may have been having some positive impact. The overall increase in damage throughout the blocks could have been in part due to the increasing drought conditions in the district, which would have been impacting on the health and vigour of the trees.

The damage levels between the 2005 and 2006 assessments showed no significant differences between treatments. There was an increase in recorded borer damage in all trial blocks except two. In those two blocks in Orchard 2, borer damage actually decreased. This would have been very encouraging had both blocks been release blocks, but one was a release and one a non-release block.

There are several possible explanations for the level of borer damage decreasing in Orchard 2. The decrease in the release block (Block C) supported the view that the *Trichogramma* contributed to suppression of new borer damage. This block was located in close proximity (within 50 m) to the non-release block (Block B) which also showed a reduction in borer damage. It could be argued that perhaps due to *Trichogramma* searching capabilities and ability to disperse up to 700 m from a release site (Kuske *et al.*, 2003), the parasitoids in the release block had an effect in the non-release block. This was not the trend, though, for the other release block (Block D), which was in closer proximity to Block C, and which had an increase in
recorded borer damage. The prevailing westerly winds may also have been a factor in this result. There is a general recommendation to apply more capsules on the windward side of a release area (Llewellyn, 2004). Block D was west of Block C and Block B so wind may have aided in the dispersal of *Trichogramma* into those blocks.

Another possible explanation lies in the way damage was recorded. Damage was assessed on the presence of frass. If there was frass, either old or new, it was assumed there was a borer present and was recorded as such. It is possible that this method may have over-estimated the number of larvae present. In the final assessment there were a number of trees, especially in Block B of Orchard 2, which clearly had signs of previous borer damage but there was an absence of frass around those damage sites (i.e. the holes were visible but there was no frass). Therefore those trees were not recorded as having damage. One possible reason for this scenario could be that the borers had pupated and emerged from their tunnels and therefore there was no continued accumulation of frass. However, it is unclear why this scenario was so noticeable in this particular year, in that particular block, and not the others.

The *M. melanostigma* life cycle may also help to explain some of the results assessing the active (new) frass sites. While my studies were inconclusive as to the length of the life cycle, they generally supported previous observations of the life cycle being greater than one year. If it is assumed that the life cycle of *M. melanostigma* is at least two years in the Young district (Lloyd, 1972; Hely *et al*, 1982) then direct effects of egg parasitoid releases may take longer to be observed. If a proportion of larvae in the trees pupated, with subsequent adult moth emergence
and oviposition during the same season as the *Trichogramma* were released, the number of active frass sites would decrease if the parasitoids were eliminating the pest, in the egg stage, before it emerged as a larva to cause further damage.

While conducting the damage assessments on trees it was difficult to see frass in the limbs, especially in taller branches. Also, some of the damage may have been removed by pruning, which was undertaken on a limited basis during the years of this trial. Every effort was made to record damage in a consistent manner, but given the nature of this type of visual assessment some damage sites could have been overlooked. Damage in the crotch area was very visible but there was generally only one crotch in a tree, although some of the larger branch forks had damage and were recorded as crotch damage in the assessments. The trunk area was the most easily assessed as damage sites were very apparent (Figure 9). Considering these parameters, it was not surprising that the majority of frass sites in the trees were recorded as located in the trunk region of the tree (33%), followed by the crotches (20%), and then by damage in limbs (11%). These data were not analysed further.
5.2 *Trichogramma* and parasitism levels

Early in the third season (2005/06) of using *Helicoverpa* egg cards to assess parasitism in the trial blocks, results from Orchard 2 showed presence of three species of *Trichogramma*. These parasitised *Helicoverpa* eggs were recovered from the random block, adjacent to the two release blocks. The two non-released species of *Trichogramma* (*T. nr brassicae* and *T. pretiosum*) were not recovered from any of the previous or subsequent *Helicoverpa* egg applications.

