The potential for greenhouse biocontrol by generalist predators *Hippodamia variegata* and *Micromus tasmaniae*

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Certificate of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Abstract

Generalist predators *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) and *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae) are predators known to consume a variety of arthropod pests. A key step in determining the potential of such predators for use in a biocontrol program is to identify the prey range and the suitability of various pest species as prey. Other gaps in the knowledge investigated in this thesis are the photoperiod and temperature conditions that may initiate dormancy and whether biocontrol agents are adversely affected by the pesticides commonly used in greenhouses.

No-choice experiments were conducted to determine the effects of five different diets on larvae of *H. variegata* and *M. tasmaniae*. The five diets were (*Myzus persicae* Sulzer (Homoptera: Aphididae), *Tetranychus urticae* Koch (Acari: Tetranychidae), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and *Aphis craccivora* Koch (Hemiptera: Aphididae)). The predators were reared on a diet of *A. craccivora* which were used in these experiments as a standard. Development from first instar larva to adult was significantly faster on aphid diets than on other prey species for both predators. *Hippodamia variegata* that were fed *F. occidentalis* failed to reach pupation and pre-imaginal survival of *M. tasmaniae* were significantly lower on a diet of *F. occidentalis* than on either *M. persicae* or *A. craccivora*. *Micromus tasmaniae* could not complete development on a diet of *T. urticae*. Both predators may have
potential against aphid species whilst *M. tasmaniae* may also have utility against *T. vaporariorum*.

Dormancy was not detected in *M. tasmaniae* adults exposed to 16L:8D and 8L:16D at 18°C and 25°C. A second experiment determined that *H. variegata* adults undergo a diapause at 18°C but not at 25°C. The five photoperiods used showed no effect on *H. variegata*. Temperature also had a statistically significant effect on mean daily oviposition and pre-oviposition period at 18°C. At the end of the experimental period, the ovaries of all *H. variegata* held at 25°C were mature while most of the ovaries of the insects held at 18°C were not mature. The cold-induced diapause response of *H. variegata* may aid storage and transportation.

When sprayed with abamectin, chlorpyrifos or imidacloprid, all *H. variegata* larvae died in less than 24h. Applications of bifenthin, buprofezin, maldison, botanical oil and pirimicarb resulted in survival from 0.30-0.72 after 24h, but were not significantly different from each other. Larvae of *M. tasmaniae* sprayed with bifenthin, chlorpyrifos, imidacloprid and maldison died in less than 24h. Treatments of abamectin, botanical oil and pirimicarb gave survival from 0.44-0.61 after 24h and were not significantly different from each other. Buprofezin did not cause significantly higher mortality in *M. tasmaniae* over 24h. The importance of more selective chemicals in integrated pest management (IPM) needs to be considered in order to improve the use of biocontrol agents with the spraying of pesticides.
The results presented in this thesis contribute towards the body of knowledge on *H. variegata* and *M. tasmaniae* and their future use as biocontrol agents in greenhouses.
CHAPTER ONE – General introduction

*Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) and *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae) are generalist predators of pestiferous arthropods and potential biocontrol agents for use in the greenhouses in the Sydney Basin. *Hippodamia variegata* is an amber-red ladybird beetle with a characteristic white collar on the pronotum, which differs with gender. *Hippodamia variegata* (formerly *Adonia variegata*) was first recorded in Queensland in November 2000 (Franzmann, 2002). *Micromus tasmaniae* is a native species of Australia but has since colonised New Zealand, New Hebrides, New Caledonia, Chatan Island, Antipodes and Auckland Island (Hussein, 1984).

1.1 Greenhouses and protected cropping

To understand the context in which the experiments reported in this thesis were constructed, the context of greenhouse grown crops and the control methods they utilise need be considered. There are few peer reviewed sources describing the greenhouse industry in New South Wales. Further, no study has comprehensively catalogued the range of crops and technologies that growers use. This section makes use of non peer-reviewed references from industry professionals in an attempt to describe the industry in the absence of this material.
Greenhouses can reduce the environmental risks associated with arable production by providing optimal growing conditions, protect the crop environment from adverse environmental conditions, extend growing seasons, grow crops out of season, grow different varieties of crops, and exclude pests from the crop (Badgery-Parker, 1999). Greenhouses can have coverings of glass, plastic or screening fabrics (Badgery-Parker, 1999).

The greenhouse vegetable industry in Australia has been categorised into three main divisions based on the technology which they use (Badgery-Parker, 2005). Low technology greenhouses have poor crop management due to lack of proper environmental controls and overuse of spraying of pesticides (Badgery-Parker, 2005). In contrast, high technology greenhouses utilise appropriate environmental, crop and pest management practices (Badgery-Parker, 2005). Medium technology greenhouses compromise between cost and productivity.

The optimal average temperature range for photosynthesis in a greenhouse is 18-28°C over a 24h period, with the day and night temperatures directly affecting the leaf size, fruit growth rate, the maximum height of the plant and the life of the plant (Nederhoff and Houter, 2009). Some crops may need lower temperatures at certain times, such as capsicum crops needing a temperature below 18°C for fruit set during the night, but requiring temperatures in the 18-
28°C range during the day (Nederhoff and Houter, 2009). Growers in medium and low technology greenhouses may not have the equipment to optimise their greenhouses to this range, and as a result, their greenhouses lose efficiency, and may exceed the lethal temperature threshold of biocontrol agents on hot days.

1.2 Integrated Pest Management

When implemented correctly, Integrated Pest Management (IPM) is the most scientifically rigorous pest management system. It is important to understand where *H. variegata* and *M. tasmaniae* fit into the IPM methodology, by first defining the accepted definition of IPM. IPM involves the utilisation of the most financially and ecologically effective means to combat a pest problem, leading to a stable and satisfactory solution (Kogan, 1998). Integrated control requires the application of chemical control used in a manner least disruptive of biological control (Stern et al., 1959). Integrated pest management schemes in Australia have been shown to produce a 2-11% increase in net returns compared with conventional farm management approaches (Goldie, 1997).

Since the appearance of the first article on IPM and the terms associated with it by Stern et al. (1959), more than 64 definitions of IPM have come to exist (Kogan, 1998). The Prokopy (2003) definition of IPM is “a decision-based process involving coordinated use of
multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner”. The definition is adopted and expanded upon by Ehler (2006), proposing that this statement of IPM includes “simultaneous management of multiple pests, regular monitoring pests, natural enemies and antagonists; use of economic thresholds for pesticide application; and integrated use of multiple suppressive tactics to control pests”. The term ‘integrated’ in IPM implies the use multiple strategies of decision-based approaches to control by considering levels of natural enemies compared to antagonist levels and providing a non-disruptive solution to a pest problem (Ehler, 2006). At a grower level, IPM is relevant to all crops, IPM requires monitoring and better record-keeping, better hygiene, choosing better chemicals and using fewer chemicals (Goodwin et al., 2002).

Kogan (1998) identifies several steps in the process of integrating IPM components into a conventional farm system based on the need to quantify the adoption of IPM by growers. Starting at level zero IPM is the treatment of crops by broad spectrum pesticides based on set calendar dates (Kogan, 1998). The first step towards IPM is to start field scouting for pests and use field thresholds to determine when to spray (Pizzol, 2010). At level one of integration, field scouting is expanded to search for natural enemies rather than just pests and thresholds for action are established based on the presence of natural enemies in the crop (Sterling, 1984). Also at this level more
selective chemicals may be considered in order to reduce the impact of pesticides on natural enemies (Deligeorgidis et al., 2005). At this level of integration, crop rotation over several years may be used to reduce insect pests (Sexion and Wyman, 2005) and plant diseases present in the soil (Peters et al., 2003). Additional progress towards IPM integration leads to full biocontrol of pest organisms including augmentative and inundative releases of pest organisms (van Lenteren, 2000). Pest and disease resistant varieties of crops and cultural pest control techniques may be used to reduce the impacts of pests and diseases without spraying pesticides (van Lenteren, 2000). From level two onwards, a range of tactical components and strategies are employed with increasing levels of sophistication. Multiple-pest interactions can be exploited to improve pest control (Abrams et al., 1998) and plant habitats can be managed more effectively. Dynamic crop/pest models and plant community level processes start to be used to achieve optimal pest control (Tang et al., 2010). Finally, at level three, all of the above is implemented at a farm-wide scale with multi-crop level interactions that improve overall productivity (Kogan 1998).

1.3 Pesticides and biocontrol agents

Biocontrol agents fare poorly when they are used in conjunction with conventional broad spectrum pesticides. The most immediate effect is chemicals causing direct mortality on the biocontrol agents (van
Driesche et al., 2008), but natural enemies also suffer sub-lethal harm too. Sub-lethal doses of pesticides can cause reduced foraging efficiency, reduced fecundity, extended development time and can repel biocontrol agents (van Driesche et al., 2008). Ultimately, these effects lead to biocontrol agents being less effective in combination with conventional pesticides.

Certain formulations of toxic pesticides may prove less damaging to certain biocontrol agents by avoiding biocontrol agents, such as systemic chemicals which do not leave a foliar residue, and would not affect biocontrol agents that do not consume phloem sap or as taxa specific pathogens such as *Bacillus thuringiensis* (Lacey et al., 2001). Selective application of pesticides to alternate rows of crops can reduce overall pest levels, while still maintaining an acceptable level of control, so long as the biocontrol agents are able to disperse between rows (van Driesche et al., 2008).

The most effective way to minimise the harm to biocontrol agents caused by pesticides is to restrict use of broad spectrum pesticides and supplement them with alternative control techniques such as cultural methods, trapping and autocidal controls. Chemicals with more selective modes of actions can be used to reduce these negative effects but not eliminate them entirely. Even chemicals with selective action may still have sub-lethal impacts including shortened lifespan, reduced number of offspring, increased preoviposition period, weight loss and mutation in offspring (Stark and Banks 2003).
These effects can in turn lead to an impact on overall population-level characteristics such as net reproductive rate, intrinsic rate of increase and generation time (Stark et al., 2007). If these effects are severe enough, over several generations, they can lead to a failure of biocontrol.

1.4 Greenhouse biocontrol

1.4.1 Functional and numerical responses

When biocontrol agents and prey interact density dependant predation or parasitism may arise. Density dependant responses to prey can be in the form of a numerical response or a functional response (Solomon 1949). The numerical response is an increase in predator density in response to an increase in prey density, caused by an increase in reproduction and survival as a result of more prey or more hosts. It may also occur from predators or parasitoids being attracted to sites of high density prey. Without a numerical response to prey density, it is unlikely that a predator can stabilise a prey population. The functional response is an increase in the number of prey consumed per predator as prey density increases. The rate of this increase starts to slow towards an upper maximum as the predators’ capacity to consume more prey decrease. At this point the predator spends more time handling prey rather than searching for prey (Holling 1959, Holling 1965). Above this limit, additional prey density does not cause higher consumption. This is called a type II functional response. Under certain conditions a functional response
can lead to positive density-dependant predation. When an increase in prey density results in a change in the foraging behaviour of a predator, such as focusing more efficiently on one prey species, predation rate will increase with increasing host density. In this scenario both the number of prey and the proportion of prey consumed by the predator will increase. This is called a type III functional response (Holling 1965).

1.4.2 Biocontrol in Australia

There were some early successes in biocontrol with control of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae) using the Australian beetle *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) in 1888. There has also been success in Australia itself with control of the prickly pear *Opuntia* spp. using pyralid *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) from Argentina in the 1920s. The coconut moth *Levuana iridescens* (Lepidoptera: Zygaenidae) has been controlled in Fiji using the Malaysian tachnid fly *Besa renita* (Diptera: Tachinidae) (Caltagirone, 1981). *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is one of the widely distributed predators of mealy bugs. Endemic to Queensland and New South Wales, it has been used as a biocontrol agent in a variety of countries worldwide including North America (Garcia and O’neil 2000), Mexico (Rosas-Garcia et al, 2009), Micronesia (Reddy et al., 2009), Egypt (Al-Khateeb and Asslan, 2009) and India (Baskaran, 2002). Both adults
and larvae consume mealy bugs but dense populations of mealy bugs are needed to sustain a population of *C. montrouzieri* (van Driesche, 2008).

There is a gap in the literature surrounding the use of native coccinellids in augmentative or inundative strategies. Native ladybirds *Cleobora mellyi* Mulsant (Coleoptera: Coccinellidae) and *Harmonia conformis* Boisduval, (Coleoptera: Coccinellidae) have been used in an inundative augmentative approach against Eucalyptus leaf beetle *Chrysophtharta bimaculata* Olivier (Coleoptera: Chrysomelidae) (Baker et al., 2003). In this example, the abundance of ladybirds returned to pre-release levels seven days after release (Baker et al., 2003), making them a poor comparison to a greenhouse scenario with closed or screened exits, where it is much harder for the ladybirds to migrate to other areas. There are no currently published studies using Australian native coccinellids in field crops or greenhouses. There have been more than 150 species of pests targeted in biocontrol schemes, with 70 of these pests being targeted by specific projects. Of these 70, 30 of the pests have good control, and 20 are no longer important pests. Almost all of these successes have been on exotic species, with only one success on an indigenous pest (Waterhouse and Sands, 2001).

Australia was the first country in the world to regulate the release of biocontrol agents. The *Biological Control Act 1984* was formed after
a dispute arising over the exotic weed species *Echium plantagineum* Nox (Boraginaceae), also known as ‘Paterson’s Curse’ or ‘Salvation Jane’. Objections arose to controlling this weed because of its contribution to honey production at the time, which lead to the *Biological Control Act 1984* being passed (Cullen and Delfosse, 1985)

### 1.4.2 Biocontrol in Greenhouses

It is likely that greenhouse biocontrol will increase over time, as more managers seek to overcome pesticide resistance (Pilkington et al., 2010). Climate control available to growers can optimise the efficacy of biocontrol agents (van Lenteren, 2000), as well as the growth of the crop (Nederhoff and Houter, 2009). The first attempt at greenhouse biocontrol is attributed to Réaumur in 1734, who advised growers to use predatory lacewings to control aphids (Luck and Forster, 2003). Another early example is by Erasmus Darwin, who proposed the use of syrphid larvae to control aphids in greenhouses (van Lenteren, 2008). Perhaps the key example of greenhouse biocontrol is *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) which is an efficient parasitoid of *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) (van Lenteren et al., 1996). Another more recent success story is specialist predator *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), which is so successful against *T. urticae* that it is used in greenhouse biocontrol worldwide (van Lenteren, 1995).
The release of commercially produced natural enemies is called augmentation biocontrol (van Driesche et al., 2008). The term augmentation biocontrol applies to inoculation biocontrol and inundative biocontrol, which are discussed in the following sections.

1.4.3 Inoculation Biocontrol

Inoculation biocontrol is the intentional release of a biocontrol agent to control a pest population over an extended period of time, but not permanently (Eilenberg et al., 2001). Establishment of a breeding population of hymenopteran parasitoid *E. formosa* in a greenhouse, along with alternative food sources, is an example of inoculation biocontrol. Inoculation biocontrol is more often used in greenhouses, where a population of biocontrol agents reproduce throughout the lifetime of the crop, but does not survive once the crop is harvested and the greenhouse is cleaned (Eilenberg et al., 2000). When the next crop generation is planted, a new release of the biocontrol is necessary. Unlike inundative control, low levels of the biocontrol agents are often introduced, insufficient to control a pest, often due to the expense of supplying the organism. It is the subsequent generations of the agent that control the pest. Inoculation biocontrol can also be used in an ‘augmentative’ approach in a greenhouse, adding extra individuals to an already established biocontrol population in order to shift the level of control towards inundative control (Eilenberg et al., 2001).
1.4.4 Inundative biocontrol

Inundative biocontrol is the mass release of large numbers of biocontrol agents to reduce a pest population without necessarily achieving continuing impact or establishment in the process. This form of control can provide quicker control than classical biocontrol when a higher density of arthropods are released, and can be useful in conjunction with other control strategies such as pesticides (van Driesche et al., 2008). Mass release is important in short-term crops that have a low tolerance for pests. In this situation an inoculative release would take too long to achieve control (van Driesche et al., 1999). For example, *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) has been mass released against *Bemisia tabaci* Gennadius Biotype B (Hemiptera: Aleyrodidae) in greenhouse poinsettias in north-eastern USA (Hoddle and van Driesche, 1999). Whitefly populations are maintained at low levels by up to 14 weekly releases of one female per two plants (van Driesche et al., 2008).

1.4.3 Banker plants

Banker plants are a solution to the problem of maintaining biocontrol agents in a crop environment when prey or host species are insufficient to provide adequate food to the biocontrol agent. A banker plant scheme utilises non-crop plants infested with a herbivorous insect that is not a pest for the host crop to provide food
and reproductive resources for parasitoids or predators (Yano, 2006). Banker plant schemes have been successfully implemented for parasitoids *E. formosa* (Pickett et al., 2004) and *Aphidius colemani* Viereck (Hymenoptera: Braconidae, Aphidiinae) (Yano, 2006) but there are few references to the use of predators in combination with banker plants in the literature, with the majority focusing on parasitoids (Matteoni, 2003, Pickett et al., 2004, Pratt and Croft, 2000). Typically, larger predator species of the Neuroptera and Coccinellidae are released curatively in a banker plant scheme rather than preventatively, with the predators on the banker plant providing additional food and reproductive resources (Yano, 2006). Pratt and Croft (2000) investigate the properties that affect banker plant systems using predatory mites to control spidermites in plant nurseries. They conclude that the success of a banker plant system is site-specific, dependant on the plants being cultured and the layout of the nursery. Banker plants can be part of the hedgerow that surrounds an agricultural site (Pratt and Croft, 2000). In comparison to cheap and easy to use parasitoid controls, use of predators against pests such as aphids can be impractical and expensive when predators die out at low aphid densities, allowing aphid populations to recover (Vansteenis, 1992).

### 1.5 Biocontrol agents

The greenhouse environment provides unique conditions for the control of pests although the extent to which environmental
conditions inside the greenhouses are controllable are largely dependent on the technology investment available to the grower. Temperature and humidity are the two critical variables for growth of greenhouse crops as well as establishment of plant pathogens (Paulitz and Belanger, 2001). Temperature and humidity are also critical for controlling development of natural enemy populations and allowing prediction of pest population parameters (van Roermund et al., 1997).

Biocontrol agents have a host range that is part of a continuum of monophagy or polyphagy, also known as specialist or generalist. At one end of this continuum are ultra-specialist arthropods that select only one species to prey upon or use as a host, while at the other end of the continuum, more polyphagous arthropods choose from a range of host species (Symondson et al., 2002). Some predatory species described in the literature as ‘predators’ can survive on either arthropod prey or plant based foods and could be considered omnivorous in addition to being described as polyphagous or monophagous. Jacometti and Jorgensen (2010) apply this term “omnivory” to *M. tasmaniae* because the lacewings can survive and reproduce on plant-based food sources. Omnivorous arthropod predators can simultaneously produce conflicting direct and indirect effects on the same species or trophic level, making it hard to predict the exact effects of the omnivore on the environment (Ho and Pennings, 2008).
Specialist predators and parasitoids, those with a narrow prey or host range, are useful in biocontrol because they have evolved to seek out specific targets without being distracted by the presence of alternative prey (Symondson et al., 2002). Generalist predators consume a wide range of prey. Suites of different generalist predators can be responsible for top-down control of native pests in native environments (Hawkins et al., 1999) and are important in many agricultural systems. In the context of early season rice fields, generalist predators can build up high numbers by feeding on detritus-feeding and plankton-feeding insects present in organic matter (Settle et al., 1996). This allows the predators to obtain a ‘head start’ at controlling pest species when they arrive in the crop (Settle et al., 1996). Generalist predators have been shown to control spider mites, mealy bugs or scale insects in peach crops (James, 1990), grapes (James and Whitney, 1993), and apples and almonds (AliNiazee and Croft, 1999). Native coccinellid *C. montrouzieri* provides control against different mealy bug species around the world (Garcia and O’neil 2000; Rosas-Garcia et al, 2009; Reddy et al., 2009; Al-Khateeb and Asslan, 2009; Baskaran, 2002). However, in the context of classical biocontrol, regarding exotic plants hosting exotic pests in cultivated habitats, a single parasitoid species has been most frequently found to cause top-down control (Hawkins et al., 1999)."
1.5.1 Parasitoids in biocontrol

Parasitoids are one of the most commonly used forms of greenhouse biocontrol agent used against insect pests (van Driesche et al., 2008) and have been used in predominantly inundative releases in a variety of field and greenhouse crops over the last 40 years (New, 2002a). Parasitoids are insects that have an immature life stage that develops either inside or attached to a single immature host (Hoffmann and Frodsham, 1993), killing the host as part of its developmental cycle (van Lenteren and Godfray, 2005).

