Interactions between *Plutella xylostella*, *Diadegma semiclausum* and some generalist predators of Brassica crops

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Abstract

While much is known about the diamondback moth, *Plutella xylostella* (L.) (Plutellidae) (DBM), the most important pest of brassica crops worldwide, there is little understanding about the dynamics of predatory invertebrates in brassica systems. Thus, the main objective of this work was to identify and study some interactions that occur among the parasitoid *Diadegma semiclausum* Hellen (Ichneumonidae), some commonly found predators and DBM, which may impact on biological control of this pest.

Some novel key interactions were identified. First, predation of early DBM instars by *Coccinella undecimpunctata* Linnaeus, *Coccinella transversalis* (Fabricius) (Coccinellidae) and *Micromus tasmaniae* (Walker) (Hemerobiidae) was verified, even in the presence of *Myzus persicae* Sulzer (Aphididae). However, consumption decreased with the increasing availability of the aphid, suggesting these predators may display a low but consistent consumption of DBM, which may increase in periods of scarcity of alternative prey. Second, this study elucidated the modification in the behaviour and movement of larval DBM caused by *D. semiclausum*. Despite coincidental intraguild predation on the parasitoid, an increase in predation on DBM was observed when *D. semiclausum* and either of two hemipteran predators, *Oechalia schellenbergii* Guérin-Méneville (Pentatomidae) or *Nabis kinbergii* Reuter (Nabidae), coexisted. This probably resulted from the higher movement rate of DBM in the presence of *D. semiclausum*, which made it less cryptic. And third, it was observed that DBM larvae parasitised by *D. semiclausum* became more vulnerable to predation by *C. transversalis*, probably as a result of the specific hunting and attacking mode of this predator.

These results indicate that among the predatory species studied the predatory bugs and parasitoids may have a synergistic interaction that enhances biological control. Only coccinellids might disrupt biological control. However, their low level of predation on DBM and preference for aphids in the field would make this unlikely.

Although the short-term experiments reported in this thesis were conducted under laboratory conditions, important mechanisms resulting from the interaction between DBM larvae, alternative prey, a larval parasitoid, and generalist predators have been identified. Understanding the impact of these mechanisms under real crop conditions and in the long-term will help developing sustainable pest management strategies in Australian vegetable crops.

Declaration

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institutions. To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except where due reference has been made in text.

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Chapter 1 General Introduction

All terrestrial communities based on living plants are composed of at least four interacting trophic levels: plants, herbivores, natural enemies of herbivores, and decomposers (Price *et al.* 1980). Research on biological control has historically focused on simple vertical trophic interactions among these levels (Cardinale *et al.* 2003; Brodeur and Boivin 2006), considering only one enemy species or different enemies, but one at a time (Cardinale *et al.* 2003). However, in the last three decades, studies have been extended to multi-trophic interactions (Brodeur and Boivin 2006) showing that the effect of natural enemies acting together on the target prey population can be quantitatively and qualitatively different from the impact of each species acting on its own, with a range of outcomes for biological control (Rosenheim *et al.* 1995; Riechert and Lawrence 1997; Sih *et al.* 1998; Losey and Denno 1998a; Schellhorn and Andow 1999; Symondson *et al.* 2002; Cardinale *et al.* 2003). This happens because food webs in most ecosystems range from a few to hundreds of species, with high connectance and omnivory, which creates varied and multidirectional links to other species in the same or different trophic levels and in the same or different strata in the habitat they share. Thus, most consumer populations are linked to multiple resource populations that can occur at different trophic levels (Polis and Strong 1996). And because insect pests normally have a patchy distribution, natural enemies congregate in these areas of high resource density establishing multiple and complex interactions (Schellhorn and Andow 1999)

Historically, biological control has mainly concentrated on classical strategies with specialists, commonly parasitoids, in part because their dynamics and those of the target prey are closely linked (Symondson *et al.* 2002). However, more recently experts are also considering the use of generalist predators as biological control agents. They present desirable characteristics, such as their ability to quickly establish populations in annual crops, which are highly disturbed ecosystems (Rosenheim *et al.* 1999; Symondson *et al.* 2002). Conversely, specialists are more vulnerable and likely to disappear following the natural population oscillations of the prey and due to periodical disruptions in the form of harvesting, rotation and cultivation of the crops and the application of agrochemicals (Symondson *et al.* 2002).

Due to their polyphagous nature generalist predators can persist in the crops, or in surrounding patches or fields, feeding opportunistically on many types of prey, and therefore their population dynamics do not rely on any particular prey species (Polis and Strong 1996;

Limburg and Rosenheim 2001; Symondson *et al.* 2002; Colfer *et al.* 2003; Madsen *et al.* 2004). Specialists may take a long time to arrive and establish in the crops early in the season when pest densities are still low, while generalists may be already present subsisting on non-target or non-preferred prey. The presence of generalist predators may help suppressing the pest early, delaying or preventing the rapid growth phase that results in a serious pest attack, and giving a background control before specialists cause substantial mortality (Sabelis 1992; Symondson *et al.* 2002). And when conditions change, generalist predators can switch rapidly to an emergent food resource, such as herbivores reinvading the crops (Polis and Strong 1996; Symondson *et al.* 2002). However, generalist predators can also engage in interactions that may reduce the effectiveness of biological control. For example, intraguild predation occurs when two heterospecific predators share a given host and also engage in some sort of trophic interaction (predation) (Rosenheim *et al.* 1995). Their trophic interactions with other predators can interfere with effective biological control (Snyder and Ives 2001).

The work presented in this thesis focuses on some multi-species interactions among generalist predators commonly found in brassica crops, the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and a key larval parasitoid of this lepidopteran pest, *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) (Figure 1-1).

DBM is the most important insect pest of brassica crops worldwide (Shelton *et al.* 1988; Muckenfuss *et al.* 1992; Talekar and Shelton 1993; Shelton 2001), consuming all brassicaceous crops and weeds (Barker *et al.* 2001), with weeds sustaining DBM populations in periods when crops are absent (Talekar and Shelton 1993; Sayyed *et al.* 2002). Besides high voracity and reproductive potential this species has a broad geographical distribution (Talekar and Shelton 1993). The effects of DBM infestation in crops can vary from a reduction in yield or quality, to making the crops unmarketable with losses close to 100% when no control is undertaken.

The control of DBM relied for several decades on the use of chemical insecticides (Talekar and Shelton 1993). This has resulted in serious negative consequences such as insecticide residues in crops toxic to human health and the environment and disruption of the natural enemy complex of insect pests (Shelton 2001). Besides, DBM has an enormous capacity to develop resistance to insecticides (Talekar and Shelton 1993) and several studies have shown that populations of DBM around the world have developed resistance to all major groups of insecticides (Feng *et al.* 2001; Heisswolf and Bilston 2001; Liu *et al.* 2001; Shelton 2001; Sivapragasam 2001; Walker *et al.* 2001). Therefore, the need to find alternative solutions for

the control of this serious pest has been a high priority for field entomologists around the world. This has led to the development of integrated pest management programs including non-insecticidal methods, such as biological control, in order to reduce the pest status of DBM in brassica crops (Lim 1986; California 1987; Shelton *et al.* 1988; Talekar and Shelton 1993; White *et al.* 1995; Heisswolf and Bilston 2001; Liu *et al.* 2001; Löhr 2001; Sastrosiswojo *et al.* 2001; Sivapragasam 2001; Walker *et al.* 2001; Sayyed *et al.* 2002; Furlong *et al.* 2004; Hamilton *et al.* 2004).

While much is known about the life history of *P. xylostella*, there is not much understanding of its predators in terms of species present, abundance, seasonality, basic biology and ecology. Likewise, interactions among them and with parasitoids and other fauna present in brassica crops have not been thoroughly studied. Although the processes that influence predatory activity are mainly unknown, there is evidence that predation can be extremely significant, having a big impact on DBM populations (Furlong *et al.* 2001; Furlong *et al.* 2004; Wang *et al.* 2004). For example, predatory activity accounted for 2 to 85% of the mortality of DBM in a study in Australia (Furlong *et al.* 2001) and 90% in a study outside Australia (Ullyet 1947). In South Carolina, more than 20 predatory species were found in collard fields with 42 and 72% mortality of DBM eggs and larvae respectively, attributed to the action of these predators (Muckenfuss *et al.* 1992).

A rich local arthropod fauna, including pests and natural enemies that interact in food webs, has been recorded in brassica crops around the world. Some of these species are non-native but are already established, becoming part of the local system (Oatman and Platner 1969; Lim 1986; California 1987; Alam 1992; Muckenfuss *et al.* 1992; White *et al.* 1995; Flint and Dreistadt 1998; Kirk *et al.* 2001; Löhr 2001; Walker *et al.* 2001; Wu *et al.* 2003; Furlong *et al.* 2004; Wang *et al.* 2004). For example, in South Australia there are at least six brassica pest species and numerous predatory arthropods from at least three classes, seven orders and 18 families (Hosseini 2007). And in Pukekohe, New Zealand predatory species have been recorded in brassica crops from at least two classes, six orders and ten families (Walker[1] 2005, personal communication). In addition, in Australia there are about 20 species of parasitoids (Waterhouse and Sands 2001), the most important being *D. semiclausum*, *Diadegma rapi* (Cameron), *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), *Apanteles ippeus* Nixon and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) (Wilson 1960; Goodwin 1979; Sarfraz *et al.* 2005). *Diadegma semiclausum* is the most common parasitoid species in South Australia and has been successfully used in management programs. This parasitoid is also

considered as a model species for biological control studies. All these arthropod natural enemies are a valuable resource for growers that can contribute to the control of pests in their crops.

Figure 1-1 Adult diamondback moth (aprox. 12 mm long) (top left), larval DBM (aprox 7 mm long) (top right) and adult female *D. semiclausum* (aprox. 8 mm long) (bottom).

In brassica systems there are opportunities for a variety of interactions among DBM, other herbivores and natural enemies, and these interactions and how they impact on DBM populations are largely unknown. The factors that affect predatory activity on DBM need to be better understood for improved biological control in brassica crops. In order to have a thorough understanding of the system and for successful biological control, it is critical to identify the important predators in the system and elucidate the nature and strength of interactions among them (Losey and Denno 1998a; Schellhorn and Andow 1999; Symondson *et al.* 2002; Denno and Finke 2005). Few studies have disentangled the roles of each group of natural enemies in these interacting communities, but already it is becoming clear that interactions can have negative as well as positive implications for biological control, and that the net effect of the interactions can vary with crop and season (Symondson *et al.* 2002).

Given that there is limited knowledge about the interactions between natural enemies that attack DBM, especially those that include predators, the overarching objective of this project was to identify and study some interactions that occur in brassica systems, which may impact on DBM populations. Chapter 2 examines whether three common predators found in brassica crops feed on DBM. When assessing the potential of biological control agents, it is necessary to find out the voracity and feeding preference of predators (Lucas *et al.* 1997), so this chapter also evaluates the effect of the presence of an aphid as alternative prey on the predation of DBM by these generalist predators. In chapter 3, two experiments were conducted considering the escape behaviour of DBM. The first evaluates how the presence of the parasitoid *D. semiclausum* affects behaviour and movement rate by DBM, and the second evaluates the outcome of the multi-species interaction in a system composed of DBM larvae, *D. semiclausum* and the hemipteran predators *Oechalia schellenbergii* Guérin-Méneville (Pentatomidae) or *Nabis kinbergii* Reuter (Nabidae). Chapter 4 evaluates whether parasitism by *D. semiclausum* influences predation of larval DBM by three generalist predators, the hemipterans *N. kinbergii* and *O. schellenbergii,* and the coccinellid *Coccinella transversalis* (Fabricius) (Coccinellidae). And finally Chapter 5 reviews and integrates the main findings of this work. It also presents a general discussion on the contribution these results should have on future research on the role of generalist predators on *P. xylostella*. Understanding these impacts could be important in the development of sustainable management strategies for this pest.

Chapter 2 Feeding voracity of *Coccinella undecimpunctata*, *Coccinella transversalis* and *Micromus tasmaniae*: impact on DBM

2.1 INTRODUCTION

In agro-ecosystems there is a wide variety of food types that generalist predators can utilize. As many generalist predators are omnivorous and feed on both animal and plant-based food (Eubanks and Denno 2000a; Harmon and Andow 2002; Wäckers and Fadamiro 2005; Lundgren 2009), it is unlikely that a generalist predator will have a strong interaction with only one prey species (Harmon and Andow 2002).

Many authors agree that the polyphagous nature of generalist predators can result in the partial or total rejection of a target pest in favour of other preferred available prey (Koss *et al.* 2004; Harwood and Obrycki 2005), affecting pest consumption rates in the field (Eubanks and Denno 2000b; Harper *et al.* 2005) and reducing their capacity for effective biological control. For example, Koss *et al* (2004) and Hazzard and Ferro (1991) found that in laboratory experiments the impact of the predators *Geocoris* spp (Hemiptera: Lygaeidae) and *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), respectively, on the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Crysomelidae) was disrupted in the presence of the green peach aphid (GPA), *Myzus persicae* Sulzer (Hemiptera: Aphididae), as both predators preferred to feed on aphids, and switched from eating Colorado potato beetle eggs. Similarly, the increased density of non-pest collembolan prey due to added compost in cucurbit crops decreased the consumption of herbivores by wolf spiders and carabid beetles even though the density of these predators was increased due to the abundance of alternative prey (Halaj and Wise 2002). Also, Gavish-Regev *et al.* (2009) observed that the density of additional collembolan prey reduced aphid predation by erigonid spiders in wheat fields.

On the other hand, the consumption of different food, especially arthropod prey, allows generalist predators to subsist on these sources of food when the target pest is not abundant (Settle *et al.* 1996; Harwood and Obrycki 2005). Thus, the presence of other prey early in the season should allow predators to colonize habitats prior to the arrival of a target pest, or

before this becomes abundant (Settle *et al.* 1996; Harwood and Obrycki 2005), and to remain in the fields despite fluctuations of the pest population density during the crop season (Harmon and Andow 2002).

The effects of predator-prey interactions on biological control are dynamic and there may be a time lag before they effectively influence the target pest population. Harmon and Andow (2002) argue that, although the use of multiple resources may make generalist predators more beneficial, this behaviour may also be an immediate complicating factor in understanding their effectiveness. Furthermore, Holt and Lawton (1994) suggest that when determining whether other available prey improves or reduces biological control, the time scale being considered might be important, because in the short term it might distract generalists from feeding on target pests and weaken biological control, but in the long term, if it enhances predator density, biological control could be improved.

Figure 2-1 The role of non-pest prey in regulating mechanisms of biological control by generalist predator populations (Harwood and Obrycki 2005).

In a similar analysis Harwood and Obrycki (2005) (Figure 2-1) propose that the result of consuming non-pest species by generalist predators can have different outcomes. On one

hand, feeding upon nutritious non-pest food items generally enhances fecundity, and improves predator population growth. However, the authors suggest that the presence of this food, especially during times when pest regulation is required, may also reduce the level of pest consumption per individual predator. On the other hand, an increased density of natural enemies can counteract this reduction in pest consumption and exert significant levels of biological control.

There is evidence that the process of prey selection by a predator can be influenced by one or more factors, including nutritional quality (Madsen *et al.* 2004), productivity of the predator population (Venzon *et al.* 2002), palatability (Bilde and Toft 1994), availability of prey (Del Bianco and Conde 2001), mobility (Eubanks and Denno 2000b), hunger level (Stephens and Krebs 1986) and prey defensive behaviour (Roger *et al.* 2000), among others. According to Lang and Gsödl (2001) prey preference consists of two behavioural elements, not necessarily mutually exclusive: active choice (i.e. selectivity of the predator among prey differing in nutritional value or profitability) and passive selection (i.e. prey differing in vulnerability, which determines the outcome of encounters with predators).

Based on the nutritional needs of predators, food can be classified as essential (i.e. those that support both immature growth and development, and adult reproduction) or alternative (i.e. those that serve only as a source of energy and nutrients to maintain the predator, but do not permit development or reproduction) (Evans *et al.* 1999; Cabral *et al.* 2006). For instance, none of the cereal aphids *Metopolophium dirhodum* (Wlk.), *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) allowed development of the linyphiid spider *Erigone atra* (Bl.) either in single or mixed-species diets, while fruit flies *Drosophila melanogaster* (Meig) (Diptera: Drosophilidae) sustained egg production and hatching and survival of spiderlings until adulthood (Bilde and Toft 2001). Similarly, a single-species diet of either *Aphis fabae* Scopoli or *My. pesicae* supported development to adult and reproduction of *Coccinella undecimpunctata* Linnaeus (Coleoptera: Coccinellidae), whereas survival of larvae of this coccinellid fed only *Aleyrodes proletella* L. (Hemiptera: Aleyrodidae) was extremely low (Cabral *et al.* 2006). Likewise, spiderlings of *Schizocosa* sp. (Araneae: Lycosidae) fed *Tomocerus bidentatus* Folsom (Collembola: Tomoceridae) sustained the highest overall rates of survival, growth, and development. However, when fed on single-species diet of either the collembolans *Folsomia candida* Willem, *Isotoma trispinata* Mac Gillivray, or the aphid *Aphis nerii* Boyer de Fonscolombe, the spiderlings did not grow and died without moulting (Toft and Wise 1999). Also, Eubanks and Denno (2000b) observed that when fed eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), the predatory bug

Geocoris punctipes (Say) completed development and reached adulthood, which could not be achieved when fed the aphid *Acyrtosiphum pisum* (Harris).

However, non-essential foods may supplement essential foods when consumed together in a mixed diet, which can enhance larval growth or adult reproduction of predators (Evans *et al.* 1999). For example, *Coccinella septempunctata* L. and *C. transversoguttata* Brown (Coleoptera: Coccinellidae) fed on essential prey, the aphid *A. pisum* and an alternative prey, the alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), produced significantly more eggs than when consuming only the aphids, and did not produce any eggs at all when consuming only weevils (Evans *et al.* 1999). Similarly, a mixed-species diet of *My. persicae* and *A. fabae* was more advantageous than a single-species diet for the fecundity and fertility of the coccinellid *Harmonia axyridis* (Pallas) (Soares *et al.* 2004). Also, Harwood and Obrycki (2005) found that spiderlings of *Erigone autumnalis* (Emerton) could not survive to adult on single-species diets of the alfalfa pest species *A. pisum* or *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae). However, when provided with non-pest Collembola or Diptera, given in single-species diet or as part of a mixed diet, most spiders survived to adult.

Besides the nutritional suitability of prey, other factors that may influence prey selection by predators are the energetic value of a prey, and the cost associated with capture and ingestion of different kinds of prey (Roger *et al.* 2000; Lang and Gsödl 2001). Foraging predators face several constraints that may influence their net energy gain and consequently prey profitability, such as predator age, prey size and their escape responses may play an important role in prey utilization for predators facing different prey types in their habitat (Roger *et al.* 2000). For instance, when offered different live prey, the carabid beetle *Poecilus cupreus* (L.) selected the prey species that was easiest to catch, the aphid *R. padi*. However, when prey was offered dead, the highest consumption was recorded on the house cricket *Acheta domestica* (L.) (Orthoptera: Gryllidae), which displayed the most effective escape response when alive, and at the same time was the most profitable prey in terms of prey weight (Lang and Gsödl 2001).

Another important factor influencing prey consumption is availability, in cases where all prey are equally acceptable options (Medal *et al.* 1997; Eubanks and Denno 2000a; Del Bianco and Conde 2001; Lang and Gsödl 2001). Related to this, Saint-Cry and Cloutier (1996) found that maternal induction could also affect prey consumption. In their work these authors found that prey consumed by adult females can induce acceptance for such prey in their progeny. Thus, juveniles focus on a preferred prey when it is available, but exhibit no strong preference for it

when alternative suboptimal prey is temporarily available, which they can recognise, accept and switch to.

Characterising potential alternative prey and the mechanisms through which they affect predator-prey systems is an important step towards developing predictable and effective management strategies for maximising conservation biological control with generalist predators (Harmon and Andow 2002). Understanding predator-prey relationships requires that other food resources used by the predator be taken into account (Robinson *et al.* 2008).

Along with DBM, five other species of arthropods are considered major pests of brassicas in South Australia: *Pieris rapae* L. (Lepidoptera: Pieridae), *Helicoverpa punctigera* Wallengren (Lepidoptera: Noctuidae), *Hellula hydralis* Guenee (Lepidoptera: Crambidae), *Brevicoryne brassicae* L. (Hemiptera: Aphididae) and *My. persicae*. However, more than 30 arthropod species attack brassica crops in Australia (Hely et al. 1982). Thus, some species of aphids, such as *My. persicae* and DBM may be simultaneously present in brassica crops (Hely et al. 1982; Blackman and Eastop 2000). *Myzus persicae* is a polyphagous aphid that can be found worldwide attacking many agricultural crops (Blackman and Eastop 2000) and it is a common and widespread pest of brassica crops in the world (California 1987). According to the definition given earlier (Evans *et al.* 1999; Cabral *et al.* 2006), this aphid is essential food for the three predators studied, the brown lacewing, *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) and the coccinellids *C. undecimpunctata* and *Coccinella transversalis* (Fabricius), as they are able to complete their life cycle for several generations on only this species, and with low mortality (Hodek and Honek 1996 and references within; Cabral *et al.* 2006). DBM and *My. persicae* exhibit different feeding habits, size, morphology, mobility, nutritional value and escape behaviour.

Micromus tasmaniae is widespread and abundant in Australasian agroecosystems (Horne *et al.* 2001). Larval stages are polyphagous and consume a range of small soft-bodied arthropods (Hosseini 2007). Apart from sharing the diet with juveniles, adults are also omnivorous, consuming pollen and nectar as well (Silberbauer *et al.* 2004; Robinson *et al.* 2008). This species is considered to be a significant biological control agent in lucerne (Leathwick and Winterbourn 1984; Milne and Bishop 1987; Horne *et al.* 2001) and it was one of the generalist predator species most frequently collected in a broccoli field in South Australia (Hosseini 2007).

Coccinella undecimpunctata and *C. transversalis* are frequently found in vegetable (including brassica) crops in the Auckland, New Zealand, and Adelaide Regions, respectively. For instance, both species were frequently collected from a broccoli field in South Australia (Hosseini 2007). However, little is known about their prey range and potential impact on DBM populations. There is evidence that other coccinellid predators feed on DBM. For example, *C. maculata lengi* consumed this species preferentially, when compared to other lepidopteran larvae of similar size that attack brassica crops (Roger *et al.* 2000). According to some authors (Raimundo and Alves 1986; Soares *et al.* 2004; Soares *et al.* 2005), despite being polyphagous, coccinellids are highly specific with respect to their essential prey. For example, *C. undecimpunctata* prefers to feed on aphids (Hodek and Honek 1996).

Through molecular analysis, DBM-specific DNA was detected in gut contents of *C. transversalis* and *Mi. tasmaniae* collected from a broccoli field in the Adelaide region (Hosseini 2007; Hogendoorn[3] and Juen[4] 2008, personal communication), but their predation efficacy on this species has not been quantified and the details of the interaction between these predators and prey are not well understood.

To advance on the understanding of these interactions, two key objectives are addressed in this chapter:

1. To verify predation of DBM by three generalist predators commonly found in brassica crops, the eleven-spotted ladybird, *C. undecimpunctata*, the transverse ladybird, *C. transversalis,* and the Tasmanian brown lacewing, *Mi. tasmaniae.*
2. To evaluate the effect of the presence of an alternative prey, the green peach aphid *My. persicae*, on the consumption of DBM by these predators.

2.2 MATERIALS AND METHODS

Cultures of *C. undecimpunctata* and *Mi. tasmaniae* were maintained and experiments conducted in the New Zealand Institute for Crop & Food Research (now Plant & Food Research) insectary facility, located at the Mount Albert Research Centre in Auckland, New Zealand, between February and August of 2006. The experiments with *C. transversalis* were conducted in the Waite Campus insectary facility, University of Adelaide, Australia, between November 2006 and May 2007.

2.2.1 PLANT AND INSECT CULTURES

New Zealand cultures

For New Zealand insect cultures, all lighting was provided by two fluorescent tubes per shelf (Philips TL-D Graphica Pro Triphosphor T8 58 Watts) on a time switch and electronic ballast. These provided a photoperiod of 16L:8D. Temperature was maintained at 20±1 °C, except where noted otherwise.

2.2.1.1 Plants

Insecticide-free plants of the species *Brassica campestris* subsp. *chinensis* (Pak Choi, var. "Riko") and *B. oleracea* cv. capitata (cabbage, var. "Sugarloaf") were grown under natural light in a shaded glasshouse. The plants were grown individually in black plastic bags (12 x 12 x 12 cm) with a standard fertilized potting mix of pumice and peat. Plants were used in the cultures or experiments after they had grown eight to ten leaves.

2.2.1.2 Plutella xylostella

Stock culture: The culture was established using larvae collected from an insecticide-free cabbage field in Pukekohe (36°1'60S, 174°13'0E), in the Auckland Region in February 2006. To avoid releasing any parasitoids or diseases into the culture, each larva was placed individually in a plastic vial (8 x 1.2 cm) and covered with a cotton ball until adult emergence. A fresh piece of cabbage leaf was put in the vial every day until pupation. Vials were cleaned or replaced as required. Newly emerged DBM adults were transferred to a gauze-covered rearing cage (60 x 60 x 60 cm) containing four to six cabbage plants. In each cage a 100 ml cup of 10% sugar solution coloured with yellow food colouring (6 drops/100 ml solution) provided food for the moths. A 5 cm long cotton wick embedded in the liquid dispensed the solution through the perforated lid of the cup. Plants were replaced periodically and every three to four weeks a new cage was established using adults of the culture to replace the oldest cage. Between one and three of these cages were kept according to need (Figure 2-2).

Experimental culture: To obtain enough larvae of the same age for the experiments, 80-100 DBM adults were removed from the rearing cages with an aspirator and transferred to a glass jar (20 x 10 cm) lined in gauze and covered with a lid with three openings (MacDonald[5], Walker[1] and Workman[6] 2005, personal communication): (1) a 3 cm diameter opening covered

with a vented lid through which adults were introduced in the jar; (2) a 3 x 0.3 cm rectangular opening holding a slightly crumpled 3 x 5 cm piece of aluminium foil; and (3) a 1 cm round opening containing a 5 cm long cotton wick embedded in 10% sugar solution. The jars were kept in partial darkness. The aluminium foil was used by the moths as an ovipositing substratum and was replaced daily. The piece of aluminium foil with eggs could be incubated at 9°C to delay egg hatching until larvae were needed. To obtain larvae, the pieces of aluminium foil with eggs were put on a cabbage leaf in a plastic container (20 x 20 x 15 cm) with a vented lid at 25 °C. New leaves were added to the container daily, until the desired larval stage was reached. Larvae that were not used were put back into the stock culture (Figure 2-3).

Figure 2-2 Cage with the stock DBM culture.

2.2.1.3 Myzus persicae

The GPA culture was established using aphids from a long-term culture maintained by the Horticulture and Food Research Institute of New Zealand Limited, Auckland. The aphids were reared on Pak Choi plants inside gauze rearing cages (60 x 60 x 60 cm) at 25±1°C. These cages were placed near a large window so they received additional natural light. The culture consisted of two to three cages and four to six plants inside each cage. Plants were replenished with new ones when necessary, and cages were replaced by clean ones approximately every 10 days or more often if necessary.

Figure 2-3 Glass jar for DBM egg laying (top left); Lid with three openings for mass production of DBM (top right); DBM eggs on piece of aluminium foil (bottom left); Container for mass production of DBM larvae (bottom right).

2.2.1.4 Coccinella undecimpunctata

Coccinella undecimpunctata egg masses were collected from insecticide-free lettuce (*Lactuca sativa* L.) and cabbage fields at Pukekohe in February 2006 and kept individually in 5 cm Petri dishes until egg hatching. As there is high incidence of cannibalism in this species, newly emerged larvae were transferred individually to 5 cm diameter Petri dishes with a filter paper disc (Whatman® n.2, 4.25 cm) and GPA as food. The Petri dishes were arranged on trays and were cleaned daily, removing old aphids and aphid's corpses and fresh aphids were given to the ladybird larvae. The filter papers were replaced every 3-4 days. In order to have non-related adults for reproduction, ladybirds coming from different egg masses were identified and reared separately. Newly emerged adults were placed in transparent vented plastic containers (15 x 15 x 13 cm) (Figure 2-4) in groups of approximately 10 males and 10 females, making sure males and females had a different origin. At first, these containers were maintained at 20±1°C, but as no egg laying was observed, the reproductive individuals were moved to a room at 25±1°C. The bottom of each jar was lined with a sheet of absorbent

paper. Food was provided by a 100 ml plastic cup with water and a perforated lid holding a Pak Choi leaf infested with GPA. The stem of the leaf was pushed through the perforation and cotton wool wrapped around the stem to seal the lid, to prevent ladybirds and aphids drowning. In addition, some honey drops were spread on the absorbent paper as food. A crumpled tissue paper was located in a corner of the container as an oviposition substrate.

The culture of *C. undecimpunctata* was maintained daily. This included replacement of absorbent paper, adding new honey drops and checking the filter and tissue papers for eggs. New aphids were added to the leaf (about 40 aphids/ladybird), and the whole leaf was replaced by a new one every four to five days. Egg masses were placed individually in dated Petri dishes, which were kept in an incubator at 9°C, to be used when needed. To obtain ladybirds for the experiments egg masses were put at 20±1°C and 16L:8D photoperiod. Newly emerged larvae were transferred individually to Petri dishes and fed daily with fresh GPA.