It was not unexpected to recover *T. carverae* from the *Helicoverpa* eggs, as that was the species which was inundatively released. It was surprising, though, to recover *T. nr brassicae* and *T. pretiosum*. Although *T. pretiosum* is commercially available it is unlikely that it had been released in that area due to the nature of neighbouring non-farming properties. *T. nr brassicae*, while not commercially available, is a native species and must therefore be assumed to be present in that area, if only in low numbers. This is of particular interest given that it was this latter species which was responsible for reducing damage caused by *M. melanostigma* in pecans in Moree, NSW (Seymour and Crouch, 1993). It also supports the likelihood that there are parasitoids present in the orchards, but not in sufficient numbers to noticeably reduce pest populations.

One factor possibly contributing to finding different species of *Trichogramma* was the time of year they were recovered. It was early in December, before there were
sustained high temperatures. There had also been some early summer rain so humidity levels were elevated. These two factors could have contributed to this unexpected result. By the next *Helicoverpa* egg application one week later, temperatures had increased and the likelihood of finding non-released species of *Trichogramma* decreased.

While there were a number of *Helicoverpa* eggs recovered which had been parasitised, there was a noticeable loss of *Helicoverpa* eggs (between 20 – 100%) from the cards when they were collected from the field 24 h after application. Simpson and Cavallaro (1998) reported similar observations in their investigations. This was most likely due to predation by other insects. Kuske *et al* (2003) reported similar results from their research surveying *Trichogramma* presence in field sites. They reported between 31 – 90% egg loss from sentinel cards containing lepidopteran eggs (either *Ephestia kuehniella* Zeller or *Mamestra brassicae* L.). Future investigations could look at applying a physical barrier, such as Tree Tanglefoot Pest Barrier around the trees, to reduce predation by crawling insects such as ants and earwigs.

While there were a number of arthropods which may have preyed upon the *Helicoverpa* eggs in this investigation, the most likely were ants, spiders and ladybirds (Coleoptera: Coccinellidae) (Mansfield and Lawrence, 2002), all of which were present in the trial orchards. Predation seemed to increase as the season continued. This could likely be attributed to the increase in temperature and reduction in humidity, making food and water sources more scarce for foraging insects.
When Herz and Hassan (2006) tested a number of *Trichogramma* species for control of lepidopteran pests (*Prays oleae* Bern. and *Palpita unionalis* Hübn.) in olives, they found that under the most favourable conditions (25°C & 70% RH) the fecundity of *T. brassicae* was the highest of all the species they tested. When the different strains of *Trichogramma* were exposed to high temperature and low humidity (25°C/35°C & <40% RH), most of the strains had a significant reduction in survival and fecundity, with the species originating from temperate regions, such as *T. brassicae*, the most severely affected. Many authors have reported heat and low humidity as limiting factors in the efficacy of *Trichogramma*. These findings add weight to the possibility that the effectiveness of *T. carverae* in Young, could have been reduced because of the in-field weather conditions of high temperatures and low humidity.
5.3 Factors impacting on project outcomes

There were a number of factors impacting on the outcomes of this project, which could not be accounted for in the trial design. Two of these were environmental and one technical.

Drought effects impacting on project

The first of the environmental factors was drought. This trial was carried out during severe drought conditions in the Young district. The district was declared to be in Exceptional Circumstances (EC), due to drought, in December 2003. While drought, for EC purposes, is declared when 50% of farmers in a Rural Lands Protection Board are hand-feeding stock for survival, in the case of stone fruit growers the criteria were different. Orchardists were required to show that they had sustained 25% loss of trees in their orchards, which could be directly attributed to decreased rain events.

The drought did impact on the trial in a number of ways, yet these impacts cannot be fully determined because the length of these conditions is unprecedented and scientifically difficult to quantify. It is also difficult to assess the real effects of drought because it is a condition which is unstable, as droughts can ‘break’ at any time. Therefore, issues are discussed in relation to the trial dynamics, as opposed to a statistical analysis (Underwood, 1993). Because this was a field trial, the different
variables were interconnected to each other in a circular manner, rather than in a linear fashion where one variable sequentially affected the next.

