

ROSELYNE M. LABBÉ

**INTRAGUILD INTERACTIONS OF THE
GREENHOUSE WHITEFLY NATURAL ENEMIES,
PREDATOR *DICYPHUS HESPERUS*, PATHOGEN
BEAUVERIA BASSIANA AND PARASITOID
*ENCARSIA FORMOSA***

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Résumé

Les organismes constituant des assemblages d'ennemis naturels des herbivores peuvent interagir de façon variable, influençant ainsi l'efficacité de la lutte biologique. En serriculture, la mouche blanche, *Trialeurodes vaporariorum* est effectivement contrôlée par une gamme d'ennemis naturels dont le prédateur zoophytophage, *Dicyphus hesperus*, le champignon entomopathogène *Beauveria bassiana* et le parasitoïde *Encarsia formosa*. Cette étude explore les interactions entre ces agents afin d'identifier celles qui sont efficaces. En laboratoire, la capacité discriminatoire du prédateur *D. hesperus* envers des proies saines, parasitées ou infectées a été étudiée. Le prédateur attaque tant les proies saines que les proies parasitées par *E. formosa*, mais rejette les proies présentant des symptômes d'infection avancée par *B. bassiana*. En serre expérimentale, l'impacte de *B. bassiana* sous sa formule commercialisée de BotaniGard® sur la lutte biologique a été évaluée pendant une saison de production de huit semaines. Une réduction de la prédation des mouches blanches par *D. hesperus* suggère que ce pathogène interfère avec le prédateur, ce qui pourrait être évité par l'aménagement de refuges dans l'espace et dans le temps pour le prédateur, lors de l'utilisation de *B. bassiana*.

Abstract

In Canada, an increasing diversity of natural enemies, which includes generalist predators, is available in the control of greenhouse pests such as the greenhouse whitefly on tomato crops. Successful whitefly suppression is now achieved through the concurrent use of the specialist parasitoid *Encarsia formosa*, the native zoophytogphagous predatory bug *Dicyphus hesperus* and products such as biological insecticide BotaniGard®, an entomopathogenic formulation based on the conidia of fungus *Beauveria bassiana* strain GHA. In this study, experiments conducted in the laboratory and greenhouse evaluated the impact of interactions among these organisms on non-target organisms. In the laboratory, we identified an important decrease in predation of infected whitefly at late infection stages, but not of parasitized whitefly suggesting that *D. hesperus* may interfere with parasitoid biological control. In the greenhouse, reduced predation of *B. bassiana* infected whitefly by *D. hesperus* may be avoided by applying temporal or spatial separation between these two agents.

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To Zoë, Andréa and Brian

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Chapter 1. General Introduction

1.1. Introduction

The production of greenhouse vegetables in Canada is a rapidly growing sector of agriculture. Canada is the second most important user of high technology greenhouses, covering 8.76 million m² in 2002 (BC Government Fact sheet, 2003). Within this highly controlled environment, the greenhouse tomato is the leading crop as it covers 4.82 million m², over half of the total high tech greenhouse area. This fast growing sector of the greenhouse industry in North America has resulted from the increase in demand for higher quality produce on which little or no pesticides have been applied. In Canada, biological control is the primary pest control strategy in the greenhouse vegetable industry (Murphy *et al.*, 2002). Vegetable growers take advantage of the benefits achieved through the use of biological control including potential yield increases from pollinators, reduced pesticide residues as well as the marketing advantages of selling organic produce (Murphy, 2002). Growers who do not resort to the use of pesticides gain a lucrative advantage over conventional producers while satisfying the demands of vigilant consumers. In Canada, approximately 83% of greenhouse vegetable growers make use of biological control on their crops (Murphy *et al.*, 2002). Of those greenhouses growing tomatoes, 93% use biological control. For these purposes, many natural enemies have become commercially available. These natural enemies may each suppress herbivore populations in their own way and they may also be combined. Thus, it is important to understand the nature and consequences of the interactions that may exist between these organisms. By studying the community ecology of such artificial food webs, it may be possible to achieve an optimal pest management strategy.

One of the major hurdles encountered by greenhouse tomato growers is the control of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Aleyrodidae: Homoptera). This pest species is well known for its ability to reduce plant vigour, facilitate the growth of mould on leaves, transmit viral diseases and consequently reduce crop quality and yield (Johnson *et al.*, 1992). It is a generalist pest that has been reported to feed on over 250 plant species throughout the world (Osborne and Landa, 1992). Growers must continually monitor for this pest, which can achieve explosive population levels when left unattended.

Pest outbreaks may occur quickly in greenhouse ecosystems because of the inherent simplicity of artificial food webs and the absence of natural enemies (Brodeur *et al.*, 2002). During pest outbreaks, growers should be aware of the tools available for biological control that will enable effective management of whitefly populations.

1.2. Zoophytophages in biological control

Traditionally, polyphagous insect predators known to consume not only pest insects but also potentially any other insect species present in their environment have taken second place to specific natural enemies used in biological control. Characteristics associated with specialists including a high degree of prey specificity, short development time relative to prey, and high reproductive potential have traditionally been seen as important attributes of effective biocontrol agents (Snyder and Ives, 2003). It is also most likely that past success in biological control involving predators has resulted from the use of specialists whose life histories resemble those of specific parasitoids rather than typical predators (DeBach and Rosen 1991). While specialists normally avoid direct interaction with other natural enemies, generalists commonly engage in intraguild predation, which is defined as the killing and eating of species that use similar resources and thus, are potential competitors (Polis *et al.*, 1989, Polis and Holt, 1992; Rosenheim *et al.*, 1993, 1995; Rosenheim, 1998). However, a number of studies have proven that biological control by generalist predators may be successful in various agro-environments (Reichert and Bishop, 1990, Settle *et al.*, 1996, Snyder and Wise, 2001) and may make up for the shortfalls of specialist biocontrol agents, such as their limited host range or their tendency to disappear once the pest resource is depleted. In comparison, while the scheduled release of pest-specific natural enemies may be costly and complicated, generalist predators are increasingly appreciated for their long-term establishment in crops. The polyphagous nature of certain generalist predators allow populations to become established on a crop more easily than specialists, subsequently providing consistent control of pest populations (Gillespie and McGregor 2000). Furthermore, these predators may attack a number of different unrelated prey species (Albajes and Alomar, 1999). Generalist predators also occur naturally in most areas of the world and therefore reduce the need to import exotic biological control agents (Khoo, 1992).

By definition, zoophytophagous predators are omnivores, meaning that they may feed at more than one trophic level (Pimm and Lawton, 1978). Recently, zoophytophagous predators have become increasingly studied for their potential as biological control agents. They may withstand periods of prey scarcity, due to their capacity to feed on plant biomass in addition to animal prey (Naranjo and Gibson 1996, Wiedenmann *et al.*, 1996). Zoophytophagous organisms have the ability to exploit both food types through certain biochemical, morphological and physiological adaptations such as their production of digestive enzymes and detoxifying compounds, or by their mouthparts which allow for the structural breakdown of both insect and plant materials (Coll and Guershon, 2002).

The hemipterans (Heteroptera) in particular vary greatly in their ability to feed on plants or animals. Their evolution is characterized by a divergence between the predominantly carnivorous cimicomorphs and the phytophagous pentatomorphs (Figure 1-1, Schuh, 1979; Wheeler *et al.*, 1993 and Cohen, 1996). Interestingly, phytophagy in mirids is a new trait, which is quite distinct from phytophagy by ancestral groups (Cohen, 1996). It is generally assumed that hemipteran lineages arose from a phytophagous ancestor that exhibited a secondary ability to feed through predation (Sweet, 1979). Zoophytophagous hemipterans with the capacity to exploit both food sources are said to exhibit «trophic switching», allowing them to survive in the absence of prey (Cohen, 1996).

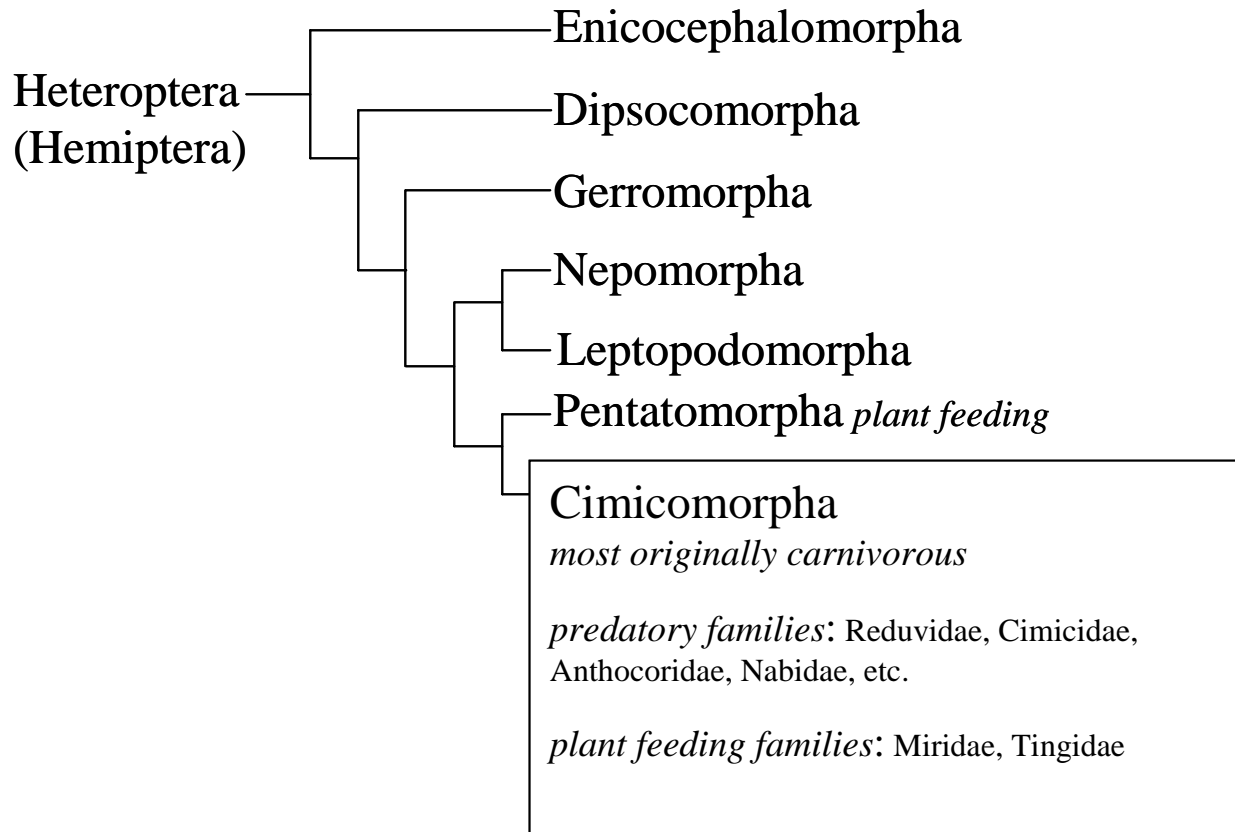


Figure 1-1 Phylogeny and feeding strategies of insects belonging to the order Hemiptera. Based on, Schuh, 1979, Wheeler *et al.*, 1993 and Cohen, 1996.

Among zoophytophagous hemipterans, plant feeding may vary in importance from insignificant to essential to their survival and reproduction. For some predators, plants provide important nutrients and moisture that are less abundant in prey (Coll and Ruberson 1998). In such cases, plant feeding aids in maintaining predator populations and to improve individual life history traits such as survival, developmental time, fecundity and longevity (Cohen and DeBolt, 1983; Naranjo and Gibson, 1996; Coll, 1998). For others, plants are considered a sub-optimal food source that is only used when prey is not available (Gillespie and McGregor 2000). In this case, plant feeding serves as a means to escape starvation or as a strategy for colonizing crops before prey arrival (Alomar and Wiedenmann 1996; Coll and Ruberson 1998). Plant material is merely a complementary food source to animal prey, which is the major source of nutrients. And in yet another group of extra-orally digesting hemiptera, plant material may simply provide the necessary liquid that enables prey feeding and may not represent an important source of nutrients (Cohen 1995, 1998). These predators acquire the necessary protein, fat and cholesterol from solid tissue and haemolymph from prey, which is used to complete development.

These different feeding strategies may differentiate between zoophytophagous predators that are suitable as biological control agents from those that are not. The degree and type of plant feeding expressed by zoophytophages must be considered *a priori*. Plant injury has been observed for some predator species such as *Macrolophus caliginosus* Wagner, which is known to damage cherry tomato crops (Sampson and Jacobson, 1999). Plant feeding has also been documented for predaceous bugs of the genus *Geocoris* that are responsible for unacceptable levels of plant damage, even in the presence of potential prey (Alomar and Albajes, 1996). However, when combinations of predators and tolerant crops are correctly chosen, effective pest suppression may be achieved. For example, *M. caliginosus* on many tomato cultivars will not injure fruits or plants (Lucas and Alomar, 2002). In such cases plant feeding results in the removal of a minimal quantity of plant biomass and is therefore less concerning (Lalonde *et al.*, 1999). As a result, the use of zoophytophagous predators that are highly effective at prey feeding, has become increasingly common worldwide (Table1-1).