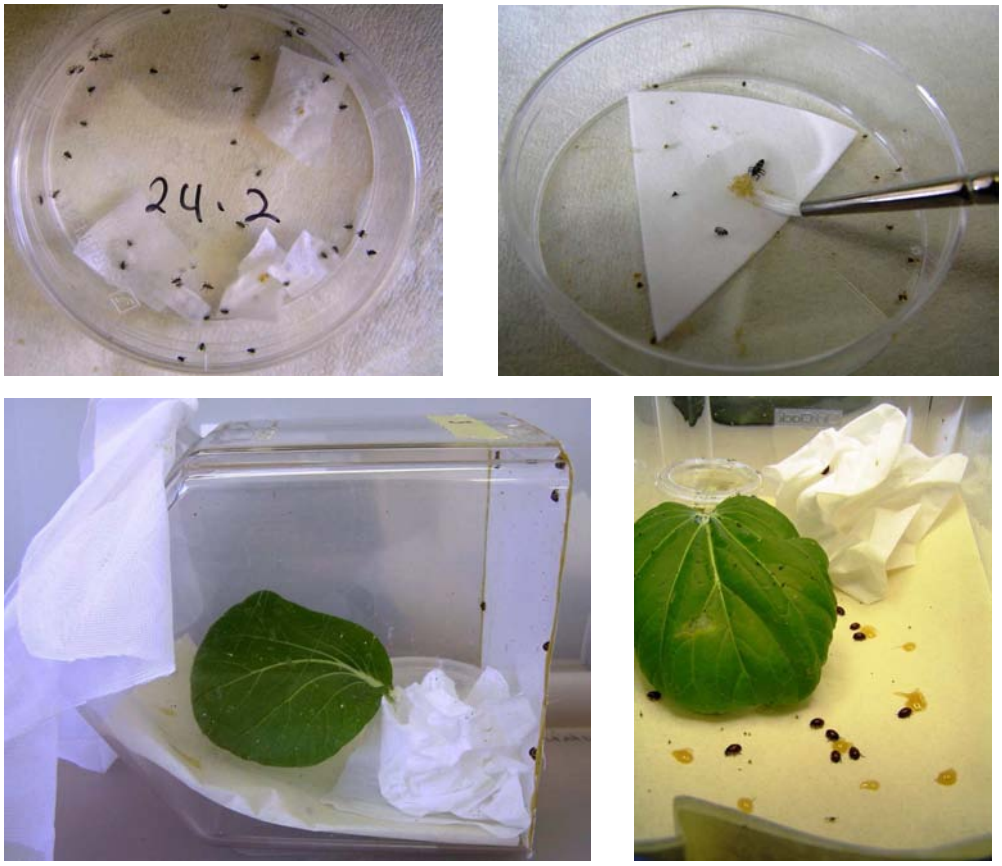


Figure 2-4 Ladybird larvae recently emerged from eggs on tissue paper (top left) are placed individually on Petri dishes and fed with aphids (top right). Adults are fed aphids and honey (bottom).

2.2.1.5 Micromus tasmaniae

A culture of *Mi. tasmaniae* was established from eggs that were collected from insecticide-free lettuce and cabbage fields at Pukekohe in February 2006 and kept in 5 cm Petri dishes until egg hatching. As there is high incidence of cannibalism in this species, newly emerged larvae were transferred individually to 5 cm diameter Petri dishes with a filter paper disc (Whatman® n.2, 4.25 cm) and GPA as food. The Petri dishes were arranged on trays and were cleaned daily, removing old aphids and aphid corpses and fresh aphids were given to the lacewing larvae. The filter papers were replaced every 3-4 days, and after pupation Petri dishes were kept for about 12 days until emergence of adults started. Newly emerged adults were sexed and transferred to a transparent plastic jar (500 ml) covered with gauze (Figure 2-5). Inside the jar a 50 ml plastic cup with water and a perforated lid held a Pak Choi leaf with GPA. Around the hole of the lid the stem of the leaf was wrapped with non-absorbent cotton wool to avoid insects drowning.

Figure 2-5 The cotton strip in the jar serves as an oviposition substrate (left) and can be kept at 9°C to delay egg hatching; (B) newly hatched lacewing larvae are reared individually in Petri dishes.

A 2 cm wide strip of a white coarse-textured fabric was hung loose from the border of the container making a loop to act as an ovipositing substrate (Figure 2-5). This strip was replaced by a new one every day and the one containing eggs was dated and kept in an incubator at 9° C

to be used when needed. New aphids were added to the leaf every day and the leaf was replaced when necessary. When lacewings were needed for experiments, eggs were incubated in a transparent plastic container (10 x 7 x 2cm) with a vented lid at 20±1°C and 16L:8D photoperiod. Newly emerged larvae were placed individually in 5 cm Petri dishes and were reared as described above (Figure 2-5).

Australian cultures

2.2.1.6 Plants

Insecticide-free Pak Choi and cabbage plants were produced under natural light in a shaded glasshouse. The plants were grown individually in black plastic pots (18 cm tall x 12 cm diam) with a standard UC soil mix (Matkin and Chandler 1957). Plants were normally used in the cultures or experiments when they had at least eight to 10 leaves.

2.2.1.7 Plutella xylostella

Stock culture: The DBM culture was established using adult moths from a long-term culture maintained by the South Australian Research and Development Institute (SARDI) and with additional larvae collected from a cabbage field in the Adelaide Region in December 2006. To avoid releasing parasitoids or diseases into the culture, each larva was placed individually in a plastic vial (6 x 1 cm) until adult emergence (as explained previously). Newly emerged adults were transferred to a gauze rearing cage (60 x 50 x 60 cm) containing four to six cabbage plants. A 10% sugar solution was offered as food to the adults (as described previously). A new cage was started with adults from the culture every 3-4 weeks to replace the oldest cage. The culture consisted of one to four cages according to need and was kept at 20±1°C and 14L:10D photoperiod.

Experimental culture: To obtain large numbers of larvae of the same age for the experiments, the system used in New Zealand was slightly modified because Australian DBM did not lay enough eggs in the glass jar (see New Zealand DBM culture). For this, 80 to 100 DBM adults were removed from the rearing cages with an aspirator and transferred to a gauze rearing cage (30 x 30 x 20 cm) which contained a 100 ml cup with sugar solution (as described previously). Another plastic cup held a cabbage leaf as ovipositing substrate which was replaced daily (Figure 2-6). Using this system, eggs could not be kept in an incubator to delay egg hatching because leaves would dehydrate or decompose. To obtain larvae for the experiments the egg-infested leaves were placed in a plastic container (20 x 20 x 15 cm) with a vented lid in a room

at 20±1°C. New leaves were added to the container daily, until the desired larval stage was reached.

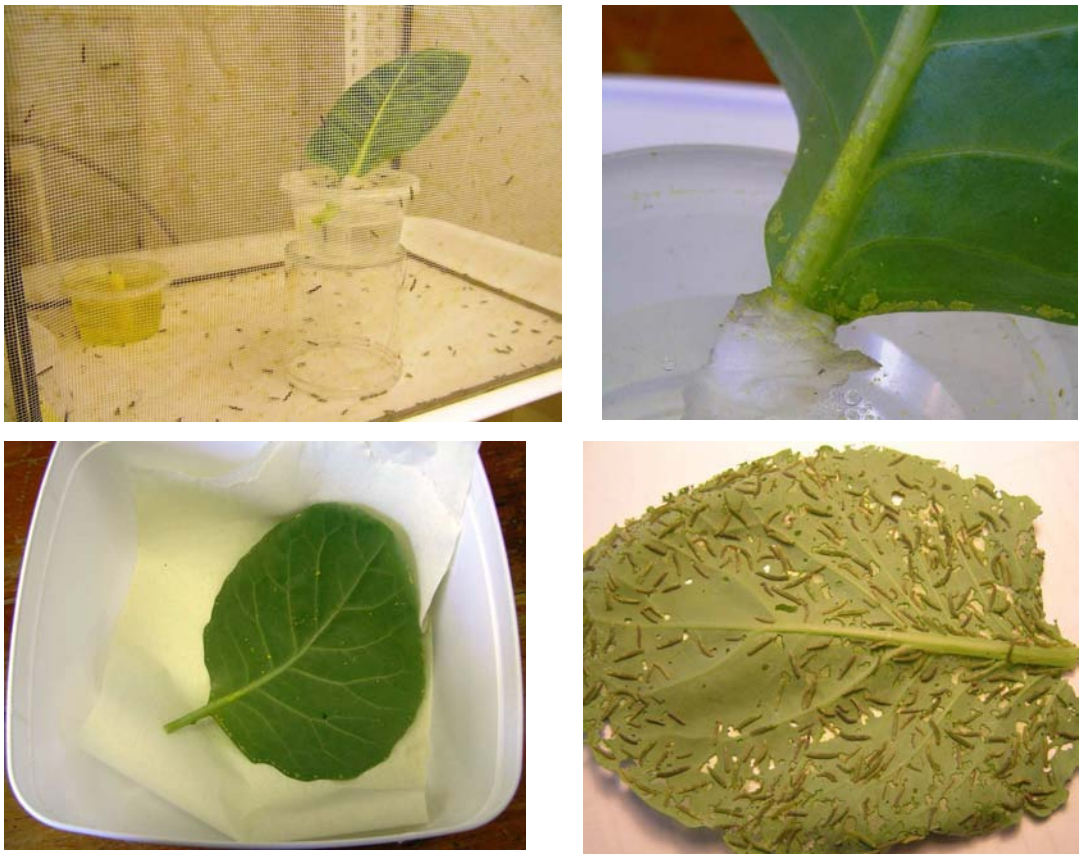


Figure 2-6 Adults in the DBM egg laying cage (top left) lay eggs on the leaf (top right), which is kept in a plastic container (bottom left) to obtain large numbers of larvae of the same age (bottom right).

2.2.1.8 Myzus persicae

The GPA culture was established using aphids obtained from a greenhouse that contained brassica plants infested with this species at the Waite Campus, University of Adelaide. The culture was reared on Pak Choi plants, in gauze cages (60 x 60 x 60 cm) in rooms with no temperature control (26±6°C) with natural light. Plants were replenished every 4-5 days.

2.2.1.9 Coccinella transversalis

The culture of *C. transversalis* was established using adults collected from a cabbage crop in Currency Creek, South Adelaide in December 2006. To avoid releasing parasitoids into the

culture, adults were placed in individual 5 cm Petri dishes with GPA as food. After 7 days those not parasitised were put in plastic containers, as described in the New Zealand ladybird culture. The containers with reproductive adults were kept at 24±1°C and 14L:10D photoperiod. Egg masses produced in these containers were placed in dated Petri dishes and at hatching larvae were transferred individually to 5 cm diameter Petri dishes. These contained a disc of filter paper (Whatman® n.2, 4.25 cm) and a piece of Pak Choi leaf and GPA and were maintained at 20±1°C and 14:10 L:D photoperiod. Petri dishes were cleaned daily, removing old aphids and aphid corpses and fresh aphids were given to the ladybird larvae. Filter papers were replaced when dirt or mould had built-up.

Lighting for Australian DBM and *C. transversalis* was provided by a control system that simulated dusk and dawn conditions. Daytime lighting was provided by four fluorescent lamps (GE Tri-Tech F36T8/840) powered by solid state ballasts that reproduced near-natural lighting by flickering at 40-100 Hz (PCA ECO 18-58W 220-240 V 50/60/0 Hz dimmable ballast, Tridonic.Atco GmbH & Co KG, Dornbirn, Austria), which is greater than the flicker fusion frequency of insect eyes (Shields 1989). An electronic ballast controller (DDBC1200; Dynalite, Mascot, NSW, Australia) operated by an astronomical time clock (DTC602 Dynalite, Mascot, NSW, Australia) provided dimming functions. Full lighting was provided when the controller delivered the maximum 255 units of power. Dusk conditions were simulated by decreasing the lamp power by 1 unit every seven seconds such that the lamps went from full power to off over a 30 minute period. Relative light levels were not linear; the 50% light level occurred 5 minutes after the dusk cycle commenced. At the end of the dusk period, there was an abrupt change from low level lighting to darkness. Dawn conditions were the reverse of dusk. The photoperiod was considered to last from when lights went on at the start of the dawn period until they were completely off at the end of simulated dusk.

Insects do not behave normally in complete darkness because they cannot see in the dark (Shields 1989). Hence a low-power night lamp (0.1 A minilamp; 4 mm diam x 10 mm long) provided "moonlight". It was powered by a 6 V sealed lead-acid battery that was continuously recharged by a 6 V, 500mA fully automatic sealed lead acid battery charger (Powertech Cat MB-3516; Jaycar Electronics, Silverwater, NSW, Australia). This delivered continuous low-level flicker-free lighting day and night to promote normal insect behaviour.

2.2.2 EXPERIMENTS

Experiments were conducted in rooms at 20±1ºC and a photoperiod of 16L:8D (New Zealand) and 14L:10D (Australia). In order to standardise their condition, prior to the beginning of the experiments, predators were starved individually for 24 h in 5 cm diameter Petri dishes with a filter paper disc (Whatman® n.2, 4.25 cm) moistened with two drops of tap water. In all cases prey were allowed to settle for about two hours before the predator was introduced in the arena. All the replications began at approximately 13.00 hr and ran for 48 h (exp. 1) or 24 h (exp. 2). Preliminary tests allowed determining the number of DBM and GPA that should be provided in each treatment (maximum number that could be consumed) and prey were offered in excess to reduce the effect of prey availability on prey consumption. Body mass of DBM instars and aphids was determined by weighing 20 lots of 10 live individuals of each prey type utilised before offering them to the predators in the first replicates.

Two experiments were conducted:

2.2.2.1 Experiment 1: Predation on DBM by C. undecimpunctata, Mi. tasmaniae and C. transversalis.

A non-choice experiment was conducted to establish which stages of the prey and predators are more likely to participate in trophic interactions. In addition, from this experiment the most suitable life stages of the predators and DBM were selected for experiment 2.

Second and third instars, as well as adult *C. undecimpunctata* and *Mi. tasmaniae* were placed individually in the presence of either second, third or fourth instar DBM. The arena used was an empty vented 5 cm Petri dish, and after 24 h surviving DBM larvae were counted and removed and the same original number of larvae was offered for another 24 h to the surviving predators. Partially consumed prey was counted as consumed prey. Predators that did not survive were counted but not replaced. Adult *C. undecimpunctata* and *Mi. tasmaniae* were sexed and half the repetitions were made with males and the other half with females. In the case of *C. transversalis*, only adults of this predator and second instar DBM were used, because this was the most voracious stage in *C. transversalis* (as shown in preliminary studies). In addition, because adult *C. transversalis* are difficult to sex, individuals were chosen randomly from a total of 70 adults. To verify mortality of the tested instars of DBM in the absence of predators, control treatments were included in each block (Table 2-1).

A complete randomized block design was used. Each block included all predator-prey combinations with replications in time. Sixteen replicates were conducted for every prey-predator stage combination with *C. undecimpunctata* and *Mi. tasmaniae* but in cases where there was high predator mortality, and according to the availability of the right stages of predators and prey, more replicates were conducted. Twenty replicates were conducted for the treatment with *C. transversalis*. Every replicate was performed with new individuals.

Table 2-1 Number of replicates per treatment (n) and number of *P. xylostella* (DBM) larvae of each instar offered to predators in each treatment in non-choice experiment.

Predator	Predator stage	DBM instar					
		2nd		3rd		4th	
		N	larvae	n	larvae	n	larvae
C. undecimpunctata	2nd instar	18	40	34	10	16	5
	3rd instar	16	40	16	10	16	5
	adult	16	40	16	10	16	5
Mi. tasmaniae	2nd instar	16	40	29	10	19	5
	3rd instar	16	40	18	10	16	5
	adult	16	40	16	10	16	5
C. transversalis	adult	20	40	-	-	-	-
Control (no predator)		20	40	16	10	16	5

2.2.2.2 Experiment 2: Effect of the presence of alternative prey on the consumption of DBM.

A choice experiment was conducted to evaluate the effect of the availability of the green peach aphid (GPA) as an alternative prey on the consumption of DBM by adult *C. undecimpunctata* and *C. transversalis* and third instar *Mi. tasmaniae*. For this, a mixed diet of second instar DBM and large fourth instar nymphs to wingless adult GPA was offered to the predators placed individually in two different experimental arenas with different levels of complexity. Arena 1 consisted of a vented 5 cm Petri dish with a 2 cm diameter Pak Choi leaf disc. The leaf disc was used to avoid DBM larvae consuming aphids in the absence of plant material (as was observed in pilot experiments). The leaf disc was suspended by a cotton thread, as it was previously observed that when left loose on the bottom of the Petri dish, it dehydrated and flattened down after a short period, and both aphids and DBM larvae hid

underneath, out of the reach of predators. Arena 2 consisted of a vented transparent plastic container (8 cm deep x 11 cm diameter) with a Pak Choi leaf, with the stem inserted in the bottom of the container and sealed with a disc of high density foam. The stem was immersed in water to prevent leaf desiccation and the container was covered with a piece of gauze held with a rubber band to avoid insect escape. The leaf was not in contact with the sides or bottom of the container, nor with the gauze, so insects did not have access to hiding places (Figure 2-7).

Predators confronted different challenges in the two arenas. Arena 1 imposed few difficulties for attack and capture of prey. Arena 2 on the other hand, was bigger and more complex so prey were more dispersed and difficult to find and the elevation of the leaf allowed DBM larvae to elude predators by using a normal escape response, dropping off the leaf and hanging from a silk thread when attacked. By testing the predators in the different arenas, the effects of the arenas *per se* could be evaluated, and the effect of the presence of an alternative prey on predation of DBM could be validated. Both arenas were used in experiments with *C. undecimpunctata* and *Mi. tasmaniae* but only arena 2 was used with *C. transversalis*.

Four treatments combined a fixed number of DBM larvae with an increasing number of GPA, so that the proportion of DBM in the diet decreased gradually (treatments A, B, C and D). In addition, one treatment consisting of only GPA (treatment E) allowed comparison of the consumption of this prey by the predators in the presence and absence of DBM (Table 2-2). Because in experiment 1 there was no difference in the consumption of DBM larvae by these predators between day 1 and day 2, this experiment lasted only 24 h.

A complete randomized block design was used. Each block included all predator–prey combinations with replications in time. Ten replicates were conducted for each diet combination in the experiments using *C. undecimpunctata* (five with females and five with males) and *Mi. tasmaniae* (these juveniles were not sexed but selected randomly from a pool of about 100 individuals), and thirteen for *C. transversalis* (not sexed, but selected randomly from a pool of 70 adults). Control treatments were included in each block to verify mortality of both prey species in the absence of predators (Table 2-2). Every replicate was performed with new individuals.

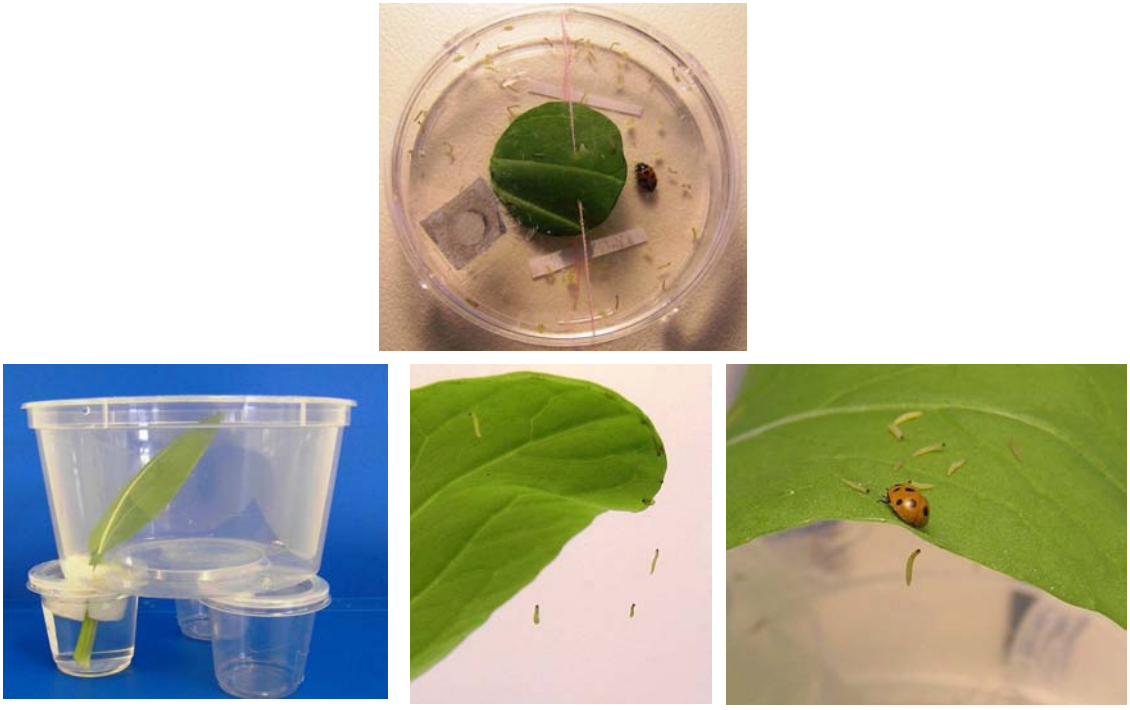


Figure 2-7 Arena 1 (top) is a simpler environment with no places for the prey to escape or hide, whereas arena 2 (bottom) offers a much more complex environment, and DBM larvae can escape the predators by dropping from the leaf on a silk thread.

Table 2-2 Number of replicates of each treatment (n) and number of 2nd instar *P. xylostella* (DBM) and *My. persicae* (GPA) (large 4ths and wingless adults) offered to predators in a choice experiment.

Predator	Arena	n	Treatment - number DBM/GPA in Arena				
			A	B	C	D	E
C. undecimpunctata	1	10	35/0	35/15	35/30	35/60	0/30
C. undecimpunctata	2	10	35/0	35/15	35/30	35/60	0/30
C. transversalis	2	10	35/0	35/15	35/30	35/60	0/30
Mi. tasmaniae	1	13	20/0	20/8	20/16	20/32	0/16
Mi. tasmaniae	2	13	20/0	20/8	20/16	20/32	0/16
Control (no pred.)	1	10	35/0	-	35/30	-	0/30
Control (no pred.)	2	10	35/0	-	35/30	-	0/30

DATA ANALYSIS

Two aspects were analysed in experiment 1:

The survival of different stages of predators after 48 h in treatments with one DBM instar was compared using a Chi-square approach (Zar 1999; Dytham 2003).

Consumption of larvae/day within treatments with one DBM instar was compared by Student *t*-test (SAS-Institute 2000). Comparisons were made only between day 1 and day 2 (in all predator stages) and between females and males (in the case of adults). Only those predators that survived after 48 hr in each treatment were used in these comparisons and the data for number of larvae consumed/day were tested for normality using the Shapiro-Wilk test and log-transformed when necessary (Zar 1999; SAS-Institute 2000).

Three aspects were analysed in experiment 2:

Total consumption of prey was calculated on a weight basis. For this the number of prey consumed (GPA + DBM) was transformed to weight consumed by multiplying the average weight of each prey type by the number of prey totally or partially consumed by each predator. Consumption of prey in all treatments that included DBM (treatments A, B, C and D in Table 2-2) was analysed by non-linear regression using an exponential curve as the model (SAS-Institute 2000). The fitted model for the function used is given by:

$$\textbf{Total eaten} = m + (M - m) * (1 - \text{Exp}(-\beta * \text{GPA}))$$

Total eaten = sum of DBM and GPA consumed (mg)

m = estimated minimum consumption of prey (mg)

M = estimated maximum consumption of prey (mg) or satiation level (asymptote)

β = slope of the curve

GPA = Number of GPA offered in each treatment

To visualise the results, the fitted curves were plotted together with the mean consumption of DBM expressed in milligrams (mg $\pm$ SE). The relationships between number of DBM consumed by the predators and the number of GPA in the Arena (treatments A, B, C and D in Table 2-2) were analysed using linear regression (SAS-Institute).

The number of GPA consumed in the presence and absence of DBM (treatments C and E) and the consumption of GPA in both arenas in the presence and absence of DBM were compared using Student *t*-test (SAS-Institute 2000). In addition, Student a *t*-test was used to make an exploratory comparison between the consumption of GPA in both the presence and absence of DBM by coccinellid species in arena 2 (because experiments with each species were conducted in different seasons, as explained previously). In relation to the utilisation of the alternative prey, it is important to mention that the scores for consumption of aphids in this experiment reflect the minimum consumption, since some aphids produced offspring during the 24 hr period the experiment lasted. Predators may have consumed small nymphs along with adults, making the count of the actual number of aphids consumed inaccurate. No control treatment was conducted to evaluate this.

2.3 RESULTS

2.3.1 EXPERIMENT 1

2.3.1.1 Mortality of DBM in control treatment

No mortality was observed in any DBM instar in the control treatments during 24 h.

2.3.1.2 Survival of predators after 48 h

Coccinella undecimpunctata While 100% of third instar and adult ladybirds survived in all treatments, only 83% of second instar ladybirds survived in the treatment with second instar DBM, but this difference was not significant. In contrast, survival of second instar *C. undecimpunctata* was significantly lower in treatments with third (44%) and fourth (75%) instar DBM ($P<0.05$; Tables 2-3 and 2-6). No significant differences were observed between survival of females and males of *C. undecimpunctata* so the data were pooled ($P>0.05$).

Micromus tasmaniae In treatments with second instar DBM, survival of all stages of lacewings was over 80%. However, in treatments with third and fourth instar DBM there was significant differences in survival between lacewing stages ($P<0.05$). Survival of second instar lacewings decreased as the size of DBM increased, dropping from 81% (second instar DBM) to only 16% (fourth instar DBM). In treatments with third instar DBM the survival of second and

third instar lacewings was significantly lower than survival of adult lacewings (41, 61 and 100% respectively). In treatments with fourth instar DBM the survival of third instar and adult lacewings were similar and significantly higher that second instar lacewings (81.3, 81.3 and 16% respectively; Tables 2-4 and 2-6). No significant differences were observed between survival of females and males of *Mi. tasmaniae* either, so data were pooled ($P>0.05$).

Coccinella transversalis No predator mortality was observed in the experiment with *C. transversalis* (Table 2-5).

Table 2-3 Number of replicates (n) and survival of *C. undecimpunctata* after day 1 (n_1) and day 2 (n_2) in treatments with (A) second, (B) third, and (C) fourth instar DBM. In tables 2-3 to 2-5, the percentage of survival for day 1 is based on the number of insects that started the treatment (n), while for day 2 it is based on the number of predators that survived after day 1 (n_1).

(A) 2nd instar DBM

Predator stage	n	Survivors day 1		Survivors day 2		Total survival after 48 h (%)
		n_1	%	n_2	%	
2nd instar	18	17	94.4	15	88.2	83.3
3rd instar	16	16	100	16	100	100
Adults	16	16	100	16	100	100

(B) 3rd instar DBM

Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 h (%)
		n_1	%	n_2	%	
2nd instar	34	28	82.4	15	53.6	44.1 [a]
3rd instar	16	16	100	16	100	100 [b]
Adults	16	16	100	16	100	100 [b]

(C) 4th instar DBM

Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 h (%)
		n_1	%	n_2	%	
2nd instar	16	15	93.8	12	80.0	75 [a]
3rd instar	16	16	100	16	100	100 [b]
Adults	16	16	100	16	100	100 [b]

Different letters within a DBM instar indicate statistically significant differences ($P<0.05$)

Table 2-4 Number of replicates (n) and survival of *Mi. tasmaniae* (mean number and percentage) after day 1 (n_1) and day 2 (n_2) in treatments with (A) second, (B) third and (C) fourth instar DBM.

(A) 2nd instar DBM

Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 hr (%)
		n_1	%	n_2	%	
2nd instar	16	15	93.8	13	86.7	81.3
3rd instar	16	16	100	15	93.8	93.8
Adults	16	16	100	16	100	100

(B) 3rd instar DBM

Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 hr (%)
		n_1	%	n_2	%	
2nd instar	29	21	72.4	12	57.1	41.4 [a]
3rd instar	18	16	88.9	11	68.8	61.1 [a]
Adults	16	16	100	16	100	100 [b]

(C) 4th instar DBM

Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 h (%)
		n_1	%	n_2	%	
2nd instar	19	12	63.2	3	25.0	15.8 [a]
3rd instar	16	16	100	13	81.3	81.3 [b]
Adults	16	16	100	13	81.3	81.3 [b]

Different letters within a DBM instar indicate statistical significant differences ($P<0.05$)

Table 2-5 Number of replicates (n) and survival of *C. transversalis* after day 1 (n_1) and day 2 (n_2) in treatment with second instar DBM.

2nd instar DBM						
Predator stage	n	survivors day 1		survivors day 2		Total survival after 48 h (%)
		n_1	%	n_2	%	
Adults	20	10	100	10	100	100

Table 2-6 Calculated X^2 and *P* values for the comparisons of survival between predator life stages in treatments with either second, third or fourth instar *P. xylostella* (DBM).

Predator	DBM instar	Predator stages compared	X^2 calc.*	P
C. undecimp.	2nd	2nd – 3rd – adult	5.673	0.058
	3rd	2nd – 3rd – adult	25.111	<0.0001
		2nd – 3rd	14.421	0.00015
		2nd – adult	14.421	0.00015
		3rd – adult	-	-
	4th	2nd – 3rd – adult	8.727	0.0127
		2nd – 3rd	4.571	0.032
		2nd – adult	4.571	0.032
		3rd – adult	-	-
Mi. tasmaniae	2nd	2nd – 3rd – adult	3.818	0.148
	3rd	2nd – 3rd – adult	15.031	0.00054
		2nd – adult	15.073	0.00013
		2nd – 3rd	-	-
		3rd – adult	7.835	0.0051
	4th	2nd – 3rd – adult	20.826	<0.001
		2nd – 3rd	14.99	0.0001
		2nd – adult	14.99	0.0001
		3rd – adult	-	-

* Critical $X_1^2{}_{(1-0.05)}$=3.841; $X_2^2{}_{(1-0.05)}$=5.991 (Zar 1999).

