The potential of *Hippodamia variegata* (Coleoptera Coccinellidae) and *Micromus tasmaniae* (Neuroptera: Hemerobiidae) as biological control agents for arthropod pests in Brassica crops

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TABLE OF CONTENTS

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

CERTIFICATE OF AUTHORSHIP

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

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Viliami Heimoana

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DEDICATION

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ABSTRACT

The ladybird beetle, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a recent arrival in Australia, and a native brown lacewing, *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae), were being investigated as potential biological control agents for arthropod pests in brassica crops.

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*.

This thesis has contributed useful information on the potential use of *H. variagata* and *M. tasmaniae* as biological agents for brassica crop pests. It also demonstrated the importance of non-crop areas in agro-ecosystems as alternative and source habitat for natural enemies.

CHAPTER ONE - GENERAL INTRODUCTION

1.1. INTRODUCTION

Fruit and vegetable production is a necessity to feed a country's population not only in Australia but worldwide. For this reason agriculture has been intensified, mechanised and industrialised (Primdahl, 1990; Winfrey & Darity Jr., 1997; Gockowski & Ndoumbe, 2004). Horticultural production of high quality fruit and vegetables generally involves the use of pesticides, often in large amounts. Yet as our population has become more health conscious and environmentally aware in recent years, the demand for food products grown with minimal or no chemicals has increased. In order to satisfy this market, producers have adopted integrated pest management (IPM) approaches into their production systems to varying degrees (Tilman et *al*., 2002; Jat et *al*., 2006). These are characterized by more mechanical, biological and ecological techniques and less reliance on chemicals. By accepting IPM approaches, producers and researchers alike have come to realise the complexity of healthy ecological systems. One approach to facilitate IPM is to create a farmscape environment that encourages biological control agents to flourish. Less synthetic pesticide use, more biological pesticide use, establishment of shelter belts, wooded areas, cover crops and food crops for insects are just some ways in which habitats can be manipulated to create a

farmscape more conducive to beneficial insects than the normal situation on commercial farms. In Australia, habitat manipulation of farming environments to maintain an environment favourable to beneficial insects has been emphasized by authors such as Gurr and others. Habitat manipulation will support the effective use of natural enemies within an integrated pest management (IPM) program in field grown vegetables. White et al. (1995), Schellhorn and Silberbauer (2003), Gurr et al. (2004) and Stephens et al. (2006) all stated that establishment and maintenance of suitable habitat on farm or in the surrounding landscape can enhance the survival of natural enemies and their efficacy as biological control agents (BCAs). Use of natural enemies as biological agents is a more sustainable insect pest control option with benefits such as minimizing the use of insecticides; slowing down the increasing incidence of insecticide resistance; producing reduced chemical or chemical free produce for the consumers; lessen production cost for the growers and support overall agricultural sustainability.

In Australia, vegetable growers from the Bathurst area in the Central West of New South Wales (CW-NSW) account for 26% of the vegetable production of the state of NSW (Naughton, 2002). They grow diverse brassica crops as well as sweet corn. Recognising the unsustainability of relying on pesticides the industry body Horticulture Australia Ltd (HAL) has supported work on the potential for biological control to be enhanced, particularly with the newly-arrived, exotic ladybird beetle *Hippodamia variegata* (Goeze) (syn. *Adonia variegata* (Goeze)) (Coleoptera: Coccinellidae). Several earlier studies (Belikova & Kosaev, 1985; Pyke & Brown,

1996; Franzman, 2002); Kavallieratos et al., 2002) have identified *H. variegata* and the native brown lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae), as potentially important biological control agents, particularly of aphids and the eggs and larvae of Lepidoptera. Since species of Aphididae and Lepidoptera are the key pests of brassicaceous vegetable crops in fields, and also because both predators are active in Australia (New & Boros 1983; Pyke & Brown 1996; Franzman 2002; New 2002), *H. variegata* and *M. tasmaniae* were investigated for their potential usefulness as biological control agents in the vegetable crop pests in CW–NSW. Currently Lepidoptera and aphid pests are controlled by applying a variety of insecticides, however, the continued reliance on insecticides will not be viable due to resistance build-up and safety concerns. Therefore, there is a need in the vegetable industry for an alternative insect pests control method.

While biological control may be a favoured alternative control method, and growers in CW-NSW are supportive of its use, it is not currently either widely or successfully practiced (Campbell AJ 2006, pers. com). The degree to which biological control agents such as predators, parasites and pathogens can be utilised varies between crops and areas. The implementation of biological control of specific pests requires a detailed knowledge of the crop situation, the pest itself and the potential biological control agent or agents (BioResources 2006).

The entomophagous insects *H. variegata* and *M. tasmaniae* are commonly present in the Central West region, however, their biology, relative abundance and preferred prey throughout the year have so far not been studied. To assess the potential of such generalist predators for application as BCAs in the vegetable industry, more information about their biology, hosts and predatory capacity is required. This project aims at gathering such information so as to include these two predators in IPM strategies for the vegetable industry in CW-NSW.

This chapter gives a brief introduction to the Australian vegetable industry and its pest problems and IPM practices, an overview of conservation biological control and habitat management, landscape scale issues in biological control of agricultural pests and also provides an overview of the structure for the thesis to follow.

1.2. Vegetable production systems

1.2.1. Study Area

The Central West region of NSW (Figure 1) is situated west of the Blue Mountains, and extends from Lithgow (latitude: 33.4901 S, longitude: 150.1498 E) to Condobolin (latitude: -33.0896, longitude: 147.1439) Bathurst and Orange are the

two major cities in the region. Bathurst District consists of the Shire of Evans, the city of Greater Lithgow, Oberon, city of Bathurst and a section of Blayney Shire (Figure 1.1). The area includes 11,744 km² of which 44% is used for agricultural activities (Naughton 2002). About 45,000 tonnes of vegetables are grown on about 2200 ha by 75 growers (Wade 2005).

Figure 1.1: The Central West region of New South Wales
Source: Australian Bureau of Statistics (2002)

Vegetables are primarily grown on the Central Tablelands, which include the Bathurst Plains and the Orange and Oberon Plateau. The fertile black clay loam of the Bathurst Plains is irrigated by the Macquarie River while the red clay loam of the plateau is supplied by farm dams and bores (Wade 2005).

1.2.2. Climate

The temperate climate of the Central West region is distinguished by cold winters and warm to hot summers. Rainfall is spread fairly evenly throughout the year. The annual rainfall in Bathurst is 631mm (Wade 2005). Annual evaporation is 1341 mm and 1468 mm, respectively in Bathurst and its rate exceeds that of rainfall during every season except winter. Therefore, irrigation is necessary to grow vegetable crops successfully. Temperatures range from 13 to 28° C in January to 1 to 11° C in July (Table 1.1). Frosts may occur during 9 months at Bathurst (Wade, 2005).

Table 1.1. The Bathurst climate monthly averages (Bathurst Agricultural Station 1908-2004)

Season	Spring			Summer			Autumn			Winter		
Month	S	O	N	D	J	F	M	A	M	J	J	A
Max. temp. (˚C)	16	20	23	26	28	27	26	25	16	12	11	13
Min. temp. (˚C)	3	6	9	12	13	13	11	7	4	2	1	1
Frost (days)	7	2	0.1	0	0	0	0.1	2	8	12	16	13
Rainfall (mm)	46	61	58	62	68	57	50	43	43	44	49	50
Evaporation (mm)	81	124	156	205	211	165	140	87	50	33	34	56

Source: Wade, 2005.

1.2.3. Crops

The Central West NSW grows about 6% of the vegetable crop of NSW. A wide range of summer and winter vegetables including asparagus, beans, beetroot, cucumbers, lettuce, peas (27% of the NSW crop), potatoes, pumpkins, rockmelons, silverbeet, sweet corn, tomatoes, watermelons and zucchinis are grown for the fresh and processing markets (ABS, 2002; Wade, 2005). Of this, processing vegetables such as potatoes, sweet corn, beans and beetroot (56% of the NSW crop) are principal industry efforts. Bathurst is also the major centre for the production of brassica crops in New South Wales including Brussels sprouts, cabbages, turnips, cauliflowers and broccoli (26% of the NSW crop) (Naughton, 2002).

Cabbages are planted in the Bathurst area in the spring and summer months between September and March and are harvested between January and August (Wade, 2005). Green Coronet and Warrior are the common varieties that go to fresh-product markets and the processing industry, respectively, yielding an average of 41 t/ha. Three varieties of cauliflower are planted between September and February and are harvested between December and August. Cauliflower yields an average of 24 t/ha (Wade, 2005). Broccoli, another major brassica crop, is planted between September and March and is harvested between November and May. It yields much lower than cabbage or cauliflower, averaging 6 t/ha. Lettuce for the fresh market is sown from August to February and is harvested

between November and May, averaging 30 t/ha. Sweet corn is one of the key vegetables for the processing industry. Corn is either canned or frozen (Wade, 2005).

Sequential planting for a continuous and extended supply of vegetables is common practice. This gives the grower better management options and avoids flooding the market with one specific produce at one time. There are early, mid and late plantings which are also dictated by the processing plants that require reliable and consistent supplies of produce for processing (Wade, 2005; Camenzuli, pers. comm.)

1.2.4. Factors affecting vegetable production in the Central West

The region has been very successful in vegetable production due to a number of factors (Wade, 2005):

- climatically it is suited to grow cool season vegetables such as the brassicas in the summer months, therefore it can supply the markets during times when other regions are not competitive
- local processors purchase produce under contract giving growers an income other than from the fresh produce market

- access to the central markets of Sydney is only a relatively short distance (225-350 km) away, keeping transport costs down and travel times short. This is an important factor for perishables.
- water availability has in the past not been a problem, however, the past four years of drought have taken their toll on water availability for irrigation. Current dam levels stand at 55% capacity for Ben Chifley Dam (Macquarie River Catchment) (Bathurst Regional Council, 2007) and 11% and 8% capacity for Carcoar and Wyangala Dams (Lachlan River Slopes Catchment), respectively (Waterinfo, NSW Gov., 2007). Current and future developments in the vegetable industry will depend on trends in climate change, the availability and reliability of water supplies and the government's water policies.

1.3. Brassica Pests

Vegetable crops growing in the Central West region have various pest complexes. Some crops are attacked by similar pests, which create problems when cropping cycles overlap and the pests move from a finishing crop to an emerging crop. Sequential planting of one crop also ensures that specific crop pests can always move to fresh hosts. For example, the aphid species *Aphis gossypii* Glover (Homoptera:Aphididae), *Aphis fabae* Scopoli (Homoptera:Aphididae), *Aphis craccivora* Koch (Homoptera:Aphididae), *Macrosiphum euphorbiae* (Thomas) (Homoptera:Aphididae)and *Myzus persicae* (Sulzer) (Homoptera:Aphididae) are all pests of peas and beans (Blackman and Eastop, 2000). *Myzus persicae*, which is a highly polyphagous species, also infests brassicas, corn, onions, potatoes and beets. Most of the species of *Aphis* readily feed and breed on cucurbits, beets, corn and potatoes. This *Aphis* example illustrates the flexibility of only one group of pests in vegetable cropping systems. Since this project is primarily concerned with the control of pests in brassica crops, only the pest species specific to this family are further discussed.

1.3.1. Major pests

The most severe damage to growing brassicas is usually inflicted by the diamond back moth (DBM) (*Plutella xylostella* L. (Lepidoptera:Plutellidae)) and the cabbage white butterfly (CWB) (*Pieris rapae* L. (Lepidoptera:Pieridae)). The mealy cabbage aphid (*Brevicoryne brassicae* L. (Homoptera:Aphididae)), the false cabbage aphid (or turnip aphid) (*Lipaphis pseudobrassicae* syn. *erysymi* Kaltenbach ((Homoptera:Aphididae)) and the green peach aphid (*Myzus persicae* Sulzer) also inflict severe losses (Hamilton and Toffolon, 1987; Murison and Napier, 2006). These insect species occur on all brassica crops Australia wide.

Throughout Australia, diamondback moth is possibly the most damaging pest (Learmonth et al., 2003; Liu et al. 2006). Reports from the United States also list Lepidoptera larvae, in particular DBM, as a major pest of brassica crops (Mossler, 2005). *Pl. xylostella* is a small moth (about 10mm long) with long antennae and a distinct cream coloured diamond pattern on its back when its wings are folded. It appears grayish-brown and its wing tips turn up at the ends (Plate 1.1).

Plate 1. 1: An adult diamond back moth, *Plutella xylostella,* the major pest of brassica vegetable crops

Eggs are laid in small groups on any part of a brassica plant but more commonly on the upper leaf surface in cauliflowers (Learmonth et al., 2003). Hatched colourless larvae mine and feed on leaves producing a scribbly pattern across the leaf. If disturbed, they drop from the leaf suspended on a strand of silk. Later instars are light green and may feed from 10 and 30 days, depending on the atmospheric temperature (Mossler, 2005). Pupation occurs in silk cocoons on leaves. The development time from egg to adult ranges from 14 days at 28°C to 4

months at 12°C (SARDI, 2005). *Pl. xylostella* can attack plants of all growth stages. Damage is severe with large areas of mesophyll eaten except the cuticle (Talekar, 1996). Larvae also attack the developing cabbage heads or floral heads of broccoli and cauliflower, deforming them and providing entry points for pathogens. Uijtewaal (2006) states that crop losses without the use of insecticides can reach up to 90%, but that even with application of insecticides, losses can be substantial: the estimated cost of controlling this pest worldwide is about US$ 1 billion per annum (Uijtewaal, 2006).

Cabbage white butterfly is another major lepidopteran pest of brassica crops. Eggs are laid on leaves and larvae hatch to feed on leaves. While cabbage white butterfly larvae do not tend to feed on the heads of cabbages and cauliflowers, their frass may contaminate the harvestable parts and reduce their commercial value (Learmonth et al., 2003). Pupation also occurs on leaves and pupae remain attached to leaves by a silken thread. While damage by DBM is considered important, damage inflicted by cabbage white butterfly appears even more severe. Liu et al. (2001) listed some action thresholds for Lepidoptera in brassica crops in China and converted different pests to 'standard insects' scale: 1 standard insect was equivalent to 1 *Pi. rapae* or 5 *Pl. xylostella*. At times of pre-heading to early heading, the action threshold was 1 standard insect/plant, which increased to 4 standard insects/plant between heading and maturity. These thresholds were based on the finding that cabbages were able to endure some defoliation without reduction in head weight at harvest.

Cluster caterpillars such *as Spodoptera litura* (Fabricius) (Lepidoptera:Noctuidae) and *Crocidolomia pavonana* (Fabricius) (Lepidoptera:Pyralidae) can also be severe pests of brassicas, especially throughout summer and autumn during hot and dry spells. They may destroy the hearts of cabbages and leave large holes in the leaves (Hamilton and Toffolon, 1987).

Plate 1. 2: Cabbage plant damage by aphids
(Photo: V. Heimoana)

Aphids reduce plant growth and crop quality (Collier, 1999) by sucking carbohydrate rich assimilates from the phloem of their host plants. This feeding damage results in weak and stunted plants with curled and distorted leaves (Plate 1.2). Seedlings may show wilting, chlorosis and may die (Li et al., 2001). Van Emden (1990) found that *B. brassicae* decreased the total dry weight of Brussels sprouts by 25 -73%**.** Specific figures for crop losses are very rare in the literature but there is no doubt that even low aphid infestations cannot be tolerated. The ability of aphid colonies to foul leaves and buds, and hence reduce the marketability of the crop, renders growers intolerant of even low thresholds (Wilson et al., 1983; Hines and Hutchison, 2002). Foster and Flood (1995) defined aphid thresholds on brassica crops: a rating of two (low), where 4-10 aphids were present on a plant, already warranted the recommendation of an insecticide spray. If heavy rain was predicted or many predators and parasites were present, spraying was not recommended but regular monitoring was advised.

Apart from causing obvious physical damage to brassica crops, aphids have further significance as vectors of at least 20 viral diseases (Blackman and Eastop, 2000). Latham et al. (2003) reported the incidence of three viruses, Beet western yellow virus – (BWYV), Cauliflower mosaic virus (CaMV) and Turnip mosaic virus (TuMV) in vegetable and wild brassica plantings in Western Australia. Virus detections occurred in wild radish (BWYV at 65%; TuMV at 2%) but not in vegetable brassicas.

A consequence of phloem feeding by aphids is the high secretion of honeydew, a sticky residue on leaves which acts as a medium for sooty mould fungal growth. Leaves contaminated with sticky black mould patches reduce the marketability of the crop.

1.3.1.1. Seedling pests

Crop establishment can be affected by a number of species of snails, slugs, and various species of weevil larvae and cutworms (Learmonth et al., 2003). Seedling pests may not occur every season, but during outbreaks, a substantial part of the crop can be destroyed. Slugs and snails eat the leaves of young seedlings but can also feed on the harvestable parts such as cabbage heads and cauliflower curds. Usually they are controlled by placing snail bait in the field at any time. Weevil (Coleoptera) larvae kill seedlings by chewing through the stem below the ground. They are more difficult to control as they live below ground and usually require cultural control and chemical application. Cutworms (Lepitoptera: Noctuidae) are the larvae of moths and often feed nocturnally on seedlings (Neilson, 2001). They can damage seedlings in different ways such as by severing the stems and feeding on leaves or roots. Preplant herbicides that reduce vegetation can reduce the attractiveness of the field as an oviposition site thus reducing the number of emerging larvae (Dively, 2006). Since larvae live and pupate in the soil, cultural practices may also control cutworms. Autumn “pupa busting” (ploughing the top 10 cm of soil to destroy lepidopteran pupae or their emergence tunnels) is a technique

used for moth pests in cotton systems (Farrell and Johnson, 2005) and may be applicable when ploughing out brassica fields.

1.3.2. Minor pests

Hamilton and Toffolon (1987) and Murison and Napier (2006) noted that the following insects damage brassicas in Australia on occasions: *Helicoverpa* spp., cabbage centre grubs *Hellula hydralis* Guenée (Lepidoptera:Pyralidae), looper caterpillars *Chrysodeixis* spp. (Lepidoptera), onion thrips *Thrips tabaci* Lindeman (Thysanoptera:Thripidae), seedling maggots *Hylemya cilicrura* (Rond.), (Diptera, Anthomyiidae), vegetable weevils *Listroderes costirostris* Schoenherr (Coleoptera: Curculionidae), black beetles *Heteronychus arator* (Fabricius) (Coleoptera: Scarabaeidae) and Rutherglen bugs *Nysius vinitor* Bergroth (Hemiptera:Lygaeidae).

1.4. Insect Pest Management

1.4.1. Current practices and problems

Currently almost all growers are relying solely on insecticides for insect pest management. The use of pesticides is the usual pest management strategy against

all brassicas pests since damaged products are not easily marketed. There are many risks associated with the use and misuse of pesticides in commercial vegetable production. Some of them include, a minor pest becoming a major pests, or resurgence of pest populations, due to a reduction of natural enemies; insect resistance buildup; adverse impacts on crop growth; pollution of ground and surface waters; unwanted chemical residues in foods; reduced farm profitability; inadvertent chemical exposure of the public or farm workers, and land devaluation due to real or perceived hazards. Therefore there is a need to investigate other control methods such as biological control which can be used in an IPM approach.

1.5. Biological Control

1.5.1. Principles

Biological control employs natural enemies of pests to reduce the adverse effects these pests have on humans, animals and plants. Callan (1969) described biological control as being a part of applied ecology where an introduced natural enemy is used to control a pest in a new area. Predators, parasites and pathogens are all considered natural enemies of various pest populations that may contribute to bringing a particular pest species below the economic threshold. Essentially three approaches can be used in biological control. These are not mutually

exclusive but may be combined and integrated into a comprehensive control program:

1.5.1.1. Importation

The classical method of biological control relies on the importation of a beneficial species from its native range, which is usually a different geographical location, often another continent (Alston, 1996). Since many exotic pests were accidentally introduced from overseas on exotic crop plants, their natural enemies would most likely be found in their native habitat. Once such an agent has been found it needs to be quarantined and rigorously tested for its specificity to the pest before it can be released safely in the new location (Landis and Orr, 1996). For example, many different species of Hymenoptera, such as *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae), *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and *Aphidius ervi* Haliday (Hymenoptera: Braconidae), have been introduced into Australia in an attempt to control several aphid species (Waterhouse and Sands, 2001). Ferrar et al. (2004) reviewed the biosecurity risks associated with the importation and release of biological control agents and found that when conducted scientifically, biological control was a safe and cost effective practice. It was strongly supported by both quarantine managers and end users as it was seen as safe, economical and environmentally friendly.

1.5.1.2. Augmentation

Augmentation involves mass production and periodic colonization; or genetic enhancement of natural enemies to increase their effectiveness in controlling the pest (Landis and Orr, 1996). It is most often used in situations where natural enemies are lacking or their populations respond too slowly to be effective on the pest. Because the natural enemy cannot sustain its population at effective levels, it needs to be replenished periodically. An inundative release is a single release of large numbers of a natural enemy with the aim of a one-time reduction in pest numbers. An inoculative release involves multiple releases of small numbers of natural enemies over a period of time with the expectation that the enemy will spread and become established in a given area (Alston, 1996). Natural enemies for these releases may be commercially mass reared or they can be collected and released (Sloderbeck et al., 1996). They may be generalist predators such as various coccinellid beetles (lady-bird beetles), e.g., *Chilocorus* spp., *Adalia bipunctata* L. (Coleoptera: Coccinellidae), *Coccinella* spp., *Harmonia* spp. and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), or specific parasitoids such as brachonid wasps, e.g., *Aphidius* spp., *Diaretiella rapae* Mc'Intosh (Hymenoptera: Aphidiidae) and *Lysiphlebus* spp. (Waterhouse and Sands, 2001).

While augmentation is the least sustainable among biological control methods, it can be highly effective, economical and environmentally sound in specific

situations. For example, releases of the hymenopteran parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) to suppress populations of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) have been very effective in greenhouse situations (Parrella, 1990).

1.5.1.3. Conservation

For natural enemies, indigenous or exotic, to continue to be effective, they need to be supported by a management program that avoids practices that harm them and implements practices that benefit them (Sloderbeck et al., 1996). To implement such systems, specific factors must be identified, understood and integrated for the best possible outcome (Landis and Orr, 1996). There is no easy formula and one of the most critical factors is the application of broad spectrum pesticides that will also affect non-target species such as predators and parasites. Even if they do not affect the natural enemy, they may deprive it of its food source by killing the specific pest species it feeds on (Sloderbeck et al., 1996). The use of target specific or soft insecticides and biological agents such as *Bacillus thuringisensis* (*Bt*) can greatly reduce the impact on beneficial insects and support integrated pest management (IPM) programs. For example, the West and South Australian Department of Agriculture has developed and implemented a number of approaches to conserve beneficial insects as long as possible into the season. By testing the effects of a wide range of chemicals on beneficial non-target species

they have developed chemical charts (Figure 1.2) with ratings from “very low impact” to “very high” impact for various pesticides that give growers the opportunity to make an informed choice when they need to spray opting for target-specific, low beneficial impact (or soft option) insecticides early in the season.

IMPACT OF INSECTICIDES ON NATURAL ENEMIES FOUND IN BRASSICA VEGETABLES

Information provided is based on the current best information available from research data.
Users of insecticides should check the label for registration in their particular Crop & State, and for rates, pest spectrum, safe handling and application details.

