Development of Hippodamia and Micromus biocontrol agents for use in Brassica and other vegetable crops

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Department of Primary Industries

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(August 31 2011)

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Submitted August 31 2011

The purpose of this report is to summarise a series of trials that have were a part of two dovetailing postgraduate studies. One study examined the utility of two generalist predators in an inland setting and their potential use as a part of conservation biological control. The other study examined the same generalist predators' utility in a greenhouse context. Both students completed successfully with one student attaining a Doctor of Philosophy award and the other being awarded a Masters of Philosophy. These two studies have contributed to the understanding and future use of two very important biological control agents.

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Media Summary

Complete reliance on chemical control in vegetable crops is an unsustainable approach to pest management. For some time there have been difficulties experienced with managing pest thrips, particularly western flower thrips, and aphid numbers. In the lettuce industry, for example, there is the ever-present threat of the lettuce aphid *Nasonovia ribis-nigri*.

The predatory white-collared ladybird (*Hippodamia variegata*) which is an introduced species and widespread throughout Australia was targeted as an industry priority for development and one focus for the PhD project. Another beneficial insect, the Tasman's lacewing (*Micromus tasmaniae*), a native to Australia and also widespread, also has great potential for this purpose.

Both species were studied in simultaneous postgraduate research projects that yielded no only important information about the biological control agents, but also produced two industry professionals that will remain an important part of the industry now that their studies are complete.

These two postgraduate studies conducted research to develop essential laboratory information and field data on these natural enemies and effective integrated pest management strategies for use in field Brassica and greenhouse vegetable crops. Limitations, such as climate, that may interfere with the year-round use of either or both of the predators in different vegetable growing districts was examined and recommendations made for their best-use practices.

Technical Summary

Generalist predators *Hippodamia variegata* and *Micromus tasmaniae* 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 project are the photoperiod and temperature conditions that may initiate dormancy and whether biocontrol agents are adversely affected by the pesticides commonly used in greenhouses, habitat range in field situations, range of wild prey species and knowledge of the beneficial insects' movements.

Monthly surveys of various habitats on vegetable farms in the Central West of New South Wales, Australia showed that both species are an important numerical component of the natural enemy fauna. Numbers increased during spring but were not uniformly high over summer months possibly reflecting broad-spectrum pesticide use and lack of available prey. Predator densities in non-crop habitats were relatively high in the period leading up to Brassica crop planting and may be an important source of natural enemies.

A 'mark – capture technique' study was conducted to quantify movement of predators between crop and non-crop habitats. This involved the non-crop vegetation adjacent to Brassica crops being sprayed with a dye mixture developed by the South Australian Research and Development Institute (SARDI). Arthropods were sampled 20, 40, 80 and 100 m into the crop 2, 5, 7 and 10 days after treatment. That work showed that the proportion of wild predators that was marked (therefore was in the non-crop habitat at the time of dye application) was relatively high; approximately 2/3 for *H. variegata* and 1/3 for *M. tasmaniae*. Marked ladybirds and lacewings remained a very significant portion of the insect catches even 10 days after dye application. This finding is important in establishing that both predator species move into crops from the adjacent non-crop habitats. The spatial sampling in that study further demonstrated that marked predators were recovered even at the farthest point of the crop transect, 100m from the non-crop vegetation. It is noteworthy that the total catch trends (ie marked plus unmarked) for both predators declined with distance from the field margin, further evidence that in-crop biological control by lacewings and ladybirds is likely to be influenced by edge effects and more effective in crop margins.

A follow-up re-population experiment was established to investigate how the two target predators would re-populate an insecticide sprayed field over time and whether re-population of the field was from neighbouring cropland and non-crop vegetation or via longer range movement. This experiment again demonstrated the significance of non-crop habitats as source habitats from which natural enemies re-populate Brassica fields immediately after disruption by insecticide spraying. It also demonstrated that remnant croplands can act as a refuge for predators when fields are sprayed with insecticides and a source habitat for re-population of the sprayed field. Many factors influence insect behaviour within a crop and movement out of a crop such as microclimate, prey availability, competition between predators or intraguild predation.

DNA gut analysis of field collected predators was carried out to examine the dietary habits and the relationships between predators *H. variegata* and *M. tasmaniae*. Results showed that both were generalist predators with a broad diet range. This included for both predators the primary Brassica pests *P. xylostella*, *B. brassicae* and *P. rapae*. Therefore both *H. variegata* and *M. tasmaniae* could be good potential predators for use in a Brassica IPM system. One of the main practical problems in such a system would be intraguild predation which was shown to be highly asymmetrical and in favour of *H. variegata*, with a high percentage of its gut contents testing positive for *M. tasmaniae*.

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*, *Tetranychus urticae, Trialeurodes vaporariorum*, *Frankliniella occidentalis* and *Aphis craccivora*. 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 bifenthrin, 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 bifenthrin, 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 IPM needs to be considered in order to improve the use of biocontrol agents with the spraying of pesticides.

This project contributes towards the body of knowledge on *H. variegata* and *M. tasmaniae* and their future use as biological control agents in greenhouses and against Brassica crop pests. It has demonstrated the importance environmental control in greenhouses and the importance of non-crop areas in agro-ecosystems as alternative and source habitat for natural enemies.

Introduction

Growers continue to rely on chemicals for the control of a range of insect pests in vegetable crops. Since its accidental arrival in Australia in 2000, the ladybird *Hippodamia variegata* has demonstrated to vegetable growers that it is potentially a very useful predator for aphid control, and possibly control of some other small, soft-bodied insect pests such as thrips and caterpillar eggs and small larvae.

Properly managed, this natural enemy could significantly reduce, or possibly even replace, the use of chemicals against these pests. In addition, the native brown lacewing *Micromus tasmaniae* has long been regarded as having similar potential as an effective natural enemy for the same purposes.

To date there has been no ecological study on these species as the basis for the development of an effective pest management strategy for targeted vegetable crops in Australia. In New Zealand, *Micromus* is being effectively used against the invasive lettuce aphid, *Nasonovia ribis-nigri*. This aphid pest is now present in Tasmania and could possibly make its way on to the mainland and become a significant problem for the lettuce industry.

Hippodamia variegata is originally a Palaearctic species that has spread worldwide with records from India, Africa, North and South America and, most recently, Australia. Adult ladybeetles are 4 – 5 mm long, of convex-oval shape and basic red colour with 3-15 black spots and spot fusions are common. The pronotum is white around the outer edges with a more or less symmetrical black pattern. In males, the front of the head has more of the white pattern than the female (Figure 1). Adults have fully developed hind wings and readily fly.

Figure 1: Female and male *Hippodamia variegata* (Photo courtesy of Brendan Nolan, QDPI)

Eggs are yellow and conical and are laid on leaves in clusters of 10-50 near prey (Figure 2). The grey-black larvae are elongate and somewhat flattened with angular legs. They are covered with small tubercles or spines resembling well armoured alligators (Figure 3).

Figure 2: Eggs of *Hippodamia variegata*

Figure 3: Larva of *Hippodamia variegata*

The length of the lifecycle is determined by climatic factors, such as temperature and humidity, as well as ecological factors such as food supply. Under optimal conditions the lifecycle from egg to adult may take about two weeks, however, cooler conditions can extend this to six weeks.

Micromus tasmaniae has been described as a predator of aphid species on a number of crops. In cotton it is considered an important predator of aphids, mites, *Helicoverpa* eggs and other soft bodied insects. In New Zealand, it was reported to feed on mealybugs on pipfruit (apples, pears and nashi) but was not deemed an important control agent of this pest. In lettuce and celery, brown lacewings fed on aphids, mealybugs, scale insects and whiteflies and both adults and larvae are predacious. They are common throughout Australia and are often found on native vegetation, especially when it is in flower.

Micromus tasmaniae adults (Figure 4) are up to 12 mm long and are delicate and slender with prominent eyes and lacy brown wings. The wings are narrowly oval with a slightly pointed apex. Larvae are smaller than those of green lacewings and do not cover themselves with debris. They are slim and elongate with the abdomen tapering towards the end. The most prominent features of the square head are the sickle-shaped pincers, which are used to catch and hold prey. *M. tasmaniae* larvae are greyish-brown in colour with dark bands along the edges of the body. Eggs are elongate ovoid and usually attached by a long dorsal axis to a substrate such as a leaf. They are 0.78 mm ± 0.02 long and 0.34 mm in width and whitish-pink in colour when freshly laid. Towards hatching they darken to a pale grey.

Figure 4: Adult *Micromus tasmaniae*

Materials and Methods

Influence of Non-Crop Vegetation and Insecticides on the Presence/Absence and Abundance of Predators

Introduction

This study aimed to determine the relative temporal and spatial abundance of *H. variegata* and *M. tasmaniae* in Brassica crops and the adjacent habitats that may serve as source habitats. It also assessed the field impact of pesticide use on the predators' potential as biological control agents for Brassica pests. Predator densities are predicted to be higher on surrounding land than on crops sprayed with pesticide.

Methods

To establish the temporal and spatial distribution of *H. variegata* and *M. tasmaniae*, monthly surveys were carried out using a suction sampler. The surveys included different non-crop habitats (bushland, pasture and riparian areas) as well as different crop habitats that included monocultures of cabbage, broccoli and cauliflower planted at different times in the season (early season plantings in September–October, mid-season plantings in November–December, and late-season plantings in January–February). To assess the effect of insecticide usage on *H. variegata* and *M. tasmaniae*, the spray records for each crop were analysed by using a weighting and scoring system for chemical type and application frequency in relation to *H. variegata* and *M. tasmaniae* catches.

Movement from Non-Crop Vegetation to Brassica Crops

Introduction

This study aimed to test the hypothesis that predators are moving from adjacent non-crop vegetation areas to Brassica crops and to identify if there are any differences between non-crop vegetation types such as remnant bushland, pasture or riparian. The study also explored the temporal and spatial abundance of *H. variegata* and *M. tasmaniae* as they moved in Brassica crops. The SARDI yellow fluorescent pigment dye (South Australian Research and Development Institute) was used to mark the predators in non-crop habitat adjacent to the Brassica crop field and then monitor their movement into the crop field.

Methods

Marking is a method used in arthropod ecology to estimate population size, survival, growth and movement. Such a method was used to investigate the temporal, spatial and magnitude of *H. variegata* and *M. tasmaniae* movement into Brassica crops from adjacent non-crop vegetation.

The experiment was carried out on three commercial Brassica farms. These farms grew Brassica crops that were located adjacent to non-crop habitats of pasture, remnant bushland and/or riparian areas (i.e. three Brassica fields per farm, each bordered by one of the three types of non-crop vegetation). At each site a 10 m wide strip of non-crop vegetation along the field edge bordering each Brassica cop field was sprayed with a dye mixture of SARDI yellow fluorescent pigment at a rate of 100 litres per hectare.

The dye was applied at a rate of two litres per 100 litres of water. For sampling, a blower-vacuum shredder (STIHL® BG 85 blower-vac, Andreas Stihl Ag & Co. Waiblingen, Germany) was used to collect arthropods at 20, 40, 60, 80 and 100 m into the crop from the edge bordering the non-crop vegetation. The inner tube of the blower-vacuum shredder was fitted with a removable voile bag to intercept arthropods. At each distance, four strips of row 20 m long were sampled at 0, 2, 5, 7 and 10 days after treatment (DAT) with dye. Four samples of 20 m were taken instead of one 80 m sample as the voile bag in the blower had limited capacity. As soon as each sample was collected it was placed in a covered 20 L plastic bucket which held an uncovered 100 ml beaker filled with 20 ml of chloroform to kill arthropods quickly and prevent predation or contamination of samples due to movement of marked or un-marked predators.

Processing of the samples involved the separation of the arthropods from debris by carefully picking *H. variegata* and *M. tasmaniae* out and transferring them to individual 5ml vials The predators were counted as marked or un-marked under UV-light in a dark room. The SARDI yellow fluorescent pigment dye appeared as a bright yellow mark on any marked predator. Preliminary testing of the dye was carried out to establish the time frame for detecting marked insects in the field. The test showed that the marked predators were found for up to 10 days.

Repopulation of Insecticide-Sprayed Brassica Crops

Introduction

This study is an extension of the experiment in the previous chapter, but in contrast considers re-population by predators from adjacent non-crop vegetation immediately after an insecticide spray event. The objectives to be examined considered:

- whether re-population of the field was from neighbouring cropland and non-crop vegetation or via longer range movement
- how the two target predators would re-populate an insecticide sprayed field over time
- whether surrounding vegetation had an edge effect on population abundance of the two target predators
- if blue or yellow sticky traps were more efficient at attracting insects and whether their aspect on the supporting stakes (high or low) affected catch and
- the usefulness of Malaise traps in determining the movement of *H. variegata* and *M. tasmaniae*.

Methods

All farms were managed according to the farmer's standard practices including irrigation, weeding and chemical application. The testing of recolonisation of Brassica crops after spraying took place on an opportunistic basis by sampling fields after the farmer applied an insecticide of his own choice as part of their normal farm operations. Arthropod sampling started two days after treatment. Sampling was repeated six times at two day intervals (2, 4, 6, 8, 10, and 12 days).

Samples were taken from four sampling positions at 20, 40, 60, 80 and 100 m into the crop from the edge of the non-crop vegetation or cropland. Predators were intercepted with 30 cm x 16 cm yellow and blue sticky traps. While it was known that yellow sticky traps are more efficient at attracting certain insects than blue ones, yellow sticky traps were not available initially. Blue traps were therefore used with yellow traps also used once they became available. Each trap consisted of a sticky, coloured strip which was placed either at the top of the stakes or immediately below the top strip on the stakes (Figure 5) and traps were placed in four alternate rows across the field. At each sampling point, *H. variegata* and *M. tasmaniae* were recorded every second day for 12 days after the initial insecticide application, then marked with a paint marker or removed to avoid double counting at the next sample check. In addition to trapping, four bi-directional Malaise traps (Figure 6) were set up, two at each end of the field.

Figure 5: Sticky trap stations in a Brassica field in Bathurst, CW-NSW.

Figure 6: Malaise trap in a Brassica field in Bathurst, CW-NSW.

DNA Gut Analysis of Field-Collected Predators

Introduction

This study investigated the prey range of *Hippodamia variegata* and *Micromus tasmaniae* in a Brassica field production system and surrounding vegetation by assessing their gut contents in DNA barcode-based assays. Prey tested for included the main Brassica insect pests: diamond back moth (DBM) *Plutella xylostella* (L.), cabbage white butterfly (CWB) *Pieris rapae* (L.), cabbage aphid (CA) *Brevicoryne brassicae* (L.) and Rutherglen bug (RB) *Nysius vinitor* Bergroth which had been identified as the most abundant possible prey species during the surveys described in earlier studies.

Identification of prey DNA in the gut content allowed a more detailed study and quantification of trophic relationships and a better understanding of the temporal and spatial dynamics of predation. Analysing the gut content also provided information on the extent of any intra-guild predation between the two BCAs studied. Since predators were collected at various distances from the field edge as well as in non-crop vegetation, the relationship between predator type and distance was also tested.