2.3.1.3 Consumption of DBM

All *C. undecimpunctata, Mi. tasmaniae* and *C. transversalis* stages evaluated successfully attacked and were able to consume all instars of DBM offered, during at least one day of the experiment (Figures 2-8 and 2-9). However, the proportion of individuals that utilised prey was higher in those treatments that combined older stage predators with younger stage prey. In addition, all predators consumed second instar DBM during day 1 and over 87% during day 2 of the experiment. In general, utilization of DBM larvae decreased with increasing age (size) of the prey (Table 2-7).

Figure 2-8 *Mi. tasmaniae* larvae of all developmental stages could attack and eat all instars of larval DBM, including the biggest and heaviest larvae.

Figure 2-9 Adult and larval eleven-spotted ladybird eating DBM larvae.

2.3.1.4 Mean consumption of prey/day:

No significant differences were observed between mean number of larvae consumed in day 1 and day 2, so the data were pooled. In the case of *C. undecimpunctata*, within one prey instar, predation level increased with increasing predator life stage. In addition, consumption of third instar DBM by females was significantly higher than that of males ($P<0.05$; df=7). On the

other hand, third instar *Mi. tasmaniae*, demonstrated a higher level of predation than adult lacewings within one prey instar (Table 2-8).

Due to their high survivorship, the higher proportion of individuals that effectively killed and consumed DBM, and the prey consumption rate, adult *C. undecimpunctata* and *C. transversalis* and third instar *Mi. tasmaniae* in combination with second instar DBM were selected for experiment 2.

Table 2-7 Percentage of (A) *C. undecimpunctata,* (B) *Mi. tasmaniae,* and (C) *C. transversalis* that effectively attacked and consumed DBM on each day of the experiment (based on the number of predators that survived each day, see Tables 2-3 to 2-5).

Predator stage	2nd instar DBM		3rd instar DBM		4th instar DBM	
	day 1	day 2	day 1	day 2	day 1	day 2
(A) *C. undecimpunctata*						
2nd instar	100	100	8.8	21.4	31.3	6.7
3rd instar	100	93.8	68.8	81.3	18.8	25
Adult ♀	100	100	100	87.5	87.5	75
Adult ♂	100	100	100	87.5	50	75
(B) *Mi. tasmaniae*						
2nd instar	100	100	13.8	19.0	5.3	50.0
3rd instar	100	93.8	72.2	62.5	18.8	12.5
Adult ♀	100	100	62.5	12.5	37.5	0.0
Adult ♂	100	87.5	25.0	12.5	12.5	25.0
(C) *C. transversalis*						
Adults	100	100	-	-	-	-

Table 2-8 Mean daily consumption of second, third and fourth instar DBM by larvae and adults of *C. undecimpunctata, Mi. tasmaniae* and *C. transversalis* when offered either second, third, or fourth instar DBM (number ± 95 CI).

Predator	Pred. stage	DBM instar (number offered/day)		
		2nd (40)	3rd (10)	4th (5)
C. undecimpunctata	2nd instar	8.8 ± 1.7	0.13 ± 0.11 *	0.25 ± 0.21 *
	3rd instar	20.5 ± 4.8	1.6 ± 0.6 *	0.22 ± 0.15 *
	Adult ♀	33.3 ± 4.8	4.25 ± 1.57 [a]	1.30 ± 0.6 *
	Adult ♂	30.4 ± 4.8	2.25 ± 1.03 [b]	0.80 ± 0.45 *
	Adult mean	31.8 ± 3.56	3.25 ± 1.04	1.10 ± 0.38 *
Mi. tasmaniae	2nd instar	5.38 ± 1.04	0.3 ± 0.25 *	0 *
	3rd instar	20.17 ± 3.71	1.23 ± 0.46 *	0.19 ± 0.17 *
	Adult ♀	8.63 ± 2.13	0.38 ± 0.16 *	0.33 ± 0.32 *
	Adult ♂	9 ± 2.9	0.19 ± 0.17 *	0.07 ± 1.14 *
	Adult mean	8.81 ± 1.74	0.28 ± 0.12 *	0.19 ± 0.1 *
C. transversalis	Adult mean	28.17 ± 2.39	-	-

* Data contained an abundance of zeros, therefore could not be analysed, but were averaged to present approximate values for these instars.

[a b] Different letters within one DBM instar indicate statistical significant differences ($P<0.05$)

2.3.2 EXPERIMENT 2

2.3.2.1 Control treatment

No mortality was observed in DBM or GPA in control treatments during 24 h.

2.3.2.2 Survival of predators

Survival of predators in this experiment was 100%.

2.3.2.3 Total consumption of prey

The total consumption by all predators in both arenas tended to increase with prey availability, until a satiation level was reached after which the consumption stabilised (Figures 2-10 and 2-11). This increase was driven by an increased consumption of GPA, since the consumption of DBM with increasing numbers of GPA in the diet either decreased (*C. undecimpunctata* arena 1,

Mi. tasmaniae, both arenas) or was constant (*C. undecimpunctata* and *C. transversalis*, arena 2) (Figures 2-10 and 2-11 and Table 2-9).

Coccinella undecimpunctata consumed more prey in arena 1 than in arena 2 (8.31±1.77 and 5.89±1.14 mg of total consumption respectively) and satiation was approached when approximately 30 and 60 GPA were provided respectively.

The estimated satiation level for *C. transversalis* was higher than for *C. undecimpunctata* (9.5±3.08 mg) and within the prey range studied this predator did not approach it (the slope of the curve is lower than for *C. undecimpunctata*, Figure 2.10). According to the equation, the extrapolated estimate of the satiation level would be approached when a number of GPA available higher than 180.

For *Mi. tasmaniae* the estimated parameters in both arenas were very similar (Figure 2-11 and Table 2-9) and the level of total consumption was not affected by the complexity of the arena.

Estimated maximum amounts of prey biomass that could be consumed varied among predator species, and depended on the type of arena in the case of *C. undecimpinctata* (Table 2-9).

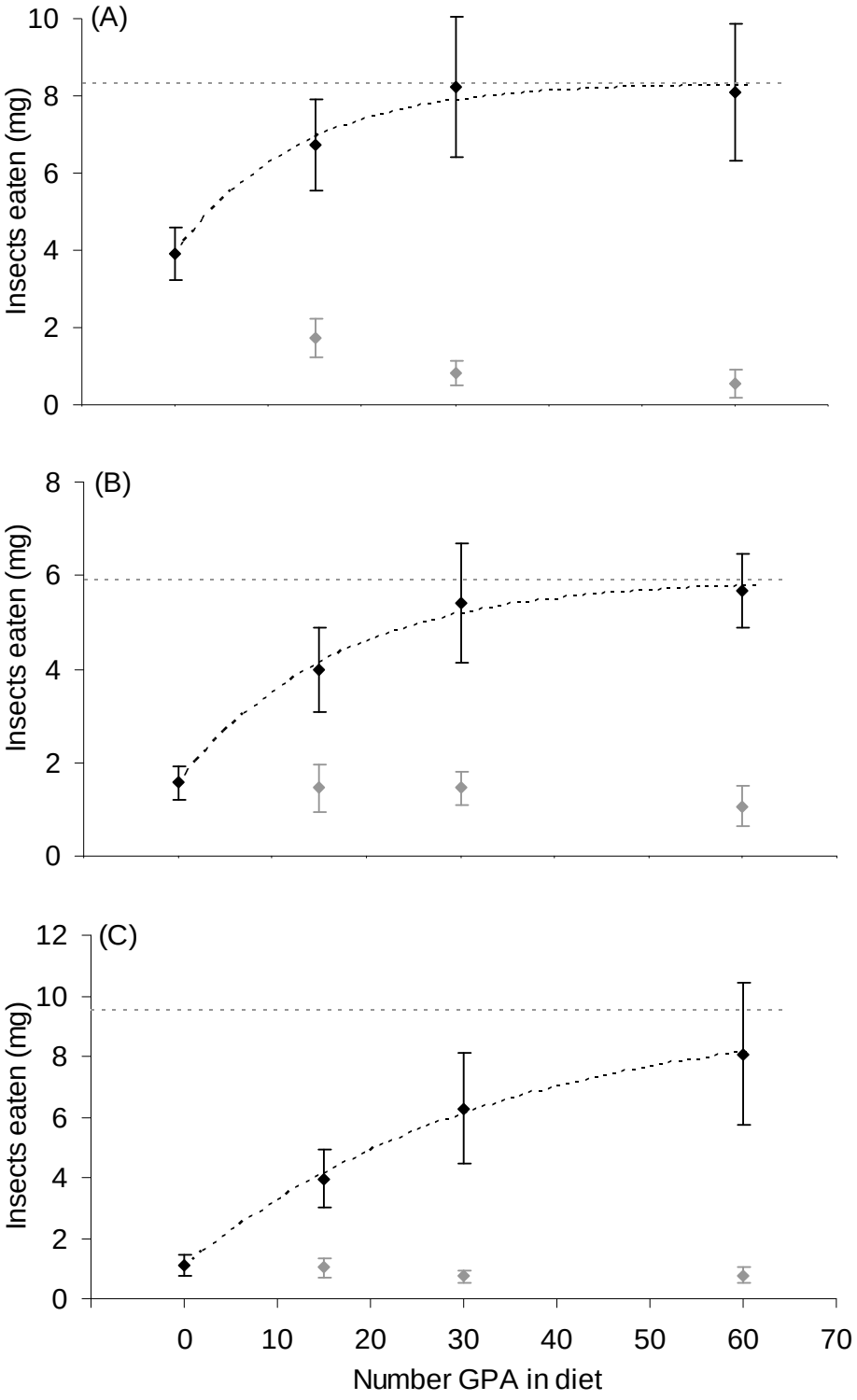


Figure 2-10 Total consumption (2nd instar DBM + large GPA) (black figures), 2nd instar DBM consumption (grey figures), fitted model (black dotted line) and asymptote (grey dotted line) by (A) *C. undecimpunctata* in arena 1, (B) *C. undecimpunctata* in arena 2 and (C) *C. transversalis* in arena 2 (mg ± 95 CI). Insect's mean weight: DBM = 0.19 mg, GPA = 0.39 mg.

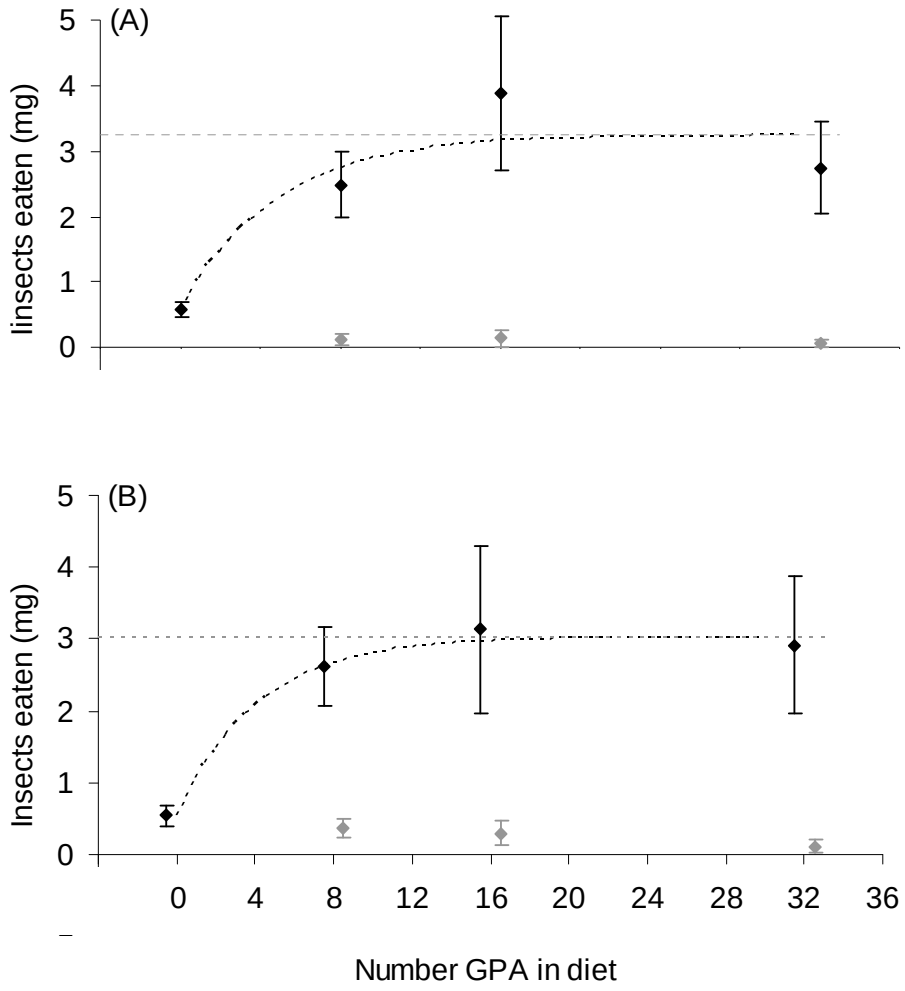


Figure 2-11 Total consumption (2nd instar DBM + large GPA) (black figures), 2nd instar DBM consumption (grey figures), fitted model (black dotted line) and asymptote (grey dotted line) by *Mi. tasmaniae* in (A) arena 1 and (B) arena 2 (mg ± 95 CI). Insect mean weight: DBM = 0.19 mg; GPA = 0.39 mg.

Table 2-9 Estimated values for Max, Min and exponent parameter fitted model for the total consumption of prey (2nd instar DBM + large GPA).

Predator species	Arena	Min ± SE (mg)	Max ± SE (mg)	Exp. par ± SE
C. undecimpunctata	1	3.88 ± 0.73	8.31 ± 1.77	0.08 ± 0.05
C. undecimpunctata	2	1.53 ± 0.45	5.89 ± 1.14	0.06 ± 0.02
C. transversalis	2	1.04 ± 0.78	9.5 ± 3.08	0.03 ± 0.2
Mi. tasmaniae	1	0.56 ± 0.4	3.25 ± 0.92	0.21 ± 0.13
Mi. tasmaniae	2	0.54 ± 0.41	3.02 ± 0.94	0.24 ± 0.19

2.3.2.4 Consumption of DBM at different densities of the alternative prey GPA

The general trend of all predators in both arenas was to consume fewer DBM as the number of GPA in the diet increased. While this relationship was highly significant for *C. undecimpunctata* in arena 1 and *Mi. tasmaniae* in both arenas, it was not significant for *C. undecimpunctata* and *C. transversalis* in arena 2 (Figure 2-13). In addition, regardless of the proportion of the DBM in the arena there was always consumption of this species in both arenas by all predators (Figure 2-17).

The consumption of DBM by *C. undecimpunctata* in arena 1 dropped drastically from around 20 larvae when this was the only food available to only three in the treatment with the highest number of GPA (Figure 2-13A). For *C. undecimpunctata* and *C. transversalis* increasing the number of GPA in arena 2 did not result in a significant difference in DBM consumption, which were approximately seven and five larvae respectively for each species (Figure 2-13A and B).

In the complex arena the consumption of DBM by *Mi. tasmaniae* decreased gradually as the availability of GPA increased (Figure 2-14). In contrast, in arena 1 the consumption of DBM larvae decreased abruptly from approximately three larvae when this was the only prey available, to less than one when there was an alternative prey (treatments B, C and D). Under these conditions not all lacewings consumed DBM, even though the linear regression indicated a very significant relationship when considering all treatments.

2.3.2.5 Consumption of GPA in the presence/absence of DBM (treatments C and E)

Consumption of GPA by *C. undecimpunctata* was affected by the arena, as this predator consumed a significantly greater number of aphids in arena 1 than in arena 2 either in the presence or absence of DBM ($P<0.05$; df=9). In arena 2 *C. transversalis* consumed significantly less GPA in the presence of DBM ($P<0.05$; df=12). Exploratory comparisons between the consumption of GPA in arena 2 by the coccinellid species show that *C. undecimpunctata* tended to eat fewer aphids than *C. transversalis* in both treatments, but the difference was only significant in the absence of DBM ($P<0.0001$; df=21) (Figure 2-15).

No significant differences in the predation of GPA by *Mi. tasmaniae* were observed between arena 1 and 2 in either case (Figure 2-16).

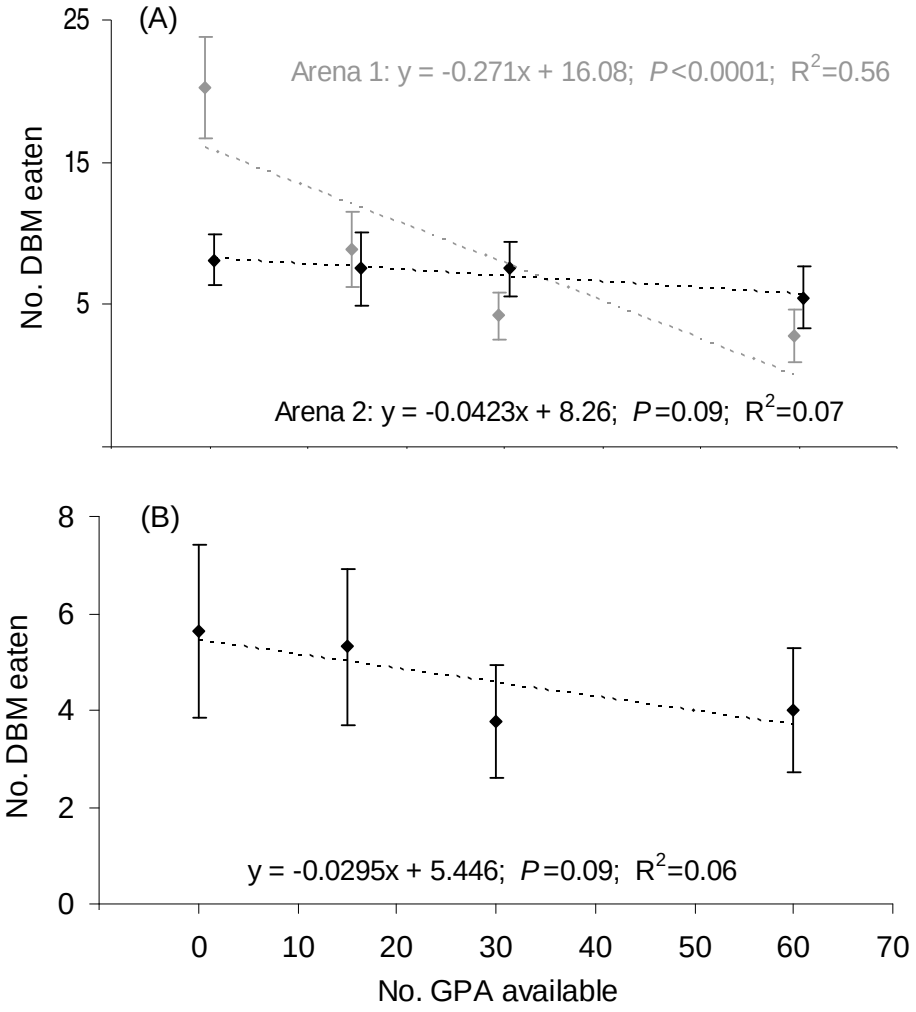


Figure 2-12 Mean number of 2nd instar DBM eaten at different GPA densities by (A) *C. undecimpunctata* in arena 1 (grey figures) and arena 2 (black figures) and by (B) *C. transversalis* in arena 2 (number ±95 CI). To facilitate visualisation of data, x values for arena 1 and arena 2 in (A) have been displaced -0.5 and +0.5 units respectively from the original value (0, 15, 30, 60).

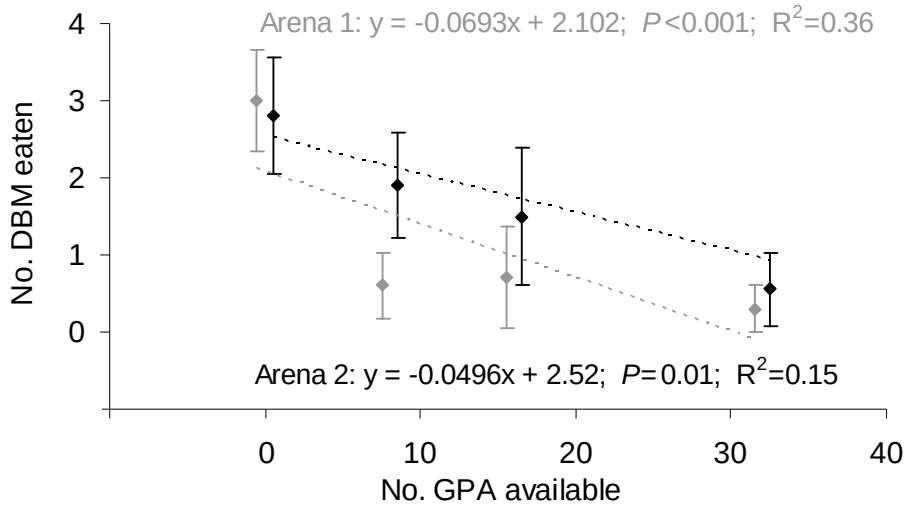


Figure 2-13 Mean number of 2nd instar DBM eaten by *Mi. tasmaniae* at different GPA densities in arena 1 (black figures) and arena 2 (grey figures) (number ± 95 CI). To facilitate visualisation of data, x values for arena 1 and arena 2 have been displaced -0.5 and +0.5 units respectively from the original value (0, 8, 16, 32).

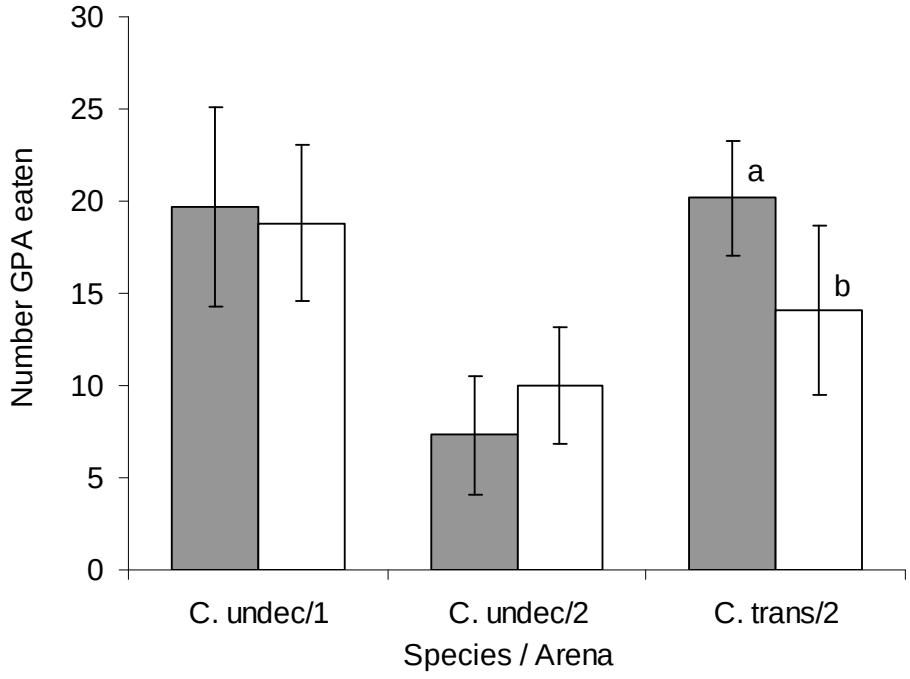


Figure 2-14 Number of large GPA consumed by *C. undecimpunctata* in arena 1 and 2 and *C. transversalis* in arena 2 in the absence (grey bars) and presence (white bars) of 2nd instar DBM (number ± 95 CI). Different letters on columns indicate statistical significant differences (*P*<0.05).

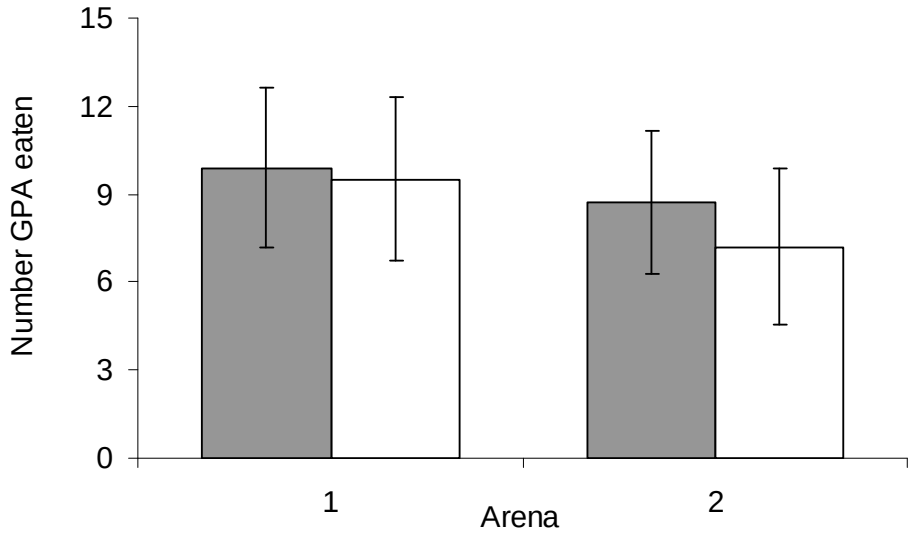


Figure 2-15 Number of large GPA consumed by *Mi. tasmaniae* in arena 1 and 2 in the absence (grey bars) and presence (white bars) of 2nd instar DBM (number ± 95 CI).

Figure 2-16 Eleven-spotted ladybird eating a DBM lava in the presence of green peach aphids.

2.4 DISCUSSION

The results of the first experiment suggest that all DBM instars are potential prey for all the stages of *C. undecimpunctata*, *C. transversalis* and *Mi. tasmaniae*, as the predaceous adults and immatures were able to kill and eat all stages of DBM offered.

Most predators are limited to some extent in their prey choice by physical, physiological or behavioural factors, and body size is one very important factor in determining the prey range of a predator (Symondson *et al.* 2002). Adult neuropterans and coleopterans have orthopteroid mouthparts that allow them to ingest the entire body of their prey (Cohen 1995; Canard 2001). And thanks to extra-oral digestion, the feeding strategy that larval Hemerobiidae and Coccinellidae use (Cohen 1995), the very small young predators could attack and kill all sizes of larval DBM, even those that were larger in size than the predator. For instance, in the treatment with second instar lacewings and fourth instar DBM, at the end of the experiment some prey were dead, partially crushed and surrounded by fluids (personal observation), possibly the result of digestive enzymes acting on body tissues. Extra oral digestion is an adaptation that gives small predators the ecological advantage of utilising relatively large prey that cannot be swallowed whole or ingested piecemeal (Cohen 1995).
According to some authors, relative predator and prey body sizes are a key factor in understanding the dynamics of predator-prey systems (Sabelis 1992; Halaj and Wise 2002). Despite their capacity to kill and consume all sizes of prey studied, a low survival of second instar predators, particularly lacewings, was observed as prey size increased, especially during the second day of the experiment. While mortality among third instar *C. undecimpunctata* was nil, a percentage of third instar *Mi. tasmaniae* died in each treatment. Among predatory adults, mortality was nil in coccinellids in all treatments and low in lacewings in treatments with fourth instar DBM. This may suggest that in real crop systems it is more likely that these predators consume DBM larvae at an early stage, when they are still small.

The high mortality level of small predators with large prey, in conjunction with personal observations, suggest that subduing bigger prey imposed various difficulties and had more associated risks. Thus, some attacks may have resulted in injury due to the defensive behaviour of DBM, involving a violent wiggling and moving away from the attacker. In the restricted space of a small Petri dish, tiny predators were hit and probably physically injured. Besides, not all small predators (both *Mi. tasmaniae* and *C. undecimpunctata*) effectively utilised

prey during 48 hr, which may have resulted in some degree of mortality due to dehydration and starvation.

Low utilization of prey was also observed in treatments that combined adult lacewings and third or fourth instar DBM, where the percentage of individuals that consumed prey was generally below 50%. Previous studies have shown that adult hemerobiid lacewings have predatory habits and a variety of prey types have been found in the gut content of adult brown lacewings, including aphids (Robinson *et al.* 2008), coccids, pseudoccocids, spider mites, dipteran (Canard 2001 and references within), lepidopteran (Samson and Blood 1980), pollen and honeydew (Robinson *et al.* 2008). However, their true feeding requirements are poorly understood (Canard 2001).

From the results obtained it seems unlikely that small *C. undecimpunctata* larvae and *Mi. tasmaniae* small larvae and adults utilize older DBM instars in natural conditions, when they can find an alternative, preferred, and easier to catch prey. According to Roger *et al.* (2000) prey age, size and their induced escape behaviour are factors that may play an important role in prey utilization for a predator when facing different prey types in their habitat. Evidence suggests that more suitable prey for small coccinellids and brown lacewings may include eggs or younger larvae of DBM or other lepidoptera, aphids and other small slow-moving soft-bodied arthropods (Hodek and Honek 1996; Canard 2001 and references within), other small predators such as coccinellid larvae (Sengonca and Frings 1985; Lucas *et al.* 1997), or cannibalization of conspecific eggs or larvae (Hodek and Honek 1996; Canard 2001). One may think then, that the DBM-specific DNA found in guts of adult lacewing may be the result of consuming eggs or very small DBM larvae.

There was an inverse relationship between number of prey killed and prey size, which has already been observed in laboratory studies conducted with coccinellids (Giroux *et al.* 1995; Roger *et al.* 2000). In a study by Roger *et al.* (2000) predation rate on lepidopteran larvae by *C. maculata lengi* was higher on small instars. The author reported that despite this, energy intake was optimised when predators consumed intermediate-sized larvae because they represented the best trade-off regarding predation costs and instantaneous rate of energy gain. Roger *et al.* (2000) suggests that consuming smaller prey may be adaptive if larger prey are costly in terms of injury risks. Coccinellids and lacewing larvae and adults detect prey mostly by physical contact (Storch 1976; Ferran and Dixon 1993; Hodek and Honek 1996; Harmon *et al.* 1998; Canard 2001) and touching larval DBM triggers a more vigorous reaction in later instars, since

their escape is more efficient. In addition, they are bigger and heavier. The average weight of DBM in different instars was approximately 0.2 mg for second, 3 mg for third and 6 mg for fourth instar, which means that in terms of body mass 30 2nd instars are equivalent to two 3rd instars which are equivalent to one 4th instar. Along with the escape behaviour, this may also explain the utilization of prey sizes, where the difference of consumption per capita between second and third instar was larger than the difference between third and fourth instar DBM.