The two primary aspects which need to be taken into consideration are the effects of the drought on the trees, as well as on the pest. The trees were under a great deal of water stress in all trial sites for the duration of this investigation. While all sites were under drip irrigation, the quality of the water was of concern. Bore water in Young tends to be saline and the concentration of salts can be high enough to reduce production. Salt tolerance varies in fruit trees. In *Prunus* species, there is no expected loss of production when salt levels are 1.1 dS m\(^{-1}\) or below. When salt levels reach 1.8 dS m\(^{-1}\), there is an expected 25% reduction in yield (Salt Action, 1999). Prior to the drought, bore water was pumped into farm dams where it ‘shandied’ with collected rain water. This practice helped to reduce salt concentrations to a level which did not affect production. Due to the low rainfall (*Appendix 5*), growers during the trial years either pumped from the bores into the empty dams and then to irrigation lines; or straight from the bores to the irrigation lines. This resulted in higher than optimal levels of salt in the irrigation water, increasing salinity impacts on tree health. Higher levels of salt increase the stress on trees which makes them more prone to pest and disease attack (Anon., 1996). This appears to be illustrated by the levels of damage recorded in Orchard 3 over the investigation period where bore water had a recorded salt level of 3.2 dS m\(^{-1}\) (*Figure 17*).

In the base year (2004), Orchard 3 had relatively low levels of borer damage (mean 32% damage across blocks compared with 56% in Orchard 1 and 22% in Orchard 2). In the second year of the trial (2005), the owner ceased watering midway through the
season due to costs of pumping water from the bore. In the third year (2006), with the orchard now under a lease agreement, the manager did not irrigate due to high salt levels in the bore water and the damage levels were elevated quite considerably (mean 59% damage across blocks compared with 72% in Orchard 1 and 40% in Orchard 2). Overall, total damage in Orchard 3 increased by 27% over the trial years, compared with 16% in Orchard 1 and 18% in Orchard 2. This appears to demonstrate the importance water stress on trees can play in borer damage, as well as on overall tree health.

While the other two orchards followed their normal irrigation schedule throughout the trial years, lack of rain also increased tree stress, particularly through higher than optimal salt accumulation in the root zone of the trees. Without water to decrease salt concentrations in the root zone, the vigour of the trees would have been compromised (Anon., 1996).

It is unknown what effects the drought may have had on the life cycle of *M. melanostigma*. Hely *et al* (1982) and Lloyd (1972) state that the life cycle of this wood-boring lepidopteran pest is at least two years. According to Gillespie & Fletcher (Pers. comm.) the larvae of many species of wood boring insects can stay cryptic until conditions are favourable for their emergence. It was unclear from the investigations in this trial whether the drought shortened, or extended, the *M. melanostigma* life cycle, although the quality of the wood does have a bearing on the rate of development in a number of insect species (M. Davison, Pers. comm.). The wood from drought-affected trees is probably of lesser quality than from irrigated trees, and therefore a wood boring insect’s growth and development may be delayed.
Quality of wood may also have had a bearing on the difference between the numbers of emerged moths recorded in Young during this trial, as compared with Moree during previous research (Seymour and Crouch, 1993). It may be that the condition and quality of pecan trees was more favourable for rate of development of *M. melanostigma* given that pecans were grown under flood irrigation.

Change in length of the life cycle is likely to influence effectiveness of control strategies for this pest. If the life cycle was shortened, the effectiveness of inundative releases of egg parasitoids such as *Trichogramma* should not be compromised. But if drought conditions delayed moth emergence as a result of the life cycle remaining in the larval or pupal stage within trees, inundative releases over two seasons would be much less likely to reduce subsequent borer damage. Therefore, releases could ultimately be a waste of resources for a commercial producer.