Table 1-1 Predaceous mirids identified and studied for their potential as biological control agents on vegetable crops (Lucas and Alomar, 2002; Tanda and Holdaway, 1954)

Species	Countries
<i>Macrolophus caliginosus</i> Wagner (<i>M. melanotoma</i> Costa)	Many in Europe
<i>M. pygmaeus</i> Rambur	Greece
<i>Dicyphus hesperus</i> Knight	Canada, USA
<i>D. hyalinipennis</i> Burmeister	Hungary
<i>D. cerastii</i> Wagner	Portugal
<i>D. errans</i> Wolff	Italy
<i>D. tamaninii</i> Wagner	Spain
<i>Nesidicoris tenuis</i> Reuter	Canary Islands, Philipines, Italy
<i>Cyrtopeltis (Engytatus) modestus</i> Distant	Hawaii, USA

Predatory mirids from the Dicyphini tribe are frequently used in Europe as control agents for pests on field vegetables (Alomar and Albajes, 1996; Malausa and Trottin-Caudal, 1996). Plant-feeding predators investigated for their potential in biological control such as *M. calignosus* and *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) are good candidates in crops tolerant to small levels of herbivory (Gillespie & McGregor 2000). In fact, *D. tamaninii* is an efficient predator of whitefly pupae when found at high densities on tomato plants (Montserrat *et al.*, 2000). The predator *Dicyphus hesperus* is also an efficient biological control agent of many small insect species (Gillespie *et al.*, 2000). This mirid bug is native to and is widely distributed across North America (Kelton, 1980; Henry and Wheeler, 1988). *Dicyphus hesperus* provides a particularly good control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) and of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) on greenhouse tomato crops (McGregor *et al.*, 1999).

The recent success observed by *D. hesperus* in biological control can be attributed to its zoophytophagous nature whereby plant feeding provides this predator water that allows it to consume its prey (Gillespie and McGregor 2000). Yet, this zoophytophage is all too often associated with fruit damage caused when the stylets are inserted within a fruit, subsequently rendering it less commercially viable. In reply, McGregor *et al.* (2000) have assured biological control practitioners that unlike certain zoophytophagous mirids, *D. hesperus* prefers tomato leaves and will resort to fruit feeding only when other plant material is unavailable.

Dicyphus hesperus is well suited for use within the greenhouse environment, and its use has become increasingly common on greenhouse tomato crops in Canada. However, as a generalist predator, it is important to understand the interaction it may have with other currently applied natural enemy species. This generalist predator may interact with other predator or parasitoid species in ways that are not yet completely understood, and may have an important impact on the population dynamics of these species (Polis *et al.*, 1989, Polis

and Holt, 1992; Rosenheim *et al.*, 1993, 1995; Rosenheim, 1998). Successful biological control by *D. hesperus* depends on factors such as the specificity of the predator for prey, and spatial distribution, which is an important characteristic that may affect the outcome of an interaction. The role of these two factors is examined in this study.

1.3. Greenhouse ecology

In a greenhouse agro-ecosystem, the first response of biological control practitioners to pest population outbreaks is the use of specific natural enemies that have a direct impact on the target pest species. Specialists hold a tight relationship with their prey and are able to reduce or suppress herbivore outbreaks (Hassel and May, 1986; Murcoch, 1994; Turchin *et al.*, 1999). Specialist biological control agents are usually the strongest contributing factor to the reduction of a prey resource. However, while highly effective pest suppression is achieved in the short term, the specificity of these organisms will cause them to rapidly decline in numbers following reduction in the abundance of prey. In contrast, generalist predators are released only as the second line of defence, due to the possibility that they may have a negative impact on specialist predators or parasitoids that are susceptible to intraguild predation (Brodeur *et al.*, 2002). In any situation where two or more natural enemy species are concurrently applied in biological control, their respective population dynamics, and subsequently that of the pest species may vary according to their characteristics. Such complex interactions may result in the dampening of herbivore population fluctuations, subsequently stabilizing a system, or may disrupt the activity of specialists (McCann *et al.*, 1998). Successful biological control may only be achieved when interactions among organisms are known and predictable.

Biological control is a continually improving field of scientific research (van Lenteren, 2000). It requires an understanding of complex interactions existing not only between one natural enemy and the herbivore but of all subsequent guild interactions that may result from the use of a cocktail of natural enemies. Not all biological control agents afford practitioners the same result in identical conditions. Thirty natural enemy species were available in Canada in 2002, of which 23 predator species are used for biological control on the greenhouse tomato (Brodeur *et al.*, 2002). These belong to six insect and two mite

families. When selecting and integrating the use of these biological control organisms within the framework of a pest management program, practitioners may invest a good deal of time and energy. Yet, results are not necessarily guaranteed. For this reason, it is not surprising that many producers resort to the use of pesticides. The frequent failure of biological control emphasizes our poor understanding of the nature of interactions between natural enemies. Better choice of the constituents of a biological control ‘cocktail’ will reduce the number of biocontrol organisms needed to control herbivores giving growers a financial incentive to invest in such strategies (Brodeur and Rosenheim, 2000).

Intraguild predation is considered to be one of the major factors affecting the structure, distribution and abundance patterns of multispecies assemblages and may contribute to determining the effectiveness of a given pest management strategy (Polis and McCormick, 1987). In the next section, I define intraguild interactions. I explore the possible outcomes of these relationships and provide examples in natural food webs to display the breadth of their applicability. I then examine the implication of feeding habits to intraguild interaction and look at the subsequent impact they may have on biological control.

1.4. Intraguild interactions

1.4.1. What is Intraguild Predation (IGP)?

A guild is a group of species that use identical and potentially limited resources and thus compete for it (Root, 1967). Intraguild predation is therefore defined as the killing and eating of species that use similar resources and are therefore potential competitors (Polis and McCormick, 1987). Polis *et al.*, (1989) have categorized the relationships that may exist between two interacting species as: competition (-,-), predation/parasitism (+,-), mutualism (+,+), commensalism (+,0), or amensalism (-,0). Along this continuum, IGP consists of lethal forms of competition or predation/parasitism. In biological control, a superior competitor that reduces exploitation competition for a limited resource may be desirable. Such is the case when pest insect density is low. However, when a superior predator preys preferentially upon other predator species, the effectiveness of biological control may be reduced.

1.4.2. Examples of IGP in various natural ecosystems

Intraguild predation occurs in many different food webs in aquatic, marine and terrestrial systems. To describe the breadth of IGP, two natural food webs are presented. In a first example, Polis and McCormick (1987) examined the interactions between four species of desert scorpions in the Coachella Valley, California. While these species shared similar prey, they also engaged in IGP by feeding on one another. In fact, the other scorpion species constituted between 8 and 21.9 % of an individual species dietary intake. The specific mortality caused by the dominant predator scorpion *Paruroctonus mesaensis* Stahnke (Scorpiones: Vaejovidae) generally held an inverse relationship to the densities of the smaller scorpion species, *P. luteolus* and the *Vaejovis confusus* Stahnke (Scorpiones: Vaejovidae), but not of third intraguild prey species *Hadrurus arizonensis* Ewing (Scorpiones: Vaejovidae). Two important factors affecting IGP in this and other food webs are the size of individuals as well as the availability of extraguild prey. Larger scorpions of any species were always the predator and IGP was greatest when insect availability was low.

In another natural food web, that of a littoral lake community, Diehl (1995) manipulated the density of an omnivorous top consumer, the benthivorous perch, *Perca fluviatilis* L. to study the importance of IGP on the abundance of benthic invertebrate predators, larvae of alderfly *Sialis lutaria* (Megaloptera: Sialidae), intermediate odonate consumers, and of their common prey, chironomid larvae. In the absence of the perch, *S. lutaria* predator densities were constant over time. However, in its presence both *S. lutaria* and odonate predator densities experienced an important reduction. In addition, although chironomid abundance was strongly reduced at the lowest perch density, it levelled off as perch density was increased. In both of these systems, IGP played a key role in structuring the observed patterns of distribution and abundance of species.

1.4.3. Qualifying IGP

IGP exists when one of several predators is not restricted to feeding only on their common prey organism. It is further characterized by the reciprocal feeding habits of guild members

(Polis *et al.* 1989; Lucas *et al.*, 1998). First, IGP may be symmetric or asymmetric. In asymmetric IGP, one dominant species is always the predator of the other. Symmetric IGP occurs when there is mutual predation between both species. Furthermore, the vulnerability of organisms to predation may change with growth and development. Intraguild predation may be a more important factor for certain lifestages of an organism.

1.4.4. Impact of intraguild interactions on biological control

Many research projects have identified intraguild interactions including IGP as interesting as desirable outcomes when multiple biological control organisms are applied together (Rosenheim *et al.*, 1995; Ferguson and Stilling, 1996). Ideally the result of a given species 'cocktail' or assemblage is cooperation and more specifically, synergism in which the two species may actually exceed the additive control achieved by each agent separately. When intraguild predation exists, the net effect of interactions between natural enemies may range from reduction in the quality or quantity of plant material due to the predation between natural enemies, or rather the suppression of the herbivore through a reduction in interspecific competition.

In one case, the infection of the aphid, *Metopolophium dirhodum* Walker by the generalist fungus, *Erynia neoaphidis* Remaudière & Hennebert (playing a top consumer) may have had important implications on aphid reproduction and control by the aphid parasitoid *Aphidius rhopalosiphi* De Stefani Perez as well as on parasitoid populations, which were negatively correlated to hosts infected by the entomopathogen (Brobyn, 1988). During the early stages of infection, the parasitoid does not discriminate between healthy and infected prey and therefore may oviposit in unsuitable hosts. Only at successively later stages during the development of infection in aphid hosts does the parasitoid detect infection and subsequently reduce ovipositions in them. Biological control practitioners can avoid such antagonistic intraguild interactions by temporally or spatially separating aphid parasitoid introductions from the fungal treatment. In contrast, intraguild interactions between biological control organisms can lead to synergistic interactions. Such is the case where control of the Colorado potato beetle (Coleoptera: Chrysomelidae) using both the predatory stinkbug *Perillus bioculatus* (Hemiptera: pentatomidae) and *Bacillus thuringiensis* var.

tenebrionis is greater than applying either of these natural enemies separately (Cloutier and Jean, 1998). And in yet another case, *Bacillus thuringiensis* used to control white grubs *Cyclocephala hirta* Le Conte (Coleoptera: Scarabaeidae), and *C. pasadenae* Casey (Coleoptera: Scarabaeidae), may actually facilitate entomopathogenic nematodes *Heterorhabditis bacteriophora* Poiner (Nematoda: Heterorhadditidae) or *Steinernema glaseri* Steiner (Nematoda: Steinernematidae) in attacking scarab grubs when *B. thuringiensis* is applied at least seven days prior to the introduction of nematodes (Koppenhöfer and Kaya, 1997). Finally, Levin *et al.* (1983) also demonstrated that the parasitoid *Apanteles glomeratus* L. markedly improved the transmission of the granulosis virus (PrGV) to butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). The outcome of such interactions depends on a number of factors including behaviour, physical or morphological characteristics and the role played by each organism in a given trophic web.

1.4.5. Whitefly biological control

In certain situations, biological control involving the concurrent use of more than one agent may provide an advantage over the use of a single specific natural enemy. In the greenhouse environment, whitefly suppression by the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) may be limited in cool climate (Perdikis and Lykouressis, 2002). For this and other such limitations, to use multiple natural enemy species may be a more effective strategy. In certain parts of the world, the predaceous mirid bugs *Macrolophus caliginosus* Wagner and *Nesidiocorsis tenuis* Reuter have been investigated for their ability to control whitefly populations and for their potential interference with the parasitoid *E. formosa* (Naninni, 2000). While predators and parasitoids successfully controlled whitefly infestations in April-May, parasitoid abundance became heavily reduced further in the season due to predation of developing stages of *E. formosa*. As a result, the timing and number of predator and parasitoid releases were considered important factors in the success of whitefly control. For yet another generalist predator, the coccinellid *Delphastus pusillus*, predation of parasitized hosts is affected by parasitoid ontogeny (Hoelmer *et al.*, 1994). While the predator will readily feed upon sweet potato whiteflies parasitized by first-instar aphelinid parasitoids, it will avoid feeding on whiteflies containing third-instar and pupal parasitoids. These contrasting studies demonstrate the

breadth of predator response to parasitized hosts.

Studies of the interactions between parasitoids and pathogens of whiteflies have been conducted that further explain the role of insect behaviour in pest suppression. Landa (1984) examined the concurrent application of the parasitoid *E. formosa* with the fungal entomopathogen *Aschersonia aleyrodis* and identified this interaction as effective as it did not cause a negative interaction. Whitefly control was superior when both natural enemies were used together compared to when they were applied separately (Landa, 1984). This results from the selection of uninfected prey for parasitism by *E. formosa*. In the US and Europe where the use of the hyphomycete *Beauveria bassiana* Balsamo (Vuillemin) for the control of the greenhouse whitefly has become increasingly frequent, it will be important to identify the outcome of interactions that may exist between the pathogen and other biological control organisms (Butt *et al.*, 2001; Inglis *et al.* 2001).

BotaniGard® (Emerald BioAgriculture Corp.) is a leading product on the biocontrol market, that is based on the conidia of *B. bassiana*. In the advent of registration of such products in Canada, one of the primary concerns that must be investigated is the potential impact of this pathogen on non-target organisms. In the greenhouse environment, *B. bassiana* is likely to interact with whitefly natural enemies including the parasitoid, *E. formosa* as well as the predator *D. hesperus*. In this study, the effectiveness or impediments resulting from these interactions are described (Figure 1-2). In this framework, the importance of predator behaviour *versus* pathogen infection and parasitism to the outcome of interactions is considered. Through their characterization, pest suppression may be optimized through the intelligent use of the right density and type of organism.