Hymenopteran and dipteran parasitoids have applications against a wide range of insect families, with hymenopteran species attacking Lepidoptera, Coleoptera, Coccoidea, Diptera, and Homoptera, while dipteran parasitoids attack Lepidoptera, Coleoptera and Homoptera (New, 2002a). Use of *E. formosa* against greenhouse whitefly (*T. vaporariorum*) is considered a great success of greenhouse biocontrol after the rise of pesticide resistance in the 1970s (van Lenteren et al., 1996). In 1996, more than half of countries with greenhouse industries used *E. formosa* (van Lenteren et al., 1996). Some pest species, such as leaf mining *Lyriomyza* spp., may not have other biocontrol agents available (Salvo and Valladares, 2007). Differences in parasitoid host species can affect the larval mortality rate, the host preference of adult offspring, and the body size of adults (Ode et al., 2005). Access to supplemental food sources for
adult parasitoids increases adult longevity and egg production (McDougall and Mills 1997).

1.5.2 Predators in biocontrol

For arthropods, the terms ‘generalist predator’ and ‘polyphagous predator’ are largely unspecific labels that are used to describe the predatory behaviour of a wide group of arthropods such as coccinellids (Harmon et al., 2000), mites (Solomon et al., 2000), carabids (Goldschmidt and Toft, 1997) staphylinids (Dennis et al., 1991) and lacewings (New, 2002b). More specific definitions define a predator as a generalist rather than a specialist if it feeds on prey in different taxonomic families (Van Driesche, 2009). Because a large proportion of predators collected from the field show empty guts (Greenstone, 1979, Sunderland, 1975), predators can often be in a state of suboptimal nutrition (van Dijk, 1996, Anderson, 1974), and generalist predators have developed behaviour to cope with prey scarcity, eating anything they can subdue in order to survive (Bilde and Toft, 1998), and exploitation of alternative food (non-arthropod) sources that extend survival. There may be only a small subsection of prey species that support immature development and adult reproduction by a given predator (Evans et al., 1999). The prey of generalist predators can be split into essential foods that facilitate reproduction and maturation of larvae and supplemental foods which maintain the predator until it can obtain essential foods.
1.5.3 Diapause and quiescence in biocontrol agents

Photoperiod and temperature are key factors in the regulation of seasonal cycles in insects (Orlova, 1998). Individuals in wild insect populations are exposed to decreasing photoperiods and lower temperatures in autumn and winter months, which may induce a state of dormancy (Tadmor and Applebaum, 1971), a generic term that refers to a state of suppressed or arrested development (Danks, 1987). Dormancy, which may also be induced at higher temperatures and photoperiods (Katsoyannos et al., 2005), is usually accompanied by metabolic suppression (Danks, 1987) and can be adaptive either ecologically or in the evolutionary sense.

Quiescence is an immediate response to the decline of a limiting environmental factor below a physiological threshold with immediate resumption once conditions return to above the threshold (Saunders, 1982). Diapause, in contrast, is a programmed response to environmental factors, indirectly shifting the course of physiological development away from direct morphogenesis (Danks, 1987) and into an alternate sequence of physiological events (Kostal, 2006). Development does not always stop during this alternate sequence of events, but may continue at a slower rate, growth may continue without development, or extra instar stages may be added to the larval development of the insect (Kostal, 2006).
Dormancy can encompass quiescence and diapause and should be used when neither of those terms can be applied to the physiology of a given arthropod with certainty (Kostal, 2006). A biocontrol agent undergoing dormancy may not provide as good a level of control, or may not reproduce under certain conditions. The presence of dormancy in an agent can also be used advantageously to transport them more effectively and minimise mortality (Chang et al., 2000).

1.5.4 Supplemental food sources

Naturally occurring plant material exploitable as food for predators and parasitoids can be described as either ‘direct’, with sustenance coming from plants, or indirect, with the food source coming from plant feeding insects. Direct food sources include nectar and pollen (Spellman et al., 2006), extrafloral nectaries (Spellman et al., 2006), fruits (Jander, 1998), plant sap (Wellenstein, 1952) and leaking photoassimilates (Mercier and Lindow, 2000). However, floral resources only provide pollen and nectar for a limited time and can sustain pest lepidopteran adults as well as biocontrol agents, potentially leading to increased crop damage (Burleigh, 1972). Indirect food sources such as honeydew are produced by homopteran insects such as aphids, mealybugs, scale insects and whiteflies (Jervis and Kidd, 1996). However honeydews are discouraged in most cropping systems (Baggen and Gurr, 1998).
Supplementary foods can also be provided to the biocontrol agents in the form of food sprays composed of a mixture of complex carbohydrates and protein supplements in a liquid formulation (Mensah, 1997). Food sprays have the potential to encourage immigration by natural enemies, reduce emigration away from pest containing areas, increase the reproductive capacity of biocontrol agents and reduce the mortality of biocontrol agents (Wade et al., 2008). Despite these benefits, use of food sprays has been extremely low, suggesting that the performance of food sprays have been inconsistent (Wade et al., 2008). Wade et al., (2008) reviewed the prospects for use of food sprays and conclude that food sprays may have scope for use with other IPM components including narrow spectrum insecticides, floral resources, refugia and herbivore induced plant volatiles.

Insects can have an intimate relationship with certain host plants or alternative food sources, and can be split into generalist and specialist feeders of plant resources (Jervis and Kidd, 1996). Hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae) is an extreme generalist, reacting to its nutritional requirements, changing profitability, abundance and dispersion of plant species (Jervis and Kidd, 1996). In some cases, the availability of alternative food sources can actually reduce the predation on target pests in *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Stephens et al., 1998). Provision of multiple food sources to a biocontrol agent may decrease the control provided by the agent (Koss and Snyder,
In the case of *M. tasmaniae* feeding on aphids and buckwheat, aphid predation is decreased but this reduction is offset by an increase in fecundity (Robinson et al., 2007).

### 1.5.5 Intraguild predation, competition and biocontrol

Intraguild predation (IGP) is when two predator species share the same food source but one predator attacks and feeds upon the other predator as well (Kindlmann and Houdkova, 2006). To clarify, intraguild predation is not cannibalism (which would be intraspecific predation), nor is it obligate secondary predation. A secondary consumer does not compete with the primary consumer in secondary predation. The relationship between parasitoids and hyperparasitoids, for example, is not IGP (Rosenheim et al., 1995). In a crop environment, the occurrence of IGP raises the question as to whether multiple biocontrol agents can improve control of an insect pest, or have a neutral or negative effect upon pest control overall. Even when there is alternative prey available, the incidence of IGP does not change (Snyder et al., 2004b).

In microcosm experiments examining incidences of IGP on these insects, it was determined that alone, *A. aphidimyza* slows the build up of *A. glycines*, but cannot stop economically injurious levels of the pest building up. However, once introduced, *H. axyridis* controlled of the aphid in 3h. When both *A. aphidimyza* and *H. axyridis* are both present, there a significant level of intraguild predation of *A. aphidimyza* by *H. axyridis*, but aphid levels for both predators were not significantly different from aphid population levels exposed to just *A. aphidimyza*. In contrast to this example, *C. carnea* provides control of aphid levels on its own. However, when *H. axyridis* is introduced to the microcosm, there was a significant reduction in *C. carnea* and in this situation IGP could potentially release the aphid from control. Even so, the two predator species maintained control throughout the experiment (Gardiner and Landis, 2007). Overall, experiments show the importance of introduced predators in pest control. A transient coccinellid predator can be introduced to a crop environment and achieve control in a small period of time, if weak predators such as *A. aphidimyza* are present. If strong predators such as *C. carnea* are present, transient predators may be less important for control, but may reduce the population of established biocontrol agents and jeopardise pest control. This is not always the case, however, as other studies have shown that generalist coccinellid predators have a neutral effect on neuroptaran predator populations outside of the lab (Brown, 2003, Lucas et al., 1998, Chang, 1996).
While *H. axyridis* frequently exhibits IGP behaviour, there is some evidence of complimentary biocontrol with *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae), parasitoid of aphid *Macrosiphum euphorbiae* Thomas (Homoptera: Aphididae) that attacks roses (Snyder et al., 2004a). Although *H. axyridis* attacks aphid mummies when presented with both, the coccinellid ate twice as many aphids as mummies. When both predator and parasitoid where present, aphid infestation levels were significantly smaller than when only one biocontrol was present (Snyder et al., 2004a). This complementarity between generalist intraguild predators and specialist predators seems to be the exception rather than the rule, and there are many case studies where generalist predators disrupt biocontrol by a specialist parasitoid. The generalist predator *Anthocoris nemorum* L. (Heteroptera: Anthocoridae) exhibits IGP on the aphid parasitoid *A. colemani*. The predator does not discern between aphid mummies and healthy aphids (Meyling et al., 2004), nor does generalist predator, *Delphastus catalinae* Horn (Coleoptera: Coccinellidae), discern between whitefly larvae parasitised by *Encarsia sophia* Girault & Dodd (Hymenoptera: Aphelinidae) (Zang and Liu, 2007). Parasitised pests can be more vulnerable to predation than healthy individuals when prey species have anti-predator behaviour that ceases when the prey is mummified, such as in the case of the predation of *Acyrthosiphon pisum* (Homoptera: Aphididae) by carabid beetles in alfalfa (Snyder and Ives, 2001). These studies illustrate the problem of using multiple biocontrol agents against the same prey and show that more biocontrol species does not simply lead to a
higher level of control, indeed it can give less. In multi-trophic ecosystems, an increase in the number of predator species that exhibit IGP, or an increase in the abundance of those species, may lead to higher herbivore density and lower plant productivity. In apple orchards, the invasion of *H. axyridis* significantly increased the abundance of other coccinellids (Brown, 2003). Diverse communities where IGP predators are rare provide maximum productivity (Finke and Denno, 2005). In greenhouses, IGP may be less of an issue than in other agricultural systems (Brodeur et al., 2002).

While IGP does occur between greenhouse biocontrol agents, particularly those that prey upon aphids, generalist predators released to control high densities of pests may disappear from the system once the pest is gone, stopping further predation (Brodeur et al., 2002). Parasitoids can then be reintroduced to the system.

Although no papers have been published on IGP interactions in *H. variegata*, based on the reactions of other coccinellids it is likely that *H. variegata* larvae and adults are likely to engage in some level of IGP with other biocontrol agents. The relative size and relative developmental stage of a coccinellid larva may be a factor in determining the extent to which IGP occurs between *H. variegata* and other coccinellids that might be used as biocontrol agents in the same crop environment (Sato et al., 2009). While *H. variegata* may have some level of compatibility with other coccinellids, and
compliment the use of them, there will also be some level of IGP occurring between them.

1.6 Hippodamia variegata

A survey of *H. variegata* in Australia published in Franzmann (2002) found *H. variegata* feeding on the aphids *Rhopalosiphum maidis* Fitch, *Aphis gossypii* Glover, *Macrosiphum rosae* L., *Acyrthosiphon pisum* Harris, *Metopolophium dirhodum* Walker, *Macrosiphum euphorbiae* Thomas, *Hyperomyzus lactucae* L., *Lipaphis pseudobrassicae* Davis, *Aphis nerii* Boyer de Fonscolombe, *Uroleucon sonchi* L., *Brachycaudus persicae* Passerini and *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae). Its recent arrival in Australia has prompted interest in its potential against common greenhouse pest species in its predicted climatic range from Carnarvon Esperance (WA) and from Ceduna (SA) to Bega (NSW) to Cape York (Qld) (Nolan, 2007). *Hippodamia variegata* has been deliberately introduced in Chile (Zúñiga et al., 1986) and the North Eastern USA (Ellis et al., 1999)). This is not the case in Australia where *H. variegata* is thought to have been unintentionally released in Queensland, Australia, in November 2000 (Franzmann, 2002). Ladybirds such as *Hippodamia* have a complete metamorphosis lifecycle, going from egg, through four larval instars before pupating and emergence as an adult (Figure 1.1).
Figure 1.1 Life stages of *H. variegata*

Clockwise from top: Adult, Right: Eggs, Bottom: early and late instar larvae, Left: pupa. Scales are approximate.
*Hippodamia variegata* adults lay clusters of 10-20 eggs packed closely together in one oviposition bout. The mean generation time is 34 days at 25°C and adult females are known to have an oviposition period of up to 70 days (Kontodimas and Stathas, 2005). Over this period adult females can produce an average of 960 eggs (Kontodimas and Stathas, 2005), while only 73% of eggs hatch (Lanzoni et al., 2004). The lower developmental threshold of *H. variegata* is around 11°C and development time decreases with increasing temperature and has been shown to develop at temperatures as high as 34°C, but suffers from high larval and egg mortality (El Habi et al., 2000). A temperature of 26-27° has been shown to provide maximum survival and growth rate (El Habi et al., 2000, Michels and Bateman, 1986).

There are no dormancy studies on *H. variegata* in the literature but several other *Hippodamia* species exhibit some form of diapause, as do many coccinellids. Adult *Hippodamia convergens* Guerin (Coleoptera: Coccinellidae) enter reproductive diapause that affects adult reproduction when fed a suboptimal diet of sunflower stalks or Lepidoptera eggs, with more than 50% of the female population failing to produce viable eggs (Michaud and Qureshi, 2005). *Hippodamia tredecimpunctata* Say (Coleoptera: Coccinellidae) enters diapause when day length falls below a certain level and is initiated at 12L:12D (Storch and Vaundell, 1972). *Hippodamia undecimnotata* Schneider (Coleoptera: Coccinellidae) has been shown to enter a summer diapause when transferred laboratory with high temperature
conditions (25°C) and long day photoperiod (16L:8D), as well as a quiescence in the winter (Katsoyannos et al., 2005). Other genera of coccinellids also exhibit diapaus, including *Coccinella septempunctata* L. (Ricci et al., 2005), *Epilachna admirabilis* Crotch (Takeuchi et al., 1999) and *H. axyridis* (Sakurai et al., 1992). After consideration of other studies of diapause in congenerics, it is possible that *H. variegata* may undergo some form of dormancy.

IPM Technologies Pty Ltd (Hurstbridge, Victoria) is currently the only supplier of *H. variegata* in Australia but is unable to provide the quantities of the coccinellid at the levels that will be required for commercial control in greenhouses or field crops.

### 1.7 *Micromus tasmaniae*

In contrast to *Micromus tasmaniae*, which is currently only available for home garden use, *Mallada signata* Schneider (Neuroptera: Chrysopidae) is currently for sale as a biocontrol agent for use by commercial growers. *Mallada signata* is a commercially available biocontrol agent that has very little representation in the literature. This creates a gap concerning the basic biology of *M. signata*. Preliminary studies have started to investigate the use of *M. signata* in field trials (Horne et al, 2001) and New (2002) has discussed the
possibilities for control using Australian lacewings including *M. signata*. These studies have yet to be followed up with large-scale studies on *M. signata*.

*Micromus* sp. (brown lacewings) have been reported feeding on *A. gossypii*, Lepidoptera larvae, cicadellids, psyllids (Stelzl, 1991) tetranychid spider mites, aleyrodids and Coccoidea (Sato and Takada, 2004). Prey attacked by lacewings are comparatively sessile and relatively small with thin cuticles that are easily pierced by the sucking mouthparts of lacewings (Davidson et al., 2006).

The developmental threshold of *M. tasmaniae* is just under 6°C, while the upper temperature limit is around 30°C (Syrett and Penman, 1981). The optimal temperature for *M. tasmaniae* is around 25°C (Syrett and Penman, 1981). *Micromus tasmaniae* has a complete metamorphosis lifecycle, going from egg, through larval instars before pupating and emerging as an adult (Figure 1.2). Egg-adult development takes 21 days at 25°C (Syrett and Penman, 1981). *Micromus tasmaniae* is, however, active in cool periods when few other predators are and is particularly effective in controlling aphid populations as they build up in spring. (Walker et al., 2007)

At present there are no published studies showing a dormancy response in Hemerobiidae. The related aphidophagous predator *Boriomyia subnebulosa* (Stephens) (Neuroptera: Hemerobiidae) shows no dormancy under 8L:16D photoperiod at 25°C or 15°C and...
does not exhibit dormancy under winter conditions in south-west France (Laffranque and Canard, 1975). Another hemerobiid, *Hemerobius pacificus* Banks (Neuroptera: Hemerobiidae), did not show winter dormancy below 5°C under short day photoperiods of 8L:16D. Summer dormancy in *H. pacificus* could not be induced (Neuenschwander, 1976). Unlike brown lacewings, some green lacewings (Neuroptera: Chrysopidae) undergo diapause as larvae (Nakahira and Arakawa, 2005) or adults (Tauber et al., 1997). *Chrysopa pallens* Rambur (Neuroptera: Chrysopidae) has diapause induced at a photoperiod shorter than 14L:10D (Nakahira and Arakawa, 2005). Under a long day photoperiod, diapause is not induced by temperatures in the range of 16-33°C (Orlova, 1998). It is less likely that *M. tasmaniae* exhibits dormancy than is the case for chrysopids because of the lack of studies demonstrating dormancy onset in Hemerobiidae. If *M. tasmaniae* does not exhibit dormancy, it will be able to be active as a biocontrol agent throughout the year.
The larvae of *M. tasmaniae* are very active and readily disperse between plants (Horne et al., 2001, New, 2002b) which can be a useful trait for a biocontrol agent. A biocontrol agent that readily disperses reduces costs for treatment of an area over which the agent can disperse to, which is unusual in Hemerobiidae, giving the lacewing high potential for use in an IPM scheme (New, 2002b).
Micromus tasmaniae has limited resistance to pesticides such as neem extract (Hamd et al., 2005) and certain insect growth regulators (Rumpf et al., 1997, Rumpf et al., 1998), though sublethal effects are present in subsequent generations. This allows for an inundative biocontrol approach to be used, but would seriously harm establishment of the predator in the mid-long term.

In the absence of a specific study considering how M. tasmaniae will contribute to biocontrol, the biology of other lacewings must be considered for comparison. Brown lacewing (Hemerobiidae) adults are predatory which means that unlike green lacewings they do not require honeydew for egg production and can reproduce at lower aphid densities than green lacewings (Hagen et al., 1999). There is also some evidence that Micromus angulatus Stephens has been mass cultured and used in greenhouses to control aphids on cucumbers at a ratio of 1:1 or 1:3 when released every 7 to 9 days (Khloptseva, R.I. 1991). If M. tasmaniae can show comparable control of aphids in Australian greenhouses, it may be a valuable biocontrol agent. A spray dispersal mechanism has been designed for eggs of M. tasmaniae using a 206kPa compressed air sprayer in a medium of agar, sucrose, gelatine, plant glue and xanthan gum (Hussein, 1984). Although it is likely that this system will decrease the number of eggs hatching, Hussein (1984) found that it reduced aphid populations by 70% compared to untreated plants. This system
might allow for easy distribution of *M. tasmaniae* in greenhouses. To summarise, both adult and larvae *M. tasmaniae* should have functionality as biocontrol agents. Larvae will be able to be used in a similar fashion to green lacewings, but the predatory adults should have higher reproductive capacity at low prey levels. A greenhouse study using another *Micromus* species has been successful overseas and in addition there may be some capacity for distribution of *M. tasmaniae* eggs by spraying.