The utilization of young larvae by these predators is probably positive for biological control. A phytophagous larva killed at an early stage is prevented from doing the potential damage that would result from all the feeding needed to complete larval development. As Roger *et al.* (2000) point out, because prey size is positively correlated with prey age, it is expected that the age structure of the population of *P. xylostella* in the field influences the level of predation. This could favour predation of young lepidopteran larvae by the predators at the beginning of a crop season during colonization by DBM, and whenever there are small larvae present.

Previous studies using *My. persicae* as prey (Cabral *et al.* 2006; Cabral *et al.* 2009) showed that adult *C. undecimpunctata* were less voracious than the fourth instar. However, in the present study adult coccinellids were more voracious than the larval stages. This may be the result of differences in physiological state, age or mating status of the insects utilised in each case. In contrast, third instar *Mi. tasmaniae* were more voracious than adults in treatments with second and third instar DBM, which may reflect the advantage of using extra oral digestion used by the larval stages of this predator. That is why these were the developmental stages chosen for the second experiment of this study.

Total consumption of prey

In the second experiment general consumption by all predators increased with total availability of prey until a satiation level was reached. This increase was the result of more GPA utilisation, since the consumption of DBM either decreased or was maintained with respect to the treatment with only DBM available. However, all predators studied killed and consumed DBM in the presence of the alternative prey, GPA, regardless of their density and the complexity of the arena.

General consumption by *C. undecimpunctata* was higher in arena 1 (simple) than in arena 2 (complex). It was probably easier for the predator to find prey in the simpler arena, both GPA and DBM, because they were mostly located on the leaf disc (personal observation).

Maximum consumption was approached by this predator when approximately 30-35 aphids were provided. In a study by Cabral *et al.* (2009) adult *C. undecimpunctata* reached maximum consumption when 90 GPA were provided as a single-species diet. This difference is probably due to particular characteristics of the experimental arenas used in each case, and to the fact that in the present study aphids were offered in combination with larval DBM. At the maximum consumption level, an average of 4.2 second instar DBM was part of the total food ingested. This suggests that, having the option, this coccinellid replaces some aphids with DBM despite the fact that GPA is essential prey for this predator.

In the complex arena *C. undecimpunctata* reached maximum consumption when approximately 60 GPA were provided, reflecting the extra difficulty of finding prey. Again, at this density of aphids, consumption included an average of 5.5 DBM larvae. In the complex arena as well, despite having a similar consumption level in all treatments compared to *C. undecimpunctata*, *C. transversalis* did not reach satiation within the range of prey density studied, which indicates that this predator is capable of consuming more prey biomass.

General consumption did not vary between arenas for *Mi. tasmaniae*. As in arena 1, it should be easier to find and capture prey, the lack of difference in consumption may indicate that the amount offered exceeded by far the maximum eaten and the predator reached satiation. So, the complexity of the arena did not influence predation, as there was plenty of food for the needs of this species.

In general, in comparison with the GPA single-species diet (30 aphids), providing a combination of DBM and aphids reduced consumption of aphids, suggesting that predators exploited both resources as they were available. For *C. undecimpunctata*, complexity of arena affected the level of predation on aphids. However, this was not observed with *Mi. tasmaniae*, suggesting that there was an excess of prey over the consumption needs in both arenas for this species. The higher consumption of aphids by *C. transversalis* compared to *C. undecimpunctata* in the complex arena suggests once more that this species has the capacity to consume more prey biomass.

Consumption of DBM in the presence of GPA

Consumption of DBM in general decreased with increasing GPA density in both arenas. In the simple arena consumption of DBM by *C. undecimpunctata* decreased from almost four 2nd instar larvae in the single-species diet treatment to 0.5 larvae in the treatment with the

maximum density of GPA. This result may be expected because aphids are reported to be essential prey for *C. undecimpunctata* (Cabral *et al.* 2006). Due to their hunting mode, prey consumption by coccinellids depends on the frequency of encounters with prey, or availability of prey (Carter and Dixon 1982), and on the relative vulnerability of prey (Wratten 1973). The change in aphid density modified these two factors. On the one hand, the increasing abundance of aphids allowed more encounters with aphids, as both types of prey tended to concentrate on the leaf disc. On the other hand, GPA were the "easier-to-catch" prey, as they did not perform the violent and effective escape behaviour of DBM (personal observation). Thus, it is likely that this predator switched from extensive to intensive search behaviour on the disc (Hodek and Honek 1996), having an opportunistic preference towards these aggregated aphids. According to Lang and Gsödl (2001), generalist predators may forage opportunistically, "taking what they can get", and some may consume aphids in high numbers, because they are easier to subdue than other prey. As Evans *et al.* (1999) suggest, many predatory insects appear highly opportunistic in attacking certain species and kinds of prey, but such behaviour may be misleading as to prey suitability and the nutritional requirements of the predator.

In contrast, in the complex arena the level of consumption of DBM by both *C. undecimpunctata* and *C. transversalis* did not vary significantly among treatments, despite the increase in aphid density. This could be the result of the random distribution of prey in this arena (personal observation), which did not offer the chance for the opportunistic predation on aphids in particular. In this arena predators foraged for prey in the whole space (personal observation), therefore the chance of encountering different prey was in direct relation to the density of each type. In addition, an encounter with DBM larvae had fewer chances to result in a successful attack because they escaped frequently by wriggling vigorously and throwing themselves off the leaf to which they remained attached by a silk thread, while aphids did not display any active defensive behaviour (personal observation). For these reasons a constant level of predation on DBM in all treatments may suggest that both coccinellids searched actively for this prey, or they encountered this prey type in a regular manner.

Likewise, *Mi. tasmaniae* always showed some level of DBM utilisation. While the consumption of DBM by this predator decreased as aphid density increased in the complex arena, in the simple arena the big difference between the consumption of DBM in the single-species diet and any of the combined-species diet treatments may be the results of the effect of all the prey being concentrated on the leaf disc, as with the coccinellids in the same arena.

The results obtained with all predators suggest that they prefer GPA, because they consumed more as its availability increased. However, these results along with evidence that they consume DBM in natural conditions (Hosseini 2007) suggest that DBM or small lepidopteran larvae are part of their normal diet. It is noteworthy that approximately 20 % of adult *C. transversalis* (Hosseini 2007) and 50 % of adult *Mi. tasmaniae* (Hosseini 2007; Hogendoorn[3] and Juen[4] 2009, personal communication) found in brassica crops in South Australia had specific-DNA of DBM in their gut content (Hosseini 2007).

There may be more than one reason why predators consumed DBM in the presence of high density of GPA. For instance, nutritional requirements may be better achieved by several prey species rather than by only one (Canard 2001), and evidence suggests that predators choose to eat certain prey to balance their aminoacid requirements (Symondson *et al.* 2002). For example, Dean and Schuster (1995) observed that *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) performed better when fed a diet consisting of the aphid *Macrosiphum euphorbiae* (Thomas) and the aleyrodid *Bemisia argentifolii* Bellows & Perring, than on single-species diet of either species. In addition, Evans *et al.* (1999) suggest that predatory insects frequently face low food supplies. Thus, the tendency of generalist predators to consume essential prey in conjunction with alternative prey, may improve their ability to capitalise on short-lived and scattered opportunities as they seek out suitable sites in which to reproduce. Moreover, Bilde and Toft (1998) found that in the field generalist predators are frequently in a state of hunger. As a result, simply because pests are suboptimal prey items does not necessarily translate to little or no biological control in the field, because they may readily consume these prey due to hunger (Harwood and Obrycki 2005).

According to Symondson *et al.* (2002) classifying non-specialist predators as either stenophagous, oligophagous or polyphagous is an uncertain process since the dietary breadth of most species is not known completely. In addition, the relationship between a predator and a prey species may be influenced by several variables, such as functional and numerical responses to prey density, the availability of alternative food resources, prey choice and the degree of polyphagy. The results from this study suggest that all three predator species may exploit aphids along with alternative prey, which may be part of their normal, or required, diet.

Generalist predators encounter a variety of prey types with different energetic values and costs associated with their capture and ingestion (Roger *et al.* 2000). Although alternative prey can improve growth parameters and biological control by some generalist predators, the

availability of it can detract biological control agents from feeding on the target pest if populations overlap temporally and spatially (Harwood and Obrycki 2005). The results from this study indicate that even though the presence of GPA in brassica crops may influence the consumption of DBM, the predators studied may display a low but consistent consumption of DBM, and may increase their consumption of this species in periods of scarcity of their preferred prey. As Harwood and Obrycki (2005) point out, when the availability of non pest food overlaps with pests, the potential role in controlling target pests may be reduced due to diverting their feeding efforts towards alternative prey.

Being so polyphagous, GPA is found widely in brassica and other crops and surrounding areas. In the South Australian region, both GPA and DBM are present in brassica crops all year round, although the population density of both species decreases in winter (Keller[7] 2009, personal communication). GPA tends to be more abundant in autumn, and generally associated with the basal, older leaves (Baker[8] 2009, personal communication). In the Auckland region GPA and DBM are present all year although populations of both species are very low in winter in vegetable crops (Walker[1] 2009, personal communication). Thus, during the whole year in South Australia and in the Auckland region the presence of GPA in brassica crops and surrounding areas probably allows the arrival, establishment and population growth of these and other generalist predators. In an analogous situation Eubanks and Denno (2000a) observed that when *G. punctipes* consumed a high quality alternative food, lima beans, their individual consumption on the target insect pests, *A. pisum*, was reduced. However, they also reported that numerical response of the predator population was positive and the predation rate on the target prey increased when the predator fed on the beans.

One question arising from this study is whether seasonal dynamics of GPA and other alternative prey contribute to or disrupt biological control of DBM by these and other similar generalist predators. For instance, a guild of generalist predators effectively suppressed *L. decemlineata* on potatoes only when the density of the alternative prey, GPA, was less than 5 aphids/plant, equivalent to that observed at the beginning of the season (Koss 2003, cited by Koss *et al.* 2004; Koss and Snyder 2005).

Furthermore, being essential prey for the predators studied, the presence of aphid species such as GPA may also explain why these predators arrive in crops. Sometimes generalist predators are attracted to crops by an alternative food source and consume a target pest that occurs in the same area. For instance, in alfalfa plots containing dandelion with high *C. maculata*

densities aggregating on pollen resources was associated with low densities of the pea aphid, *A. pisum*, resulting from increased predation (Harmon *et al.* 2000). Furthermore, according to Evans and England (1996), many aphidophagous predators aggregate in response to aphid density, but feed both on aphids and other co-occurring phytophagous insects. This was observed by Östman and Ives (2003), where *A. pisum* attracted nabids (*Nabis* spp.; Hemiptera: Nabidae) into fields that also contained a second prey, the leafhopper *E. fabae*. Similarly, the aphid *R. padi* on grasslands attracted coccinellids that also consumed nettle aphids *Microlophium carnosum* Buckton in the vicinity of the grasslands (Müller and Godfray 1997). And Evans and England (1996) observed increased consumption of the alfalfa weevil *H. postica* resulting from aggregation of the seven-spotted ladybird *C. septempunctata* in response to high density of pea aphids in alfalfa fields. Similarly, Ullyet (1947) suggested that many generalist predators, such as staphylinids, wasps of the genus *Polistes*, syrphids, crysopids, hemerobiids and anthocorids were attracted initially to brassica crops by aphids and switched to DBM as the aphid population declined. Thus, the presence of a preferred prey, GPA, may attract predators to brassica fields, which could result in increased utilization of DBM as an additional prey.

The simplified experimental arenas used in both experiments may have reduced the searching time and resulted in a higher consumption rate of both DBM and aphids by predators compared to natural environments, because encounters of the predators with prey were probably more frequent than in natural conditions. In addition, the small size of the Petri dish used in experiment 1, and especially in experiment 2 where there was a leaf disc, which most insects used as substrate, simulated an aggregated distribution of DBM larvae in densities much higher than might be found in commercial crops. In New Zealand, for example, the action threshold for DBM in cabbage is reached when 15% of the plants in a crop are infested by lepidopteran larvae (Beck and Cameron 1990b; Beck *et al.* 1992). In Australia the action threshold for this pest in broccoli and cauliflower crops varies according to the stage and commercial destiny of the crop and parasitism level, but it has an upper limit when 60% and 70% of plants in the crop contain at least one larva respectively (Hamilton *et al.* 2004).

Nevertheless, the results obtained in this study suggest that all the predators studied contribute to the control of DBM in brassica fields. Further studies under field conditions, considering the complexities of the environment, longer experimental periods and other species present in crops, will provide necessary information to understand and predict population dynamics of *P. xyllostella* and generalist predators commonly present in brassica crops.

Chapter 3 The presence of *Diadegma semiclausum* affects predation rate of diamondback moth by *Nabis kinbergii* and *Oechalia schellenbergii*

3.1 INTRODUCTION

The ecological mechanisms that regulate biological control are very complex and may involve a great variety of coexisting species from different trophic levels, which interact directly and/or indirectly (Price *et al.* 1980; Rosenheim *et al.* 1995; Rosenheim *et al.* 1999). For example, a variety of interactions occur between predatory species and parasitoids. Even though these interactions may have important implications for biological control, most of them are poorly understood (Price *et al.* 1980; Sih *et al.* 1998; Symondson *et al.* 2002).

An example of a direct interaction between natural enemies, where there is a direct lethal effect of one natural enemy on others (Schmitz *et al.* 1997; Preisser *et al.* 2007) is intraguild predation (IGP hereafter), *i.e.*, when two species of natural enemies that share a prey species, and therefore are potential competitors, also engage in predator-prey interactions with each other (Polis *et al.* 1989). Because IGP is a widespread interaction in nature (Arim and Marquet 2004), one of the risks of using generalist predators in biological control is that they may interfere with each other or with parasitoids (De Clercq 2002). For a start, endemic generalist predators may reduce the efficiency of augmentative biological control programs if they consume some individuals of the natural enemy that has been released (Symondson *et al.* 2002). For instance, IGP of lacewing larvae (*Chrysopa* spp.) by the hemipteran predators *Nabis* spp. (Nabidae), *Geocoris* spp. (Lygaeidae) and *Zelus* spp. (Reduviidae) resulted in an increased density of *Aphis gossypii* Glover (Hemiptera: Aphididae) in cotton, because predatory bugs were unable to compensate fully for the loss of lacewing predation (Rosenheim *et al.* 1993). Also, Snyder and Ives (2001) recorded a three-fold reduction in the parasitism rate due to IGP, and an increase in aphid population growth in the host-parasitoid-predator system that included the pea aphid *Acyrthosiphon pisum* (Harris), the braconid parasitoid wasp *Aphidius ervi* (Haliday) and generalist predatory carabid beetles in alfalfa. Furthermore, augmentative release of predatory mite populations established and grew by more than 60% on cotton after naturally occurring predators had been removed. However, in the presence of naturally

occurring hemipteran generalist predators the population density of this predatory mite was greatly reduced, but despite this, biological control of spider mites was not disrupted (Colfer *et al.* 2003).

Interactions between natural enemies can also be indirect, for example where the presence of a natural enemy alters the behaviour of prey in terms of foraging or habitat use, among other traits (Preisser *et al.* 2007), and the prey becomes more vulnerable to a second natural enemy (Losey and Denno 1998a; Losey and Denno 1998b). The reason for this is that mortality caused by natural enemies constitutes one of the most important forces of natural selection driving the evolution of herbivores (Sih 1980). Herbivorous insects have developed a variety of defensive strategies that involve both morphological and behavioural traits (Losey and Denno 1998b) to minimise the risk of being eaten while feeding (Sih 1980). For instance, different species of aphids remain motionless or drop to the ground when approached by their natural enemies or in response to alarm pheromones emitted by other conspecifics on the plant (Clegg and Barlow 1982). Lepidopteran larvae wriggle vigorously, thrash, roll or curl in response to the touch of a natural enemy (Gross 1993). For example, larvae of the pyralid *Uresiphita reversalis* (Guenée) spin down from a silk thread when attacked by an anthocorid predatory bug (Bernays 1997). Dropping behaviour has also been observed in the generalist predator *Coccinella septempunctata* (L.) when attacked by another generalist predator, the coccinellid *Harmonia axyridis* Pallas, which successfully escape predation by the latter (Sato *et al.* 2005).

Changes in behaviour induced by the presence of a natural enemy can affect ecosystems in several ways. For example, the presence of the parasitoid *A. ervi* facilitated the dispersal of the pea enation mosaic virus through inducing the escape behaviour of its vector, the pea aphid *A. pisum* (Hodge and Powell 2008). When attacked, this aphid dropped from the plant, moved away from the feeding site and colonised a new uninfected host plant, thereby spreading the virus within the crop. Another case of change in behaviour induced by a natural enemy was reported by Losey and Denno (1998a), where *A. pisum* dropped to the ground while escaping from the foliage predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), and became more vulnerable to the ground-foraging predator *Harpalus pennsylvanicus* (DeGeer) (Coleoptera: Carabidae). Also, Finke and Denno (2003) found that when the wolf spider *Pardosa littoralis* Banks (Araneae: Lycosidae) and the predatory bug *Tytthus vagus* Knight (Hemiptera: Miridae) were present in a system simultaneously, there was an increase in the population density of their common prey, the planthoppers *Prokelisia* spp. (Hemiptera: Delphacidae), in comparison

to when each natural enemy acted alone. The authors suggested that this population growth may be due to unidirectional intraguild predation on the mirid bug by the spider, since the mirid population density decreased. The authors suggest that there may be a behavioural component as well, consisting of mirid bugs emigrating from the habitat due to the perceived risk of predation by spiders.

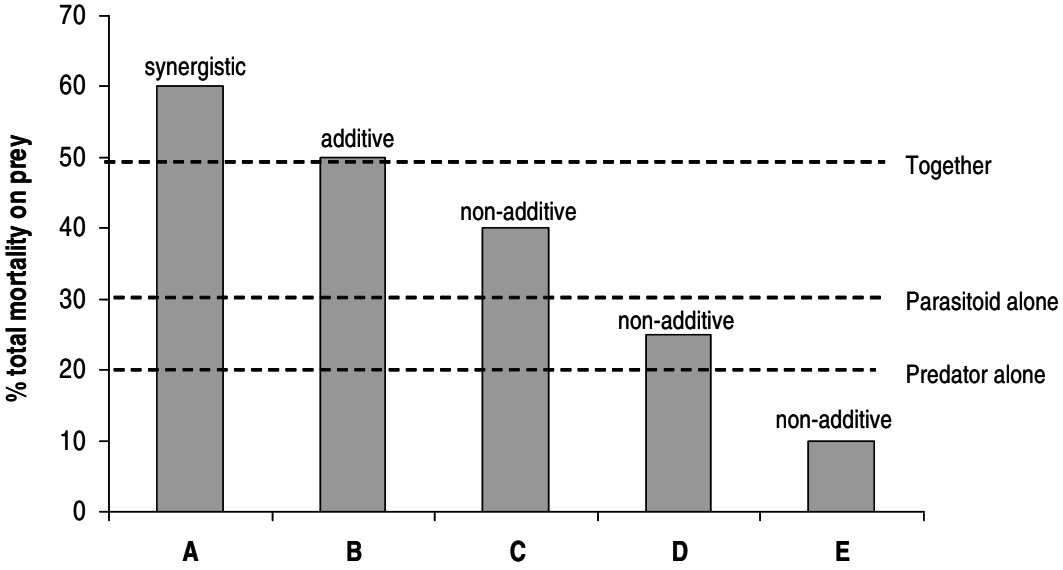


Figure 3-1 Possible outcomes when two natural enemies, for example a predator and a parasitoid, act together (Ferguson and Stiling 1996).

Whether it is by direct-lethal or behavioural factors, the interaction between natural enemies acting simultaneously may either improve (Sih *et al.* 1998; Cardinale *et al.* 2003; Snyder and Ives 2003), or disrupt biological control (Polis *et al.* 1989; Rosenheim *et al.* 1993; Sih *et al.* 1998; Finke and Denno 2003). Ferguson and Stiling (1996) described at least five recognizable outcomes when more than one natural enemy is released in the field (Figure 3-1). (A) Enemies could act synergistically, resulting in a higher than expected rate of mortality to the prey population. (B) Enemies might not interact so that the total level of mortality is equivalent to the individual mortalities combined (additive mortality). (C) Enemies could interact with one another to produce a level of total mortality less than additive mortality (non-additive mortality). (D) Total mortality might be less than that caused by one natural enemy alone, but not the other. (E) Total mortality could be less than when either natural enemy acts alone. So, we need to understand how the species present in brassica crops interact, and how this may

influence biological control of DBM, in order to manage the crop-systems in a way that those interactions that enhance control of the pest will be favoured.

Brassica crops host a diverse number of species of natural enemies of DBM. In a study by Hosseini (2007), numerous generalist predators, from at least two classes, six orders and ten families, were found in a broccoli field in the Adelaide region in South Australia, which could be potential predators of DBM. In addition, in Australia there are about 20 species of parasitoids (Waterhouse and Sands 2001), the most important being *Diadegma semiclausum* Hellen, *D. rapi* (Cameron), *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), *Apanteles ippeus* Nixon and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) (Wilson 1960; Goodwin 1979; Sarfraz *et al.* 2005). *Diadegma semiclausum* and *C. plutellae* have been successfully used in biological control programs of DBM in Australia (Wilson 1960; Goodwin 1979; Waterhouse and Sands 2001) and are used as model species for biological control studies.

DBM presents a characteristic defensive behaviour when attacked by natural enemies, including parasitoids, consisting of vigorously wriggling and moving backwards and sometimes spinning down from the leaf on a silk thread, remaining suspended for a while. Larvae may respond to vibrations of the leaf caused by parasitoids landing or other kind of movement, since larvae on the underside of leaves that could not have visual contact with the parasitoids on the topside of leafs showed the same behaviour (Wang and Keller 2002). Sometimes parasitoids wait for the host near the silk and attack it when it climbs back up to the leaf. Other times, parasitoids walk down on the thread and start a "fight" with the larva, which can result in both the parasitoid and the larva dropping to the ground. The parasitoid takes off and leaves the ground rapidly but it takes considerably longer for the larva to return to the foliage (personal observation). Despite the defensive behaviour of larvae, parasitoids of DBM are successful. For example, Wang and Keller (2002) recorded that 79% of encounters between *D. semiclausum* and DBM larvae resulted in hosts being parasitised and 65% of the stings were made at the first attack, while only 35% occurred after the first attack. DBM shows the same behaviour when attacked by other natural enemies, such as predators (personal observation) and the parasitoid *C. plutellae* (Wang and Keller 2002).

Oechalia schellenbergii Guérin-Méneville (Hemiptera: Pentatomidae) and *Nabis kinbergii* Reuter (Hemiptera: Nabidae) are two generalist predators frequently found in vegetable crops (including brassicas) in the Adelaide Region (Hosseini 2007). Nymphs and adults of both

predatory species consume lepidopteran larvae (Awan 1981; Siddique 1985). Through molecular analysis, DBM-specific DNA was detected in gut contents of over 90% of adult specimens of *O. schellenbergii* and *N. kinbergii* collected from a broccoli crop in the Adelaide region (Hosseini 2007). Despite their abundance in brassica crops, the interactions these predators establish with other natural enemies and their influence on DBM populations are unknown.

The objective of this work was to determine if, and how, the presence of a parasitoid affects behaviour of DBM larvae in ways that could affect their susceptibility to predation by *O. schellenbergii* and *N. kinbergii*.

For this, experiments were conducted to answer the following questions:
- How does the presence of a parasitoid (*D. semiclausum*) affect movement of DBM and their distribution on the plant?
- Is mortality rate of DBM additive when predators (*O. schellenbergii* and *N. kinbergii*) and *D. semiclausum* are present?
- Is the rate of coincidental IGP on *D. semiclausum* by *O. schellenbergii* and *N. kinbergii* through consumption of parasitised DBM within the first 24 hr of parasitism by *D. semiclausum* random or influenced by parasitism?

3.2 MATERIALS AND METHODS

Experiments were conducted in facilities of the Waite Campus, University of Adelaide, Australia, from October 2007 through April 2008.

3.2.1 PLANT AND INSECT CULTURES

3.2.1.1 Plants

See Chapter 2, Australian cultures.

3.2.1.2 Plutella xylostella

Culturing of DBM is described in Chapter 2, Australian cultures, for the experiments that included *C. transversalis* (Dec-May 2007). A sanitary problem arose in this DBM culture after

several generations due to the confinement of larvae with cabbage leaves in plastic containers. This favoured the development of pathogens in the experimental and stock cultures. Therefore these cultures were terminated and the following season (Oct 2007-Apr 2008) a new experimental culture was established using a different rearing method, which is described below.

Culture *P. xylostella* 2007-2008: The DBM culture was established using larval DBM collected from a cabbage field at Currency Creek (35°26'S, 138°46'E), South Australia in October 2007. Larvae were reared individually until adult emergence, as described in Chapter 2. The culture consisted of a screened cage (60 x 60 x 60 cm^3) for egg laying that always contained 100-150 adult moths and was kept at 20±1°C and in a 14L:10D photoperiod. Lighting in the rearing rooms was provided by the solid state ballasts and lamps described in Chapter 2. A single cabbage plant served as an oviposition substrate, which was replaced daily. Food was provided to the moths through a 100 ml cup filled with 10% sugar solution (Chapter 2). Periodically new adults were added as they were produced and the cage was replaced every 10-15 days. The plants bearing moth eggs were placed on a dated plastic tray (40 x 20 x 5 cm) on a bench in the same room. As DBM eggs hatched and small larvae emerged from mines, new plants were added to the tray. Each tray was periodically cleaned, new plants were added when needed, and the original plants were removed as the larvae ate most of the leaves and moved to new plants. Each tray was kept for the duration of the DBM lifecycle. This system allowed the production of a continuous supply of larvae of different ages. All larvae that were not used and became adults were placed in the egg laying cage (Figure 3-2). This system was more time consuming than the one used previously, especially as approximately 10-15 new plants were needed every day, but there were no further disease problems.

Figure 3-2 Eggs were laid by *P. xylostella* on the plant in the cage for 24 h. Then the potted plant was kept on dated trays to allow development of larvae until they reached the right size for the experiments.

3.2.1.3 Diadegma semiclausum

This culture of *D. semiclausum* was established using adults and parasitized DBM larvae collected from a cabbage field at Currency Creek (35° 26' S, 138° 46' E), South Australia in October 2007. Adults were identified and placed in a gauze rearing cage (60 x 50 x 60 cm) at 20±1°C and 14L:10D photoperiod. DBM larvae collected from the field were kept individually in 5 x 1 cm transparent plastic vials with a cotton wool plug and a fresh piece of cabbage leaf, which was replaced daily until formation of the parasitoid pupae. After verifying the identity of newly emerged adult parasitoids in the vials, they were added to the rearing cage. This cage contained two to four cabbage plants infested with DBM larvae in different stages; new non-infested plants were added as needed as old plants were being consumed by the larvae. A 100 mL cup of 20% sugar solution (Chapter 2) provided food for the adult parasitoids. When the first parasitoid pupae were observed on the plants, these were moved to an empty cage, and the cage with the parasitoid adults was replenished with new plants infested with non-parasitised DBM. To avoid inbreeding, all old *Diadegma* adults were replaced periodically by new ones collected in the field every three to four weeks, approximately.

For the experiments, cocoons were collected and placed individually in 5 x 1 cm transparent vials arranged on trays and left in the same room. Newly emerged *Diadegma* adults in the vials were sexed and transferred to 15 x 15 x 13 cm transparent vented plastic containers, separated by sex (Figure 3-3). A thin film of honey (1 x 2 cm) on one of the internal walls of each container and a cup of 20% sugar solution provided food to the parasitoids.

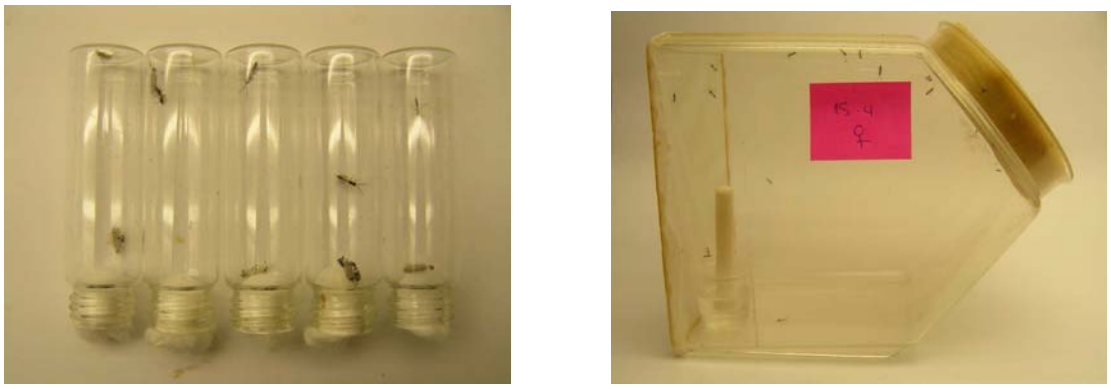


Figure 3-3 Adult *D. semiclausum* freshly emerged from individually kept pupae (left) were transferred to a container with a sugar solution as food, and separated by sex (right).