INSECTICIDES	TOXIC EFFECT ON SPECIFIC NATURAL ENEMIES*						RATING OF INSECTICIDE IMPACT ON NATURAL ENEMIES OVERALL*
	Parasitic wasps		Predators				
Active Ingredient	Egg Parasitoid *Trichogramma*	Laval and Pupal Parasitoids	Predatory Beetles	Predatory Bugs	Lacewings	Spiders	The Natural Enemies Assessed; Larval, Egg and Pupal Parasitoids, Predatory Beetles, Predatory Bugs, Lacewings, Spiders
Bacillus thuringiensis (Bt)	VL	VL	VL	VL	VL	VL	★★★★★ SOFTEST
pirimicarb (Pirimor®)	H	VL	VL	L	VL	VL	★★★★★
pymetrozine (Chess®)	L	L	L	L	L	L	★★★★
spinosad (Success®, Entrust®)	VH	M	VL	M	VL	VL	★★★★
emamectin benzoate (Prodaim®)	M	M	L	H	L	M	★★★½
imidacloprid (Confidor®) soil	L	L	L	M	L	L	★★★½
indoxacarb (Avatar®)	L	M	H	L	VL	VL	★★★½
chlorfenapyr (Secure®)	VH	M	M	M	L	L	★★★
endosulfan	VH	M	M	M	L	M	★★★
fipronil (Regent®)	VH	H	L	M	VL	M	★★½
imidacloprid (Confidor®) foliar	VH	M	H	H	L	L	★★½
organophosphates	H	H	H	H	L	M	★★½
methomyl (Lannate®, Marlin®, Nudrin®, Electra®)	H	H	VH	H	H	M	★★
synthetic pyrethroids	H	VH	VH	VH	H	VH	★ HARDEST

Figure 1. 2: The impact of insecticides on natural enemies commonly found in brassica vegetable crops.

Source:http://www.sardi.sa.gov.au/pdfserve/ento/dbm/publications/project_newsletters/toxicity_chart_v2.pdf

Other techniques that minimise the impact of insecticides on natural enemies include a change in the timing of applications to avoid exposing predators or placing insecticides in a location that avoids contact with natural enemies, though these techniques may not always be practical or very effective (Sloderbeck et al.,

1996). Repeated tillage, mowing and harvesting all can interrupt the lifecycle of some natural enemies. The cultivation of field edges, application of herbicides and/or the removal of all weeds around the farm (advocated as good farm hygiene) can all impact on the survival of natural enemies by altering a favourable environment (Landis and Orr, 1996; Sloderbeck et al., 1996). Van Mele and van Lenteren (2000) reported that citrus orchard hygiene in the Mekong Delta of Vietnam was practiced so diligently that it possibly aggravated some pest problems. Farmers completely destroyed the weed flora in their orchards thereby robbing natural enemies of alternative food sources and habitats to sustain their populations. The authors suggested that small adjustments to the weed management techniques and the utilisation of non-crop trees such as *Eucalyptus tereticornis* Sm and *Ceiba pentandra* (L.) Gaertn would offer pollen and nectar to beneficial insects during crucial times in the cropping season.

Growers must decide if there is a need to provide shelter belts, food supplements and alternate crop hosts that serve as over wintering habitats and food sources to offset the effects of good farm hygiene, which may prevent pest problems but consequently also prevent the persistence of natural enemies.

1.5.1.4. Habitat Manipulation

Rather than radically altering and continually disturbing cropping environments, habitat management or manipulation of agricultural areas and their surroundings

has aimed at conserving or augmenting populations of natural enemies (Hoffman and Frodsham, 1993, Landis et al., 2000). Gurr and Wratten (1999) stressed the importance of key ecological resources such as nectar for adults, suitable microclimates and alternative host prey in the successful establishment of biological control agents. In principle, habitat manipulation underscores conservation of natural enemies – either local or introduced - by providing them with such resources (Landis et al., 2000). While the theory of habitat manipulation appears to be a logical method for increasing biological pest control in crops, it is a complex situation to achieve and has not been fully tested, particularly in vegetable crops (Skirvin, 2006). Reviewers of the subject consistently recognise the importance of temporal and spatial factors that affect the biodiversity, mobility and trophic interactions of populations (Landis et al., 2000; Tscharntke and Brandl, 2004; Bianchi et al, 2006).

A number of studies have investigated various aspects of local habitats that are thought to contribute to enhanced biocontrol. Bugg (1993) reviewed the requirements of syrphids for aphid control on Californian farmlands. He reported that syrphids performed poorly in hot as well as in wet, cold and windy conditions but that shelterbelts, windbreaks and hedgerows offered a protected area that harboured more syrphids than insects of other taxa (from data by Lewis, 1965). Bowden and Dean (1977) showed that hedgerows could have confounding effects of shelter and flowers. Since hedgerows often have an abundance of nectar and pollen bearing plants, it was unclear whether syrphids were attracted to them

because of the shelter they provided or because of the supply of food sources in the form of pollen and nectar. Suction trap sampling showed that syrphids prevailed on the side that was more floristically diverse, indicating a preference for food sources. Early experiments by van Emden (1965), Pollard (1971) and Chandler (1968) all investigated the oviposition behaviour of syrphids with respect to their preference for shelter or food. All studies were inconclusive due to either inadequate experimental design, lack of replication or poor experimental procedures. These issues hint at some of the difficulties researchers have in designing representative and unbiased experiments in complex systems with many variables.

The effect of landscape and food cannot always be strictly separated. Crops have a pre-flowering period during which they are not able to attract predators with nectar and pollen. Lack of these energy and protein sources affects not only the abundance but also the fitness of predators. Dyer and Landis (1996) showed that the parasitoid *Eriborus terebrans* (Gravenhorst) (Hymenoptera: Ichneumonidae) was more abundant and active near the wooded edges of cornfields early in the season because they required shelter and a sugar source, which the woodland habitat provided. *E. terebrans* also survived longer in caged woodlots than in caged cornfields. In the laboratory, wasp longevity was increased by sugar supplementation and was higher at 25°C than at 35°C. The authors concluded that perennial habitats were important components of annual agricultural landscapes as

they provided the resources and stability required for the successful conservation of BCAs. Bianchi et al. (2004) also demonstrated that woody habitats were supportive of predators and parasites controlling the cabbage armyworm, *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), in organic Brussels sprout fields in the Netherlands. Predation rates were positively correlated with woody habitats which were assumed to provide alternative food sources and shelter for predators. Frequently disturbed non-crop habitats such as channels, road edges and hedgerows did not support predation. In contrast, parasites correlated positively with pasture areas, which were considered permanent habitats. These habitats provided lepidopteran hosts as well as overwintering opportunities for wasp parasites.

The establishment of field margins and buffer areas to conserve vertebrate species or reduce pesticide drift and wind erosion may also provide other ecological services. Olson and Waeckers (2007) investigated whether the diversity of insect species in a field margin designed to support the conservation of the northern bobwhite quail (*Colinus virginianus* Ridgwayi) would carry over into an adjacent cotton field. They assessed insect species and their predation and parasitism rates and found that two year old field margins had greater abundance of insect species than cotton fields with the exception of staphylinid beetles and aphids. Equal abundance in the cotton field was only matched by thrips and their pirate bug predator, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Other predatory species such as *Trichogramma*, lygus bugs and tachinid flies seemed to prefer the

field margin to the cotton field. The authors suggested that field margins established for the vertebrate species were inadequate in botanical composition to sustain the invertebrate fauna. They supported this suggestion with the low sugar content found in a parasitoid of lepidoptera larvae, which indicated an energy limitation in the system.

Matthews et al. (2003) manipulated the floors of apple orchards to study the effect on ground dwelling predators. They evaluated organic mulch, synthetic mulch, dead weeds and no weeds. The abundance of predators was assessed using pitfall traps while predation was measured by exposing the larvae of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) to predators. The latter were consistently more abundant in the organic mulch than in the other treatments. This was correlated with a correspondingly higher abundance of alternative prey in the organic mulch. However, predation of *C. pomonella* in that treatment was significantly lower. The authors did not speculate whether the reason for this was habitat structure, a preference for non-pest prey or competition from an abundance of other prey.

Apart from weed tolerance and preserving woodland communities or hedgerows, refuge strips and cover crops are other forms of habitat manipulation. Marr et al. (1998) suggested the use of such in vegetable production systems to help conserve natural enemies while providing side benefits by trapping and holding nutrients, protecting soil from erosion and fixing nitrogen. Carmona and Landis

(1999) investigated the effect of refugia and cover crops on the abundance of carabid beetles in a soybean, oats and corn rotation. Three meter wide refuge strips were centred in 30x30 m plots and contained flowering plants as well as some grass and clovers. The cover crop was red clover and the experiment was conducted over two years. In the first year, carabid beetle numbers were greater in the refuge strips than in crop areas, however, the presence or absence of strips did not affect beetle abundance in the surrounding plot area. In the second year, no significant differences were observed. However, plots with a cover crop had significantly higher beetle numbers than plots without a cover crop. The authors concluded that the use of cover crops and refuge strips could both increase carabid beetle density and diversity, but speculated on whether associated concentration effects would limit beetle movement by being a sink rather than a source of beetles (after Corbett and Plant, 1993).

The principle of managing cover crops well would also be relevant to the vegetation surrounding farms whether they be pasture, weedy strips or native vegetation. Pasture is subject to being grazed, in some systems intensively, which would in effect be similar to regular cutting. However, livestock grazing would not be uniform so crop cover would not be removed in one operation. Pasture regrowth, with the right management, would be young and vigorous therefore making the crop more attractive to invertebrates. Weed strips are generally not irrigated or fertilized and under dry conditions can decline rapidly in quality

providing a poorer habitat for insects and arachnids. Refuge strips of native vegetation may require the least attention and would perhaps be a more suitable option for a refuge area as many native species generally have a higher tolerance of dry conditions and low fertility soils.

If lucerne is used as a refuge strip in a crop rather than a cover crop, the objective of cutting or withholding irrigation techniques would be to force beneficials into the more attractive main crop. Pearce and Zalucki (2005) experimented with cut lucerne strips in soybeans and used suction samples to determine the abundance of insects at six 5 m intervals into both cut lucerne and soybeans from their interface. They found that predator abundance was markedly reduced in the cut lucerne to a distance of 30 m from the crop/strip interface. Abundance of predators in the soybeans was not affected at the same distances. While arthropod numbers gradually recovered as the lucerne regrew, their numbers in soybeans fluctuated irrespectively. The cutting of lucerne strips alone did not force predators into the soybean crop. That study demonstrates that habitat management is not an easy accomplishment where rules and recommendations guarantee a particular effect.

Scale remains a largely unknown factor in the dynamics of insects within agricultural landscapes. While woodlands may be of benefit, most agricultural areas contain sparse stands with most of the fertile and productive area being allocated to cropping. It is difficult to determine how insects exist and interact in such systems in small seasonal experiments. Holland et al. (2005) considered this

problem by setting up a large scale study (64 ha) over a three year period and collected a total of 7784 pitfall trap samples to evaluate the spatial distribution of four species of carabid beetles. They found that populations appeared in patches and that the spatial extent for a population patch was species-specific. Species overwintering in sites near field boundaries would also remain in those areas throughout summer while species that overwintered mid-field spread wider during the summer. In general, population patches remained stable within years but showed variation for some species over the duration of the experiment. The authors concluded that different carabid populations were to a large extent confined by field boundaries and that the frequent disturbance of fields contributed to the isolation and periodic decimation of these populations. Re-invasion of fields from beetles surviving in surrounding non-crop habitats would be correlated with the degree of field disturbance. Hence, the number and type of farm operations should be spatially and temporally desynchronised to conserve beetle populations in fields.

Since experiments of the scale described above carry so many variables, it is understandable why the interpretation of results is not straight forward or precise. The spatial patterns of carabid beetles could be influenced by any number of factors including crop type, soil factors, prey abundance, moisture gradients, organic matter content and pH of the soil and interconnection of fields. Tscharntke and Brandl (2004) also list the trophic level of a species, its body size, resource specialisation, population size variability and rarity. They state that while

metapopulation theory manages to frame the complexity of spatial plant-insect systems, the theory is often ahead of the research and therefore landscape context may be inadequately explored and explained.

While habitat manipulation is undoubtedly an important factor in the success of biological control agents, there are no guaranteed recipes for success. Rather, there are indications of factors from a number of experiments that need to be considered before implementing studies in horticultural systems, as is intended in this project. The provision of shelter and food sources on farms may need to be considered. With respect to brown lacewings, McEwen et al. (2001) noted that hedgerows, windbreaks, weedy strips and riparian areas may serve as a reservoirs or ecological corridors for brown lacewings and other natural enemies. Szentkiralyi (1992) and Szentkiralyi and Kozar (1991) also found more brown lacewings in higher levels of forest patches and uncultivated field margins than in low levels of vegetation in lowland areas**.** Apart from just considering their inherent characteristics as predators, it would be advisable to determine the habitat requirements both *of M. tasmaniae* and *H. variegata* in order to maximise their efficiency in field experiments.

1.5.2. Biological control in brassica IPM production systems

In Australia biological control is one of the techniques incorporated into an IPM strategy to control arthropod pests of vegetable crops. In brassicas, IPM programs

have been implemented in Victoria, South Australia, Queensland, Western Australia and New South Wales. Various agricultural departments regularly publish brassica IPM newsletters to extend the latest research and development, e.g., Better Brassicas (WA Department of Agriculture), Brassica IPM (SA and VIC Governments). These departments have also developed chemical charts (e.g. Figure 3) that growers can use to implement better IPM systems such as similar to that used by the Australian cotton industry.

In Queensland, the brassica industry combines biological and cultural methods with chemical control and monitoring. A reduction in the use of synthetic pesticides has enabled more growers to adopt IPM strategies and a recent survey indicated that 70% of growers employed IPM to some degree while 10% were using “advanced IPM” (Walsh, 2006). Techniques used included:

- Use of biological pesticides (e.g. *Bacillus thuringiensis*, Nuclear Polyhedrosis Virus)
- Crop monitoring
- Use of narrow spectrum insecticides
- Rotation of chemical groups
- Deliberate protection of natural enemies
- Summer fallows
- Use of sticky traps and pheromone traps (as monitoring tools)

Increasing levels of resistance of major brassica pests such as *Pl. xylostella* to synthetic pyrethroids and organophosphates have also led to the introduction of IPM systems in New Zealand (Walker et al., 2001). Control failures were detected in three regions in 1997 and subsequent IPM focused on reduced spray programs, crop monitoring, insecticide resistance management rotation strategies, selective insecticides and action thresholds. Training of crop scouts and managers involved an accreditation system that also emphasized on record keeping. Within three years, 80% of growers had adopted these IPM strategies and 96% were monitoring their crops regularly. When comparing records from IPM crops with those of conventional crops, the authors noted a 50% reduction in insecticide use for IPM crops.

Sastrosiswojo (1996) reported on the impact of IPM systems in brassica crops in Indonesia. After a major ban on 57 broad-spectrum chemicals used in agriculture, the Indonesian Government introduced national IPM programs for vegetables in 1991. An evaluation three years later showed significant benefits to the growers including:

- A 61-81 % reduction in insecticide use
- A 64-79 % reduction in the cost of insecticides
- Up to 80% increase in the parasitism of *P. xylostella*.
- An 8-16 % increase in marketable yield
- A profit increase of $834/ha when using the IPM system

The environmental benefits were greater fauna diversity and higher numbers of soil microorganisms in IPM systems (Sastrosiswojo et al., 2001).

In Malaysia, IPM programs also rely on the generic components of monitoring, use of threshold levels, the reduction of synthetic insecticides and the incorporation of other non-chemical control measures (Sivapragasam, 2001). While the benefits of IPM usually became obvious rather quickly, grower adoption rates in some areas were slow. A number of factors that posed major constraints to the adoption of IPM strategies were identified:

- Difficulty in complying with the procedures that determine economic threshold levels (ETL)
- Focus on *Pl. xylostella* management without regard for other pests
- Lack of confidence in the technology
- Lack of time for monitoring
- Lack of understanding of the benefits IPM can provide
- Poor support from extension agencies

(Sivapragasam, 2001).

Some of these constraints may be overcome with more education and extension while some may require more government backing and political impetus. In Australia, brassica growers in different regions have adopted some or all of the IPM strategies and have realised the benefits. This adoption has sometimes been out of necessity due to the failure of chemical sprays as insect

pests became resistant to them. There are working examples of brassica IPM in Australia (Walsh, 2005) which should encourage growers in other vegetable growing regions to consider adopting these techniques.

1.6. Selection of Biological Control Agents

1.6.1. Considerations for the successful use of biological control agents

Government protocols require extensive information about potential biological control agents to ensure that they are a safe option for the importing country. For example, Biosecurity Australia (DAFFA, 2007) lists non-target organisms at risk, interactions with other biological control programs and host specificity testing – amongst other information - as mandatory criteria when applying for an import permit for potential biological control agents. While this information is important from a biosecurity aspect, the success of a potential biological control agent is evaluated by somewhat different criteria. Ideally it must be free living and mobile, it must consume many prey insects during their life and it should usually be as large as or larger than its prey (Strand and Obrycki, 1996). More specific factors have been listed by other authors. Minkenberg and van Lenteren (1990) listed completeness of development and quality of the offspring, synchronisation of generations, population growth and searching efficiency as selection criteria for

hymenoptan parasitoids but added that this list was incomplete. Drost et al. (1996) used a number of bionomic parameters to rank species of biological control agents against whitefly. They included longevity without hosts, development time, fecundity, parasitism rate and longevity with hosts.

1.6.2. Generalist versus specialist predators

A biological control agent's host range includes all the insect species that it can feed and reproduce on. Of the predators, very few are monophagous or specialist predators which specifically feed only on one or two species (Strand and Obrycki, 1996). Takahashi et al. (2001) described the movement of the specialist predators *Scolothrips takahashii* Priesner (Thysanoptera: Thripidae), *Oligota kashmirica* benefica Naomi (Coleoptera: Staphylinidae) and *Stethorus japonicus* Kamiya (Coleoptera: Coccinellidae) between two pear orchards infested with the mite *Tetranychus kanzawai* Kishida (Acarina: Tetranychidae). These predators disappeared rapidly from one orchard with declining mite numbers only to be found in suddenly increased numbers in the other orchard. The authors interpreted this movement as an indication that the predators followed their favoured prey.

Most of the predators employed as biological control agents are polyphagous and thrive on a variety of prey. For example, of the two common hemerobiid species in Australasia, the less common *Drepanacra binocular* Newman is a relatively specialised feeder on psyllids, usually found in trees. The very abundant *M.*

tasmaniae, however, is a generalised polyphagous feeder with a wide host range usually found on understorey shrubs and crop plants (Horne et al., 2001). The host range of a predator is influenced by factors such as reproduction, foraging behaviour and feeding habits.

1.6.3. Searching capacity

The way predators forage is affected by hunger level, prey distribution and density as well as by competition from other predators. Baumgaertner et al. (1981) found that with increasing hunger level, larvae of *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) searched more widely for aphid prey on lucerne plants. A reduviid predator, *Rhynocoris marginatus* (Fabricius) (Hemiptera: Reduviidae) - starved for 1 day, however, travelled longer distances (215.57 cm) for *S. litura* prey than one starved for 4 days (92.14 cm) in experiments carried out by Claver & Ambrose (2001).

The distribution of prey on a plant can change a predator's foraging behaviour. Nakamuta (1982) observed that after consuming one *M. persicae* aphid, the searching behaviour of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) became more convoluted, concentrating on the spot where the aphid had been consumed. Kalushkov (1999) also saw this behaviour in *Adalia bipunctata* L. feeding on *Phorodon humuli* (Schrank) (Hemiptera:Aphididae) and described it as a change from extensive to intensive searching behaviour. Dixon & Wratten (1971)

explained this behaviour with the fact that most aphid species existed in colonies, therefore, it would be likely that another aphid would be found close to where the first one was found. Guershon & Gerling (2006) also stated that searching for their prey, coccinellids may use two different patterns of movement: ‘extensive search’, constituting a rapid linear movement from one prey patch to the other, or ‘intensive (or area concentrated) search involving slow and sinuous movement inside each of the patches switching from extensive search to intensive search.

Prey density affects the distance travelled and the travel speed of a predator searching for suitable prey. Omkar & Srivastava (2001) reported that consumption of *M. persicae* (Sulzer) , *Ropalosiphum maidis* (Fitch) (Hemiptera: Aphididae) and *Macrosiphum rosae* (Linnaeus) (Hemiptera: Aphididae) by *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae) and *C. septempunctata* L. resulted in decreased searching capacity as either prey or predator density increased. Claver & Ambrose (2001) also investigated how the prey density of *S. litura* would affect the searching capacity of *R.marginatus* (Fabricius). At a density of one *S. litura* larva, *R. marginatus* travelled 230.43 cm at a speed of 0.345 cm/second while at a density of four larvae, it travelled 92.14 cm at a speed of 0.607 cm/second. The study does not mention if the searching speed increased after the first larva in the four larvae density arena was consumed. Nevertheless, it would appear that at lower densities searching was more extensive, slower and more thorough while at higher densities it was more intensive and faster. This study

would support Dixon and Wratten's explanation that the predator would expect to find more prey close to where it previously found some prey.

1.6.4. Predation capacity

Predation capacity is an important criterium for biological control agents and can be assessed in several ways. Braccamontes et al. (1995) evaluated the ability of different larval stages of *H. convergens* to decimate defined populations of *M. rosae* on rose bushes. They found that 4 predators completely reduced their prey of 150 aphids in 16 days, while 6 predators took only 13 days, and recommended a prey: predator ratio of 8:1 for 100% control. An assessment based on kill rate (kills/minute) was employed by Tommasini et al. (2004) when they assessed the predation capacity of four species of *Orius* (Hemiptera) feeding on the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Kill rate took into account the age specific predation of both nymphal and adult stages and ranged from 0.19-0.25 kills/minute. The possibility of assessing predatory capacity by measuring weight gain of the predator was explored by Djavan-Moghaddam (1976) using larvae of *C. septempunctata*. The weight gain was more pronounced during the later larval stages and larval predation was 191 times greater than adult predation. The authors did not draw a conclusion as to whether the methodology was useful but it would warrant consideration if the predatory capacity of confined *H. variegata* and *M. tasmaniae* were to be assessed.

Another study that considered the predation capacity of several phenotypes of *Adalia decempunctata* L. feeding on *Toxoptera aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae), also related it to the gut capacity of the predator and considered times allocated to resting, walking, feeding, predation, prey consumption and grooming (Smaili et al., 2006). Overall, predation rates ranged from 45.3 to 71.6% though gut capacity of the species was relatively small and a large portion of the insects' time was allocated to resting. Samson and Blood (1980) assessed the voracity of *M. tasmaniae* fed on *Helicoverpa punctigera* (Wallengren) larvae. While *M. tasmaniae* consumed relatively few larvae when compared to other predators and preferred aphid prey, its voracity increased with each successive larval instar. First instar larvae of the predator consumed 0.9 first instar prey larvae but consumed 17.3 by the time they had become third instars.

1.6.5. Overwintering capacity, reproduction and population buildup

Most insects are capable of at least one type of quiescence: hibernation or true diapause and/or aestivation, a facultative dormancy (Bazzocchi et al., 2004). Hibernation is most commonly prompted by temperature and photoperiod changes (Hunter and McNeil, 1997) while aestivation is often a response to low prey availability or an avoidance of extreme weather conditions and occurs during summer (Jervis, 2005). As facultative diapause affects the number of generations produced by a population, it is a critical factor for the success of a potential BCA, especially with respect to population build-up.

Hippodamia convergens, for example, exhibits summer aestivation when aphid prey is low but shows normal summer development when abundant aphids are present in irrigated crops (Stewart et al. 1967). *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) another ladybird beetle, also exhibits aestivation (Sakurai et al., 1988). Since hibernation is broken by temperature increases, a low thermal threshold would be of advantage in a biological control agent (provided that some of the prey also has a low thermal threshold, otherwise there would be no food). *M. tasmaniae* is able to develop at temperatures as low as -2.9°C (Samson and Blood, 1979) while *H. variegata*'s thermal threshold is 12.76 (Jafari et al. 2002). *M. tasmaniae* would therefore be active for most of the year in all but the coldest parts of Australia.