1.8 Project Objectives

**OVERALL OBJECTIVE**

Assess the utility of *H. variegata* and *M. tasmaniae* as biocontrol agents in a greenhouse environment by determining what prey species they consume, whether dormancy occurs, and to what extent pesticides affect them.

**SPECIFIC OBJECTIVES**

1. Determine the effects of consuming different pest species on *H. variegata* and *M. tasmaniae*.

2. Determine if dormancy occurs in either species, and if so, what the conditions for the onset of dormancy are.
3. Test the side-effects of commonly used greenhouse pesticides.
CHAPTER TWO - Potential for using larvae of generalist predators *Hippodamia variegata* (Coleoptera: Coccinellidae) and *Micromus tasmaniae* (Neuroptera: Hemerobiidae) against common greenhouse pests

2.1 Introduction

A large proportion of field-collected predators show empty guts (Sunderland, 1975) and are often found to be in a state of suboptimal nutrition (van Dijk, 1996). Generalist predators have developed behaviour to cope with prey scarcity such as eating anything they can subdue in order to survive (Bilde and Toft, 1998) and exploiting plant-derived food sources such as pollen or nectar that extend survival (Van Rijn et al., 2002). Notwithstanding this plasticity, for any predator species there may be only a small subsection of prey species that support immature development and adult reproduction (Evans et al., 1999).

Generalist predators consume a range of prey types although some level of specialisation is common. Accordingly, diet range studies are important to ascertain the likely biocontrol utility of a given predator species. Greenhouse biocontrol uses a variety of agents to achieve control, such as *Nabis kinbergii* Reuter (Hemiptera: Nabidae),
polyphagous predator of aphids, mirids and other arthropods (Siddique and Chapman, 1987) or Australian native *Transeius montdorensis* Schicha (Acari: Phytoseiidae) which feeds upon thrips species (Steiner et al., 2003). *Encarsia formosa* is a very successful parasitoid of *T. vaporariorum* (van Lenteren et al., 1996), while specialist predator *P. persimilis* is so successful against *T. urticae* that it is used as a greenhouse biocontrol worldwide. Further, parasitoids such as *A. colemani* are known to have a diverse range of host aphid species that differs in different parts of the world (Messing and Rabasse, 1995), including *M. persicae* and *A. gossypii* (Martinou and Wright, 2007).

The green-peach aphid, *M. persicae*, is a major pest of a wide range of agricultural plants, causing damage by phloem feeding and vectoring plant viruses (Martinou and Wright, 2007), with pesticide-resistant strains disrupting chemical control (Gillespie et al., 2009). Western flower thrips, *F. occidentalis*, are resistant to a range of insecticides and reduce crop yields by direct feeding damage and by transmitting tospoviruses (Pablo, 2008). Greenhouse whitefly, *T. vaporariorum*, is a phloem feeding pest of many horticultural and greenhouse crops and is so resistant to commonly used pesticides that alternative control strategies must be sought (Jian and Nick, 2007). The two spotted mite, *T. urticae*, is a pest of a wide range of crop plants. The mites feed on chlorophyll, water and nutrients inside leaf cells, causing foliar damage, discolouration and plant death (Rott and Ponsonby, 2000).
A survey of Australia published in Franzmann (2002) found *H. variegata* feeding on the aphids *R. maidis, A. gossypii, M. rosae, A. pisum, M. dirhodum, M. euphorbiae, H. lactucae, L. pseudobrassicae, A. nerii, U. sonchi, B. persicae* and *T. citricida* as well as Cicadellidae (Singh et al., 1991). Its recent arrival in Australia has prompted interest in its potential against common greenhouse pest species in its predicted climactic range from Carnarvon (WA) to Esperance (WA) and from Ceduna (SA) to Bega (NSW) to Cape York (Qld) (Nolan, 2007).

*Micromus* sp. (brown lacewings) have been reported feeding on *Aphis gossypii* Glover (Homoptera: Aphididae), Lepidoptera larvae, cicadellids, psyllids (Stelzl, 1991) tetranychid spider mites, aleyrodids and Coccoidea (Sato and Takada, 2004). Prey attacked by lacewings are comparatively sessile and relatively small with thin cuticles that are easily pierced by the sucking mouthparts of lacewings (Davidson et al., 2006).

Under greenhouse conditions both predators may only have access to one prey species and if this diet is sub-optimal there will be a reduction in the development rate, larval survival (Mayntz and Toft, 2001, Mayntz et al., 2003), fecundity and egg viability (van Driesche et al., 2008). The performance of *H. variegata* and *M. tasmaniae* on
common pest arthropods is critical for using these species in the biocontrol decision-making process.

In this study *F. occidentalis, M. persicae, T. urticae* and *T. vaporariorum* were tested for suitability for *H. variegata* and *M. tasmaniae* development. A fifth prey species, *A. craccivora*, is not typically a pest of greenhouse crops but was included as a diet known to support growth and reproduction of both predator species and upon which both predators were reared. Although *A. craccivora* is not considered a major pest of greenhouse crops and its host range is limited to Leguminosae, it is a potential vector of several plant viruses including *cucumber* mosaic virus (Berlandier et al., 1997). Transient *A. craccivora* may therefore transmit stylet-borne diseases though probing behaviour on non-host greenhouse crops (Hooks and Fereres, 2006).

This study aimed to identify the suitability of several key greenhouse pests as diets to support the development of *H. variegata* and *M. tasmaniae* and to assess their potential suitability as biocontrol agents in greenhouse crops.

### 2.2 Materials and methods

**Source of predators**
Founder insects were from IPM Technologies, Hurstbridge, Victoria where they were cultured in plastic food containers with ventilation holes cut in the top. Once the founder material was received, they were transferred into wooden framed cages with mesh-covered ventilation holes and a Perspex door in my labs at Gosford. The cultures were started in March 2007 and last used in December 2009. New individuals were introduced at least twice a year to maintain the genetic diversity of the culture and reduce adaptation to a caged environment. The cultures were maintained with the help of a part-time technical assistant solely for use in this project. Cultures were fed using a greenhouse population of *A. craccivora* reared on trays of faba bean stalks (*Vicia faba* L.) in aluminium cages surrounded by mesh. Faba bean stalks were cut from the aphid culture and provided to the predator cultures twice a week. At the same time, the cages were cleaned of dead plant and insect material. Cultures were maintained at 25°C, 80-90% RH and 14L:10D. Predators were also provided with 10% honey-water solution.

Adult *H. variegata* and *M. tasmaniae* were taken from a laboratory culture held in a controlled environment room at 25°C and 80-90% RH with a photoperiod of 16L:8D, in Gosford, New South Wales, Australia.
Eggs of *M. tasmaniae* and *H. variegata* were placed in separate plastic tubs, with aerated lids. Inside each container was a piece of wetted 90mm filter paper and faba bean shoots infested with *A. craccivora* that were renewed daily to provide sustenance to newly emerged larvae which were used in subsequent experiments.

**Source of prey diets**

Five prey diet treatments were tested: *M. persicae*, *T. urticae*, *T. vaporariorum*, *F. occidentalis*, and *A. craccivora*. All prey species tested were immatures to avoid the complication of prey reproduction. Insects used as diet treatments were sourced from greenhouse cultures at Gosford, New South Wales.

**Predator diet experiments**

A moistened piece of 90mm diameter filter paper was placed in a 90mm plastic Petri dish and replaced every 24h. To provide aeration, a 35mm diameter hole was cut in the lid and gauze cloth with a weave of 240μm was glued across the hole. Prey individuals were placed into Petri dishes using a soft haired brush except in the case of *T. vaporariorum* larvae that could not be separated from the host plant without killing them. For this treatment, cucumber leaves with a known number of third instar *T. vaporariorum* larvae (Table 1) were used to prepare 90mm leaf discs. To avoid desiccation, discs were
embedded on 1% liquid agar jelly and a ball of moist cotton wool was placed in the centre of the leaf disc. The predators were transferred into the Petri dish with a soft haired brush. The lid was sealed in place with parafilm.

One *H. variegata* larva (L1 stage, 0-24h old) was placed onto each Petri dish and a known number of prey were added (Table 1). More prey than could possibly be eaten was provided to the predator each day to ensure an oversupply. The amount of prey provided (Table 1) was increased as predator larvae developed and the number of prey eaten per day increased. Dishes were held in a controlled environment room at 25°C, 70-90%RH, 16L:8D and laid out in a randomised block design within the controlled environment room. At 24h intervals, prey numbers remaining were recorded and each predator larva transferred into a fresh Petri dish with fresh prey. At this time the life stage of the predator was recorded as well as any predator mortality. Survival was defined as the number of predator larvae surviving from the start of the experiment until they eclosed from pupae as adults. Each Petri dish containing an insect predator and the prey diet was considered a replicate. Due to limitations in insect availability, replication was semi-temporal with three concurrent blocks followed by an additional six blocks commencing 10 days later and four blocks commencing 19 days after that making a total of 13 blocks. Thirteen predators were used in total in this experiment, one per block.
A second experiment was conducted using the previously described methods to assess performance of *M. tasmaniae*. The experiment was semi temporally replicated with blocks commencing on 14/03/08, 28/03/08, 22/04/08, 29/04/08 and 6/03/08 comprising of 3, 8, 3, 5 and 6 randomised blocks respectively, giving a total of 25 blocks. Twenty-five insects were used in this experiment, one per block.

**Statistical Methods**

Diet treatments with no predators surviving to adult for all experimental units were omitted prior to the statistical analysis; these were *F. occidentalis* in experiment 1 (*H. variegata*) and *T. urticae* in experiment 2 (*M. tasmaniae*).

**Survival**

For each predator species, a generalised linear model with binomial error distribution and logit link function was used to test the effect of diet on the proportion of predators surviving to adults (p) as follows:

\[
\text{logit} (p) = \text{block} + \text{diet} + \text{error},
\]

where logit is the logistic transformation. Diet treatments were compared on the logit scale, and back-transformed for presentation.
Development Time

We defined the larval development time as the number of days from hatching to pupation. The number of replicates for each diet was unequal due to predator deaths after pupation, so a mixed linear effects model with treatment set as a fixed effect and block as a random effect was used to analyse the number of days until pupation (development time). Inspection of residual plots confirmed that the data were normally distributed. Separate analyses were conducted for each predator. All analyses used GenStat statistical software (2008) and significance tests were carried out at the 5% level.

Daily Feeding and Estimated Weight

For each replicate and diet treatment, the average number of prey consumed by each predator per day over the course of the experiment was calculated. The weight of a group of prey individuals were recorded, with the number collected and weighed being based on their relative size, 200 *A. craccivora*, 200 *M. persicae*, 500 *F. occidentalis*, 400 *T. urticae* and 100 *T. vaporariorum* were weighed (Precisa XT220, Zurich, Switzerland, accurate to 0.1mg). The average weight per insect was then calculated from this weight. The estimated weight of prey consumed per day was calculated by multiplying the average number consumed per day by the average weight per insect. The estimated weight was used to compare the relative amount of each prey species eaten every day. Differences between the amounts of prey consumed per day were compared
using analysis of variance. Log$_e$ transformation was used prior to the analysis to stabilise the variance. For the purposes of estimated weight and to allow comparison across prey species, it was assumed that predation resulted in the entire prey insect being eaten by the predator.
Table 2.1. Approximate daily provision of prey to predators in experiments 1 and 2 by predator life stage.

<table>
<thead>
<tr>
<th>Experiment 1 H. variegata</th>
<th>Diet</th>
<th>Prey individuals provided to L1-L2 predators per day</th>
<th>Estimated weight provided per day</th>
<th>Prey individuals provided to L3-L4 predators per day</th>
<th>Estimated weight provided per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. craccivora</td>
<td></td>
<td>15-30</td>
<td>5.0-10.0</td>
<td>30-90</td>
<td>10.0-30.1</td>
</tr>
<tr>
<td>F. occidentalis</td>
<td>40-100</td>
<td>0.6-1.5</td>
<td>40-50</td>
<td>10.7-13.4</td>
<td></td>
</tr>
<tr>
<td>M. persicae</td>
<td>15-40</td>
<td>4.0-10.7</td>
<td>40-90</td>
<td>1.5-3.0</td>
<td></td>
</tr>
<tr>
<td>T. urticae</td>
<td>50-100</td>
<td>0.9-1.8</td>
<td>100-300</td>
<td>1.8-5.4</td>
<td></td>
</tr>
<tr>
<td>T. vaporariorum</td>
<td>40-150</td>
<td>1.0-3.8</td>
<td>50-150</td>
<td>1.3-3.9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2 M. tasmaniae</th>
<th>Diet</th>
<th>Prey individuals provided to L1-L2 predators per day</th>
<th>Estimated weight per day</th>
<th>Prey individuals provided to L3 predators per day</th>
<th>Estimated weight per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. craccivora</td>
<td>15-40</td>
<td>5.0-13.4</td>
<td>40-80</td>
<td>13.4-26.7</td>
<td></td>
</tr>
<tr>
<td>F. occidentalis</td>
<td>40-100</td>
<td>0.6-1.5</td>
<td>100-200</td>
<td>1.5-3.0</td>
<td></td>
</tr>
<tr>
<td>M. persicae</td>
<td>15-40</td>
<td>4.0-10.7</td>
<td>40-90</td>
<td>10.7-24.0</td>
<td></td>
</tr>
<tr>
<td>T. urticae</td>
<td>50</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>T. vaporariorum</td>
<td>50-100</td>
<td>1.3-2.5</td>
<td>100-200</td>
<td>2.5-5.0</td>
<td></td>
</tr>
</tbody>
</table>
2.3 Results

Survival

All diets resulted in more than half of the population in *H. variegata* (Fig 2.1a). Survival in *M. tasmaniae* also showed considerable mortality over the experimental period (Fig 2.1b). There was no significant effect of prey diet on survival to adult for *H. variegata* (*p*=0.06). For *M. tasmaniae*, diets consisting of *A. craccivora*, *T. vaporariorum* and *M. persicae* resulted in significantly higher survival rates (*p*<0.05) than for the *F. occidentalis* diet (Table 2.2). No *H. variegata* survived to adult on a diet of *F. occidentalis*. No *M. tasmaniae* survived longer than four days on a diet of *T. urticae*.
Figure 2.1 The effects of prey diets on survival of a) H. variegata and b) M. tasmaniae over time while fed on a diet of A. craccivora (closed circle), F. occidentalis (open circle), M. persicae (closed square), T. urticae (open square) and T. vaporariorum (closed triangle).

Larval development time

Approximate F-tests revealed significant effects of diet on the larval development time. Both predators had significantly shorter larval development time on diets of A. craccivora and M. persicae (Table 2.2). Both H. variegata and M. tasmaniae had a combination of higher survival and shorter development rates on aphid diets. Although M. tasmaniae had higher survival on T. vaporariorum, it also had slower development time compared to other diets.
Table 2.2 Effect of diet on predator survival from neonate to adult (logit scale) and larval development time from emergence as first instar larva to pupation.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival (n=13)</td>
<td>Larval development time (days)</td>
</tr>
<tr>
<td></td>
<td>n#</td>
<td>logit</td>
</tr>
<tr>
<td>A. craccivora</td>
<td>0.31</td>
<td>-0.79</td>
</tr>
<tr>
<td>F. occidentalis</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>M. persicae</td>
<td>0.23</td>
<td>-2.61</td>
</tr>
<tr>
<td>T. urticae</td>
<td>0.08</td>
<td>-1.23</td>
</tr>
<tr>
<td>T. vaporariorum</td>
<td>0.08</td>
<td>-1.23</td>
</tr>
</tbody>
</table>

LSD 5% ns 1.9 1.44 0.8

* proportion of predators surviving to adult

Daily feeding

Significantly more A. craccivora and M. persicae were consumed by H. variegata (Fig 2.1) and M. tasmaniae (Fig 2.2) on a daily basis compared to the other diets (Table 2.3). Significantly fewer T. urticae were consumed by M. tasmaniae compared to all other diets.
a) $y = -1.0529x^2 + 14.625x - 10.381$
   $R^2 = 0.4719$

b) $y = 11.21x - 11.534$
   $R^2 = 0.485$

c) $y = -0.8171x^2 + 11.67x - 8.5577$
   $R^2 = 0.4897$
Figure 2.2 Mean prey consumed per day by *H. variegata* larvae per day when fed on a diet of a) *A. craccivora*, b) *F. occidentalis*, c) *M. persicae*, d) *T. urticae* or e) *T. vaporariorum*. 
a) \[ y = -0.8281x^2 + 5.8376x - 0.65 \]
\[ R^2 = 0.8831 \]

b) \[ y = -1.1949x^2 + 9.7982x - 3.0895 \]
\[ R^2 = 0.7269 \]

c) \[ y = -0.1413x^2 + 1.611x + 2.5823 \]
\[ R^2 = 0.5898 \]
Figure 2.3 Mean prey consumed per day by *M. tasmaniae* larvae per day when fed on a diet of a) *A. craccivora*, b) *F. occidentalis*, c) *M. persicae*, d) *T. urticae* or e) *T. vaporariorum*. 
Table 2.3 Mean daily feeding over the course of neonate-pupa development for 
H. variegata and M. tasmaniae ± s.e.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Experiment 1 H. variegata</th>
<th>Experiment 2 M. tasmaniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean daily Feeding* ± s.e.</td>
<td>Estimated live weight consumption/ mg</td>
</tr>
<tr>
<td>A. craccivora</td>
<td>19 ± 3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>F. occidentalis</td>
<td>31 ± 9.8</td>
<td>0.5</td>
</tr>
<tr>
<td>M. persicae</td>
<td>14 ± 3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>T. urticae</td>
<td>35 ± 10</td>
<td>0.6</td>
</tr>
<tr>
<td>T. vaporariorum</td>
<td>20 ± 4.4</td>
<td>0.5</td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* until death or pupation

2.4 Discussion

Pest species as food sources

Based on the fact that they supported some level of larval development from larva to adult (Table 2.3), all of the pest species except F. occidentalis could potentially be considered a food source for H. variegata. If the low survival in the experiment is a true representation of how H. variegata react to these prey crop settings, this may suggest that none of the most prevalent greenhouse pests could support a high survival of H. variegata from larva to adult. If this situation were to be proven true in greenhouse trials it would reduce the potential of H. variegata as a greenhouse biocontrol agent.
There remains the possibility of a ‘better’ diet that was not tested that would give an increased survival relative to other diets. In the absence of statistically detectable differences between the survival proportions of *H. variegata* on different diets, the recommendations for which diets are more suitable than others should be treated with more caution than for *M. tasmaniae*.

The experimental protocol for providing *T. vaporariorum* to the predators was different to the other treatments so the extent to which the data from this diet can be compared to the other treatments is limited. Even so, both predators showed significantly longer larval development time on a diet of *T. vaporariorum*. Paired with longer larval development time, *M. tasmaniae* had higher survival compared to other non-aphid diets suggesting that *T. vaporariorum* may be considered a more suitable diet than *F. occidentalis* for *M. tasmaniae*.