Methods

Hippodamia variegata and *Micromus tasmaniae* specimens were collected from different Brassica farms in the Central West New South Wales (CWNSW), Australia. Collections were done manually by hand picking or using a vacuum sampler. Specimens were sampled from the non-crop edge of Brassica fields and in 20 m strips at distances of 20, 40, 60, 80 and 100 meters into the crop fields. Individual specimens were immediately placed in 5 ml vials filled with 100 percent alcohol and labelled.

DNA extraction and PCR amplification of material followed standard protocols. More detail can be found in the PhD Thesis of Mr Heimoana.

Potential Against Common Greenhouse Pests

Introduction

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. Transient *A. craccivora* may therefore transmit stylet-borne diseases though probing behaviour on non-host greenhouse crops.

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.

Methods

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 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. More prey than could possibly be eaten was provided to the predator each day to ensure an oversupply. The amount of prey provided 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* with 25 predators used..

The role of photoperiod and temperature in the onset of dormancy

Introduction

This study in 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.

Methods

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. 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.

Effect of Eight Greenhouse Crop Pesticides

Introduction

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 Australia.

Methods

Pesticide solutions were made up in 500ml volumetric flasks (Silber Brand, Brand, Germany) according to the manufacturers' label rates (Table 1). 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 at 25°C±0.5, 70%±13 RH and 16L:8D photoperiod.

Table 1: Details of pesticide treatments used in laboratory evaluation of effects on first instar *H. variegata and M. tasmaniae* larvae

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

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. The six 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 15 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.

Results

Influence of Non-Crop Vegetation and Insecticides on the Presence/Absence and Abundance of Predators

Results

Seasonal population trends of H. variegata and M. tasmaniae

Overall, *H. variegata* constituted half of the 1594 coccinelids captured from all habitats with 797 adults (50%) and 72 larvae (4.52%). The total coccinellid density trend mirrored that of *H. variegata* (Figure 7), the density of which peaked in October 2006 (number of *H. variegata* – 1.2/sample). In summer, January 2007 numbers declined to less than 0.5 *H. variegata*/sample. A second peak of 2.3/sample was evident in autumn followed by a decline in winter (July–August 2007) to less than 0.5/sample.

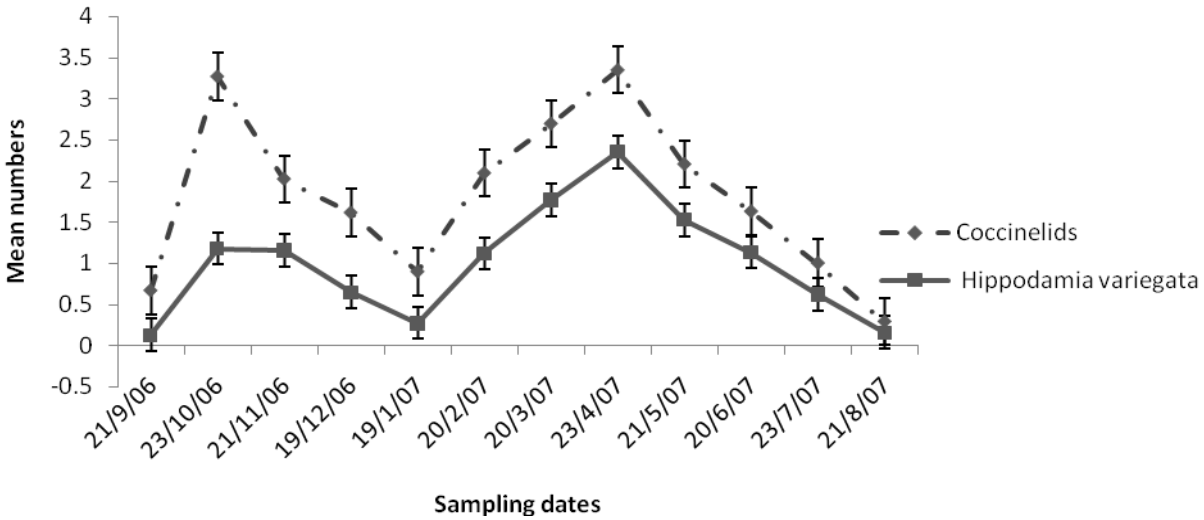

Figure 7: Temporal trend of *Hippodamia variegata* and all coccinellids on vegetable farms, Bathurst, Central West of New South Wales

Neuroptera were still more heavily dominated by *M. tasmaniae.* Of the 964 individuals captured, 4.46% and 90.66% were larva and adults, respectively, of this species. The overall temporal trend for Neuroptera exhibited three peaks during the 12 months of the survey (Figure 8). The first peak occurred in November 2006 at the rate of 1.5 *M. tasmaniae/*sample followed by two peaks in April and July with 2 and 2.1 specimen/sample, respectively.

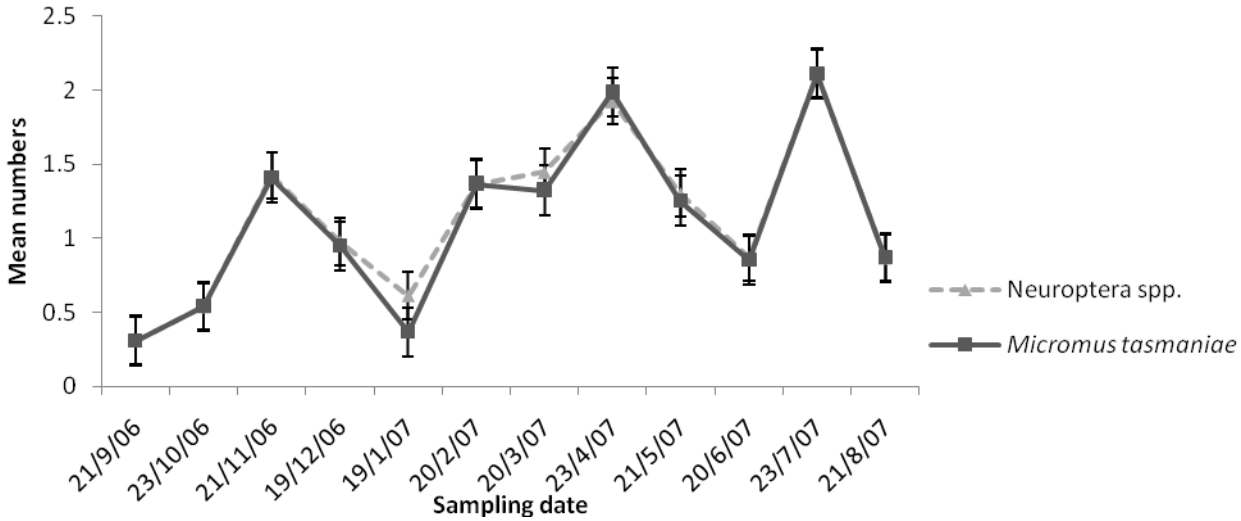

Figure 8: Temporal trend of *Micromus tasmaniae* and all Neuroptera on vegetable farms, Bathurst, Central West of New South Wales

Seasonal availability of prey species

Several possible prey species were available to *H. variegata* and *M. tasmaniae* (Figure 9). Over all habitats samples, the most abundant pests captured were Rutherglen bug (*Nysius vinitor* Bergroth, Hemiptera: Lygaeidae), jassids (species of *Austroasca* and *Amrasca*, Hemiptera: Jassidae) and thrips (species of *Frankliniella* and *Thrips*, Thysanoptera: Thripidae) with a total of 21,810, 17,008 and 13,514 individuals, respectively. Aphids were less numerous in samples, totalling 1,638, whilst lepidopterans were still scarcer (total: 332 larvae). The most prevalent aphid species found in the Brassica crops was *Brevicoryne brassicae* (L.) and in the non-crop sites *Aphis gossypii* (Glover). For subsequent analyses both aphid species were combined.

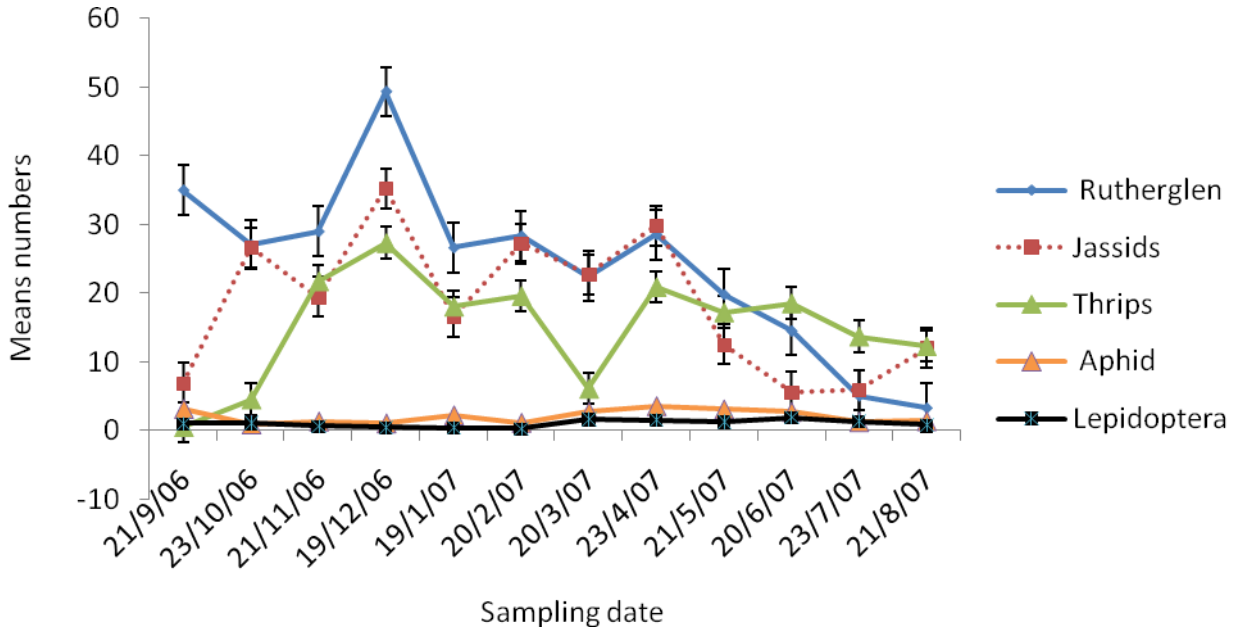

Figure 9: Mean number of potential prey individuals over all habitats and all farms in CW-NSW.

Numbers of Rutherglen bug, jassids and thrips peaked in December 2006 with as many as 49.3 Rutherglen bugs, 35 jassids and 27 thrips/sample. Aphid and Lepidoptera densities at that time were at the lowest with 1 and 0.5 specimen/sample, respectively. From December onwards, the Rutherglen bug, jassids and thrips densities gradually declined towards the coldest winter months (July - August 2007) with the exception of a decline in March 2007. In contrast, aphid and Lepidoptera densities at this time increased gradually to 3.5 aphids/sample in April 2007 and 1.9 Lepidoptera/sample in June 2007. Both species then declined over the winter months to 1.4 aphids/sample and 0.8 Lepidoptera/sample. Aphids exhibited a peak of 3.0 aphids/sample in September 2006 when the first crops were planted.

Impact of insecticide use on H. variegata and M. tasmaniae

BDI values calculated reflect the impact of pesticide regime intensity on *H. variegata* and *M. tasmaniae* (Figure 10) in crops. Regression analysis of predator abundance against BDI found a significant relationship (F=0.0023 at P=0.05) between the spray regime on a farm and the presence of *H. variegata*. The higher the BDI, the fewer *H. variegata* populate the area. This relationship was moderately strong (R^2 =0.620), i.e. the BDI accounts for 62% of the total variability of the data. In contrast, *M. tasmaniae* had a insignificant and weak relationship with the BDI (F= 0.0754, R^2 = 0.282) indicating that it is either less sensitive to chemicals or that factors other than the BDI play a more important role in the abundance of *M. tasmaniae*.

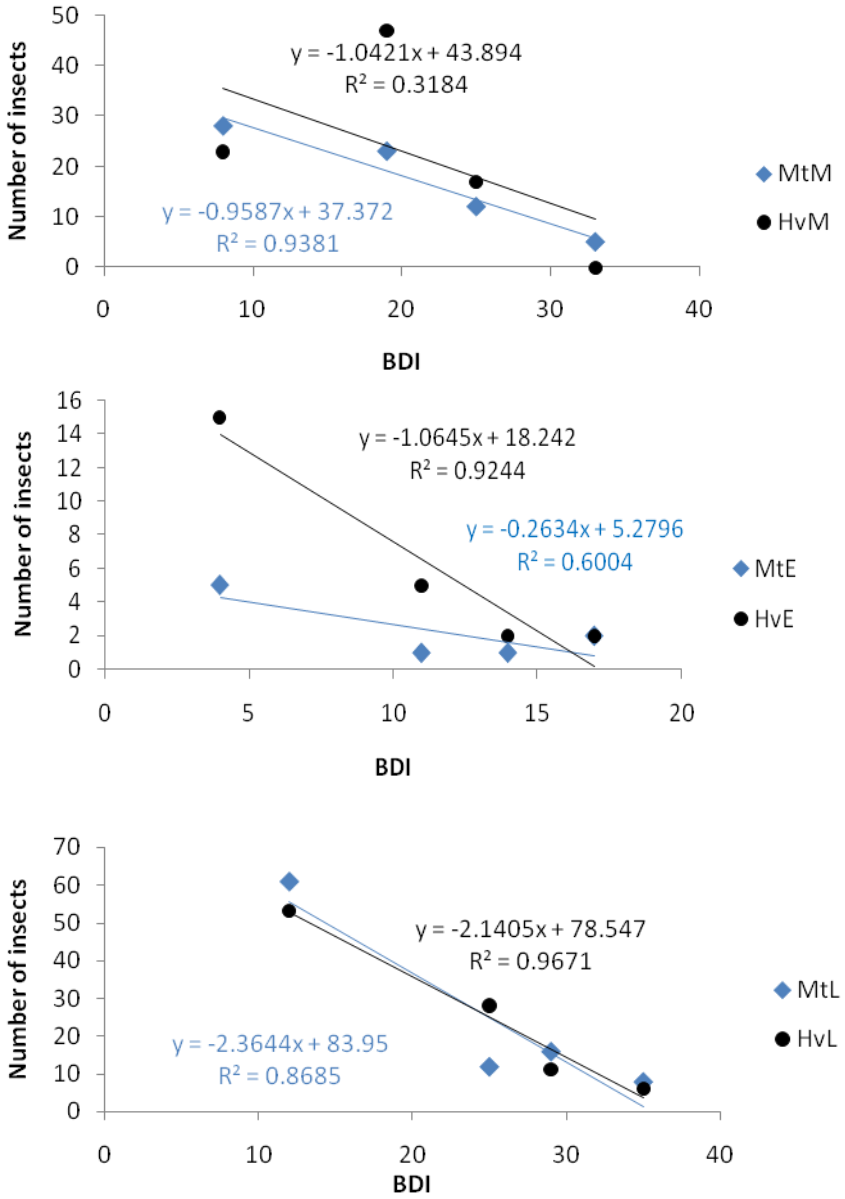

Figure 10: Relationship between the biological disruption index (BDI) of pesticide regimes and catches of *Hippodamia variegata* and *Micromus tasmaniae* in early-(HvE, MtE), medium- (HvM, MtM) and late-(HvL, MtL) planted Brassica crops

Discussion

The study showed that the exotic *H. variegata* was the dominant species of coccinellid while the native *M. tasmaniae* was the dominant neuropteran in CW-NSW. Both predators followed similar seasonal patterns, however, there was variation in numbers. *H. variegata* and *M. tasmaniae* were common in Brassica crops, especially in those planted in October (mid-season), indicating that they may already have high potential as biological control agents. *H. variegata* and *M. tasmaniae* were also found in non-crop habitats such as bushland, pastures and riparian areas showing their potential importance as reservoirs for the predators. Surrounding non-crop vegetation directly benefits beneficial arthropods by providing habitat and also indirectly provides benefits by acting as a host for their prey species. Moreover, the establishment and maintenance of suitable habitat on farm or surrounding landscape

can enhance the survival of natural enemies. This means that fence lines, ditches, hedgerows, windbreaks, weedy strips or riparian areas may serve as a reservoir or ecological corridor for *M. tasmaniae*, *H. variegata* and other natural enemies. The structure, vegetation type and shape can have a direct effect on the density of beneficial arthropods in a cropping system. Therefore, Brassica growers could manipulate or conserve the non-crop vegetation on their farms to enhance the population density of beneficial arthropods. Enhanced populations of natural enemies immigrated to neighbouring crops and attacked insect pests and reduced their population below economic threshold. The low numbers of immature predators in all habitats including the non-crop sites suggests that neither predator species reproduces extensively within crops or in any of the on-farm habitats sampled. It is likely that longer range immigration is the main source of predators to these farms. Growers need to use lower BDI insecticides for their insect pest control and maximise the amount and proximity of non-crop source habitats that are not sprayed. Softer insecticide early in the season will enable the predators to establish in their fields.