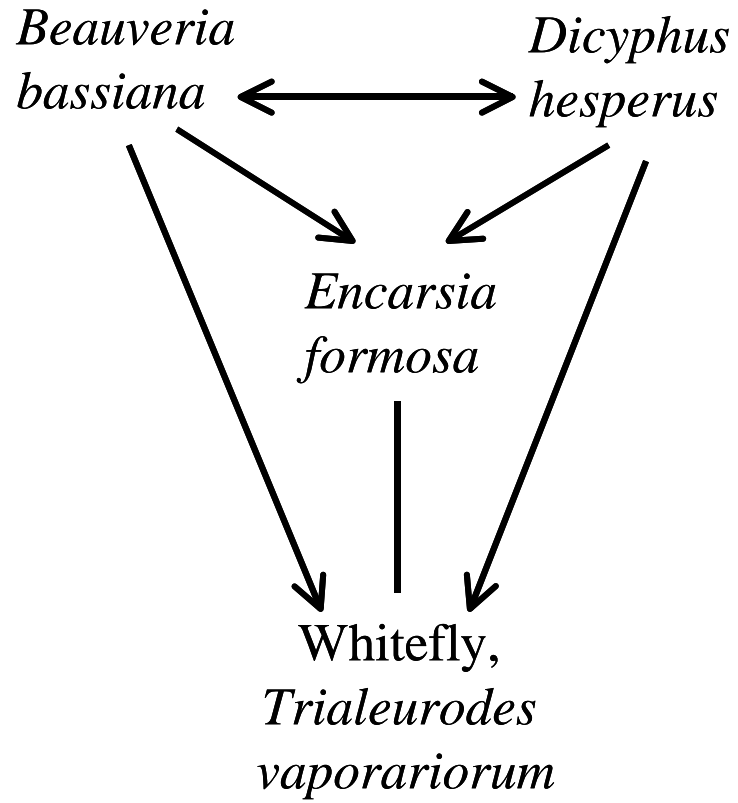


Figure 1-2 Diagram of the trophic interactions existing between the organisms studied here.

1.5. Study organisms

1.5.1. The herbivore, *Trialeurodes vaporariorum*

Greenhouse whitefly, *Trialeuroides vaporariorum* (Homoptera: Aleyrodidae), are important pests in greenhouse vegetable and ornamental crops, have achieved a worldwide distribution. They may be inconspicuously introduced via transactions between greenhouses in different regions of the world (Fransen and van Lenteren, 1994). Greenhouse whiteflies are phloem-feeding and cause economic losses through direct crop injury and by acting as a vector of plant fungal infection such as by *Cladosporium spp.* Such fungi are consequently responsible for lowering photosynthesis (Byrne *et al.*, 1990; Johnson *et al.*, 1992). Damage is caused by the whitefly's sucking mouthparts, which are used to extract sap from the host plant (van Roermund *et al.*, 1997). Chemical control of *T. vaporariorum* is considered very difficult as a result of morphological and autecological characteristics such as a waxy cuticle, the colonization of the underside of leaves and rapid population growth (Osborne and Landa, 1992). Whiteflies have developed resistance to numerous broad-spectrum insecticides including chlorinated hydrocarbons, organophosphates, and pyrethroids (Dreistadt, 2001). In addition, its multiple overlapping generations allow it to become abundant very rapidly (Dreistadt, 2001). As a result, the development of pest management alternatives has become a priority (Fargues *et al.*, 2003).

Over the course of its life cycle, the greenhouse whitefly may take one of the following forms: the egg, one mobile nymphal instar followed by three sedentary nymphal instars (also called scales), and the adult (Osborne and Landa, 1992). Whitefly eggs laid on the underside of leaves. The eggs hatch and develop into successive nymphal stages of which the first stage is mobile. Whiteflies of the final or fourth instar of whitefly, is often referred to as pupae. This instar is divided into three sub-stages. Exceptionally, whiteflies exhibit complex metamorphosis despite being within the homoptera, a group of mostly hemimetabolous insects. During the second sub-stage, the nymph thickens, turns to an opaque white and develops a waxy cuticle. Finally during the third sub-stage, the eyes of the adult whitefly become red and are apparent through the cuticle. The adult whitefly emerges from a slit in the fourth instar exoskeleton (Gill, 1990). Total developmental time

from egg to adult may last from 21 to 30 days depending on temperature (Burnett, 1949).

1.5.2. The parasitoid, *Encarsia formosa*

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is an effective parasitoid of several whitefly species including the greenhouse whitefly, *T. vaporariorum* in tomato crops for which it has demonstrated excellent control (van Lenteren and Woets, 1988; Fransen and van Lenteren, 1993; 1994; Hu *et al.*, 2002)(Figure 1-5, Plate D). Commercial use of this parasitoid, which has started in the 1970's, has since expanded to become one of the most important methods used to control whitefly. *Encarsia formosa* is now available worldwide (van Lenteren *et al.*, 1996). Successful biological control by *E. formosa* is related to its rapid development within hosts (Figure 2). In fact, van Lenteren *et al.* (1996) have shown that on many important crops, a single *E. formosa* or her offspring can kill more whiteflies per unit of time than an individual whitefly female can produce. At first detection of whiteflies in tomato greenhouses, adequate control is achieved by weekly parasitoid releases (van Roermund *et al.*, 1997).

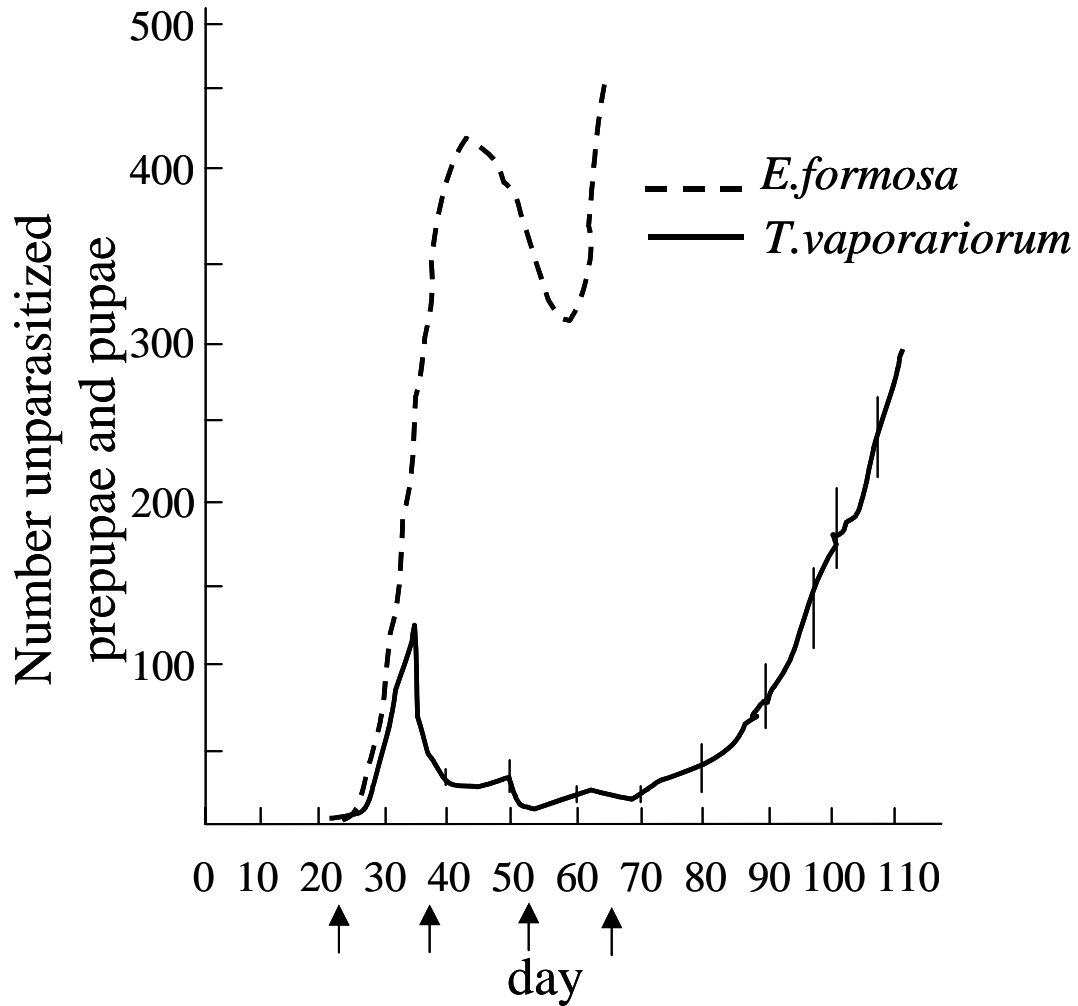


Figure 1-3 Simulated model of the population response by parasitoid *Encarsia formosa* to an increase in number of the greenhouse whitefly *Trialeurodes vaporariorum*. Arrows on x-axis represent moments of parasitoid releases. Reproduced with permission from Van Roermund *et al.*, 1997.

Adult parasitoids preferentially oviposit within 3rd and 4th instar whitefly with the greatest rate of development occurring when 3rd instar whitefly are parasitized (Hu *et al.*, 2002). Eight to ten days after parasitism, *E. formosa* larvae stop feeding and begin their pupal development, at which point parasitized whitefly pupae appear black. The adult parasitoid emerges about 10 days later. At 22.5°–25°C, development of immature *E. formosa* in fourth-instar *T. vaporariorum* nymphs takes 15 days on tomato (Woets and van Lenteren, 1976) and tobacco (Arakawa, 1982).

While successful as a preventative biological control agent, *E. formosa* is limited in its ability to control whiteflies when they have achieved high densities. This is especially true for periods of the year with peak high or low temperature. Lower theoretical threshold for development of pre-imaginal stages is 10 to 13° C, and upper lethal temperature for immature parasitoids is estimated at 39° C (van Roermund and van Lenteren 1992). When temperatures are below or above these thresholds, whitefly populations may become difficult to control by *E. formosa*. In such situations, biological control practitioners resort to the use of other control measures (Perdikis and Lykouressis, 2002).

1.5.3. The entomopathogen, *Beauveria bassiana*

White muscardine, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) is a ubiquitous fungus occurring naturally in many areas of the world (Feng *et al.*, 1994)(Figure 1-5, Plate E and F). Isolated from over 700 species of insects from nine orders, it most commonly infects Lepidopteran and Coleopteran hosts (Li, 1988). *Beauveria bassiana* was the first reported insect pathogen, originally isolated from the silkworm, *Bombyx mori* L. (Lepidoptera Bombycidae), by Agostino Bassi in 1834, and has subsequently become the most extensively studied and exploited entomopathogen (Glare and Milner, 1991). *Beauveria bassiana* is now exploited in greenhouse and outdoor crops as a tool for the control of many agricultural pest arthropods including whiteflies, aphids, thrips, psyllids, weevils and mealybugs (Shah and Goettel, 1999). It is the active component of commercially available biological control products such as BotaniGard®, Mycotrol® (Emerald Bioagriculture Corp.) and Naturalis® (Troy Biosciences) which are based on the conidia of *B. bassiana* (Butt *et al.*, 2001; Inglis *et al.* 2001). The successful commercialization of these formulated products owes its success to the fact that conidia

may be stored dry at room temperature for over a year (Goettel and Hajek 2001). These products are currently available in many regions of the world and may soon be registered for use in Canada.

Pathogenesis of insect hosts due to *B. bassiana* occurs mainly through infection via integument though it may also enter through the respiratory system (Feng *et al.*, 1994; Pehrul and Grula, 1979). Host infection consists of the germination of conidia on the cuticle surface, the penetration of hyphae through the activity of cuticle lytic enzymes produced by the fungus, proliferation inside the hemocoel and host death due to toxemia (Khachatourians, 1991). Under favourable conditions, the fungus emerges and produces aerial conidia over the host cuticle (Feng *et al.*, 1994; Gupta *et al.*, 1995; Pehrul and Grula, 1979). The virulence of *B. bassiana* is attributed to the production of toxins including beauvericin, bassianolide and oosporein (Gupta *et al.*, 1995). Infected hosts exhibiting the production of antibiotic oosporein gives hosts a red appearance making them easily detectable (Vining *et al.*, 1962). Pathogenicity of *B. bassiana* for the greenhouse whitefly *T. vaporariorum*, has been studied by Siongers and Coosemans (2003) who found that BotaniGard® on cucumber plants had the greatest impact on the first nymphal instar with subsequent instars appearing to be decreasing in sensitivity with age (Siongers and Coosemans, 2003). In comparison, James *et al.*, (2003) found that second and third instars of the silverleaf whitefly *Bemisia argentifolii* (Bellows and Perring) (Hemiptera: Aleyrodidae) were the most susceptible larval stages. While conidia of *B. bassiana* readily germinated on the cuticle of second instars (54 %), the cuticles of fourth instars were apparently less susceptible with only 7 % of conidia germination (James *et al.*, 2003). Lipids in the whitefly cuticle were found to have a toxic or inhibitory effect on conidia germination (James *et al.*, 2003).

1.5.4. The generalist predator, *Dicyphus hesperus*

Dicyphus hesperus Knight (Hemiptera: Miridae: Bryocorinae) belongs to the Dicyphini, a tribe whose members are well known predators of pest species and are therefore used in biological control on greenhouse or field vegetable crops worldwide (McGregor *et al.*, 1999)(Figure 1-5 Plates A, B and C). *Dicyphus hesperus* is native to North America and is

widely distributed across Canada (Henry and Wheeler, 1988; Maw *et al.*, 2000). It is an effective predator of several pest insects including many whitefly species, aphids, lepidopterans and mites (McGregor *et al.*, 1999). When prey is abundant, *D. hesperus* populations increase through reproduction resulting in a steady decrease in pest populations (Gillespie *et al.*, 2000b). In this way, *D. hesperus* responds well to increases in whitefly populations in greenhouse crops such as the tomato (Figure 1-4, adapted from Gillespie *et al.*, 2000a).