The way *M. tasmaniae* feeds (with piercing-sucking mouthparts) may have made visual determination of predation of *T. vaporariorum* less accurate than for other diets. As a diet for *H. variegata*, *T. urticae* could be considered a poor quality diet because despite of the large amount of mites consumed, there was low survival. Based on the very low daily predation and zero survival, *T. urticae* should not be considered a food source for *M. tasmaniae*. 
In an attempt to determine the number of predators needed to control a population of the prey, the daily predation of *H. variegata* and *M. tasmaniae* were related to the daily reproductive capacity of the prey. Taking a simple ratio of daily predator consumption and daily prey reproduction, it may be possible to obtain a rough estimate of how many larval predators would be needed to control an adult pest and its progeny (Table 2.4). In simple terms, when a predator consumes more pests per day than the pest reproduces, pest management is possible. Expressed as a simple ratio, this is the daily fecundity of the pest divided by the daily consumption of the predator (Table 2.4). If the number is greater than one, then the predator can manage multiple adult pests and their offspring per day.

The concept of predator modelling is not new. More mathematically complex approaches to modelling predator behaviour in coccinellids have already been constructed by authors such as Deligeorgidis et al. (2005). Such approaches may be more comprehensive and accurate, encompassing more aspects of predator and prey biology, but these simplistic ratios may still be of use as a basis for the decision to release *H. variegata* or *M. tasmaniae* larvae to provide control to a greenhouse based on the severity of a pest outbreak.
Table 2.4 Ratios of the mean prey predator feeding over mean prey reproduction. The ratio represents the amount of pests that one predator larva can control when factoring in pest reproduction.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Pest daily fecundity</th>
<th>Mean daily feeding by a predator larva</th>
<th>Ratio of predator feeding/pest fecundity</th>
<th>Mean daily feeding by a predator larva</th>
<th>Ratio of predator feeding/pest fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. craccivora</td>
<td></td>
<td>8 Ofuya (1995)</td>
<td>19</td>
<td>2.4</td>
<td>7</td>
</tr>
<tr>
<td>F. occidentalis</td>
<td></td>
<td>2.1 Reitz (2008)</td>
<td>31</td>
<td>14.8</td>
<td>29</td>
</tr>
<tr>
<td>M. persicae</td>
<td></td>
<td>5.7 Peppe &amp; Lomonaco (2003)</td>
<td>14</td>
<td>2.5</td>
<td>1.8</td>
</tr>
<tr>
<td>T. urticae</td>
<td></td>
<td>4.4 Bounfour &amp; Tanigoshi (2001)</td>
<td>35</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>T. vaporariorum</td>
<td></td>
<td>3.7 Jauset et al. (2000)</td>
<td>20</td>
<td>5.4</td>
<td>6</td>
</tr>
</tbody>
</table>

While the usefulness of these indices are diminished by the poor survival of the predators in this study, they may be of use in combination with the figures 2.2 and 2.3. As demonstrated in these figures, substantial predation took place across all the experimental insects in spite of the low survival to the adult stage overall. A population of H. variegata or M. tasmaniae may be able to provide a significant contribution to biocontrol even if they later die. These indices may be of use to future authors as a starting point in an attempt to determine what density of the predators can control a given pest species.
A diet that results in no survival or low survival is considered to be of low quality. In terms of greenhouse biocontrol, releasing *H. variegata* or *M. tasmaniae* against a pest which results in low predator survival is unlikely to be cost effective, but there are methods that can potentially be employed to make such a release feasible. On its own a diet consisting purely of *F. occidentalis* cannot support development of *H. variegata* or large numbers of *M. tasmaniae* to adult (fig 2.1). It is possible that the addition of a high quality food source would allow *H. variegata* or *M. tasmaniae* to develop and reproduce while still providing some level of control against another low quality pest species. To provide control in either of these scenarios, a non-pest, high quality food source would need to be provided to the biocontrol agent to sustain a viable population of biocontrol agents. Two potential options are either use of banker plants or an artificial food source. Even when being provided with a high density food supply, arthropod generalist predators still exhibit searching behaviour (Elliott et al., 2000) and will find and prey upon low quality prey even when a better food source is available (Evans et al., 1999). The issue remains as to what extent the generalist predator favours feeding on the high quality non-pest over feeding on the low quality pest, but some level of predation is likely to occur, even if it is not adequate to control the pest on its own. This approach needs to be investigated by future authors to see to see to what extent this approach controls low quality prey.
A banker plant system consists of non-crops plants infested with a prey insect that does not attack the crop. The prey insect provides reproductive resources to the predators or parasitoids introduced as biocontrol agents, acting as an open rearing system for biocontrol agents inside the crop (van Driesche et al., 2008). Typically, larger predators such as Neuroptera and Coccinellidae are released curatively in a banker plant system rather than preventatively, with the predators on the banker plant providing additional food and reproductive resources (Yano, 2006). A possible candidate for banker plant prey-source is *A. craccivora*, the primary diet of the *H. variegata* and *M. tasmaniae* used in this study. As a potential candidate, *A. craccivora* only feeds on Leguminosae and is not a host of common greenhouse crops such as tomatoes, cucumbers, capsicums and others. A laboratory-reared source population of *A. craccivora* would not have an opportunity to vector stylet-borne viruses.

Without additional food sources, control of an aphid species using a generalist predator has been described as impractical due to costs involved and the tendency for the predator to die out at low aphid density (Vansteenis, 1992), a high quality artificial diet might overcome this limitation. Artificial diets have been developed for green lacewings (Sattar et al., 2007) and Coccinellids (Sighinolfi et al., 2008, Silva et al., 2009). Predatory mirids have been reared on meat based diets which are superior to more natural diets, having higher oviposition and nymphal survival on the artificial diet (Iriarte
and Castane, 2001). Artificial diets are rarely as good as natural diets for coccinellids however. Silva et al. (2009) found that no adults of *E. connexa* could be reared on artificial diets as a stand-alone food source. Although Sighinolfi et al. (2008) were able to rear *H. axyridis* adults on an artificial diet, it was inferior to *E. kuehniella* eggs in a number of ways. Larvae reared on the artificial diet showed longer developmental time and a lower adult emergence. Upon emerging, adult weights were significantly lower on an artificial diet than for adults fed *E. kuehniella* eggs. The artificial diet also increased the preoviposition period, and lowered daily weight gain and fecundity compared to those fed on *E. kuehniella* eggs. Once emerged as adults, the artificial diet had no further adverse effects compared to *E. kuehniella* eggs. When considered for use with biocontrol agents, the consequences of using an artificial diet would have to be weighed carefully against the benefits of such a diet.

When two or more prey species are present, ‘apparent competition’ can occur between two pest species in the presence of a predator (Morris et al., 2004). Even though the pests do not interact directly, a population-level effect that resembles competition can be observed between the pest populations. A natural enemy population predates the ‘competing’ pest populations, preying on one species more than the other. This causes the population of one pest to decrease in density relative to the other pest population (Muller and Godfray, 1997). A related population-level effect known as ‘apparent mutualism’ is the opposite of ‘apparent competition’. An increase in
the density of the first prey species can cause satiation of the shared natural enemies, leading to an increase in the density of the second prey species (Muller and Godfray, 1997). Messelink et al (2008) found that when Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) or Euseius ovalis Evans (Acari: Phytoseiidae) were predating F. occidentalis and T. vaporariorum under greenhouse conditions, predator numbers were up to 15 times higher when both pest species were present than with only one. Messelink et al (2008) proposed that this was due to a higher juvenile survival and developmental rate on a mixed diet.

Future work needs to consider a number of different aspects. The effects of multiple-prey diets should be considered, especially in light of the findings of Messelink et al (2008). If multiple prey enhance the survival of generalist predators, this may make control possible against low quality diets. This study only investigated a limited range of hypotheses involving H. variegata and M. tasmaniae. Additional work is needed on the effects of different diets on adult lifespan and fecundity and prey consumption rates at different life stages. Compared to field studies, no-choice laboratory studies may overestimate prey consumption. A field study is needed to expand upon these initial experiments. It is of great importance to future laboratory work to establish the best species of prey to support a colony of H. variegata and M. tasmaniae.
Possible complementary biocontrol agents

Aphid parasitoids, (a commercially available example is *A. colemani*) may work in a complementary fashion to generalist predators (Snyder et al., 2004a). Coccinellid predators do not distinguish between parasitised aphids and healthy aphids (Snyder et al., 2004a, Bilu and Coll, 2009) and coccinellid larvae develop slower when fed on aphid mummies (Bilu and Coll, 2009). Aphid mummies are nevertheless still capable of supporting development, so the addition of parasitoids to a crop containing coccinellid predators may have a positive effect on overall control of the pests, although parasitoid efficacy will be reduced by the intraguild predation.

As biocontrol agents, *H. variegata* and *M. tasmaniae* are most likely to be of use against aphids and whitefly, and there may be some scope for complementary biocontrol with parasitoids. While other, more specific, biocontrol agents may provide better control in some situations, if generalist predators are provided with an appropriate food source they may be able to be used preventatively as well as curatively.
CHAPTER THREE - The role of photoperiod and temperature in the onset of dormancy in *Micromus tasmaniae* (Neuroptera: Hemerobiidae) and *Hippodamia variegata* (Coleoptera: Coccinellidae)

3.1 Introduction

Historically, use of the term diapause has been varied and inaccurate (Kostal, 2006). Originally used to describe periods of arrest in ontogenic development, the term later developed two definitions (Kostal, 2006). The cessation of development due to cold is now referred to as quiescence while termination in physiological or reproductive development is now referred to as diapause (Danks, 1987).

Quiescence is an immediate response to the decline of a limiting environmental factor below a physiological threshold with immediate resumption once conditions return to above the threshold (Saunders, 1982). Diapause, in contrast, is a programmed response to environmental factors, indirectly shifting the course of physiological development away from direct morphogenesis (Danks, 1987) and into an alternate sequence of physiological events (Kostal, 2006). Development does not always stop during this alternate sequences
of events, but may continue at a slower rate, or growth may continue without development, or extra instar stages may be added to the larval development of the insect (Kostal, 2006). Typically, the onset of the diapause state precedes the start of adverse conditions and is most commonly induced by reduced photoperiod and, often at the population level, may result in the synchronisation of seasonal activities of that population (Saunders, 1982). In the case of arthropod biocontrol agents, this may have serious repercussions for their efficacy as predators or parasitoids of pest species. If the conditions for the onset or termination of seasonal dormancy in a particular biological control agent are known, the controlled environment of greenhouses may offer scope to avoid, minimise, or optimise the timing of diapause to improve control efficacy.

There are no diapause studies on *H. variegata* in the literature but several other *Hippodamia* species exhibit some form of diapause, as do many coccinellids. Adult *H. convergens* enter reproductive diapause that affects adult reproduction when fed a suboptimal diet of sunflower stalks or Lepidoptera eggs, with more than 50% of the female population failing to create viable eggs (Michaud and Qureshi, 2005). *Hippodamia tredecimpunctata* enters diapause when day length falls below a certain level and is initiated at 12L:12D (Storch and Vaundell, 1972). *Hippodamia undecimnotata* has been shown to enter a summer diapause when transferred to a laboratory with high temperature conditions (25°C) and long day photoperiod (16L:8D), as well as a quiescence in the winter (Katsoyannos et al., 2005). Other
genera of coccinellids also exhibit diapauses, including

*C. septempunctata* (Ricci et al., 2005), *E. admirabilis* (Takeuchi et al., 1999) and *H. axyridis* (Sakurai et al., 1992). After consideration of other studies of diapause in congenerics, it is likely that *H. variegata* may undergo some form of dormancy. It is because most Coccinellidae overwinter in the adult stage (Takeuchi et al., 1999) that adult *H. variegata* were chosen for this experiment.

At present there are no published studies showing a diapause response in brown lacewings (Neuroptera: Hemerobiidae). Aphidophagous predator *B. subnebulosa* shows no diapause under 8L:16D photoperiod at 25°C or 15°C and does not exhibit diapause under winter conditions in south-west France (Laffranque and Canard, 1975). Another hemerobiid, *H. pacificus* (Neuroptera: Hemerobiidae), did not show winter diapause below 5°C under short day photoperiods of 8L:16D. Summer diapause in *H. pacificus* could not be induced (Neuenschwander, 1976). Unlike brown lacewings, some green lacewings (Neuroptera: Chrysopidae) undergo diapause as larvae (Nakahira and Arakawa, 2005) or adults (Tauber et al., 1997). *Chrysopa pallens* has diapause induced at a photoperiod shorter than 14L:10D (Nakahira and Arakawa, 2005). Under a long day photoperiod, diapause is not induced by temperatures in the range of 16-33°C (Orlova, 1998). There are no studies demonstrating dormancy onset in Hemerobiidae, so it is less likely that *M. tasmaniae* exhibits dormancy than is the case for chrysopids. In reference to this apparent trend, this study exposed *M. tasmaniae* to
seasonal photoperiods representative of mid-summer (16L:8D) and mid-winter (8L:16D) in order to determine if a seasonal dormancy was likely to occur. This study targeted the adult life stages of *H. variegata* and *M. tasmaniae* which, if affected by photoperiod or temperature, would impact on the reproduction of the insect.

The optimal average temperature for photosynthesis in most crops grown in greenhouses in Australia is in the range of 18-28°C which is well above the minimum temperatures for development of *H. variegata* and *M. tasmaniae* to provide biocontrol and reproduce, 11.2°C and 5.8°C respectively (El Habi et al., 2000, Syrett and Penman, 1981). Under greenhouse conditions, if a predatory biocontrol agent is able to reproduce while controlling pest populations, the resulting additional generations of biocontrol agents would be likely to enhance overall control.

Previous studies on *H. variegata* (El Habi et al., 2000) and on *M. tasmaniae* (Syrett and Penman, 1981; Samson and Blood, 1979) have already determined much about the basic effects of temperature on development, but there have been no studies comparing the effects of photoperiods and temperatures combined. This study investigated the photoperiods and temperature combinations rather than factors such as development rates and prey consumption, which have been investigated by other authors. The aim of this study was to determine if adult *M. tasmaniae* or *H. variegata* would enter a form of dormancy under photoperiods and
temperatures that they would encounter in Australian greenhouses and to provide information on the likely consequences for their utility in greenhouse biocontrol.

3.2 Materials & methods

Containers

Ten light-proofed boxes were constructed using 40L (450mm by 390m, 310mm deep) plastic containers painted black and lined with aluminium foil. The lid of each container had a 310mm light fixture with a fluorescent aquarium tube (Hagen Aqua-Glo 8W A-1580 - 12" - T5, Montreal, Canada). Photoperiod was regulated by a 24h timer switch (HPM D809/1VP2, Waterloo, NSW, Australia). The containers had two 80x80mm ventilation holes at each end with a 12V 80mm ball bearing fan (Sirocco YX2513 0.02A, Journing Blowers, Taipei, Taiwan) attached to each vent. The fans were aligned to create a flow-through of air to ensure the ambient temperature in the controlled environment room was transferred into the box. A black plastic hood was attached to each vent to avoid emission of light to other boxes while maintaining air flow. Containers were held in controlled environment rooms set at 15.0°C±0.5 and 25.0±0.5°C, 68%±13 RH respectively. Inside each box, the temperatures were 18.1°C ±3.5 and 25°C ±3.5. The controlled environment rooms were unlit and light-proofed.

Insect cultures
Individuals of *H. variegata* and *M. tasmaniae* used in this trial were taken from laboratory cultures reared in a controlled environment room at 25°C±1.7°C, 84%±12 RH at 16L:8D with a photoperiod of 16L:8D, in Gosford, New South Wales, Australia. Insects were fed on a greenhouse population of *A. craccivora* (Homoptera: Aphididae) reared on faba beans (*Vicia faba* L.) in a greenhouse. Eggs of *M. tasmaniae* and *H. variegata* were collected from the cultures and moved into 150x150mm plastic tubs containing 240μm gauze-covered holes, in order to rear the eggs through to pupae. Inside the container was a moist piece of 90mm filter paper and faba bean stalks containing *A. craccivora*.

**Experiment 1. M. tasmaniae**

Newly eclosed (0-24h) adult females were paired with a male from the culture then transferred to a 90mm plastic Petri dish containing moist filter paper and placed within the experimental containers. Every 24h for the duration of the experiment, predators were transferred to fresh Petri dishes in which *A. craccivora*, in numbers exceeding the predators’ estimated daily dietary requirements, were placed along with a droplet of 90% honey-water solution on the gauze lid. Males that died over the course of the experiment were replaced from the stock culture.

Two photoperiods, 16L:8D or 8L:16D, and two temperatures, 18.1°C ±3.5 or 25°C ±3.5, were allocated to light proof boxes, arranged in a
split-plot design with temperature as the main-plot and photoperiod as the sub-plot, temporally replicated three times. Each of the boxes, which contained multiple Petri dishes, was considered a block for the photoperiod and temperature allocated to it. Mated females of *M. tasmaniae* were observed for oviposition every day over a 21 day period, after which they were dissected by light microscope to look for evidence of ovarian maturity. The number of insects within each temporal replicate were originally uniform but became uneven due to adult deaths over the course of the 21-day period. Under a 8L:16D photoperiod at 18°C the three temporal replicates consisted of 5, 4, 4 adult females, while at 25°C, the three temporal replicates consisted of 3, 5, 3, adult females. Under a 16L:8D photoperiod at 18°C the three temporal replicates consisted of 5, 5, 3 adult females, while at 25°C, the three temporal replicates consisted of 5, 4, 3 individuals. The mean of the number of insects within each box was calculated prior to analysis to avoid pseudoreplication.

**Experiment 2. *H. variegata***

The method used in experiment 2 was used for *H. variegata* but with the photoperiods 16L:8D, 10L:14D, 12L:12D,14L:10D, and 8L:16D. These were assigned to containers using a randomised block design. Three *H. variegata* adult females per photoperiod treatment were prepared using the experimental protocol described above. This protocol was undertaken at 25°C±3.5 and 18°C±3.5, each temporally replicated three times, with a randomly assigned container for each
photoperiod for each replicate. Labelled Petri dishes were divided between the treatments evenly with multiple Petri dishes to a box.

The experiment was arranged as three replicates of a split-plot design, with the two temperature regimes as the main-plots and the five photoperiod treatments as subplots giving a 2 x 5 factorial treatment structure. To avoid pseudo replication, the assignment of temperatures to controlled environment rooms were randomised for each replicate. An experimental unit consisted of 2-5 Petri dishes. The number of days before egg-laying was recorded for each dish and the average calculated for each experimental unit. The number of insects within each temporal replicate were originally uniform but became uneven due to adult deaths over the course of the 21-day period (Table 1). The low replicates used in this experiment were insufficient to detect small changes in oviposition between treatments but large changes such, such as a large proportion of the insects having atrophied ovaries, would be detected.
Table 3.1 The number of insects used in each temporal replicate in experiment 2

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Temporal Replicate</th>
<th>18°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>8L:16D</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10L:14D</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>12L:12D</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>14L:10D</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>16L:8D</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Statistical analysis

Mean daily oviposition

Within each replicate, the mean daily oviposition over the 21 day period was calculated and ANOVA was used to analyse the data using Genstat 11.1(VSNi, 2008).
Pre oviposition

Some insects did not lay eggs at all within the 21 day time frame and these were omitted from the data which led to an unbalanced treatment structure. The factorial structure was ignored and a 10 level treatment factor, combining temperature and photoperiod, was formed. Analysis of pre-oviposition was undertaken with REML using Genstat 11.1 (VSNi, 2008).

3.3 Results

Experiment 1. *M. tasmaniae*

There was no effect on oviposition from temperature (F=15.49, df=1,2 P=0.059), photoperiod (F=0.92, df =1,2, P=0.39 or the interaction of these treatments (F=0.06, df=1,2, F=0.82). All *M. tasmaniae* had mature ovaries upon dissection. The mean daily oviposition showed a weak quadratic pattern on a daily basis (R²=0.38 and 0.59 at 16L:8D and 8L:16D) (Figure 3.1).

The interaction effect of temperature and photoperiod resulted in a significantly longer pre-oviposition period when exposed to 25°C at 8L:16D compared to 18°C at 8L:16D (3.9 days compared to 6.8 days. F=8.08, df=11, P=0.05, LSD 5%=1.9).
The number of pre-oviposition days was not affected by temperature under a 16L:8D photoperiod (5.04 and 5.35 days, F=5.64 df=11, P=0.14, LSD 5%=1.9).