Results clearly show that increase number of sprays (high BDI), decreases the number of predators, possibly for two reasons:

(1) prey is being reduced forcing predators to feed elsewhere, and

(2) predators are being killed by off target effects.

Milder spray regimes (low BDI) showed significantly higher numbers of predators, especially coccinellids. In order to develop successful IPM strategies including biological control agents, farmers in the area will need to reconsider their spraying strategies and preserve non-crop habitats as refuge and breeding areas.

Movement from Non-Crop Vegetation to Brassica Crops

Results

Movement of predators into the crop from sprayed areas

A total of 1,404 *H. variegata* were captured of which 683 (48.65%) had been marked. For *M. tasmaniae* the percentage captured was much less, 268 (33.67%) of a total of 796 captured specimen. These figures clearly demonstrated that both predators moved from surrounding non-crop vegetation into Brassica crops. There were no significant differences in the proportions of marked *H. variegata* and *M. tasmaniae* that came out of the three different vegetation types (river/pasture/bush).

Effect of different vegetation types

Vegetation type was not a significant factor in insect movement except for unmarked *M. tasmaniae* which were present in the Brassica fields next to adjacent riparian vegetation in significantly higher numbers than in crops next to bush vegetation (Table 2).

Table 2: ANOVA F-values for *H. variegata* and *M. tasmaniae* captured from crops adjacent to different vegetation types

				Adjacent vegetation		
		F-value	**LSD**	**Bush**	**Pasture**	**Riparian**
H.variegata	Marked	0.611	n.s.			
	Unmarked	0.744	n.s.			
	Total	0.886	n.s.			
M. tasmaniae	Marked	0.705	n.s.			
	Unmarked	0.022	0.0872	*0.331	0.406	0.453
	Total	0.07	n.s.			

* signifies a significant difference from riparian vegetation at P=0.05

Temporal and spatial movement of predators

Sample distance was highly significant for marked, unmarked and total *H. variegata and M. tasmaniae.* DAT was also highly significant for all captured *H. variegata*, marked and unmarked *M. tasmaniae*, but not for total *M. tasmaniae* (F= 0.864). The interactions between time and distance were significant for all captured specimen of both predators.

Two days after marking, 88.1% of the overall *H. variegata* captured were marked showing they had moved to the crop from surrounding vegetation. Their numbers declined over the 20 to 60 m sample points but were 81.3% at 80 m into the field. At 100 m into the field 60.1% of captured *H. variegata* were marked. At 7 and 10 DAT *H. variegata* moved in a similar trend except that none of the predators caught 100 m into the field at 10 DAT were unmarked. Marked *H. variegata* recaptured 5 DAT showed a somewhat different pattern with similar capture percentages over the first 60 m into the field and then a sharp decline further in. It was difficult to discern an obvious relationship as the data displayed high variability (Figure 11). By regressing the means of the distance component (Figure 12) of the interaction against the percentage of marked *H. variegata*, a significant relationship (F=0.035) could be seen. As distance from the edge increased, the proportion of marked *H. variegata* became

less, indicating that about 50% of *H. variegata* moved within the first 20 m of the crop.

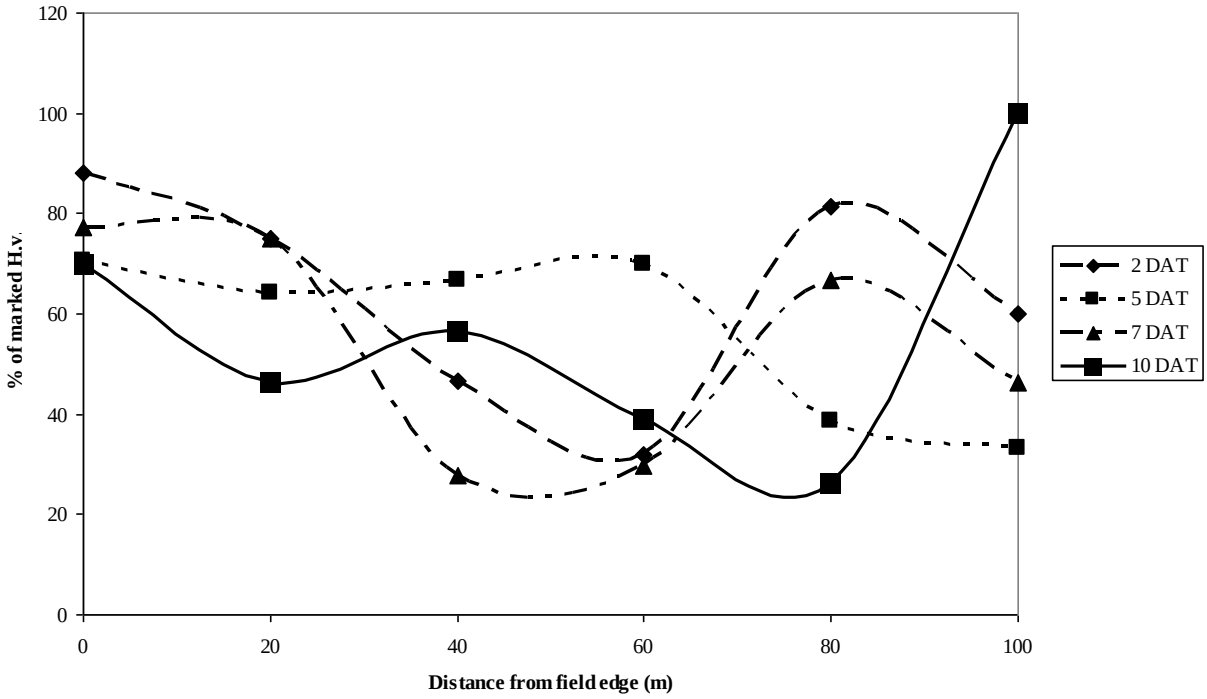

Figure 11: Movement of *Hippodamia variegata* into Brassica fields from surrounding vegetation

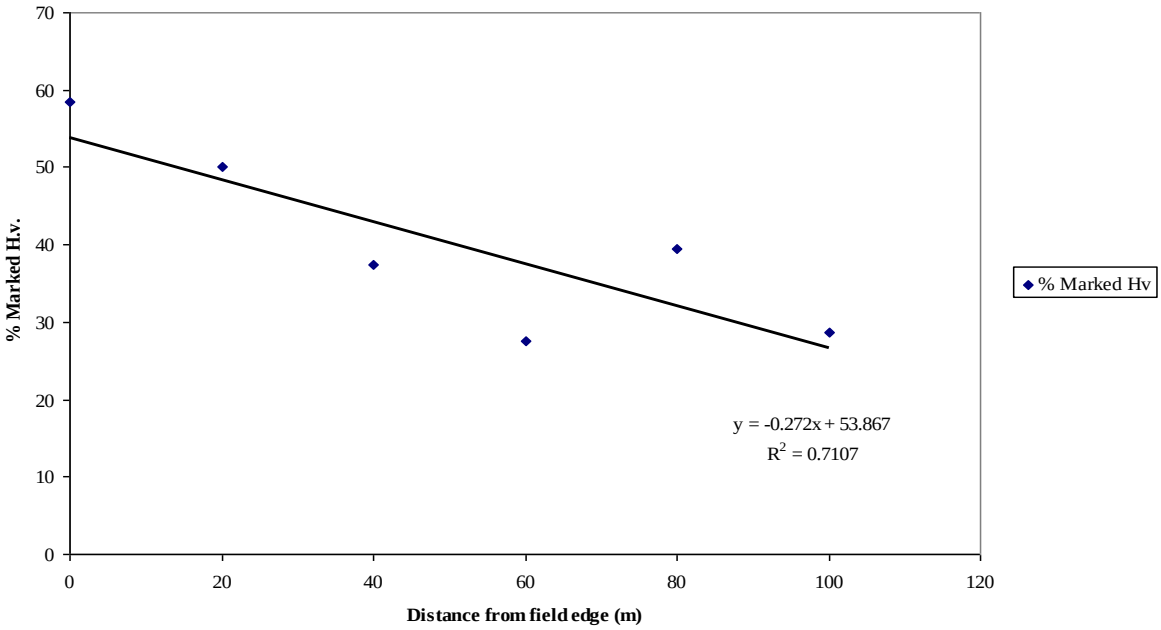

Figure 12: Regression of distance component of captured marked *H. variegata*

When regressing the time component of the interaction (Figure 13) against the percentage of marked *H. variegata*, the relationship was also significant (F=0.025) and stronger (R^2=0.9502). As time from spraying (marking the insects in the

surrounding vegetation) passed, the proportion of captured, marked *H. variegata* became less, from 61.7% at 2 DAT to 41.5% 10 DAT.

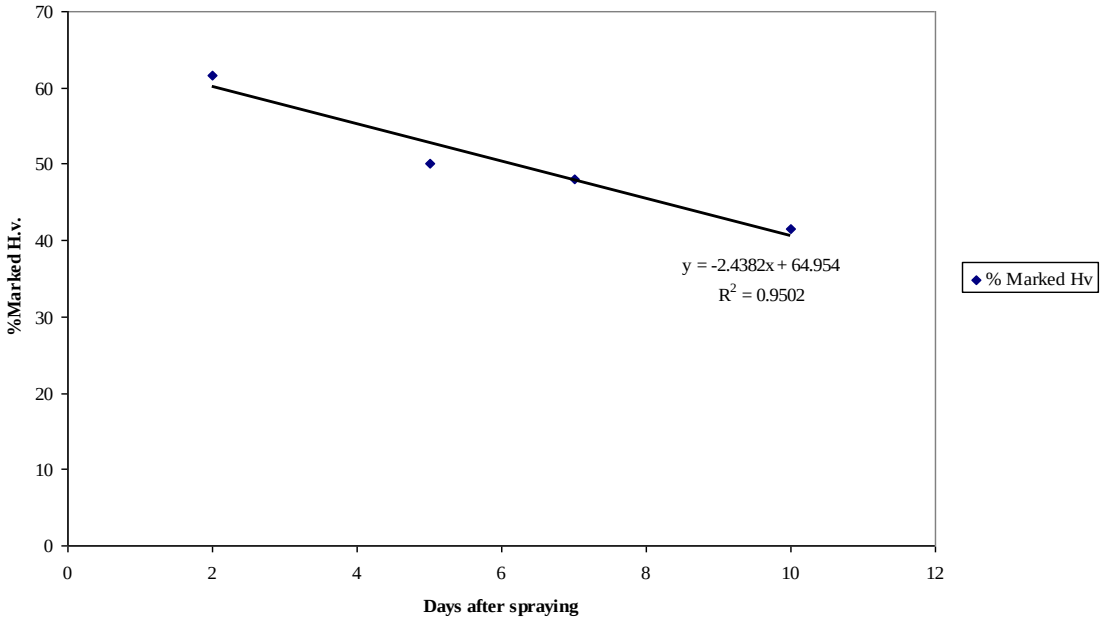

Figure 13: Regression of time component of captured marked *H. variegata*

The percentage of captured, marked *M. tasmaniae* (Figure 14) was generally lower than capture for marked *H. variegata*. There was also more variability, both over distance and time, in the movement pattern of *M. tasmaniae*. This was reflected in the regression analysis of the two components, neither of which was significant (F=0.242 and 0.238 for distance and time, respectively).

Captured *M. tasmaniae* had a weak relationship with distance (Figure 15, R^2 value = 0.3199). The correlation with time (Figure 16, R^2=0.5795), was higher than that for distance and accounted for nearly 60% of the variability.