3.2.1.4 Nabis kinbergii

The culture of *N. kinbergii* was planned to be the same as the one described later in Chapter 4. However, pilot experiments showed that adults reared in a confined space did not consume any DBM larvae when transferred to a much bigger arena. Therefore, the system was modified and newly emerged nymphs were transferred to 60 x 50 x 60 cm gauze rearing cages containing between two and four cabbage plants (sometimes mixed with Pak Choi) infested with first and second instar DBM and green peach aphids. These cages were maintained at 20±1ºC and a 14L:10D photoperiod (Figure 3-4). Newly emerged nymphs were added to one cage for one week and then a new identical cage was started. Each cage was periodically cleaned and new DBM-infested plants and non infested plants were added. The size of the DBM offered to the predators increased gradually as the predator nymphs grew, making sure to always add plants with small larvae as well, since these grow faster than the predators. After the first sight of an adult nabid in a cage, this was kept for only 20-25 days, when a new cage was started. Generally there were four cages with predators at different stages simultaneously.

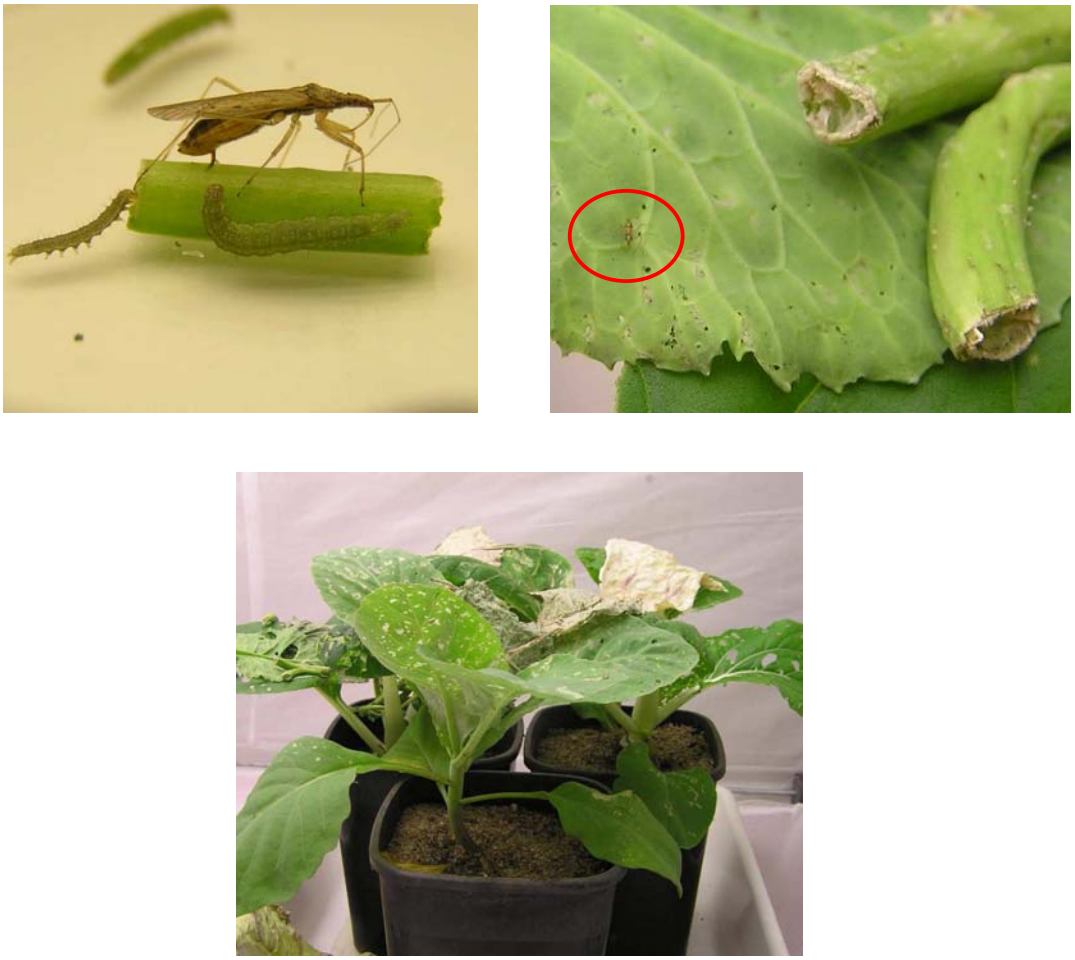


Figure 3-4 Female nabid inserting egg in cabbage stem (top left), nabid nymph on cabbage leaf with first instar DBM (red circle, top right) and cabbage plants in nabids rearing cage.

3.2.1.5 Oechalia schellenbergii

Oechalia schellenbergii did not consume larvae either when transferred from a small rearing container to a bigger arena. Therefore the rearing system for *O. schellenbergii* was also modified (also respect to Chapter 4), and small nymphs were transferred to gauze rearing cages after their first moult (Figure 3-5). The cages containing *O. schellenbergii* were also maintained in a room at 20±1°C and a 14L:10D photoperiod.

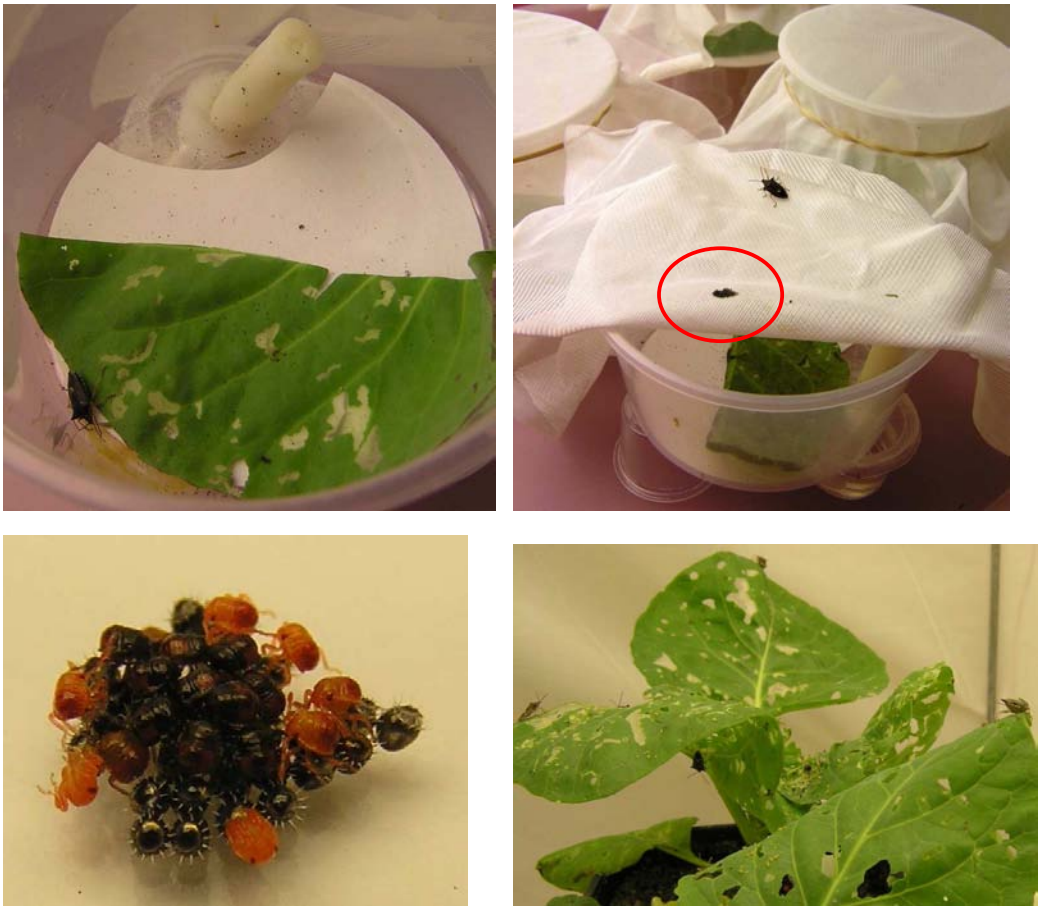


Figure 3-5 Eggs of *O. schellenbergii* were obtained in plastic containers (top) and second instar nymphs were transferred to the rearing cage (bottom).

The lighting system used in *D. semiclausum*, *N. kinbergii* and *O. schellenbergii* cultures, which simulated dusk and dawn conditions, is described in Chapter 2.

3.2.2 EXPERIMENTS

Two experiments were conducted at 20±1ºC and a 14L:10D photoperiod (see lighting system in Chapter 2, Australian cultures). To homogenize the predators' condition, prior to the beginning of the experiment, *N. kinbergii* and *O. schellenbergii* were starved individually for 24 h in 5 cm diameter Petri dishes with a filter paper disc (Whatman® n.2, 4.25 cm diameter) moisturised with two drops of tap water. All replicates were started at around 13:00 h and run for 24 h. In experiment 2, only females of *O. schellenbergii* and *N. kinbergii* were used, as

previous studies have reported that they are more voracious than males (Awan 1981; Ma *et al.* 2005; Quang 2007).

The experimental arena used for both experiments consisted of a 40 x 30 x 11 cm plastic tray containing soil and two cabbage plants covered with a 38 x 28 x 35 cm wire cage with three walls made of transparent polyethylene and one wall and the roof made of gauze. Twenty four h prior to the beginning of the experiment, leaves were trimmed to fit the cage so this could be installed and uninstalled without touching the plants (to avoid disturbing DBM larvae once they were settled on the plants). Plants within a cage did not touch each other. To standardise the size of the plants, excess leaves were eliminated, leaving each plant with only seven leaves. After setting the larvae on the plants the cage was installed and buried approximately 2 cm in the soil to prevent escape of insects. On one side of the cage a gauze sleeve allowed handling of the plants and insects (predators and parasitoids) without the need to remove the cage during the experiment. Inside one of the walls of all cages, a 2 x 2 mm thin film of honey provided food for the parasitoids, regardless of the treatment (Figure 3-6).

Figure 3-6 Experimental arena used in experiments 1 and 2.

Four h prior to the beginning of the experiment, 25 DBM larvae were placed on each plant so they could settle and start eating. Any larvae which fell from the plants during the setup process were carefully replaced with the help of a brush. In order to minimise superparasitism, preliminary experiments were conducted to establish the number of larvae that would be used in each cage, having as a criterion that about 50% should be parasitised by one *D. semiclausum* in 24 h in this arena. This number was calculated as 50 larvae/cage.

For all the treatments that included parasitoids, 24 h prior to the experiment an equal number of females and males of *D. semiclausum* (generally double of what would be needed) were taken from the culture and were allowed to mate in a 15 x 15 x 20 cm transparent plastic container for 24 h at 20±1ºC and a 14L:10D photoperiod. Inside the container, a 20 mL cup of 20% sugar solution dispensed through a cotton wick and a 2 x 1 cm thin film of honey on one of the sides served as food. After 24 h the males were removed, and 1 h prior to the beginning of the experiment the required number of females were taken randomly from the container and put individually in a plastic vial with a piece of cabbage leaf partially eaten containing DBM frass to stimulate them to search for hosts (Figure 3-7).

At the conclusion of both experiments, those larvae coming from cages with *Diadegma* were further reared for 24 h in a Petri dish with a piece of cabbage leaf and then dissected to determine parasitism rate.

Figure 3-7 Vial containing a *D. semiclausum* female and a piece of cabbage leaf with frass.

3.2.2.1 Experiment 1: Movement of DBM larvae in the presence/absence of D. semiclausum.

To evaluate whether the presence of a parasitoid modifies the behaviour of DBM larvae, leaves in different positions on the plant and larvae were identified with a colour code using a permanent ink pen (Staedler Lumocolor® 0.6 mm permanent dry safe, Art. Nr. 318 WP4, Made in Germany, ©STAEDLER Mars GmbH & Co. KG Moosaeckerstr 90427 Nuremberg, Germany), and distribution of larvae was monitored at the beginning and at the end of a 24 h period in the presence and absence of *D. semiclausum*.

Identification of plants and leaves

In each cage one plant was randomly designated "black" and the other "red". Cabbage leaves develop in a spiral manner and the smallest and curliest leaves are in the centre and the most expanded and oldest leaves are located more externally on the plant. The leaf in the centre of the plant is curled protecting the shoot apex. According to the position of each leaf in respect to the shoot apex, they were designated a colour, except for L_2 and L_7 (Table 3-1). For this, each leaf was marked with two colour dots on the base of the stem, one indicating the plant and the other indicating the position of the leaf. For example, "red-purple", was the third leaf from the shoot apex on a red plant and "black-blue" was the sixth leaf from the shoot apex on a black plant (Figure 3-8).

Table 3-1 Colour code for identification of plants and leaves.

Name of leaf	Position	Colour of leaf
L_1	Biggest curled leaf protecting shoot apex	Black/red, depending on plant
L_2	1st expanded leaf from shoot apex	No colour
L_3	2nd expanded leaf from shoot apex	Purple
L_4	3rd expanded leaf from shoot apex	Green
L_5	4th expanded leaf from shoot apex	Orange
L_6	5th expanded leaf from shoot apex	Blue
L_7	6th expanded leaf from shoot apex	No colour

Identification of larvae

The larvae were also identified by painting two dots on their dorsum: one on the extreme front end indicating the plant and another on the rear end indicating the position of the leaf on which they were placed. For example, on the red plant, leaf L_5 (red-orange), five larvae with a red and an orange dot were placed. The larvae on position L1-black plant were marked with two black dots and in the red one with two red dots. Larvae were used as early 4th instar, just after moulting, so the marks would not be lost during the experiment due to ecdysis.

The larvae corresponding to L_1 were set in the space formed by the curled leaf, while in all other positions larvae were set on top of the leaf (Figure 3-8). Five marked larvae were placed on each of the following leaves: L_1, L_3, L_4, L_5 and L_6. No larvae were put on L_2 and L_7 to avoid overcrowding the area close to the centre and also to give larvae more choices to migrate.

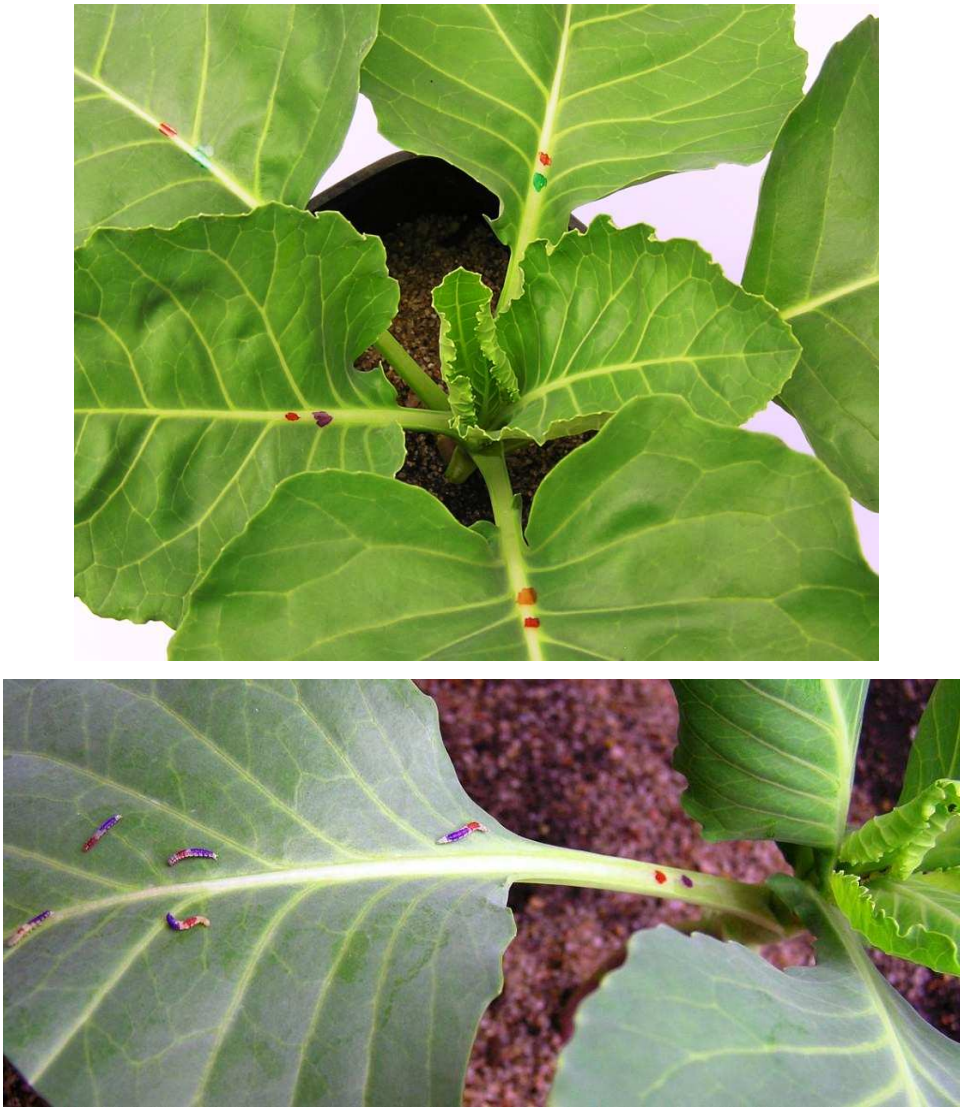


Figure 3-8 Colour code for identification of leaves in arena (top) and larvae on the "purple leaf-red plant" (bottom).

Treatments

Two treatments were applied:

1. *Diadegma* (*D* hereafter): One fertilised *D. semiclausum* female was released inside the cage.
2. No-*Diadegma* (*ND* hereafter): This was the control treatment; without a parasitoid released inside the cage.

The two treatment combinations were blocked by date and were replicated seven times (randomised complete block). For practical reasons a maximum of two blocks were done in each date. A further six replicates were conducted for the *D* treatment only. Every replicate was performed with new individuals.

The experiment began when one vial with one female parasitoid was introduced through the gauze sleeve inside each *D* cage and placed half way between the two plants. Then, the cotton ball was removed and the parasitoid was allowed to fly freely in the cage. After 24 h, cages of all treatments were carefully removed and the final position of DBM larvae was recorded according to their identification and the identification of the leaf where they were located. Those larvae coming from *D* cages were further reared for 24 h in a Petri dish with a piece of cabbage leaf and then dissected to determine parasitism rate.

Dissection consisted of grasping each DBM larva with forceps and pulling the body apart, and if necessary the body was squeezed carefully with forceps to expose all the body contents. The presence of the egg stage was then assessed using a dissecting microscope (Figure 3-9).

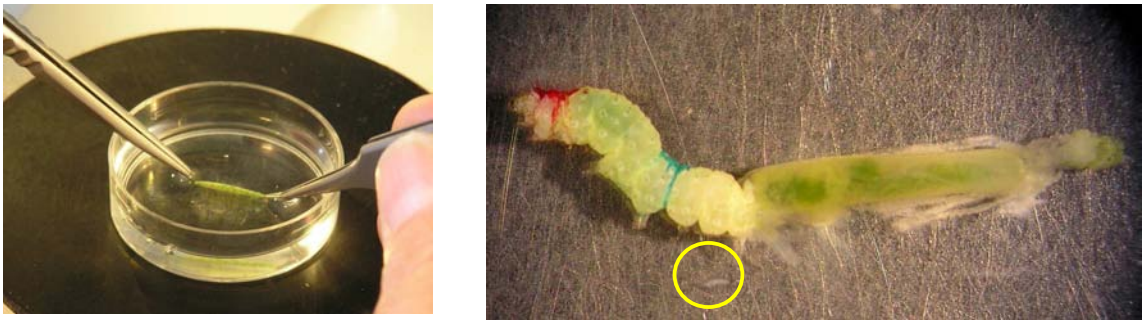


Figure 3-9 Dissection of DBM larva under the microscope (left), and dissected DBM larva and *D. semiclausum* egg (yellow circle, right).

DATA ANALYSIS

All data that consisted of percentages were arcsin-transformed (Zar 1999).

The percentage of DBM larvae that moved from their original position (leaf and plant) in cages with and without the parasitoid was compared using Student *t*-test (Zar 1999; SAS-Institute 2000).

The distribution of DBM larvae at the end of the experiment was analysed graphically by constructing charts of the distribution of larvae, which helped to identify some patterns of movement in both treatments.

Student *t*-test were used to analyse the relationship between incidence of parasitism, and the movement and position of the larvae within a cage in the *D* treatment, based on two parameters (Zar 1999; SAS-Institute 2000):

- Percentage of parasitism in DBM larvae that remained in their original position.
- Percentage of parasitism in DBM larvae that moved from their original position.

The Pearson correlation coefficient was calculated to explore whether there was any relationship between the percentage of parasitism and the percentage of DBM that abandoned their original position in *D* cages (Zar 1999; SAS-Institute 2000).

3.2.2.2 Experiment 2: Predation of DBM in the presence or absence of a parasitoid.

This experiment consisted of evaluating the predation rate of DBM by two predatory species (*N. kinbergii* and *O. schellenbergii*) in the presence and absence of *D. semiclausum*, and the parasitism by *D. semiclausum* in the presence and absence of these predators after 24 h. The studies on the two predator species with their respective combinations with the parasitoid were conducted as two separate experiments.

Twenty five DBM larvae (early 4th instar) were distributed on each plant in a similar manner as in experiment 1, but in this case larvae on L_1 were not set in the curled leaf but on top of L_1 and L_2, and neither leaves nor larvae were identified with a colour code at any position. The experiment began when the predator and parasitoid were released inside each cage through the gauze sleeve. After they had interacted for 24 h, cages were carefully removed and larvae that had survived were counted. Risk of predation was assessed by determining the number of larvae consumed (missing) at the end of the 24 h exposure period, i.e., the difference between the 50 larvae placed on each cage originally and the number of larvae found alive at the end of the experiment. Those larvae coming from *D* cages were further reared for 24 h in a Petri dish with a piece of cabbage leaf and then dissected to determine parasitism rate.

Treatments

The experiment consisted of six treatments that combined presence and absence of one of the predator species and the parasitoid (Table 3-2). The six treatment combinations were blocked by date and were replicated ten times (randomised complete block). For practical reasons only one block was done on each date. Every replicate was performed with new individuals.

Table 3-2 Number of replicates for each treatment (n) and number of DBM larvae and natural enemies used in each treatment.

Treatment	n	Number of insects in each treatment			
		DBM larvae	*D. semiclausum*	*O. schellenbergii*	*N. kinbergii*
T_1	10	50			
T_2	10	50	1		
T_3	10	50		2	
T_4	10	50	1	2	
T_5	10	50			3
T_6	10	50	1		3

DATA ANALYSIS

The predation rate was assessed by determining the number of larvae consumed at the end of the 24 h exposure period. The consumption of DBM larvae by each predator in the presence or absence of the parasitoid was compared using logistic regression. Before applying regressions, the raw data from treatments T_2 - T_6 were adjusted for missing DBM in the control treatment (T_1), using Abbott's formula (Abbott 1925).

No comparisons were done between the consumption of DBM larvae by *N. kinbergii* and *O. schellenbergii*, being beyond the scope of this work.

The percentage of parasitised larvae in the treatments that included only *D. semiclausum* (T_2) and those containing the parasitoid and either predator (T_4 and T_6) were compared using Logistic regressions (Genstat10.2 2007).

3.3 RESULTS

3.3.1 EXPERIMENT 1

3.3.1.1 Movement of DBM larvae on the plants

DBM larvae were observed to move to a new position on the plants in four ways (Figure 3-10):

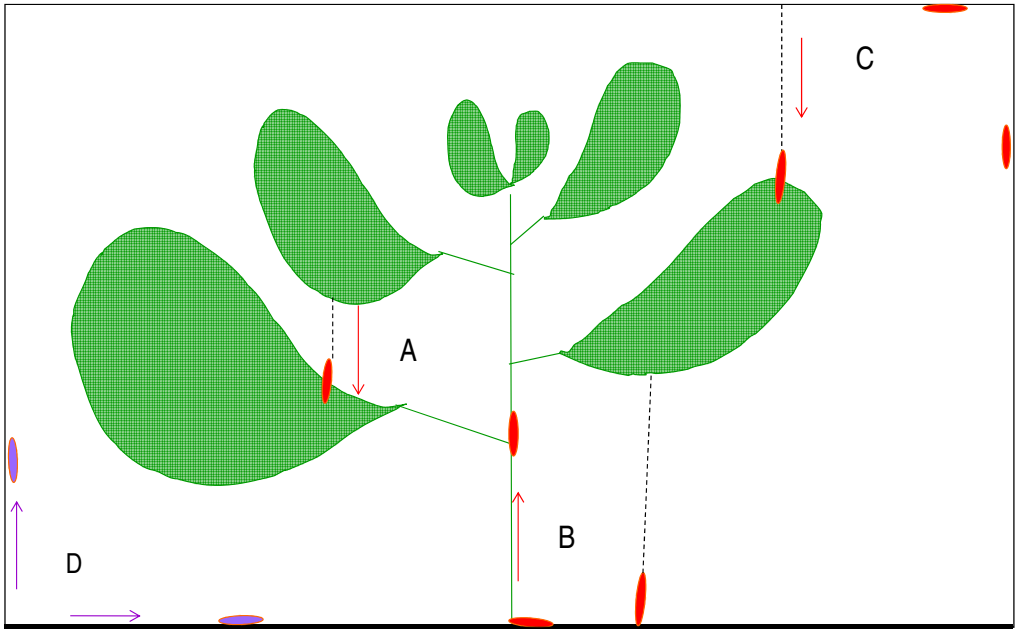


Figure 3-10 Scheme representing a cabbage plant and the observed ways in which larvae moved to other leaves within one plant (red figures, A, B and C), or to the other plant within a cage (purple figures, D).

- They hung from a silk thread landing on a leaf below.
- They hung from a silk thread and landed on the ground. Then they crawled back to the main stem of the plant, climbed up on it and got to a new position, generally close to L_1.
- Some larvae crawled in a different direction, reached an internal wall of the cage and climbed up to the ceiling of it, from where they dropped back to a plant, landing mainly on external, bigger leaves.
- Larvae from the other plant within the cage moved either via the ground or the cage walls.

In both treatments DBM larvae also reacted to the movement caused by other larvae approaching. Especially in the centre of the plant, where there was limited space, there was a

fast circulation of larvae caused by these larvae climbing a plant via the main stem. This occurred more frequently in *D* cages because the attack of the parasitoids triggered a chain reaction, causing more larvae to move and disturb others (personal observation).

3.3.1.2 Movement of DBM larvae from its original position in the presence and absence of a parasitoid

Larvae moved from their original position in both treatments. The majority of migrations occurred within the same plant, but a smaller proportion moved to the other plant within the cage. The presence of the parasitoid caused an incremental in the activity of DBM larvae and a significantly higher percentage of these larvae had left their original position (either leaf or plant) after 24 h in cages with *D. semiclausum* compared with those without the parasitoid (79 and 40 % respectively) (Figures 3-11 and 3-12).

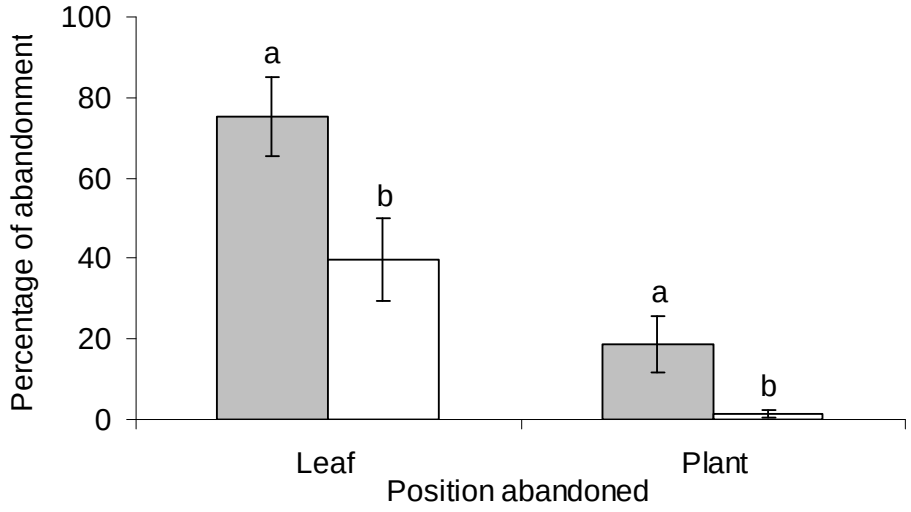


Figure 3-11 DBM larvae (% ± 95 CI) that had moved from the original leaf/plant after 24 h in cages with (grey bars) and without (white bars) *D. semiclausum*. Different letters on the bars within the same position indicate significant differences (*P*<0.0001, df=6).

3.3.1.3 Redistribution of larvae on plants

From the 25 DBM larvae that were set on each plant at the beginning of the experiment, an average of 24.1 and 24.7 larvae were recovered after 24 h on plants in the *D* and *ND* treatments, respectively. The original distribution of larvae had changed at the end of the experimental period in both treatments.

Larvae left the most external leaves and moved towards the centre of the plant, especially in the presence of the parasitoid. At the beginning of the experiment only 20% of larvae on a plant were located on leaves L_1-L_2 (Figure 3-13B). After 24 h, 40% were found on those positions in the *D* treatment, whereas only 26% occupied this position in the *ND* cages (Figure 3-13C and D). In addition, in both treatments larvae had occupied L_2 and L_7, but while 23 and 15% moved to L_2 (internal leaf), only 2 and 1% moved to L_7 in the treatments *D* and *ND*, respectively (Figure 3-13C and D, Table 3-3).