Figure 3.1 The effects of temperature and photoperiod on the mean daily oviposition of *M. tasmaniae* at 18°C (open circle) and 25°C (open square) and cumulative mean daily eggs count at 18°C (closed circle) and 25°C (closed square) over 21 days.

Experiment 2. *H. variegata*
Female *H. variegata* showed highly significant differences ($F=287.43$, $df=1,2$, $P=0.003$) in mean oviposition between temperature treatments of 18°C and 25°C (Table 1). The effect of photoperiod on daily oviposition was not significant ($F=0.38$, $df=1,2$ $P=0.82$). All predators at 25°C showed fully developed ovaries and contained mature eggs in the abdominal cavity, while the majority of those held at 18°C showed atrophied ovaries. The effects of photoperiod and temperature showed significant interaction effects on pre-oviposition period of *H. variegata*. Pre-oviposition period of *H. variegata* was shown to be significantly different between 18°C 8L:16D and 25°C 8L:16D, between 18°C 12L:12D and 25°C 12L:12D ($F=36.40$, $df=12$, $P=0.015$, see Table 1). At 18°C 8L:16D, the pre-oviposition period was significantly longer than other photoperiods, and 14L:10D was significantly shorter than other photoperiods at 18°C. Differences between photoperiods at 25°C were not significant (Table 2.1). The mean daily oviposition did not show a strong quadratic relationship ($R^2=0.66$, 0.23, 0.20, 0.35 and 0.48 at 8L:16D, 10L:14D, 12L:12D, 14L:10D, 16L:8D) (Figure 3.2).
Table 3.2 The effects of photoperiod on pre-oviposition period and daily mean daily oviposition of *H. variegata* at 18° and 25°C at 18° and 25°C over 21 days. Values with letters following them denote that they are significantly different from values with different letters.

<table>
<thead>
<tr>
<th>Temperature/ °C</th>
<th>Photoperiod</th>
<th>Pre-oviposition period</th>
<th>Mean daily oviposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>8L:16D</td>
<td>12.9 a</td>
<td>0.7 a</td>
</tr>
<tr>
<td>18</td>
<td>10L:14D</td>
<td>4.5 bcd</td>
<td>2.0 a</td>
</tr>
<tr>
<td>18</td>
<td>12L:12D</td>
<td>8.3 b</td>
<td>3.1 a</td>
</tr>
<tr>
<td>18</td>
<td>14L:10D</td>
<td>1.1 d</td>
<td>1.7 a</td>
</tr>
<tr>
<td>18</td>
<td>16L:8D</td>
<td>8.0 bc</td>
<td>3.6 a</td>
</tr>
<tr>
<td>25</td>
<td>8L:16D</td>
<td>2.5 d</td>
<td>23.7 b</td>
</tr>
<tr>
<td>25</td>
<td>10L:14D</td>
<td>2.8 d</td>
<td>31.1 b</td>
</tr>
<tr>
<td>25</td>
<td>12L:12D</td>
<td>3.7 cd</td>
<td>24.5 b</td>
</tr>
<tr>
<td>25</td>
<td>14L:10D</td>
<td>4.0 bcd</td>
<td>22.1 b</td>
</tr>
<tr>
<td>25</td>
<td>16L:8D</td>
<td>4.2 d</td>
<td>26.5 b</td>
</tr>
</tbody>
</table>

LSD 5% 4.6 8.4
**Figure 3.2** The effects of temperature and photoperiod on the mean daily oviposition of *H. variegata* at 18°C (open circle) and 25°C (open square) and cumulative mean daily eggs count at 18°C (closed circle) and 25°C (closed square) over 21 days.
3.4 Discussion

The significantly longer pre-oviposition period in *M. tasmaniae* at 8L:16D between temperatures of 18°C and 25°C may be accounted for by the direct effect of temperature on insect physiology slowing the initial maturation of ovaries and vitellogenesis. If the study was expanded to include more *M. tasmaniae*, differences in the pre-oviposition periods between temperatures of 18°C and 25°C might become statistically significant, although even if that were the case it would still be unlikely that a dormancy would be identified in *M. tasmaniae* based on the mean daily oviposition and dissection data. At present there are not enough degrees of freedom to conclusively determine if there was an effect of temperature on oviposition of *M. tasmaniae*. Mean daily oviposition showed a slight trend, rising towards the middle of the experimental period, but confidence intervals ranged from 38%-69% (Figure 3.1), suggesting that the egg laying followed a very loose pattern over the 21 day period. Egg laying may not have peaked over the 21 day period. The results of experiment 1 indicate that it is unlikely that dormancy occurs in *M. tasmaniae* in response to photoperiods or temperatures used in this experiment.

There were no significant effects of photoperiod or temperature in *M. tasmaniae* which suggests this predator may be able to reproduce all year round, provided that the average temperature is above a minimum threshold and there is sufficient food available. In the
context of greenhouse biocontrol, this study suggests that photoperiodic seasonality will not affect the reproductive performance of *M. tasmaniae*.

The differences in pre-oviposition period in *H. variegata* are statistically significant at some photoperiods at 18°C, but in these cases, only one female from the dataset was usable in the analysis at photoperiods of 8L:16D and 14L:10D. Other females under these conditions did not lay eggs over the experimental period and could not be included in that analysis. Accordingly the observed differences in pre-oviposition period at 8L:16D and 14L:10D need to be treated with caution.

Ovarian atrophy is considered to be an indication of diapause (Danks, 1987) and has provided a practical way by which diapause in beetles may be determined. There are numerous studies that make use of this method to determine a state of diapause (Katsoyannos et al., 2005, Sakurai et al., 1992, Hodek, 1970). El Habi et al. (2000) found that the minimum developmental threshold for *H. variegata* was 11.22°C, suggesting that *H. variegata* is above its developmental threshold at 18°C. The combination of this observation, the dissection data showing atrophied ovaries, the statistically significant effects of temperature on pre-oviposition and the reduced oviposition at 18°C suggest that a diapause occurred. Although relatively low numbers of *H. variegata* were used in the
experiment, following the highly significant results between temperature treatments, it was decided that additional temporal replicates would not benefit the study further. Further research is needed to define the exact characteristics of diapause in *H. variegata* at low temperatures, particularly the termination conditions as it is not known what conditions might restore the ovaries of the insects or how long the diapause might last.

For optimum photosynthesis and growth, most greenhouse crops, including tomato, capsicum and cucumber, require a properly managed greenhouse to stay in the temperature range of 18-28°C on average, with a more typical range of 19-24°C (Nederhoff and Houter, 2009). The average temperature over a 24h period needs to conform to this range (Nederhoff and Houter, 2009).

In a greenhouse, temperatures above 28°C can harm fruit set and photosynthesis rate (Nederhoff and Houter, 2009). Higher temperatures increase fruit production at the cost of new leaf formation, wearing out plants faster and reducing area of new leaves, reducing overall productivity (Nederhoff and Houter, 2009). In greenhouses where temperatures for optimal growth are maintained, *M. tasmaniae* and *H. variegata* are likely to be able to survive. Care must be taken not to deviate from the optimal range because at 30°C or above *M. tasmaniae* larvae very rarely survive beyond the first instar (Syrett and Penman, 1981), while at 18°C *H. variegata*
undergoes reproductive diapause. Certain crops which need lower temperature conditions may prove problematic for control by *H. variegata*. Greenhouse crops such as capsicum require special night time growing conditions. Capsicum requires a night temperature less than 18°C during the fruit set period, while still maintaining the 18-28°C 24h average needed for optimum photosynthesis in the day, which may induce diapause in *H. variegata* during the cool night period (Nederhoff and Houter, 2009).

Exploiting diapause as a method to improve transport of biocontrol agents can be beneficial by improving the survival of stored biocontrol agents at lower temperatures. Biocontrol by predators such as *C. carnea* (Chang et al., 2000) and certain parasitoids such as *Chrysocharis pubcomis* (Zetterstedt) (Hymenoptera: Eulophidae) (Larios and Ohno, 2007) have been enhanced in this way, and control using *H. variegata* may benefit from this technique too. This study did not determine the termination conditions for the diapause in *H. variegata* which, if left unaddressed, might lead to diapausing females being transported to a control site where the predators might not lay eggs. In the absence of diapause termination data, Sakurai et al. (1992) found that the topical treatment of diapausing *H. axyridis* with juvenile hormone analogues have been shown to initiate ‘immediate’ ovarian development.
While *M. tasmaniae* did not appear to exhibit this response, the predator is somewhat cold-hardy, having a minimum egg-adult developmental threshold of 5.8°C, suggesting that a mass-rearing scheme involving reduced temperature transport may also be successful. Although storage of biocontrol agents for transport to growers is essential to the commercialisation of a biocontrol agent, long term cold storage does have an adverse effect on biocontrol agents. It is important to minimise the time spent in transit. Exposure to low temperatures over the entire preimaginal development period has been demonstrated to lower the survival rate from egg to adult in *M. tasmaniae* (Syrett and Penman, 1981). Reduced hatching and survival also occur in other biocontrol agents exposed to cold storage (Coudron et al., 2007, Chang et al., 1996). Coudron et al.,(2007) found that survival of *Podisus maculiventris* Say (Hemiptera: Pentatomidae) starts to drop sharply after four weeks, though further studies are required on other biocontrol agents.

Lack of a photoperiodic response in either predator allows these predators to be used in greenhouses without supplementary lighting during winter months, where predators that exhibit a photoperiodic dormancy might suffer an overall reduction in control efficacy. This study did not detect any low temperature thresholds or barriers to the reproduction of *M. tasmaniae* in greenhouses, though the upper temperatures limits of the species may be exceeded in some greenhouses, reducing overall survival. The onset of the diapause response of *H. variegata* appears to be dependant on temperature
rather than photoperiod and may not be a barrier to greenhouse biocontrol with this species. The temperature induced response may be useful for mass-distribution of the species.
CHAPTER FOUR - Effect of eight greenhouse crop pesticides on *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) and *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae) larvae

4.1 Introduction

The spraying of broad spectrum pesticides have been the most prevalent method used to manage arthropod pests on high value crops since the 1950s, increasing in use through the 60s and 70s before peaking in 1982 (National Research Council, 1996). The occurrence of pesticide-resistant pests has emerged as a consequence of overuse of pesticides, forcing growers to use more pesticide, often ineffectually, or take a more integrated approach to pest management (Kogan, 1998). The adoption of integrated pest management strategy enables a grower to reduce pesticide use by integrating biological, chemical, cultural, autocidal and other methods (Stern et al., 1959).

Conventional pesticides have a variety of different modes of action to kill insects. These pesticides are nerve toxins that kill by overstimulating the insect nervous system in a variety of different ways. Abamectin is a chloride channel inhibitor while bifenthrin is a
sodium channel modulator, both of which cause a build up of sodium ions in nerves, leading to overstimulation and death (Kim et al., 2006). Neonicotinoids such as imidacloprid mimic neurotransmitter chemical acetylcholine and acts as an agonist, creating hyperexcitation of the nervous system, leading to death (Mullins, 1993). The neurotransmitter acetylcholine is broken down by acetylcholinesterase enzymes, but, if this process does not occur then hyperexcitation will kill the insect. Organophosphates such as chlorpyrifos and pirimicarb and carbamate such as maldison all inhibit Acetylcholinesterase in order to cause overstimulation of the nervous system, leading to death (Zhao et al., 2005; Gutierrez et al., 2005; Fukuto, 1990).

Reduced risk pesticides have different modes of action to broad spectrum chemicals and are less persistent in the environment or are less toxic to non-target animals and plants. Unlike chemical pesticides, reduced risk pesticides are less likely eradicate pest populations, but rather suppress them to levels that do not cause economic damage (Crump et al., 1999). Reduced risk pesticides generally take longer to act upon pests than conventional synthetic chemicals. Spray oils reduce pest populations primarily by suffocation of larvae and eggs of insects. Soft bodied adults are also affected by spray oil. Spray oils may also affect oviposition and feeding behaviour as shown in Helicoverpa (Mensah et al., 1995). There is some evidence that some spray oils do not affect predatory beetles, bugs, lacewings or spiders (Mensah et al., 1995). Insect
growth regulators (IGRs) are insect juvenile hormone analogues that effect the development of immature insects, stopping reproductive development, eventually leading to death. Chitin synthesis inhibitors (CSIs) block chitin production, preventing exoskeleton creation and moulting, killing the insect (Brown, 2006).

In the absence of a viable biocontrol agent for a particular pest a grower may need to resort to using a chemical pesticide. Pests may have no local biocontrol agent when the pest is new in the geographical area, or when there is no known biocontrol agent that attacks the pest. In this situation growers must use other control methods, including cultural controls, resistant varieties, early or late seasonal planting and effective weed control (Summy and King, 1992) trap crops (Buitenhuis et al., 2007), pheromone trapping (Wakamura and Takai, 1995) or chemical pesticides.

_Hippodamia variegata_ was originally a native of the Palaearctic region but has since colonised South Africa (Aalbersberg et al., 1988), Kenya (Ogengalatigo, 1994), India (Singh et al., 1991), Canada (Gordon, 1984), China (Fan and Zhao, 1988) and Chile (Grez and Villagran, 2000). Recently _H. variegata_ has been recorded in Gatton, Queensland in November 2000 (Franzmann, 2002) and has been observed in large numbers in Victoria (Horne, Pers. Comm. 2007) and New South Wales (Heimoana, unpublished). The native predator _M. tasmaniae_ is found on roses (_Rosa_ sp L.), lucerne
Medicago sativa L.), brassicas and cereals, as well as other crops. It consumes aphids with a preference for wingless over winged forms, but also consumes mealy bugs. Micromus tasmaniae is active in cool periods when few other predators are and is particularly effective in controlling aphid populations as they build up in spring (Walker et al., 2007).

Studies on H. variegata in Europe, Africa and Asia have investigated the lethal and sublethal effects of pyrethroids (permethrin, deltamethrin, fenpropathrin, alpha-cypermethrin and bioresmethrin (Kalushkov, 2000)), esfenvalerate (Hamd et al., 2005), chlorpyrifos (Al-Doghairi et al., 2004), entomopathogen Beauvaria bassiana, (Al-Doghairi et al., 2004) and azadirachtin (Al-Doghairi et al., 2004, Hamd et al., 2005).

There are several New Zealand studies on the effects of pesticides on M. tasmaniae. The pesticides pirimicarb, imidacloprid and pymetrozine cause high mortality to M. tasmaniae over a period of 24h when used on lettuce (Walker et al., 2007). Insect growth regulators fenoxycarb, diflubenzuron and tebufenozide have been tested on M. tasmaniae alongside organophosphates methyl-parathion, azinphos-methyl, and cypermethrin over a 24h period (Rumpf et al., 1998). Pesticides have impacts at both the individual and population levels, causing direct mortality to individuals, but also causing sublethal effects on the population affecting life-table
parameters such as intrinsic rates of increase. While organophosphates killed greater numbers of *M. tasmaniae* through direct contact, insect growth regulators fenoxycarb and diflubenzuron had severe impacts on longevity, daily oviposition and total number of eggs laid (Rumpf et al., 1997, Rumpf et al., 1998). The effects of insect growth regulators on biocontrol agents are often less lethal than conventional pesticides but can have serous deleterious effects on biocontrol agents in integrated pest management (Hattingh, 1996). Repellent properties of pesticides may cause pests or biocontrol agents to avoid areas where a chemical has been sprayed, but a study using organophosphates diazinon and chlorpyrifos did not observe that response in *M. tasmaniae* (Hodge and Longley, 2000).

While there are studies of the effects of pesticides on *H. variegata* and *M. tasmaniae*, the literature lacks studies using Australian greenhouse pesticide application rates. This study compares the majority of chemicals available for use in greenhouse vegetable crops simultaneously so a direct comparison could be made. The study uses first instar larvae of *H. variegata* and *M. tasmaniae* to determine which chemicals have the greatest effect on mortality 24h after spraying. The aim of this study was to determine which pesticides might have a detrimental effect on *H. variegata* and *M. tasmaniae* when formulated according to guidelines for use in New South Wales, Australia.
4.2 Materials and methods

Insect cultures

Predator colonies were founded from individuals collected from Hurstbridge, Victoria, Australia and reared at Gosford, New South Wales. Both *H. variegata* and *M. tasmaniae* were fed on laboratory reared *A. craccivora*. *Aphis craccivora* were sourced from greenhouse cultures at Gosford, New South Wales. The predators were reared in a controlled environment room at 25°C±1.5 and 80%±15 humidity with a photoperiod of 16L:8D. To obtain first instar larvae for the experiments, egg clusters of both predators were removed and isolated in a sealed plastic container with a 2cm ventilation hole covered with 280μm gauze. These containers held faba bean (*Vicia faba* L.) stalks and were placed inside the controlled environment room and checked every 24h until hatching. The predators were isolated in sealed plastic vials, 5cm long and 1cm wide at room temperature (approx. 23-25°C) while the leaf discs and treatments were prepared.

Bioassay arenas

A 200ml, 1% agar jelly solution was prepared by placing 2g of agar 750 gel powder (Chem-supply, Gillman, South Australia) into a laboratory beaker and filling to the 200ml mark with tap water. The mixture was then boiled using a microwave oven (NEC N707M,
Seoul, Korea), stirred and left on the laboratory bench to cool for five minutes. 3ml of the liquid agar jelly was then transferred by pipette (Gilson Pipetman® P1000) into 35mm Petri dishes (Sarstedt, Mawson Lakes, South Australia). A leaf disc, cut using a 34mm wad punch (Boker, Germany), from a three week-old cucumber plant (Cucumis sativus L.) was embedded on the surface of the liquid agar jelly. The same cucumber plant was used as a source for all leaf discs from the same replicate. A H. variegata larva was gently tapped out of the plastic vials onto the surface of each Petri dish immediately prior to being sprayed. Ten A. craccivora adults were added to the Petri dish after spraying.

Potter tower calibration

The Potter tower (Burkard manufacturing co., Rickmansworth, Hertfordshire, England) was calibrated immediately prior to the application of treatments by spraying 2ml of tap water onto a 22x50mm glass slide (Superior Marienfield, Marienfield, Germany). The slide was weighed on an electronic balance (Precisia XT220A, Bacto Laboratories, Liverpool, New South Wales) before and after the application of tap water, then cleaned. The process was repeated until six readings of 2.100g ±0.001of water were deposited on the slide. The balance observations were recorded and the spray deposition was calculated.

Pesticide Selection
The pesticides used in this experiment were selected using the Infopest 2009 database to determine which chemicals were registered for use in greenhouse crops.