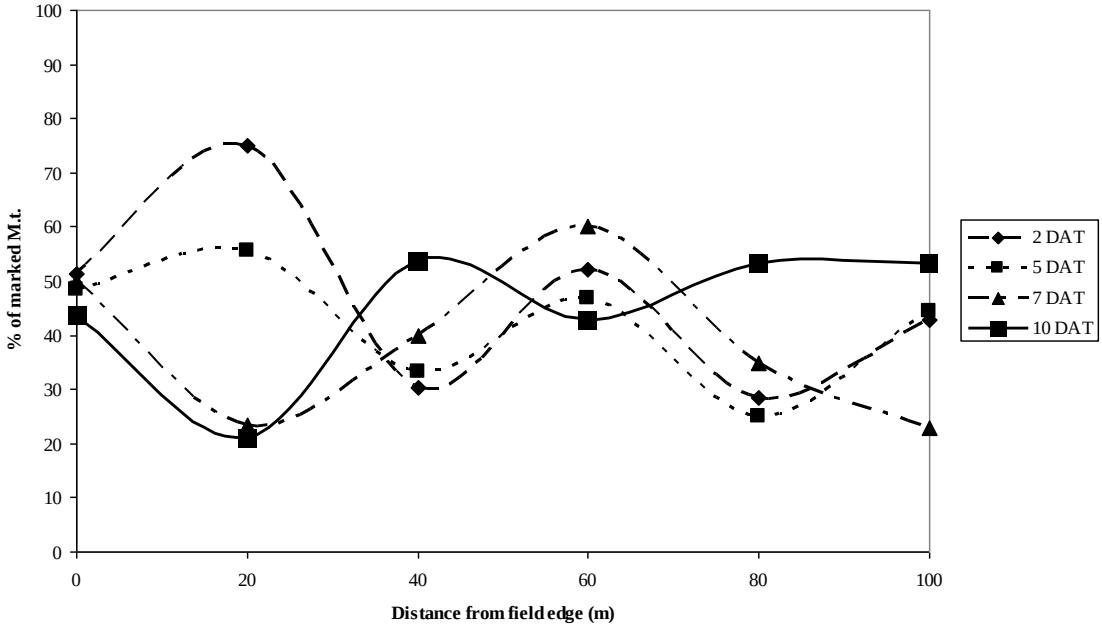

Figure 14: Movement of means of *Micromus tasmaniae* into Brassica fields from surrounding vegetation

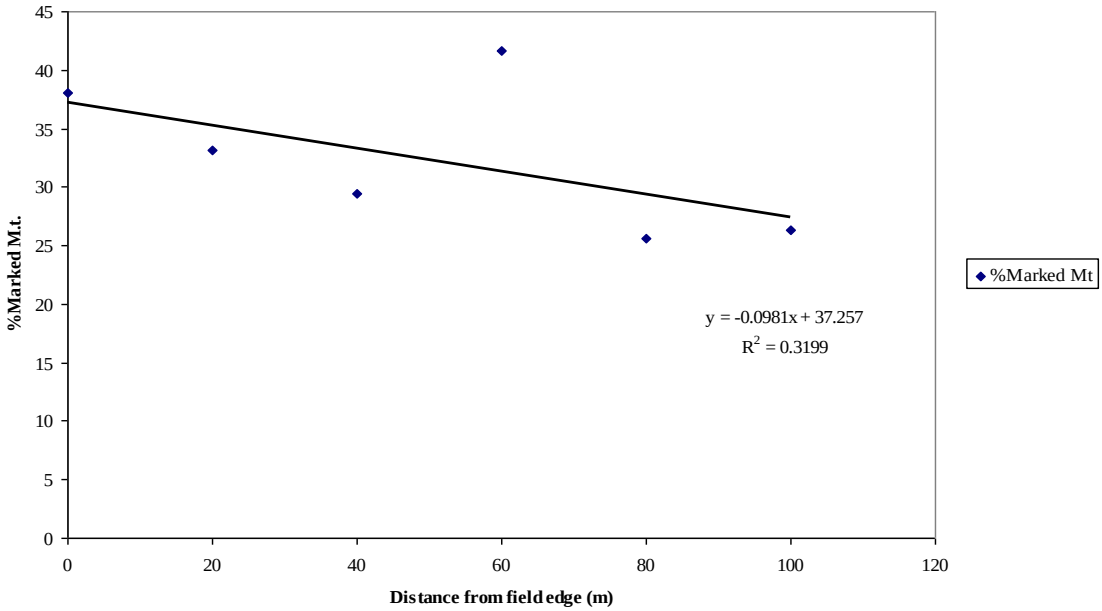

Figure 15: Regression of distance component of captured marked *M. tasmaniae*

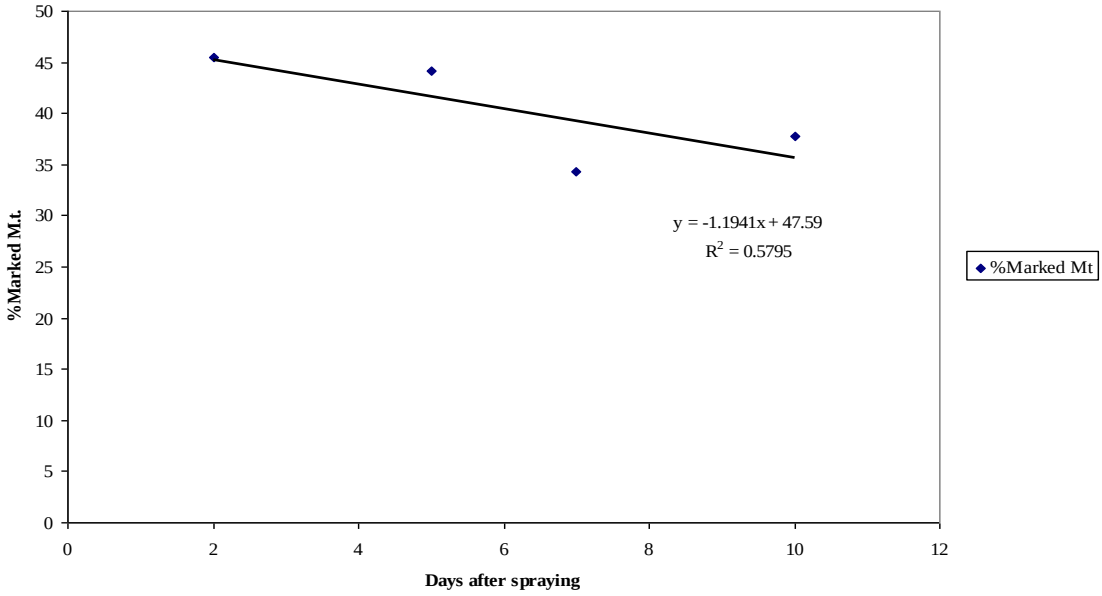

Figure 16: Regression of time component of captured marked *M. tasmaniae*

Discussion

By marking predators in non-crop vegetation strips along the crop, their movement within their environment could be tracked. Less than half of *H. variegata* and one third of *M. tasmaniae* captured were marked. This experiment showed that both *M. tasmaniae* and *H. variegata* moved into Brassica crops from surrounding vegetation. It is generally difficult to track movement over longer distances or from different source areas but with a range of different coloured marking dyes and intensive sampling this could be achieved.

In this experiment movement was only demonstrated in one direction, i.e. by the percentage of captured, marked predators into the Brassica fields. Movement also occurs out of the field and into surrounding vegetation but this was not captured in the design. It would require a second experiment that reverses the sprayed (marked) area and sampling in the surrounding vegetation to determine how much movement out of the field occurs.

Beneficial insects move through habitats at a broad range of spatial scales and non-crop vegetation enhances population of beneficial insects for crop pest control. Earlier, it was shown that bush, pasture and riparian habitats surrounding farmland acted as reservoir habitats for predators and this was confirmed in this experiment. Numbers of captured, marked *H. variegata* and *M. tasmaniae* did not reveal any preference for bush, pasture or riparian areas as source areas. Both species moved into the crop from all vegetation types to similar extent. The only significant influence of non-crop vegetation type was for unmarked *M. tasmaniae*, which were found in significantly greater numbers in crops near riparian vegetation than in crops near bush vegetation. This may reflect the relative importance of the different non-crop habitats to the lacewing over a time scale greater than the present experiment. For example, crops adjacent to riparian vegetation my have had relatively high lacewing densities because of an earlier period of dispersal from this largely perennial vegetation to the establishing crop. It is known that *M. tasmaniae* is associated with perennial

vegetation. This could be due to the type and quality of plant species in that habitat, micro climate, moisture situation, food sources and other factors.

Marking predators showed that their movement was affected by temporal and spatial factors. While there was much variability in the data, a strong and clear relationship emerged for *H. variegata* over time. As time from marking passed, fewer of these marked coccinellid predators were found in the crops. There are a number of possible explanations: the predators were eaten, missed in the sampling procedure, depleted by sampling or they moved out of the crops, or the dye may have been rubbed or groomed off. While coccinellid diets in general include a wide range of prey and non-prey food sources, some groups are specialized in particular prey species such as aphids or mites. *H. variegata* may prefer aphid prey commonly found in Brassica crops over other food sources found in the surrounding habitat. As the preferred prey species became exhausted – due to feeding or chemical intervention – *H. variegata* may have moved out of the crop and back into surrounding vegetation which provided sources of nectar and other prey species. The relationship between captured *M. tasmaniae* and time was less defined and captured marked *M. tasmaniae* numbers remained relatively steady over time. They were less abundant than *H. variegata* for unknown reasons, but some factors could be a preference for a different habitat, insufficient prey, or predation by other predators.

The relationship of both predators with distance showed the same trend as that with time. *H variegata's* presence showed a relatively steep decline in numbers further away from the field edge suggesting that significant movement occurs from the non-crop vegetation to the field margins. This indicates an advantage of smaller fields that allow predators to move further into the crop. Large fields therefore, are at a disadvantage in terms of predator movement into the field, however, provision of vegetated strips could help maintain predators within fields. The use of hedgerows and beetle banks has been shown to increase the abundance of predators through the provision of nectar and pollen from insectary plants, alternate prey and overwintering and dispersal sites**.** *M. tasmaniae* was less affected by distance from field edge and was found right across the field in variable numbers without a definite trend. This is an indication of the wide feeding range of *M. tasmaniae*; rather than relying on a particular pest species, it feeds perhaps more opportunistically on a range of crop pests. Previous work at the same location of this experiment showed that during fallows abundant numbers of the two target predators were already present in the non-crop vegetation at the edges of the field. Overall, the distance measurements did not give conclusive information of predator movement from beyond the distance of the marked strip. Any distance studies would be more complex and involve colour coded marking dye that could be related to different distances. As both *H. variegata* and *M. tasmaniae* adults are winged, they are highly mobile and can actively seek for food. The study showed that, irrespective of its vegetation type, non-crop habitat is an important source of *H. variegata* and *M. tasmaniae* as a breeding and feeding habitat. The findings from this study and from Chapter 2 supported the suggestion that it was important to manage non-crop habitats to conserve natural enemies in order to enhance biological control.

Understanding the characteristics of vegetation is important in the manipulation of vegetation that provides shelter and food for natural enemies. For example, hedgerows, wind breaks, weedy strips and riparian areas could serve as a reservoir or

ecological corridors for brown lacewings and other natural enemies. Specific plants can provide nectar and pollen or serve as alternate hosts of prey for natural enemies which may increase their activity at field edges. Natural enemies can move over to an adjacent crop and control pests and therefore provide direct benefits to the growers by offsetting costs. For growers to adopt a positive attitude to the maintenance of non-crop areas, they should first be shown the benefits. Non-crop areas should also require low input and maintenance and therefore natural areas such as riverbanks would be the most suitable. However, not every field is located near water sources and growers may have to create suitable habitats by planting windbreaks, cultivating hedgerows or planting strips of flowering plants.

The experiment demonstrated movement of predators into crops, however, it was carried out in the absence of any disturbance to the system. In reality Brassica production systems are commonly sprayed. Insecticide applications deny predators food sources and usually have direct non-target effects so impact on the effectiveness of predators as biological control agents.

Repopulation of Insecticide-Sprayed Brassica Crops

Results

Repopulation from cropland, non-crop vegetation and long range movement

Adjacent vegetation type played a significant role in the repopulation of the sprayed fields (F= <0.001). *H. variegata* repopulated significantly more strongly from the cropland side, while *M. tasmaniae* migrated more strongly from the non-crop vegetation side. There were no interactions between orientations, days after treatment (DAT) and distance from field edge.

There were no significant differences for *H. variegata* and *M. tasmaniae* between the mean number of insects captured at various distances from the field edge (F-values of 0.143 and 0.129, respectively).

When regressing the distance component (Figure 19) against the means of trapped *H. variegata*, no relationship was evident (R^2=0.0031, F=0.9294). The number of trapped *H. variegata* was highly variable with distance into the sprayed crop. For *M. tasmaniae* regressing the distance component (Figure 20) also showed no significant relationship (R^2=0.079, F=0.6461).

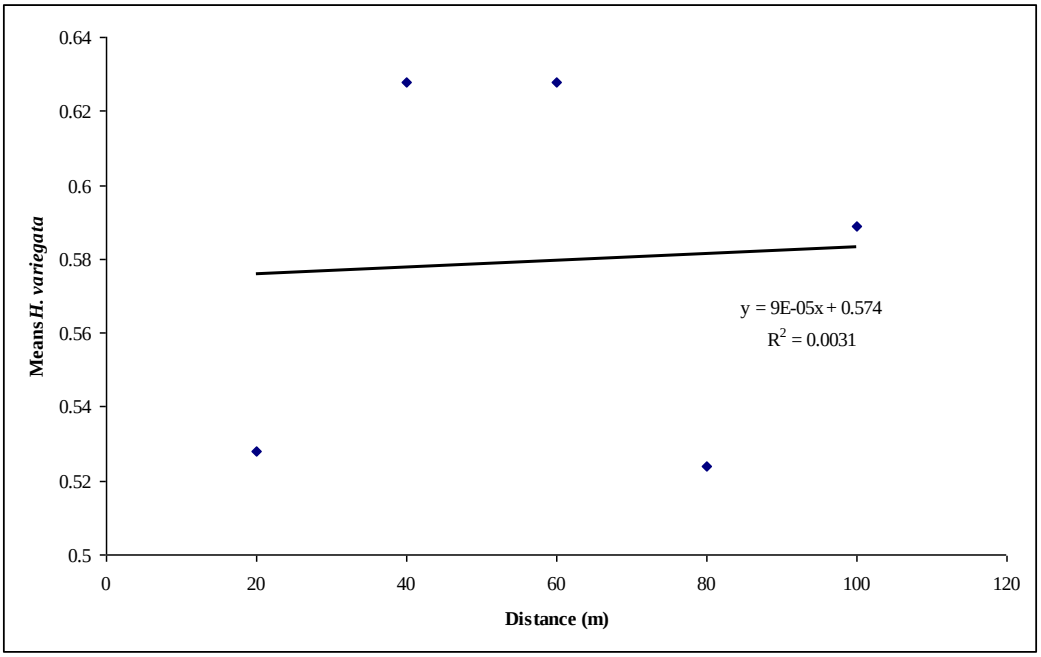

Figure 19: Regression of mean of distance component of recaptured *H. variegata*

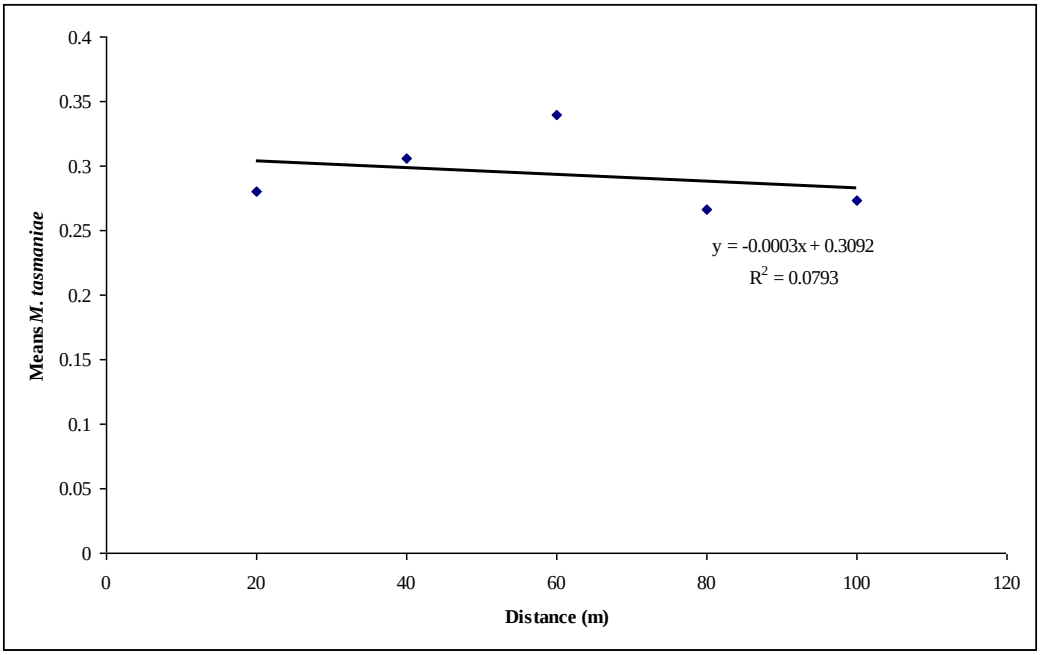

Figure 20: Regression of mean of distance component of recaptured *M. tasmaniae*

Repopulation over time

Time was a significant factor in the number of *H. variegata* and *M. tasmaniae* caught (F=<0.001). Both species repopulated the field within two days of spraying. Numbers increased gradually as time from the spray date increased. By 12 days from treatment, the number of *H.variegata* had increased 3.1 times since the first check on day 2 after treatment while the number of *M. tasmaniae* had increased 3.3 times.