While the drought had likely effects on the trees and pest, temperature and humidity within the trial orchards played a major role in regard to the effectiveness of *Trichogramma* releases. There is extensive data from previous research to show that *Trichogramma* are adversely affected by high temperatures and low humidity (Seymour *et al.*, 1994; Smith, 1996), although temperature plays the more critical role (Kalyebi *et al.*, 2006). They also require a food supply and an appropriate microclimate to maximise their effectiveness (Landis and Orr, 2004). The efficacy of *Trichogramma* as an egg parasitoid, as determined by its activity and fecundity, significantly diminishes at temperatures below 20°C and above 29°C. The optimal relative humidity for most *Trichogramma* spp survival is 40-60% (Smith, 1996). These types of favourable conditions can be present in the Young district in the
spring months (generally October and November). By mid-December, though, humidity levels have dropped considerably and day time temperatures can range between 30°C - 42°C (Appendices 3 & 4). These conditions are not favourable for *Trichogramma* searching activity or survival. From life cycle studies of *M. melanostigma* in Young, reported in Chapter 4.2, moth emergence occurs from early December to late January, with the peak of emergence in the last week of December (Table 4). With the moth of *M. melanostigma* active during summer in Young, control during this period with *T. carverae* as an egg parasitoid is not likely to be very effective.

**Orchard biodiversity**

The second environmental factor in this project was the low level of biodiversity in and near orchard sites. Much of the world’s agriculture today is grown in monoculture with low natural diversity (Smith, 1996) and prunes are not an exception to this. In the Young district many farming enterprises border other farming enterprises, with a noticeable lack of native vegetation.

One important observation emanating from this trial is the apparent lack of natural enemies of *M. melanostigma*. Because it is a native pest with a wide host range of native and introduced plant species, it was anticipated there should have been a range of native predators and parasites, able to keep it controlled to a greater extent than was observed. It is possible that vertebrates, such as birds and bats, may be predators but vertebrates were not considered in this study. One possible reason as to why this control is not observed, could be due to the ecosystem which surrounds orchards at Young. Pests and their natural enemies require a place where they can interact and
where levels of pests and predation/parasitism can naturally occur (Bugg and Pickett, 1998). If there is no undisturbed place for this to happen, then it is likely to take place in the orchard. Much of this balance can be maintained or restored on the orchard floor but current horticultural practice, including in prune orchards, commonly involves slashing or spraying any vegetative sward in the tree rows. This leaves the tree canopy as the next likely place for the interactions to occur. This type of dynamic increases pest damage levels in the tree, to the point where natural control can become difficult (Horton et al., 2003). If insects are causing economic damage growers may be faced with using insecticides in their orchards. While this strategy aims to reduce target pest numbers, beneficial insect populations are also reduced, thereby limiting further the chance of natural biological control.

While this scenario can be self-perpetuating, there are practices which may help to improve it. One recommendation to come from this project is for orchardists to plant native trees and shrubs in areas in, or near, their orchards. There is evidence from this trial, based on the *Trichogramma* species recovered from the *Helicoverpa* egg applications, that native and introduced beneficial species do inhabit orchard areas but their populations tended to decline when temperatures increased (Figures 11-13), humidity decreased and food and water supplies diminished. Providing an area of native vegetation is likely to encourage arthropod diversity in a more protected and sustaining environment (Bugg and Pickett, 1998; Gurr and Wratten, 1999; Lavandero et al., 2006). Also, according to Smith (1996), *Trichogramma* are more habitat-specific than host-specific, so by providing more optimal habitat conditions than currently exist in prune orchards, the parasitoid is likely to better maintain its reproductive and parasitic activity.
The research carried out in this trial has implications not only for the prune industry, but for a number of other horticulture industries such as cherries and plums. These industries are also interested in an economically viable and sustainable control of *M. melanostigma*. While cherry and plum growers can obtain some level of control using broad spectrum insecticides, such as the organophosphate chlorpyrifos, prune growers have tended to utilise IPM and therefore are less likely to spray such insecticides.