Bazzocchi et al. (2004) compared the overwintering ability of the exotic *H. axyridis* to that of the native *A. bipunctata* in Northern Italy. The overwintering mortality of *H. axyridis* was 31.9% compared to that of *A. bipunctata* which was 61.3%. Consequently the post diapausal rate of increase of *H. axyridis* was also higher though its fecundity was only slightly higher at 783.8 eggs/female cf 720.2 eggs/female of *A. bipunctata*. Both species completed four generations. In comparison, another native species, *Propylea quatuordecimpunctata* (L.) (Coleoptera:Coccinellidae), had a mortality of 68.9%, a fecundity of 193.7 eggs/female and completed only three generations. While the authors decided that *H. axyridis* possessed high potential for establishment in Italy, they did not consider the effects that the exotic competitor could have on the native species.

Few articles are available on the overwintering habits of lacewings. Weidner (1971) reported that green lacewings (*Chrysopa carnea* (Stephens)) (Neuroptera: Chrysopidae) in Germany often overwintered in houses and were considered pests by tenants. He urged that their role as important predators needed to be emphasized to encourage people to collect them and place them in a safe overwintering place.

1.6.6. Release options

Ideally biological control agents should be released only once and then become naturalised in the environment. In Australia, both *M. tasmaniae* and *H. variegata* are relatively abundant in crop areas (Horne et. al., 2001; Franzmann, 2002). Since relative abundance of the two predator species in the CW-NSW was not known, there was a need for a survey of their presence and abundance before any supplementary release of the predators (since *M. tasmaniae* is native it is already there and *H. variegata* had already been reported as a new species). Eggs of *H. variegata* are commercially available on tape for distribution in aphid infested fields and orchards (Goodbugs, 2007). Eggs of brown lacewings are also supplied, however, release rates and strategies are still being tested. In situations where early pests occur, it may be a good strategy to supplement the natural population with strategic releases.

For example, recommended release rates for green lacewing eggs and larvae vary from 500-1000 eggs per release for a garden situation to 2000-4000 eggs/acre twice a week for commercial situations (Garrett, 2007). Rincon-Vitova (2007), a commercial insectary supplying BCAs in the United States, suggested to start releases of 50,000 green lacewing eggs per acre early in the season and then to continue at weekly intervals. Eggs are commonly mixed with corn grits, rice hulls or vermiculite, however, these materials can dehydrate eggs, cause poor adherence to the leaf and injure hatching larvae (Mahr, 2000). Improved technologies, such as the use of agar solution to disperse eggs through commercial sprayers, not just reduced egg mortality, aided in better adherence of eggs to leaves but also had the advantage of reducing attractiveness to ants (Mahr, 2000).

1.6.7. Susceptibility to insecticides

Most biological control agents are also susceptible to a number of commercially used insecticides aimed at target pests. By choosing chemicals that have low impact on certain beneficial insects, those impacts can be minimised and beneficial insects may gain an advantage over pest species. In real life this scenario is not always achievable. However, biological control agents may develop insecticide resistance just as pest species do. For example, in the United States a number of stored grain pests are controlled with malathion. Baker and Throne (1995) tested the natural enemies of grain moths and weevils and found significant levels of malathion resistance in the warehouse pirate bug (*Xylocoris flavipes* (Reuter)

(Hemiptera: Anthocoridae)) as well as in two wasp parasitoids (*Anisopteromalus calandrae* (Howard) (Hymenoptera:Pteromalidae)) and *Bracon hebetor* Say (Hymenoptera:Braconidae)) of grain caterpillars and weevils.

Amongst proponents of IPM strategies, there is a general understanding that “soft” chemicals, which also includes insect growth regulators (IGRs), are less damaging to beneficial insects than traditional chemistry (eg. organophosphates, pyrethroids). Reeve and French (1978) tested the toxicity of 19 commercial pesticides and three insect growth regulator on the brown lacewing *Sympherobius barberi* Banks . They concluded that most organophosphates and carbamates were highly toxic to *S. barberi* but that the insect growth regulators diflubenzuron, kinoprene and epofenonane caused low mortality. This experiment only tested the acute effects of the insecticides but did not consider the effects on subsequent generations. Rumpf et al. (1998) used several generations of *M. tasmaniae* to test the effects of conventional insecticides (methyl parathion, azinphos-methyl and cypermethrin) against the effects of IGRs (fenoxycarb, diflubenzuron, tebufenocide). They found that some of the IGRs had more severe impacts on the life-table parameters of *M. tasmaniae* than the conventional chemicals. Some of these differences, however, did not show up until the second and third generations when egg production and fecundity was reduced. This research shows the need for longer term studies of new chemistry before it is recommended as suitable for IPM systems.

1.7. Target predators

1.7.1. Hippodamia variegata

Hippodamia variegata is an important predator of aphid pests that globally infest a large number of crops. Being a generalist predator, other prey includes cicadellids (Singh et al., 1991) and the larvae of the curculionid *Hypera postica* Gyllenahl (Sadeghi and Esmaili, 1992). Kavallieratos et al. (2002) reported that *H. variegata* represented 64.5% of the predators feeding on *A. gossypii* in cotton in Greece. In Turkmenistan, Belikova and Kosaev (1985) also found them to be the most abundant aphid predators of cotton. Natskova (1973) commented on the abundance of *H. variegata* as a predator of aphids on pepper and roses in Bulgaria but noted that they were only highly effective as long as the aphid population was relatively low. In the Ukraine, *H. variegata* competed with other coccinellid species and only became a significant aphid predator in some years (Gumovskaya, 1985).

The detection of *H. variegata* in Australia is very similar to the detection of the multicoloured ladybird beetle *Harmonia axyridis* (Pallas) (Coleoptera:Coccinellidae) in South America. *H. axyridis* is a voracious predator and therefore effective in biological control (Koch et al. 2006). Koch et al. (2006) suggested that *H. axyridis,* like most exotic predators (such as *H. variegata* in Australia), not only has beneficial impact as a biological control agent but may threaten native organisms. Several authors have described the negative impact of *H. axyridis* on indigenous

biodiversity in the USA (Nault & Kennedy 2000; Michaud 2002; Alyokhin & Sewel 2004). *H. axyridis* is a great threat for indigenous aphid predator species. Studies have shown that it is very aggressive and able to dominate *Coccinella septempunctata* L. and *Adalia bipunctata* (L.) (Coleoptera:Coccinellidae) in terms of competition for food resources (Kajita et al. 2000; Yasuda et al. 2001). Also densities of native predators have been known to decrease while the abundance of *H. axyridis* was increasing and researchers believed that this was due to intraguild predation (Brown 2003; Saini 2004; Sato & Dixon 2004). The impact of *H. variegata* on native predator species in Australia is unknown at this stage.

1.7.1.1. Description

Hippodamia variegata (Goeze) syn. *Adonia variegata* (Common name: Amber spotted ladybeetle or white collar ladybeetle) (Coleoptera: Coccinellidae, Suborder Coccinellinae) is originally a Palaearctic species that has spread worldwide with records from India, Africa, North and South America and, most recently, Australia (Franzmann, 2002). Adult ladybeetles are 4 – 5 mm long, of convex-oval shape and basic red colour with 3-15 black spots. 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 (Plate 1.3). Adults have fully developed hind wings and readily fly (Helyer et al., 2003).

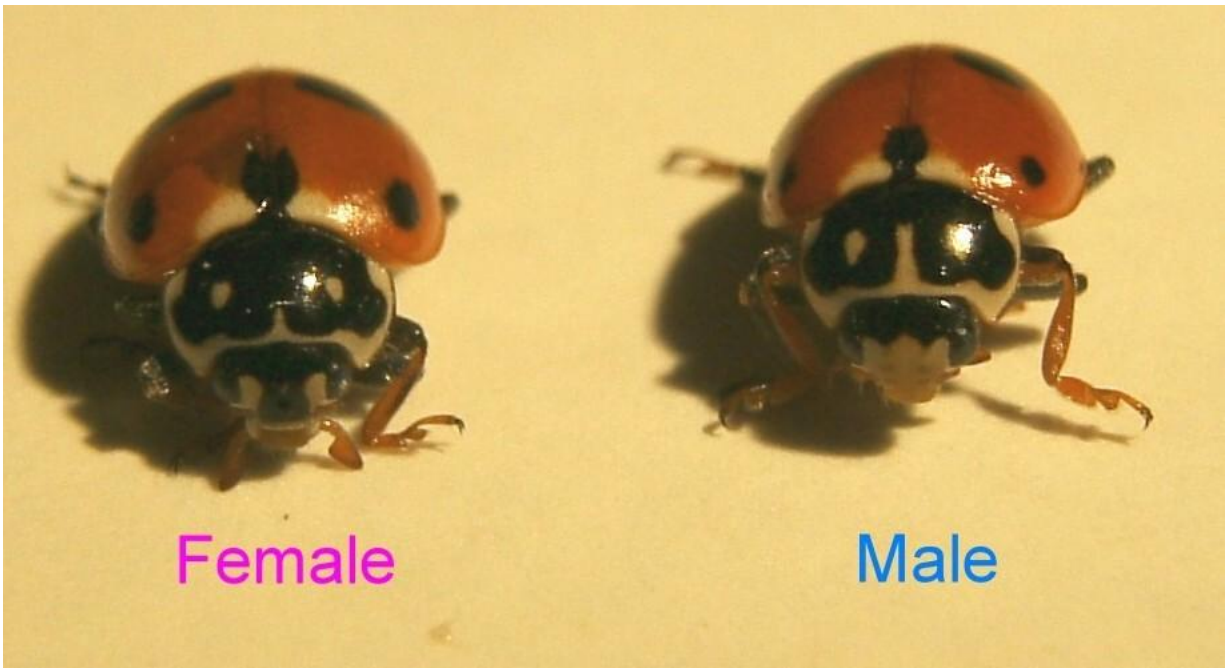


Plate 1. 3: Female and male *Hippodamia variegata*
(*Photo courtesy of Brendan Nolan, QDPI*)

Eggs are yellow and conical and are laid on leaves in clusters of 10-50 near prey (Plate1.4). The grey-black larvae are elongate and somewhat flattened with angular legs. They are covered with small tubercles or spines resembling well armoured alligators (Plate 1.5). Their mandibles are sharp and sickle shaped (Lyon, 2002).

Plate 1. 4: **Eggs of *Hippodamia variegata***
(*Photo: V. Heimoana*)

Plate 1. 5: **Larva of *Hippodamia variegata*** (*Photo: V. Heimoana*)

1.7.1.2. Life cycle

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 (Lyon, 2002).

Ladybird beetle larvae complete their development in three instars before attaching themselves to a solid surface (often a leaf) to pupate (Cranshaw, 2001). Each instar may take 10-14 days before it moults while the pupal stage typically lasts 5-8 days. Cranshaw (2001) estimates that 2-3 generations may be produced during winter (in North America), however, in warmer climates with long summers this number may increase. Kontodimas and Stathas (2005) reared seven generations of *H. variegata* between April and November in a northern hemisphere Mediterranean climate.

During autumn ladybird bird beetles congregate to diapause in crevices and plant refuse until spring when they mate, feed and begin to lay eggs. *H. variegata* is distinguished from other coccinellid species in that it does not diapause (Kontodimas and Stathas, 2005). Some coccinellid species migrate to higher altitudes for winter diapause but Sadeghi and Esmaili (1992) and Hodek (1973) give mention of the overwintering habit of groups of *H. variegata* in lowland fields.

1.7.1.3. Fecundity

General information on ladybird bird beetles cites an egg production of 50-300 in a lifetime, usually in clusters of 10-50 (Lyon, 2002). Kontodimas and Stathas (2005) reared *H. variegata* on *Dysaphis crataegi* (Kaltenbach) in the laboratory at 25°C and at 65% R.H. with a 16:8 (L: D) photoperiod to determine fecundity. The total fecundity ranged between 789 and 1255 eggs with a mean of 959.6 eggs. Of these eggs, 45% were oviposited in clutches of 11-20 eggs with a mean clutch size of 17 eggs. Steward et al. (1991) reported an egg clutch mean of 20. Kontodimas and Stathas (2005) recorded a mean generation time of 34 days and each female produced 425.9 female offspring. They concluded that *H. variegata* females produced eggs for most of their lifetime beginning 4-7 days after emergence and ceasing 5-7 days prior to death.

Day length and wavelength of light may be critical factors in reproductive performance. Omkar and Pathak (2006) found that day length and wavelength affected the life cycle of the coccinellid *Coleophora saucia* (Mulsant). Females maintained at long photoperiods (16:8, L: D; as compared to 8:16, L: D) and under white light had a significantly higher reproductive performance with a total egg production of 784.6 as compared to 310.8. Females under the longer daylight regime also consumed more prey. Such studies have not been conducted for *H. variegata*, however, they may need to be considered when determining the suitability of this predator for a particular location

1.7.1.4. Prey

Ladybird beetles are diurnal in habit and are able to locate their prey visually from a very short distance. *H. variegata* feeds on a large number of aphid species including *D. crataegi* (Kontodimas and Stathas, 2005) and *L. erysimi* (Kalra, 1988). Isikber (2005) noted that the response of coccinellids as a predator to aphids is a Type II functional response, i.e. the more aphids available the more aphids consumed by the coccinellid.

While *H. variegata* reputedly prefers aphid prey (Franzmann, 2002), it is considered a generalist predator and therefore it can be assumed that its prey range in nature is much wider than recorded in the literature. Franzmann (2002) also listed psyllids as one of its prey species while Helyer et al. (2003) listed scale insects, mites, mealybugs and whitefly as prey species generally consumed by coccinellids. In cotton, coccinellids have been observed to prey on *Helicoverpa* eggs and small larvae (Pyke and Brown, 1996).

1.7.2. *Micromus tasmaniae*

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 (Pyke and Brown, 1996). In New

Zealand, it was reported to feed on *Phenacoccus graminicola* Leonardi mealybugs on pipfruit (apples, pears and nashi) but was not deemed an important control agent of this pest (Anon. (a), 2007). Fondren (1976) considers the brown lacewing's predatory capacity as important as that of the green lacewing in the United States, but it is less common. In lettuce and celery, brown lacewings fed on aphids, mealybugs, scale insects and whiteflies (Anon. (b), 2007). Both adults and larvae are predacious. They are common throughout Australia and are often found on native vegetation, especially when it is in flower (CSIRO, 2007). New and Boros (1983) and Samson and Blood (1980) reported that they are particularly common on low vegetation including field crops.

Plate 1. 6: Adult *Micromus tasmaniae*
(*Photo: V. Heimoana*)

1.7.2.1. Description

The brown lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) has several synonyms – *Hemerobius tasmaniae* (Walker), *Micromus australis* Froggatt, *Micromus froggatti* Banks and *Micromus perkinsi* Banks, however, the current name in use is *M. tasmaniae* (DEH, 2007).

Micromus tasmaniae adults (Plate 1.6) are up to 12 mm long and are delicate and slender with prominent eyes and lacy brown wings (CSIRO, 2007). The wings are narrowly oval with a slightly pointed apex (Fondren, 1976). 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 (Anon., 2006). *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. Detailed morphological descriptions of the larval stage can be found in New and Boros (1983).

1.7.2.2. Life cycle

No specific data on the lifecycle of *M. tasmaniae* has been found in the literature, however, reports of *Hemerobius stigma* Stephens, a cold tolerant North American species, give some indication of the lifecycle of brown lacewings. *H. stigma* overwintered as an adult or as a pre-pupa and became active in early spring (Fondren, 1976). Adults mated and deposited single eggs about two weeks later, which hatched in about 11 days and developed through three larval stages into pupae. Pupae remained in a silken cocoon for about 9-14 days. Fondren (1976) estimated that two generations per year were common, however, Australian species tend to have 3-5 generations per year (CSIRO, 2007) which indicates higher environmental temperatures and/or shorter lifecycles. Rumpf et al. (1998) determined the longevity of female *M. tasmaniae* in New Zealand to be 46.4 days. Samson and Blood (1979) gave a lifespan of 27 days for *M. tasmaniae* at 28°C. Sato and Takada (2004) reported the lifecycle ranges of three brown lacewing species found in Japan. – *Micromus numerosus* Navás*, Micromus angulatus* Stephens *and Micromus linearis* Hagen – as ranging from 49.4 to 83.7 days at 10°C to 16.3 to 27.8 days at 25°C.

At 10°C a complete cycle from egg to adult took 81 days while the cycle was shortened to 21 days at 25°C (Syrett and Penman, 1981). The authors noted a linear relationship up to 20°C, however, beyond that temperature, the relationship began to deviate and mortality increased significantly. Samson and Blood (1979)

calculated even lower threshold figures for *M. tasmaniae* in Queensland. Fondren (1976) noted that North American hemerobiids were quite cold tolerant and Neuenschwander et al. (1975) cited thresholds of 4.1 and 0.4 °C for larval and egg development, respectively, of *Hemerobius* spp. in California.

The implications of developmental thresholds are very important to the suitability of a predator as a biological control agent. The developmental threshold of the pea aphid *Acyrtosiphon pisum* (Harris), a common prey species of *M. tasmaniae* is 4°C (Syrett and Penman, 1981) while that of *H. variegata* is 12.76 °C (Jafari et al., 2002) A polyphagous predator with a developmental threshold lower than that of some of its main prey species and competitors can be more abundant earlier in the season than the target pest and at the same time compete earlier against other predators.

1.7.2.3. Fecundity

Samson and Blood (1979) reported that *M. tasmaniae* in Queensland produced an average of 19.1 eggs/day at 23°C while Rumpf et al. (1998) recorded 14.6 eggs/day at 19°C in New Zealand. Results from Sato and Takada (2004) for *M. numerosus, M. angulatus* and *M. linearis* reared at 25 °C showed daily egg productions of 18.65, 10.14 and 15.58, respectively. There is wide variation in the daily egg production of various *Micromus* spp. depending on temperature and other factors. Female nutrition was shown to be an important factor by Hussein

(1984) who found that the egg production of females deprived of protein (in the form of aphid prey) was greatly reduced. They produced an average of 0.65/day eggs compared to females fed on aphids who averaged at 67.55 eggs/day over an 8 day period.

1.7.2.4. Prey

Micromus tasmaniae feeds on soft bodied arthropods and insect eggs which provide the nutritional protein required for reproduction. These include aphids, mites, scale insects and whiteflies. Fondren (1976) listed a number of conifer feeding aphids (*Adelges* spp.) as primary prey in North America and in Australia *M. tasmaniae* reportedly also fed on longtailed mealybugs (Anon (b), 2007). While insect protein is the most common food source, pollen and honeydew may be used as a diet supplement by the adults of some lacewing species (CSIRO, 2007).

1.8. Aim of the study

The overall aim of this project is to investigate the suitability of *H. variegata* and *M. tasmaniae* as biological control agents of brassica pests. Many coccinellids, including *Hippodamia* spp., are effective biological control agents for aphids (Strand and Obrycki 1996; Obrycki and King 1998; Musser *et al*. 2004). *H.*

variegata was first discovered in Australia feeding on *Rhopalosiphum maidi*s (Fitch) (Hemipetra: Aphididae) on sorghum in November 2000 (Toowoomba, Queensland). Since then, *H. variegata* has been recorded to feed on 12 species of aphids and a psyllid (Franzmann 2002), indicating its potential in biological control. The performance of *H. variegata* could potentially be augmented by the native predatory lacewing *M. tasmaniae.* Sampson and Blood (1980), New (2002) and Sato and Takada (2004) reported that *M. tasmaniae* possesses high potential for managing aphids. The potential of these two predators is strongly affected by temperature, availability of host plants, other predators and farming systems, factors that can influence the performance of any biological control agent (Landis *et al*. 2000; Schellhorn and Silberbauer 2002; Schellhorn and Andow 2005; Burgio *et al*. 2006). Therefore, a need exists to investigate the performance of *H. variegata* and *M. tasmaniae* under the field climatic conditions of the Central West's brassica and other field vegetable crop ecosystems. Results from this study will contribute to the formulation of an improved pest management strategy for brassica field crops.

1.8.1. Objectives

1. To investigate the temporal and spatial distribution of the two target predators *H. variegata* and *M. tasmaniae* in the Central West, New South Wales cropping and non-cropping areas. Chapter two reports on this study.

2. To monitor the movement of *H. variegata* and *M. tasmaniae* from non-cropping areas such as bush, pasture or riparian to cropping areas to assess whether these neighbouring non-crop areas act as a sink or source of the predators. This study is reported in chapter three.

3. Examine the impact of insecticide treatment on *H. variegata* and *M. tasmaniae* re-population of a cropping area. This study is reported in chapter four.

4. Use of DNA techniques to analyse gut contents of *H. variegata* and *M. tasmaniae* field populations. The molecular work is not the main focus of this thesis, but rather it is hoped that it would give additional information on a topic that has been the subject of little research that has attempted to use molecular methods to understand field dynamics of insects. The molecular chapter aimed to provide (i) a list of which prey species each predator consumed and (ii) tentative information on relative predation. This will also investigate the potential for interspecific competition (intraguild predation) between *H. variegata* and *M. tasmaniae*. Chapter five reports on this study.

The final chapter will summarises the findings from the whole project and discuss the possibility of using the predators *H. variegata* and *M. tasmaniae* in an integrated pest management strategy which can be useful to Brassica growers in Central West, New South Wales and beyond.

CHAPTER TWO - INFLUENCE OF NON-CROP VEGETATION AND INSECTICIDES ON THE PRESENCE/ABSENCE AND ABUNDANCE OF PREDATORS *Hippodamia variegata* (Goeze) AND *Micromus tasmaniae* (Walker)

2.1. INTRODUCTION

In Australia, as in many other parts of the world, insect pests inflict considerable economic damage to crops and growers rely heavily on chemical control (Tran et al. 2004). Growing environmental concerns coupled with the rising cost of pesticides and increasing pesticide resistance (Edward and Lawrence 2006) of insects in recent years, have encouraged the industry to explore the use of biological control agents.

Vegetable growers from the Bathurst area, in the Central West of New South Wales (CW-NSW) in eastern inland Australia, account for 26% of the vegetable production of the state of NSW (Naughton 2002). They grow diverse brassica crops as well as sweet corn. Recognising the unsustainability of relying on pesticides the industry body Horticulture Australia Ltd (HAL) has supported work on the potential for biological control to be enhanced, particularly with the newly-arrived, exotic ladybird beetle *Hippodamia variegata* (Goeze) (syn. *Adonia*

variegata (Goeze)) (Coleoptera: Coccinellidae). Several earlier studies (Belikova &Kosaev 1985; Pyke &Brown 1996 Franzmann 2002; Kavallieratos et al. 2002) have identified *H. variegata* and the brown lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae), as potentially important biological control agents, particularly of aphids and the eggs and larvae of Lepidoptera. Because species of Aphididae and Lepidoptera are the key pests of brassica vegetable crops in fields, and also because both predators are active in Australia (New & Boros 1983; Pyke &Brown 1996; Franzmann 2002; New 2002), *H. variegata* and *M. tasmaniae* were investigated for their potential usefulness as biological control agents of the vegetable crop pests in CW–NSW.

Originally a Palaearctic species, *Hippodamia variegata* has now spread widely, having been recorded in south Asia, Africa, North and South America and since 2000 in Australia (Franzmann 2002). The first record of *H. variegata* in Australia was as a predator of sorghum-feeding *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) in south-eastern Queensland in November 2000. Since then, *H. variegata* has been found feeding on several other aphid species (Franzmann 2002) indicating its potential as a biological-control agent. Many Coccinellidae (including species of *Hippodamia*) are established biological control agents (BCA) of aphids (Strand & Obrycki 1996; Obrycki & King 1998; Musser & Shelton 2003). Although *H. variegata* preferentially feeds on aphids (Franzman 2002), it is a generalist predator because it also preys on psyllids (Franzman 2002), scale insects, mites, mealybugs and whitefly (Obrycki & King 1998).