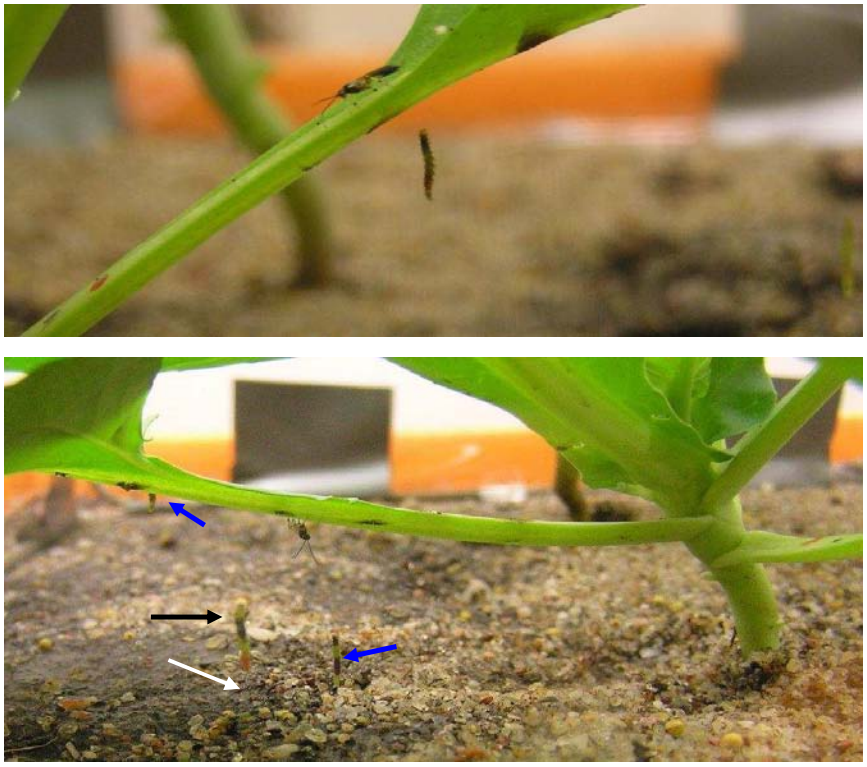


Figure 3-12 Larval DBM hanging from a silk thread in the presence of the parasitoid (top). *D. semiclausum* and DBM larvae that left their original position on plants. Two larvae are hanging from the plant (blue arrows), one is crawling on the internal wall of the cage (black arrow) and one is on the ground (white arrow) (bottom).

In the *D* treatment the positions that lost the biggest percentage of larvae with respect to the original number were L_6 (-16%), followed by L_5 (-7%) and L_1 (-3%). In the *ND* cages it was L_1 (-9%) followed by L_6 (-8%) and L_2 (-5%). For both treatments, the positions that gained the biggest proportion of larvae was L_2 (23 and 15% for *D* and *ND*, respectively) followed by L_3 (2 and 3%) (Figure 3-13).

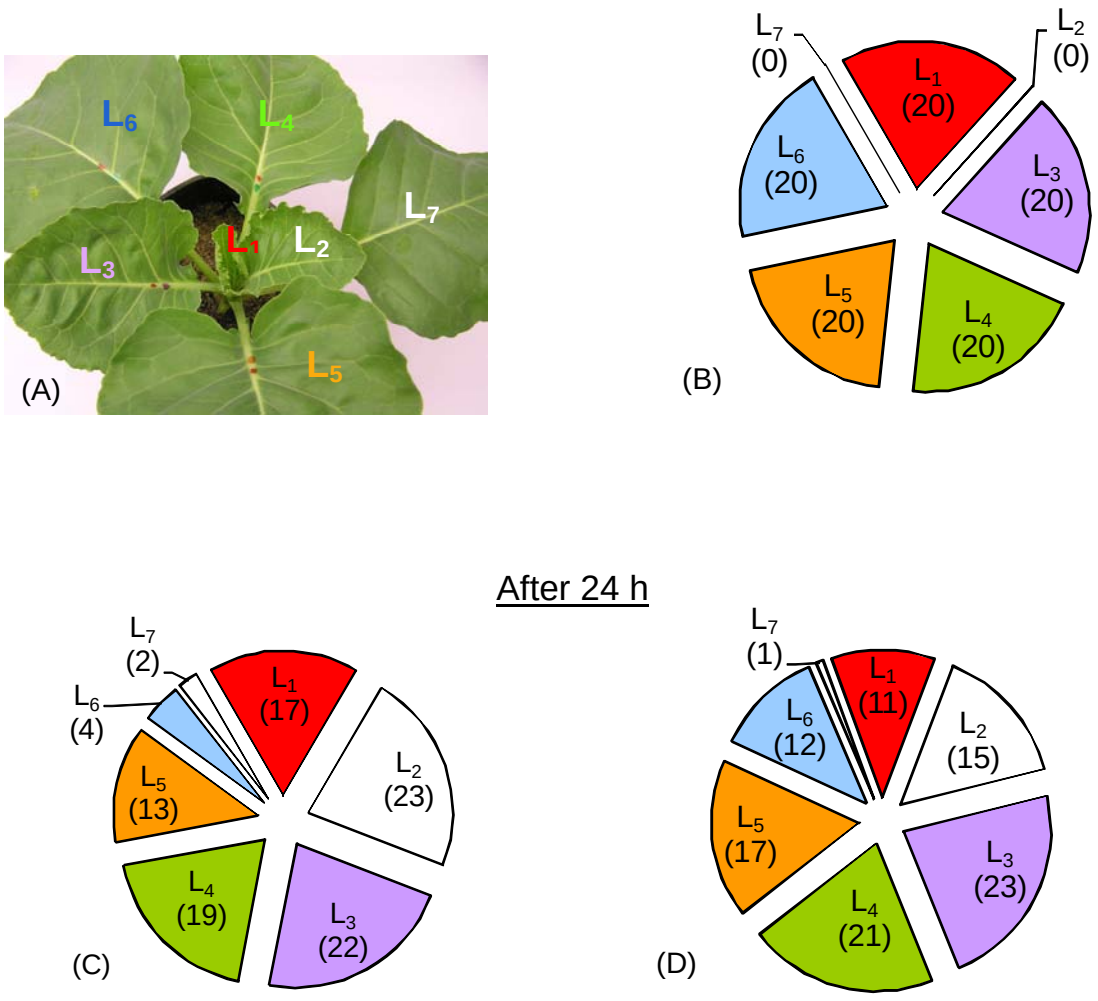


Figure 3-13 Position of leaves on experimental plant (A), and distribution of DBM larvae on leaves L_1 - L_7 on plants at the beginning of the experiment (B), after 24 h on plants from the *Diadegma* treatment (C) and after 24 h on plants from the without *Diadegma* treatment (D). The numbers in brackets indicate the percentage that larvae found on each position represent out of the total number of larvae found on a plant.

Figure 3-14 represents the composition of DBM larvae on each position according to their origin. While in *ND* cages an average 36% of larvae on L_1 came from a different leaf within the same plant, in *D* cages 69% of larvae on this position had migrated from either the same plant (51%) or from the other plant (18%).

In *D* cages an average of about 20% of the DBM larvae found on a plant came from the other plant within the cage, while in *ND* cages this was only 1% (Table 3-3). In addition, the percentage of DBM larvae that remained in their original position was higher in *ND* than in *D* cages in all the positions (white piece in each chart; Figure 3-14).

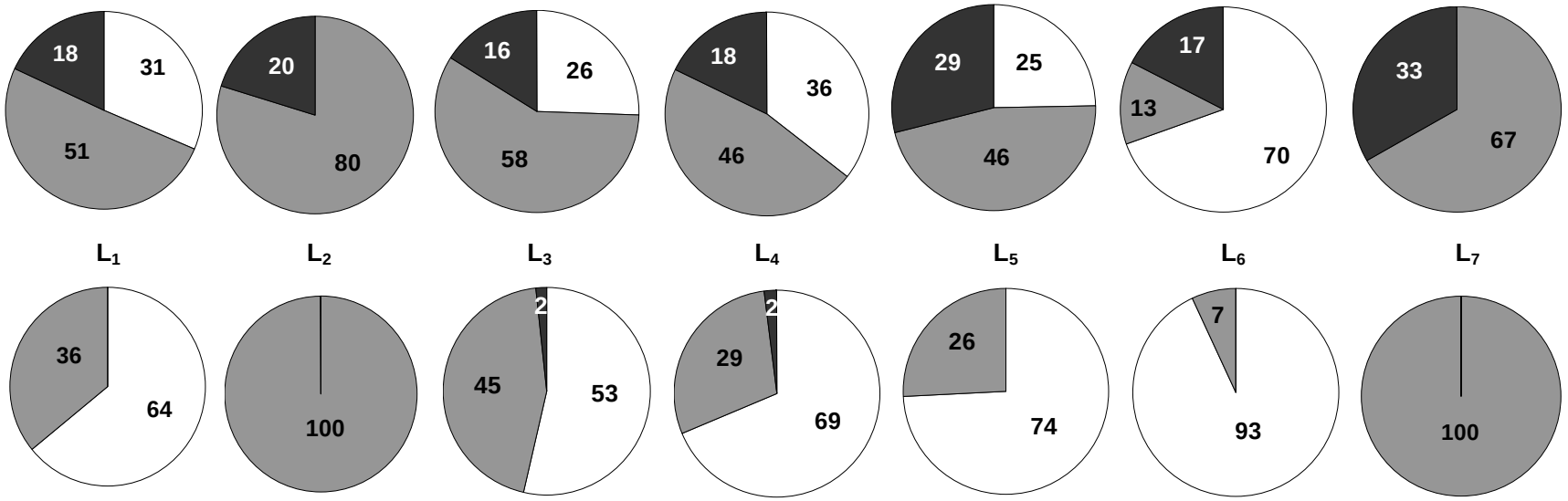
In the *ND* treatment, with the exception of L_1, the percentage of original DBM larvae on a given position increased gradually as the leaf was located more externally, and in both treatments L_6 had the biggest proportion of original larvae at the end of the 24 h period (Figure 3-14, Table 3-3).

3.3.1.4 Parasitism and movement of DBM larvae in the D treatment

In cages with the parasitoid, the percentage of DBM larvae that had moved from their original position was significantly higher than those that did not (77 and 23% respectively; $P<0.0001$), and at dissection, the percentage of parasitism among those larvae that had moved was significantly higher than those that had remained on their original position (53 and 33%, respectively; $P=0.0001$; df=12).

Parasitoids in different cages had different levels of activity, and also, different levels of parasitism were observed when dissecting larvae from cages with the parasitoid. Parasitism varied from 8.3% to 91.7% (mean 49.8 ± 11.7 95CI). A Pearson correlation coefficient of 0.85 indicated a correlation between the percentage of DBM larvae that were parasitised and the percentage of larvae that moved from their original position in *D* treatment cages (n=13).

With *Diadegma*



Without *Diadegma*

Figure 3-14 **Composition of DBM larvae according to their original position on leaves L_1 – L_7 after 24 h on plants from the *Diadegma* treatment (top row of charts) and without *Diadegma* treatment (bottom row of charts). The numbers indicate the percentage of the total of larvae found on a leaf, which either remained at the original position (white), migrated from another position within the same plant (grey), or that migrated from the other plant in the cage (black).**

Table 3-3 Number of DBM larvae on each position at the end of the experiment (rows) according to their original position (columns) in cages from *Diadegma* and *No Diadegma* treatments (number ± SD).

Position at end	Position at beginning					
	L_1	L_3	L_4	L_5	L_6	other plant
Diadegma treatment						
L_1	1.27 ± 1.27	0.72 ± 0.88	0.45 ± 0.67	0.41 ± 0.50	0.45 ± 0.73	0.72 ± 0.98
L_2	1.27 ± 1.03	0.77 ± 0.68	0.72 ± 0.98	1.04 ± 0.89	0.5 ± 0.74	1.09 ± 1.19
L_3	0.50 ± 0.80	1.36 ± 1.29	0.77 ± 0.61	0.95 ± 0.99	0.86 ± 0.88	0.86 ± 1.28
L_4	0.45 ± 0.59	0.59 ± 0.79	1.63 ± 1.13	0.54 ± 0.67	0.54 ± 0.85	0.81 ± 1.05
L_5	0.30 ± 0.56	0.45 ± 0.59	0.22 ± 0.48	0.77 ± 0.86	0.45 ± 0.59	0.91 ± 1.11
L_6	0.00	0.04 ± 0.21	0.00	0.09 ± 0.29	0.72 ± 1.03	0.18 ± 0.39
L_7	0.13 ± 0.46	0.00	0.09 ± 0.29	0.05 ± 0.21	0.09 ± 0.29	0.18 ± 0.50
No-*Diadegma* treatment						
L_1	1.80 ± 1.61	0.20 ± 0.42	0.40 ± 0.51	0.20 ± 0.42	0.20 ± 0.63	0
L_2	1.60 ± 1.17	0.90 ± 0.87	0.20 ± 0.42	0.70 ± 0.67	0.40 ± 0.69	0
L_3	0.80 ± 1.03	3.00 ± 1.49	0.30 ± 0.48	1.70 ± 0.82	0.70 ± 1.05	0.10 ± 0.31
L_4	0.30 ± 0.67	0.40 ± 0.69	3.50 ± 1.26	0.10 ± 0.31	0.70 ± 1.25	0.10 ± 0.31
L_5	0.50 ± 0.97	0.22 ± 0.44	0.20 ± 0.42	3.20 ± 1.03	0.20 ± 0.42	0.00
L_6	0.00	0.20 ± 0.42	0.00	0.00	2.70 ± 1.49	0.00
L_7	0.00	0.00	0.20 ± 0.42	0.00	0.00	0.00

3.3.2 EXPERIMENT 2

3.3.2.1 Predation rate in the presence and absence of the parasitoid

As in experiment 1, there was a small proportion of larvae missing from treatments T_1 (control) and T_2 (*Diadegma* only), where on average the recovery was 98.8 and 96.8%, respectively. However, in the treatments that included the predators, the percentage of larvae missing was higher. Moreover, in the presence of the parasitoid, the predation rate increased, and significantly more individuals were consumed when a parasitoid and a predator were present at the same time (P<0.001, df=4; residual mean deviance=1.4, df=36; Figures 3-15 and 3.16). In T3 an average of 6.7 larvae were consumed by each *O. schellenbergii*, while in T4

(*O. schellenbergii* + *D. semiclausum*) this number significantly increased to 10.5 larvae (60% more, 3.35 and 5.25 larvae/predator, since the cage had two individual predators) (*P*=0.01) (Figure 3-15). In T_5 an average of 7.6 larvae were consumed by *N. kinbergii,* while in T_6 (*N. kinbergii* + *D. semiclausum*) this number significantly increased to 12.6 larvae (70% more, 2.5 and 4.2 larvae/predator, since in this case there were three individual predators in each cage) (*P*=0.002) (Figure 3-16).

3.3.2.2 Parasitism rate in the presence and absence of predators

Despite the significantly higher consumption of DBM larvae by both predators in the presence of *D. semiclausum*, the parasitism rate by this parasitoid when either predator was present (means of treatments T_4 and T_6) was similar to that obtained in the absence of any predator (T_2, 56%) (t=0.29, df=1, *P*=078, Residual df=18, residual deviance= 3.73). The parasitism rate in the presence of *O. schellenbergii* (T_4, 53.6%) was similar to that in the presence of *N. kinbergii* (T_6, 55.4%) (t= -0.27, df= 1, *P*=0.79) (Figure 3-17).

3.3.2.3 Combined effect of predation and parasitism

In cages with *D. semiclausum* (T_2, T_4 and T_6), approximately 55% of larvae that were recovered were parasitised. If all parasitoids completed development within the parasitised hosts and eventually killed them, then the total mortality in each treatment would be the result of the predation (or larvae missing after 24 h) plus the percentage that would die later due to parasitism. In cages with *O. schellenbergii* and *N. kinbergii* alone, total mortality reached 13.4 and 15.2%, respectively. If all parasitoids killed the parasitised hosts, mortality would reach 57% when *D. semiclausum* acted alone and 63.2 and 66.6% when the parasitoid acted in combination with *O. schellenbergii* and *N. kinbergii*, respectively (Figure 3-18).

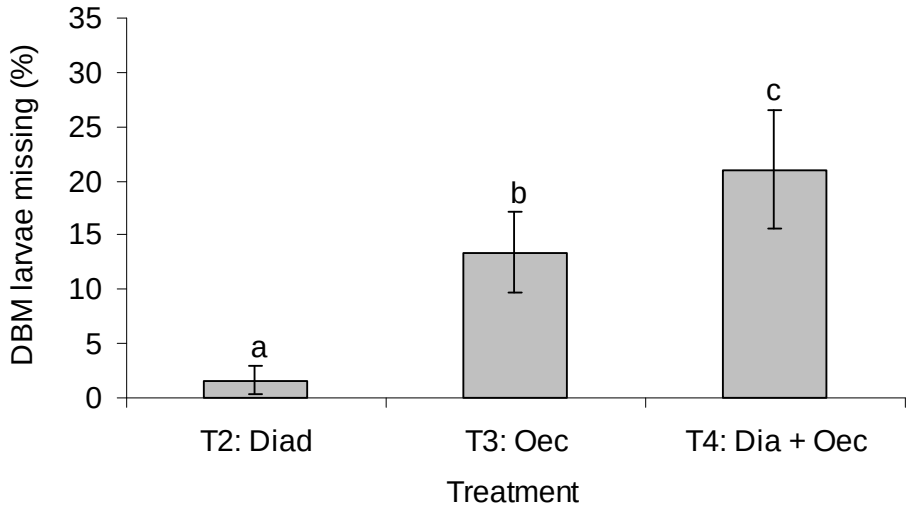


Figure 3-15 DBM larvae missing at the end of the experiment in treatments that included *D. semiclausum* and/or *O. schellenbergii* (% ± 95 CI).

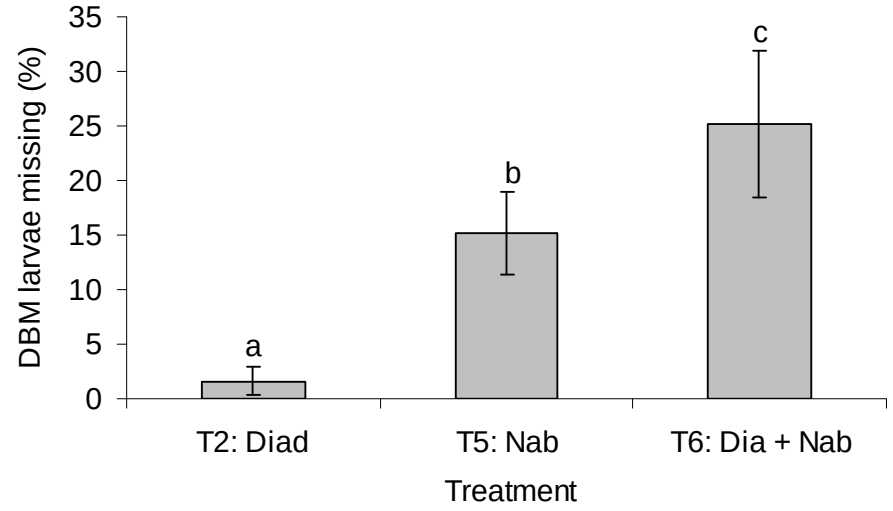


Figure 3-16 DBM larvae missing at the end of the experiment in treatments that included *D. semiclausum* and/or *N. kinbergii* (% ± 95 CI).

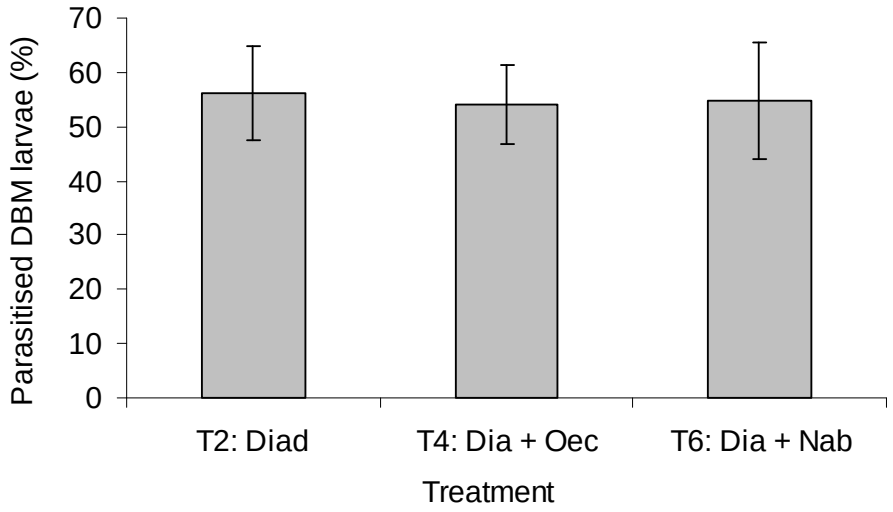


Figure 3-17 Parasitism rate of DBM larvae in treatments that included only *D. semiclausum* or *D. semiclausum* with *O. schellenbergii* or *N. kinbergii* (% ± 95 CI).

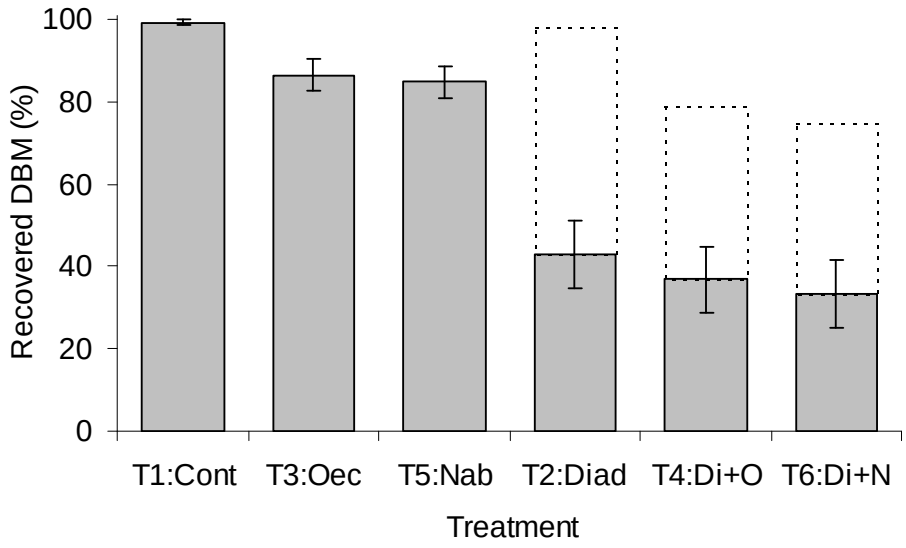


Figure 3-18 Non parasitised (grey bars) and parasitised DBM larvae (white bars with dotted line) recovered from treatments that included only *D. semiclausum* (T2), *O. schellenbergii* (T3) or *N. kinbergii* (T5), or *D. semiclausum* with *O. schellenbergii* (T4) or *N. kinbergii* (T6) (% ± 95 CI). The lowest percentages of recovery (or higher total mortality) would be achieved in the cages with both a parasitoid and a predator together (T4 and T6).

3.4 DISCUSSION

The co-occurrence of the parasitoid and the predators in this multi-species system resulted in two main interactions. On the one hand there was a behaviourally-mediated interaction, as the higher movement activity caused by the presence of the parasitoid made DBM larvae more vulnerable to predation. On the other hand there was coincidental IGP on juvenile *D. semiclausum*, as both *N. kinbergii* and *O. schellenbergii* consumed parasitised DBM larvae. The final outcome of the coexistence of these natural enemies was enhanced pest mortality above and beyond the sum of predation and parasitism alone.

Movement and redistribution of larvae on the plants in the presence and absence of *D. semiclausum*

In the first experiment, even though the movement rate of DBM larvae was significantly higher in the presence of the parasitoid, larvae also tended to move and redistribute on the plant in the absence of *D. semiclausum,* although somewhat less frequently. In both treatments larvae tended to move mainly towards the central leaves. Plants used in this study had only seven leaves and the area most protected was the shoot apex. However, this structure was still small and could only harbour a few larvae, so the rest of the larvae had to occupy neighbouring leaves. Thus, L1-L3 started with 20% of larvae and ended with 40% and 26% in treatments with and without the parasitoid, respectively. This plant architecture only lasts a few days and it is likely that in a more developed plant the forming head would offer more space and protection for larvae.

The observations reported here are consistent with the behaviour of DBM in the field. Beck and Cameron (1990a) observed a similar trend in larval location on cabbage, broccoli and cauliflower plants in the field. They reported that on cabbage plants the movement of larvae started after the plant had more than seven leaves and before head formation was initiated, and throughout the growing season 50-90% of DBM larvae were located in the shoot apex/head area, depending on the growth stage of the plant. In the same study, on cauliflower plants, at least 50% of DBM larvae moved to the shoot apex/inner leaf area when the plants had grown eight-ten leaves. On broccoli the movement of larvae towards the centre of the plant started later, and only when the florets were harvestable were more than 50% of the larvae found in this structure. In addition, after floret formation in either broccoli or cauliflower, large DBM larvae were found mainly in the floret area, whereas small larvae were distributed preferentially on mid leaves (Beck and Cameron 1990a). Momanyi *et al.* (2006) also

observed a strong tendency of DBM larvae to move towards the tender part of cabbage plants. The authors suggested this could be due to a better nutritional quality of the growth area, at least when the plants are small.

In the cages with the parasitoid, more DBM larvae moved to the other plant in the arena than in the cage without the parasitoid. In the cage without the parasitoid, where more larvae tended to remain in their original position, this trend was more pronounced on the external leaves. Observation revealed that the higher rate of physical contact between DBM larvae in the more central leaves in both treatments elicited the escape response more frequently in this part of the plant where larvae tended to move more. Momanyi *et al.*(2006) suggested that parasitoid interference with DBM larvae is responsible for more larval mortality than just those killed by parasitism, because leaving the plant may cause larvae to perish. In fact, in real crop conditions, a higher movement rate would probably expose more larvae to ground predators or lead to mortality related to very dry, wet or hot soils.

Predation rate in the presence and absence of the parasitoid

In the presence of the parasitoid both predators consumed almost twice the number of DBM larvae compared to predators alone. It is likely that mobility of the prey makes it more easily detectable, as the parasitoid elicits frequent escape behaviour in DBM. *Diadegma semiclausum* is the most common parasitoid of DBM larvae in South Australia. However DBM displays the same behaviour towards other natural enemies (personal observation). According to Sih *et al.* (1998), the effect of multiple natural enemies generally results in risk enhancement for the prey when its defences against one predator puts the prey at greater risk of being killed by another predator.

Different predatory arthropods utilise different ways to locate their prey: olfaction, vision and physical cues such as silk webbing of host or prey, or leaf mines made by a suitable prey. Madsen *et al.* (2004) observed that the spider *Pardosa prativaga* (L. Koch) was unable to locate *Tomocerus bidentatus* (Folsom) due to the motionless behaviour of this collembolan prey. Furthermore, planthoppers reduced their mobility in the presence of *Pardosa* sp. spiders (Finke and Denno 2005). Finke and Denno (2003) suggest that the specialist predatory mirid *Tytthus vagus* Knight are under bigger risk of predation than planthoppers by lycosid spiders because these spiders are visually oriented and detect their prey by movement and vibration. And while planthoppers remain motionless for long periods feeding from the plant, these predatory mirids are active foragers searching for prey to eat. Also, the predatory bug *Geocoris punctipes*

(Say) (Heteroptera: Geocoridae) consumed preferentially pea aphids (*A. pisum*) over eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), even though the aphid was the nutritionally inferior prey. In addition, this predator attacked mobile aphids preferentially when offered both mobile and immobilised aphids (Eubanks and Denno 2000b).

Movement seems to be important in perception of prey by *O. schellenbergii*. In a study by Awan (1981), this predator took significantly less time to locate and orientate towards a moving caterpillar than a stationary one and attacked moving larvae significantly more often than stationary ones. When offered moving caterpillars these predators seldom used their antennae to touch the prey prior to attack. However, when presented to stationary caterpillars they used their antennae more frequently to perceive their prey. In the case of *N. kinbergii*, this predator mainly uses olfactory stimuli to locate and recognise prey from a long distance, although they also use vision and tactile cues for short range perception (Siddique 1985). When not in motion DBM larvae were very cryptic on the leaf background (personal observation), and a cryptic animal can avoid visually oriented predators as long as it remains motionless (Lima and Dill 1989). However, when moving, DBM larvae were much more visible against the ground, the cage screen or even on the leaves (personal observation).

It is important to note that this experiment was performed in arenas with a simplified vegetation structure, and few layers of leaves overlapped. In real crop conditions more leaves would overlap and larvae that dropped from a leaf would have more chances to land on another leaf from the same or a neighbouring plant, without the need to go back to the plant, and therefore move less. In addition, a more complex plant structure would provide DBM larvae with more refuge from predators or parasitoids. So these results should be considered as habitat dependent and influenced by the growth stage of the crop. In a study with different brassica species, Beck and Cameron (1990a) found that cabbage, the brassica species with comparatively the most closed and protected framework, harboured the highest number of DBM pupae and had the lowest rate of parasitism of those pupae, whereas broccoli, the species with the most open framework, harboured the least number of DBM pupae, and had the highest rates of parasitism. Beck and Cameron (1990a) conclude that because DBM pupae are sessile (unlike larvae that are highly mobile), the open florets of broccoli plants would give pupae less protection against natural enemies. Another similar case is that recorded by Finke and Denno (2003), who observed high predation in experiments with the cordgrass *Spartina* spp. performed in artificial arenas with no litter. However the same species performed differently in studies with a more complex vegetation structure, which included litter and

therefore refuge, decreasing the amount of predation (Finke and Denno 2002). Also, Snyder and Ives (2001) observed a strong interaction between ground carabids, aphids and parasitised aphids when alfalfa plants were short (just after cutting), while interactions were weaker when plants had grown and the structure of the crop was more complex.

Combined effect of predation and parasitism

Despite the higher predation rate in the presence of *D. semiclausum*, the parasitism rate recorded suggests that there is no preference or different vulnerability to predation of DBM larvae within the first 24 h of parasitism. This was also described by Stark and Hopper (1988), who reported that larvae of *Chrysoperla carnea* (Stephens) did not feed preferentially on larval *Heliothis virescens* (F.) that were parasitised by *Microplitis croceipes* (Cresson) with respect to unparasitised larvae, at least within the first 72 h of parasitism.