The recommendation to physically control *M. melanostigma* by clearing away the frass and infiltrating the tunnel beneath with a small wire to pierce and kill the larva is probably the practice which still has the greatest level of success. If this is done methodically over an entire orchard, adequate control should occur for up to four years, especially where this species has a life cycle of two years or longer. While prune growers were hoping for a control strategy consistent with IPDM which was as effective, yet less labour intensive than this physical control, this project was unable to provide that under the climatic conditions which prevailed.

**Technical impact**

The major technical aspect which impacted on the investigations was the difficulty in rearing *M. melanostigma*. Although a number of options were assessed for rearing this pest, none was successful in being able to complete the life cycle in captivity, to the stage of oviposition. There is no proven medium on which to rear this insect other than wood. As they are often in the trunk of the tree it is difficult to remove them from the tree uninjured, and to grow them in a suitable substrate outside of the
whole tree. Also, in areas where the life cycle is longer than one year, larvae can be in a number of different instars within the tree (Lloyd, 1972). If limbs with damage are removed from the tree to monitor moth emergence, it is not possible to determine the stages of development of the larvae in the limb, and whether or not they would emerge in that current season. Also, the dying wood becomes a much less sustainable location for them to develop as the quality of their growing medium dictates the quality of the insect during its life cycle stages.

The adult moths proved to be elusive, and although other researchers reported success in capturing them with light traps at night (Seymour and Crouch, 1993) this was not the case in this project. There are several possible reasons why this was so. One is the quality of pecan wood compared with prune wood. Pecan trees are commonly flood irrigated and therefore under less physical stress than prune trees in Young. It could be argued that the wood of the pecan trees was of better quality than the wood of the prune trees and therefore more sustainable for the pest to complete its life cycle. While the life cycle of this pest is temperature (degree-day) dependent, this could also possibly explain why the life cycle was observed as definitely one year in Moree.

Another reason moths may not have come to the light traps was the light spectrum of the globes used. Perhaps a different spectrum would have yielded different results (A. Gibb, Pers. comm.). Even if moths were captured by the light traps or in the field cages, it was unlikely that they would have successfully completed their life cycle under laboratory conditions (M. Davison, Pers. comm.). It is likely that, in the absence of a suitable in vitro growing medium, successful rearing of M.
*melanostigma* would require whole trees to be enclosed in mesh cages in order for the life cycle to be completed in natural optimal conditions. Therefore, if this species cannot be artificially reared successfully through its life cycle, and a quantity of eggs cannot be collected for preference testing of, for example, egg parasitoids, it will be very difficult to conduct detailed investigations to screen for suitable candidates for its biological control in prune trees.
5.4 Economics

The industry needs to weigh up the cost of labour to inspect and treat individual trees against the loss in production, and therefore income, if the pest is left untreated. This project was intended to provide a biocontrol option to add another element to IPDM programs already utilised by prune orchardists. The control options investigated in detail in this project, though, appear to be no less labour-intensive than locating and penetrating individual borer tunnels, and were also possibly less effective.

Assuming that the use of *Trichogramma* was an effective control option, a person would need to be employed to scout for moth emergence. They would also need to distribute the capsules at least four times in a season (more in the beginning of a control program and possibly less as control was achieved). The following is a summary of projected costs for an orchard to be treated four times a year with *Trichogramma* capsules (Table 6), on a per hectare basis.