Micromus tasmaniae is a predator of many pestiferous aphids and allegedly possesses a high potential to manage these pests (Sampson & Blood 1980; Hodge &Longley 2000; Horne et al. 2001; New 2002; Sato & Takada 2004). Further, it is considered a key predator of aphids, mites, eggs of *Helicoverpa* spp. and many other soft-bodied insect pests of cotton (Pyke &Brown 1996). In New Zealand, it was found to feed on *Phenacoccus graminicola* Leonardi (Hemiptera: Pseudococcidae) which attacks apple and pear trees (Anon 2007). Both adults and larvae of *M. tasmaniae* are predatory and they occur commonly throughout Australia, usually on native low vegetation (Sampson & Blood 1980; New & Boros 1983), especially during flowering (CSIRO 2007).

The potential of the predators such as *H. variegata* and *M. tasmaniae* is strongly affected by factors such as temperature (Jafari et al. 2002), availability of host plants, other competing predators and farming techniques (Schellhorn & Silberbauer 2002; Schellhorn & Andow 2005; Burgio et al. 2006). Most vegetable production systems require insecticides to achieve profitable yields and market–quality produce and insecticides on brassica crops are usually applied with a ground rig to control different species of pestiferous aphids and Lepidoptera. Since these pests are the main prey for *H. variegata* and *M. tasmaniae*, it was considered useful to assess how insecticide spray practices affected these predators.

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.

2.2. MATERIALS AND 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.

2.2.1. *Site sampling*

Survey were carried out on all commercial brassica farms in the CW-NSW (Bathurst region; 149˚11´ E; 33˚31´) area. Monthly surveys were conducted from September 2006 to August 2007.A total of 119 sampling sites on these farms were selected from crops as well as non-crop habitats of pasture, remnant bushland and riparian areas. The early-, mid- and late-season plantings of broccoli, cabbage and cauliflower crops were separately sampled. At each site, a 20 m x 1 m x 4 times area was sampled randomly from each of the brassica fields and non-crop sites.

Since not every farmer had every crop of every planting time at all times, there was no even replication for each crop type, however, each farm had an area of pasture, bushland and riparian vegetation. Samples were collected with a blower-vacuum shredder (STIHL® BG 85 blower-vac, Andreas Stihl Ag & Co. Waiblingen, Germany). The inner tube of the blower-vacuum shredder was fitted with a removable voile bag to intercept arthropods as described in Hamilton et al. (2004) and Schellhorn & Silberbauer (2003).

As soon as a sample was collected it was placed in a covered 20 l plastic bucket that included an uncovered 100 ml beaker filled with 20 ml of chloroform to kill arthropods quickly and prevent predation. Processing of the samples involved the separation of the arthropods from debris by sieving and brushing, transfer to Petri dishes and storage in a freezer for sorting at a later date. A stereo-binocular dissecting microscope (10x) (Leica, SE305-A, Mel Sorbel Microscope Ltd., New York, USA), mounted with counting grids was used for counting and identification of the arthropods.

2.2.2. *Pesticide impact*

To compare the population densities of *H. variegata and M. tasmaniae* in relation to insecticide regime, a modified beneficial disruption index (BDI) was employed, which incorporated the key principles of the BDI (a generalized measure of insecticide impact on beneficial insects in cotton crops after insecticide application)

(Hoque et al. 2002) and of a toxicity chart (TC) (a measure of the impact of insecticides on beneficial insects in brassica crops) (Walsh 2005). The impacts of each insecticide on beneficial insects according to these references were scored on a scale of 1–5 (1 - very low impact to 5 – very high impact): impact refers to the toxicity to beneficial insects of a specific insecticide). Chemicals were graded into five classes depending on their impact. For example, biological insecticides such as Gemstar® were scored 1 while an organophosphate (OP) insecticide was scored 5. The total BDI for each crop was then calculated by multiplying the number of applications for each type of insecticide applied to each crop by the corresponding BDI score (Table 2.1) for that insecticide and by summing the BDIs.

Table 2.1: The beneficial disruption index (BDI) scores given for each pesticide used by surveyed brassica growers in the Bathurst area, Central West New South Wales.

Beneficial Disruption Index (BDI)	Chemical Trade names (®)	Active Ingredients (a.i.)	Target Pest
1	Delfin	*Bacillus thuringiensis* (sub. Kurstaki SA-11)	Lepidoptera
1	Dipel	*Bacillus thuringiensis* (sub. Kurstaki HD-1)	Lepidoptera
1	Gemstar LC	2000 million/mL polyhedral occlusion bodies of NPV	Lepidoptera
1	Vivus	$5x10^9$ polyhedral inclusions of NPV of *H. armigera*	*Helicoverpa* spp.

1	Azamax	11.82 g/L azadirachtin A and B	Aphids, mites, whitefly, thrips, caterpillars
2	Success Success II	120-240g/L spinosad	Lepidoptera, Western Flower thrips
2	Tracer	125g/L spinosad	Lepidoptera
2	Aphidex WP	500g/kg pirimicarb	Aphids
3	Avatar	300g/kg indoxacarb	Lepidoptera, sucking pests (GVB, mirids, thrips)
3	Indoxacarb (Steward)	400g/kg indoxacarb	Lepidoptera, sucking pests
3	Proclaim	44g/kg Emamectin (emamectin benzoate)	Lepidoptera, mites
3	Dicofol Miti-Fol Kelthane	240 g/L Dicofol1,-bis (chlorophenyl2, 2-trichloroethanol)	Mites
4	Confidor 200 SC	200g/L imidacloprid	Aphids
5	Axe Ambush Permethrin	500g/L permethrin	Lepidoptera
5	Alpha	100g/L alpha-cypermethrin	Lepidoptera, sucking pests
5	Lorsban 500 EC	500g/L chlorpyrifos	Aphids, wireworm
5	Methomyl Lannate 225	225g/L methomyl (anticholinesterase compound)	Lepidoptera
5	Pyrethrum	40g/L pyrethrin, 160g/L piperonyl butoxide	Lepidoptera, aphids, jassids

2.2.3. *Statistical analysis*

The monthly abundance of both predators and possible prey species were graphed for the survey period and their trends were discussed. The counts of *H. variegata* and *M. tasmaniae* were conditionally Poisson distributed with log (mean) modelled to include additive effects due to time, farm and vegetation. The effects due to vegetation were broken down into differences between crops and non-crop vegetation types. The vegetation types within crops were further broken down to type of crop, stage of crop and an interaction between these two. The modified BDI was analysed against abundance of each predator species using a simple linear regression model.

2.3. RESULTS

2.3.1. *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 2.1), 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.

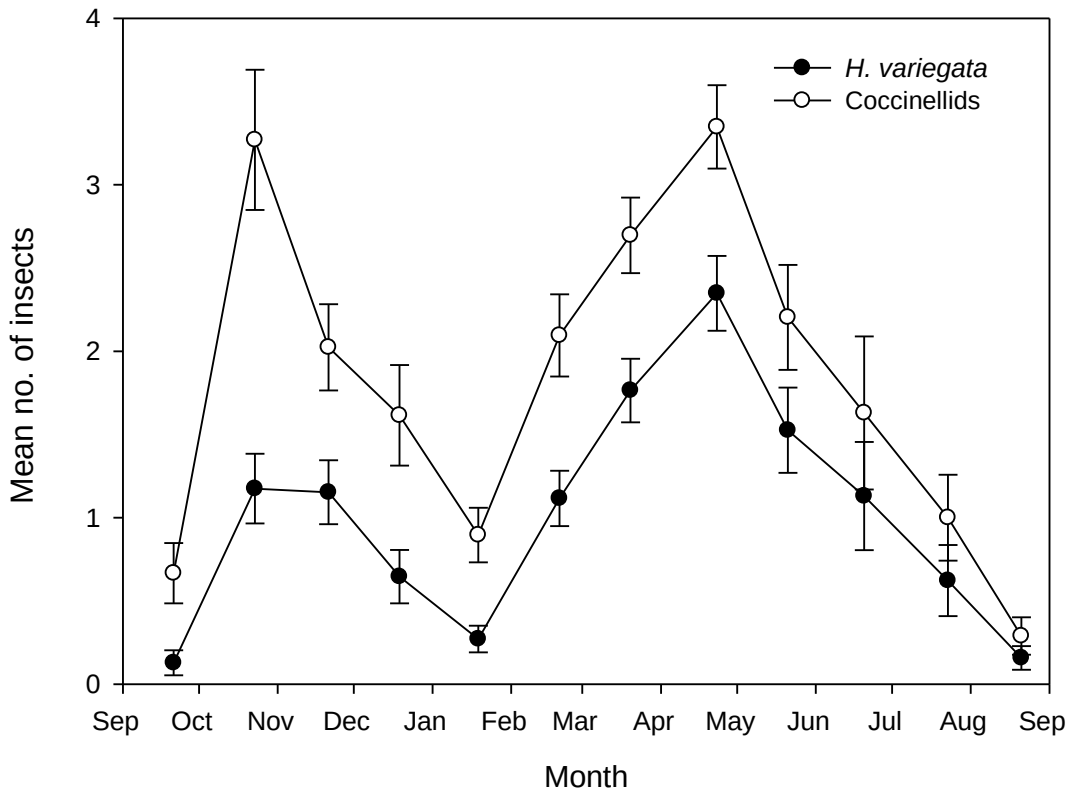


Figure 2. 1: Temporal trend of *Hippodamia variegata* and all coccinellids on vegetable farms, Bathurst, Central West of New South Wales. Error bars indicate standard error of means.

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 2.2). 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.

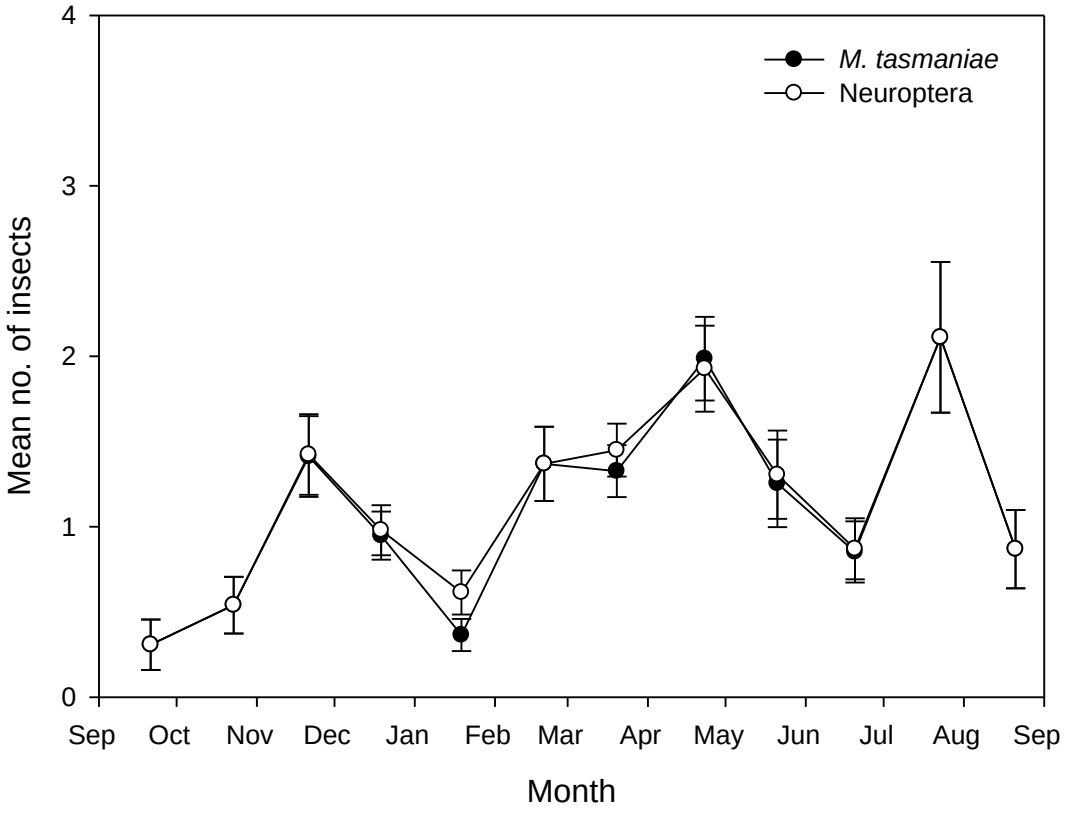


Figure 2. 2: Temporal trend of *Micromus tasmaniae* and all Neuroptera on vegetable farms, Bathurst, Central West of New South Wales. Error bars indicate standard error of means.

2.3.2. *Seasonal availability of prey species*

Several possible prey species were available to *H. variegata* and *M. tasmaniae* (Figure 2.3). 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.

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.

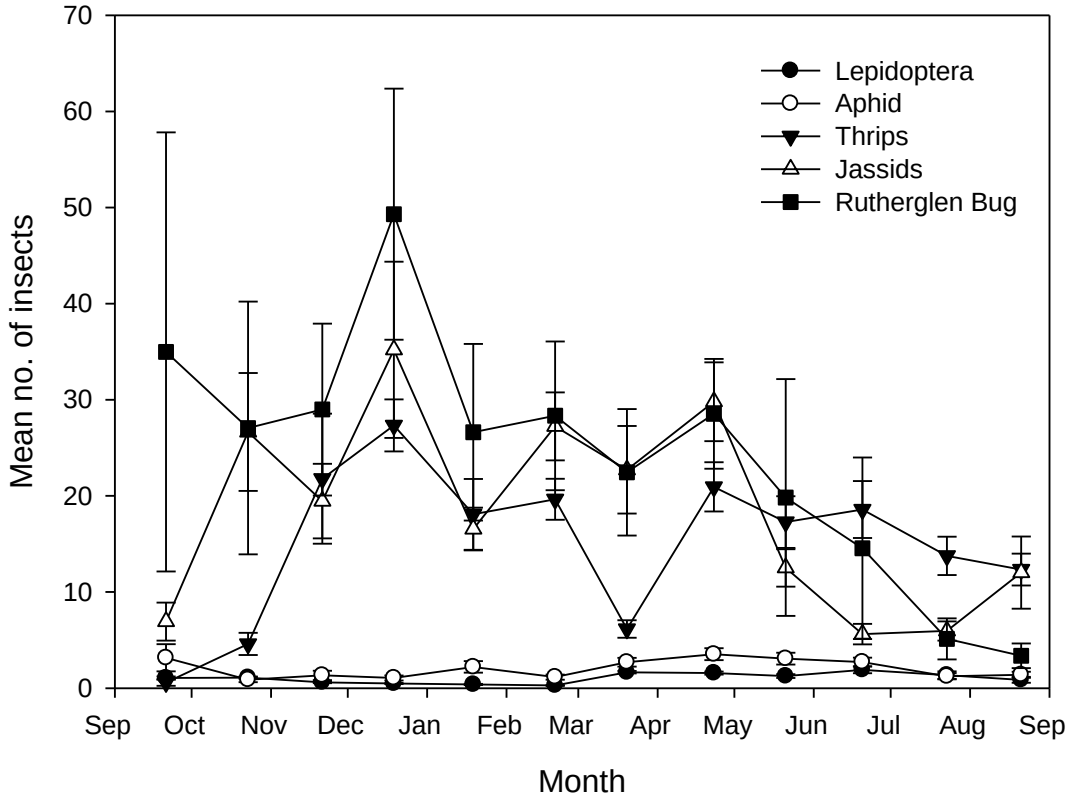


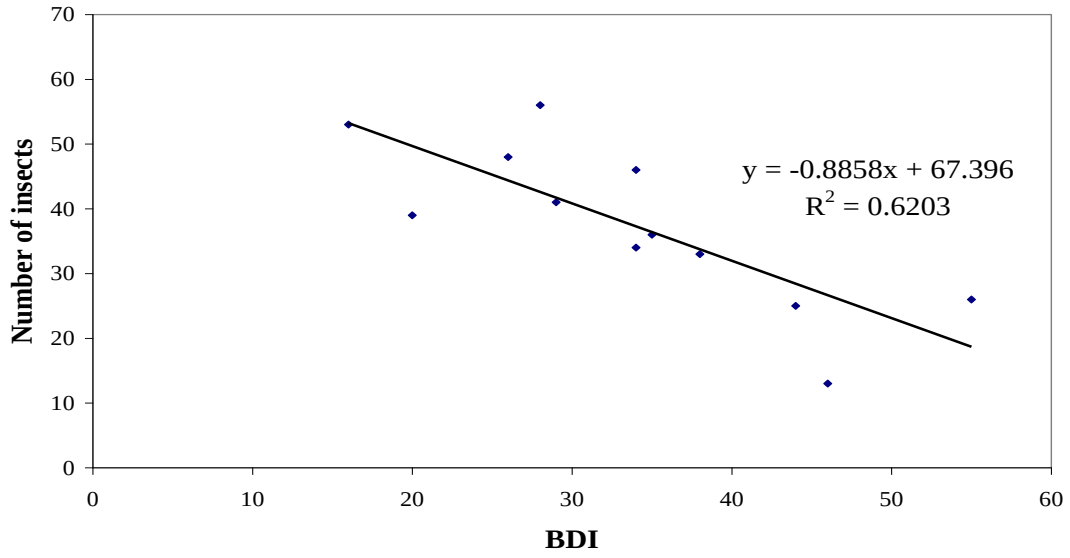
Figure 2. 3: Mean number of potential prey individuals over all habitats and all farms in CW-NSW. Error bars indicate standard error of means.

2.3.3. *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 2.4 (a) & (b)) 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*.

(a)



(b)

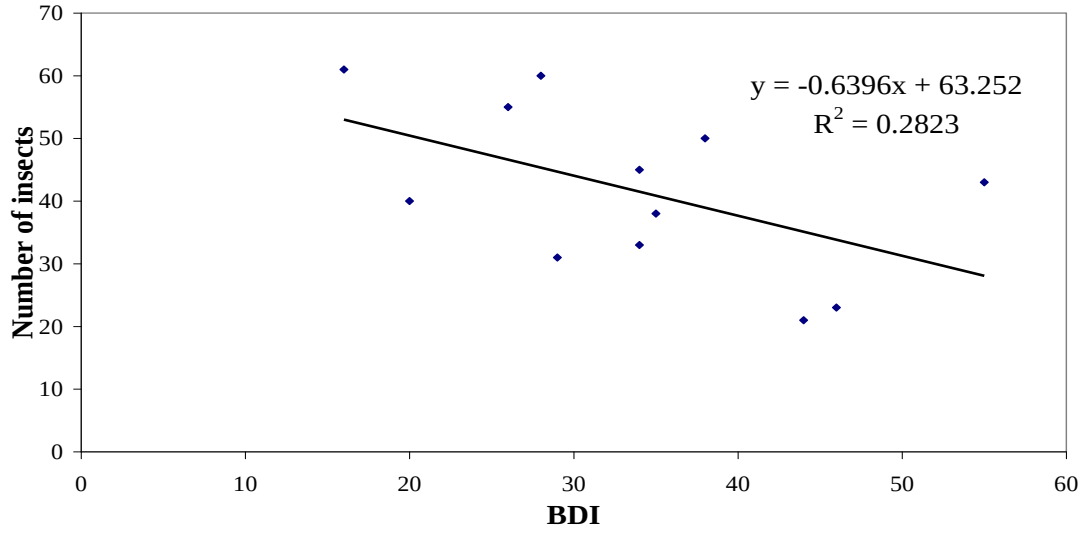


Figure 2.4: Relationship between the biological disruption index (BDI) of pesticide regimes and catches of (a) *Hippodamia variegata* and (b) *Micromus tasmaniae* on vegetable farms in New South Wales, Australia.

2.3.4. Influence of different vegetation types on the abundance of *H. variegata* and *M. tasmaniae*

REML analysis of the effects of time of season (date) and vegetation type showed that vegetation type alone had no significant impact (F= 0.451, df= 208, P=0.05) on the numbers of *H. variegata* sampled over all sites each month. The means for crop and non-crop vegetation were 1.027 and 0.957, respectively. Time of season was a significant factor (F<0.001) as population numbers in both vegetation types fluctuated throughout the year. The interaction between time of season and vegetation type was also highly significant (F= 0.004, Figure 2.5) with *H. variegata* populations in crops exceeding those in non-crops in October, December and from March to May.

Time of season (F< 0.001, df= 829, P=0.05) and vegetation type (F= 0.004) significantly affected *M. tasmaniae* populations. Means for crop and non-crop vegetation were 1.098 and 0.948, respectively. *M. tasmaniae* populations were significantly higher in crops in September, December, February, April and May. There was no significant interaction between time of season and vegetation type for *M. tasmaniae* (F=0.214).

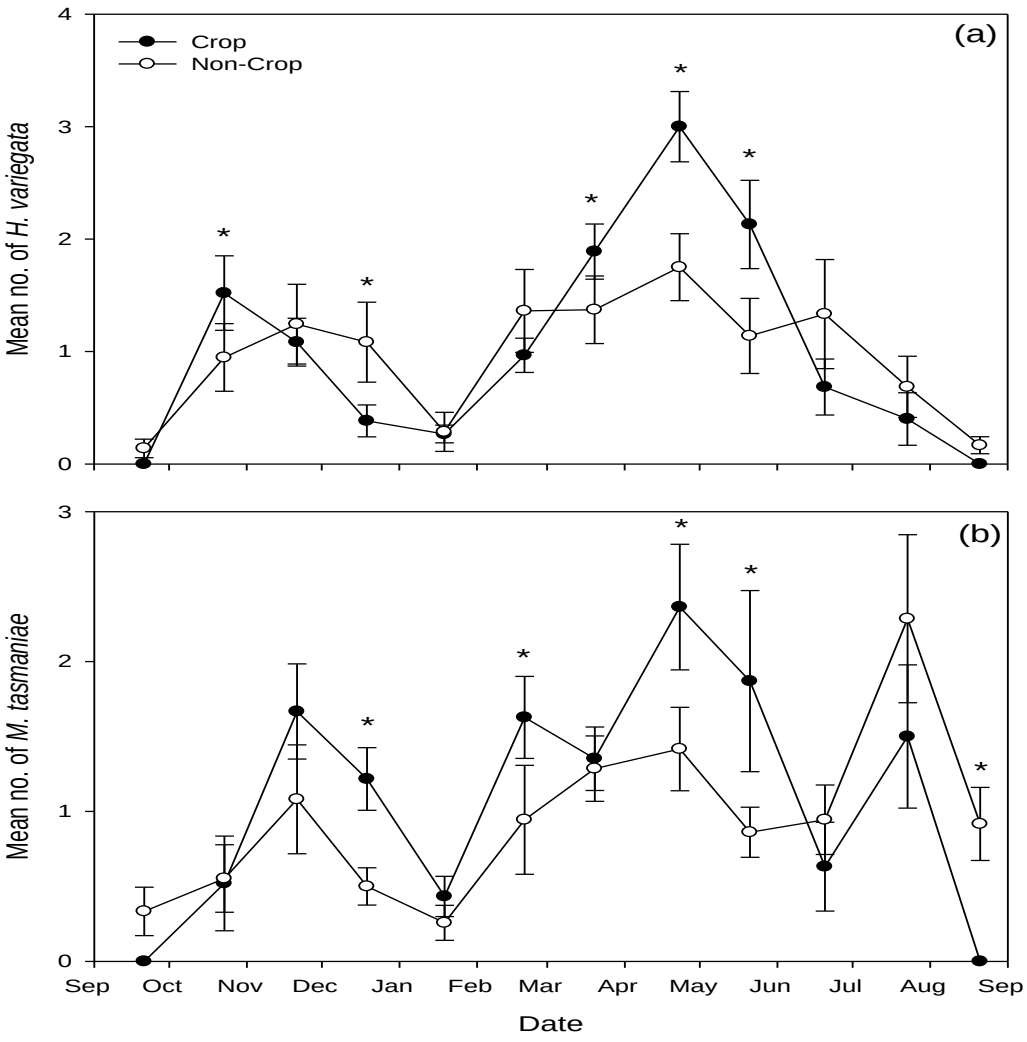


Figure : 2.5: Mean number of *Hippodamia variegata* and *Micromus tasmaniae* in crop and non-crop vegetation over all brassica farms Bathurst, New South Wales. Error bars indicate standard error of means.