Rosenheim and Harmon (2006) suggested that coincidental IGP has less potential to disrupt biological control than omnivorous IGP because in coincidental IGP there are less chances that the IG-predator can distinguish between parasitised and unparasitised prey, especially at an early developmental stage of the parasitoid. These authors suggest that coincidental IG-predators should impose similar or lower levels of mortality on the intermediate predator population than on the herbivore population. For example, despite the negative impact of the coccinellid *Hippodamia convergens* Guérin–Menenville on aphid parasitoids through coincidental IGP, aphid population suppression was higher when both natural enemies acted simultaneously than alone (Colfer and Rosenheim 2001). The lack of differences in parasitism between treatments with and without predators in our study suggests that predators do not feed preferentially on parasitised larvae, therefore coincidental IGP on the parasitoid should not disrupt biological control by *D. semiclausum*.

According to Losey and Denno (1998b), in agroecosystems where prey defensive behaviour towards one natural enemy species affects its susceptibility to other natural enemies, the potential for interactions between natural enemies in significantly suppressing pest populations will be higher. For example the authors observed that *A. pisum*, an aphid species that drops off its host plant in the presence of a foliar predator, was consumed significantly more by both ground-foraging and foliar-foraging predators when both were present, compared to when only the foliar foraging predator was present. On the other hand, *A. kondoi* Shinji, which is much less likely to initiate escape behaviour, was under more risk to be consumed by a foliar-foraging predator when both natural enemies were present.

For synergistic predation to occur by members of a predator complex, three key elements are necessary (Losey and Denno 1999): (1) escape behaviour by the prey induced by the attack of predators and habitat shifting; (2) synchrony of predators in the habitat; and (3) minimal negative interactions between predators (intraguild predation or interference). In our study, habitat shifting is probably not the reason why DBM larvae were attacked by predators more frequently in the presence of *D. semiclausum,* but the fact that these larvae became less cryptic while moving. DBM larvae would probably be under a higher risk of mortality if the system included ground-foraging predators that could attack larvae before they climbed back to a plant. The effects of additional kinds of trophic interactions like this require further investigation.

Even though it is positive that the presence of the parasitoid increased mortality by predation, the movement of DBM larvae towards the centre of the plant may increase the risk of these larvae consuming the growth tips. This could be potentially very detrimental to the development of the plants, causing increased economic losses to certain crops (Walker[1] 2008, personal communication). This is an issue that would also require further study.

The larval density used in these experiments were established to ensure that parasitism by *D. semiclausum* would not exceed 50% on average, avoiding excessive superparasitism, thus excluding other factors that may affect DBM mortality or parasitism. The number of larvae per plant used in this study is much higher than what would normally be found in field crops. In New Zealand, for example, the economic threshold for DBM in cabbage is reached when 15% of the plants in a crop are infested by caterpillars (Beck and Cameron 1990b; Beck *et al.* 1992). In Australia the action threshold for this pest in broccoli and cauliflower crops varies according to the stage and commercial destiny of the crop and parasitism level, but it has an upper limit when 60% and 70% of plants in the crop contain at least one larva respectively (Hamilton *et al.* 2004). It would be expected that results would differ at lower DBM densities as a result of changes in the behaviour of both natural enemies and pests. Functional responses of predators (Ma *et al.* 2005) and less frequent meetings of natural enemies and larval DBM would probably elicit the escape behaviour less often, allowing these to remain less visible.

This study looks at the interaction between *D. semiclausum* and predators. However it only considers the first 24 h of parasitism. This interaction may change and there may be different levels of intra-guild predation on the parasitoid as it develops inside the DBM larvae over the

number of days before pupation. In Chapter 4, it was observed that more advanced parasitism makes DBM more vulnerable to predation by a coccinellid. Higher levels of intraguild predation by some predatory species could be quite negative to, not only this, but also populations of other species of parasitoids. For example, in Gisborne, an isolated region in New Zealand, *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) has failed to establish because there have not been enough parasitised *P. rapae* larvae to overwinter the parasitoid population successfully, probably due to the high levels of predation in autumn devastating the parasitoid life stage inside the caterpillars. This resulted in not enough diapausing cocoons of *C. rubecula* to establish a population the following spring and entomologists have failed to establish this parasitoid in this region (Walker[1] 2009, personal communication).

There is no doubt that this work should be repeated under natural conditions and for a longer period, because monitoring brassica crops through several host and pest generations would allow better understanding of the impact of multiple natural enemies on DBM population dynamics. However, an important outcome resulting from the interaction between parasitoids and predators has been identified, being increased predation in the presence of parasitoids, probably because of increased movement of prey. Understanding the impact of this mechanism under real crop conditions would allow the incorporation of this information into management strategies for DBM.

Chapter 4 Does advanced parasitism by *Diadegma semiclausum* affect predation of DBM by *Coccinella transversalis*, *Nabis kinbergii* and *Oechalia schellenbergii*?

4.1 INTRODUCTION

Several studies show that among the diverse effects that parasitism can have on phytophagous insects, the vulnerability of mobile hosts to predation by natural enemies may be modified. For example, parasitised aphids, *Chromaphis juglandicola* (Kaltenbach), were consumed preferentially by the Argentine ant, *Iridomyrmex humilis* (Mayr), over unparasitised aphids (Frazer and Van den Bosch 1973), and larvae and adults of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) consumed more *Aphis fabae* (Scop) (Hemiptera: Aphididae) parasitised by *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphidiidae) than unparasitised conspecifics (Meyhöfer and Klug 2002). In addition, ants preferentially consumed larval *Pieris rapae* L. (Lepidoptera: Pieridae) parasitised by the braconid *Cotesia glomerata* L. (=*Apanteles glomeratus* L.) than unparasitised larvae (Jones 1987). And parasitised larvae of the sawfly *Neodiprion swainei* Middleton (Hymenoptera: Diprionidae) were consumed preferentially by the predatory pentatomid *Podisus modestus* (Dallas) (Tostowaryk 1971). Thus, the evidence suggests that parasitised insects can become more susceptible to predation by their natural enemies.

According to Brodeur and Boivin (2004), parasitised hosts are biochemically, physiologically and ecologically different from unparasitised conspecifics. The effects of parasitism are numerous and diverse. These range from modification in the host's behaviour (Thomson 1990; Godfray 1993; Adamo and Shoemaker 2000; Brodeur and Boivin 2004; Thomas *et al.* 2005) and the host's response to the environment (Thomson 1990; Godfray 1993; Whitfield 1998; Brodeur and Boivin 2004; Thomas *et al.* 2005), to physiological and biochemical changes (Thomson 1990; Brodeur and Boivin 2004). As a result, parasitism can impact directly or indirectly on host survival.

Through the sequence of events that take place in the process of parasitisation, there are many instances where parasitoids can influence the vulnerability of their hosts to predation. For example, during oviposition, some parasitoids inject venoms that may cause local or total temporary paralysis (Vinson and Iwantsch 1980 and references within; Godfray 1993). Some parasitised hosts move to microhabitats more or less protected against natural enemies or unfavourable climatic conditions (Tostowaryk 1971; Stamp 1981; Brodeur and McNeil 1989), or modify their period of activity or foraging patterns (Brodeur and Boivin 2004). In addition, a developing parasitoid may affect the host's immune (Salt 1968; Thomson 1990; Godfray 1993; Whitfield 1998; Brodeur and Boivin 2004) or endocrine systems (Godfray 1993; Adamo and Shoemaker 2000; Thomson and Redak 2008). Development and metamorphosis can also be altered (Vinson and Iwantsch 1980; Godfray 1993; Brodeur and Boivin 2004; Thomson and Redak 2008) as well as the host's food consumption (Sisterson and Averill 2003; Thomson and Redak 2008), digestive function, assimilation, metabolic conversion efficiency, nutritional status (Thomson 1990; Brodeur and Boivin 2004), and growth and weight (Jones and Lewis 1971; Thomson and Redak 2008). A parasitised host can also have physiological (Jones and Lewis 1971) and biochemical modifications (Vinson and Iwantsch 1980; Thomson and Redak 2008) and tissues not directly attacked by the ovipositing female may suffer alterations (Vinson and Iwantsch 1980). Some authors agree that many of these processes can be weakening for the host (Thomson 1990; Godfray 1993; Adamo and Shoemaker 2000; Thomas *et al.* 2005).

While many of these modifications are adaptive and beneficial for either the parasitoid or the host (Thomson 1990; Brodeur and Boivin 2004; Thomas *et al.* 2005), some may be also non-adaptive, side-effects of parasitoid attack (Godfray 1993; Brodeur and Boivin 2004). , Distinguishing between active host manipulation and traumatic effects of parasitism may be very difficult (Godfray 1993).

Parasitism of the diamondback moth

Previous studies reveal that some parasitoids oviposit preferentially on early instars of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (DBM). For example, Cai *et al.* (2005) found that in a non-choice situation *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) parasitised almost twice as many second or third instars compared to the early fourth instar, and as the larvae grew within the fourth instar, the parasitoid attack rate decreased more than 50% after three days. In a choice experiment by the same researchers,

parasitism rate by this parasitoid was not significantly different between second or third, but significantly higher compared to that observed on fourth instar DBM. In addition, Talekar and Yang (1991) observed that *D. semiclausum* (=*D. eucerophaga* Horstmann) parasitised preferentially second and third instar DBM followed by first instar, and did not parasitise fourth instar larvae at all. Another larval parasitoid of DBM, *Cotesia vestalis* Haliday (=*Apanteles plutellae* Kurdjmov) (Hymenoptera: Braconidae), parasitised preferentially second, followed by first and third instars, and the fourth instar was the least preferred (Talekar and Yang 1991). In a non-choice experiment, another ichneumonid, *Diadegma mollipla* (Holmgren), parasitised approximately 70% more second than third, and 71% more third than fourth instar DBM, and in a choice situation it parasitised significantly more third than second and more second than fourth instar DBM (Nofemela and Kfir 2008). *Diadegma* spp. begin to develop soon after oviposition, starting as hemolymph feeders, and reaching the final instar after the host larva has pupated (Brodeur and Boivin 2004; Nofemela and Kfir 2008). Like most campoplegine ichneumonids, *D. semiclausum* pupate after consuming most or all host tissues (Harvey and Strand 2002).

There is not much detailed knowledge about the exact modifications *D. semiclausum* or other parasitoids induce in DBM, but Cai *et al.* (2005) found that development of parasitised larvae took significantly longer than unparasitised ones, and that DBM parasitised at second instar had eaten less than unparasitised ones at the end of the larval stage. Yang *et al.*(1994) also found that DBM larvae parasitised by *D. semiclausum* ate less than unparasitised larvae, and that the earlier the parasitisation occurred, the bigger the difference in food consumption between parasitised and healthy larvae. In addition, Choh *et al.* (2008) observed that, during the four-day period immediately after parasitism, DBM parasitised by *C. vestalis* in the second instar ate less than half of what non parasitised larvae did.

Because *D. semiclausum* and other parasitoids preferentially oviposit in early instars of DBM and they do not immediately kill the host, there is a time lag between the attack of the parasitoid and the death of the host where parasitised and unparasitised larvae coexist in the field. As DBM are attacked by both predators and parasitoids, an increase in the vulnerability of parasitised DBM to predation could have ecological consequences by affecting parasitoid populations through coincidental intraguild predation (IGP), (Rosenheim *et al.* 1995; Snyder and Ives 2008), i.e., when a predator consumes a herbivore that harbours developing parasitoids (Rosenheim and Harmon 2006). For example, Colfer and Rosenheim (2001) observed intense predation on immature parasitoids by the coccinellid *Hippodamia convergens*

Guérin-Méneville, which consumed almost 100% of mummies of *Aphis gossypii* Glover containing juvenile braconid wasps, *Lysiphlebus testaceipes* (Cresson). Also, Snyder and Ives (2001) recorded a threefold reduction in parasitism rate due to IGP, and an increase in aphid population growth in the host-parasitoid-predator system that included the aphid *Acyrthosiphon pisum* (Harris), the braconid parasitoid wasp *Aphidius ervi* (Haliday) and generalist predatory carabid beetles in alfalfa. And Snyder and Ives (2003) reported that in the presence of a complex of generalist predators such as nabids, spiders, carabids and coccinellids the density of mummies of *A. pisum* parasitised by *A. ervi* was reduced by 50%.

Understanding the effects of parasitism on the vulnerability of larval DBM to predation will allow improved prediction of the impact of other natural enemies on the biological control of DBM by *D. semiclausum* in brassica crops. This information is important to select those practices that favour the survival and activity of the most effective natural enemies that complement the action of parasitoids when planning and implementing conservation or augmentative biological control of this pest.

The aim of this chapter is to evaluate the possible effect of 5-day advanced parasitism by *D. semiclausum* on the vulnerability of DBM to predation by three generalist predators commonly found in brassica crops in South Australia: *Oechalia schellenbergii* Guérin-Méneville (Hemiptera: Pentatomidae), *Nabis kinbergii* Reuter (Hemiptera: Nabidae) and *Coccinella transversalis* (Fabricius) (Coleoptera: Coccinellidae).

4.2 MATERIALS AND METHODS

Experiments were conducted in two stages at the University of Adelaide, Australia. Experiments involving *C. transversalis* were conducted between December and May 2007 and those with *N. kinbergii* and *O. schellenbergii* between October 2007 and April 2008.

4.2.1 PLANT AND INSECT CULTURES

4.2.1.1 Plants

See Chapter 2, Australian cultures.

4.2.1.2 Plutella xylostella

See Chapter 3, Culture 2007-2008.

4.2.1.3 Diadegma semiclausum

See Chapter 3, Plant and Insect Cultures.

4.2.1.4 Myzus persicae

See Chapter 2, Australian Cultures.

4.2.1.5 Coccinella transversalis

See Chapter 2, Australian Cultures.

4.2.1.6 Nabis kinbergii

The culture of *N. kinbergii* was established using adults collected from alfalfa (*Medicago sativa* L.) crops at the Waite campus (34°58'4"S, 138°38'E) in October 2007. Adults were sexed and allowed to mate in groups of equal numbers of females and males (between five and eight of each) in vented transparent plastic containers (8 cm x 11 cm diam.) at 20±1°C and in a 14L:10D photoperiod. The lighting system used, which simulated dusk and dawn conditions, is described in Chapter 2.

A piece of pak Choi leaf with green peach aphids *My .persicae* and larval DBM was placed inside the container as food for the adults, and this was replaced daily. After two days, females only were placed into individual 5 cm plastic Petri dishes. Each Petri dish contained a piece of filter paper on the bottom, two to three 3 cm pieces of cabbage stem and approximately 10 larval DBM and green peach aphids for food. The stems were an oviposition substrate, which were replaced every day, along with additional fresh food (Figure 3-4, Chapter 3). Stem pieces were examined under the microscope and those that contained eggs were put in 5 cm dated Petri dishes in the same room. After approximately 10 days, newly emerged nymphs were put individually in 5 cm Petri dishes with a piece of filter paper on the bottom and a piece of cabbage leaf infested with green peach aphids and first and early second instar DBM (within mines and just emerged from mining). Petri dishes were housed in a 9 L air tight transparent plastic box, where a 100 ml plastic cup of saturated salt solution (Winston and Bates 1960)

was placed in order to create a humid atmosphere and prevent desiccation of nymphs (Figure 4-1). Every second day the Petri dishes were cleaned or replaced and fresh food was provided. The size of the aphids and DBM provided was increased as the nymphs grew. As the original egg-laying females died or ceased ovipositing, they were replaced with new ones collected from the field.

4.2.1.7 Oechalia schellenbergii

This culture was established from a single fertilised female collected from a white clover field at the Urrbrae Agricultural School in Adelaide, South Australia (34°57'56"S, 138°37'32"E) in October 2007. This female was placed in a transparent plastic container with a 10 cm long cotton wick saturated with water (Figure 3-5, Chapter 3). Everyday, a piece of cabbage leaf with about 20 third or fourth instar DBM provided food to the predator. The container was covered with gauze and kept at 20±1ºC and in a 14L:10D photoperiod. Lighting in the rearing rooms was provided by the solid state ballasts and lamps described in Chapter 2. The container was checked for eggs, and a new piece of cabbage leaf and DBM larvae were added daily. Egg masses were put in dated 5 cm Petri dishes inside a 2 L air tight transparent plastic box, with a 100 ml plastic cup of saturated salt solution (Figure 4-1). Since first instar *O. schellenbergii* are not carnivorous (Awan 1981) when eggs had hatched after about 8 days, a piece of cabbage leaf was added to the Petri dish as food and replaced every day with fresh material until first moulting. After the first moult, second instars were put individually in 5 cm Petri dishes with a filter paper on the bottom and larval DBM that had been killed with hot water (Awan 1981) were supplied as food (Figure 4-1). Petri dishes were cleaned or replaced every second day when filter paper was changed, old DBM larvae were removed and fresh larvae were added. Another *O. schellenbergii* egg mass was found in the field in December 2007, and reared until adulthood. Then, five females from the established culture were mated with males from this new field-collected brood, to replace the original female whose egg production was decreasing. The new females produced eggs for about three months.

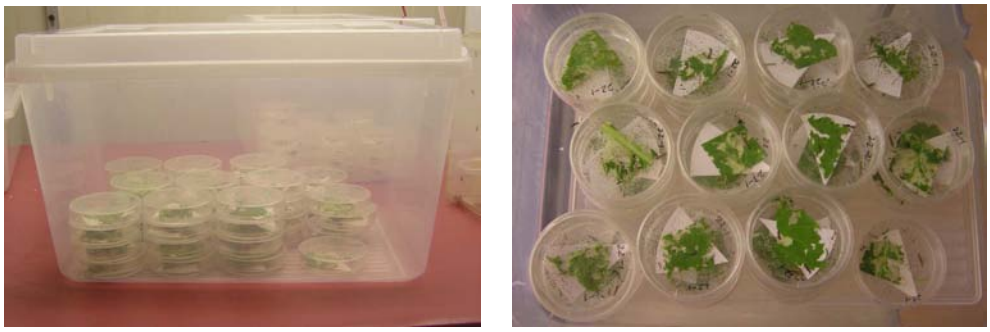


Figure 4-1 Air tight plastic box for keeping *N. kinbergii* (left) and *O. schellenbergii* reared individually in Petri dishes (right).

4.2.2 EXPERIMENTS

All experiments in this chapter were conducted at 20±1°C and a photoperiod of 14L:10D (see lighting system in Chapter 2, Australian cultures). In order to standardise the predators' condition, prior to the beginning of each experiment, they were starved individually for 24 h in a 5 cm diameter Petri dish with a filter paper disc (Whatman® n.2, 4.25 cm) moistened with two drops of tap water. All the experiments began at approximately 13:00 h and ran for 24 h. Only female *O. schellenbergii* and *N. kinbergii* were used as personal observations and previous studies have suggested that they are more voracious than males (Awan 1981; Quang 2007). However because of the difficulty in sexing adult *C. transversalis*, these were chosen randomly from a group of 80 individuals. Preliminary tests allowed determining the number of DBM that should be provided and prey were offered in excess to reduce the effect of prey availability on prey consumption.

This experiment involved three steps: Evaluation of the effect of marking the larvae on predation; parasitising DBM larvae and evaluating of parasitism efficacy; and conducting a choice experiment with parasitised and unparasitised larvae as the prey options in two different arenas.

4.2.2.1 Effect of marking larvae on predation.

The effect of marking larvae on predation was evaluated once at the beginning of the experiment, to verify that marking would not affect prey acceptance by predators. For this, six fourth instar DBM marked with a permanent black ink pen (Staedler Lumocolor® 0.6 mm

permanent dry safe, Art. Nr. 318 WP4, made in Germany, STAEDLER Mars GmbH & Co. KG Moosaeckerstr 90427 Nuremberg, Germany) and six non-marked larvae of the same age and size were offered to adult *C. transversalis* in a 5 cm diameter Petri dish. None of these larvae had been exposed to parasitoids. After 24 h, the remaining larvae were counted and the number of larvae consumed was calculated. Ten replicates of this experiment were carried out.

4.2.2.2 Paraitising DBM.

The process of parasitising DBM consisted of allowing approximately 10 female and 10 male *D. semiclausum* to mate in a transparent vented plastic container (15 x 15 x 20 cm) containing a 20 ml cup of 10% sugar solution dispensed through a cotton wick (as in Figure 3-3, Chapter 3). After 24 h, males were removed and a piece of cabbage leaf partially eaten by DBM and containing DBM frass was placed in the container to stimulate female parasitoids to start searching for hosts. One hour later females were assessed as ready to start the parasitising process. Then, a group of second instar DBM, homogeneous in size, were divided approximately in half. Larvae from one half were parasitised by offering them, one at a time and with the help of a fine paintbrush, to *D. semiclausum* in the plastic container (Figure 4-2). Only after a parasitoid was seen stinging a larva with its ovipositor was the latter considered parasitised. This process was repeated with each larva.

The parasitised larva was then placed in a plastic box (20 x 20 x 15 cm) containing a fresh cabbage leaf. A similar container was used to keep the other half of the larvae, which were not parasitised. Both containers were covered with vented lids and left for five days at 23±1° C. Daily, fresh cabbage leaves were added and the containers were cleaned. At the end of the five-day period DBM had grown to fourth instar. Oviposition activity by *D. semiclausum* was variable. Some days females were more active, efficient and faster than other days. For these reasons, the number of larvae parasitised in a day varied with a maximum of approximately 200. Therefore parasitising was done over several days resulting in different "sets" of parasitised and correspondingly unparasitised larvae.

Despite observing all larvae from each parasitised set being stung by *D. semiclausum*, sometimes parasitoids failed to deposit an egg, or the host larva may have encapsulated and killed the parasitoid (Salt 1968; Godfray 1993). Therefore, each set was checked for parasitism. For this, larvae from each set were randomly divided into groups of 10 and, depending on the total number of larvae in the set, two or three groups were randomly selected for larval dissection

(as explained in Chapter 3). Despite the fact that some sets may have not have been 100% parasitised (Table 4-1), these larvae will be referred to as parasitised to distinguish them from the half that were not exposed to *D. semiclausum*, which are referred to as unparasitised.

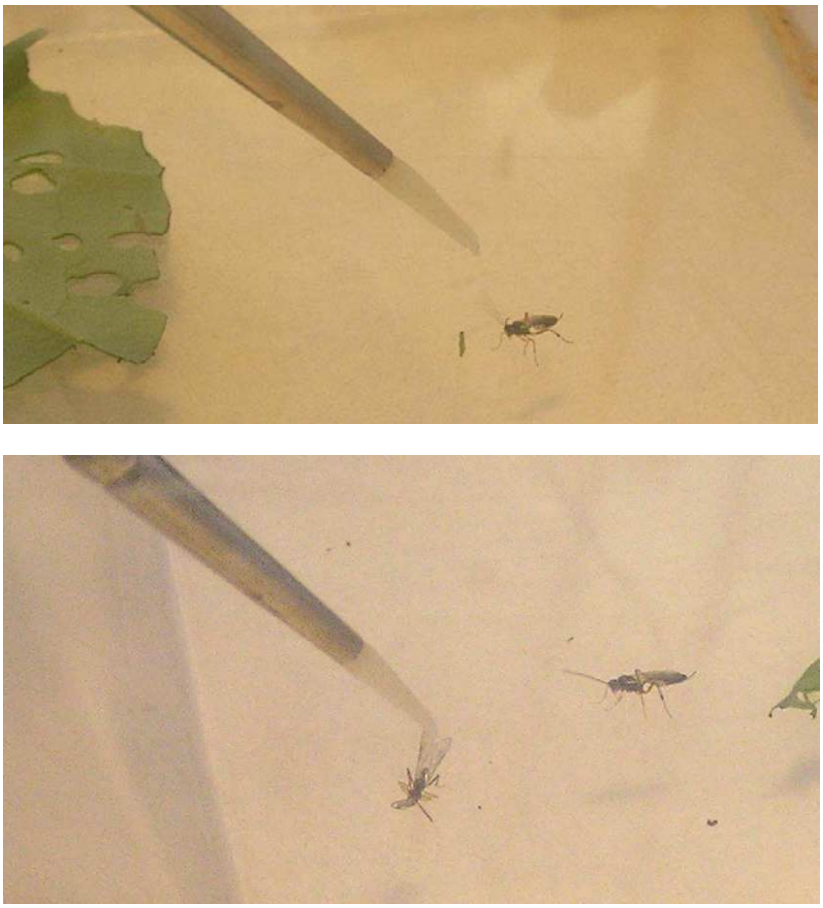


Figure 4-2 A DBM larva is offered to the wasp with the help of a paintbrush (top) and the larva is considered parasitised only when it has been stung by a wasp (bottom).

4.2.2.3 Predation of parasitised and unparasitised DBM.

The choice experiment consisted of offering equal number of parasitised and unparasitised fourth instar DBM (marked with a red and a black permanent ink pen respectively, Figure 4-3) to each predator in two arenas for 24 h. Arena 1 consisted of a vented Petri dish with a 1 cm piece of cabbage leaf to provide food for larvae. Preliminary observations revealed that the number of larvae eaten by *O. schellenbergii* was greater than eaten by the other predators. For this reason bigger Petri dishes were used to reduce overcrowding. Five cm diameter Petri dishes were used for *C. transversalis* and *N. kinbergii* and 10 cm Petri dishes for *O. schellenbergii*.

Arena 2 consisted of a vented transparent plastic container (8 cm deep x 11 cm diameter) with a Pak Choi brassica leaf, with the stem inserted in the bottom of the container and sealed with a disc of high density foam. The end of the stem was immersed in water to prevent leaf desiccation and the container was covered with a piece of gauze held with a rubber band to avoid insect escape. The leaf was not in contact with the sides or bottom of the container, nor with the gauze, so insects did not have access to hiding places (Figure 2-7, Chapter 2).

Figure 4-3 Petri dish arena (left) and leaf arena (right) with a female adult *O. schellenbergii* and equal number of parasitised (red) and unparasitised (black) DBM larvae.

Predators confronted different challenges in the two arenas. Arena 1 imposed few difficulties for attack and capture of prey. Arena 2 on the other hand, was bigger and more complex so prey were more dispersed and difficult to find and the elevation of the leaf allowed larvae to escape by hanging from a silk thread when attacked. By testing the predators in the different arenas, the effects of the arenas *per se* could be evaluated, and the effect of parasitism on susceptibility to predation could be validated.

This experiment was blocked by date (randomised complete block). For practical reasons the number of blocks completed at each date depended on the availability of parasitised larvae in each set. On each date, and for each predator species, there was the same number of replicates of both arenas. A control treatment was also conducted to evaluate mortality of parasitised and unparasitised DBM in the absence of predators. Every replicate was performed with new individuals.

DATA ANALYSIS

The vulnerability of marked and non-marked DBM to predation by *C. transversalis* was analysed using Student *t*-test (Zar 1999; SAS-Institute 2000).

The parasitism rates across larval sets used for the three predator species was analysed using a generalised linear model with binomial error distribution (Genstat10.2 2007).

The vulnerability of parasitised and unparasitised larvae to predation by *C. transversalis*, *N. kinbergii* and *O. schellenbergii* in two different arenas was analysed using Student *t*-test. After the first 10 replicates were completed for each treatment, a power analysis was conducted to establish the levels of replication that were needed to rigorously test the hypotheses using this method (Zar 1999; SAS-Institute 2000). Additional replicates were added to achieve a high statistical power.

4.3 RESULTS

4.3.1 PARASITISM ACROSS PARASITISED DBM SETS OF LARVAE

The parasitism rate of the sets used with the predators varied between 83.3 and 89.2%, but these differences were not significant (P=0.61, df=15; Residual mean deviance=1.3; Table 4-1).

Table 4-1 Parasitism of sets of DBM larvae by *D. semiclausum* used in experiments with predators (% ± SE).

Predator	Parasitism
C. transversalis	89.2 ± 4.1
N. kinbergii	83.3 ± 3.9
O. schellenbergii	85.7 ± 3.4

4.3.2 EFFECT OF MARKING LARVAE ON PREDATION

There was no statistical difference in the number of marked and unmarked larval DBM consumed by *C. transversalis* (P=0.45, df=9; Residual mean deviance=1.08; Table 4-2).

Table 4-2 Consumption of marked and unmarked DBM larvae by *C. transversalis* (number ± 95 CI).

No. of larvae eaten		
Marked	Unmarked	Marked : unmarked
2.2 ± 1.04	1.7 ± 0.71	1.3

4.3.3 EFFECT OF PARASITISM ON PREDATION OF DBM

There was a general trend for all predators to consume more larvae in arena 1 (Petri dish) than in the more complex arena. *Oechalia schellenbergii* consumed the greatest number of larvae (a mean of 20.7 and 8.5 in arenas 1 and 2, respectively), followed by *N. kinbergii* (13.4 and 3.3) and *C. transversalis* (4.4 and 2.9).

The analysis showed no significant differences in the consumption of parasitised and unparasitised DBM in both arenas by *N. kinbergii* and *O. schellenbergii*. However, *C. transversalis* consumed a significantly greater number of parasitised than unparasitised larvae in both arenas (65% and 61% more parasitised than unparasitised larvae in arena 1 and arena 2 respectively; $P<0.05$; Table 4-3).

Table 4-3 Number of replicates per treatment (n) in each arena, number of parasitised (P) and unparasitised (UP) DBM larvae offered in each treatment and number of parasitised and unparasitised larvae consumed by *N. kinbergii*, *O. schellenbergii* and *C. transversalis* in arena 1 and arena 2 (number ± 95 CI). Different letters in a row indicate significant differences in the consumption of parasitised/unparasitised larvae by each predator in one arena ($P<0.05$).