| Cost of capsules (as of 2006) | = $48/sheet |
| Need 2 sheets/ha for release rate of 120,000 wasps/ha | = $96/ha |
| x 4 releases/season | = $384/ha |
| Cost of labour | |
| 3 hours of insect scouting/week for 12 weeks @ $17/hr | = $612/season |
| 1 hr/ha to release capsules @ $17 x 4 weeks of releases | = $68/ha |
| **Total costs for a season of releases per hectare** | = $452 |
| **Total costs for seasonal scouting** | = $612 |
For each individual enterprise the cost-effectiveness of this biological control strategy would have to be considered in comparison to the manual labour of infiltrating the borer tunnels with wire. Fruit growers are innovative and if the results from this project had been more encouraging, there is little doubt that more cost-effective methods for monitoring and release would be developed. This type of innovation was evidenced following the introduction, then wide-spread adoption by Australian apple growers, of biological control of mites and of codling moth mating disruption (W.G. Thwaite, Pers. comm.).
5.5 Other control options

Entomopathogenic nematodes were also considered as possible biological control agents. The reported efficacy of ENs, in both laboratory and field assessments, is variable. According to Begley (1990), initial nematode infectivity tests for most insects in petri dish experiments recorded high levels of mortality. These levels, though, were often inconsistent under field conditions. Other trials have shown that petri dish screenings may not always provide evidence of efficacy of ENs because in some cases laboratory bioassays may underestimate their potential activity in the field (R. Spooner-Hart, Pers. comm.). In studying the efficacy of ENs in above-ground situations (borer holes, cryptic foliage and exposed foliage), Arthurs et al (2004) found that nematode efficacy against lepidopteran pests was greatest in borer holes. Their ability to infect *M. melanostigma* larvae in limited laboratory bioassays conducted during this investigation were inconclusive. A much larger number of larvae would be required to undertake a more extensive laboratory study to determine *M. melanostigma* larval susceptibility to nematodes.

Even though EN effectiveness in the bioassay investigations against *M. melanostigma* was inconclusive, ENs may still have potential as a control agent. Huaiwen et al (1993) trialled four application techniques in their research of tree borer control in China. Their techniques were: injection into borer galleries using an aqueous nematode suspension; blocking borer holes with nematode suspension absorbed onto sponge; coating holes with a nematode paste made of starch; and
coating holes with nematodes in a paste made of sweet potato powder. They found the injection and sponge blocking techniques to be the most efficacious. They reported that the injection of nematodes was labour intensive, and therefore costly, while the sponge blocking was not as costly and preferable in areas with less water.

In prune orchards in Australia, the nematodes would need to penetrate the frass to reach the larvae. Due to the generally hot and dry conditions in prune orchards, it would probably depend on how far, and how quickly, the nematodes were able to penetrate the frass as to the likelihood of their survival. The frass might provide the protection needed to reduce desiccation as well as toxic ultraviolet light effects on ENs. The application of nematodes would still be labour intensive and time consuming.

Kain and Agnello (1999) found that although ENs were able to infect lepidopteran larvae in laboratory bioassays and in field trials, the ENs were unable to effectively control borers. They postulated that perhaps the borer galleries were not moist enough to support the ENs, or that the target larvae were beyond the searching reach of the nematodes. Lack of moisture could also be an issue in prune trees, especially under drought conditions. Being able to assess in-field trials would be crucial in determining the ability of ENs to control *M. melanostigma* larvae and their impact on damage levels.
5.6 Further work

One of the major difficulties with the Trichogramma in this investigation was the limited range of species commercially available. The species used with success in Moree was no longer commercially available when the trial work began. Because of the difficulty in rearing M. melanostigma to produce eggs during this investigation, this project was unable to test the commercially available T. carverae and T. pretiosum against M. melanostigma eggs to check for host preference and likely parasitism. Previous researchers, though, had subjected M. melanostigma eggs to both T. carverae and T. pretiosum. While both Trichogramma species parasitised the eggs, T. carverae did so more quickly (R. Llewellyn, Pers. comm.).

If the prune industry was interested in further research into this pest, there are a number of options they could consider. One would be to rear sufficient numbers of M. melanostigma, possibly utilising larvae from other districts, to conduct host preference tests with a number of egg parasitoids as well as with predators/parasites of larvae. This would, however, require development of a suitable rearing substrate in which to rear the larvae.