2.4. 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. Over the year, *H. variegata* numbers were higher in non-crop vegetation in 8/12 months and *M. tasmaniae* numbers in 7/12 months, yet the mean number of each species for the whole year was higher in crop vegetation. This is frequency versus quantity. 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 (White et al 1995; Grez & Villagran 2000; Marshall & Moonen 2002; Langellotto & Deno 2004). Moreover, the establishment and maintenance of suitable habitat on farm or surrounding landscape can enhance the survival of natural enemies (Gurr, 2004; Stephens, 2006). 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 (McEwen et al. 2001). The structure, vegetation type and shape can have

a direct effect on the density of beneficial arthropods in a cropping system (Andow 1990; Landis et al. 2000; Sunderland & Samu 2000). Therefore, brassica growers could manipulate or conserve the non-crop vegetation on their farms to enhance the population density of beneficial arthropods. Further, Thies and Tscharntke (1999) found that 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. Migration is an important factor in seasonal occurance of predators in fields (Shimoda & Takabayashi 2001) stated that migration is an important factor in seasonal occurance of predators in fields. 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. The use of selective insecticide early in the season will enable the predators to establish in their fields. Results clearly show that an increased number of sprays (high BDI), lead to reduce predators abundance, 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 refuse and breeding areas.

CHAPTER THREE - MOVEMENT OF *Hippodamia variegata* AND *Micromus tasmaniae* FROM NON-CROP VEGETATION TO BRASSICA CROPS

3.1. INTRODUCTION

Potential use of the white collar ladybird beetle *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) and the native brown lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) as biological control agents will be determined by their biology, efficacy, optimal environmental conditions, susceptibility to insecticides and population dynamics. Therefore, tracking the movement of the two predators through various habitat types on farms is essential for understanding their biology (Hagler & Jackson 2001) and population dynamics (Nowatzki et al. 2003) to enable their successful use as biological control agents (BCAs). The role of these two predators in an integrated pest management (IPM) program may potentially be optimised by strategic manipulation of farm habitats under management recommendations that could apply over a wide range of horticultural production systems. Many experiments have shown that manipulating the farm habitat, for example by adding areas of flowering plants to the agroecosystem, can increase the abundance of beneficial insects (Wratten et al., 2002) and enhance their effectiveness as BCAs (Gurr and Wratten 1999).

Similarly, Schellhorn and Sork (1997) and Landis et al. (2000) suggest that manipulation of non-crop habitat can enhance natural enemies' abundance.

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. There are various methods of marking insects that can be used to study their movement among crop or non-crop vegetation. These methods include, marking, tagging, mutilation, radioisotopes, genetic and elemental marking. (Shellhorn et al. 2008; Shellhorn et al. 2004; Hagler and Jackson 2001 and Akey 1991). To measure the movement of *H. variegata* and *M. tasmaniae,* the mark – capture technique (Shellhorn et al. 2004) was used. The main advantage of this method was its inexpensiveness and that it can be easily applied to the insects in their natural habitat. The previously cited articles show also that it meets other important criteria: no effect on insect longevity and behavior, no environmental impact or possible risk to the crop to which it is applied and no risk to the human operator. 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.

3.2. MATERIALS AND METHODS

Marking is a method used in arthropod ecology to estimate population size, survival, growth and movement (Lavandero et al., 2004; Schellhorn et al., 2004). 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 in the CW-NSW region (Bathurst; 149˚11´ E; 33˚31´) from December 2007 to August 2008. 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 crop field was sprayed with a dye mixture of SARDI yellow fluorescent pigment at a rate of 100 litres per hectare. The dye was applied with a mistblower sprayer (STIHL® SR 450) at a rate of 2 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 as described in Schellhorn & Silberbauer (2003) and Hamilton et al. (2004). 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.

3.2.1. *Data Analysis*

The experimental design was factorial, however, it was analysed in separate ANOVAs in order to answer different questions. Site has been used as a replicate (block) in each analysis. Data were analysed in a blocked three-way ANOVA (Vegetation * DAT * Sample Distance) and using a generalized linear model (GLM) with Poisson distribution and logarithmic link function in Genstat V12.1, PC/Windows XP, 2009. Sample distance from non-crop (Distance), Days after treatment (DAT), Vegetation type and the interactions between the three were

used as the fitted terms. Marked insects were regressed against DAT and distance separately to test the strength of the relationships. Data were not transformed as residual scatter plots showed normal distribution of the means. According to statistical advice to the candidate, not all percentage data derived from count data needs to be transformed if the percentage data lies within the range of 30-70%, i.e. it is homogenous and hence no transformation is needed (Parsad, undated).

3.3. RESULTS

3.3.1. *Movement of predators into the crop from marked areas*

A total of 1,404 *H. variegata* were captured of which 683 (48.65%) had been marked. For *M. tasmaniae* the percentage marked was much less, 268 (33.67%) of a total of 796 captured specimens. 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).

3.3.2. *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 (Table3.1).

Table 3. 1: 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	0.829	n.s.	1.17	1.29	1.32
	Unmarked	0.892	n.s.	1.63	1.50	1.59
	Total	0.942	n.s.	2.83	2.79	2.91
M. tasmaniae	Marked	0.741	n.s.	0.889	.822	.933
	Unmarked	0.042	0.383	*1.32	1.62	1.81
	Total	0.088	n.s.	2.21	2.44	2.74

* signifies a significant difference from riparian vegetation at P=0.05
*df = 269

3.3.3. 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. The ANOVA derived means for movement of both predators are shown in Tables 3.2 and 3.3. Percentages represent the proportion of mean marked and unmarked *H. variegata* or *M. tasmaniae* of the total sampled insects at that date and distance from the area that was marked.

Table 3. 2: Means for temporal and spatial parameters of *H. variegata* – movement from non-crop vegetation to brassica crops

			Distance (m)											
T	Captured *H.v.*	LSD	0		20		40		60		80		100	
			Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
	Marked	0.443	0	0	0	0	0	0	0	0	0	0	0	0
	Unmarked	0.474	1.94	100	0.122	100	1.33	100	0.556	100	0.333	100	0.278	100
	Total	0.675	1.94	100	1.222	100	1.333	100	0.556	100	0.333	100	0.278	100
	Marked		2.056	88.1	[a]0.417	75.0	[a] 0.194	46.5	[a] 0.222	32.0	[a] 0.361	81.3	[a] 0.167	60.1
	Unmarked		0.278	11.9	0.139	25.0	0.222	53.5	0.472	68.0	0.083	18.7	0.111	39.9
	Total		2..333	100	0.556	100	0.417	100	0.694	100	0.444	100	0.278	100
	Marked		[b]0.861	70.5	0.694	64.0	[a] 0.194	69.8	[a] 0.278	38.5	[a] 0.278	45.5	[a] 0.111	33.3
	Unmarked		0.361	29.5	0.389	36.0	0.083	30.2	0.444	61.5	0.333	54.5	0.222	66.7
	Total		1.222	100	1.083	100	0.278	100	0.722	100	0.611	100	0.333	100
	Marked		0.750	77.2	0.667	75.0	[a] 0.139	27.8	[a] 0.222	29.6	[a] 0.278	66.7	[a] 0.167	46.3
	Unmarked		0.222	22.8	0.222	25.0	0.361	72.2	0.528	70.4	0.139	33.3	0.194	53.7
	Total		0.972	100	0.889	100	0.500	100	0.750	100	0.417	100	0.361	100
	Marked		[b] 0.389	69.9	0.333	46.1	0.222	56.6	0.194	38.8	0.139	26.3	0.111	100
	Unmarked		0.167	30.1	0.389	53.9	0.167	43.4	0.278	61.2	0.389	73.7	0	0
	Total		0.556	100	0.722	100	0.389	100	0.500	100	0.528	100	0.111	100

df = 1079

[a] represents significant difference of means compared to the edge of the field (0 metres)

[b] represents significant means compared to the first samples of marked *H. variegata* (2 DAT)

Table 3. 3: Means for temporal and spatial parameters of *M. tasmaniae* – movement from non-crop vegetation to brassica crops

DAT	Captured *M.t.*	LSD	Distance (m)											
			0		20		40		60		80		100	
			Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
0	Marked	0.359	0	0	0	0	0	0	0	0	0	0	0	0
	Unmarked	0.477	0.806	100	0.944	100	0.667	100	0.306	100	0.583	100	0.333	10
	Total	0.621	0.806	100	0.944	100	0.667	100	0.306	100	0.583	100	0.333	10
2	Marked		0.528	51.4	0.333	75.0	0.194	30.4	0.333	52.1	[a] 0.111	28.5	0.250	42
	Unmarked		0.500	48.6	0.111	25.0	0.444	69.6	0.306	47	0.278	71.5	0.333	57
	Total		1.028	100	0.444	100	0.639	100	0.639	100	0.389	100	0.583	10
5	Marked		0.389	48.3	0.417	55.6	0.194	33.3	0.389	46.7	0.083	24.9	0.111	44
	Unmarked		0.417	51.7	0.333	44.4	0.389	66.7	0.444	53.3	0.250	75.1	0.139	55
	Total		0.806	100	0.750	100	0.538	100	0.833	100	0.333	100	0.250	10
7	Marked		0.472	50.0	[a] 0.11	23.5	0.222	39.9	0.417	60.1	0.194	34.9	[a] 0.083	23
	Unmarked		0.472	50.0	0.361	76.5	0.333	60.1	0.278	39.9	0.361	65.1	0.278	77
	Total		0.944	100	0.472	100	0.556	100	0.694	100	0.556	100	0.361	10
10	Marked		0.472	43.6	[a] 0.11	21.0	0.417	53.6	0.333	42.8	0.222	53.2	0.222	53
	Unmarked		0.611	56.4	0.417	79.0	0.361	46.4	0.444	57.2	0.194	46.8	0.194	46
	Total		1.083	100	0.528	100	0.778	100	0.778	100	0.417	100	0.417	10

df = 1079

[a] represents significant difference of means compared to the edge of the field (0 metres)

[b] represents significant means compared to the first samples of marked *M. tasmaniae* (2 DAT)

Two days after marking, 88.1% of the overall *H. variegata* captured were marked showing they had moved to the crop from surrounding vegetation (Table 3.2). 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. Table 3. 2 shows the means of the interaction, however, it was difficult to discern an obvious relationship as the data displayed high variability (Figure 3.1). By regressing the means of the distance component (Figure 3.2) 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.

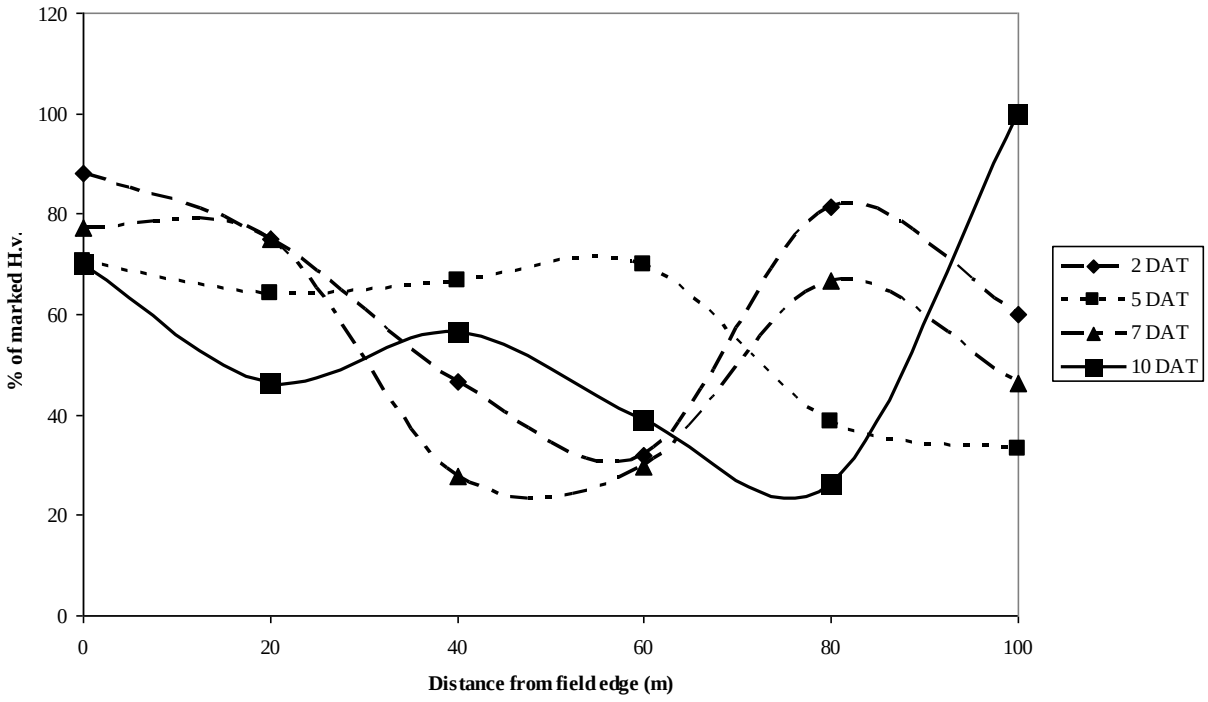


Figure 3. 1: Movement of *Hippodamia variegata* into brassica fields from surrounding vegetation

When regressing the time component of the interaction (Figure 3.3) 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.

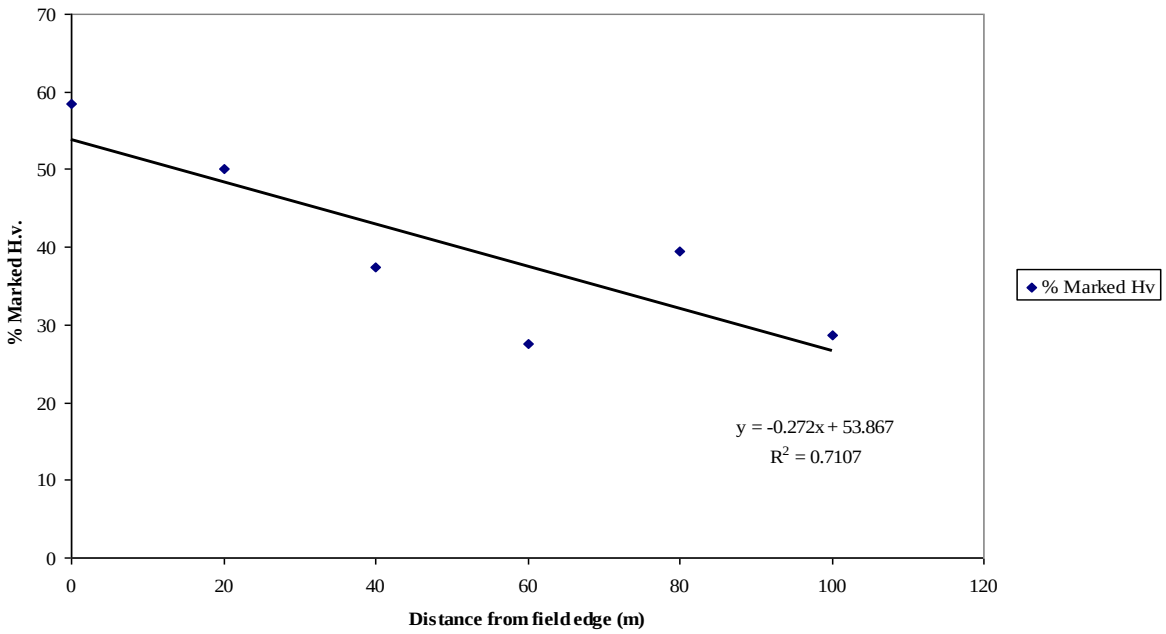


Figure 3. 2: Regression of distance component of captured marked *H. variegata*

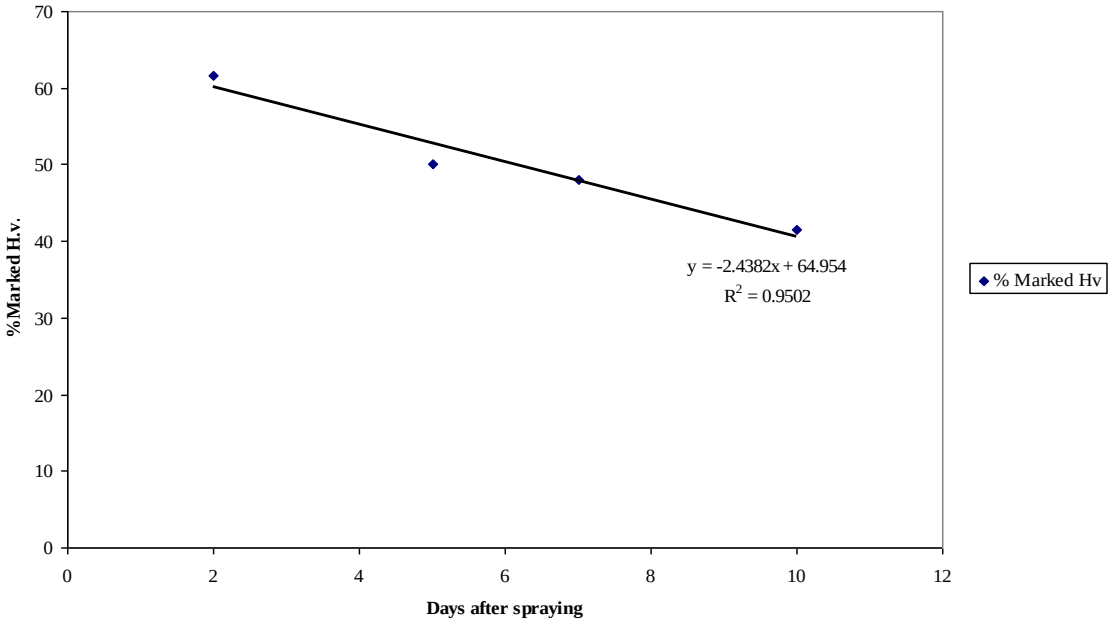


Figure 3. 3: Regression of time component of captured marked *H. variegata*

The percentage of captured, marked *M. tasmaniae* (Figure 3.4) 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 3.5, R^2 value = 0.3199). The correlation with time (Figure 3.6, R^2=0.5795), was higher than that for distance and accounted for nearly 60% of the variability.

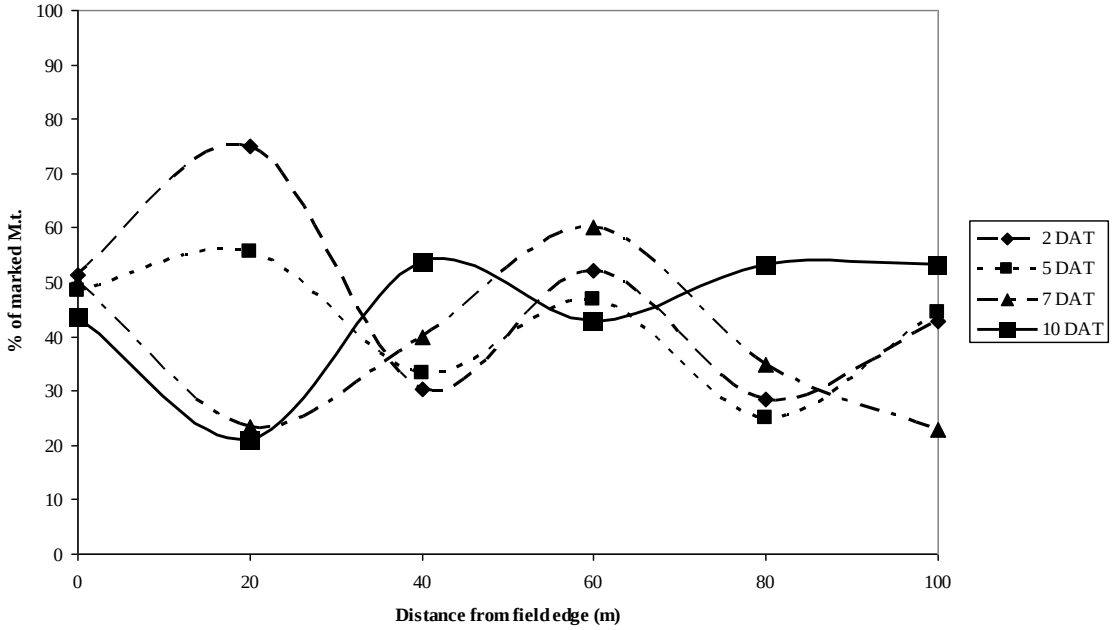


Figure 3. 4: Movement of means of *Micromus tasmaniae* into brassica fields from surrounding vegetation

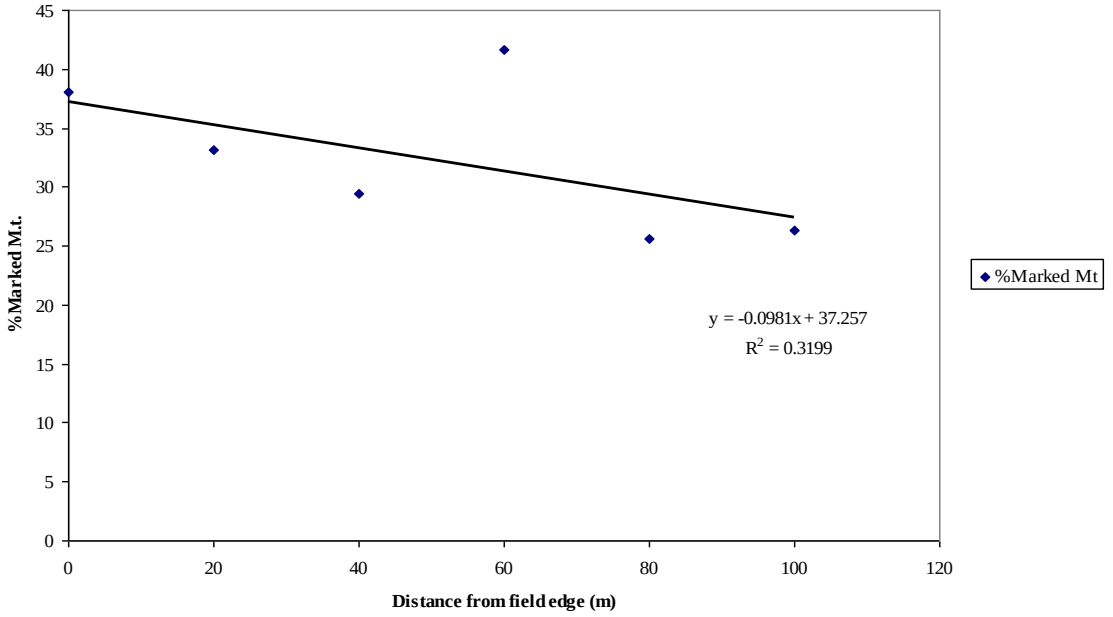


Figure 3. 5: Regression of distance component of captured marked *M. tasmaniae*

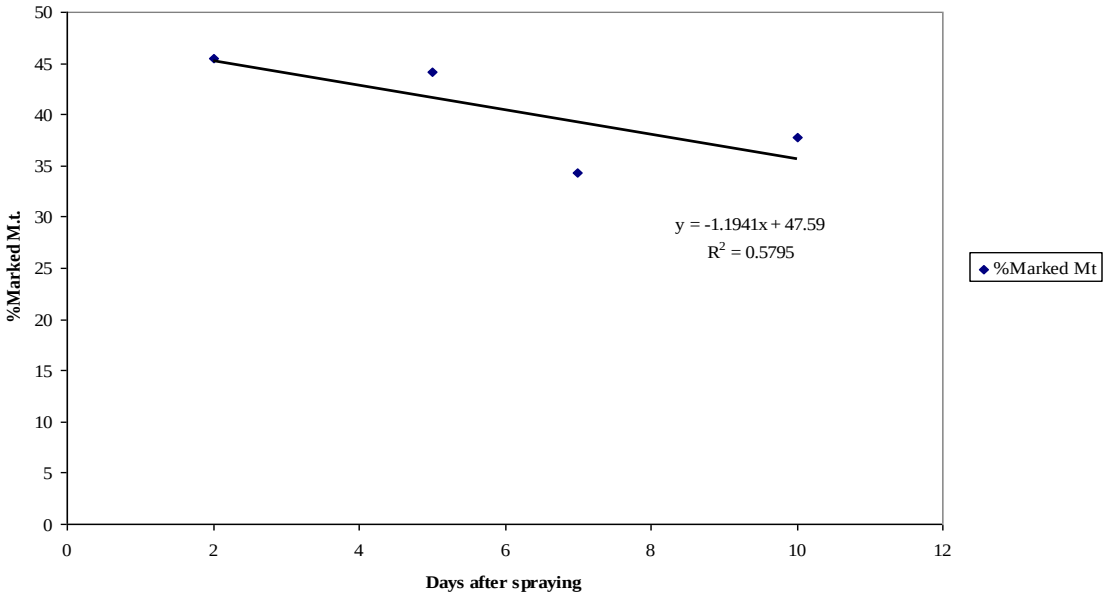


Figure 3. 6: Regression of time component of captured marked *M. tasmaniae*

3.4. 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. Schellhorn and Silberbauer (2002) have

demonstrated movement with released predators in cotton, however, such work is labour intensive and needs to be carried out on a larger scale, and for these reasons was beyond the scope of this project.