**Spraying**

Based on Herron et al. (1998), Herron et al. (1995), Herron and Barchia (2002), pesticide solutions were made up in 500ml volumetric flasks (Silber Brand, Brand, Germany) according to the manufacturers’ label rates (Table 4.1, (Infopest., 2007)). Aliquots of 2.0ml of each pesticide solutions were pipetted (Gilson Pipetman® P1000) into 12x75mm disposable ‘Borex’ glass tubes (Crown Scientific, NSW) for use in the Potter tower. A new pesticide solution was diluted from concentrate for each experimental block. The Potter tower was used to spray the pesticide solution onto the lower surface the leaf disc at 75kPa. The Petri dishes were covered with the Petri dish lid with a 2mm diameter gauze-covered (140μm gauze) ventilation hole and the rim was sealed with laboratory parafilm (Pechiney Plastic Packaging, Chicago). Petri dishes were then held in a controlled environment room at 25°C±0.5, 70%±13 RH and 16L:8D photoperiod.
### Table 4.1 Details of pesticide treatments used in laboratory evaluation of effects on first instar *H. variegata* and *M. tasmaniae* larvae

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Active ingredient concentration in product</th>
<th>Target pests</th>
<th>Target crop</th>
<th>Spray concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>abamectin</td>
<td>18g/L</td>
<td>thrips, mites</td>
<td>cucumber, tomatoes</td>
<td>0.90µl/ml</td>
</tr>
<tr>
<td>bifenthrin</td>
<td>100g/L</td>
<td>whitefly, earworm, russet mite</td>
<td>cucumber, tomatoes</td>
<td>0.60µl/ml</td>
</tr>
<tr>
<td>buprofezin</td>
<td>440g/L</td>
<td>Whitefly</td>
<td>tomatoes, cucumber</td>
<td>0.30µl/ml</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>500g/L</td>
<td>crickets, caterpillars, whitefly, mealybug, ants</td>
<td>cucumber, tomatoes</td>
<td>0.50µl/ml</td>
</tr>
<tr>
<td>imidaclorpid</td>
<td>200g/L</td>
<td>aphids &amp; whitefly</td>
<td>cucumber, capsicum, tomatoes</td>
<td>0.25µl/ml</td>
</tr>
<tr>
<td>maldison</td>
<td>500g/L</td>
<td>aphids, jassids, hoppers, Rutherglen bug</td>
<td>cucumber, tomatoes</td>
<td>1.0µl/ml</td>
</tr>
<tr>
<td>Eco oil botanical oil</td>
<td>830g/L</td>
<td>aphids, mites, thrips, whitefly, leafhoppers,</td>
<td>cucumber, tomatoes, capsicum, many other crops</td>
<td>5µl/ml</td>
</tr>
<tr>
<td>pirimicarb</td>
<td>500g/L</td>
<td>Aphids</td>
<td>cucumber, capsicum, tomatoes</td>
<td>0.5mg/ml</td>
</tr>
</tbody>
</table>

**Experimental design**

Pesticide treatments were allocated to Petri dishes containing a *H. variegata* larva according to a randomised block design. A block consisted of three replicates for each of the eight treatments plus the control (water) treatment. The experiment was semi temporally
replicated. Blocks for experiment 1 were performed on 20/08/09, 18/08/09, 24/08/09, 05/09/09 and two on 09/09/09. The data was analysed after each replicate was complete until significant differences between treatments were detected. In total 18 predators were used in this experiment.

A second experiment was conducted using the previously described methods to assess survival of *M. tasmaniae* 24h after being sprayed. Blocks for experiment 2 were performed on 20/08/09, two on 23/08/09, 05/09/09, 09/09/09 and 11/09/09. The data was analysed after each replicate was complete until significant differences between treatments were detected. In total 18 predators were used in this experiment.

**Assessment of survival**

A fine metal probe was used to gently nudge the predator in the Petri dish after 24h. If the predator showed signs of movement, the probe was used to flip the predator onto its back. If the insect could not right itself after 30s it was considered moribund and was recorded as having died.

**Data analysis**
Treatments where there was no survival were excluded from the analysis. Other treatments were analysed using a generalised linear model with binomial error distribution and logit link function in Genstat 11.1 (VSNi, 2008). After each replicate the data was analysed for significance. Once the differences between treatments emerged, no new replicates were undertaken. The variation of the data is incorporated into the presentation of the analysis in the form of the letters denoting significant differences between treatments at P=0.05.

4.3 Results

_Hippodamia variegata_ mortality

For _Hippodamia variegata_ in experiment 1 there was no survival in the abamectin, chlorpyrifos or imidacloprid treatments. Survival differed between the remaining treatments (F=3.88, df=106, P<0.001) exceeding 0.72 in the botanical oil and control treatments and significantly lower in the bifenthrin and maldison treatments where survival was lower than 0.30. Survival in the remaining treatments was intermediate (Table 4.2).

_Micromus tasmaniae_ mortality

For _M. tasmaniae_ in experiment 2 there was no survival in the bifenthrin, chlorpyrifos, imidacloprid or maldison chemical treatments.
Survival differed between the remaining treatments (F=3.39, df=89, P=0.002) exceeding 0.84 in the buprofezin and control treatments and significantly lower in the abamectin treatment. Survival in the remaining treatments was intermediate (Table 2).

The control treatment resulted in a significant difference in survival of *M. tasmaniae* compared to the lower survival for the other treatments except buprofezin. There was no significant difference in survival proportion between treatments of buprofezin, botanical oil or pirimicarb. The effect of the abamectin treatment resulted in a significant decrease in survival proportion (Table 2).
Table 4.2 The effects of pesticide treatment on total survival after 24h and corresponding IOBC classification for chemicals sprayed on first instar *H. variegata* and *M. tasmaniae*. Proportions in columns with different letters are significantly different at P=0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hippodamia variegata</th>
<th>Micromus. tasmaniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival proportion,</td>
<td>IOBC classification*</td>
</tr>
<tr>
<td></td>
<td>n=18</td>
<td></td>
</tr>
<tr>
<td>abamectin</td>
<td>0</td>
<td>Harmful</td>
</tr>
<tr>
<td>bifenthrin</td>
<td>0.30 bc</td>
<td>Harmful</td>
</tr>
<tr>
<td>buprofezin</td>
<td>0.39 b</td>
<td>Mod. Harmful</td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>0</td>
<td>Harmful</td>
</tr>
<tr>
<td>control</td>
<td>0.94 a</td>
<td>Harmless</td>
</tr>
<tr>
<td>imidacloprid</td>
<td>0</td>
<td>Harmful</td>
</tr>
<tr>
<td>maldison</td>
<td>0.17 c</td>
<td>Harmful</td>
</tr>
<tr>
<td>botanical oil</td>
<td>0.72 ab</td>
<td>Mod. Harmful</td>
</tr>
<tr>
<td>pirimicarb</td>
<td>0.61 b</td>
<td>Mod. Harmful</td>
</tr>
</tbody>
</table>

*Based on Boller et al. (2005)*
4.4 Discussion

A Potter spray tower was used in these experiments to provide a very precise dosage to the predators. The Potter tower was used to spray multiple pesticides in quick succession (after the appropriate cleaning procedure). Although a larger scale study might be useful for determining the absolute effects of these chemicals on the predators, this study may be of use in deciding which chemicals would be most useful to examine further for compatibility with the predators. Even though some of the chemicals used in the experiment have been used on the same predators previously, this study tests the majority of chemicals available for use in greenhouse vegetable crops simultaneously so that comparisons can be made directly.

The pesticides tested in the present study that led to no survival are likely to have an adverse impact on predators in a greenhouse IPM system. Accordingly, it is recommended that *H. variegata* not be considered in production systems where abamectin, chlorpyrifos and imidacloprid use is unavoidable. Similarly, *M. tasmaniae* appears unsuitable for use as a biological control agent in situations where bifenthrin, chlorpyrifos, imidacloprid and maldison are used. Of the other chemical treatments tested, only buprofezin in the case of *M. tasmaniae* and botanical oil for *H. variegata* resulted in a survival comparable to the control. The other chemicals caused intermediate
degrees of mortality to the predator species and may have scope for
careful use in an IPM program. Clearly, however, these results are
preliminary in nature and longer term experiments are required to
properly assess lethal and sub-lethal effects.

The International Organisation for Biological and Integrated Control
of Noxious Animals and Plants (IOBC) has classified chemical
pesticides based on the harm they cause to biocontrol agents. They
broadly classify harmless or slightly harmful chemicals as reducing a
population of biocontrol agents by 0-33%, classify moderately
harmful chemicals as reducing a population by 33-75%, and classify
harmful chemicals as reducing a population by more than 75%
(Boller et al., 2005). Within these broad categories, we can attempt to
classify the chemicals used in the experiments (Table 2). For
_H. variegata_, abamectin, bifenthrin, buprofezin, chlorpyrifos,
imidacloprid and maldison are harmful while botanical oil and
pirimicarb could be said to be moderately harmful. For _M. tasmaniae_,
bifenthrin, chlorpyrifos imidacloprid and maldison could be
considered harmful, while abamectin, botanical oil and pirimicarb
could be considered moderately harmful. Buprofezin could be
considered harmless or slightly harmful. Given the simplicity of the
experiments, only considering the survival of the predators over a
24h period, we cannot say with any confidence that buprofezin is
harmless to _H. variegata_, just that it causes no immediate harm.
Though the testing of the two predator species in separate experiments precludes direct comparisons it is striking that buprofezin was amongst the more hazardous treatments for *H. variegata* yet comparable to water for *M. tasmaniae*. The effects of buprofezin have been demonstrated to be incompatible for use with other coccinellids (Grafton-Cardwell and Gu, 2003, James, 2004) while having been shown to be harmless to a green lacewing (Liu and Chen, 2000). Abamectin, bifenthrin and maldison also had dissimilar impacts on survival between *H. variegata* and *M. tasmaniae*. This may be explained by the different modes of action of the different pesticides. The biology of Coccinellids and Hemerobiids may be different enough for different chemicals to affect them in different ways. For example, as a chitin synthesis inhibitor, buprofezin affects the developmental biology of different species in different ways. Few early instar larvae of *H. axyridis* or *Stethorus punctum piscipes* Casey (Coleoptera: Coccinellidae) treated with buprofezin survived to adulthood, while early instar *Orius tristicolor* White (Hemiptera: Anthocoridae) were unaffected (James, 2004). Our results are broadly comparable to Shaw et al., (1997) where treatment of apple orchards with buprofezin had more beneficial insects than an orchard sprayed with chlorpyrifos including *M. tasmaniae*. Cole and Horne (2006) found imidacloprid toxic to *M. tasmaniae* and Walker et al., (2007) found that pirimicarb resulted in 30-40% mortality of first instar *M. tasmaniae* larvae.
The results of this study confirm similar studies where nearly all commercial pesticides used were harmful to these organisms after 24h. If these pesticides are used in the same crop environment as the predators, then biocontrol strategies that require the biocontrol agents to persist in the environment are likely to fail. Strategies such as inundative biocontrol may have some scope to work with the less harmful chemicals. The less harmful chemicals would be most appropriate for use in an inundative strategy, depending on the timing of the spray. The persistence of the chemicals in the environment and the effects of toxic residues left on the leaves of the crop plants are an important consideration that needs to be explored in further studies. For an inundative biocontrol strategy to work, the insects must be able to feed on the crop pests without immediately dying from toxic spray residue that may have been applied days before the biocontrol agents were introduced.

Further research on the effects of pesticides on *H. variegata* and *M. tasmaniae* needs to target later life stages, specifically the effects of pesticides on pupal emergence and adult fecundity and egg viability, as well as the effects of pesticides at the population level. Studies on other chemicals is somewhat restricted by governmental regulations of use of pesticides in a crop, but certain reduced-risk chemicals may prove valuable in finding additional tools to compliment biocontrol rather than harming it, leading to better, longer lasting IPM solutions. Fungicides have been shown to negatively affect natural enemies, such as pyrazophos, a fungicide that has
insecticidal properties (Ledieu and Helyer, 1983). Future studies need to consider the effects of fungicides on these predators. Databases by European Biocontrol suppliers Koppert and Biobest include information on the effects of various pesticides on a range of European biocontrol agents, rating each chemical according to its compatibility with those biocontrol agents. A similar system in Australia would be a very valuable tool that would greatly benefit the uptake of biocontrol agents in Australia by providing growers with a resource for determining which chemical to use to minimise harm to natural enemies.

Nearly all of the chemicals used in the study caused significant reductions in survival, and may reduce survival even more over a longer period, or else have sublethal impacts on the predators, reducing oviposition and egg viability. Where possible, it is recommended that pesticides be avoided when these insects are being used for biocontrol, unless more selective chemicals are employed.
CHAPTER FIVE – General Discussion

5.1 The potential for greenhouse biocontrol

_Hippodamia variegata_ and _M. tasmaniae_ are generalist predators with considerable potential to be used as biocontrol agents. Previous authors’ work has examined various aspects of the biology of both predators. Factors determining the success of biocontrol are discussed in relation to the effects of different diets on _H. variegata_ and _M. tasmaniae_, greenhouse biocontrol, the possible onset of dormancy, and pesticide side effects have all been addressed in this thesis. The chapter also discusses the work needed to be done by future authors to expand upon the studies in the thesis.

A number of steps need to be taken in order to successfully develop a biocontrol agent from a concept to a potential biocontrol agent, through to a successful commercial product usable by the agricultural industry (Figure 5.1). A different methodology would be used to enable a potential biocontrol agent to be used in conservation biocontrol compared to augmentation, inundative or classical biocontrol methodologies. Conservation biocontrol does not typically require mass rearing or distribution of biocontrol agents because it makes use of wild biocontrol agents.

It is important that a biocontrol agent provides financial advantage comparable to chemical control methods. However, in a greenhouse
context a series of factors, including pesticide resistance, lack of new chemicals to replace old ones, and dangers of worker exposure are driving growers away from pesticides and towards biocontrol agents (Pilkington et al., 2010).

**Potential biocontrol agents**

Based on the work of other authors outlined in chapter one, both *H. variegata* and *M. tasmaniae* have already been deemed to have potential as biocontrol agents. Once a potential biocontrol agent has been identified, the first step is to study the biological characteristics in the lab and the field (van Lenteren, 2002). This step of figure three is explored in chapters two, three and four while the work in appendix two attempted to explore the utility of *H. variegata* and *M. tasmaniae* in an experimental greenhouse context. The following sections discuss the contribution of each of the experimental studies in the overall context of assessing the utility of the two species.

**Biocontrol agent diet range**

Chapter two observed *H. variegata* and *M. tasmaniae* larvae as they developed to adults on diets consisting of four key greenhouse pests and a control diet. The study determined the survival proportion, development times and daily feeding of the predators on each prey diet. This enabled recommendations to be made on which of the pest species could be controlled by *H. variegata* and *M. tasmaniae*. 
Figure 5.1 The steps involved in assessing the potential of a candidate biocontrol agent (after van Lenteren, 2002).
The results from chapter two suggested that *H. variegata* and *M. tasmaniae* could develop on *M. persicae* in addition to the control treatment, *A. craccivora*. *Micromus tasmaniae* could also develop to adults on *T. vaporariorum*. Providing a high quality food source in addition to a poor quality pest species may allow *H. variegata* and *M. tasmaniae* to control the pest while maintaining a reproducing population in the greenhouse.

In terms of the utility of *H. variegata* and *M. tasmaniae* to control the pest species used in chapter two, *H. variegata* may have more use as potential greenhouse biocontrol agents than *M. tasmaniae*. Although both *H. variegata* and *M. tasmaniae* had zero survival when on a diet of one pest species, *F. occidentalis* and *T. urticae* respectively, *H. variegata* still consumed an average of 31 *F. occidentalis* larvae per day while *M. tasmaniae* consumed less than one *T. urticae* nymph per day. If the predators are used against a pest species that cannot support their development, *H. variegata* may be more likely to provide control using inundative approach.

In this study, several factors may have influenced the results, causing greater mortality of the predators across all diets including the control diet. A study by Lanzoni et al. (2004) reported survival ratio of just 0.491 from egg to adult in *H. variegata*, compared to the value of 0.308 (4/13) in chapter two. Lanzoni et al. (2004) suggested the reason for low survival was the methodology used to transfer insects
between containers. Cannibalism was not an issue raised by Lanzoni et al. (2004). The study in chapter two used of a soft haired brush to transfer larvae between Petri dishes every day and it is possible this may have contributed to the mortality. Future work should use a low-impact aspiration method rather than a soft-haired brush to make sure that this is not an issue.

Another factor that may have influenced the results was the difference in protocol between the *T. vaporariorum* and other treatments. Rather than having *T. vaporariorum* as the only diet treatment with a leaf disc, it would have been better to have had all treatments presented on a leaf disc. As a result of this difference in treatments, the conclusions drawn from this prey species results must be viewed with caution when compared with other diet treatments. If the different protocol had an effect on predator biology, it would be expected to have a positive effect on the predators, improving survival and possibly decreasing larval development time in the *T. vaporariorum* treatment. Future authors should use a more consistent protocol, providing all of the diets on a leaf disc. Overall however, the study showed that *T. vaporariorum* could be a viable food source for both predators, so biocontrol against *T. vaporariorum* might be achievable.

While *H. variegata* and *M. tasmaniae* have been shown to have potential against several prey species, there is evidence they may
not be compatible with one another for simultaneous use in biological control. Heimoana (unpublished) recently found that 32.4% of *H. variegata* had traces of *M. tasmaniae* in their guts in brassica farms in Central West New South Wales, while only 2.74% of *M. tasmaniae* had traces of *H. variegata*. This finding suggests that while some level of intraguild predation occurs between both species, *H. variegata* engages in IGP more frequently than *M. tasmaniae*. *Hippodamia variegata* has the advantage in intraguild predation against *M. tasmaniae*, perhaps due to the larger size of *H. variegata* (Heimoana, unpublished). This finding suggests that *H. variegata* might be more suitable when used as the sole biocontrol agent in a greenhouse, while *M. tasmaniae* may be better suited to use with other biocontrol agents. A greenhouse trial would be needed to determine to what extent this might be true.

**Onset of dormancy**

Chapter three investigated whether photoperiod or temperature would cause dormancy in either of the predators. Although the existence of dormancy in *H. variegata* or *M. tasmaniae* might ultimately be useful for biocontrol transport, it might also limit the effectiveness of biocontrol using these species. If dormancy occurred in predators in augmentative biocontrol releases, dormancy might impact biocontrol if it could not be avoided. An inundative biocontrol agent release might not be affected by dormancy if the dormancy affected reproduction but not predatory efficiency.
The study in chapter three established that *H. variegata* does not lay eggs at 18°C. In conjunction with dissection data showing atrophied ovaries this is indicative of the coccinellid being in a state of diapause. The study did not determine the termination conditions for the diapause observed in *H. variegata*, which is an important consideration if diapause is to be exploited for transport of biocontrol agents, such as in Chang et al. (2000). Diapause is induced at some point between 18-25°C ±3.5, and further experimentation is required to determine the exact conditions under which diapause is triggered.

Even without the exact conditions for the termination of diapause there is the possibility of the application of juvenile hormones which may chemically break diapause in coccinellids (Hodek et al., 1973). This process has only been tested on two coccinellid species and needs further work to be applied to *H. variegata*. Another important topic for future work is to determine if larvae undergo diapause. This experiment did not examine larval stages of *H. variegata* but El Habi et al (2000) determined that *H. variegata* larvae are active predators when reared at 18°C so this life stage is unlikely exhibit dormancy. Chapter three did not determine if diapause occurred in *M. tasmaniae* larvae but Walker et al. (2007) noted that *M. tasmaniae* were active predators in the spring when other predators are not present suggesting that *M. tasmaniae* do not undergo dormancy. Having confirmed that *M. tasmaniae* adults do not appear to respond
to photoperiod or temperature with a diapause, this adds to the
evidence suggesting that Hemerobiidae in general do not undergo
diapause (Laffranque and Canard, 1975, Neuenschwander, 1976).

**Effects of pesticides on biocontrol agents**

Chapter four considered the immediate effects of greenhouse
pesticides on first instar predatory larvae determining which
chemicals would be the most harmful when directly sprayed onto the
insects. Buprofezin was shown to be ‘harmless’ (according to IOBC
classification (Boller et al., 2005)) when sprayed on *M. tasmaniae*,
but the duration of the experiment was not long enough to determine
if the chemicals might cause greater mortality after several days if the
insects could no longer develop. In contrast the chemicals
chlorpyrifos and imidacloprid were harmful to *H. variegata* and
*M. tasmaniae*, killing all of the insects treated with these chemicals.
Overall, all the treatments except the control and buprofezin were
harmful or moderately harmful. The reduced risk pesticide, Eco-oil,
was considered moderately harmful for both predators but is likely to
have less of an impact on biocontrol agents recolonising a crop
environment after spraying compared to conventional pesticides.