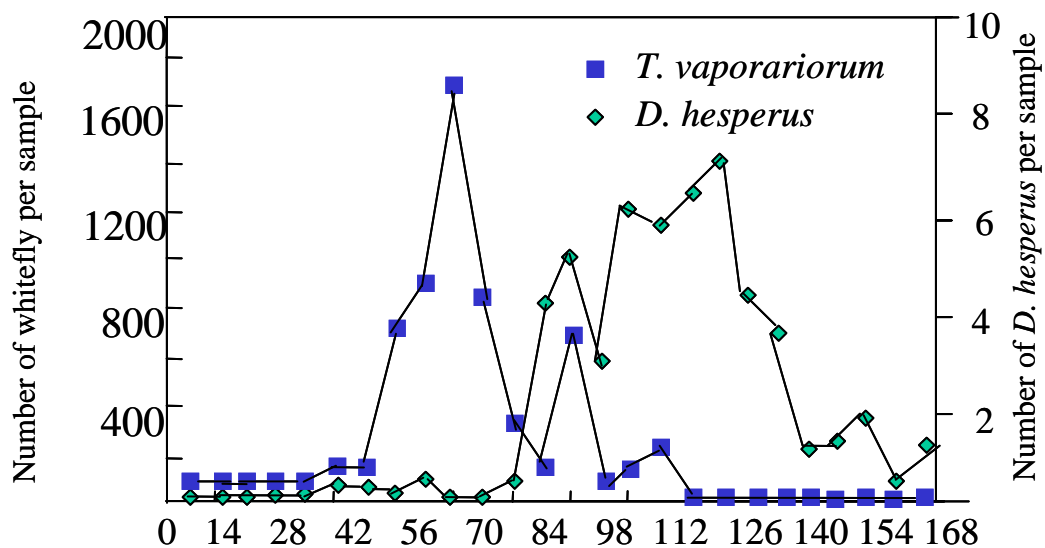


Figure 1-4 Populations of the greenhouse whitefly *Trialeurodes vaporariorum* and of the predator *Dicyphus hesperus* on tomato plants in a research greenhouse over the course of a growing season. Reproduced with permission from Gillespie *et al.*, 2000a.

McGregor *et al.* (1999) have demonstrated the effectiveness of *D. hesperus* as a predator of the greenhouse whitefly, particularly on greenhouse tomato crops. In Canada, this predator has become an interesting complement to other biological control organisms used in commercial greenhouses (McGregor *et al.*, 1999). Greenhouse growers in many provinces exploit the generalist nature of this predator as well as the possibility of long-term whitefly suppression (Gillespie, personal communication). When feeding on the greenhouse whitefly, *T. vaporariorum*, females of *D. hesperus* feed preferentially on older fourth-instars, and males on younger second- and third-instars scales (McGregor *et al.*, 1999). However neither sex is exclusively restricted to these whitefly stages. *Dicyphus hesperus* feeding is also mostly nocturnal (VanLaerhoven *et al.*, 2003).

Gillespie and McGregor (2000) demonstrated the importance of plant feeding to *D. hesperus* for whom complete nymphal development was achieved only in 6% of individuals fed a diet of *Ephesttia kuehniella* eggs alone, while 97% and 88% of nymphs completed development when this diet was supplemented with either tomato leaves or a source of water, respectively.

In the greenhouse environment, *D. hesperus* populations are maintained on a native host plant, common mullein *Verbascum thapsus* L. on which survival of the predator is optimized (Sanchez *et al.*, 2003). This predator is the next major contributor to whitefly suppression on tomato crops besides the well-established parasitoid *E. formosa*. The use of *D. hesperus* is part of a biological control strategy that aims to optimize the effectiveness and durability of greenhouse whitefly suppression. A study examining the interaction of the related mirid predators *M. caliginosus* Wagner and *Nesidiocorsis tenuis* Reuter with *E. formosa* suggests that the combined use of these predators and the parasitoid resulted in superior control of the greenhouse whitefly, whose populations were significantly reduced. However, in this same study, intraguild predation also led to an important reduction in the developing stages of the parasitoid, resulting in a decrease in its abundance (Nannini, 2000). In this light, evaluating the appropriate release period and numbers of predators and parasitoids was considered important in order to prevent unnecessary expenses in pest suppression. Also, when using an entomopathogen to control overabundant whitefly populations, it is important to know the susceptibility of *D. hesperus* and *E. formosa* to the

pathogen. *Beauveria bassiana* has been found to naturally infect mirid pests such as on the cocoa mirid, *Helopeltis theobromae* (Lim *et al.*, 2000). However, little is known of the interaction of mirids in artificial agricultural settings such as those created in greenhouses. In such situations, are mirid predators able to detect and reject whitefly hosts? Interactions resulting from the combined use of whitefly natural enemies including: the parasitoid *E. formosa*, the predator *D. hesperus* and the entomopathogen *B. bassiana* are classified as potential intraguild interactions. Such interactions may impede or increase the suppression of whitefly and thus require investigation.

1.6. General predictions

In the laboratory, the suitability of prey infected by *B. bassiana* along the continuum of fungal development for the predator *D. hesperus* was evaluated. It is possible that the predator may detect changes in the host *T. vaporariorum* when it is infected by *B. bassiana*, especially late during the process of infection. In contrast, it is hypothesized that whitefly prey parasitized by *E. formosa* would be consumed by *D. hesperus*. It is suggested that predator rejection versus acceptance of whitefly, could have important implications in applied biological control.

Understanding the role of intraguild interactions in greenhouse biological control may contribute to an effective pest management strategy to control a given pest. In the greenhouse, the effect of the pathogen *B. bassiana* on the population dynamics of the parasitoid, the predator and of the greenhouse whitefly was tested. First, it was predicted that the generalist pathogen would have a negative impact on natural enemy populations in addition to the expected reduction of whitefly abundance. It was also expected that parasitoids and predators would be less exposed to *B. bassiana* as compared with whitefly larvae or pupae. Finally, whitefly suppression afforded by the concurrent use of these three natural enemies was expected to exceed that achieved when the pathogen was absent.

1.7. Objectives

Our research objectives were:

- 1 To assess the discriminatory capacity of the predator *Dicyphus hesperus* towards *B. bassiana* infected or parasitized whitefly prey through small scale, non-choice

predation experiments.

- 2 Under greenhouse conditions, to evaluate the impact of BotaniGard® on the survival and reproduction of *D. hesperus* and *E. formosa* in relation to its efficacy as a control agent of the greenhouse whitefly.

Figure 1-5 Photographs of organisms studied in this thesis. A: *Dicyphus hesperus* adult female. Upper panel top view, B: lower panel side view; C: Third instar nymph of *Dicyphus hesperus*; D: Adult *Encarsia formosa* parasitoid



Plate A: top view; Plate B: side view of adult predator *Dicyphus hesperus*



Plate C: *Dicyphus hesperus*, 3rd instar nymph; Plate D: *Encarsia formosa* adult.



Plate E: *Beauveria bassiana* infected *Dicyphus hesperus* predator; Plate F: *Beauveria bassiana* infected fourth instar whitefly, *Trialeurodes vaporariorum*.

Description of the chapters

In the second chapter, the acceptability of infected or parasitized whitefly to the predator *D. hesperus* was assessed. The impact of interactions among guild members including the predator *Dicyphus hesperus*, the parasitoid *Encarsia formosa* and the pathogen *Beauveria bassiana* was characterized. Laboratory predator non-choice experiments were conducted which provided insight on the behavioural interactions between predators with intraguild competitors in the form of *E. formosa* parasitized or *B. bassiana* infected whitefly prey. Through these results, the outcome of interactions occurring in the greenhouse may be better understood and predicted.

In the third chapter, a greenhouse experiment explored the interactions that occur between the above biological control agents of the whitefly at the scale of the greenhouse. Such a setting allowed for a good estimation of population interactions in a large environment, which may vary widely in microclimate, arthropod dispersal and density over time. Greenhouse compartments in which *E. formosa* and *D. hesperus* were applied, were treated with a formulated suspension of *B. bassiana* GHA (BotaniGard® Emerald BioAgriculture) or a control water carrier. This experiment offered insight on the impacts of the entomopathogen on mixed biological control, specifically with regards to the maintenance of predator and parasitoid populations.

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Chapter 2. Prey selection by predator *Dicyphus hesperus* of infected or parasitized greenhouse whitefly

2.1. Résumé

Récemment, l'utilisation du prédateur généraliste *Dicyphus hesperus* Knight comme agent de lutte biologique contre la mouche blanche des serres, *Trialeurodes vaporariorum* Westwood s'est développée. Ce prédateur se joint aux autres ennemis naturels efficaces disponibles, y inclus le parasitoïde *Encarsia formosa* Gahan, et l'entomopathogène *Beauveria bassiana* Balsamo. Dans cette étude l'interaction entre ces espèces a été évaluée afin de pouvoir distinguer entre les interactions efficaces de celles à éviter. En premier, l'acceptabilité de mouche blanches parasitées ou infectées pour le prédateur *D. hesperus* à divers stades de développement des parasitoïdes ou des pathogènes dans ces hôtes a été étudiée. Les prédateurs acceptent les mouches blanches parasitées, peu importe le stade de développement du parasitoïde. Devant des mouches blanches infectées par *B. bassiana*, les prédateurs ont rejeté particulièrement celles montrant des hyphes ou de l'oosporéine. L'acceptabilité de mouches blanches parasitées par *E. formosa* suggère l'interférence du prédateur *D. hesperus* avec le parasitoïde tandis que l'interaction prédateur-pathogène ne montre aucun antagonisme.

2.2. Abstract

The mirid bug *Dicyphus hesperus* Knight, is an effective biological control agent of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood. Potential interactions between *D. hesperus*, the whitefly parasitoid *Encarsia formosa* Gahan and the generalist entomopathogen, *Beauveria bassiana* Balsamo, are of particular interest. This study investigates *D. hesperus* acceptance of parasitized and infected greenhouse whiteflies. In non-choice laboratory experiments, parasitized or infected third instar whitefly were offered as prey to second instar or adult female predators. Experiments were performed over time, that assessed the effect of time since parasitism or infection on the acceptability of whitefly prey. While predators did not reject either parasitized or non-parasitized whiteflies at any parasitoid developmental stage, the predation rate of *B. bassiana*-infected whitefly was markedly reduced when infection was manifested through the production of oosporein or hyphae on the surface of prey. The results suggest asymmetric intraguild predation of *E. formosa* and *B. bassiana* by *D. hesperus*.

2.3. Introduction

Dicyphus hesperus (Hemiptera: Miridae) belongs to the Diciphinae tribe whose members are frequently used in biological control on field and greenhouse vegetable crops (reviewed by Alomar and Wiedenmann, 1996; Coll and Ruberson, 1998). The species is native and widely distributed in North America (Maw *et al.*, 2000). It preys on many small arthropods (Henry and Wheeler, 1988; Gillespie *et al.*, 2000). *Dicyphus hesperus* is a zoophytophage as it feeds on both animal and plant biomass, whereby it is believed that the latter mainly provides water needed for prey digestion (Gillespie and McGregor, 2000). Phytophagy may also allow predators to sustain populations under conditions of prey scarcity (Naranjo and Gibson, 1996; Wiedenmann *et al.*, 1996; Coll and Guershon, 2002), thereby favouring establishment as biological control agents over the long term in agricultural crops. In Canada, *D. hesperus* is most commonly used in inoculative and inundative releases for the control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) on tomato crops in greenhouses (McGregor *et al.*, 1999).

Biological control of the greenhouse whitefly has relied for more than three decades on regular prophylactic releases of the specialist whitefly parasitoid, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (van Lenteren *et al.*, 1996; van Roermund *et al.*, 1997). Although *E. formosa* is an effective biological control agent in most situations, failure may occur (Hamdan, 1997) possibly due to environmental factors such as cold temperatures, short photoperiod and low light intensity. Nowadays, *T. vaporariorum* biological control can be improved by using *E. formosa* in combination with other parasitoid and predator species, such as *D. hesperus*. In addition, a range of entomopathogenic fungi are available for whitefly biological control (Meeke *et al.*, 2002), including *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycete), a ubiquitous fungus occurring naturally in many areas of the world (Knauf and Wright, 1994). At present, microbial agents are applied to crops as are conventional chemical insecticides, and provide a quick and substantial reduction of pest populations (Lacey *et al.* 2001).

Suppression of whitefly populations can be achieved by exploiting the characteristics of each biological control agent when two or more agents are applied concurrently. However,

experimental evidence indicates that biological control can be reduced or even disrupted by interactions between natural enemies (reviewed by Rosenheim *et al.*, 1995). The development of effective biological control strategies, in which parasitoids, predators and pathogens are integrated, requires knowledge of their interactions. In this laboratory study, the capacity of *D. hesperus* to discriminate against whitefly prey that are parasitized by *E. formosa* or infected by *B. bassiana* was explored. An ability to detect and reject parasitized and infected prey would indicate that there might be little interference between these biocontrol agents.

2.4. Materials and Methods

2.3.1. Insect rearing

The greenhouse whitefly, *T. vaporariorum* was reared on tobacco plants *Nicotiana tabacum* at 24 ± 3 °C, with a 16: 8 L: D photoperiod and a RH of 76 ± 2 %. Adult whiteflies were released onto small tobacco plants placed inside rearing cages for a 24 h period, during which time they laid eggs on the underside of leaves. Afterwards, adult whiteflies were removed, resulting in the development of a synchronized cohort of whitefly nymphs for experiments. A constant age of whiteflies used in all tests was achieved by inoculating host plants with adult whiteflies on the appropriate day previous to tests.