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. This experiment assessed the ability of insects to move from non-crop vegetation to crops. It measured the distance moved and the time period during which movement occurred. It was not possible to assess all insects present in the strips prior to spraying; hence the percentage of insects that moved from the strips could not be calculated. Marked and unmarked insects were counted and only marked insects originated in the sprayed plots with certainty, while unmarked insects could have come either out of the strips (but were missed by the spray or moved in after spraying) or came from beyond the sprayed strip. The assessment was for movement as a function of time and distance. The percentages expressed in the results refer to the marked insects out of the total captured rather than out of the total originally marked.

Lavandero et al. (2004) showed that beneficial insects moved through habitats at a broad range of spatial scales and Tscharntke et al. (2005) and Bianchi et al. (2006)

stated that neighbouring non-crop vegetation enhanced population of beneficial insects for crop pest control. In Chapter 2 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 may 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 (New, 2007). 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 (Evans, 2009, Hodek and Honek, 2009). *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 (Smith, 2008). *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 (Chapter 2) 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 (Landis et al., 2000; Gurr et al., 2003; Lavandero et al., 2006) in order to enhance biological control.

Understanding the characteristics of vegetation (Gurr et al., 2003) is important in the manipulation of vegetation that provides shelter and food for natural enemies. For example, McEwen et al., 2001 noted that 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 (Hajek, 2004) which may increase their activity at field edges. Natural enemies can move over to an adjacent crop and control pests (Landis et al., 2000) 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. The next experiment in Chapter 4 investigated the re-colonisation of crops by predators after fields had been treated with insecticides and the movement of predators within the fields.

CHAPTER FOUR - REPOPULATION OF INSECTICIDE-SPRAYED BRASSICA CROPS BY *Hippodamia variegata* AND *Micromus tasmaniae*

4.1. INTRODUCTION

The white-collared ladybird beetle *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) and the native brown lacewing *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) are two generalist predators commonly found in the brassica cropping areas in the Central west New South Wales (CW-NSW) (Chapter 2). Both species are possible biological control agents for brassica and other crop pests. Two important factors for their success are their susceptibility to insecticides and their population dynamics with respect to recolonisation of sprayed areas.

Growers in the CW-NSW area rely heavily on insecticide sprays to produce brassica crops that meet the product standard that buyers demand. Such product is to be free of insects and insect frass and should not exhibit damage. IPM options to produce brassica crops of such standard are limited at the present, necessitating the frequent use of insecticides. While a number of insecticides with low impact on non-target insects are available, insecticide resistance management strategies do

not allow consecutive use of these insecticides. Therefore, some insecticides used in brassica production can affect beneficial insects. The impact of insecticides on natural enemies can be direct or indirect (Van Humburg & Guest 1997; Dent, 2000; Walsh 2005; Walker et al., 2007). This means that not only can natural enemies be killed by directly contacting the chemical residue on the crop, but they can also be killed by feeding on insecticide contaminated prey species. Schellhorn et al. (2008) also stated that the timing and pattern of crop colonization by insects may be affected by proximity of the source and the attractiveness of the crop.

In Chapter 2, it was demonstrated that natural enemies are found in non-crop areas such as pasture, remnant bushland and riparian areas. Further, Chapter 3 showed that both predator species moved into crops from these habitats if they were adjacent to crops. There was no significant difference between the three types of non crop habitat as source areas. While *H. variegata* exhibited strong spatial and temporal trends, *M. tasmaniae's* movements were more independent. This research also agreed with the findings by Hagler and Naranjo (2004) and Prasifka et al. (2003) that insect predators move from adjacent refuge or non-crop areas to crop areas to feed on the crops' arthropod pests. The study in this chapter is an extension of the experiment in Chapter 3, but in contrast considers re-population by predators from adjacent non-crop vegetation immediately after an insecticide spray event. Hypothesis to be tested in this study includes:

- Predators from neighbouring cropland and non-crop vegetation will repopulate an insecticide sprayed field over time

- Surrounding vegetation have an edge effect on population abundance of *H. variegata* and *M. tasmaniae*
- Colour and aspect (placement) of sticky traps affects the efficacy of catching insects, *H. variegata* and *M. tasmaniae*
- Malaise traps is important in determining the movement of *H. variegata* and *M. tasmaniae*

4.2. MATERIALS AND METHODS

The study took place on three commercial brassica farms in CW-NSW (Bathurst region; 149˚11´ E; 33˚31´) from January to March 2009. Each brassica crop field was located adjacent to pasture, remnant bushland or a riparian area (non-crops) at one end and cropland on the other end (Figure 4.1). 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 (Table 4.1) 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).

Table 4. 1: Insecticides applied at three different sample sites

Site	Trade Name	Active Ingredient	Target Pest	Impact on Coccinellids	Impact on Neuroptera	Persistence
A	Avatar	300g/kg Indoxacarb	Lepidoptera, sucking pests (GVB, mirids, thrips)	Very High	Moderate	Low
B	Steward	400g/kg indoxacarb	Lepidoptera, sucking pests	Very High	Moderate	Low
C	Confidor 200 SC	200g/L imidacloprid	Aphids, mirids	High	Low	Medium

NB: Impact of insecticides on Coccinellids and Neuroptera from - Source:**http://www.sardi.sa.gov.au/pdfserve/ento/dbm/publications/project_newsletters/toxicity.pdf**

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 (Mensah, 1997; Clare et al. 2000; Gencsoylu 2007; Harman et al. 2007; Gardiner et al., 2009) 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 (Plate 4.1) 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 (Plate 4.2) were set up, two at each end of the field (Figure 4.1).

Plate 4. 1: Sticky trap stations in a brassica field in Bathurst, CW-NSW.

Plate 4.2: Malaise trap in a brassica field in Bathurst, CW-NSW.

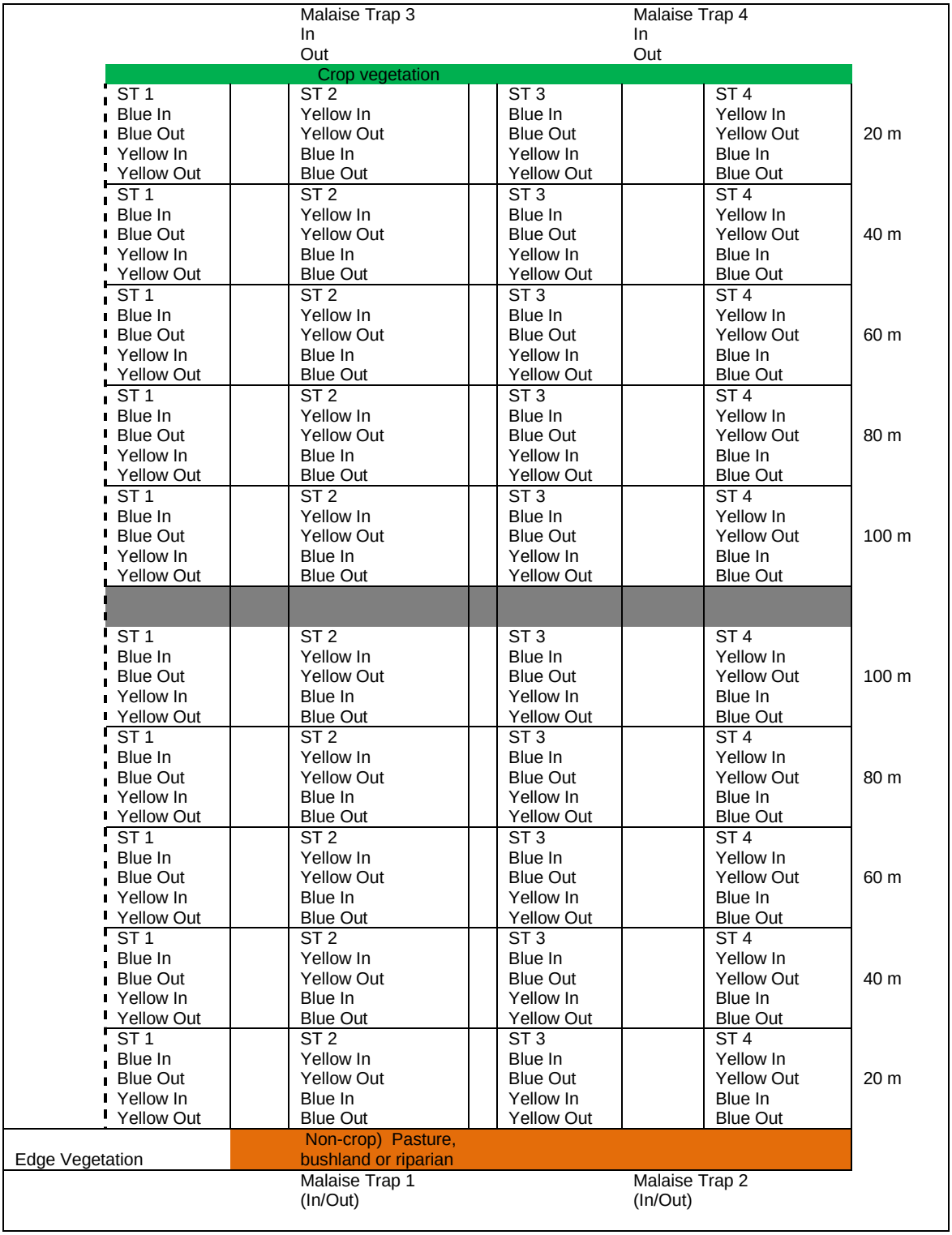


Figure 4.1: Diagram of experimental field with sticky traps stations and malaise traps, for collection of *H. variegata* and *M. tasmaniae*

4.2.1. *Data Analysis*

Data were analysed in a blocked Three-way ANOVA (Vegetation * DAT * Sample Distance) and using a generalized linear model (GLM) with Poisson distribution and logarithmic link function in Genstat V12.1 (PC/Windows XP, 2009). Sample distance from non-crop and cropland (Distance), Days after treatment (DAT) and Vegetation type and the interactions between the three were used as the fitted terms. Data were also analysed in ageneral ANOVA for Colour * Aspect * Direction. The catches on Malaise traps were analysed separately.

4.3. RESULTS

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

Adjacent vegetation type played a significant role (Table 4.2) 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.

Table 4. 2: Means for the two predators re-colonising an insecticide-treated brassica field, P=0.05

Orientation	*H. variegata*	*M. tasmaniae*
Non-crop vegetation	[a]0.512	0.327
Cropland	[a]0.647	[a]0.258
Df	2879	
F-value	<0.001	<0.001
LSD	0.0688	0.0401

[a] represents a significant difference compared to no-crop vegetation

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, Table 4.3).

Table 4. 3: Means for spatial factors of predator re-colonisation of an insecticide-treated brassica field, P=0.05

Distance	***H. variegata***	***M. tasmaniae***
20 m	0.528	0.280
40 m	0.628	0.306
60 m	0.628	0.340
80 m	0.524	0.266
100 m	0.589	0.273
Df	2879	
F-value	0.143	0.129
LSD	n.s.	n.s

When regressing the distance component (Figure 4.2) 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 4.3) also showed no significant relationship (R^2=0.079, F=0.6461).

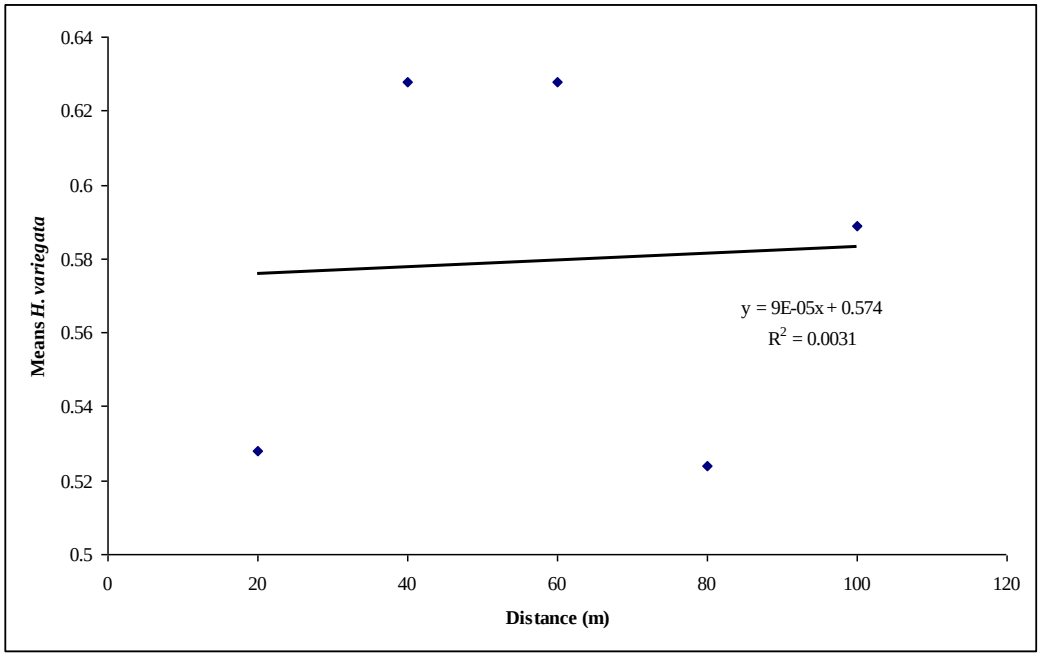


Figure 4. 2: Regression of mean of distance component of recaptured *H. variegata*

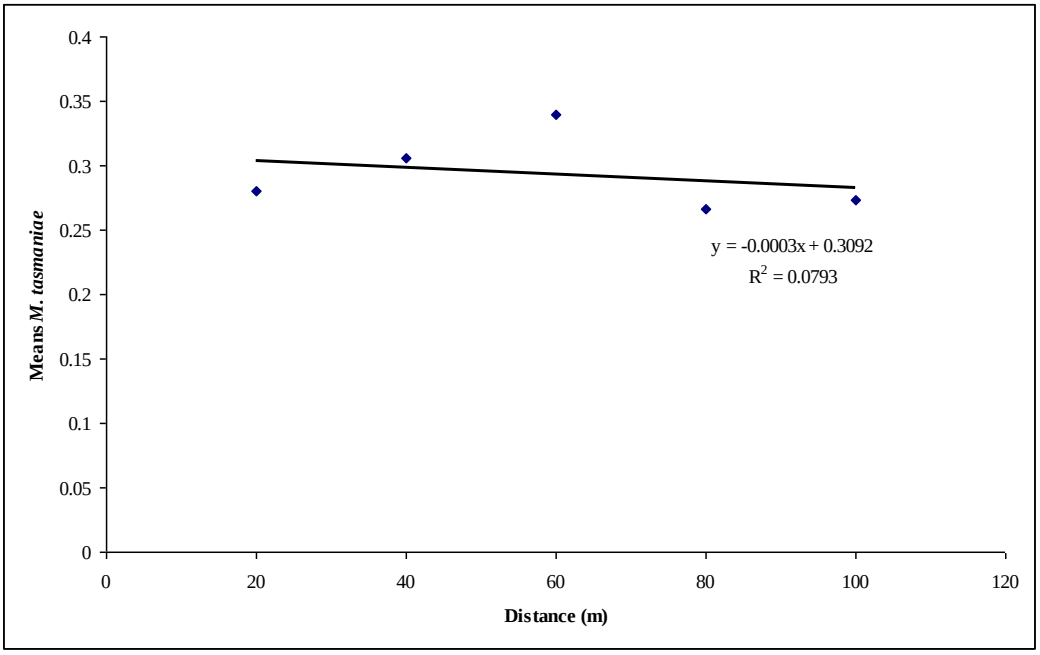


Figure 4. 3: Regression of mean of distance component of recaptured *M. tasmaniae*

4.3.2. *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 2 days of spraying. Numbers increased gradually as time from the spray date increased (Table 4.4). 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.

Table 4. 4: Means for temporal factors of predator re-colonisation of an insecticide-treated brassica field, P=0.05

Days after Treatment (DAT)	*H. variegata*	*M. tasmaniae*
2 d	0.273	0.117
4 d	[a] 0.408	[a] 0.210
6 d	[a] 0.496	[a] 0.283
8 d	[a] 0.698	[a] 0.408
10 d	[a] 0.754	[a] 0.344
12 d	[a] 0.848	[a] 0.394
Df	2879	
F-value	<0.001	<0.001
LSD	0.1193	0.0695

[a] represents a significant difference compared to 2 DAT

When regressing the time component (Figure 4.4) 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 4.5) was also seen for *M. tasmaniae* (F=0.013) but not as strongly as for *H. variegata* (R^2 = 0.8161).

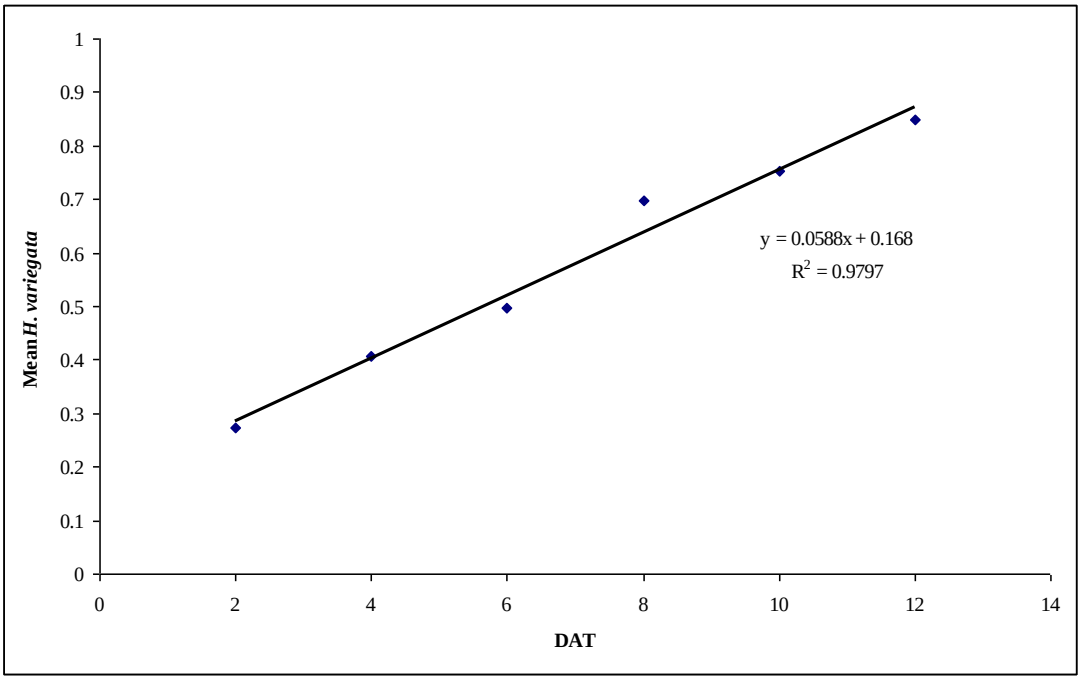


Figure 4. 4: Regression of time component of recaptured *H. variegata*

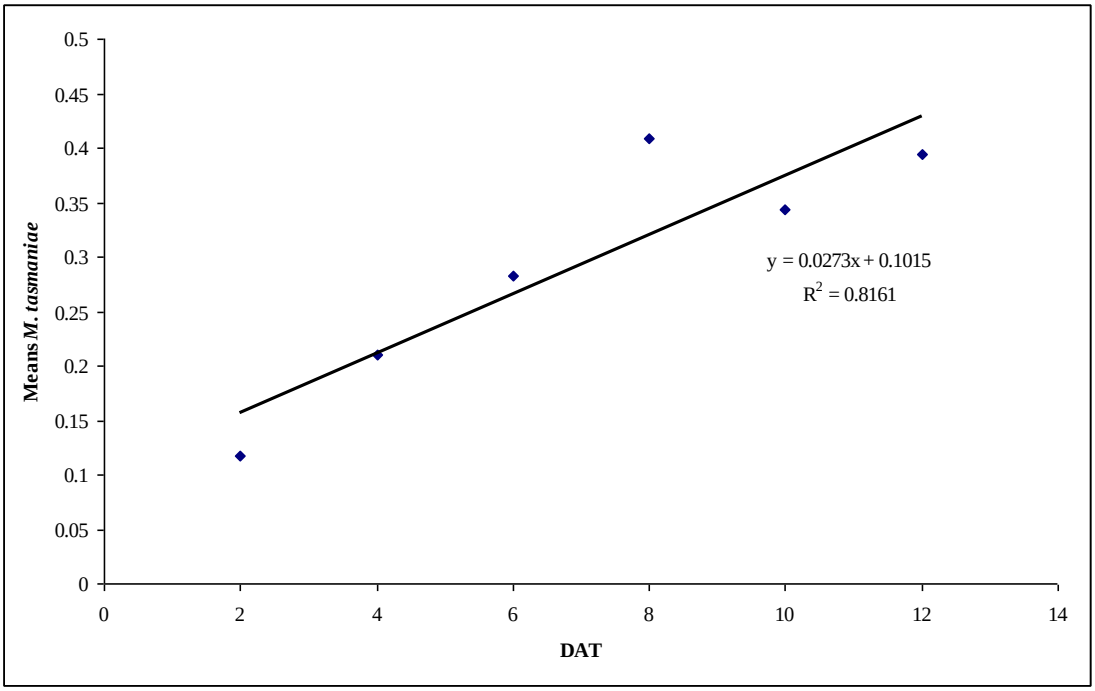


Figure 4. 5: Regression of time component of captured *M. tasmaniae*

4.3.3. *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 (Table 4.5). 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).