The methodology used in chapter four was derived from a
preliminary study. Twenty four hours after being sprayed with the
control treatment larvae in the preliminary study suffered high
mortality. After reviewing the method it was concluded that the
mortality observed was caused by not providing food to the first instar larvae. There were no problems with control mortality with the subsequent experiment after changing the method to provide the insects with *A. craccivora* after being sprayed, so this was likely to be the cause.

This study was limited by the scope of the use of only first instar larvae. Early instar larvae are likely to be the stage most vulnerable to pesticides whilst adults may be more tolerant. First instar larvae were used in the experiment because they may be the most vulnerable life stage (Yokoyama et al., 2009). First instar larvae are the life stage least likely to survive exposure to pesticides (Yokoyama et al., 2009) so they may be good candidates for determining potential compatibility. While the study cannot be used to discuss the dosage, it can be applied to the effects of the chemical on the predators when they are sprayed at commercial rates.

**Determination of release rates**

Based on the results of chapter two, a greenhouse study was conducted examining how different densities of *H. variegata* and *M. tasmaniae* control *T. vaporariorum*, one of the most prevalent greenhouse pests. This experiment, outlined in appendix two, attempted to determine whether *M. tasmaniae* could control simulated pest populations outbreaks that might occur under commercial greenhouse conditions. The first iteration of the
experiment in this study was changed after the initial protocol surveyed older leaves that senesced over course of the experiment. The method was revised to use younger plants and the protocol was carried out successfully. Results from this experiment showed the biocontrol agent having no effect on the pest populations. *Micromus tasmaniae* were rarely apparent on the plants during the experiment, and at its termination only three out of the several hundred *M. tasmaniae* released were recovered from the experimental plants. This failure of the experiment is thought to be a result of high temperatures in the greenhouse, where temperatures above 30°C occurred for over six hours on clear days, with a maximum temperature of 44.8±0.5 being recorded in the first week of the experiment. As Samson and Blood (1979) note, *M. tasmaniae* eggs hatch at 30°C, but rarely survive beyond the first instar at this high temperature. Although the average temperature in the greenhouse was around 22°C, a temperature quite reasonable for the development of *M. tasmaniae*, the extended periods of high temperature seem most likely to have resulted in the lack of biocontrol success. The failure of this experiment serves as a warning to low technology greenhouses in the Australian climate where poorly regulated summer temperatures inside a greenhouse may cause biocontrol to fail.

Reinforcing the understanding that temperatures outside of a biocontrol agent’s climatic tolerance will reduce efficacy of control (van Driesche et al., 2008), these data indicate that low technology
greenhouses with little environmental control may drastically inhibit biocontrol agents. Adequate temperature control is needed for biocontrol in greenhouses to be successful. Temperatures that are reasonable for plant growth may fall outside the range of tolerances that are needed for a specific biocontrol agent for biocontrol to be successful. Exceeding these tolerances may cause a biocontrol to be unsuitable for a particular environment. In this context, *H. variegata* is believed to have higher temperature tolerances than *M. tasmaniae*. For example, *H. variegata* adults still function as predators, developing and reproducing at 35°C (El Habi et al., 2000) while *M. tasmaniae* larvae fail to develop at 30°C (Samson and Blood, 1979). The higher heat tolerance of *H. variegata* make them more suitable biocontrol agents than *M. tasmaniae* when used in greenhouses with daily temperatures above 30°C. The recommendations are reversed in cooler greenhouses, where *M. tasmaniae* is the recommended biocontrol agent. Temperatures below 18°C may induce diapause in *H. variegata* adults, while *M. tasmaniae* larvae can complete development at temperatures as low as 5-6°C (Samson and Blood, 1980, Syrett and Penman, 1981).

Inundative biocontrol using adults may still be a possibility if extreme temperatures do not immediately kill the biocontrol agents (Omkar and Pervez, 2004). Adult predators might be the best choice in an inundative approach, but, the experiment in appendix two applied the predator as eggs rather than adults.
Before the second step on figure three, the decision upon whether the agent will be used for conservation biocontrol methodologies or other biocontrol methodologies must be made. Conversation biological control makes use of natural enemies in the environment that does not require rearing or supplies of biocontrol agents, and is not normally involved in greenhouse pest management, so will not be discussed in detail. For other biocontrol methodologies, the next step is determining the release rates to control certain pests (van Lenteren, 2002).

5.2 Broader considerations

Mass Rearing

To be commercially viable, a biocontrol agent needs to be able to be reared in sufficient quantities to provide control against the pest it is targeted against and mass rearing techniques need to be established in order to achieve this goal. The system used to rear *H. variegata* and *M. tasmaniae* in the studies reported in chapters two, three and four was extremely labour-intensive. This workload is primarily due to rearing the *V. faba* plants for two weeks prior to use, maintaining a culture of *A. craccivora* and cleaning and maintaining the predator cultures because of the reliance on arthropod food sources. This inefficient production process is unlikely to be suitable for mass production considering the workforce that would need to be involved.
The solution to this problem might be that artificial diets are provided to the biocontrol agents, which may provide a developmentally sustainable diet without the need for the tritrophic rearing of plant, prey and predator. Knowledge of the suitability of each diet for development of larvae may be useful in determining the composition of an artificial diet by future authors. Chapter two examined several potential natural prey species that may be useful for rearing *H. variegata* and *M. tasmaniae*.

**Artificial or natural diets**

Even an optimised artificial diet can cause problems for biocontrol. Natural enemies reared on an artificial diet have been shown to be less likely to react to semiochemical signals by the host or host plant because of issues arising in the searching, migration and learning behaviour of the arthropods (van Lenteren, 2002). van Lenteren (2002) proposed that where possible, natural enemies be reared on the target pest, on the plant that is to be protected, under normal climactic conditions for the crop. The goal of a mass-rearing system is to obtain large numbers of high quality biocontrol agents (Riddick, 2009). Riddick (2009) reviewed the literature on artificial diets over the past decade, noting the positives and negatives. Riddick (2009) concludes that creating an artificial diet for mass-rearing of biocontrol agents is much more likely to succeed with generalist predators than with specialist predators.
Transit considerations

Once mass rearing techniques have created a supply of *H. variegata* and *M. tasmaniae*, they will need to be transported to the growers for use in crop environments. In this context, being able to store a viable population of biocontrol agents for an extended period, from weeks to months, is highly desirable (van Lenteren, 2002). Chapter three determined that *H. variegata* adults experience cold-induced diapauses which may be useful when creating a delivery protocol. *Micromus tasmaniae* exhibit no such characteristic but is tolerant to low temperatures (Samson and Blood, 1979) which could be used to reduce appetite and movement while being transported.

5.3 Future directions

The studies reported in this thesis have advanced the basic knowledge on the generalist predators *H. variegata* and *M. tasmaniae*, however there is still a considerable amount of research to be done to determine whether they make them commercially viable biocontrol agents. Greenhouse trials need to be undertaken in a temperature-controlled greenhouse to determine how effective the predators are outside of the lab, as well as trialling potential release rates to achieve control in a cost-effective manner. Revisiting dormancy experiments may have some merit in determining whether *H. variegata* or *M. tasmaniae* larvae react to photoperiod-temperature combinations. This may yield new dormancies to exploit for transport purposes. Particularly in the case
of *M. tasmaniae*, a study exposing larvae or adults to low
temperatures might yield a protocol for storage during transit.
Another important area of future study might be to determine if
*H. variegata* adults undergoing diapause are less efficient predators.

An additional concern for using *H. variegata* as a biocontrol agent
may be the species’ poor performance on certain crop surfaces
(Nolan, 2007). Unfavourable crop surfaces, such as those caused by
high trichome density or waxy surfaces may entangle biocontrol
agents (Simmons and Gurr, 2004) or stop larvae from ascending a
plant to where pest species are present (Nolan, 2007).

Currently, *H. variegata* may have slightly more potential as a
greenhouse biocontrol agent than *M. tasmaniae*. Chapter two found
that *H. variegata* consumes more key greenhouse pests
(*M. tasmaniae* did not consume *T. urticae*) and chapter 3 found that
*H. variegata* undergoes a state of diapause that may be exploitable
for transport to growers. Other authors have demonstrated that
*H. variegata* can remain active and reproduce at temperatures as
high as 35°C (El Habi et al., 2000), while *M. tasmaniae* fail to develop
at 30°C (Samson and Blood, 1979). This does not mean that
*M. tasmaniae* cannot still be a viable greenhouse biocontrol agent,
but if future authors had to choose only one biocontrol agent based
on the results in this thesis and the data of other authors,
*H. variegata* may be a better choice.
5.4 Conclusion

Understanding the basic biology of a potential biocontrol agent is a vital part of successfully commercialising a biocontrol agent. The studies in this thesis have investigated some of important points of the basic utility of *H. variegata* and *M. tasmaniae* as greenhouse biocontrol agents but without a successful greenhouse study, these findings are limited in scope. The laboratory studies in the preceding chapters tried to replicate the conditions present in a greenhouse by using similar conditions in controlled environment rooms but the exact light, microclimate conditions or air, might be quite different in the lab compared to a greenhouse. The findings in this chapter need to be confirmed by greenhouse studies before they can be of use to growers using IPM.

A critical step in showing the potential for *H. variegata* and *M. tasmaniae* as greenhouse biocontrol agents is establishing what greenhouse pests will facilitate development and which will not facilitate development. The implications of this work important for conservation biocontrol as well as greenhouse biocontrol. Wild *H. variegata* and *M. tasmaniae* may be able to provide conservation biocontrol against the crop pests investigated, particularly *M. persicae* which a pest in both greenhouse and field crops.
Future studies

Future studies can expand on this work to investigate the performance of the predators on specific crops or against specific pest life stages.

Another important gap to be filled was the determination that *H. variegata* adults undergo diapause, and *M. tasmaniae* adults do not. Now that this information is known, greenhouses that use *H. variegata* can potentially manipulate environmental conditions to avoid it impacting biocontrol, as well as the diapauses being useful to transport *H. variegata* from supplier to grower.

Finally, the work on pesticides will be of use to future greenhouse managers, growers and crop consultants attempting to use *H. variegata* and *M. tasmaniae* in an IPM scheme.
REFERENCES


control of chrysomelid leaf beetles. *Agricultural and Forest Entomology*, 5, 97-106.


Entomology. Available: 
www.entmclasses.umd.edu/peap/leaflets/pil43.pdf [Accessed 19/10/07 43].

*Biocontrol*, 48, 141-153.


*Biocontrol*, 33, 173-183.


EL HABI, M., SEKKAT, A., EL JADD, L. & BOUMEZZOUGH, A. (2000) Biology of Hippodamia variegata Goeze (Col., Coccinellidae) and its suitability against Aphis gossypii Glov (Hom.,


HEIMOANA, V. (unpublished) The potential of *Hippodamia variegata* (Coleoptera Coccinellidae) and *Micromus tasmaniae* (Neuroptera: Hemerobiidae) as biological control agents for arthropod pests in Brassica crops. *School of Agricultural and Wine Sciences*. Orange, Charles Sturt University.


pest management in the greenhouse industry. *Biological Control*, 52, 216-220.


(Coleoptera, Coccinellidae) reared on a liver-based diet. *Archives of Insect Biochemistry and Physiology*, 68, 26-39.


rearing and storing a Mexican population. *Biological Control*, 8, 185-190.


Hemstead, Hertfordshire, UK.


**Australian Centre for International Agricultural Research (ACIAR) Canberra Australia:** 2001. 559. 1043 ref.


APPENDIX I - Preliminary evaluation of *Micromus tasmaniae* and *Hippodamia variegata* for greenhouse control of *Trialeurodes vaporariorum*

Abstract

Six different release rates of *Micromus tasmaniae* and a predator-free control were trialled for the control of *Trialeurodes vaporariorum* in order to determine the levels of control that might be achieved under greenhouse conditions and the optimal release rate of predators on cucumbers. Throughout the experiment, very few of the released *M. tasmaniae* were observed on test plants and numbers of *T. vaporariorum* were not significantly affected by any of the predator density treatments. Examination of the environmental data loggers in the greenhouse revealed that temperatures were above 30°C for over eight hours each day with a maximum temperature of 44.5°C over the course of the experiment. Other studies have found that *M. tasmaniae* cannot complete development and suffers high mortality at 30°C (Samson and Blood, 1979). The high temperatures are, therefore, almost certainly the cause of the failure of biocontrol in the experiment. A second experiment using *Hippodamia variegata* was aborted after the failure of the first experiment. Clearly, future trials in similar greenhouses at the same time of year will need better environmental controls. This experimental failure provides a graphic illustration of one of the key constraints for the use of this and other
predators in greenhouse biological control in Australia: unlike many greenhouses in Europe (where biocontrol is mainstream) those in Australia often have poor temperature control.

**Introduction**

Worldwide, growers make use of a variety of predatory biocontrol agents in greenhouses, including Cecidomyiidae (gall midges), Chrysopidae (green lacewings), Coccinellidae (ladybirds) and Anthocoridae (pirate bugs) (van Driesche et al., 2008). Most of the species that are successful in other countries are not available in Australia, creating a need for the research of alternative biocontrol agents.

Not all greenhouses in Australia will be suitable for biocontrol. Greenhouses with low levels of investment in modern technology are often characterised by lack of environmental controls, copious pesticide use and poor crop management (Badgery-Parker, 2005), all of which may contribute to the failure of biocontrol. Biocontrol in Australian greenhouses is more likely to succeed in high-technology greenhouses that have modern environmental controls, crop hygiene and appropriate pest management practices (Badgery-Parker, 2005). Growers with low-technology greenhouses that do not make use of biocontrol agents may have trouble controlling pesticide resistant species such as *Trialeurodes vaporariorum*. 
Trialeurodes vaporariorum is one of the most serious pests of greenhouse crops worldwide, causing massive damage to the crops by direct phloem feeding and as well as the transmission of plant viruses (Deligeorgidis et al., 2005). Part of the problem of controlling T. vaporariorum is the species’ resistance to commonly used pesticides. The resistance of pests such as T. vaporariorum are a factor driving greenhouse growers to biocontrol (Pilkington et al., 2010). Currently there are only two biocontrol agents, Encarsia formosa and Mallada signata, targeted for control of T. vaporariorum in Australia (http://www.goodbugs.org.au/, accessed 12/02/10). While the role of E. formosa is well documented (see Hoddle et al. (1998) for a review), the role of M. signata as a biocontrol agent is undescribed in the literature.

Generalist predators H. variegata and M. tasmaniae have the potential to fill this gap as biocontrol agents of T. vaporariorum in greenhouses. Both H. variegata and M. tasmaniae consume a range of greenhouse pest species, including Frankliniella occidentalis, Myzus persicae, Tetranychus urticae and T. vaporariorum (chapter two). Based on the study in chapter two, T. vaporariorum may sustain M. tasmaniae or H. variegata through development.
Materials and Methods

A 10x40m greenhouse was planted out with 35 cucumber plants (*Cucumis sativus* (L.)) in single pots at Gosford Primary Industries Institute (New South Wales, Australia). Fourteen day-old cucumber plants were exposed to 60 adult *T. vaporariorum* for 14 days to allow establishment of a uniform aged *T. vaporariorum* population prior to predator release. The cages were made out of Swiss voile cotton lace, 0.40x0.45x1.6m suspended from the roof of the greenhouse by string. The plants were maintained by fertigation using greenhouse cucumber nutrient mix in an NPK ratio of 5:1:10.

The experiment used five randomised blocks of seven biocontrol agent release rates including a zero rate control. Predators were released as unhatched egg clusters that were oviposited on strips of black cotton that were secured to the stem of the individual cucumber plants.

Release rates were 0, 5, 15, 20, 25, 30 and 40 eggs/plant. Observations of *T. vaporariorum* numbers and *M. tasmaniae* numbers on the bottom three leaves of each plant were taken using a x10 magnification hand lens. These observations were recorded weekly for 28 days.
Experimental design

The greenhouse was split into five blocks of the seven treatments, arranged in a balanced randomised design within each block. The count of whitefly population on the three bottom leaves were analysed using separate ANOVAs for each week Genstat 11.1 (VSNi, 2008).

Results

There was no significant effect of the predator treatments on whitefly density across all dates (df=139, F=1.11, P=0.39). The count data illustrated in Figure 1. The number of whitefly eclosed was determined by counting the empty pupal casings of *T. vaporariorum*, but these data did not show a significant effect of predator treatment densities of the number of eclosed *T. vaporariorum*. Only three *M. tasmaniae*, two larvae and one pupa, were recovered from the cages. Observations of *M. tasmaniae* over the course of the experiment were very rare.
Figure 1 Count data and average for the number of *T. vaporariorum* larvae and pupae on the lower three leaves of cucumber plants in a greenhouse when treated with different numbers of *M. tasmaniae* eggs

Discussion

Whitefly numbers were not impacted by the presence of *M. tasmaniae*. At the start of the fourth week (15/12/2010) the *T. vaporariorum* had started to eclose on the leaves samples, and had completely eclosed at the end of the experiment (23/12/2010).
The lack of significant difference in whitefly numbers between the *M. tasmaniae* treatments and the control infer that no observable biocontrol took place and the experiment was a failure. Based on the failure of experiment one, a proposed second experiment using the same method but with *H. variegata*, was aborted. Eggs of *H. variegata* were to be deposited on red plastic tape rather than black cotton.

In light of this setback the environmental data loggers were examined in an attempt to explain why biocontrol using *M. tasmaniae* failed. The average temperature was 22°C, an optimal temperature for *M. tasmaniae* (Syrett and Penman, 1981), but peaks as high as 44.8°C were recorded in the first week, with temperatures above 30°C lasting more than eight hours per day (Figure 2). These unexpectedly high temperatures are well above the environmental tolerances of *M. tasmaniae*, which ceases development and suffers high larval mortality above 30°C (Samson and Blood, 1979). The extreme temperature in the greenhouse is likely to have contributed to the lack of biocontrol success, if it was not the sole causal factor. The environmental tolerances of *H. variegata* which suffers increasingly high mortality above 35°C (El Habi et al., 2000) would have been exceeded under the same conditions that *M. tasmaniae* were exposed to and if experiment two had been undertaken then *H. variegata* is likely to have suffered some degree of larval mortality.
It was concluded that the greenhouse had inadequate cooling mechanisms for biocontrol during the Australian summer. Small differences between the environment and the climatic range of a biocontrol agent can make the difference between success and failure (van Driesche et al., 2008). Future studies should use greenhouses with adequate environmental controls that can keep temperatures within the environmental tolerances of the biocontrol agent. In the wider context of commercial greenhouse management, low technology greenhouses are less likely to have these environmental controls than high technology greenhouses suggesting that biocontrol is less likely to be successful in low technology greenhouses in the warm summer months. However,
there are a variety of low-technology modifications that can be made
to help moderate temperatures.

References

NSW. Practical Hydroponics and Greenhouses, 41-45.

DELIGEORGIDIS, P. N., IPSILANDIS, C. G., VAIPOULOU, M.,
of Coccinella septempunctata on Thrips tabaci and
Trialeurodes vaporariorum. Journal of Applied Entomology,
129, 246-249.

Biology of Hippodamia variegata Goeze (Col., Coccinellidae)
and its suitability against Aphis gossypii Glov (Hom.,
Aphididae) on cucumber under greenhouse conditions. Journal
of Applied Entomology-Zeitschrift Fur Angewandte
Entomologie, 124, 365-374.

Biology and use of the whitefly parasitoid Encarsia formosa.
Annual Review of Entomology, 43, 645-669.

PILKINGTON, L. J., MESSELINK, G., VAN LENTEREN, J. C. & LE
MOTTEE, K. (2010) " Protected Biological Control" - Biological
pest management in the greenhouse industry. Biological
Control, 52, 216-220.