When regressing the time component (Figure 21) against the means of trapped *H. variegata*, the relationship was also significant (F=<0.001) and strong (R^2=0.9797). As time from spraying the field passed, the number of trapped *H. variegata* increased. A significant relationship (Figure 22) was also seen for *M. tasmaniae* (F=0.013) but not as strongly as for *H. variegata* (R^2 = 0.8161).

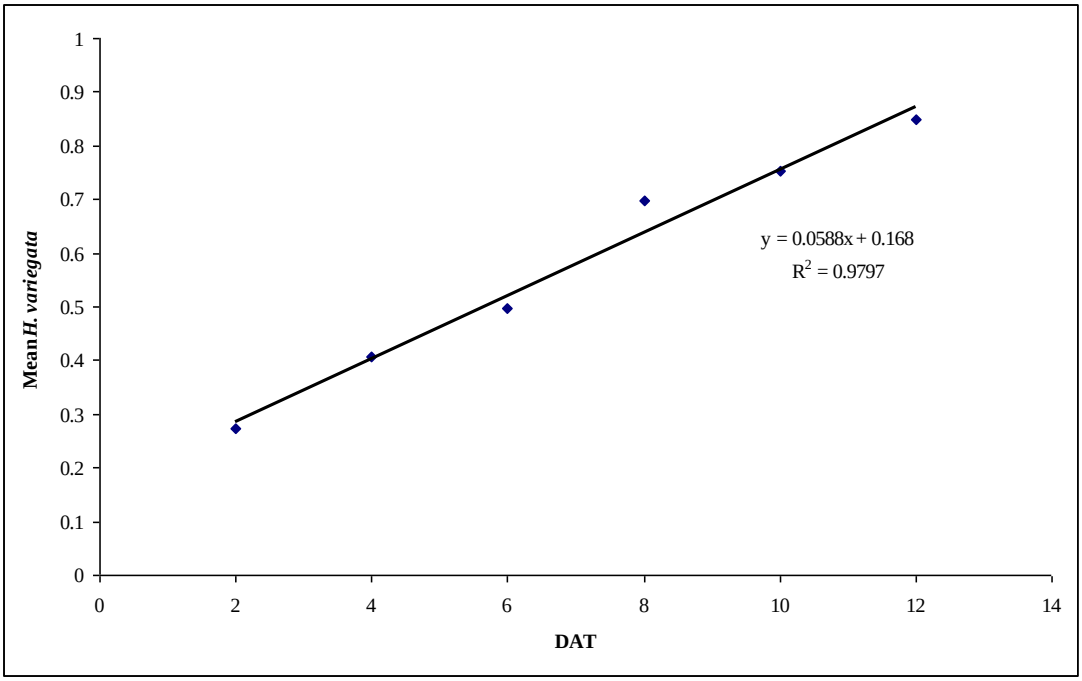

Figure 21: Regression of time component of recaptured *H. variegata*

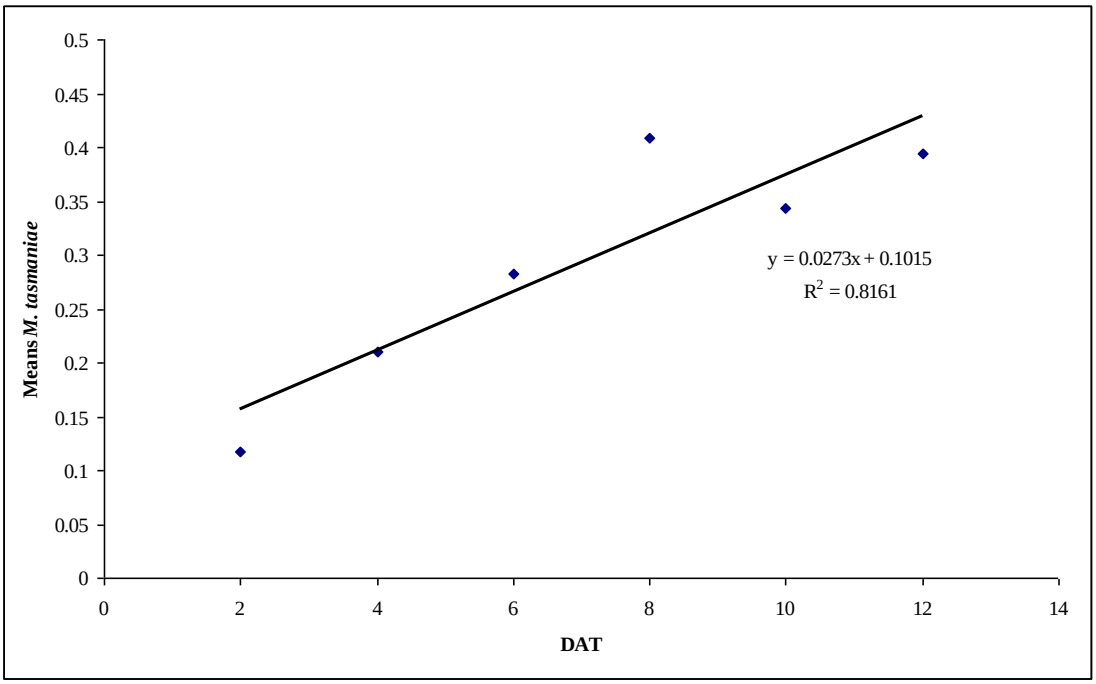

Figure 22: Regression of time component of captured *M. tasmaniae*

Edge Effects of surrounding vegetation

Testing for distance from the long field edges of the three fields (i.e. comparison between replications) did not reveal any edge effects across the experiment. No significant differences in mean trap catches were seen between all traps for either *H. variegata* or *M. tasmaniae* (F= 0.056 and 0.187, respectively).

Sticky trap colour and aspect

Sticky trap data was analysed in a general ANOVA for Colour*Aspect*Direction. Colour was highly significant with yellow sticky traps catching nearly 4.5 times as many *H. variegata* and 2.1 times as many *M. tasmaniae* than blue traps. The aspect of the sticky traps – higher or lower on the stakes - was not significant, most likely because the vertical distance between traps on the stakes was small. Direction was not significant, "Out" referring to the sticky side facing towards the centre of the field, i.e. catching insects leaving the field and "In" referring to the sticky side facing away from the centre of the field, i.e. catching insect movement into the field.

It is surprising that the in-facing sticky traps caught more insects – indicating that more insects are moving out of the sprayed field than are moving back into the field from the surrounding vegetation. For *H. variegata* direction was not significant, however, for *M. tasmaniae* there were a significantly larger number of adults moving out of the field.

The interactions between aspect, direction and colour were driven by the yellow colour of the sticky traps which caught significantly higher numbers of both insects. For both *H. variegata* and *M. tasmaniae*, yellow sticky traps caught more insects leaving the field than entering the field and more than twice as many *H. variegata* were caught than *M. tasmaniae*.

Malaise traps

There were no significant temporal differences in trap catches over the 12 days of sampling for either *H. variegata* or *M. tasmaniae* (F=0.776, 0.736). Trap opening (inward or outward catches, F=0.492 and 0.514, respectively) and orientation towards vegetation or cropland were also not significant for either species (F=0.28, F=0.514).

Discussion

Repopulation from non-crop vegetation and long range movement

Both cropland and non-crop vegetation played an important role in predator re-population of sprayed Brassica fields. This confirms earlier findings that showed that non-crop vegetation was an important source of predators. *H. variegata* repopulated more strongly from the cropland side, while more *M. tasmaniae* migrated from the non-crop vegetated side. The explanation for these results may lie in farm layout and field location. All farms had remnant Brassica fields (cropland) in the vicinity of the experiments and it is possible that *H. variegata*, being an introduced predator, prefers to feed on aphids and Lepidoptera building up in these unsprayed crop remnants, while the native predator, *M. tasmaniae* may have a wider prey range including species that are not Brassica pests and are found in non-crop habitats. Further, *H. variegata* and *M. tasmaniae* may interact competitively in areas outside the crop.

Insect movement out of the field was greater than insect movement into the field. This could possibly be due to the fact that the field had been sprayed and inwardly migrating predators did not find much prey. As a consequence they may have moved back to adjacent non-crop vegetation in search of prey.

The lack of differences in captures at the various distances over the field indicates that repopulation of the field by predators occurs at least partially as a fairly uniform movement inwards from the sheltered areas along the field edge. It was shown that *H. variegata* numbers declined significantly over distance from the field edge and that it was difficult to establish their origin. In this experiment *H. variegata* did not show this definite relationship and for *M. tasmaniae* neither experiment showed a significant relationship with distance from field edge. While both experiments demonstrated movement, there is still no clear indication of the relative importance of recolonisation directly from vegetation adjacent to sprayed fields versus longer range immigration. The demonstration of long-range movement is complicated and all methods have limitations. Mark-release-capture experiments with such small insects (aphids) have limited potential due to large dilution effects. Static 'snap-shots' of demographic population densities, using suction traps, cannot accurately distinguish local aerial density fluxes and population movements from a distance.

In general, small farm size may be a significant factor in this type of experiment. As vegetable farms tend to strip-crop smaller areas over an extended time period to allow for sequential harvesting, fields available for experimentation are generally too small for the establishment of large scale experiments that extend over wider areas. Remnants of previously harvested crops and many edges with other types of vegetation are nearby. However, small field size also lends itself to insects moving readily back into a sprayed field relatively quickly as they do not have to travel long distances to reach the crop. This effect would be supported by spraying insecticides that have a low impact on desirable predators.

Repopulation over time

Both *H. variegata* and *M. tasmaniae* were detected in sprayed fields within 2 days of spray application and showed strong positive relationships with time. Numbers increased gradually and had tripled 10 days after the first check at 2 DAT. This trend of repopulation was in total contrast to the trend seen earlier, where both *H. variegata* and *M. tasmaniae* (both marked and total numbers) declined over time in the unsprayed crop. While the earlier decline in marked insect numbers can be explained with the decline of marked specimen over time, the equivalent decline in unmarked insects can not.

Edge effects of surrounding vegetation

Edge effects on trap captures were not detected in this experiment, indicating that the fields used in this experiment were sufficiently small (40m x 300m) to avoid edge effects. While there is a well known edge effect in large monoculture fields, horticultural fields or strips are much more easily re-populated by insects as supported by the distance data. Larger fields could employ beetle banks to overcome edge effects.

Sticky traps colour and aspect

Considering that yellow sticky traps in this experiment also caught the most predators, growers wishing to employ IPM methods in their cropping system may need to consider if sticky traps would complement their pest control methods. For example, should any pheromone sticky traps become available for Lepidoptera pests of brassicas, it would be best applied in the field on green or red cards. The aspect of the

sticky traps – higher or lower on the stakes was not important possibly because the distance between different traps was too close.

Malaise traps

None of the parameters (time, flight movement in/out of field, vegetation type) measured for Malaise traps were significant. Malaise traps are commonly used for the collection of insects, and in particular insects that tend to fly upwards when encountering a barrier, eg. Diptera and Hymenoptera. Some insects tend to drop when they encounter obstacles, and coccinellids are included in this group. It would therefore have been prudent to place an ethanol filled pan underneath the traps in order to catch these insects as well. Since Malaise traps do not require any light source or suction method they were a good option for comparisons to be made over areas, crops or interesting habitats, however, at a cost of about AUD435.00 each, and with the number of traps required, sticky traps were the most economic option.

While this experiment again demonstrated insect movement into crops from non-crop and crop sources, the issue of long range movement is still speculative. As insects repopulated a sprayed crop over time, their numbers built up as the effect of the chemical wore off over time. Many factors influence insect behaviour within a crop and movement out of a crop such as microclimate, prey availability, competition between predators or intraguild predation.

DNA Gut Analysis of Field-Collected Predators

Results

A total of 172 field collected predators *H.variegata* (96 specimen, 108 detections) and *M. tasmaniae* (76 specimen, 72 detections) were screened for the presence of prey DNA (*P. xylostella*, *P. rapae*, *B. brassicae*, *H. variegata*, *M. tasmaniae* and *N. vinitor*) in their gut. Ingestion of prey was presented diagramatically for both species including all prey consumed, and again without inclusion of the other predator species and any double or triple positive detections of the predator species.

Hippodamia variegata

DNA from all four pests tested, as well as *M. tasmaniae*, was found in the gut contents of *H. variegata* confirming its reputation as a generalist predator. Of the 96 field collected specimens examined 16.7% tested positive to *P. xylostella*, 22.2% positive to *B. brassicae*, 21.3% positive to *P. rapae* and 7.4% positive to *N. vinitor* (Figure 23). Some of the tests did not show any positive results; 32.4% of H. variegata individuals no testpositive for any prey which indicates the possibility that those H. variegata were feeding on other soft bodied arthropods (e.g. other aphid species, thrips, leafhoppers and mites) present in the field. Of the tested H. variegata 37.5% tested positive to one prey species, 25% positive to two prey species and 8.3% positive to three prey species (Figure 24), further illustrating the generalist predator status of this coccinellid. With respect to intraguild predation 32.4% of the H. variegata tested had M. tasmaniae present in their gut contents.

Micromus tasmaniae

M. tasmaniae (76 specimens), was also shown to be a generalist predator with 50.7% positive detection to P. rapae, 35.60% positive to B. brassicae, 11.0% positive to *P. xylostella* (Figure 23) and 32% of did not show any positive result to any of the prey species tested. Of the *M. tasmaniae* tested 50 % were positive to one prey species, 17% positive to two prey species and only 2.6% positive to three prey species (Figure 25). Because of this overlap, percentages do not always add up to 100. Only 2.74% of M. tasmaniae tested positive to *H. variegata* which shows only a small amount of intra-guild predation between the two target biological agents in this direction.