Encarsia formosa parasitoids were obtained from Biobest®, Biological Systems (Westerlo, Belgium), in the form of parasitized *T. vaporariorum* pupae glued onto cardboard cards. Cards were maintained in a screen ventilated Petri dish with a piece of cotton swab drenched in 25 % sugar water for nourishment of emerging adult females. Parasitoids were maintained in a growth chamber at 18.0 ± 2.0 °C during the day and at 17.0 ± 0.9 °C during the night, with a 16: 8 L: D photoperiod and a RH of 55 ± 5 % until used for parasitizing test whitefly.

Dicyphus hesperus was obtained from a non-diapausing strain maintained at the Pacific Agriculture and Agri-Food Canada Research Centre (PARC) in Agassiz, British Columbia. Colonies were maintained at 24.6 ± 3.1 °C in the day and 18.3 ± 2.3 °C at night under a 16: 8 L:D photoperiod and a constant RH of 50.0 ± 5.1 %. Predators were reared on tobacco

plants *Nicotiana tabacum* and were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Ciba Bio-Logicals, Guelph, Canada).

2.3.2. Fungus infected prey

The formulated product BotaniGard® (Emerald BioAgriculture, Lansing, MI) whose active ingredient consists of conidia of *B. bassiana* strain GHA, was plated onto potato dextrose agar (PDA) plates in order to isolate formulation-free conidia. Following inoculation of plates, they were incubated for 10 days at 25°C and dried for 24 hours. Harvested conidia and mycelium were stored at 4°C for 1 day. Prior to whitefly treatment, the conidia and mycelium stock was suspended in a solution of water and Tween® surfactant (0.01% in distilled water). The suspension was then vortexed for 3 minutes and filtered using a fine fibre cloth, Miracloth® (Calbiochem, La Jolla, CA). The spore suspension was calibrated to 1×10^8 conidia/ml, a concentration giving ca. 80% whitefly mortality in pre-tests. Using a Marino® spray bottle, the fungus was applied to the underside of leaves on whitefly infested tobacco plants to runoff. Control plants were sprayed with a solution of sterilized distilled water and 0.01% aqueous Tween. Treated leaves were then individually covered with transparent polythene bags for 48 h in order to provide optimal moisture conditions for *B. bassiana* spore germination (90 ± 10 % R.H., $25.9 \pm 1^\circ\text{C}$; % \pm SEM). Plants were subsequently maintained at 24 °C, at a RH of 76 ± 6 % and a 16:8 photoperiod for 1 to 7 days, a duration that corresponds to the time required for maximal mycosis in a whitefly population (Labbe, unpublished data).

Of the *B. bassiana* treated whiteflies, an increasingly large proportion over time adopted either a red coloration, due to the production of oosporein; an antibiotic produced by *B. bassiana*, or became covered with *B. bassiana* hyphae (Figure 1-5, Plate F).

The viability of conidia was determined by applying 50 µl of the fungal solution onto a potato dextrose agar (PDA) plate amended with 0.005 % Benlate™ (Benomyl, Du Pont Agricultural Products, Wilmington, DE). This amendment facilitates counting of viable germinated cells by halting cell division, thus restricting the production of mycelium (Goettel and Inglis, 1997). After 48 h, germinated and non-germinated conidia were counted and the viability assessed at 97.8 ± 0.3 % for experiment 1 and at 96.1 ± 2.2 % for

experiment 2.

2.3.3. Parasitoid attacked prey

Based on the embryonic and larval development of *E. formosa* at 26 °C (Hu *et al.*, 2003), days 2, 6 and 12 correspond to eggs, parasitoid embryo and pupae, respectively. The following procedure was used to parasitize and rear all hosts used in the experiments. Tobacco leaves upon which approximately 50 third instar whitefly were reared, were surface sterilized by dipping them into a 5% disodium chloride solution for 3 s. They were then placed in Petri dishes filled with cooling liquid 1.5% water agar. Once the agar was set and cool, parasitoid females at a ratio of approximately one parasitoid for four whitefly pupae were introduced into the whitefly containing Petri dishes. Whitefly pupae were exposed to parasitoids for a 24 h period during which dishes were maintained at 24.8 ± 3.2 °C in the day and 18 ± 2.8 °C at night under a 16:8 photoperiod. Following parasitism, adult parasitoids were removed and dishes returned to these same conditions. After 2 and 6 days, parasitized pupae were selected for use in experiments according to the presence of oviposition scars visible on the outer surface of whitefly pupae. For 12-day-old parasitized pupae, only those whitefly hosts that were blackened due to parasitoid development were selected.

2.3.4. Experiments 1 and 2

Experiments 1 and 2 tested the acceptability of *B. bassiana* treated and untreated whitefly to *D. hesperus*. In these experiments, 1-4 day old unmated *D. hesperus* adult females, or 2nd instar nymphs of unknown sex were placed individually within 15 cm diameter Petri dishes. In order to motivate predators to attack prey, they were starved for 48 h prior to experiments, but were provided wet cotton to avoid dehydration. Following the starvation period, ten- 5 mm wide leaf disks, with a whitefly pupae of a certain treatment class on each, were introduced into the test Petri dish. During tests, predators were offered only fully distended or turgid whitefly pupae to insure that predation could be easily detected. For each of these experiments, prey were selected as followed:

In experiment one, whitefly pupae were indiscriminately chosen from the leaves on which whitefly were reared and then treated to *B. bassiana*, for 1 to 7 days and repeated following the initial application of fungus or to the control-water treatment. For this experiment, trials were carried for 7 d. in order to evaluate the acceptability of whitefly during the development of infection.

Experiment 2 was designed to compare *D. hesperus* predator acceptance of *B. bassiana* infected whitefly belonging to two specific categories: oosporein-red pupae, hyphae-covered pupae whom were also compared to uninfected control pupae. In this experiment, whiteflies were selected on the fourth day following the pathogen treatment, according to their physical appearance.

For both experiments, one *B. bassiana*-infected or uninfected control third instar whitefly (23 days old) was introduced into a predator containing Petri dish for a 12 h period (4 light: 8 dark). The 12 h trial consisted not only of a 4 h light period but also 8 h of darkness which was chosen so that predation would be favored, as *D. hesperus* is habitually a night time feeder (VanLaerhoven *et al.*, 2003). After each trial predators were discarded. In other words, for both experiments 1 and 2, predators were subjects only once so that for each trial, treatment and repetition in time (block), a new and inexperienced predator was used. These 10 repetitions for each treatment were repeated over three blocks of time. After each 12 h test period, whitefly pupae were examined for signs of predator feeding under a microscope at a 40X magnification. The empty pupal case of a consumed whitefly can be recognized as dry and fragile when probed with forceps.

2.3.5. Experiment 3

The susceptibility of unparasitized and *E. formosa* parasitized whiteflies to predation by *D. hesperus* adult females and 2nd instar nymphs was determined 2, 6 and 12 days following parasitism. For these predator acceptance tests, one *E. formosa* parasitized or unparasitized third instar whitefly pupae isolated onto a 5 mm leaf disk was placed individually into a Petri dish containing a starved predator. Predators were exposed to prey for a 12 h period.

In control trials, predators were offered unparasitized whitefly of the same age as in the treatment. In treatment trials, predators were offered prey parasitized at one of the three parasitoid developmental stages. All trials were replicated three times each. For each replication, 20 new adult and 20 new 2nd instar predators were tested. Predators were subjects only once so that in each dish, treatment and repetition, a new and inexperienced predator was used. After the 12 h test period, whitefly pupae were examined for signs of predator feeding under a microscope at a 40X magnification. For control, 2 and 6 day old parasitized whitefly pupae, predation was recognizable as an empty pupal case that was dry and fragile when probed with forceps. In the case of 12-day-old parasitized whitefly pupae, consumption of the blackened, sclerotized pupae was identified by removing the entire top surface of the pupal case with a needle or fine forceps. In the case of predator fed pupae, this revealed an empty compartment. When not consumed, the top of the pupal case was not as easily removable and discharged much liquid content when crushed.

2.3.6. Statistical analysis

The proportion of whitefly fed upon by *D. hesperus* was arcsine or square-root transformed prior to analysis to normalize data. The homogeneity of variance was assessed using the Brown-Foresythe test and the fixed effects were analyzed with an ANOVA using the GLM procedure (SAS, 1999). In the first experiment, the effects of prey treatment, time following infection, and predator stage were examined using a three-way ANOVA. For experiments 2 and 3, two-way ANOVAs were used to test for treatment and predator stage effects. Following a significant difference, means were compared by Sheffe's multiple comparison tests (SAS, 1999). Significance levels for interpretation purposes were $P = 0.05$ for all tests.

2.4. Results

2.4.1. Fungus infected prey

In experiments, the incidence of predation of control whitefly was significantly greater than that of *B. bassiana* infected whitefly for both second instar nymphal and adult *D. hesperus*

predators (Table 2-1; Figure 2-1). The proportion of whitefly consumed by *D. hesperus* did not differ over time (Table 2-1). The significant block effect suggests variability between repetitions, and its inclusion allowed to correct for it.

In the second experiment, control whiteflies were more susceptible to predation by nymph and adult predators than infected whiteflies (Table 2-2; 2-3). No differences in the proportion of predator-consumed whitefly were observed between oosporein red, and hyphae covered prey.

2.4.2. Parasitized prey

In experiment 3, the incidence of predation was similar for unparasitized and parasitized whiteflies (Table 2-4; Fig. 2-2). This pattern was consistent regardless of predator instar and time since parasitism.

Table 2-1 Results of a three-way analysis of variance for effects of *Beauveria bassiana* treatment (Trt), predator developmental stage (S), time following whitefly infection (T) and of blocks (repetitions in time) on *Trialeurodes vaporariorum* predation by *Dicyphus hesperus*.

Source	DF	MS	F value	P value
Treatment (Trt)	1	2.2390	22.24	<0.0001
Predator instar (S)	1	0.0163	0.16	0.6890
Block	2	0.3433	3.41	0.0403
Time (T)	6	0.1343	1.33	0.2582
Trt*S	1	0.0164	0.16	0.6880
Trt*T	6	0.0261	0.26	0.9531
S*T	6	0.0191	0.19	0.9786
Trt*S*T	6	0.0897	0.89	0.5082
Error	54	0.1007		

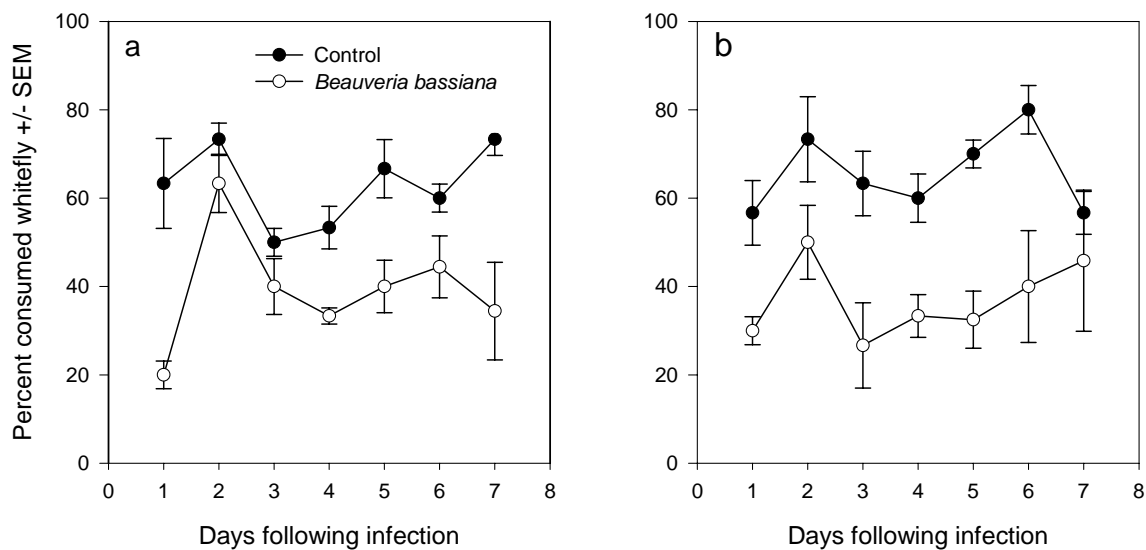


Figure 2-1 Percent ($X \pm \text{SEM}$) of *Beauveria bassiana* treated vs. control whitefly prey *Trialeurodes vaporariorum* consumed over time by 2nd instar (a) and adult female (b) *Dicyphus hesperus* during 12-h predation test for each time (n = 30, tests of 1 prey exposed to 1 predator).

Table 2-2 Results of a two-way analysis of variance for effects of *Beauveria bassiana* treatments (Trt) and predator developmental stage (S) and of blocks (repetitions in time) on *Trialeurodes vaporariorum* predation by *Dicyphus hesperus*.

Source	DF	MS	F	P
Treatment (Trt)	2	0.9794	49.07	<0.0001
Predator instar (S)	1	0.0001	0.00	0.9592
Block	2	0.0500	2.51	0.1312
Trt*S	2	0.0030	0.15	0.8638
Error	10	0.0200		

Table 2-3 Percentage of control and *Beauveria bassiana* treated *Trialeurodes vaporariorum* pupae at two late stages of infection, consumed by 2nd instar and adult female *Dicyphus hesperus* predators.

Treatment	Percent consumption ($X \pm \text{SEM}$)	
	2nd instar	Adult females
Control prey pupae	66.67 \pm 1.71 a ¹	61.67 \pm 1.29 a
Pupae with fungus hyphae	10.00 \pm 1.12 b	15.00 \pm 3.35 b
Red pupae with oosporein	3.33 \pm 0.65 b	5.00 \pm 1.12 b

1. Within columns, values with the same letter are not significantly different (Sheffé's *a posteriori* test, $P > 0.05$).