SAMSON, P. R. & BLOOD, P. R. B. (1979) Biology and temperature
relationships of Chrysopa Sp, Micromus tasmaniae and Nabis


APPENDIX II “Protected Biological Control” –

Biological pest management in the greenhouse industry

This paper was co-authored over the course of the candidature by the PhD candidate. This paper makes use of an early draft of Chapter One.
Review

"Protected Biological Control" – Biological pest management in the greenhouse industry

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ABSTRACT

This paper briefly describes the foundations and characteristics of biological control in protected cropping and what drivers are behind adoption of this management system within this industry. Examining a brief history of biological control in greenhouses and what makes it a successful management strategy within the industry, the authors describe the rapid growth of biological control in parts of Europe and what this may mean for the industry in other parts of the world. The reaction of the greenhouse industry to several consumer-led campaigns aimed at reducing the incidence of pesticides in the marketplace may be replicated in many other parts of the world. The size and robustness of the biological control industry in greenhouses, which is a reflection of the inherent characteristics of this industry that lends itself to biological control, is strong and growing with indications that this trend will be followed in many areas of the world.

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1. Introduction

The focal point of this review will be to examine the size, both in land-use and in economic terms, and placement of the biological control industry in protected cropping in a global context with some emphasis on the industry in Europe where much of the greenhouse industry is being driven. Although biological control in the greenhouse industry is not a new concept, there have been several recent advances and changes to the manner in which growers facilitate this pest management system and several reasons for a recent expansion of its use in Europe. This review will examine the size and scope of the biological control industry in protected cropping, examine recent events that have provided impetus for the industry to adopt biological control and speculate on the impact of these drivers on the industry as a whole.

2. Protected agriculture

The protective environment that greenhouses offer is now extensively utilised by primary producers around the world. Estimates of production in greenhouses of all materials reach as high as 2,600,000 hectares worldwide with approximately 45,000 hectares under glass structures (van Lenteren, 2006). This figure has increased markedly in recent times with estimates only seven years earlier being 307,000 hectares (Gollino et al., 1998). In an agricultural context, greenhouses enable growers to mitigate the negative effects of climatic variations such as light intensity, temperature and water availability (Manrique, 1993), increase growers’ proximity to the marketplace and, to a certain degree, exclude pests (Hanan et al., 1978). The extent to which environmental conditions inside the greenhouses are controlled are largely dependent on the technology available to the grower; the costs of which are generally high (Manrique, 1993).

The pests common in greenhouses are often not specific to any one level of technology and are typically polyphagous, having a series of different host plants that they can infest and are generally more problematic in greenhouses than in the field. This is typically because of an ideal warm and moist abiotic environment, isolation from natural enemies or pathogens, or by tending to favor development of resistance to conventional pesticides through the typical pesticide use patterns observed in protected agriculture (Bielza, 2008).

Use of biological control agents in the greenhouse environment has been shown to be a viable alternative to pesticide use from both an environmental and economic perspective (van Lenteren, 2000a). There has been a recent increase in the uptake of biological control of, predominantly, vegetable pests but also seen in ornamental greenhouse production in Europe which has been attributed to a campaign designed to reduce the levels of pesticides found in vegetables tested in European markets (Krautter,
3. Biological control in greenhouses

Use of biological control in greenhouses is likely to increase markedly over time as more managers seek to reduce the effects of pesticide resistance and change the impendiments to pest control. Protected agriculture is, at times, more susceptible to pest and disease pressure than conventional/field production systems. Greenhouses have high plant host density with non-stop production systems which lends itself to the spread of pests and diseases, and often well fertilized and irrigated crops are more sensitive to outbreaks of this nature (Gullino et al., 1999). The climate control that is often available to greenhouse production systems, however, lends itself readily to optimizing conditions for the implementation of biological control (van Lenteren, 2000b) through benefits such as the effective prediction of pest and natural enemy development under controlled conditions (van Roermund et al., 1997). The adoption of biological control in the industry, by the paucity of pesticide registration for use in protected agriculture in many countries, consumer expectations regarding the presence of pesticide residues in market products and the inherent health risks to workers in greenhouses from the application of pesticides in enclosed places. In greenhouses, once a successful move is made to biological control and growers gain the knowledge and experience needed to manage pests in this way, it is often recognized that there are no phytotoxic effects associated with biological control, the release of natural enemies is far more amenable to workers, there is no withholding period after their release, and there is no resistance build-up (van Lenteren, 2000b).

Biological control is not a new concept with evidence of organized biological control being used for as long as 1700 years (Gurr et al., 2000). Our ability and understanding of augmentative releases, however, is considerably younger with the first augmentative release in Europe attributed to Réaumur who, in 1734, advised growers to release lacewings (Neuroptera) in greenhouses for the control of aphids (Homoptera) (Lueck and Forister, 2003). Erasmus Darwin suggested that aphids in greenhouses could be controlled through the augmentative release of hoverfly (Syrphidae) larvae (van Lenteren, 2007a). Kirby and Spence (1815) continued the assault on greenhouse aphids by suggesting ladybird beetles (Coccinellidae) could also be used.

Overall, while certain generalities over greenhouse pests can be drawn, no one strategy can be recommended to combat them all. Over 50 years of pesticides use have been shown to create a series of far reaching environmental problems. Biological control agents for pest organisms have typically been specialists over the short history of greenhouse biological control use, although research on generalist predators is increasing (Sabelis et al., 2008). Specialist predators can be regarded in certain systems as being inefficient at managing populations of fast breeding pests and the use of generalist predators suggests that alternative food sources are available prior to problem pest outbreaks (Snyder et al., 2004) which is often not the case in greenhouse systems. Generalist predators often perform much better when provided with supplemental foods provided by crops that have large amounts of pollen resources such as sweet pepper (Eubanks and Stensky, 2005). This abundance of pollen allows generalist predatory arthropods to build-up in numbers in the absence of pest populations and is a valuable trait for biological control in greenhouses.

4. Parasitoids in greenhouses

Whilst evidence of insect predation dates the 4th century, insect parasitoids, defined as insects that are parasitic only in their juvenile life stages and kill their hosts outright (van Lenteren and Godfray, 2005), are attributed to a later discovery. First reported in China in the late 11th century, in Europe in the 17th century and in much of the rest of the world in the 18th century (van Lenteren and Godfray, 2005), they have grown to be the most common type of biological control agents introduced against insect pests (van Driesche et al., 2008).

The first success of inoculative biocontrol in protected cropping was seen using Encarsia formosa Gahan for the management of Trialeurodes vaporariorum (Westwood) before 1939 in the US (Spreyer, 1927). Whitefly nymphs and adults are sap-sucking phloem feeders, and mainly cause injury through indirect damage by excreting honeydew encouraging sooty mould growth. Importantly, in addition to causing phytotoxic effects in some cucurbit crops (Brodsgaard and Allajas, 1998), whiteflies are also efficient vectors for economically important plant diseases and some biotypes are known for their ability to quickly develop insecticide resistance in conventional management programs.

The use of E. formosa as a biological control agent is often considered to be, historically, one of the biggest success stories in greenhouse biological control (van Lenteren et al., 1996). During the 1930s E. formosa was sent to other parts of Europe as well as Canada, Australia and New Zealand, although after World War II, use of pesticides caused this practice to be discontinued. With the rise of pesticide resistance in the 1970s, interest in whitefly parasitoids was renewed. In 1996, 20 out of 35 countries with a greenhouse industry were using E. formosa (van Lenteren et al., 1996).

In many cases of pest management, such as the biological control of Lygus spp., parasitoids are the best and often only form of biological control available (Salvo and Valladares, 2007). Natural enemies that are highly specific are also considered a smaller risk with respect to non-target impacts (Lunda et al., 2005). Parasitoids, particularly those released in greenhouse systems, are often highly specialized in their hosts (Lynch et al., 2001) and it is possible that non-target risk may be mitigated through the use of these organisms.

5. Predators in greenhouses

Among the predators used for biological control in greenhouses, there are specialists and generalists. The most important specialist predator is the phytoseiid predatory mite Phytoseiulus persimilis Athias-Henriot (Acari: Phytoseiidae), which is a foundation biological control agent for the historical success of biological control of spider mites in greenhouses (Bravenboer and Dosee, 1962) with a high rate of predation (Takahashi and Chant, 1986). This arthropod is an early addition to the arsenal of commercially available biological control agents and since its first discovery in the 1950s, it has grown dramatically as a tool used by many growers in greenhouses (van Lenteren, 1995) as well as in the field.

For arthropods the terms 'generalist predator' and 'polyphagous predator' are largely unspecific labels that are used to describe the predatory behavior of a wide group of arthropods (Symondson et al., 2002). This group of predators is generally not limited to arthropod prey, also eating plant-provide food and fungi leading to additionally being referred to as 'generalist omnivores'.

Because a large proportion of predators collected from the field are found to have empty guts (Greenstone, 1978; Sunderland, 1975), predators are often in a state of suboptimal nutrition (Anderson, 1974; van Dijk, 1996). As a result of this, generalist pre-
dators have developed behavior to cope with prey scarcity, eating anything they can subdue in order to survive (Bidle and Toft, 1998) and exploitation of alternative food (non-arthropod) sources that extend survival (Sabbatini and Denno, 2000). Many of these alternate food sources may not support predator development and adult reproduction (Evans et al., 1998) and so the prey of generalist predators can be split into "essential foods" that facilitate reproduction and maturation of larvae and supplemental foods which maintain the predator until it can obtain essential foods.

One danger of using generalist predators in isolation or with other agents is that the predator may attack other biological control agents with whom it shares prey (intraguild predation) or not (higher-order predation) (Polis et al., 1988; Rosenheim, 1988). The effect of intraguild predation, however, is widely varied across many systems and there are observations that suggest interaction between biological control agents can have a positive effect on control, no effect or a negative impact (Rosenheim et al., 1995).

The use of generalist predators for biological control in greenhouses has to be evaluated in each specific context of pests and other environmental conditions. It is not only the species of predators, but also the presence of more than one pest species. Having multiple prey species may increase the level of control delivered by generalist biocontrol agents by providing ample food sources to maintain populations and enhance predator performance due to the mixed diet effects of an agent (Messemer et al., 2008). It has further been reported that cannibalism among predators may allow the predator to remain at effective population levels if prey density decreases to a level precluding effective population growth (Budlo, 2007).

Notwithstanding several barriers that are needed to be overcome in each case before a single generalist predator is able to be used effectively for a range of different pests in a crop, there is great interest and increasing focus being placed on, for example, a generalist predator such as Amblyseius swirskii Athias-Henriot in the management of a range of pests including the major pest species whiteflies and thrips (Messemer et al., 2008; Nomikou et al., 2002). A clear benefit in utilizing a predatory mite for a number of different pests compared to the use of predatory bugs such as Orius spp. is the greatly reduced commercial production costs of mites, especially for those species that can be reared on wheat bran with storage mites (Ramakers and Lieburg, 1982).

Of particular interest in the growing use of generalist predators is the increasing drive for development of mite species for the management of whitefly populations (Nomikou et al., 2001). Generalist predators have become extremely important for the greenhouse industry and receive increasing attention in research (Sabelis et al., 2008) but are not without problems as they appear to be more problematic for registration (van Lenteren et al., 2006).

6. Pesticides as a driver for biological control?

Across agriculture as a whole, there is an extremely large, and mostly effective, synthetic pesticide market worth in excess of US$1 billion (AGROW, 2006) that greenhouse growers can draw on. Uptake of biological control will be slowed in many greenhouse growing areas of the world while these pesticides are readily available, easy to use and relatively cheap. While the major focus often reverberates the negative impacts of pesticides, growers are often forced to weigh up the cost of reducing their reliance on pesticides with the benefits and strengths of intensively used pesticides (Cooper and Dobson, 2007).

Many countries have adopted strategies to reduce reliance on pesticides and encourage, or in some instances force, growers to consider other strategies in managing their pests. For example, the Pesticides Safety Directorate in the United Kingdom lowered the registration fees associated with the development of biostetic pesticides in 2006 in an effort to increase availability of reduced-risk pesticides to growers (Economic & Social Research Council, 2008).

In recent years there has been an extremely active campaign pitched against the use of pesticides in agriculture in Europe which has driven, along with increasing demands on efficient use of irrigation water in many parts of the world, the increase of production area and market share of the greenhouse industry and use of biological control in these systems. These campaigns have been successful in encouraging the use of IPM in the greenhouse industry because they have, in large part, targeted the greenhouse industry and because greenhouse structures lend themselves very well to the use of IPM and, in particular, use of biological control agents.

The success of biological control in greenhouses can be attributed to many factors. By their very nature, greenhouses allow the grower to exclude many pests after a rigorous sterilization process where pest organisms may be cleansed from the system (van Lenteren, 2006b). As a result of unfavorable conditions for the pest outside the greenhouse, once the pest is removed from the system and a new crop initiated, it is also possible that the pest will not re-establish within the crop at there is no local population in the surrounding area (van Lenteren, 2006b). Further, the establishment of biological control organisms may be optimized because some pesticides, such as fungicides for example, may be reduced due to cultural practices that are inherent in greenhouse horticulture. If pesticide applications are necessary they are applied locally, limiting impact on adjacent growing units because of their isolation (van Lenteren, 2006b).

This style of campaign was also used in Austria (Buetscher, 2006) and The Netherlands (Maierman, 2006). These campaigns are often considered successful due to the increase in public awareness and, importantly, the increased pressure that is placed on the entire industry from farm through farm-gate to the suppliers and retailers. This pressure to self-regulate, placed on the industry as a whole, is imperative for the successful and widespread uptake of integrated pest management and biological control in any agricultural system.

The formulation of documents such as the Sixth Environmental Action Programme, written by the European Commission, reinforces and validates consumer sentiment and assists pesticide manufacturers and suppliers in this regulation by suggesting actions aimed at substituting dangerous active ingredients with safer or non-chemical substances. The document also actively encourages the uptake of low input or pesticide-free agriculture for the industry as a whole. The document goes on to encourage the use of integrated pest management strategies and limiting use of dangerous pesticides by creating monetary drivers such as taxes and duties to drive up the cost of these chemicals (Eurostat, 2007; Neurois, 2003).

Whilst these are important drivers to the uptake of biological control in the greenhouse industry, there is a complex network of extra motivations for growers to consider increasing their use of biological control. Few new insecticides are becoming available on the marketplace for greenhouse growers and those that are available are suffering an increase in resistance leading to growers seeking to reduce the impacts of the pesticide treadmill (van Lenteren, 2007a). Further to these reasons, the reduced exposure of workers, and the environment, to extremely toxic chemicals is far more attractive to managers and the comfort of releasing natural enemies is far greater for workers (van Lenteren, 2006b). Biological control in greenhouses is often timed to coincide with downtime for growers and the similar costs when compared to conventional pest management can make it an attractive management strategy (van Lenteren, 2006b).
7. The uptake of greenhouse biological control

In essence, there has been a large shift to the use of IPM in greenhouses in many parts of the world, with perhaps the most striking one recently in Spain. Prior to 2006, biological control was only used on a small scale, something that has changed dramatically in the European greenhouse season of 2007/2008 when more than 75% of the 8000 hectares of sweet pepper in Almeria started to implement biological control as a pest management strategy (Van der Blom et al., 2008). The role of organized and well orchestrated campaigns has helped facilitate this change with different management systems in food crops being demanded, often, by consumers and retailers. For this drive for change to be successful through the entire supply chain and, ultimately, has a persistent alteration of pest management practices on the other side of the farm gate, it must be supported by the availability of efficient biological organisms, that are able to be mass reared in a cost effective and efficient fashion, that can take the place of conventional pesticides in greenhouses. The availability of these agents, in turn, must be supported by an effective and non-obtrusive progression through any regulatory processes that may be present in any area. All too often promising biological control agents, and in particular entomopathogens, are stalled in the registration process preventing the use of what is often an excellent tool for the management of pests that will relieve growers’ reliance on synthetic pesticides.

The changes required in greenhouse pest management in many regions of the world, like all other agricultural systems and indeed other disciplines, are often driven by crisis. The campaigns delivering information to consumers and creating a reaction in a part of the market that creates a ‘market pull’ has provided such a crisis for several parts of the world leading to vast and sweeping changes in the way growers have viewed the use of pesticides. This crisis is yet to come to many parts of the world, and perhaps serves as a warning to many systems suggesting that, if change is on the way, understanding and developing strategies including biological control in greenhouses is something that should be started early to avoid the disruption that sudden change is sure to bring.

8. The scale of biological control use in greenhouses

While biological control has been increasingly used in many cropping systems for pest management with more than 150 species of natural enemies available to growers around the world (van Lenteren, 2007), only approximately 30 species constitute 90% of total global sales (Beldmann, 1999). It is estimated that 80% of the global commercial revenue generated by biological control agents may be attributed to their use in greenhouses (van Lenteren, 2007) where commercial biological control agents are used in approximately 32,000 hectares of greenhouse structures around the world (van Lenteren, 2006). This figure might be higher now as the authors understand that, while there is no official report, 20,000 hectares of greenhouses in Spain are now routinely using biological control. In greenhouse systems, *E. formosa* accounts for 25% of the total greenhouse revenue (van Lenteren, 2007) followed by the predatory phytophaga *P. persimilis* (Athias-Henriot) and *Amblyseius cucumeris* (Oudemans) at 12%. This division of agents available to the greenhouse industry will, however, have changed significantly in very recent years with the success of another the *Phytoseiid*, A. swedii (Athias-Henriot) increasing in Europe and North America. The market for biological control in Almeria, Spain, is estimated to value 30 million Euros, which is more than the total market in the rest of Europe as a whole. The two most important natural enemies in this area are *Orius laevigatus* (Fieber) and *A. swedii* (Van der Blom, personal communication).

There is considerable disparity between regions when examining the use of IPM and biological control. In the Netherlands, for example, 90% of greenhouse vegetable production is under an IPM system, substantially higher than the 5% global average (van Lenteren, 2000b). In 1970, European greenhouse production that utilized biological control was a mere 200 hectares and was serviced by two commercial producers of agents (Dent, 1995) but by the turn of the century, the use of biological control agents has grown to 15,000 hectares (van Lenteren, 2000b) and this has been rising steadily since.

9. Conclusion

The greenhouse industry is reacting to several drivers such as efficient utility of a scarce water-supply and to increasing production through appropriate climate controls. Greenhouse production in Spain, for example, has grown exponentially in recent years and this trend may be reflected in other parts of the world. This is highly relevant due to the fact that the greenhouse environment provides unique opportunities for the management of pests through use of characteristics that are synonymous with greenhouse production. This is true regardless of which pest biological control agents has grown with the size of the industry and expenditure on biocontrol agents in greenhouses represents the majority of sales of commercial biological control agents globally.

Greenhouse production systems lend themselves well to biological control but this industry is also reacting strongly to external influences on the use of pesticides which is driving up the use of commercial biological control agents. There are a number of drivers for greenhouse growers to decrease their reliance on synthetic pesticides. In many countries worker safety, paucity of registrations for use of pesticides within greenhouses, and consumers driving retailers to demand pesticide-free produce from their suppliers has led to a decrease in pesticide use, and an increase in utilization of biological control agents in greenhouses.

There is no doubt that the biological control industry in greenhouse production is strong and there is evidence that it is still growing. With external drivers and internal support, it is possible that pesticide use in greenhouses may be further minimized and biological control will become the cornerstone of pest management strategies in greenhouse production systems, as it is already in many greenhouse vegetable crops. With much research being conducted on these strategies in greenhouses, the use and efficacy of biological control is only set to continue to grow.

References


Snye, R.E., 1957. An important parasite of the greenhouse whitefly [Myzus persicae (Sulzer)]. Bulletin of Entomological Research 47, 301-308.


Van Lenteren, J.C., 2006c. The area under biological control and IPM in greenhouses is much larger than we thought. Chapter 30, 7.