The issue of secondary predation is an important one. As both *H. variegata* and *M. tasmaniae* consumed each other, they may have inadvertently also consumed whatever other insect species was in their meal's stomach. This would distort the real predation of each species on the other. Therefore the data was re-examined to remove any possible incidences of secondary predation via the other predator species thereby showing with a greater degree of certainty cases of pest consumption. The caveat to this is that secondary consumption via other predators that were not assayed could not be removed. Similarly scavenging by *H. variegata* or *M. tasmaniae* on pest cadavers (killed by other causes) remains a possibility. This re-examination brought the samples tested for *H. variegata* from 96 to 56 and for *M. tasmaniae* from 76 to 54. Figure 24 illustrates how the proportions of ingested prey species have changed. Intraguild predation by *H. variegata* on *M. tasmaniae* decreased by 33.85% while predation by *M. tasmaniae* on *H. variegata* increased by 35.04 %. Again the added proportions exceed 100% for *M. tasmaniae*.

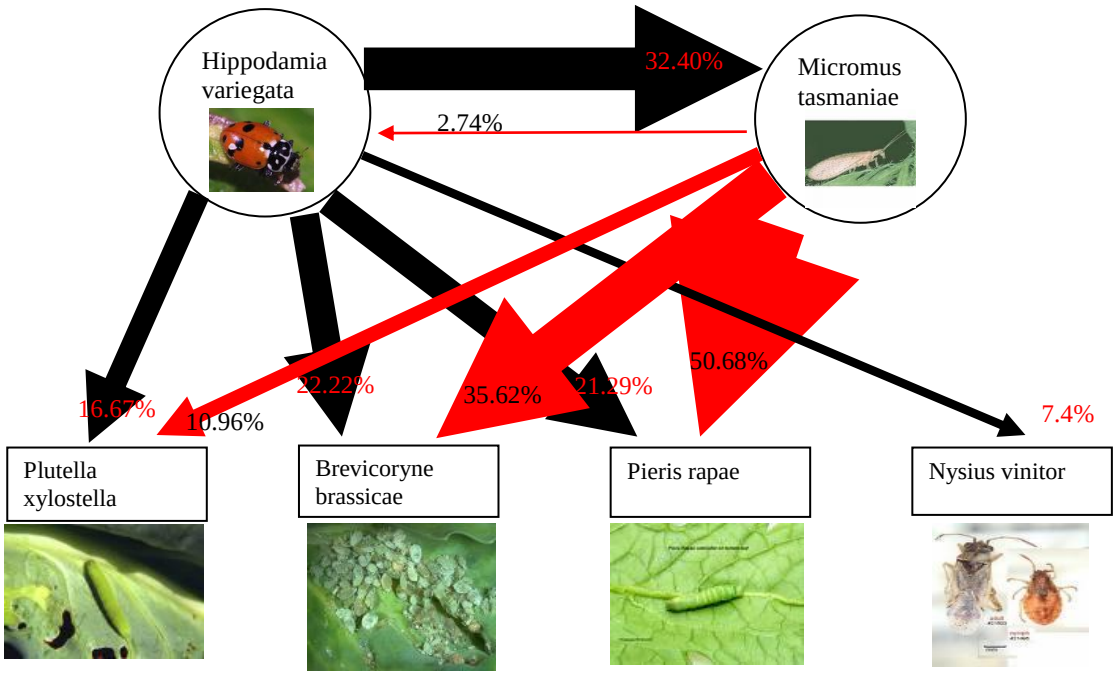

Figure 23: Percentage detection of different prey species in field collected. *Hippodamia variegata* and *Micromus tasmaniae*. Arrows indicate the percentage of prey detected in the two predators.

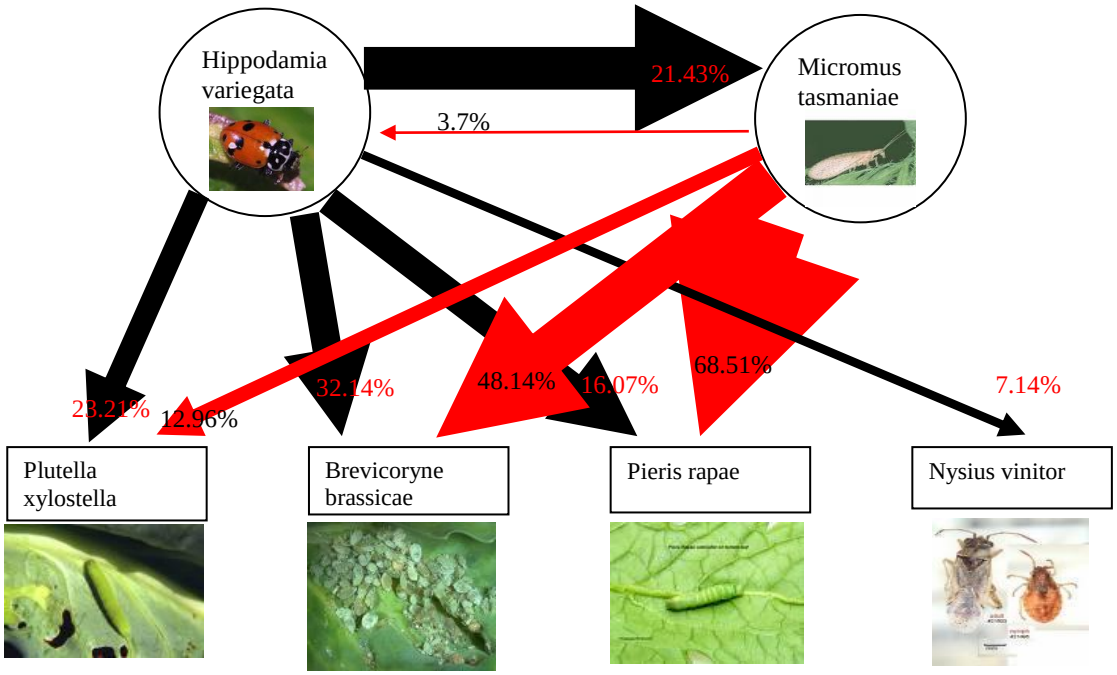

Figure 24: Percentage detection of different prey species in field collected. *Hippodamia variegata* and *Micromus* tasmaniae after the removal of double or triple positive detections of *H. variegata* or *M. tasmaniae* as well as negative detections. Arrows indicate the percentage of prey detected in the two predators.

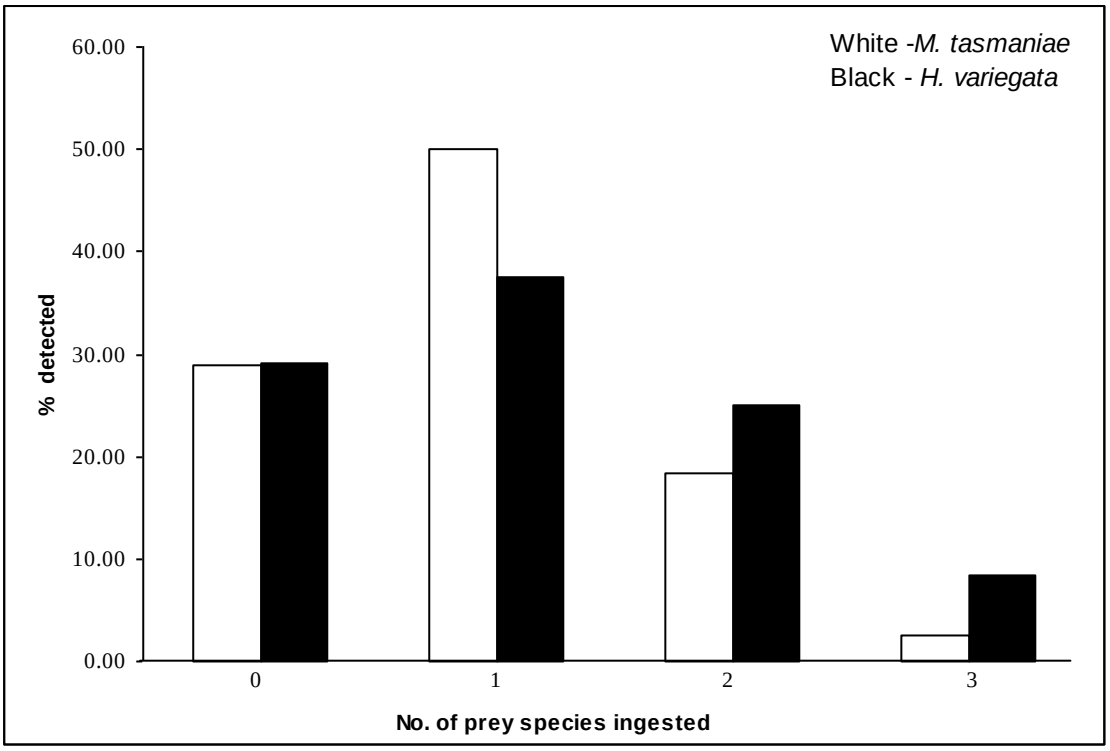

Figure 25: Positive detections for the number of prey species ingested by *H. variegata* and *M. tasmaniae*

Effects of predator species and distance from field edge

For *H. variegata* at 80 m into the crop there were significantly more detections of *P. xylostella* than at other sampling positions. This spatial effect was not significant for other prey species.

M. tasmaniae prey detection also was affected by distance from non-crop vegetation, particularly for *P. xylostella* and *B. brassicae*. At 100 m into the crop less *P. xylostella* were detected than in the non-crop vegetation. Detections of *B. brassicae* appeared to be more evenly distributed through the crop, however, numbers were still significantly lower than for non-crop vegetation.

REML analysis showed significant differences between the predator species for incidence of detection of *P. rapae* and *N. vinitor*. *M. tasmaniae* had significantly more detections for *P. rapae* than *H. variegata* (0.4579 vs. 0.2398) while *H. variegata* had significantly more detections of *N. vinitor* and *M.tasmaniae* (0.0833 vs 0.0082 and 0.3646 vs 0). *P. xylostella* and *B. brassicae* had significant distance effects as was found in the ANOVA for *M. tasmaniae*. *B. brassicae* as found in gut contents was found mostly in non-crop vegetation but also throughout the crop. *P. xylostella* in predator guts was found significantly more often in predators collected from non-crop vegetation and at 40, 80 and 100m into the crop.

Discussion

Prey range

The analysis of gut contents of the predators *H. variegata* and *M. tasmaniae* provided information on trophic relationships and the dynamics of predator-prey interaction for specimens collected from Brassica fields and non-crop vegetation. *H. variegata* proved to be a generalist predator feeding on at least 5 different prey species that commonly occur in and around Brassica crops. Three of these species, *B. brassicae, P rapae* and *P. xylostella* accounted for about 60% of ingested prey. While such statistics appear to qualify *H. variegata* as a suitable biological control agent for Brassica pests, one third of its diet constituted the predator *M. tasmaniae.*

The diet of *M. tasmaniae* included about 97% of *Brassica* pests and very few *H. variegata*. *M. tasmaniae* showed a preference for feeding on *P. rapae*, which constituted 50.68% of its diet followed by *B. brassicae* which constituted 35.62%. This also qualified *M. tasmaniae* as a useful potential biological control agent for *Brassica* pest species. Thirty percent of detections for both predators were negative, indicating that those *H. variegata* and *M. tasmaniae* had either not eaten (empty guts) or that these predators may consume a wider range of prey such as thrips, jassids, other aphids and any soft-bodied or immature insect, testing of which was beyond the scope of this project.

Intra guild and secondary predation

Secondary predation was an important issue in this experiment. Since both *H. variegata* and *M. tasmaniae* consumed more than one prey species in 20-30% of detections there was a high probability that detections were positive for prey species in the gut of a consumed predator (intraguild predation). Data were re-assessed after any detection which included a predator and another prey species were removed from the data set. Negative detections were also discounted. Proportions of detections shifted between 3.52 and 44.64 %. *M. tasmaniae* consumption by *H. variegata* decreased by 33.85% followed by decreases in *P. rapae* (31.25%) and *N. vinitor* (3.51%).Consumption of *B. brassicae* and *P. xylostella* increased by 44.64 and 39.23%, respectively. The proportionate consumption of *H. variegata* by *M. tasmaniae* increased by 35.03% and was followed by increases in all other consumed prey; 35.18% for *P. rapae*, 35.14% for *B. brassicae* and 18.24% for *P. xylostella*. Secondary predation skewed the actual detection for singular prey species in this experiment by an average of 30%. This must be taken into consideration when analysing field data rather than controlled laboratory experiments.

It must be noted that detection percentages do not appear to add up to 100%. This is due to the difference between the number of specimen tested (96) and the number of prey species detected (108) as well as the negative detections. Both *H. variegata* and *M. tasmaniae* consumed up to three different prey species but such detections were rare. Most commonly one species was ingested while two were less common.

The most noticeable result with respect to intraguild predation between *H. variegata* and *M. tasmaniae* is the asymmetry in detection. While 32.4% of *M. tasmaniae* were detected in *H. variegata* gut contents, only 2.74 % of *H. variegata* were detected in *M. tasmaniae*. Lacewing larvae are not at a disadvantage with similarly or smaller-sized coccinellids. It must therefore be assumed that they have poor chances against

adult coccinellids as they were detected in their gut contents in relatively large proportions. Therefore, an abundance of *H. variegata* in the crop should also reduce the abundance of *M. tasmaniae*. Survey data has confirmed that *M. tasmaniae* abundance was generally less than *H. variegata.* Some authors advocate habitat management to enhance coccinellid presence in the surrounding environment of agroecosystems in order to improve biological control. While this may be useful for native coccinellid species, introduced species could, with such support systems, more rapidly impact on or displace native predators.

Predator and vegetation effects

Detection of prey in predator gut contents can give an indication of the distribution of prey within the environment. Differences between distance into the crop and non-crop were not significant except for predation of *P. xylostella* by *H variegata* where most *P. xylostella* were detected at 80m into the crop. For *M. tasmaniae*, detections for *P. xylostella* were significantly higher in non-crop vegetation. It must be noted that there were not many *P. xylostella* detected in gut contents for the other distances in the crop. Since *P. xylostella* is a primary pest of Brassica crops, this may indicate movement of *M. tasmaniae* out of the crop after feeding, or that *P. xylostella* may also breed and survive on alternate weed hosts in non-crop areas. For both predators detections of *P. rapae* were not significant over crop distance or compared to non-crop vegetation, but the means indicated again the distribution of this prey species through all habitats and distances.

While no significant differences were seen for *H. variegata* consumption of *B. brassicae*, the means gave an indication of their distribution throughout the crop. For *M. tasmaniae* consumption of *B. brassicae*, significantly more detections were found in non-crop vegetation, though the means showed that *B. brassicae* was distributed throughout the field. Aphids are known to form hot spots due to the migration habits of their apterae, but in this experiment they were consumed in many locations throughout the field. Both predators also consumed *P. rapae* throughout the crop without any significant differences for location. *M. tasmaniae* detections in *H. variegata* were also not significant, but again their means showed consumption throughout the crop. This reinforced earlier findings where *M. tasmaniae* did not show a relationship with distance from field edge but rather, was distributed throughout. Initially, total detections showed that *B. brassicae* was the second most consumed prey species by *H. variegata* after *M. tasmaniae*, but after effects of secondary predation were removed, it became the favoured species. Therefore a proportion of *B. brassicae* was also consumed by *M. tasmaniae* over the same locations where *B. brassicae* was found in *H. variegata*. Both of these results supported the findings that showed that the predators in this case had no relationship with distance from field edge, possibly because there was abundant favoured prey throughout the field (in the case of *M. tasmaniae* it was *B. brassicae*, and in the case *of H. variegata* it was *M. tasmaniae* and *B. brassicae*). The results, however, contradicted other findings where *H. variegata* showed a distinct negative relationship with distance from the edge of the field.