Table 2-4 Results of a two-way analysis of variance for effects of parasitism by *Encarsia formosa* (Trt), as related to parasitoid age, predator developmental stage (S) and of blocks (repetitions in time) on *Trialeurodes vaporariorum* predation by *Dicyphus hesperus*.

Source	DF	SS	MS	F value	Pr > F
Treatment (Trt)	3	0.0115	0.0038	0.16	0.9217
Predator instar (S)	1	0.0551	0.0551	2.29	0.1524
Block	2	0.2246	0.1123	4.67	0.0279
Trt*S	3	0.0071	0.0024	0.10	0.9600
Error	14	0.3366	0.0240		

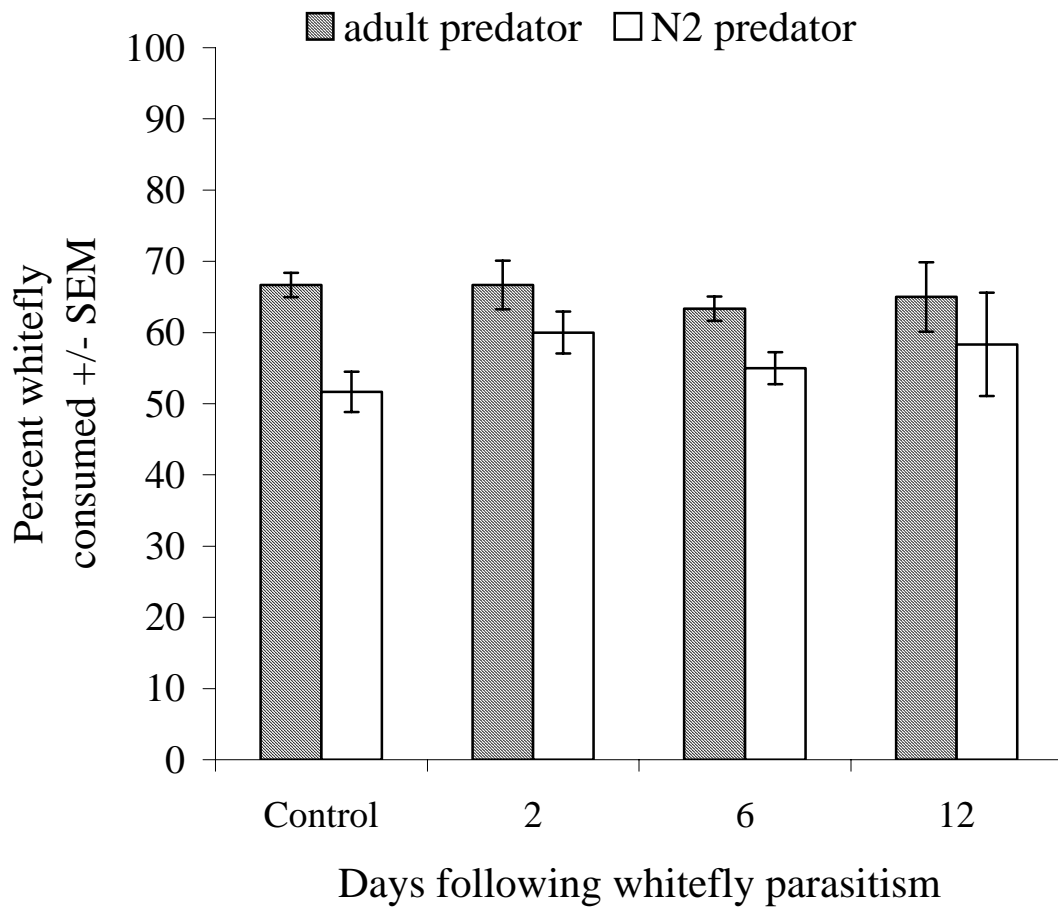


Figure 2-2 Proportion ($X \pm \text{SEM}$) of parasitized vs. control *Trialeurodes vaporariorum* prey consumed by adult and second instar (N2) *Dicyphus hesperus* predators during a 12-h test. Susceptibility of parasitized prey was determined 2, 6 and 12 days following parasitism by *Encarsia formosa* (n= 20).

2.5. Discussion

Predators have evolved the ability to select and exploit prey that support their growth, survival and reproduction. Our results suggest that the mirid *D. hesperus* has a wide host range towards *T. vaporariorum* whitefly prey that were healthy, infected or parasitized. Immature and adult females fed readily upon healthy, *E. formosa* parasitized and *B. bassiana* infected whiteflies in the laboratory. However, they generally rejected infected prey that had produced either hyphae or oosporein. Hemipterans may detect subtle changes in the chemical or physiological makeup of prey (Cohen 1995). Mirids have sensory setae on their labial tip that serve as chemoreceptors with which predators may probe and assess suitable prey (Hatfield and Frazier, 1980). Furthermore, saliva secreted by hemiptera during feeding is almost immediately reingested and exposed to gustatory receptors (Miles, 1972). And finally, though they belong to a different order than does *D. hesperus*, it was shown that phytophagous insects of genus *Lygocoris* and *Lygus lineolaris* possessed visual capacities allowing them to discriminate between white and peach colored sticky cards (Legrand and Los, 2003).

2.5.1. Predator-pathogen interaction

Rejection of *B. bassiana* infected whitefly by *D. hesperus* may be due to visual changes that occur in whitefly hosts and/or chemical cues associated with biochemical changes to hosts during infection. In experiment 1, though a *B. bassiana*-treatment effect was observed, time post-application was not a significant factor, which was unexpected. In experiment 2, which tested predation of infected hosts, showing oosporein or hyphae of fungal origin, prey was significantly rejected.

In most studies examining the interaction between entomopathogens and other natural enemies, the pathogen almost always dictates the population dynamics of other guild members. For example, oviposition by the parasitoid *E. formosa* of *Aschersonia aleyrodinis* infected whitefly was assessed by Fransen and van Lenteren (1993). This study showed that *E. formosa* only discriminated against infected hosts late during fungal development. Thus, by ovipositing infected whitefly hosts early during the procession of infection, immature parasitoids were likely to become infected (Fransen and van Lenteren, 1993). In

such a circumstance, the intraguild interaction between fungus and parasitoid was asymmetric (Lucas *et al.*, 1998), and the pathogen caused more harm to parasitoids than parasitoids did to the fungus. In our study, the susceptibility of *D. hesperus* to infection by *B. bassiana* was not evaluated, but infection may have been an important factor in predator mortality. In the greenhouse, *D. hesperus* may come in direct contact with conidia of *B. bassiana* through spray applications or horizontal transmission by feeding on infected whitefly prey. However, this study suggests that *D. hesperus* may be able to avoid *B. bassiana* infected prey at a late stage of infection.

A pathogen may also be considered the target of intraguild predation. Pell *et al.* (1997) and Roy *et al.* (1998) showed that the seven-spotted ladybeetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and the carabid *Pterostichus madidus* Fabricius (Coleoptera: Carabidae) interfere with the fungus *Erynia neoaphidis* Remaudière and Hennebert (Zygomycetes: Entomophthorales) as they consumed prey aphids at a late stage of infection. In our study, the discriminate behavior of *D. hesperus* towards whiteflies producing oosporein or hyphae suggested that there was less interference between the predator and the pathogen.

2.5.2. Predator - Parasitoid interaction

Regardless of visible changes in *T. vaporariorum* parasitised hosts by day 12 of parasitoid development such as the blackening of the host pupal exoskeleton, predators consistently consumed these prey. Such consumption of *E. formosa* parasitised whitefly by *D. hesperus* may suggest that *D. hesperus* is an intraguild predator of the parasitoid. However, this study does not determine whether or not *D. hesperus* will feed on intraguild prey when a choice of prey is available. Two different scenarios may exist in the greenhouse, which will determine the effectiveness of biological control using these two natural enemies. If parasitized and healthy pupae were indiscriminately fed upon, intraguild predation would reduce the effectiveness of biological control. In comparison, if given the choice of many prey types, predators may be more likely to select unparasitized pupae. A laboratory study by Castañe *et al.*, (2000) showed that while discrimination of *E. formosa* parasitized whitefly was observed by predators *Dicyphus tamaninii* Wagner and *Macrolophus*

caliginosus Wagner, in Mediterranean area tomato greenhouses and in the laboratory was assessed, an effective whitefly control strategy may still exist between predators and *E. formosa*. In the laboratory, predators consumed more unparasitized than parasitized pupae when these were offered in the same proportion (Castañe *et al.*, 2000). In the greenhouse however, the regular release of *E. formosa* was considered complementary to the use of a predator if it was done before the predator became fully colonized (Castañe *et al.*, 2000). The joint release of the predatory bugs *M. caliginosus* or *Nesidiocoris tenuis* with *E. formosa* was identified as an effective strategy for the control of whitefly populations (Nannini, 2000). This result holds true even though predation on developing parasitoids was observed (Nannini, 2000). In light of these studies, though *D. hesperus* will feed on *E. formosa* parasitized whitefly, this interaction in the greenhouse environment may appear quite different.

2.5.3. Inference from laboratory to field environment

Given the reduced spatial scale of the Petri dish environment, it is impossible to make definitive conclusions on interactions between the predator *D. hesperus* and the parasitoid *E. formosa* or the fungus *B. bassiana* on the effectiveness of greenhouse biological control. Goettel and Johnson (1994) show that spatial separation in field experiments may play a role in the dynamical linkage between organisms. Janssen *et al.*, (1998) explain that spatial scale in confined laboratory studies may suppress avoidance/attraction behavior and provide misleading results. A complementary greenhouse study should therefore ensue that would describe the in field host range of *D. hesperus*.

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**Chapter 3. Interactions between a predator, a parasitoid,
and a fungus: consequences on greenhouse whitefly
populations**

3.1. Résumé

La lutte biologique est un des moyens plus efficaces de contrôle de la mouche blanche des serres, *Trialeurodes vaporariorum*. Cette étude examine l'interaction entre différents ennemis naturels de la mouche blanche y inclus le prédateur *Dicyphus hesperus*, le parasitoïde *Encarsia formosa* et l'entomopathogène *Beauveria bassiana* dans les cultures de tomates en serres. Cette étude avait comme but particulier de décrire l'impact de *B. bassiana* sur la lutte biologique par *D. hesperus* ou *E. formosa*. En serre expérimentale, des populations de la mouche blanche, du parasitoïde et du prédateur ont été manipulées. En compartiments traités, trois applications du pathogène d'une concentration moyenne de 5.13×10^3 conidia/mm² ont été effectuées en 27 jours. Le pathogène *B. bassiana* a réduit significativement le nombre de mouches blanches saines sans diminuer le nombre de parasitoïdes ou de prédateurs. Par contre, l'entomopathogène a eu un effet néfaste sur le taux de prédation, ce qui pourrait être réduit par l'aménagement de refuges spatiaux ou temporels pour la protection du prédateur.

3.2. Abstract

Biological control is one of the most effective strategies of greenhouse whitefly, *Trialeurodes vaporariorum* control. This study investigates the outcome of interactions among whitefly natural enemies including the predator *Dicyphus hesperus*, parasitoid *Encarsia formosa* and entomopathogen *Beauveria bassiana* on greenhouse tomato crops. Our objective was to determine whether *B. bassiana* would disrupt biological control by interfering with *D. hesperus* or *E. formosa*. In experimental greenhouses, whitefly, parasitoid and predator populations were established on tomato crops. In treated compartments, three applications of the entomopathogen, at the dose of 5.13×10^3 conidia/mm², were made over the course of a 27-day period. Overall, *B. bassiana* treated compartments had fewer immature whitefly and neither parasitoid nor predator populations were significantly reduced by the pathogen. However, in *B. bassiana* treated compartments, whitefly predation by *D. hesperus* was markedly reduced.

3.3. Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) is an important pest of field and greenhouse crops worldwide (Byrne and Bellows, 1991). It causes yield reduction in many plants as a result of phloem sap extraction by feeding and of honeydew excretion that serves as a medium for the growth of sooty mold (Byrne and Bellows, 1991). Typically, populations of the greenhouse whitefly are characterized by overlapping generations, ensuring a rapid rate of increase (Dreistadt, 2001). Extensive use of insecticides has led to the development of resistance in whiteflies (Gorman *et al.*, 2002), thereby favouring the development of biological control as an alternative control strategy (Hoddle *et al.*, 1997, van Lenteren, 2000).

Biological control of the greenhouse whitefly has for many decades depended on inundative releases of the specialist parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Fransen and van Lenteren, 1993). However, under certain environmental conditions, such as in low temperatures, the efficacy of *E. formosa* is limited (Perdikis and Lykouressis, 2002). In Canada, a large proportion of greenhouse tomato production uses *E. formosa* in combination with the native and widely distributed predator, *Dicyphus hesperus* Knight (Hemiptera: Miridae), who though recently introduced is an effective natural enemy on both field and greenhouse vegetable crops (McGregor *et al.*, 1999). *Dicyphus hesperus* is particularly appealing as a biological control agent due to its zoophytophagous nature that allows it to feed partly on plants as well as on prey. Plant feeding may permit predators to become established onto crops when prey density is low (Gillespie and McGregor, 2000). Furthermore, being a generalist also enables *D. hesperus* to prey upon many species of small insect pests (Kelton, 1980; Henry and Wheeler, 1988; Gillespie *et al.*, 2000). Application of *D. hesperus* onto greenhouse crops represents a long-term control strategy as the introduced predator establishes and reproduces on a crop so long as adequate resources and environmental conditions are available (Sanchez *et al.*, 2003).