Table 4. 5: Means for trap positions to test edge effects of predator re-colonisation of an insecticide-treated brassica field, P=0.05

Replication (distance from long edge)	*H. variegata*		*M. tasmaniae*
1 – (10 m)	0.664		0.294
2 – (20 m)	0.550		0.262
3 – (30 m)	0.564		0.287
4 – (40 m)	0.540		0.326
Df		2879	
F-value	0.056		0.187
LSD	n.s.		n.s.

4.3.4. *Sticky trap colour and aspect*

Sticky trap data was analysed in a general ANOVA for Colour *Aspect*Direction. Colour was highly significant (Tables 4.6 & 4.7) 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 insect – 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.

Table 4.6: Means for trap parameters for *H. variegata* catches, P=0.05

	Colour		Aspect		Direction	
H. variegata	**Blue**	**Yellow**	**High**	**Low**	**Out**	**In**
Mean	[a] 0.212	0.947	0.572	0.587	0.603	0.556
df			2879			
F-value		<0.001		0.660		0.160
LSD		0.065		n.s.		n.s.

[a] represents a significant difference compared to yellow sticky traps

Table 4.7: Means for trap parameters for *M. tasmaniae* catches, P=0.05

	Colour		Aspect		Direction	
M. tasmaniae	**Blue**	**Yellow**	**High**	**Low**	**Out**	**In**
Mean	[a] 0.186	0.399	0.288	0.298	[b] 0.335	0.250
df			2879			
F-value		<0.001		0.609		<0.001
LSD		0.0399		n.s.		0.0399

[a] represents a significant difference compared to yellow sticky traps
[b] represents significant differences compared to insects 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 (Tables 4.8 & 4.9). 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*.

Table 4.8: Means for trap parameters interactions for *H. variegata* catches, P=0.05

		Colour*Aspect		Colour*Direction	
H. variegata		**Blue**	**Yellow**	**Blue**	**Yellow**
Aspect	**High**	[a] 0.239	0.906		
	Low	[a] 0.185	0.989		
Direction	**Out**			[a]0.181	1.025
	In			[a] 0.243	[b]0.869
df		2879			
F-value		0.038		<0.001	
LSD		0.0919			

[a] represents a significant difference compared to yellow sticky traps
[b] represents significant differences compared to insects moving out of the field

Table 4.9: Means for trap parameters interactions for *M. tasmaniae* catches, P=0.05

		Colour*Aspect		Colour*Direction	
M. tasmaniae		**Blue**	**Yellow**	**Blue**	**Yellow**
Aspect	**High**	0.179	0.396		
	Low	0.193	0.403		
Direction	**Out**			[a] 0.200	0.471
	In			[a] 0.172	0.328
df			2879		
F-value			0.864		0.005
LSD			n.s		0.0564

[a] represents a significant difference compared to yellow sticky traps

4.3.5. *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).

4.4. DISCUSSION

4.4.1. *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 the findings in Chapter 3 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. Chapter 5 will discuss the implications of competition between the two predators in more detail.

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. In Chapter 3 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. Reynolds and Riley (2002) have reviewed various techniques including visual, optical and acoustic methods amongst more complex methods, however, none of these would be easily applicable to small scale field movement. Loxdale et al. (2008) stated that "meteorological backtracking has also indicated long-distance movement, but the accuracy of such predictions is dubious unless the altitude of transport is known. 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.

4.4.2. *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 in Chapter 3, where both *H. variegata* and *M. tasmaniae* (both marked and total numbers) declined over time in the unsprayed crop. While the decline in marked insect numbers in Chapter 3 can be explained with the decline of marked specimen over time, the equivalent decline in unmarked insects cannot.

4.4.3. *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 (Bianchi, 2006; Tscharntke et al., 2007), 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, however, Collins et al. (2002) examined the role of beetle banks in aphid suppression in Britain and found that level of aphid suppression decreased with distance from the bank, but also that most of the aphid control was exerted by carabids, staphylinids and spiders without any records of coccinellids.

4.4.4. *Sticky traps colour and aspect*

Yellow sticky traps were the most effective colour for trap captures. Clare et al., (2000) tested sticky trap colour on pollinators (bees) and pome fruit pests (light brown apple moth and codling moth) in apple orchhards in New Zealand and found that white, blue and yellow traps caught most bees (in that order). Colour did not make a difference to the target insects since traps were also impregnated with pheromones which most likely overshadowed any colour preference. Spectral reflectance was highest for white, yellow and blue traps, followed by red and green. Wigglesworth (1972) suggested that insects have trichromatic colour vision and

primarily see yellow, blue and ultraviolet colours and their mixtures. 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.

4.4.5. *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. Chapter 5 will examine the dietary habits and the relationships between predators by using PCR analysis to determine what prey have been consumed by *H. variegata* and *M. tasmaniae*.

CHAPTER FIVE: DNA GUT ANALYSIS OF FIELD COLLECTED *Hippodamia variegata* AND *Micromus tasmaniae*

5.1. INTRODUCTION

The use of predators in insect pest management (IPM) is becoming more popular in agricultural production. However, for the efficient use of predators there is a need to accurately identify the dietary range of prey species that predators consume. Assessing the diet of predators can provide useful information about the contribution of different foods to the nutrition profile of the insect and the role of intraguild predation in insect communities (Weber and Lundgren, 2009).

Historically, the identification of insects was based on morphology and often required an expert taxonomist. The process was time consuming, especially when immature specimens had to be reared in the laboratory before identification (Maitland, 2007). While morphological taxonomy remains an essential aspect of insect taxonomy and identification of intact specimens it has important limitations such as it does not allow for the identification of gut contents from predators to determine their dietary range. Some of the techniques for identifying predator gut contents include direct observation, microscopic analysis of predator gut contents, development of prey-specific protein antibodies and prey-specific electrophoretic

analyses of predators including polymerase chain reaction (PCR) based assays (Hoogendoorn & Heimpel 2001). The advent of molecular techniques such as DNA barcoding (Herbert *et al.*, 2003) has enabled not only rapid and reliable species identification, but also identification of prey remnants in gut contents of predators by non-taxonomic researchers (Maitland, 2007). The sequence of the 5-half of the mitochondrial gene cytochrome oxidase I (COI) coding strand forms the basis of DNA barcoding in animals (Mitchell, 2008).

DNA barcoding involves five main steps:

- taxonomic identification of the insect specimen
- isolation of its genomic DNA
- amplification of the barcode region (≥500 bp) of COI by polymerase chain reaction (PCR) (where the use of shorter than full-length DNA barcodes increases amplification success)
- sequencing of the PCR product and
- sequence analyses to determine whether the barcodes are diagnostic for the species in question (Ivanova et al. 2007)

PCR-based assays are very sensitive in detecting minute amounts of DNA, since PCR amplifies DNA exponentially at the rate of 2^n, where n = number of PCR cycles; thus 1 copy of DNA after 35 PCR cycles could result in 2^{35} (34 billion) copies of a PCR amplicon (Saiki *et al*, 1988). Each cell contains 80-600 mitochondria, each of which contains 2-10 copies of the mitochondrial genome; thus mtDNA-based techniques are highly sensitive and can detect even picogram

amounts of template DNA. Mitochondrial DNA is therefore well suited for analyses of predator gut contents (Foltan et al. 2005; Juen and Traugott 2004; Symondson, 2002). However, the method cannot discriminate between primary and secondary predation, as prey DNA in the gut of a predator is potentially detectable within the gut of a predator that consumed the original predator (Sheppard et al., 2005)

Intraguild predation (IGP) occurs when one species in a predatory guild feeds on another predatory species within the guild (Lucas et al., 1998). Bampfylde & Lewis (2007) described IGP as the interaction between species that eat each other and compete for shared resources. IGP has been documented to occur across a wide range of taxonomic groups and ecosystems with particular reference to non-indigenous species and agricultural pests. Predatory insects such as coccinellids and neuropterans may become prey themselves. Pressure from intraguild predators affects the spatio-temporal distribution of coccinellids on many levels and affects their capacity as predators and their defensive and reproductive behaviour (Seagraves, 2009).

While intraguild predation is not necessarily symmetrical, some natural enemy species are more likely than others to be preyed on by coccinellids. For example anthocorids and predatory larvae of some Diptera as well as immature parasitoids often are consumed by coccinellids (Santi and Maini, 2006; Lucas et al., 1998; Gardiner and Landis, 2007; Snyder et al., 2004). In contrast, Pentatomids can prey on coccinellid larvae (Mallampalli et al., 2002) while neuropteran larvae were

reportedly not disadvantaged against similarly or smaller sized coccinellids (Lucas et al., 1998; Michaud and Grant, 2003; Santi and Maini, 2006; Gardiner and Landis, 2007). While intraguild predation of coccinellids may be prevalent in the absence of preferred prey species, eg if aphids are scarce, the nutritional value of their alternative prey may be insufficient to supply their dietary needs (Royer et al, 2008) and hence may reduce as their preferred prey species become available again. Another danger of intraguild predation is the presence of entomopathogens such as *Neozygites fresenii* (Nowakowski) which may affect the health and fitness of coccinellid species ingesting infected prey (Simelane et al., 2008) as well as reducing the effectiveness of the fungus on the target species by removing it from contact (Pell et al., 2008).

Since most of the studies cited in Weber and Lundgren's (2009) review of the trophic ecology of coccinellids were carried out in laboratories, they provide insight into the potential capacity of a predator in unnatural conditions and should be interpreted in that context. The authors point out that in the field, however, the parameters that govern insect survival and interactions include climatic and environmental variation, alternative food sources, insect activity levels and behavioural patterns, escape and avoidance strategies from other predators. Further, most of the field studies have concentrated on cropland rather than non-crop vegetation and indicated that intraguild predation can affect biological control in insect communities (Pell et al., 2008).

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 Chapter 2. 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 (Agusti et al.2003). 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.

5.2. Materials and Methods

5.2.1 Predator guts content analyses

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 (as described previously in Chapter 2). During any vacuum sampling, candidate applied gentle vacuum and directed vacuum nozzle to pick solitary predators (ie. not when they are among a mass of prey) both of which would minimize/eliminate contamination. 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.

5.2.2 *DNA extraction from reference specimens*

Genomic DNA was extracted from adult specimens of the two predators *Hippodamia variegata* and *Micromus tasmaniae* and the four pest species, *Plutella xylostella, Pieris rapae, Brevicoryne brassicae,* and *Nysius vintor*. DNA was also extracted from two coccinellids species, *Coccinella transversalis* and *Diomus notescens*, which were present in the study area and are found commonly in Central West NSW crops. Legs of individual insects were used for DNA extraction to avoid any contamination with extraneous DNA in their guts. DNA extraction was performed with a Qiagen DNeasy® kit (Qiagen, Doncaster, Australia) following the manufacturer's instructions.

A single insect leg was placed in a 1.5 ml microcentrifuge tube containing 180 µl of lysis buffer T and homogenized using a disposable microtube plastic pestle. A 20

µl aliquot of proteinase K (20 mg ml^{-1}) was added to the lysate and mixed thoroughly by vortexing. Digestion was performed at 55˚C overnight. A 200 µl aliquot of lysis solution C was then added to the digest, vortexed and incubated at 70˚C for 10 minutes. Following incubation, 200 µl of ethanol (95%) was added to the mixture which was then vortexed for 10 seconds. The mixture was transferred to a binding column, placed in a 2 ml collection tube and centrifuged at 13, 000 rpm for 1 minute. The column was transferred to a new collection tube and washed with 500 µl wash buffer AW1 and spun at 6k for 1 minute. It was washed again with 500 µl wash buffer AW2.

5.2.3. *DNA extraction from predators for gut content analyses*

Genomic DNA was extracted from field collected specimens (adults only) of the two predator species for gut analysis using the Corbett® DNA extraction kit. Whole insects were digested in 240µl digestion buffer containing 2.4µl Proteinase K (20 mg ml^{-1}) at 55˚C for 15 hours. The digest was spun at 5k for 5 minutes and 220µl of the supernatant was transferred to a lysis plate, which was then placed in a Corbett® CAS-1820 Xtractor Gene robotics system for DNA extraction.

A 440µl aliquot of binding buffer was mixed with the digest and 600µl was transferred to a column of a Whatman® capture plate. The liquid was drawn through the column by vacuum filtration leaving the DNA bound to the column glass fibres. The DNA bound to the column was sequentially washed with 200 µl

binding buffer, 2 x 600 µl propanol+binding buffer and 600 µl ethanol wash buffer, and eluted in 120 µl elution buffer.

Genomic DNA obtained from whole insect digests (which would also include gut DNA) of the two predator species were analysed by PCR using the species-specific primers developed to detect pests being consumed. For the detection of *N. vinitor* and *M. tasmaniae* the forward primers NvF and MtF, respectively, were used with the conserved reverse primer Scar3RDm (Table 5.1).

5.2.4. *Polymerase chain reaction (PCR)*

PCR was conducted in a reaction volume of 15µl containing 1.5 µl 10x PCR buffer, 2-3 mM Mg^{++}, 200 µM dNTP, 0.4 units Platinum *Taq* polymerase (reagents supplied by Invitrogen, Mt Waverly, Australia) 0.1 µM forward and reverse primers, and 3-6 µl template DNA. The PCR reactions were set up in 96-well PCR plates using a Corbett® CAS-1200 robot. Reaction conditions were optimised using gradient PCR. The PCR thermal profile consisted of an initial denaturation at 94°C for 1minute, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50-55°C for 30 seconds and extension at 72°C for 1 minute and a final extension at 72°C for 5 minutes.

5.2.5. *Gel electrophoresis*

PCR reactions were electrophoresed in 1.5-2% Tris-Acetic acid-EDTA (TAE) agarose gels pre-stained with SYBR safe DNA-binding dye. Electrophoresis was conducted in A MII-DEAR gel unit (Fisher Biotec, Subiaco, Australia) at 230 volts for 7-15 minutes. The size-separated PCR amplicons were visualised with a BioRad® gel imaging system.

5.2.6. *Species-specific PCR Primers*

Barcode sequences were obtained for the two predators *Hippodamia variegata* (n=3) and *Micromus tasmaniae* (n=3), the four main pest species *Plutella xylostella* (n=2)*, Pieris rapae* (n=2)*, Brevicoryne brassicae* (n=2) and *Nysius vintor* (n=7), and the coccinellids *Coccinella transversalis* (n=9) and *Diomus notescens* (n=8). PCR amplifications used degenerate primers targeting the ends of the barcode region (BC1Fm and Scar3RDm, Mitchell (2008)) and were performed using 3mM $MgCl_2$ and an annealing temperature of 50^{o}C. PCR products were sequenced in the forward and reverse directions to confirm their sequence.

The resulting 36 COI sequences were aligned using BioEdit v.7.0.9 (Hall, 1999) and species-specific primers were designed from visual comparison of the sequences, choosing a region showing least homology among species. For *H. variegata*, *M. tasmaniae* and *N. vinitor*, a species-specific forward primer was designed but the reverse primers used were the same degenerate primer for all

species (Table 5.1). For *P. xylostella, P. rapae* and *B. brassicae* we used the primers used by Hossaini (2007). $MgCl_2$ concentration and annealing temperature were optimized using gradient PCR with annealing temperatures from 50-60˚C and $MgCl_2$ concentration ranging from 1.5-3 mM. The optimised PCR conditions are given in Table 5.1.

Table 5. 1: mt*CO1* species-specific primers and optimized PCR conditions

Species	Primers	Mg^{++}	$T_{annealing}$	amplicon size
H.variegata	HvF	3mM	50˚C	258 bp
	Scar3RDm			
M.tasmaniae	MtF	3mM	50˚C	239 bp
	Scar3RDm			
N.vinitor	NvF	1.5mM	50˚C	257 bp
	Scar3RDm			
P. xylostella	DBM F2	3mM	50˚C	293 bp
	DBM R1-1	3mM	50˚C	
P.rapae	PR-F	3mM	55˚C	222 bp
	PR-R	3mM	55˚C	
B.brassicae	BBF1	3mM	55˚C	307 bp
	BBR	3mM	55˚C	

5.2.7. *Data Analysis*

Each predator species was analysed in a blocked ANOVA to test for spatial effects. Individuals that were not positive for any predation was removed from the analysis. Since the detections of prey species in the two predator species was unevenly distributed, REML analysis was used to show differences between the predators at different distances into the crop and in non-crop vegetation.

5.3. 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 (*Pl. xylostella, Pi. rapae, B. brassicae, H. variegata, M. tasmaniae* and *N. vinitor*) in their gut (Table 5.2). 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.

Table 5. 2: Summary of prey species detected in the gut contents of field collected *Hippodamia variegata* and *Micromus tasmaniae*.

	Prey					
Predator	*P. xylostella*	*P.rapae*	*B.brassicae*	*N. vinitor*	*M. tasmaniae*	*H. variegata*
H. variegata	+ve	+ve	+ve	+ve	+ve	no test
M. tasmaniae	+ve	+ve	+ve	no test (lost sample)	no test	+ve

5.3.1. *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 *Pl. xylostella*, 22.2% positive to *B. brassicae*, 21.3% positive to *Pi. rapae* and 7.4% positive *to N. vinitor* (Figure 5.1). Some of the tests did not show any positive results; 32.4% of *H. variegata* individuals none tested positive 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 5.3), 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.

5.3.2 *Micromus tasmaniae*

M. tasmaniae (76 specimens), was also shown to be a generalist predator with 50.7% positive detection to *Pi. rapae*, 35.60% positive to *B. brassicae*, 11.0% positive to *Pl. xylostella* (Figure 5.1) 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 5.3). 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 5.2 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*.

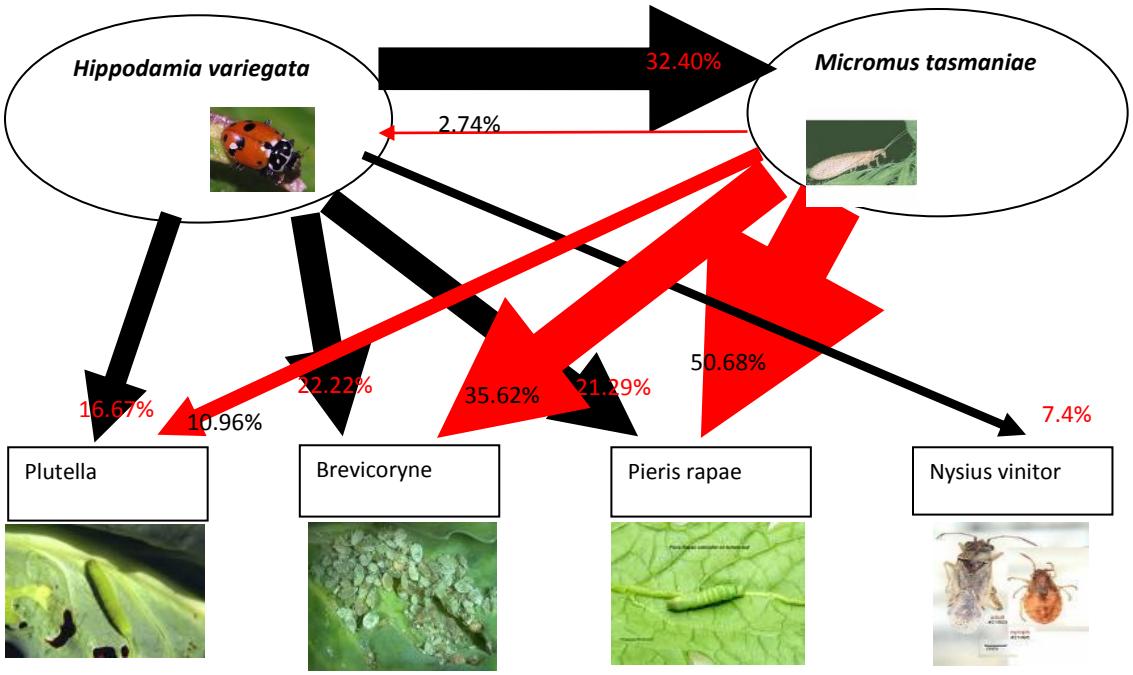


Figure 5. 1: Percentage detection of different prey species in field collected *Hippodamia variegata* and *Micromus tasmaniae*. Arrows indicate the percentage of prey detected in the two predators.

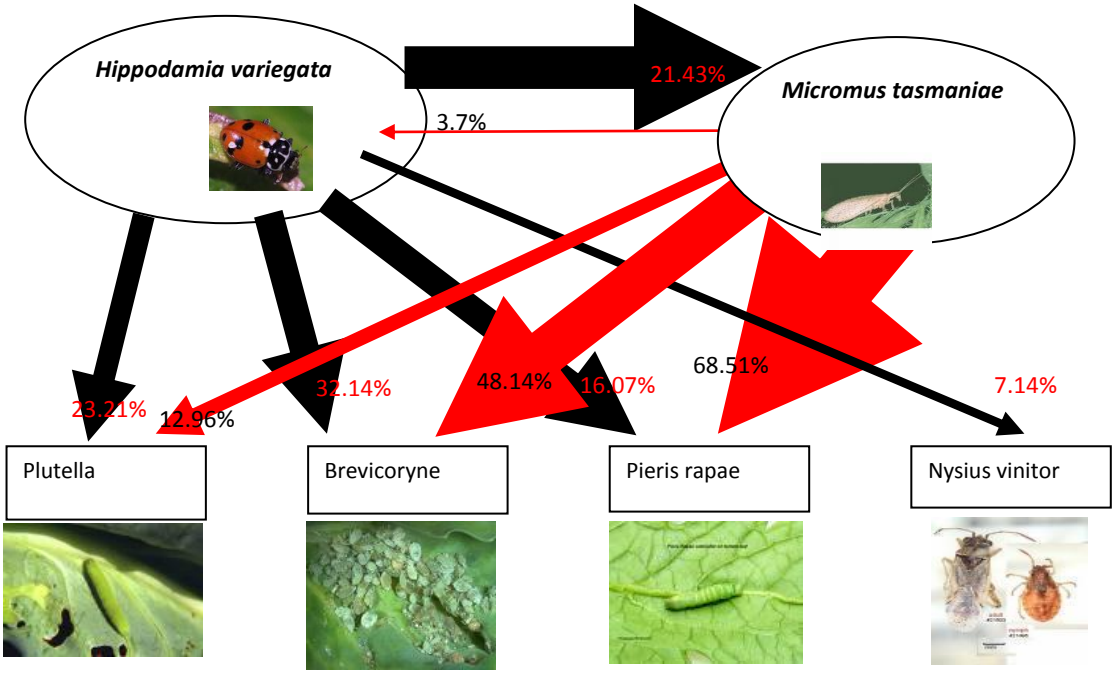


Figure 5. 2: Percentage detection of different prey species in field collected *Hippodamia variegata* and *Micromus* tasmaniae after the removal of double or triple positive detections of *H. variegata* or *M. tasmaniae* as well as negative detections. Arrows indicate the percentage of prey detected in the two predators.