Another possibility is to isolate a pheromone from the female moth which would attract male moths. This could then be used to determine emergence dates of moths more accurately, allowing for better timing of Trichogramma releases or other biological control options. There are a number of pheromone-based lures
commercially available in Australia and overseas for other pests in other horticultural
crops, such as codling moth in apples and lightbrown apple moth (*Epiphyas
postvittana* (Walker)) in a range of fruit and vine crops (Thwaite, 2004).

Mr Andrew Gibb, a research scientist with HortResearch, Lincoln New Zealand, has
completed pheromone work on a number of pests, including lepidoptera. He has
indicated his interest to work in this area to find a pheromone solution for attracting
moths of *M. melanostigma*. In my talks with Gibb, it was apparent that the single
limiting factor in this work was the number of pupae needed. It was decided to
investigate the possibility of growing larvae to pupation on an artificial diet
manufactured at HortResearch (Singh, 1983). This diet, contained in small plastic
specimen tubes (75 mm × 10 mm), was imported into Australia from New Zealand
by this project’s researcher. If this diet is successful in rearing *M. melanostigma*
through to pupation, the insect in the pupal stage can be shipped, with appropriate
approvals, to NZ for pheromone work. Several shipments of 100 larvae are needed so
this part of the process will be on-going. As *M. melanostigma* is a native Australian
species, the economics of developing a pheromone-based monitoring or control
system would need careful consideration.

In March 2005, while carrying out larval searches for the entomopathogenic
nematode studies during the investigations, it was noted that a number of larvae
found were intact but inactive. While it was recorded at the time, no further
observations were made. While carrying out the larval searches for the preliminary
pheromone work in March 2007, one larva was collected which was fully intact yet
inactive. From observation of the head capsule width and length of body, it appeared
to be in its second or third instar. Although inactive, it was deposited into one of the specimen tubes with artificial diet. All larvae collected that day were stored in a drawer, at ambient temperature, in the researcher’s office. Upon inspection the next day, it was noted that there were two small larvae, apparently emerged from the inactive *M. melanostigma* larva. These were reared through and identified as *Ligulibracon* sp (Hymenoptera: Braconidae) by Peter Gillespie (NSW DPI). This is the first record of this species parasitising *M. melanostigma*. This finding emphasises the importance of IPDM in orchards as pesticides can eliminate whatever natural controls may otherwise be present.

Both these searches were in the same block in the same orchard. This was also the same orchard where the non-released species of *Trichogramma* were recovered. This evidence indicates that there are native biological controls present, but most likely in numbers too small to be effective in pest control.

It would also be worthwhile conducting further field surveys for egg parasites and predators present in the field in prune growing regions, and to determine what time of year they are active. This would involve surveillance using trap eggs, such as *Helicoverpa*, similar to the methods carried out in this trial. A more extensive trapping system could be used to gain a broader understanding of the beneficial species ecosystems in prune orchards.

Maintaining tree health is also crucial in decreasing borer damage. Ideally, orchard practices should provide optimal growing conditions such as: efficient irrigation; maintenance of inter-row vegetative swards to increase biodiversity; alternate
slashing of rows to allow for pest/beneficial systems to be maintained away from tree canopies; improving soil health; improving pruning techniques; using wider limb angles to minimise crevices; reducing shaker damage and therefore reducing wound sites for borer tunnelling; et cetera (Lloyd, 1972; Bugg and Pickett, 1998; Gurr and Wratten, 1999). While not all of these techniques are suitable for established orchards, they should be considered and utilised for new plantings.