In situations where whitefly population levels become excessively large and out of control, pathogenic fungi could also be used as an effective means of drastic pest reduction (Kanagartnam *et al.*, 1982; Mead and Byrne, 1991; Siongers and Coosemans, 2003). In

particular, it has been shown that the ubiquitous and generalist hyphomycete *Beauveria bassiana* (Balsamo) Vuillemin may effectively reduce whitefly populations (Wraight *et al.*, 2000; Kirk *et al.*, 2001). The success of products formulated with the conidia of *B. bassiana*, such as BotaniGard® (Emerald BioAgriculture Corp., Lansing, MI, USA), is due to the long term (1-year) storage capacity of the conidia based powder or suspension at room temperature (Goettel and Hajek 2001). *Beauveria bassiana* is characterized as a generalist pathogen, having been isolated from over 700 invertebrate host species around the world (Feng *et al.*, 1994; Butt *et al.*, 2001). It is therefore expected that *B. bassiana* may infect non-target natural enemies (Shah and Goettel, 1999).

Increasing experimental evidence indicates that biological control can be disrupted by direct and indirect interactions such as competition, apparent competition, intraguild predation, and behavioral interference between natural enemies (Rosenheim *et al.*, 1995; Symondson *et al.*, 2002). Understanding and exploiting interactions among natural enemies are therefore meaningful for implementing effective pest control strategies.

In this study, we examine the compatibility of BotaniGard® a formulation based on the conidia of *B. bassiana*, strain GHA, with other biological control organisms. We assess the survival of whitefly natural enemies including the parasitoid *E. formosa* and the predator *D. hesperus* following applications of the entomopathogen BotaniGard® and measure the effect of pathogen applications on whitefly parasitism and predation. This study was designed to evaluate species interactions at the large-scale greenhouse level. The effect of *B. bassiana* on whitefly biological control is determined through a comparison of control versus pathogen treated compartments in which there are existing populations of the greenhouse whitefly, the parasitoid *E. formosa* and the predator *D. hesperus*.

3.4. Materials and Methods

3.4.1. Experimental design

Tomatoes *Lycopersicon esculentum* Mill. (Solanaceae, cv. Rhapsodie, Syngenta Seeds, Boise, ID), were grown in two, 12 x 6.4 m glasshouses at the Pacific Agriculture and Agri-Food Research Centre (PARC) in Agassiz, British Columbia. Each glasshouse stood as a

block within which four replicates of each of two treatments were applied on 10 plants each, giving a total 80 plants per house. In each glasshouse, eighty tomatoes were seeded in mid December 2002 and seedlings planted on rockwool slabs on January 15, 2003. Plants were arranged in two central rows and two lateral single rows in each glasshouse. Plants within rows were 40 cm apart, central rows being separated by 50 cm, and central and lateral rows by 100 cm. Each house was subdivided longitudinally in two and latitudinally in four sections, to give eight 3 x 1.6 m compartments with 10 plants each. Compartments were separated from each other using a perforated plastic Visqueen fabric (Oxfordshire, UK) to allow airflow, yet prevent movement of introduced organisms including *B. bassiana* spores. This fabric was suspended from a ceiling infrastructure in order to separate compartments as well as to cover their ceilings. Only natural lighting was used. Temperature was set at 22°C during the day and 18°C at night, and relative humidity (RH) at 70%. As of August 16th, or 8 months after seedlings were planted, the hourly temperature and RH were recorded using a 2-channel temperature and relative humidity (HOBO Onset Computer Corp. Bourne, MA) data logger. Humidity levels during the day ranged from 60-80% (House 1) and 56-85% RH (House 2). The mean temperature and % RH (\pm SEM) were of 23.0 ± 0.1 °C and 57.9 ± 0.3 % RH, respectively.

Table 3-1 Release schedule of greenhouse whitefly and natural enemies over the course of the study in 2003. Indicated are the numbers of organisms released at each date and the concentration of conidia within applied *Beauveria bassiana* suspensions.

Organism	Release Date	Corresponding Week number	Number /Concentration Released / Compartment
	2003		
<i>Trialeurodes vaporariorum</i> (pupae and adults)	August 16	0	300
	August 29	0	300
	September 1	0	100
<i>Encarsia formosa</i> (pupae)	September 24	3	200
	October 8	5	400
<i>Dicyphus hesperus</i> (adults)	September 24	3	100
	October 9	5	50
	October 15	6	50
<i>Beauveria bassiana</i> (BotaniGard ®)	September 25	3	7.31 x 10 ⁷ conidia/ml
	October 1	4	8.60 x 10 ⁷ conidia/ml
	October 22	7	8.85 x 10 ⁷ conidia/ml

Three preliminary whitefly releases allowed for a rapid and large build-up of pest populations (Table 3-1). Whiteflies, obtained from Applied Bionomics Corporation, B. C., Canada, were introduced either by placing fourth instar pupae onto the lowest leaf of tomato plants as for the first introduction (August 16) or by direct introduction of adults in the case of the later two introductions (August 29, September 1). Together, these introductions ensured the presence of a large developing whitefly population. By the third whitefly introduction, whitefly had colonized the entire height of the tomato plants. On September 24th, whitefly densities were judged sufficiently large to support the persistence of natural enemies that were introduced either that day or the following day. An initial release of *E. formosa* and *D. hesperus* made in late September was followed by one larger parasitoid and two smaller predator releases in October (Table 3-1). *Encarsia formosa* was first released as adults received from Biobest® Biological systems (Westerlo, Belgium) and subsequent introductions were made by hanging parasitized whitefly cards (Applied Bionomics).

Dicyphus hesperus insects were obtained from a laboratory colony at the PARC research centre, which was initially established from field collected individuals in 1999 from the foothills of the Sierra Nevada Mountains (elevation ca. 300 m, near Woody, CA, USA). These insects were originally found on stem hedge nettle, *Stachys albens* A. Gray (Lamiaceae). Colonies were maintained in screened wooden cages (38 x 51 x 53 cm) containing tobacco plants at $24.8 \pm 1.5^{\circ}\text{C}$ (\pm SD), and under a 16 h photoperiod, and were fed eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) from Biobest® Biological systems (Westerlo, Belgium). In experiments, four day old adult *D. hesperus* were added in a 50/50 ratio of males and females.

The first application of BotaniGard® followed the first release of parasitoids and predators (Table 3-1). A second and third application of this pathogen was followed 5 and 26 days after the initial application. This third and final application was applied during a period of high whitefly densities.

Before treatment, in order to determine fungal deposition rate (conidia per mm²), 5% water agar blocks were pinned on the underside of three randomly selected leaves from two plants in each of the eight BotaniGard® compartments. Pinned agar blocks held in place by a piece of plasticine were collected after microbial application and the number of conidia in a set ocular area counted. Entomopathogen applications were made using a hand held pressurized sprayer (11.4 L Model # 65010, Hudson & Industrial, Chicago IL, USA). A small quantity of the fungal suspension was taken from the sprayer early, mid and late during the course of each treatment in order to determine the actual conidial concentration of a suspension through their enumeration using a haemocytometer (Table 3-1). Conidia viability was also determined by spraying the spore suspension onto three 0.005% Benlate® (benomyl, wettable powder 50%, E.I. duPont de Nemours and Co., Wilmington, DE) amended potato dextrose agar (PDA) plates during application. Benlate®-amended plates facilitate counting germinated conidia as benlate halts cell division during mitosis that otherwise causes overgrowth to occur (Goettel and Inglis, 1997). Plates were sealed with Parafilm and incubated at 25°C for 2 days, after which the proportion of 500 conidia that had germinated from each sample was determined. Viability of conidia was established to be of 97.5, 96.3 and 93.2 % for BotaniGard® treatments 1, 2 and 3, respectively.

3.4.2. Sampling

Non-destructive sampling of insects on tomato plants was visually performed with the help of a hand lens on a bi-weekly basis starting on September 6th, 22 days before the first BotaniGard® treatment, in order to determine pre-treatment conditions. Two plants within each compartment were randomly selected, on which a leaf from each of the four canopy levels was taken (= 4 leaves x 2 plants/sampling unit). The vertical stratification and sampling of leaves on greenhouse tomato plants provided an accurate account for the abundance of all organisms at each of the life stages as related to leaf age. Plant canopies were delineated by the enumeration of leaves along a plant starting from the top, where canopy 1 = leaves 1 to 5; canopy 2 = leaves 6 to 9; canopy 3 = leaves 10 to 12; canopy 4 = leaves 13 to 15. Entire leaves, composed of several leaflets, were sampled by counting all

organisms, even when high densities were observed. The following life stages and species of insects were sampled: whitefly eggs, nymphal stages N1 and N2 (N1-2) of whiteflies, nymphal stages N3 and N4 (N3-4) whiteflies, adult whiteflies, adult parasitoids, *E. formosa* parasitized whiteflies, adult predators, *D. hesperus* preyed upon whitefly pupae, infected N1-2 whiteflies, infected N3-4 whiteflies, infected and parasitized whiteflies.

Each class has specific morphological characteristics that we used for identification. Whitefly eggs were identified as small black dots from a distance, that appear spindle shaped when examined with a hand lens. N1-2 whiteflies were also identified using a hand lens. N1 and N2 whitefly instars had characteristic small (<0.5 mm), white, flattened bodies. N3-4 whiteflies were larger in size, and were characterized by thickened bodies and a fringe of hairlike protrusions around the periphery of their waxy cover. When infected with *B. bassiana*, the colour of immature whitefly generally changed from opaque white to red or brown. In certain cases infected whitefly pupae were identified by the presence of mycelium or hyphae, which protruded around their periphery. Late in the infection process, whiteflies appeared dehydrated and flattened. Adult whiteflies were mostly found concentrated at the top of tomato plants, but were also occasionally found on lower leaves following emergence.

Adult *E. formosa* parasitoids measure approximately 0.6 mm in length and are easily detected with the naked eye. Parasitized whiteflies appeared black as they normally do two weeks following the deposition of the parasitoid egg within the host pupae. Early parasitoid development could not be visually detected.

Adult *D. hesperus* are relatively large insects with a length of approximately 7 mm and are therefore easily detectable at a distance. *Dicyphus hesperus* consumed prey are left behind on leaves, and thus could be counted by observing the empty whitefly exoskeletons. Though predator consumed whitefly could be confounded with the emptied pupal exoskeletons of emerged adult whitefly, the top surface of empty pupae resulting from predation remained intact whereas emerging adult whitefly from pupae cause a distinct tear or dislocate the top surface of the pupal shell.

The proportion of infected whitefly was determined under laboratory conditions by sampling one leaflet with at least 30 immature whiteflies from each of the 8 compartments per glasshouse. These leaflets were placed within a 15 cm diameter Petri dish on a moistened filter paper, and incubated at 25°C. Leaflets were kept moist by regularly adding water as needed. Following a 5-day incubation period, infected whitefly that were either sporulating or were red in colour due to *B. bassiana*'s production of oosporein, were enumerated and the proportion of infected whitefly calculated over the total number of whitefly per leaflet was determined.

3.4.3. Statistical Analysis

The effects of treatment and time (sample date) were evaluated on the following dependent insect density variables: whitefly egg, N1-2 nymph, N3-4 nymph, and whitefly adult, adult parasitoid, adult predator, parasitized whitefly, predator consumed whitefly and infected whitefly using a repeated measures ANOVA (SAS, 1999). The potential spatial dependency within the same house was accounted for by including the house effect as a random block factor in the model. The inclusion of this term in the ANOVA model removed the variation due to the main plot error or House error. The dependence of observations between experimental units, the compartments, was taken into account within this model. The Compound Symmetry structure specific to the house level was chosen based on the AIC, Akaike information criterion, on the basis of its smallest value among all structures tested. Following a significant treatment or time effect, multiple comparisons were done to identify differences between levels. To achieve normality assumptions, the dependent variables were square root transformed. The homogeneity of variance was verified by graphical visualization of residuals against predicted values.

3.5. Results

A house effect was observed for the dependent variables N3-4 *T. vaporariorum* whitefly, *E. formosa* parasitoid abundance, parasitized whitefly and *B. bassiana* infected + parasitized whitefly (Tables 3-2 and 3-4), probably resulting from slight environmental variability

between houses. Since only these dependents were affected by the house factor, we conclude that, there was only a minimal house effect.

Over the course of this experiment, dead *E. formosa* parasitoids and *D. hesperus* predators were occasionally found on leaves following the application of both water or pathogen treatments (Labbe, personal observation). Since these insects were normally found after the application of water or BotaniGard® in control and treated compartments respectively, drowning was considered an equally important factor in all compartments.

3.5.1. Effect of BotaniGard® on whitefly population dynamics

A treatment by time interaction was observed for N3-4 and adult whitefly classes, whereas a treatment effect alone was observed for N1-2 and egg classes (Table 3-2). Over the course of the experiment whitefly populations were lower in *B. bassiana* treated compartments than in control compartments, with an average of $50.04 \pm 1.5\%$ fewer immature whiteflies (Table 3-2 and Figure 3-1). The treatment by time interaction observed for N3-4 and adult whitefly stages probably originated from high mortality of early whitefly instars (Table 3-2 and Figure 3-1), which depleted subsequent cohorts.

Within all BotaniGard® treated compartments, the density of infected immature whitefly was significantly different over time compared to control compartments (Table 3-3). Infected whitefly were consistently more abundant in treated compartments, and increased in proportion as soon as the first sample was taken following an initial BotaniGard® treatment (Figure 3-2). Infected pupae were either red in colour, had developed fungal hyphae or displayed both of these characteristics.