Predator type was significant for the consumption of *P. rapae*, *N. vinitor* and *M. tasmaniae*. Significantly more *P. rapae* were consumed by *M. tasmaniae* than by *H. variegata* but *H. variegata* consumed more *N. vinitor* and *M. tasmaniae*. This result was no surprise as *P. rapae* was the favoured prey species of *M. tasmaniae.*

This study demonstrated for the first time, the use of DNA markers to detect secondary and intra-guild predation of the exotic *H. variegata* and the native *M. tasmaniae.* While the technique was useful in determining what prey had been consumed, it did not give any information about prey movement, feeding patterns and behaviour or opportunistic prey preference. Yet these results were far more realistic than results obtained under controlled and limited laboratory conditions. While choice tests give an idea about what a predator may consume if offered, it does not mean that the preference holds true when circumstances change. This study has been very innovative in using the new method, DNA with field collected specimens to understand actual biocontrol effects. Although these findings are tentative, especially for spatial effects and non-crop vegetation, they are uncharted territory.

Potential Against Common Greenhouse Pests

Results

All diets resulted in more than half of the population of *H. variegata* not surviving past day 15 (Figure 26a). Survival in *M. tasmaniae* also showed considerable mortality over the experimental period (Figure 26b). 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. 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.*

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*. 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.

Discussion

Pest species as food sources

Based on the fact that they supported some level of larval development from larva to adult, 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*.

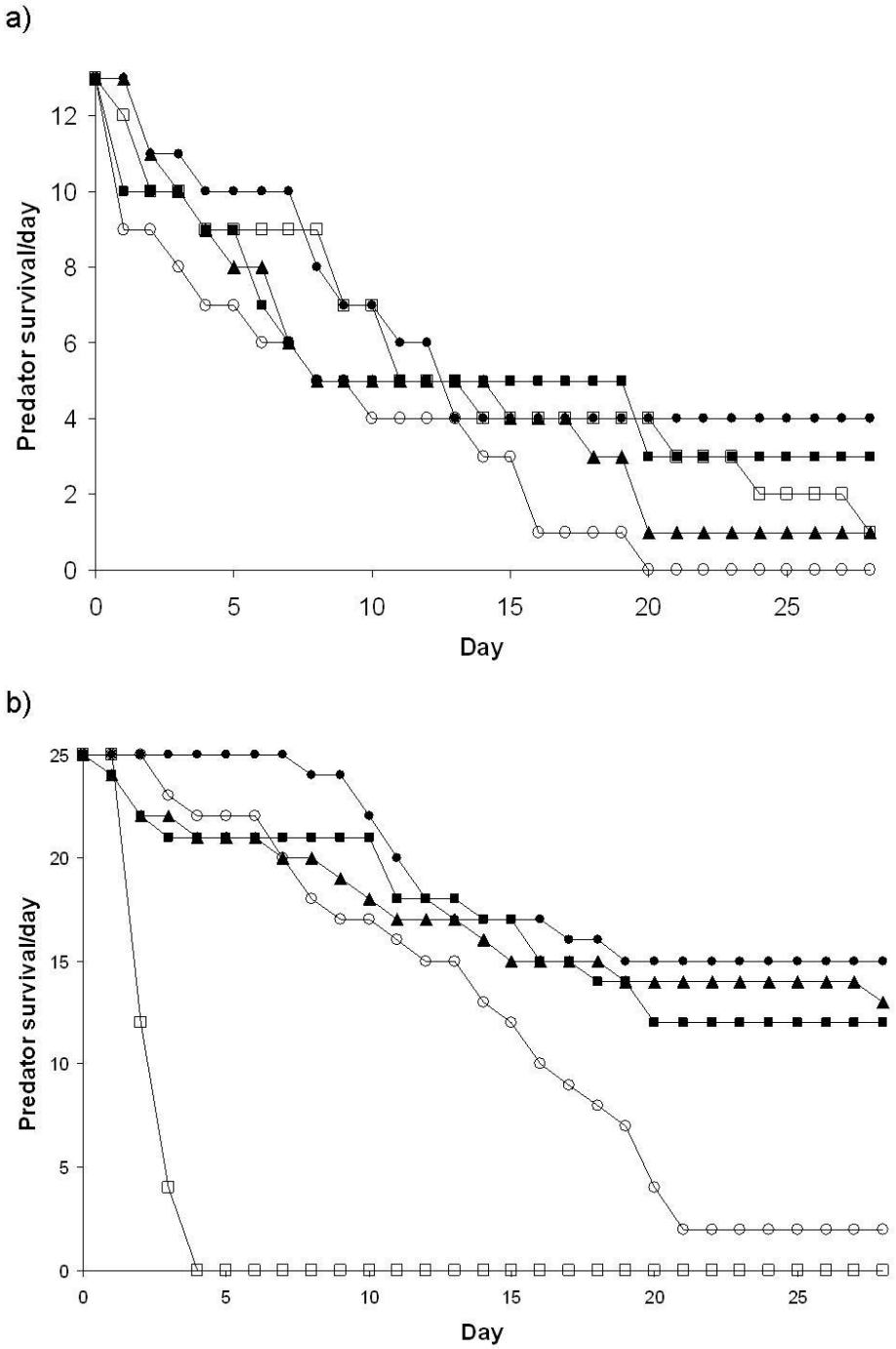

Figure 26: The effects of prey diets on survival of a) *H. variegata and b*) *M. tasmaniae* over time while fed on a diet of *A. craccivora* (closed circle), *F. occidentalis* (open circle), *M. persicae* (closed square), *T. urticae* (open square) and *T, vaporariorum* (closed triangle).

Larval development time

Daily feeding

Significantly more *A. craccivora* and *M. persicae* were consumed by *H. variegata* (Figure 27a, c) and *M. tasmaniae* (Figure 28a, c) on a daily basis compared to the other diets. Significantly fewer *T. urticae* were consumed by *M. tasmaniae*.

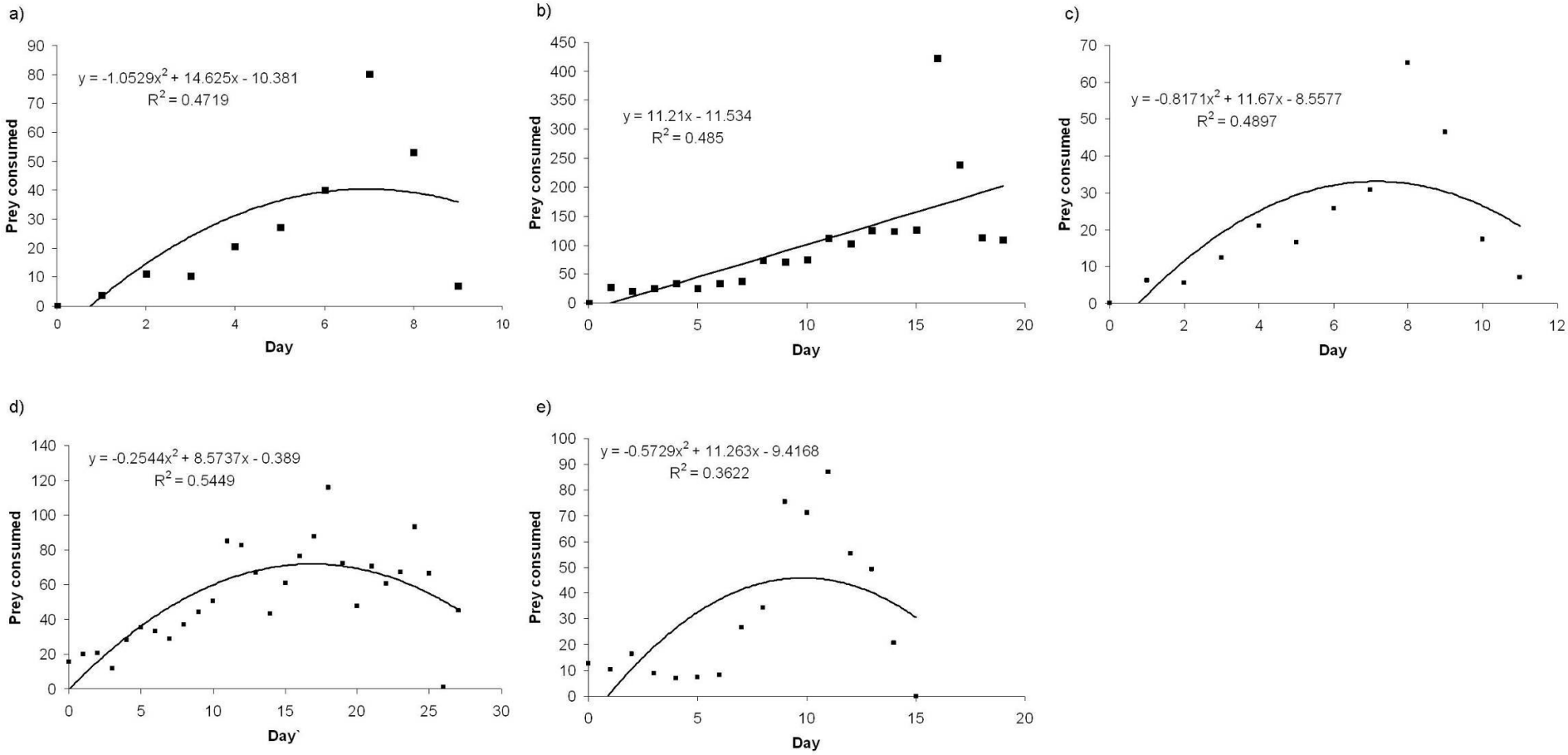

Figure 27: Mean prey consumed per day by *H. variegata* larvae per day when fed on a diet of a) *A. craccivora*, b) *F. occidentalis*, c) *M. persicae*, d) *T. urticae* or e) *T. vaporariorum*.

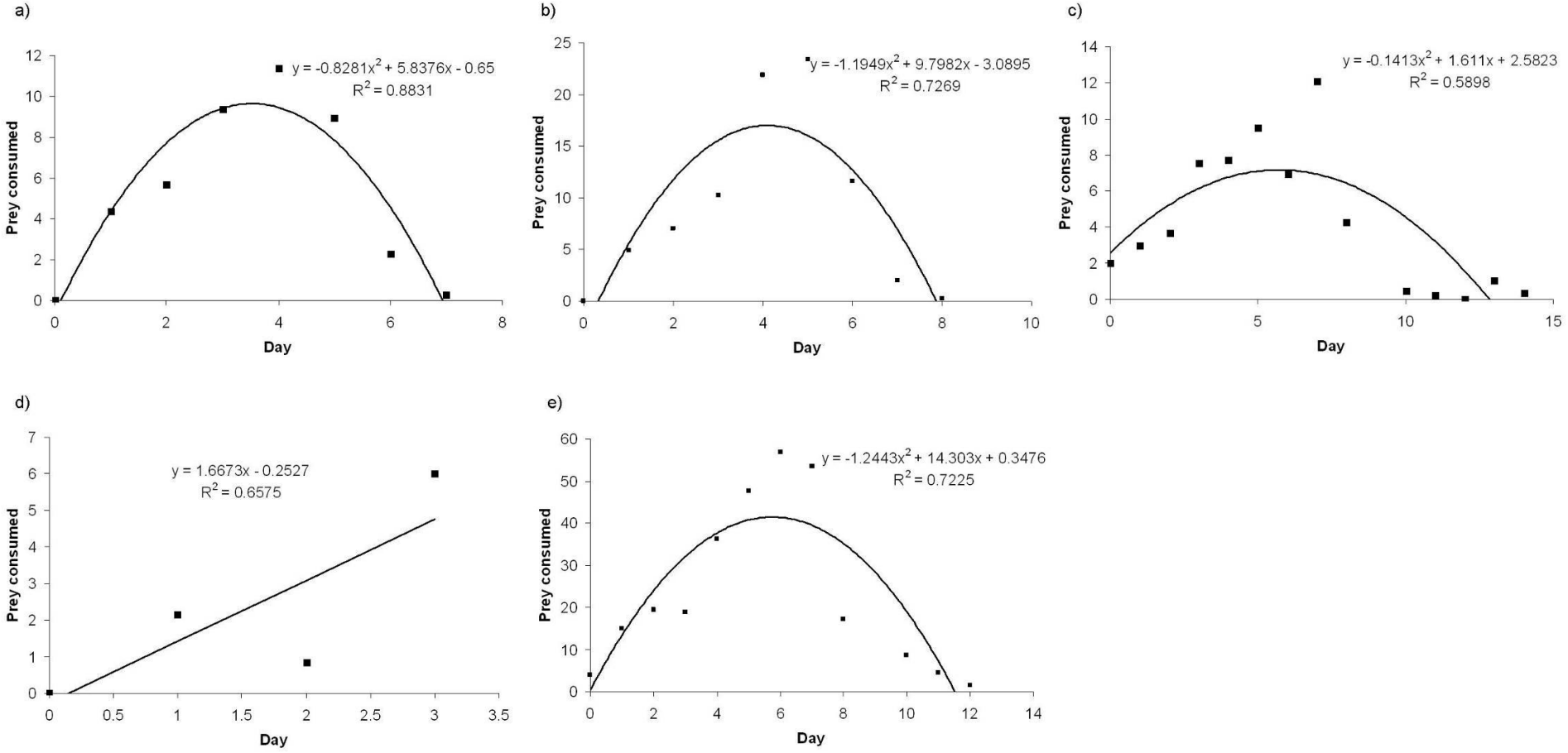

Figure 28: Mean prey consumed per day by *M. tasmaniae* larvae per day when fed on a diet of a) *A. craccivora*, b) *F. occidentalis*, c) *M. persicae*, d) *T. urticae* or e) *T. vaporariorum*.

The concept of predator modelling is not new. More mathematically complex approaches to modelling predator behaviour in coccinellids have already been constructed. 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.

While the usefulness of these indices is diminished by the poor survival of the predators in this study, they may be of use in combination with the figures 25 and 26. 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 (Figure 26). 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 and will find and prey upon low quality prey even when a better food source is available. 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. 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. 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-born 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, a high quality artificial diet might overcome this limitation. Artificial diets have been developed for green lacewings and Coccinellids. Predatory mirids have been reared on meat based diets which are superior to more natural diets, having higher oviposition and and nymphal survival on the artifical diet.

When two or more prey species are present, 'apparent competition' can occur between two pest species in the presence of a predator. 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. 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.

Future work needs to consider a number of different aspects. The effects of multiple-prey diets should be considered. 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. Coccinellid predators do not distinguish between parasitised aphids and healthy aphids and coccinellid larvae develop slower when fed on aphid mummies. 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.

The role of photoperiod and temperature in the onset of dormancy

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 (R^2=0.38 and 0.59 at 16L:8D and 8L:16D) (Figure 29).

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).

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. 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. 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. 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 30).

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 29), 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.