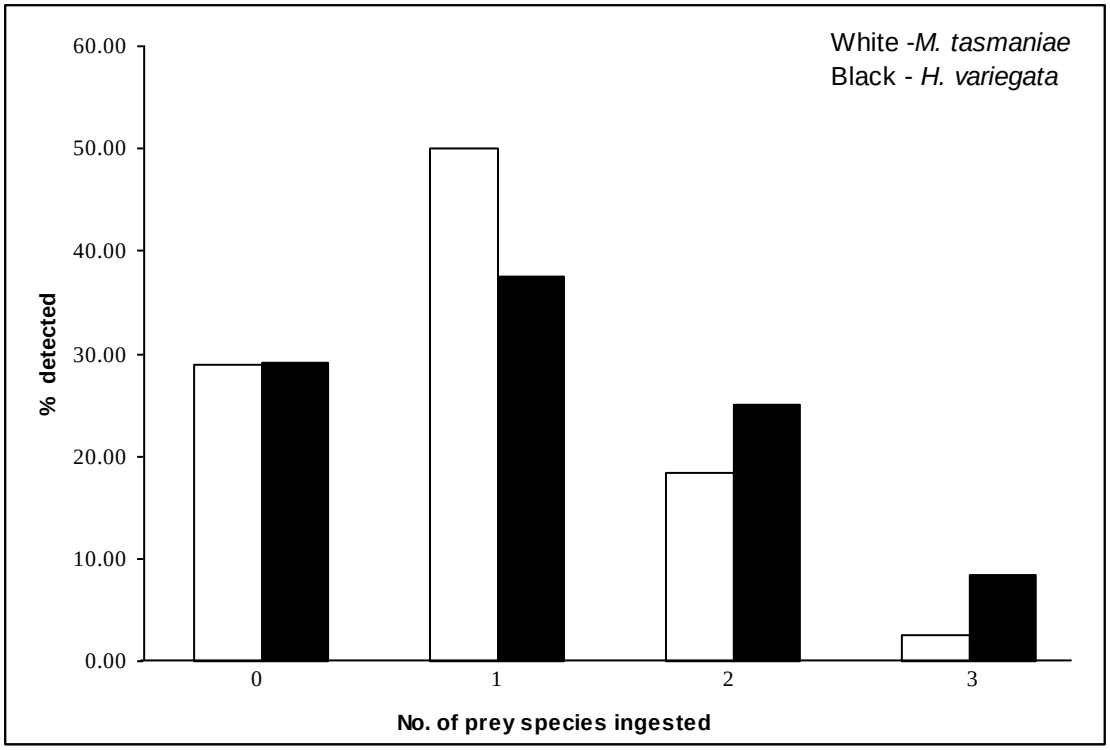


Figure 5.3: Positive detections for the number of prey species ingested by *H. variegata* and *M. tasmaniae*

5.3.3 *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 (Table 5.3). This spatial effect was not significant for other prey species.

Table 5. 3: ANOVA for *H. variegata's* PCR prey species mean detections over distance

	Prey species				
Distance	***P. xylostella***	***B. brassicae***	***P. rapae***	***N. vinitor***	***M. tasmaniae***
Non-crop	0.250	0.375	0.375	0.063	0.188
20m	0	0.312	0.250	0	0.500
40m	0.250	0.125	0.188	0.063	0.188
60m	0	0.312	0.125	0.125	0.438
80m	[a]0.375	0.250	0.438	0.063	0.562
100m	0.250	0.310	0.063	0.187	0.188
Df			95		
F-value	0.027	0.310	0.057	0.462	0.123
LSD	0.2636	n.s.	n.s.	n.s.	n.s.

[a] represents a significant difference compared non-crop

Table 5. 4: ANOVA for *M. tasmaniae's* PCR prey species mean detections over distance

	Prey species				
Distance	***P. xylostella***	***B. brassicae***	***P. rapae***	***N. vinitor***	***H. variegata***
Non-crop	0.500	0.833	0.500	0	0
20m	[a] 0	[a] 0.250	0.500	0	0
40m	[a] 0	[a] 0.250	0.583	0	0.083
60m	[a] 0	[a] 0.417	0.417	0	0
80m	[a] 0	[a] 0.083	0.333	0	0.083
100m	[a] 0.083	[a] 0.250	0.500	0	0
Df			71		
F-value	<0.001	0.002	0.722	0	0.426
LSD	0.1941	0.3506	n.s.	0	n.s.

[a] represents a significant difference compared non-crop

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* (Table 5.5). *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* (Table 5.4). *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.

Table 5.5. REML analysis of *H. variegata* and *M. tasmaniae* PCR prey species detections (df=158)

	Prey species					
F-value	***P. xylostella***	***B. brassicae***	***P. rapae***	***N. vinitor***	***M. tasmaniae***	***H. variegata***
Predator	0.059	0.171	**<0.001	*0.017	**<0.001	0.106
Distance	**<0.001	**0.004	0.309	0.443	0.120	0.453
Predator x Distance Interaction	*0.038	0.525	0.202	0.625	0.218	0.298
LSD (Predator)	n.s.	n.s.	0.0833	0.3989	0.6962	n.s.
LSD (Distance)	0.2205	0.2920	n.s.	n.s.	n.s.	n.s.
Predator Means						
H. variegata	0.1875	0.250	0.2306	0.0833	0.3646	0
M. tasmaniae	0.0737	0.317	[a]0.4579	[a] 0.0082	[a] 0	0.0278
Distance Means	**Non-Crop**	**20m**	**40m**	**60m**	**80m**	**100m**
P. xylostella	0.3125	[b]-0.0016	0.1234	[b]-0.0016	0.1859	0.1651
B. brassicae	0.5312	0.2777	[b] 0.1840	0.3611	[b] 0.1006	0.2465

[a] represents a significant difference compared to *H. variegata*

[b] represents significant differences compared to non-crop

5.4. DISCUSSION

5.4.1. *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.

In about 60% of detections, *B. brassicae, P rapae* and *P. xylostella* were part of *H. variegata's* diet. While such statistics appear to qualify *H. variegata* as a suitable biological control agent for brassica pests, one third of its detections constituted the predator *M. tasmaniae.*

In 97 % of detections, the diet of *M. tasmaniae* included brassica pests and very few *H. variegata*. *M. tasmaniae* showed a preference for feeding on *P. rapae*, (50.68% of detections) followed by *B. brassicae* (35.62% of detections).

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.

5.4.2. *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.* Research by Lucas et al., 1998; Michaud and Grant, 2003; Santi and Maini, 2006; Gardiner and Landis, 2007, determined that lacewing larvae were 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 (Chapter 2) 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 (Obrycki et al., 2009) 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.

5.4.3. *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 *Pl. xylostella* by *H variegata* where most *Pl. 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 *Pl. xylostella* detected in gut contents for the other distances in the crop. Since *Pl. xylostella* is a primary pest of Brassica crops, this may indicate movement of *M. tasmaniae* out of the crop after feeding, or that *Pl. xylostella* may also breed and survive on alternate weed hosts in non-crop areas. For both predators detections of *Pi. 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 the findings from Chapter 4 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 from Chapter 4 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 findings in Chapter 3 where *H. variegata* showed a distinct negative relationship with distance from the edge of the field.

Predator type was significant for the consumption of *Pi. rapae*, *N. vinitor* and *M. tasmaniae*. Significantly more *Pi. 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 has limitations, however, care had been taken to avoid or minimize these limitations. Feeding trials to determine how long after ingestion of a prey DNA from that prey could still be detected in predator guts were not done because

detection periods would be different for every primer pair and laboratory time granted was limited. Laboratory trials to see whether secondary predation was a significant factor were also limited by time and availability of the lab. All these type of trials would have been possible and useful in a more narrowly focused PhD. The need for such work in the future is now included in the Discussion on page 170 where future directions are dealt with.

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.

Further investigation to confirm and strengthen the results of this study would be recommended, especially to include a broader range of prey species and re-

evaluate the intra-guild predation relationship between the two predators before recommending their use as BCAs in a brassica insect pest management strategy.

CHAPTER SIX – GENERAL DISCUSSION

6.1. INTRODUCTION

Arthropod pests are an important aspect of Brassica production systems in Australia and many other parts of the world. Growers rely heavily on chemical control (Tran et al. 2004), however, the alternative use of biological control agents in an IPM system has become more attractive to growers. The exotic ladybird beetle, *H. variegata* and the native brown lacewing, *M. tasmaniae* were investigated as potential biological agents for the arthropod pests in this system. This study aimed to fill in some of the gaps in knowledge about the two predators such as habitat use, temporal population dynamics, diet range, intraguild predation and susceptibility to insecticides. Whilst the information collected from this study applied most directly to the Bathurst, CW NSW region, it also shed new light generally on the relationships between predators and prey within IPM systems.

6.2. Population dynamic and the impact of insecticides on the predators

The study showed that the exotic *H. variegata* was the dominant coccinellid species while the native *M. tasmaniae* was the dominant neuropteran in CW-NSW.

Both predators followed similar seasonal patterns with marked variation in numbers. Because both *H. variegata* and *M. tasmaniae* were common in brassica crops, especially in those planted in October (mid-season), they may have high potential as biological control agents. Significant numbers of *H. variegata* and *M. tasmaniae* were also found in non-crop habitats such as bushland, pastures and riparian areas showing the potential importance of such areas, often viewed as non-productive, as reservoirs for the predators in this farming system. Surrounding non-crop vegetation is generally acknowledged to directly benefit biological control agents by providing habitat and foods such as nectar and pollen. They may also support alternative prey and hosts in the case of parasitoids (White et al., 1995; Grez & Villagran, 2000; Marshall & Moonen, 2002; Langellotto & Denno, 2004). Moreover, the establishment and maintenance of suitable habitats on farm or surrounding landscape can enhance the survival of natural enemies (Gurr, 2004; Stephens, 2006) and in effect fence lines, ditches, hedgerows, windbreaks, weedy strips or riparian areas may serve as reservoirs or ecological corridors for *M. tasmaniae*, *H. variegata* and other natural enemies (McEwen et al. 2001). The structure, vegetation type and shape can have a direct effect on the density of beneficial arthropods in a cropping system (Andow 1990; Landis et al. 2000; Sunderland & Samu 2000). Further, Thies and Tscharntke (1999) found that enhanced populations of natural enemies immigrated to neighbouring crops and attacked insect pests and reduced their population below economic threshold. Therefore, brassica growers could potentially manipulate or conserve the non-crop

vegetation on and around their farms to enhance the population density of beneficial arthropods.

The low numbers of immature predators found in all habitats including the non-crop sites suggests that neither predator species reproduced extensively within crops or in any of the on-farm habitats sampled. It is therefore most likely that longer range immigration is the main source of predators to these farms. Despite this evidence for non-crop habitats being unimportant for predator reproduction the other benefits of such habitat set out above are likely to apply. The value of non-crop habitat as a source of adult predators will be especially important after a disruption to the within-crop community of natural enemies. Most commonly this will be caused by insecticide application.

Results clearly showed that an increased number of insecticide sprays (high BDI), decreased the number of predators, possibly for two reasons. Firstly, prey was being reduced forcing predators to feed elsewhere, and more importantly, predators were being killed by non-target effects. Spray regimes that gave a lower BDI showed significantly higher numbers of predators, especially coccinellids. Growers should therefore use insecticides with a lower BDI for their insect pest control and maximise the amount and proximity of non-crop source habitats that are not sprayed in order to attract more predators to their fields. To develop successful IPM strategies that include biological control agents, brassica farmers in the area will need to reconsider their spraying strategies and preserve non-crop habitats as refuge and breeding areas.

6.3. Movement of predators from non-crop vegetation to crops

To address the issue of predator movement, a mark–capture method was employed to establish whether movement of predators from non-crop to crop areas occurred and to determine the temporal and spatial movement of predators. Both *H. variegata* and *M. tasmaniae* moved from non-crop habitat to crops and their movement was affected by time and distance. This supported findings by Schellhorn et al., (2008) and Thomson and Hoffmann (2009), which showed that non-crop vegetation, increased the density of predators and their readiness to move into neighbouring crop fields. There was no differentiation in the present study between different non-crop vegetation types, bush, pasture and riparian areas. Geiger et al. (2009) stated that such areas served as good habitats for insects and therefore acted as reservoirs for arthropods. While there was high variability in the data, the experiment showed that *H. variegata* had a distinct negative relationship with time and distance from field edge. *M. tasmaniae* did not show this type of relationship, but moved into crops more evenly over time and distance indicating perhaps either a wider feeding range or greater vagility. *H. variegata* was more numerous than *M. tasmaniae* possibly due to intraguild predation by the former, a phenomenon explored in more detail below. Both predators moved into the field from surrounding vegetation demonstrating the advantage of smaller fields that may be subject to better biological control by predators. The experiment did not give a good assessment of long range movement but confirmed findings by Tscharntke et al. (2005) and Bianchi et al.

(2006) which showed that neighbouring non-crop vegetation enhanced the population of beneficial insects for crop pest control. Provided that brassica growers were given sufficient management guidance in the manipulation of non-crop areas on their farms and made more use of IPM compatible insecticides that have less impact on beneficials, they could increase their natural predation rates within their crops.

6.4. Repopulation of the crop field after insecticide application

The repopulation experiment in Chapter 4 confirmed the previous finding that non-crop vegetation was an important source of predators and that movement into the field occurred from surrounding vegetation. It also demonstrated that remnant croplands can act as an alternative environment for predators when fields are sprayed with insecticides and a source habitat for repopulation of the sprayed field. *H. variegata* repopulated to a greater extent from the cropland side than did *M. tasmaniae*, which tended to move back into the field from non-crop vegetation. Repopulation was relatively uniform over distance into the crop and, contrary to the mark-recapture experiment, increased over time. No edge effects were seen for the sticky traps indicating that fields were small enough to dissipate such effects which are commonly seen in larger fields (Tscharntke et al., 2007; Bianchi, 2006) where they need to be accounted for in experimental design. This experiment did not measure any parameters that would reliably indicate long range movement.

The best indication of such movement were obtained from the survey data in Chapter 2 which found few larval stages indicating that predators did not breed in the crop but moved into it to feed. Considering the frequent disrupting chemical treatments that are characteristic of horticultural fields, this result was not surprising. Of the two colours of sticky traps tested, yellow ones were more efficient in capturing both *H. variegata* and *M. tasmaniae.* Clare et al. (2000) found that bees were more strongly attracted to blue traps but made no mention of predators in their study. In this experiment, aspect of sticky traps was not significant and possibly traps were set too low to assess any higher flying insects. It would be prudent to increase the distance between high and low set traps for any subsequent experiments of this kind or, alternatively, use Malaise traps.

6.5. Prey range of predators

The molecular Chapter (5) is intended to serve as complementary information. It is hoped to give additional information on a topic that has not been researched extensively. Thus, it is a section of the PhD that provides useful, conclusive information on diet range and (with caveats) and further information on relative rates of predation by *H. variegata* and *M. tasmaniae*. DNA gut analysis of *H. variegata* and *M. tasmaniae* 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*. This, however, does not necessarily mean a definite preference for *M. tasmaniae*, since a *H. variegata* specimen that showed multiple positives for the consumption of different prey species could have consumed only a tiny portion of *M. tasmaniae* and much more of another species during the 48 hours prior to capture. Further, data from field collected predators should consider the detectability half-life for a single prey specimen (Greenstone and Hunt, 1993) because positive detectability of prey DNA in a proportion of tested predators alone does not reliably indicate the relative importance of that predator taxon (Greenstone and Shufran, 2003). Chen et al. (2000) gave the example of the aphid *Rhopalosiphum maidis* (Fitch) which had respective detectability half-lives of 3.95 hours in the green lacewing *Chrysoperla plorabunda* and 8.78 hours in the coccinellid *Hippodamia convergens*. Prey detectability half-life was not tested in this study.

The asymmetrical intraguild predation between *H. variegata* and *M. tasmaniae* could indicate a more complex relationship between these predators in the field. For example, if the numbers of *H. variegata* in a particular location were high, then the numbers of *M. tasmaniae* in the same location could be affected unless there was a numerical aggregative response by one or both of the predators. An

aggregational response is characterized by an increase in predator numbers which is due to an increase in prey density (Holling, 1959). This type of response is desirable in predators selected for biological control so that they are able to suppress prey populations and stabilize a spatially distributed predator-prey system (Sharov, 1996). If such a response is assumed for both predators, then *M. tasmaniae* attracted to *B. brassicae* prey would increase in numbers and then in turn attract *H. variegata*. However, *H. variegata* is also a predator of *B. brassicae* so it would be difficult to determine whether it was attracted to the high density of aphids or the increased density of lacewings. Crawley (1975) considered models of the numerical responses of coccinellid predators on aphids and found that predator abundance was influenced by factors other than prey density. If predators were not food limited, then the aggregated response relationship would not necessarily apply to them. However, even if they were food limited, population growth could be independent from the number of ingested prey and be influenced by such functional factors as maximum egg quotas laid by females, starvation tolerance and larval survival mechanisms. This illustrates that more studies on the relationships and interactions between *H. variegata*, *M. tasmaniae* and their prey species are required before their effectiveness as biocontrol agents can be asserted.

Considering the relatively high detection rate of the native predator *M. micromus* in *H. variegata,* it could be surmised that the latter species may also impact on other beneficial insects including native coccinellids to varying degrees. Mizell (2007)

reported on the impact of the introduced coccinellid *Harmonia axyridis* (Pallas) on the local predator fauna in Florida crape myrtles and pecan nut plantations 8-9 years after its arrival in the U.S. *H. axyridis* was first detected in Florida in 1993 feeding on crape myrtle aphids (*Sarucallis kahawaluokalani* (Kirkaldy)) but moved rapidly into pecan plantations where it effectively reduced populations of the yellow pecan aphid complex. Samples collected in 2001 were compared to baseline data from 1984 (Mizell and Schiffhauer, 1987; Tedders, 1978), where common aphid predators found in samples included five coccinellid species (*Hippodamia convergens* (Guerin-Meneville), *Olla v-nigrum* (Mulsant), *Coleomegilla maculata* (DeGeer), *Cycloneda sanguinea* L. and *C. munda* (Say)), three Neuroptera species (*Chrysoperla rufilabris* (Burmeister), *Micromus posticus* (Walker) and *Hemerobius stigma* (Stephens), a mirid (*Deraeocorus nebulosos* (Uhler), two reduviids (*Zelus exsanguis* (Stahl) and *Sinea spinipes* (Herrich-Schaeffer)), two syrphids (*Allograpta obliqua* (Say) and *Mesograpta* sp.), an anthocorid (*Orius insidious* (Say)), many spiders as well as the parasitoid *Aphelinus perpallidus* Gahan. By 2001 crape myrtle aphid numbers were greatly reduced and chemical sprays for pecan aphids declined from 5-8 applications per season to 0-3 applications per season. Of the native predator species virtually all had disappeared, even in the absence of *H. axyridis* at some sample locations. The two reduviids and spiders were still present in detectable numbers while green and brown lacewings were recorded only occasionally in pecan. Eubanks (2001) reported that these species were likely to engage in intraguild predation with *H. axyridis*. Further, the previously common parasitoid *A. perpallidus* was severely affected with no parasitized aphid mummies

observed during the 2001 study (cf. 120 parasitoids/sample in 1984). In 1987 Mizell and Schiffhauer touted crape myrtle as a plant that would potentially provide a suitable habitat for *H. axyridis*, which –at the time- was thought to be a good generalist predator to augment the native predator complex. By 2001, however, it had become clear that, despite the benefits of reduced aphid populations and associated chemical applications, *H. axyridis* had a putative negative effect on native predators and parasitoids and their range of behaviours, biologies and life histories, and consequently their importance in wider food webs.

Secondary predation skewed the data by 30% and this illustrates the importance of taking this effect into consideration when interpreting field data in DNA studies of trophic relationships. In this study predators commonly ingested only one prey species but up to three were detected in a few instances. While DNA analysis of predator gut contents has been used for various reasons such as the estimation of time from ingestion (Hogendoorn and Heimpel, 2001), the detectability of fresh or carrion prey (Juen and Traugott, 2005) or prey specificity (Greenstone and Shufran, 2003), only one other reference regarding the effect of habitat manipulation on field biological control as detected through predator gut analysis has been found in the literature. Szendrei et al. (2009), accepted for publication but as yet unpublished) assessed the gut contents of various predators preying on Colorado potato beetle (CPB) (*Leptinotarsa decemlineata*) in either tilled or mulched (rye or vetch) potato fields. They found that mulched crops had lower pest numbers (recorded data) and lower detections of prey in predator guts. However,

the lower number CPB larvae in mulched plots was not due to higher predator numbers or increased feeding since predator species abundance and diversity and prey consumption rates could not be accounted for by habitat complexity treatments. Predator gut content analysis showed highest positive pest detections in tilled plots where prey abundance was the highest. Therefore there was a direct effect of the mulch treatment on the pest species followed by an indirect effect of pest species abundance on predator feeding. The most abundant predator species was *Coleomegilla maculata* but it also had the lowest prey detections, while *Podisus maculiventris, Perillus biocalculatus* and *Lebia grandis* were less abundant but had higher prey detection rates. When the authors adjusted the proportions of positive prey detection in each of the predators according to how fast they digested their prey (detection half-life) they were able to rank the predators by their importance and found that the two predatory stink bugs (*P. maculiventris* & *P. biocalculatus*) and the carabid beetle (*L. grandis*) stood out as the most important predators of *L. decemlineata*.

The study described above (Szendrei et al. (2009) used DNA analysis of predator guts to determine the importance of crop habitat manipulation on pest predation and predator ranking. In contrast, the present project used the technique to determine prey range and looked for the first time at intraguild predation between two predators - one introduced the other native. It is important to understand the feeding relationships between different predators that share common prey species so that realistic estimates of their efficacy and interaction in the field can be made.

As Mizell's (2007) report on *H. axyridis* illustrated, the loss of native predator species by introduced predators – either because of displacement, competition or intraguild predation- can be ecologically disastrous and irreversible. Further, this study differed in that it compared non-crop vegetation to crops rather than different crop treatments. Mulched fields would not provide the same alternative prey species and food sources, shelter and absence of chemical sprays for beneficial species that non-crop vegetation can provide.

Vegetation effects did not stand out in the DNA- based diet investigation except for *P. xylostella* which was consumed by *M. tasmaniae* in higher numbers in non-crop vegetation. This could indicate in-crop feeding and subsequent movement by *M. tasmaniae*, or *P. xylostella* presence on alternate weed hosts in non-crop vegetation and subsequent movement of *M. tasmaniae* into the crop in search of more prey. The only difference between predator species that stood out were the effect of *M. tasmaniae* on *P. rapae* (50-68% of its diet) and *H. variegata* on *M. tasmaniae* and *N. vinitor* (21-32% of its diet and no competition by *M. tasmaniae* for *N. vinitor*).

The study showed which prey species were consumed by *H. variegata* and *M. tasmaniae* but could not account for feeding patterns, opportunism and behaviour or prey preference. It essentially provided a snapshot of what can happen under field situations and as such, this study has resulted in a far more realistic

investigation of predator prey relationships in crops than any laboratory food preference study could have done.

6.6. Conclusion

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.

The results reported herein do not offer all the necessary background information needed for a predator's use as a BCA, however, they have provided answers to some of the important questions: both predators are spatially and temporally present in numbers in crops and non-crops all year in the region; both predators move from non-crop to crop and feed on major pests, and intraguild predation occurs between the two predators to dramatically different extents. 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.

Therefore the survey carried out in this project provides important ecological data of the status of *H. variegata* in cropping systems and environment. While not providing baseline data, it gives an insight into the ecological impact that *H. variegata* has made over the last 8-10 years and will be useful for comparison to future monitoring.

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APPENDIX One:

Table 1: Summary of survey schedule of d-vac sampling 2006-2007. Note that not all crops and planting times (early, medium, late) were surveyed each month. The numbers under the vegetation types indicate the total number of months in which each particular type was sampled. At each sampling event, the sample size in each crop was a 1*20m suction sample.

Site	Sampling schedule	Vegetation type											
		Crop									Non-crop		
		Broccoli			Cabbage			Cauliflower			Bushland	Pasture	Riparian
		Early	Med	Late	Early	Med	Late	Early	Med	Late			
1	Monthly Sampling From 21st Sept. 2006 to 21st July 2007				5	5	5	3	4	5	12	12	12
2					5	5	4	4	5	5	12	12	12
3					4	5	5				12	12	12
4								3	4	6	12	12	12
5					4	5	5	3	5	5	12	12	12
6		3	5	7	5	6	7	3	5	7	12	12	
7					5	5	5	5	5	6	12	12	12
8					5	5	6	5	5	7	12	12	12
9					5	5	4	4	5	4	12	12	12
10		4	5	4	4	6	3				12	12	12
11		4	5	5	4	6	7	3	4	6	12	12	
12		4	5	4	4	6	8	4	5	6	12	12	