This three-year investigation could not unequivocally demonstrate that inundative releases of insectary-reared *T. carverae* will reduce damage caused by *M. melanostigma* in prune trees at Young, NSW. However, even under the extreme environmental conditions that prevailed, there was at least an indication that biological control is worth pursuing. By the third season, there was evidence of a reduction in larval activity where inundative releases of *Trichogramma* releases were made. The investigation also detected, for the first time, evidence of a larval parasite in a *M. melanostigma* population found under an IPDM program. If moth monitoring techniques can be improved through isolation and identification of the pheromone, the Australian prune industry is well on the way to adding sustainable biological control of *M. melanostigma* as a component to its existing IPDM program.
Appendix 1. Graph showing proportion of damage by quadrant direction (including crotch damage and damage to all of tree)
Appendix 2. *Procedures for counting entomopathogenic nematodes*

To count nematodes, 1.7 g nematode/cellulose mixture is added to 400 mL tap water. The solution is stirred with an electric bar mixer for about 40 seconds to get the nematodes into suspension. While keeping the solution in suspension by blowing bubbles with a pipette into that solution, 1 mL from that solution is removed and added to 30 mL water. After the 1 mL is added to the 30 mL beaker of water, the solution is kept in suspension by blowing bubbles through a pipette into the beaker. From this solution 1 mL is removed and spread across a petri dish. The petri dish has parallel lines marked on it. Under magnification, the number of nematodes are counted within each lined area and added together to get total number of nematodes/1 mL solution. Only hooked nematodes are counted, as flat ones are assumed to be dead. This procedure is repeated three times. The counts are then entered into an equation \(((\text{count} \times \text{dilution} \times \text{beaker (mL)}) / \text{sample (g)}) = \text{total})\) to determine the weight each bag of nematodes needs to be to reach the optimal rate of infectivity.
Appendix 3. Temperature data for trial sites over three years

Orchard 1 (Sept 2003 – Dec 2003)

Orchard 1 Temperature 2003

Orchard 1 (Jan 2004 – Dec 2004)

Orchard 1 Temperature 2004
Orchard 1 (Jan 2005 – Dec 2005)

Orchard 1 Temperature 2005

Orchard 1 (Jan 2006 – Feb 2006)

Orchard 1 Temperature 2006

Orchard 2 Temperature 2003

Orchard 2 (Jan 2004 – Dec 2004)

Orchard 2 Temperature 2004
Orchard 2 (Jan 2005 – Dec 2005)

Orchard 2 Temperature 2005

Orchard 2 (Jan 2006 – Feb 2006)

Orchard 2 Temperature 2006
Orchard 3 (July 2004 – Dec 2004)

Orchard 3 Temperature 2004

Orchard 3 (Jan 2005 – Dec 2005)

Orchard 3 Temperature 2005
Orchard 3 (Jan 2006 – Feb 2006)

Orchard 3 Temperature 2006

<table>
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Appendix 4. Relative humidity (%) in the three trial sites during Trichogramma releases (Dec – Jan) over the two release seasons. Note that irrigation affects humidity levels.

**Orchard 1 (Dec 2004 – Jan 2005)**

![Orchard 1 2004/05 Relative Humidity Graph]

**Orchard 1 (Dec 2005 – Jan 2006)**

![Orchard 1 2005/06 Relative Humidity Graph]
Orchard 2 (Dec 2004 – Jan 2005)

Orchard 2 2004/05 Relative Humidity

Orchard 2 (Dec 2005 – Jan 2006)

Orchard 2 2005/06 Relative Humidity
Orchard 3 (Dec 2004 – Jan 2005)

Orchard 3 2004/05 Relative Humidity

Orchard 3 (Dec 2005 – Jan 2006)

Orchard 3 2005/06 Relative Humidity
Appendix 5. Rainfall data (mm) of the long term median (monthly data from January 1889 – February 2003) in Young, NSW; and comparison data of monthly means during the trial years 2003 – 2005.

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REFERENCE LIST


Davidson, J. (1931) Insects observed on crops in South Australia during period June 1928 to June 1930. *Journal of the Department of South Australia* **34**, 741-745.