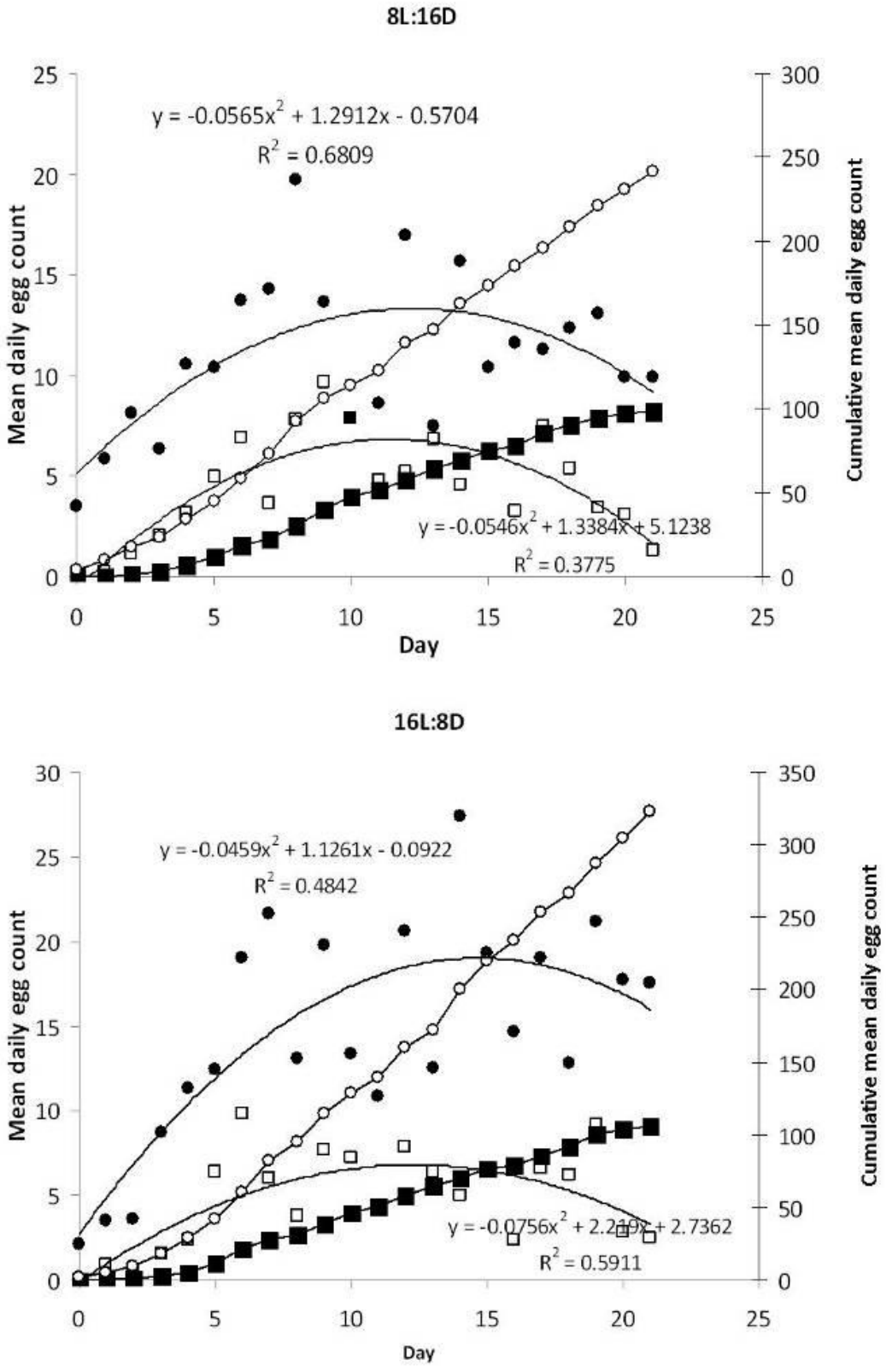

Figure 29: The effects of temperature and photoperiod on the mean daily oviposition of *M. tasmaniae* at 18°C (open circle) and 25°C (open square) and cumulative mean daily eggs count at 18°C (closed circle) and 25°C (closed square) over 21 days.

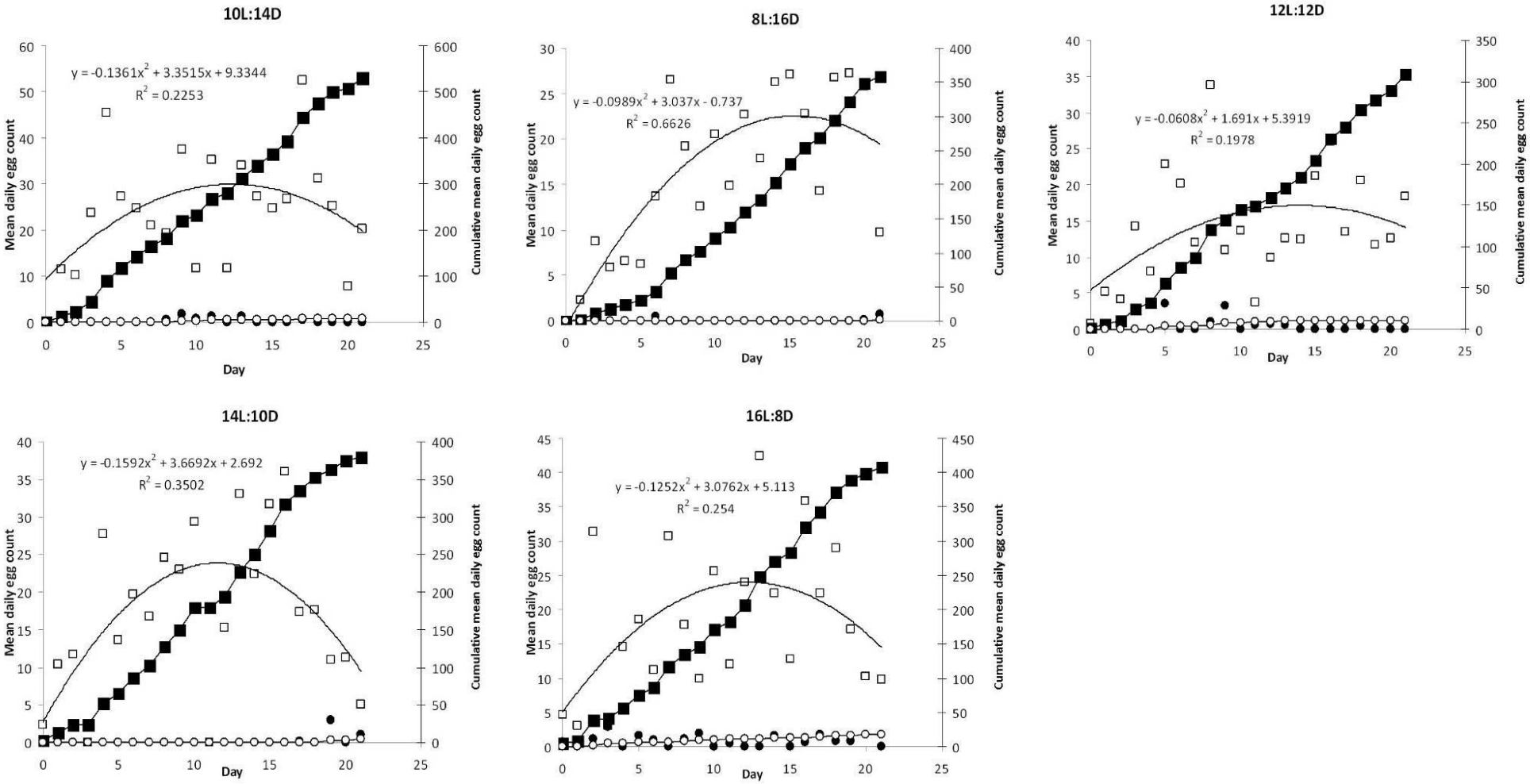

Figure 30: The effects of temperature and photoperiod on the mean daily oviposition of *H. variegata* at 18°C (open circle) and 25°C (open square) and cumulative mean daily eggs count at 18°C (closed circle) and 25°C (closed square) over 21 days.

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 and has provided a practical way by which diapause in beetles may be determined. 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. The average temperature over a 24h period needs to conform to this range.

In a greenhouse, temperatures above 28°C can harm fruit set and photosynthesis rate. 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. 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, 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.

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* and certain parasitoids such

as *Chrysocharis pubcornis* 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, the topical treatment of diapausing *H. axyridis* with juvenile hormone analogues have been shown to initiate 'immediate' ovarian development. Future work needs to focus on two areas. Firstly, to address the ending of this diapause response in *H. variegata* by exposing adult females to low temperature conditions to induce diapause, then exposing these individuals to a range of photoperiods and temperatures. Secondly, to investigate the transport of these biocontrol agents by exposing them to cold temperatures for extended periods to determine if they can survive transportation to a commercial grower.

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*. Reduced hatching and survival also occur in other biocontrol agents exposed to cold storage. Survival of *Podisus maculiventris* 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.

Results

Hippodamia variegata survival

For *Hippodamia variegata* in experiment 1 there was no survival in the abamectin, chlorpyrifos or imidacloprid treatments. Proportion of survival differed between the remaining treatments ($F=5.13$, $df=5, 79$, $P<0.001$) exceeding 0.67 in the botanical oil and control treatments and significantly lower in the bifenthrin and maldison treatments where survival was lower than 0.36. Survival in the remaining treatments was intermediate (Table 3).

Micromus tasmaniae survival

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 ($F=4.14$, $df= 4, 80$, $P=0.002$) exceeding 0.84 in the buprofezin and control treatments and significantly lower in the abamectin treatment. Survival in the remaining treatments was intermediate (Table 3).

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 3).

Table 3: 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.

Treatment	*Hippodamia variegata*		*Micromus. tasmaniae*	
	Survival proportion, n=18	IOBC classification*	Survival proportion, n=18	IOBC classification
abamectin	0	Harmful	0.44 c	Moderately Harmful
bifenthrin	0.36 bc	Harmful	0	Harmful
buprofezin	0.40 b	Moderately Harmful	0.83 ab	Harmless
chlorpyrifos	0	Harmful	0	Harmful
control	0.93 a	Harmless	0.94 a	Harmless
imidacloprid	0	Harmful	0	Harmful
maldison	0.20 c	Harmful	0	Harmful
botanical oil	0.67 ab	Moderately Harmful	0.61 bc	Moderately Harmful
pirimicarb	0.73 b	Moderately Harmful	0.61 bc	Moderately Harmful

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%. Within these broad categories, we can attempt to classify the chemicals used in the experiments. 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 while having been shown to be harmless to a green lacewing. 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 picipes* treated with buprofezin survived to adulthood, while early instar *Orius tristicolor* were unaffected.

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. 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.

Discussion

This study on the potential use of *H. variagata* and *M. tasmaniae* as biological agents for Brassicaceous crop pests has contributed useful information on the two predators for their inclusion in an IPM system. It showed that:

- *H. variegata* and *M. tasmanaie* were abundant in both brassica crops and non-crop vegetation and that their populations varied seasonally
- Both *H. variegata* and *M. tasmaniae* moved from non-crop habitat to crops and their movement was affected by time and distance
- Non-crop vegetation was an important source of predators for the repopulation of fields after the application of chemical sprays. *H. variegata* exhibited stronger repopulation from croplands than *M. tasmaniae*, which tended to move back into fields from non-crop vegetation
- DNA techniques were useful in identifying the predators' prey range and uncovered highly asymmetrical intraguild-predation activity as well as secondary predation.

There is scope to use the paint marking technique to investigate the alternative distance sources and movement of the two predators. The inclusion of other soft bodied arthropod species (possible prey) in a DNA gut analysis is warranted as is more specific work on the intraguild predation between the two predators.

While this study has shown supporting data for the potential use of the two predators as BCAs, it has not fully explored the negative effects that *H. variegata* may have on Australia's native arthropod predators. The extent to which *H. variegata* preys on *M. tasmaniae*, is concerning as it may suppress biological control by eliminating competitive predators from food systems. Since its detection in south-east Queensland in 2002 *H. variegata* has become widespread in the Australian environment. The survey of the Bathurst area has shown that it comprises about 50% of the coccinellid fauna in that area and has not yet completely displaced other coccinellids as *H. axyridis* did in Florida.

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.

Technology Transfer

Theses

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, Phd Thesis, Charles Sturt University, 2010

Le Mottee K., unpublished, The Potential for Greenhouse Biocontrol by Generalist Predators *Hippodamia variegata* and *Micromus tasmaniae*, MPhil Thesis, Charles Sturt University, 2010

Conference Proceedings

Heimoana, V., Gurr, G. M., Raman, A., Goodwin, S., Steiner, M., Pilkington, L. J., Campbell, A. J., and McDougall, S. J. (2006). The Potential of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) and *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) as biocontrol agents for insect pests in brassicas and other field vegetable crops. Australian and New Zealand Entomological Societies' Conference. Adelaide, South Australia, September 24-27, 2006. 65.

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Le Mottee, K., Gurr, G. M., Pilkington, L. J. and Raman, A. (2008). Effects of Pesticide Exposure on the Generalist Predator *Hippodamia variegata* (ColeopteraL Coccinellidae). Australia and New Zealand Biocontrol Conference. Sydney, New South Wales, February 10-14, 2008. 90.

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Le Mottee, K., Pilkington, L. J., Gurr, G. M. and Raman, A. (2008). The effect of photoperiod and temperature on reproduction of generalist predators *Hippodamia variegata* (Coleoptera: Coccinellidae) and *Micromus tasmaniae* (Neuroptera: Hemerobiidae). Australian Entomological Society's 39th Annual General Meeting and Scientific Conference, Orange, New South Wales, September 28 – October 1, 2008. 27.

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Heimoana, V., Gurr, G. M., Raman, A., Mitchell, A. and Pilkington, L. J. (2009). The two-edged sword of *Hippodamia variegata* invasion of Australia: biological control bonanza or ecological disruption? International Congress on Biological Invasions. Fuzhou, China, 2-6 November, 2009.

Manuscripts prepared for submission to journals

Four manuscripts are being prepared for submission from the work completed in this project. They will be published in various peer reviewed journals.

Recommendations

This project has provided an understanding of the conservation biological control for two generalist predators in outdoor crops and their possible utility in greenhouse horticulture. These findings provide an additional level of pest management tools that are available to vegetable growers in Australia. The recommendations generated from this project are as follows:

- That commercial rearing protocols be developed for the two biological control agents. Evidence has been presented indicating that the two organisms have great utility in pest management but as yet there is no commercially viable rearing protocols to allow a commercial producer to provide the quantities that would be needed for industry.

- Transportation protocols for the two organisms to be developed for use in a commercial supply situation. All studies to date have focussed on the biological control agents' ability to prey on pest insects, but no work has been completed on how to transport the agents. This will be natural progression, and potentially a part of, any future work looking at rearing protocols.

- The biological control agents efficacy in a commercial greenhouse production facility needs to be confirmed in large scale trials. It is recommended that extensive work be completed in greenhouses, and in the field, when rearing protocols are established.

- Industry workshops and extension material should be prepared for the effective use of these biological control agents and their use with other complementary organisms when they are commercialised.

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