Predator	Arena (n)	P/UP	Number of larvae consumed (missing)			
			Total	Parasitised	Unparasit.	P/UP
N. kinbergii	1 (18)	10/10	13.4 ± 2.3	6.7 ± 0.9	6.7 ± 1.5	0.99
	2 (15)	5/5	3.3 ± 0.8	1.5 ± 0.4	1.8 ± 0.5	0.85
O. schellenbergii	1 (15)	20/20	20.7 ± 2.8	10.5 ± 1.9	10.2 ± 1.5	1.03
	2 (15)	10/10	8.5 ± 1.9	4.8 ± 1.3	3.7 ± 0.9	1.3
C. transversalis	1 (12)	10/10	4.4 ± 1.5	2.8 ± 0.8 a	1.7 ± 0.8 b	1.65
	2 (21)	10/10	2.9 ± 0.8	1.8 ± 0.6 a	1.1 ± 0.4 b	1.61
Control	1 (10)	10/10	0	0	0	
	2 (10)	10/10	0	0	0	

4.4 DISCUSSION

The period parasitoids had to develop within the host was the maximum that this experiment allowed. Since several studies show that some parasitoids of DBM, including *D. semiclausum*, oviposit preferentially in early host instars (Talekar and Yang 1991; Cai *et al.* 2005; Nofemela and Kfir 2008), larvae were parasitised in early second instar as soon as their size was big enough to manipulate them without excessive injury. Then parasitoids were allowed to develop and larvae were used in the early fourth instar and the experiment finished just before the process of pupation started and DBM larvae become immobile and spin a cocoon in which to pupate. While *O. schellenbergii* and *N. kinbergii* did not preferentially prey on parasitised DBM, this period seems to have been long enough to notice differences in the case of *C. transversalis*, which consumed over 60% more parasitised than unparasitised larvae regardless of the arena. Probably the difference in susceptibility was due to the combination of the predators' hunting mode in the restricted arena and a weakening side-effect of parasitism, although no change in behaviour was observable in parasitised larvae.

According to De Clercq (2000) predatory pentatomids use visual, chemical and tactile cues to locate and recognise the prey, but the most important sense is vision. They are able to react to prey at distances up to 10 cm. The author observed that once the prey is located, they can spend "from several minutes up to an hour stealthily approaching the prey". In the case of *O. schellenbergii*, Awan *et al.* (1989) concluded that this species used vision and olfaction to locate prey from a relatively long distance. In the present study it was observed that once the prey was located, *O. schellenbergii* moved directly to the larva, sometimes stopping for a few seconds, especially as it was getting close to the victim. Then, once it was just a few millimetres from a larva, it extended the mouthparts and inserted them into the body of the larva. This was achieved with a very sharp and precise movement, with an almost total efficacy of capture. After this, the larva started to move vigorously, probably in an attempt to release itself from the predator, and after a few seconds it stopped moving completely. During this engagement the only contact between the predator and the victim was the mouthparts of the predator inserted in the body of the larva. *Oechalia schellenbergii* did not use the front legs or any other part of the body to hold or immobilise the prey (personal observation, Figure 4-4).

The other hemipteran predator, *N. kinbergii*, searches the habitat at a low speed (personal observation), and mainly uses olfactory stimuli to locate and recognise prey from long distance, although they also use vision and tactile keys for short range perception (Siddique

1985). In the current study it was observed that once located, this predator captured the prey with a characteristic precise and fast ambush-like jumping movement, grabbing the larva with the front legs while it simultaneously inserted the mothparts into the body of the prey, with a high efficiency of capture (personal observation, Figure 4-4).

In the case of both *O. schellenbergii* and *N. kinbergii,* most times their approach to the prey was very directed and precise, and once they had inserted the mouthparts into the body of the prey, there were very few occasions when the larva escaped (especially for *O. schellenbergii*). After a few seconds larvae did not show any signs of activity (personal observations), which was likely to be caused by the venom these hemipterans inject in the body of the victim (Cohen 1995; Cohen 2000; De Clercq 2000).

Adult coccinellids may use visual or olfactory cues to locate prey from long distance on some occasions, but they generally search for prey by moving quickly and randomly and can perceive and recognise prey visually or by olfaction only when they are a few millimetres away or contacting their prey with their prolegs or maxillary palps (Ferran and Dixon 1993; Hodek and Honek 1996 and references within; Harmon *et al.* 1998). In the current study it was frequently observed that this species searched actively, moving fast and turning frequently and apparently undirected. This resulted in very frequent encounters with DBM larvae but most of the times the coccinellid failed to capture the prey (personal observations). Because of their low efficiency in capturing their prey, coccinellids induced the characteristic escape behaviour of DBM very often. Some larvae arena 2 even escaped before the contact between them occurred (personal observation), and it is likely they could perceive the vibration of the leaf when the predator was getting closer. Once a coccinellid had successfully captured a larva, it held the prey with the front legs and started chewing on it until it was immobile and finally died (Figure 4-4). However, some larvae struggled and released themselves from the predator (personal observation). Because of the high frequency of escape behaviour induced by coccinellids, it is likely that healthy unparasitised DBM larvae could escape more easily than parasitised ones, which may have been weakened by parasitism and been an easier target. On the other hand, the very effective strategy used by the hemipteran predators to locate, capture and kill the prey did not allow DBM to escape easily and therefore healthy unparasitised larvae did not appear to have an advantage over parasitised ones for escaping.

Figure 4-4 These images show capture and feeding behaviour of three insect predators. *Nabis kinbergii* hold the prey with the front legs while inserting the stylet in its body (top). *Oechalia schellenbergii* do not touch the prey with the front legs, only with the mouthparts (middle). Coccinellids (in the photo *C. undecimpunctata)* hold the prey with the front legs while chewing on it until the prey is dead (bottom).

Because the level of suppression of DBM achieved by *D. semiclausum* is potentially very high (Muckenfuss *et al.* 1992; Furlong *et al.* 2001; Sarfraz *et al.* 2005), coincidental IGP on this parasitoid by generalist predators, could disrupt biological control by this parasitoid. However, for several reasons, and based on the level of IGP recorded in this work, it seems unlikely that this is the case. *Diadegma semiclausum* is very efficient in searching and parasitising DBM.

Consumption of larvae by the predators and parasitism rate measured in Chapters 2 and 3 suggests that in the absence of other natural enemies it inflicts higher levels of mortality than *C. transversalis*, *O. schellenbergii* or *N. kinbergii* acting singly. According to theory, this implies that in a three-species system, any of the species of predators studied should achieve an equilibrium density with DBM and the parasitoids, rather than excluding the latter (Polis *et al.* 1989; Polis and Holt 1992; Holt and Polis 1997; Janssen *et al.* 2006; Rosenheim and Harmon 2006)

Previous work also suggests that IGP does not always result in disruption of biological control of herbivorous arthropods (Rosenheim *et al.* 1995 and references within; Colfer and Rosenheim 2001; Janssen *et al.* 2006 and references within; Rosenheim and Harmon 2006; Snyder and Ives 2008). In addition, recently published work suggests that disruption may be even less likely to take place when IGP occurs coincidentally (Rosenheim and Harmon 2006; Snyder and Ives 2008). Previous examples from the literature, where coincidental IGP did disrupt biological control involved extreme circumstances, such as predators that consumed more immobile mummified than mobile aphids, because the latter could move and escape predation while mummies were completely incapable of escape (Snyder and Ives 2001). Those were not the characteristics of the system evaluated in the current study, where all larvae were capable of moving and normal escape behaviour (personal observation), and where the differences in the capability of escaping between parasitised and unparasitised were more subtle.

Rosenheim and Harmon (2006) suggested that biological control may be disrupted by IGP when the IG-predator imposes high levels of mortality on the IG-prey. In the current study both hemipterans consumed similar numbers of parasitised and unparasitised prey. Therefore, for each parasitoid consumed by either hemipteran predator, there were two DBM larvae consumed, one parasitised and one unparasitised, and there were always parasitised larvae untouched from which parasitoids could emerge and continue reproducing. Under these circumstances it seems likely that the complex *D. semiclausum*/*O. schellenbergii* or *D. semiclausum*/*N. kinbergii* should reduce the density of DBM populations more than each natural enemy acting singly and the presence of the predators should not disrupt biological control.

Coccinellids, on the other hand, consumed 60% more parasitised than unparasitised larvae. However, in Chapter 2 it was observed that coccinellids did not consume great numbers of large larvae (at least under laboratory conditions, where the other species of coccinellid, *C.*

undecimpunctata consumed an average of 1.10 ± 0.38 larvae/day). Only large larval DBM can harbour a developed parasitoid which may alter host's vulnerability to predation. Besides, in chapter 2 consumption of larval DBM by this predator decreased in the presence of alternative prey such as aphids, which is likely to be the actual scenario in brassica crops, where there is a wider variety of prey available than just DBM. So, it seems unlikely that coccinellids would reduce *D. semiclausum* populations through coincidental IGP.

Despite the known preference for caterpillars by *O. schellenbergii* and *N. kinbergii* (Braman 2000; Cohen 2000; De Clercq 2000; Quang 2007), the three predatory species studied are generalists. It is likely that DBM represents a fraction of their diet as they consume other available prey in crops including other caterpillars. Thus, their impact on DBM, and therefore on DBM parasitoids, is probably reduced under natural crop conditions compared to the results obtained under laboratory conditions with no alternative prey available. Also, crops are architecturally much more complex than either of the two arenas used here, which would impose more challenges in finding and capturing DBM, and predators would be likely to consume fewer DBM in crops than in the artificial arenas studied.

Snyder and Ives (2008) developed a model to estimate the relative predation rate of an IG-predator on parasitoids, relative to hosts, needed to disrupt biological control of the host. According to that model, if predation rate on the parasitoid is more than half the predation on the host ($P_{parasitoid}>0.5P_{host}$), then IGP should disrupt herbivore control by the parasitoid. In the current study $P_{D.semiclausum}>0.85P_{DBM}$ for all the predators studied. However, the authors considered systems that reach equilibrium in the long term, which is rarely the case for short-term growing crops such as vegetable brassicas, which are regularly subjected to disruption and do not achieve equilibrium in one cropping season. Some brassica crops for seeds may stay in the ground for a year, which is still less than the period considered in their model.

In the current study each predator was offered a diet with equal numbers of each prey type. However, in natural conditions the proportion of parasitised and unparasitised larvae would change within and between seasons, and therefore the availability of each type of prey would vary. In a situation where there are more parasitised than unparasitised DBM, the predation rate on parasitised prey may increase, not because of preference but because of higher probability of encountering a parasitised larva. Therefore the pressure of coincidental IGP may also increase. Under the same logic, the presence of more unparasitised DBM should reduce the pressure of coincidental IGP on *D. semiclausum*. It is likely that the magnitude of

coincidental IGP may fluctuate naturally within and between seasons. However, even *O. schellenbergii* and *N. kinbergii*, the species more likely to feed on caterpillars, did not preferentially feed on parasitised DBM. Therefore it is unlikely that any of the species studied would take the population of the parasitoid to very low density levels in the mid to long term.

Crop systems are more complex than just the three-species systems considered here. They also present a dynamic architecture that changes in complexity as the plants develop and get larger. In addition, the proportion of parasitised/unparasitised prey used in predation experiments may affect the behaviour of predators and poorly reflect what happens in crops under natural conditions (Thomas *et al.* 2005). Further research in semi-field conditions using exclusion cages or in open systems, and extending the study through several generations of the pest and the natural enemies, would provide more meaningful information on whether the presence of predators disrupt biological control of DBM by *D. semiclausum*. In addition, another aspect that would be interesting to study is at what stage of development *D. semiclausum* starts to affect the vulnerability of DBM larvae to predators such as coccinellids. If parasitisation occurs at a later DBM instar, as may well happen in the field to at least part of the larval population, especially those in third instar (Talekar and Yang 1991; Cai *et al.* 2005), differences between parasitised and unparasitised larvae might not be large enough to enhance the risk of coincidental IGP on the parasitoid population by such predators.

Chapter 5 General discussion

This thesis has quantified the voracity of a number of predators on DBM in artificial arenas, and has highlighted several trophic interactions between the parasitoid *D. semiclausum*, and generalist predators, which will be discussed below. The importance of this work is in the context of the development of sound biological control strategies. While *D. semiclausum* may provide good control of DBM, parasitoids have to colonise the crops every season following the establishment of the pest, which takes time (Symondson *et al.* 2002). Therefore, the presence of generalist predators in brassica crops, which can provide some control early in the season before the parasitoid density has built up, could be of great advantage for control of DBM. Furthermore, any facilitation among predators or between predators and parasitoids could be beneficial. However, it is only possible to make full use of the predators in the system if we understand their behaviour, their diet breadth and voracity, and interactions among these factors, and how they may impact on the biological control of DBM.

Agricultural systems offer the opportunity for an almost infinite number of complex multi-species interactions (Rosenheim *et al.* 1995; Losey and Denno 1999; Rosenheim *et al.* 1999; Cardinale *et al.* 2003). The results from this thesis suggest that in brassica crops more than one species of natural enemies could have a range of effects on DBM suppression. While multi-species communities can often produce phenomenological patterns, it may be possible to predict the dynamics of a given system by disentangling its numerous potential direct and indirect interactions (Harmon and Andow 2002). It is very likely that most of the natural enemies studied, or related species, coexist in brassica crops in different regions in the world as well, as similar associations have been found in South Australia and Pukekohe, New Zealand (Lush[2] 2005, Walker[1] 2005, personal communication; Hosseini 2007). Therefore, some of the results should be transferable to other brassica-producing areas.

Previous studies have shown that *N. kinbergii* and *O. schellenbergii* consume lepidopteran larvae (Awan 1981; Siddique 1985; Hosseini 2007). Furthermore, Hosseini (2007) found that *N. kinbergii* and *O. schellenbergii* are abundant in brassica crops in South Australia, and specific DNA of DBM and five other selected pest species in their gut indicted that they are predators in the brassica system. Similarly, *Mi. tasmaniae* and *C. transversalis* contained specific DNA of DBM and three other brassica pest species (Hosseini 2007). For this reason one may think all

these predators are likely to be relatively important as biological control agents in brassica crops.

However, methods based on simple dissection, electrophoresis, immunology or molecular biology only allow the knowledge of the gut contents of predators and help establishing which species they have eaten, but they do not give any indication of the amount or the life stage eaten and hence do not provide data that allow accurate assessment of the importance of the predator in the system. Furthermore, there may be interactions that influence predation which are not reflected in the results obtained with these methods, such as secondary predation (Symondson *et al.* 2002). In addition, not all the species in the gut contents may be identified, due to degradation of DNA or other inhibiting conditions inside the gut. And, as Rosenheim *et al.* (1995) pointed out, understanding the complexity of interactions in agro-ecosystems involves two aspects. The first is to understand the trophic webs, which help defining the existence of interactions between species. The other aspect is the dynamical importance of those interactions, which can be established only through experimentation.

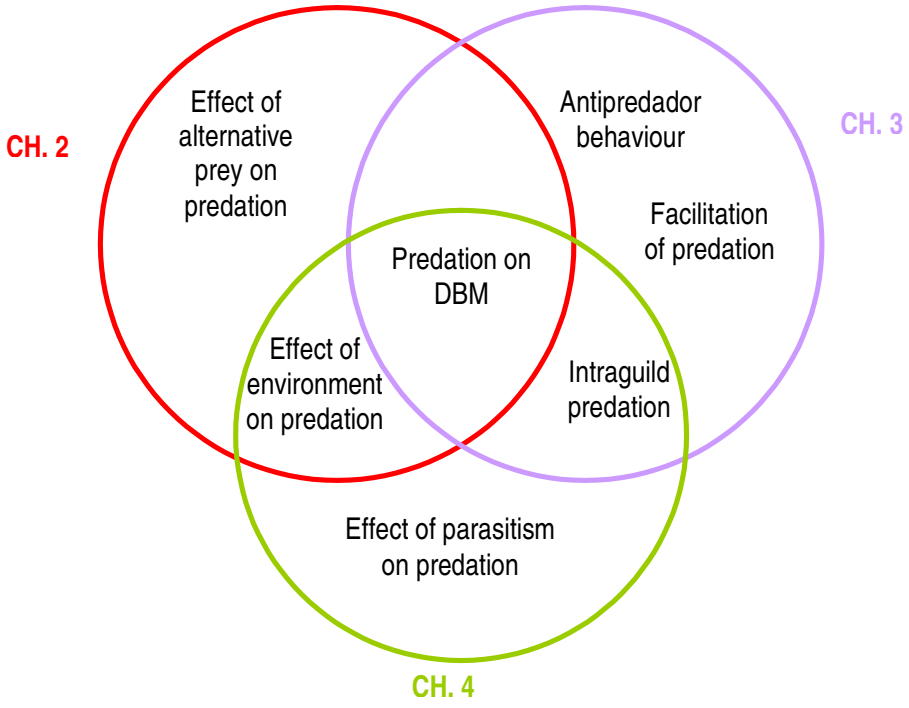


Figure 5-1 Seven key interactions were studied in this thesis.

This thesis has identified and analysed some interactions between *P. xylostella*, the parasitoid *D. semiclausum* and some generalist predators commonly found in brassica crops, which may

contribute to reducing DBM populations. All the experiments quantified predation on DBM by all the beneficial species studied. In Chapter 2, it was shown that the presence of the aphid *My. persicae* negatively influenced the predation on DBM by *C transversalis*, *C undecimpunctata* and *Mi. tasmaniae*. In Chapter 3, three main interactions were observed: escape behaviour towards *D. semiclausum* increased movement and changed the use of habitat by larval DBM; predation rate on DBM by *O. schellenbergii* and *N. kinbergii* increased in the presence of *D. semiclausum*; and these hemipteran predators did not prey preferentially on early-parasitised DBM. In Chapter 4 it was reported that more advanced parasitism on DBM increased vulnerability of parasitised DBM to predation by *C. transversalis*. In addition, in Chapters 2 and 4 it was shown that the type of arena influences the predation rate on larval DBM by all the predators studied (Figure 5-1).

The results obtained in this thesis increase our understanding of the dynamics of the interactions among natural enemies in brassica crops. It has clearly been demonstrated that the natural enemies studied do have a role in the suppression of DBM populations, and the data suggest we could take advantage of some interactions among them that may enhance biological control of this pest. For instance, in Chapter 3 it was observed that the presence of *D. semiclausum* may facilitate predation of DBM by the hemipteran predators due to the increased rate of movement of the prey, which becomes less cryptic and thus more available to the predators. The characteristic escape behaviour of DBM was elicited by all the natural enemies studied (including *Mi. tasmaniae*, although this was not mentioned in Chapter 2), and this may enhance suppression of DBM by visually oriented predators. On the other hand, all these predators would have the capacity to negatively influence parasitoid populations, because there would be coincidental intraguild predation if they consumed parasitised larvae.

Although a start has been made in understanding the interactions that occur in the brassica system, the data do not allow us to assess the effects on overall predation on DBM, due to the complex interrelations between the players in the system. If we only consider the five species of natural enemies studied, there would potentially be at least 27 different interactions among them (Figure 5-2). These include both direct prey consumption and several direct and indirect effects on the predation by other species due to competition, facilitation, and coincidental intraguild predation. Aspects of each of these effects have been highlighted in this thesis. Firstly, all predators consumed DBM larvae, and therefore they are competitors. Secondly, more advanced parasitism makes DBM more vulnerable to predators such as coccinellids, which enhances intraguild predation. Thirdly, the presence of natural enemies can increase the

movement of the prey which can increase detection rates. However, not all 27 possible interactions have been investigated, and the complex of natural enemies that attack *P. xylostella* and other herbivorous arthropods in brassica crops is diverse. So, there is an enormous potential for interspecific interactions of other types as well as those presented here.

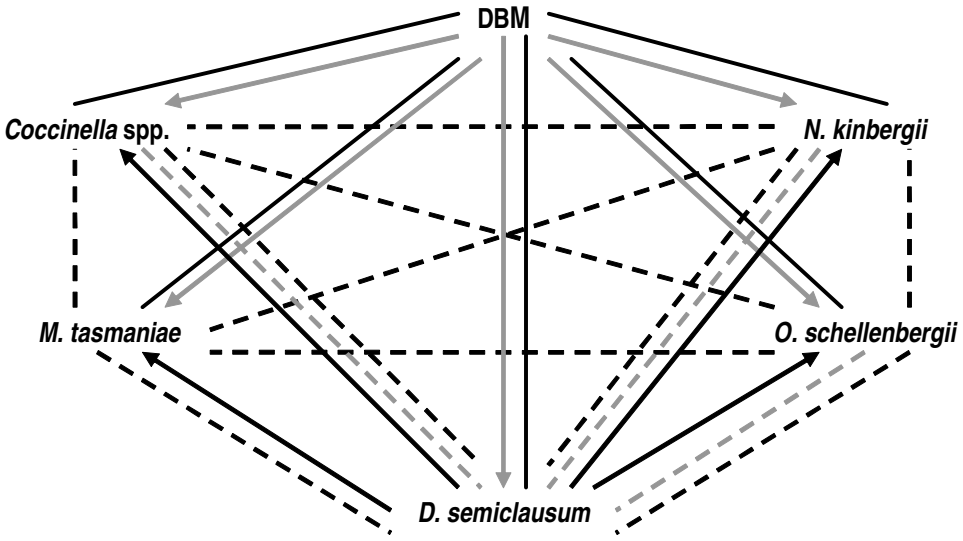


Figure 5-2 Scheme representing the interactions that are likely to take place in brassica crops among the species studied in this thesis: competition (black dotted lines), elicitation of escape behaviour (black solid lines), facilitation of predation on DBM (grey dotted lines), predation on the common prey (grey solid arrows) and coincidental intraguild predation on *D. semiclausum* (black arrows).

There are inter- or intra-specific interactions that are not included in the scheme presented in Fig. 5-2 that may also occur in a system like this, and that may contribute to the final balance on DBM population suppression, such as cannibalism or omnivorous intraguild predation among generalist predators. Also, escape behaviour may facilitate predation of DBM by ground predators (Losey and Denno 1998a; Losey and Denno 1998b). Another example is the trend of DBM larvae to move towards the centre of the plant when disturbed, which may put them under higher risk of predation by natural enemies that take refuge in this part of the plant, such as *N. kinbergii*, that tend to stay at the centre of cabbage plants during the day (personal observation). So, predation on larvae could be enhanced in the presence of parasitoids or other natural enemies. All these are interactions that still remain to be elucidated.

According to Symondson *et al.* (2002) complementary natural enemies are likely to improve control if each species attacks different life stages of a common prey. The results obtained suggest that the direct trophic interactions between each of the natural enemies studied and the different DBM instars may have different relative importance (Figure 5-3). For example, while predation of larger DBM larvae by *Mi. tasmaniae* and *Coccinella* spp. was not substantial, *Coccinella* spp. consumed many more small instars than *Mi. tasmaniae*, and both hemipteran predators consumed larger numbers of late DBM instars. Also, observations made while rearing the predators suggest that *O. schellenbergii* and *N. kinbergii* may prefer larger caterpillars. Therefore, several of the natural enemies used in this study could present complimentary predatory behaviour that affects prey regulation.

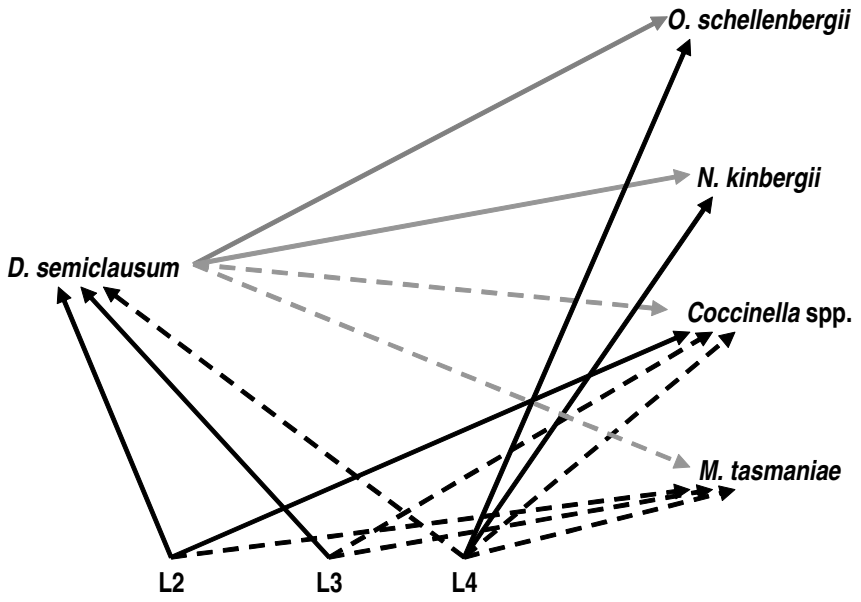


Figure 5-3 Schematic representation of trophic interactions among species studied in this thesis. The grey arrows indicate intraguild predation, and the black arrows predation on larval DBM in second (L2), third (L3), and fourth (L4) instar. Solid lines are dominant trophic links and dashed lines minor trophic links.

From observations made and reports in the literature, it is possible to infer two other interactions which were not examined in this thesis, but are also included in figure 5-3: coincidental intraguild predation of *D. semiclausum* by *Mi. tasmaniae* should not be strong, as the consumption of larval DBM was low. Also, parasitism rates of fourth instar DBM by *D. semiclausum* has been observed to be quite low (Talekar and Yang 1991; Cai *et al.* 2005).

The total impact of these natural enemies on DBM populations would be the result of the final balance between the trophic interactions that occur in this system. For example, early stages of parasitism by *D. semiclausum* (within the first 24 hr) do not seem to favour coincidental intraguild predation by *N. kinbergii* or *O. schellenbergii* (Chapter 3), nor does more advanced parasitism (Chapter 4). It is likely though, that the vulnerability of parasitised DBM to predators such as coccinellids increases the risk of coincidental intraguild predation, affecting *D. semiclausum* populations. However, as discussed in Chapter 2, it is likely that in the field the impact of coccinellids on older larval DBM is not so important, unless there is no other food choice. Also, only older DBM larvae can harbour more developed parasitoids, which could affect the host behaviour. Besides, a meta-analysis made recently by Rosenheim and Harmon (2006) suggests that adding an omnivorous intraguild predator to a herbivore/intermediate predator system often results in no changes in the density of the herbivore, whereas adding a coincidental intraguild predator to the system results in an overall improvement of herbivore suppression.

This thesis has identified several novel interactions with interesting implications for biological control of DBM that should be studied further. First, the stages of DBM which can be consumed by *Coccinella* spp. and *Mi. tasmaniae* have been identified and the observation of the fact that, even in the presence of the green peach aphid, a common pest of brassicas (which is an essential prey for both predatory species), both predators consume DBM. Second, the description of the modification in the behaviour of larval DBM and how they occupy the habitat in the presence of *D. semiclausum*, moving more within the plant and occupying the centre of the plant more often has been described. Third, a synergistic interaction was observed when *D. semiclausum* and the hemipteran predators *O. schellenbergii* and *N. kinbergii* coexist, increasing mortality of DBM. And fourth, the effect that more advanced parasitism may have on predation of DBM by predators such as coccinellids, which tend to consume more parasitised than unparasitised larvae, has been found. These interactions between DBM and its natural enemies, to my knowledge, have not been previously recorded in the literature.

Some limitations of the methods used in this study have already been discussed in this and other chapters, such as the artificial nature of the arenas, the fact that only a few species were included in the system while in natural conditions there would be a higher diversity, and the density of insects used. It is important to take into account that behavioural and predation experiments conducted under laboratory conditions must always be viewed cautiously, because they may not reflect very well what occurs in a natural environment. Caging may

increase or decrease the likelihood of species interactions relative to the open field and change foraging behaviour (Snyder and Wise 2001; Lagrue *et al.* 2007). A next step should be testing assemblages of species in semi-field or field conditions, including several combinations of these natural enemies, and evaluating the strength of the interactions and how they impact on DBM populations. This could lead to new insights due to factors that were not taken into account in this study. For example, some natural enemies that coexist in brassica crops may not overlap in space and time, which may result in a complementary action on DBM populations, or their interaction may be minor, not having any relevant impact on the common prey. For instance *N. kinbergii* (Quang 2007) and other generalist predators such as some species of spiders and formicids (Pfannestiel 2005) are more active at night, while *D. semiclausum* is practically inactive during this period (Wang *et al.* 2004). This could lead to a decrease in the expected facilitation detailed above. Harmon and Andow (2002) pointed out that despite the numerous biotic and abiotic interactions that insect ecologists and biological control practitioners have recognised, that can potentially affect the success of control efforts, we are still limited in our understanding of when and how these interactions will be important in real, inherently complex ecosystems.

Furthermore, another limitation that should also be noted is the duration of the experiments (Briggs and Borer 2005). Monitoring brassica crops through several host and pest generations would probably allow a better understanding of the impact of multiple natural enemies on DBM population dynamics. The results could also contribute to modelling of complex interactions. On one hand, modelling would be one way to extend the work to several generations, in order to generate testable predictions of what might be seen in longer, more realistic experiments. On the other hand modelling may help address key issues such as the effect of the presence of alternative prey on DBM predation, the effect that the preference for parasitised or unparasitised larvae may have on biological control, whether IGP allow coexistence of predators and parasitoids in this system, or to what extent the density of some populations of parasitoids and predators would influence the facilitation of predation observed in experimental arenas in this work.

This study has demonstrated in very simple systems how both trophic and behavioural interactions among predators and between predators and prey can lead to complex feeding webs. In addition, it has uncovered novel types of interactions between DBM, alternative prey, a parasitoid, and generalist predators. The results can be used to explore these feeding interactions both in models, and in larger scale experiments. It also complements information

obtained through molecular-biology-based work. Therefore, this study provides one of the building blocks that allow a fundamental understanding of the interactions among natural enemies in biological control. Understanding these interactions under real crop conditions will allow including this information in management strategies for DBM, ensuring the appropriate conditions for the prevalence of those combinations of beneficials that have been observed to enhance DBM suppression.

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