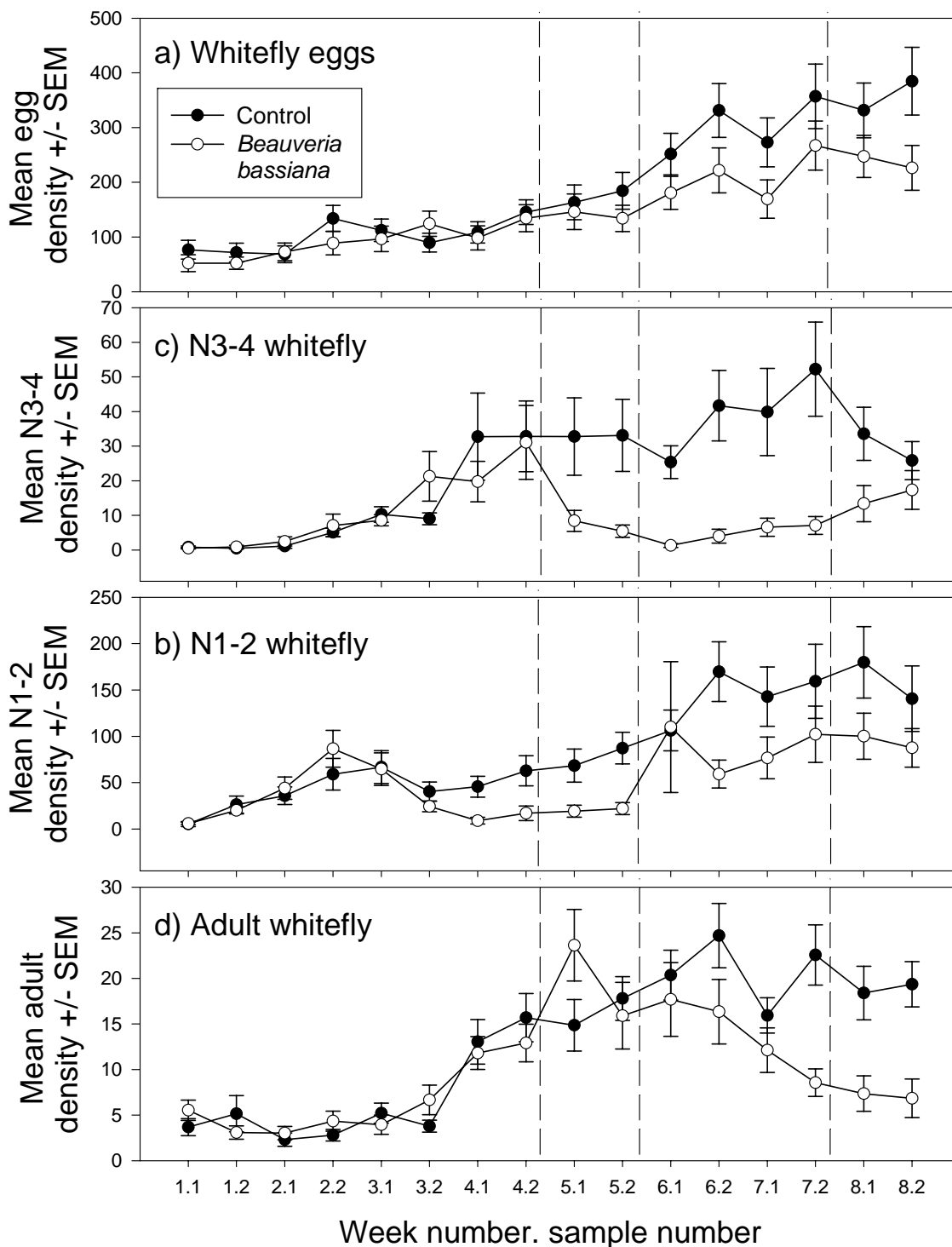


Figure 3-1 Effect of BotaniGard® treatment on the density per leaf of a) eggs; b) N1-2; c) N3-4 and d) adults of the whitefly *Trialeurodes vaporariorum*. Values represent the mean density (± SEM) of samples (N= 16) taken at each of four plant canopy levels. Vertical lines indicate BotaniGard® treatments. The x-axis represents the sample date whereby the first number stands for weeks and the second for the bi-weekly repetition of sampling.

Table 3-2 Repeated measures analysis of variance for effects of BotaniGard® treatment on the abundance of different developmental stages of the greenhouse whitefly *Trialeurodes vaporariorum* per leaf. Table reports F, DF and *P* values.

Dependent variable	Sources of variation	DF	F-value	<i>P</i>
Eggs (sqrt = square root transformed)				
	House	1	1.65	0.2215
	Treatment	1	13.04	0.0032
	Main plot error	13		
	Time	10	15.42	< 0.0001
	Treatment*time	10	1.37	0.2001
	Sub-plot error	140		
	Total	175		
N1-2 (sqrt)				
	House	1	1.58	0.2315
	Treatment	1	20.76	0.0005
	Main plot error	13		
	Time	10	12.20	< 0.0001
	Treatment*time	10	0.82	0.6109
	Sub-plot error	140		
	Total	175		
N3-4 (sqrt)				
	House	1	0.80	0.0001
	Treatment	1	32.33	< 0.0001
	Main plot error	13		
	Time	10	2.21	0.0204
	Treatment*time	10	4.05	< 0.0001
	Sub-plot error	140		< 0.0001
	Total	175		
Adult whitefly (sqrt)				
	House	1	2.33	0.1509
	Treatment	1	5.25	0.0393
	Main plot error	13		
	Time	10	10.43	< 0.0001
	Treatment*time	10	7.06	< 0.0001
	Sub-plot error	140		
	Total	175		

Table 3-3 Repeated measures analysis of variance for effects of BotaniGard® treatment on the density of *Beauveria bassiana* infected immature greenhouse whitefly, *Trialeurodes vaporariorum* per leaf.

Dependent variable	Sources of variation	DF	F-value	P
Proportion infected whitefly (sqrt)	House	1	4.12	0.0633
	Treatment	1		< 0.0001
	Main plot error	13		
	Time	10	2.12	0.0266
	Treatment*time	10	2.25	0.0179
	Sub-plot error	140		
	Total	175		

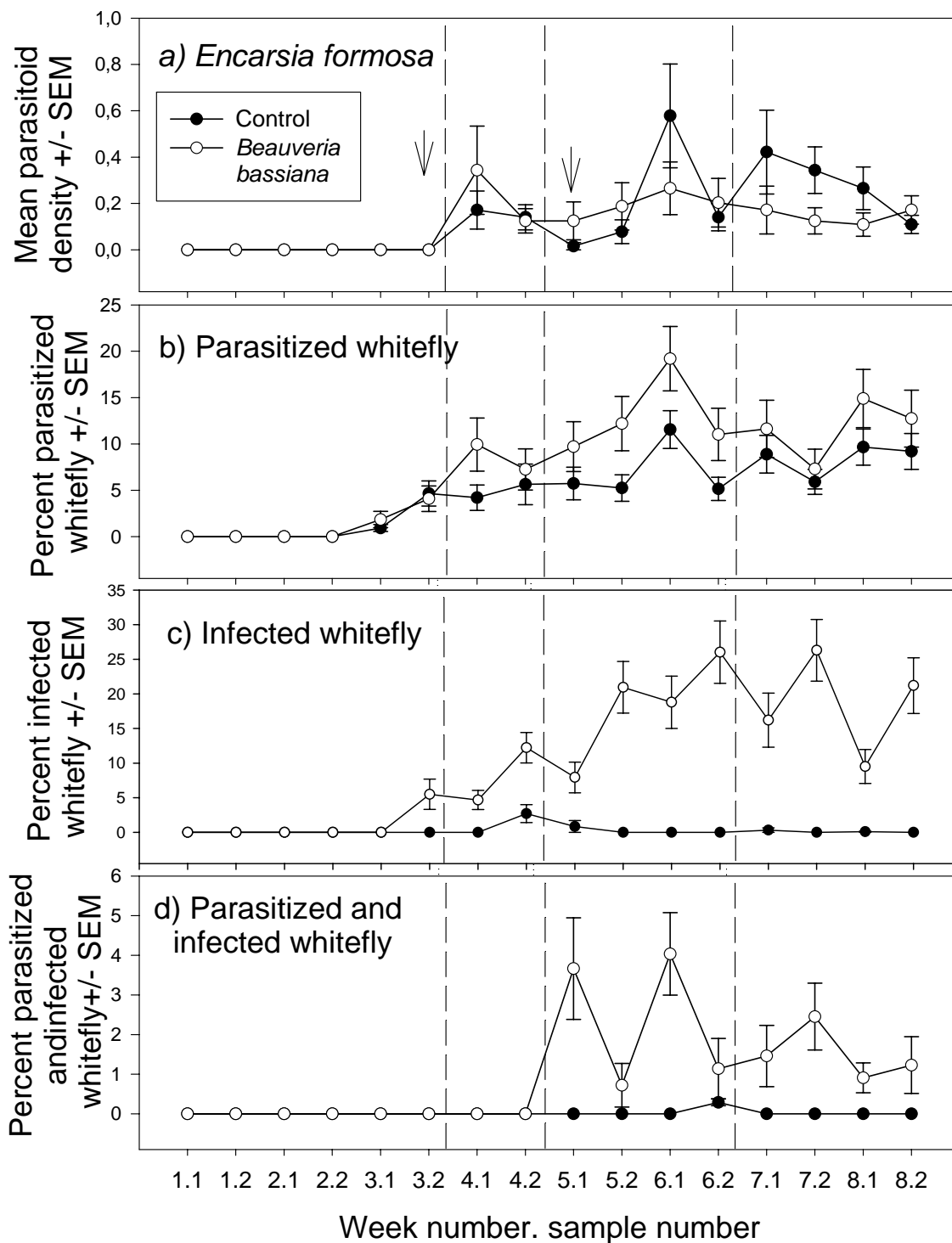


Figure 3-2 Impact of BotaniGard® treatment on a) the density of adult parasitoid *Encarsia formosa*; b) the percent of *E. formosa*-parasitized whitefly; c) the percent of *Beauveria bassiana* infected whitefly; and d) the percent of parasitized and infected whitefly per leaf. For each treatment, values represent the mean (\pm SEM) (N= 16) number of organisms per leaf sampled at 4 canopy levels, in 2 replicate compartments per house, and 2 houses.

Vertical lines indicate dates of BotaniGard® treatments. Arrows indicate dates of *E. formosa* releases.

3.5.2. Impact on parasitism

After having analysed and observed data on *E. formosa* abundance, an exceptionally higher parasitoid density in BotaniGard® treated compartments on day 6.1 (LSD $F_{1,141} = 37.44$, $P < 0.01$) suggested that values from two compartments were outliers. These values were probably due to the sampling of an exceptional aggregation of *E. formosa* parasitoids observed on the bottom canopy level of tomato plants. The presence of these data points made analysis and interpretation difficult. After having removed outlying values, it was observed that over time, *E. formosa* parasitoid density was comparable in both BotaniGard® treated and control compartments (Table 3-4; Figure 3-2a). The proportion of parasitized whitefly per leaf was significantly greater in treated compartments and remained as such during the course of this experiment (Table 3-4; Figure 3-2b). The proportion of parasitized and infected immature whitefly was also significantly greater in treated compartments compared to controls (Table 3-4; Figure 3-2d).

Table 3-4 Results of a repeated measures analysis of variance for effects of BotaniGard® treatment on *Encarsia formosa* density per leaf, and on the proportion of parasitized or parasitized and *Beauveria bassiana* infected whitefly.

Dependent variable	Sources of variation	DF	F-value	P
<i>E. formosa</i> (n/leaf) (sqrt)				
	House	1	4.68	0.0497
	Treatment	1	0.75	0.4020
	Main plot error	13		
	Time	10	4.00	< 0.0001
	Treatment*time	10	1.65	0.0979
	Sub-plot error	140		
	Total	175		
Proportion parasitized whitefly (sqrt)				
	House	1	18.15	0.0009
	Treatment	1	11.17	0.0053
	Main plot error	13		
	Time	10	6.09	< 0.0001
	Treatment*time	10	2.95	0.0022
	Sub-plot error	140		
	Total	175		
Proportion infected and parasitized whitefly (sqrt)				
	House	1	7.34	0.0179
	Treatment	1	51.81	< 0.0001
	Main plot error	13		
	Time	10	4.22	< 0.0001
	Treatment*time	10	4.64	< 0.0001
	Sub-plot error	140		
	Total	175		

3.5.3. Impact on predation

While the density of *D. hesperus* did not differ between *B. bassiana* treated and control compartments ($P = 0.0703$, Table 3-5), the proportion of immature whitefly consumed by *D. hesperus* in treated compartments was significantly lower than in control compartments (Figure 3-3). As of the second sampling date following the first pathogen treatment, a significant increase in density of whitefly killed was observed (Figure 3-3 and LSD multiple comparison on sample date 5.1, $F_{1, 141}=14.57$, $P= 0.0002$). Furthermore, a significant interaction between treatment and time was observed, indicating that a trend in the proportion of predator consumed whitefly varied in treated vs. control sampling units (Table 3-5).

Table 3-5 Repeated measures analysis of variance for effect of BotaniGard® treatment on the density per leaf of predator *Dicyphus hesperus* and on the rate of whitefly predation by *D. hesperus*.

Dependent variable	Sources of variation	DF	F-value	P
<i>Dicyphus hesperus</i> density				
	House	1	.	.
	Treatment	1	3.89	0.0703
	Main plot error	13		
	Time	10	7.41	< 0.0001
	Treatment*time	10	1.45	0.1635
	Sub-plot error	140		
	Total	175		
Whitefly consumed by predator <i>D. hesperus</i>				
	House	1	.	.
	Treatment	1	233.33	< 0.0001
	Main plot error	13		
	Time	10	14.41	< 0.0001
	Treatment*time	10	2.85	0.0030
	Sub-plot error	140		
	Total	175		

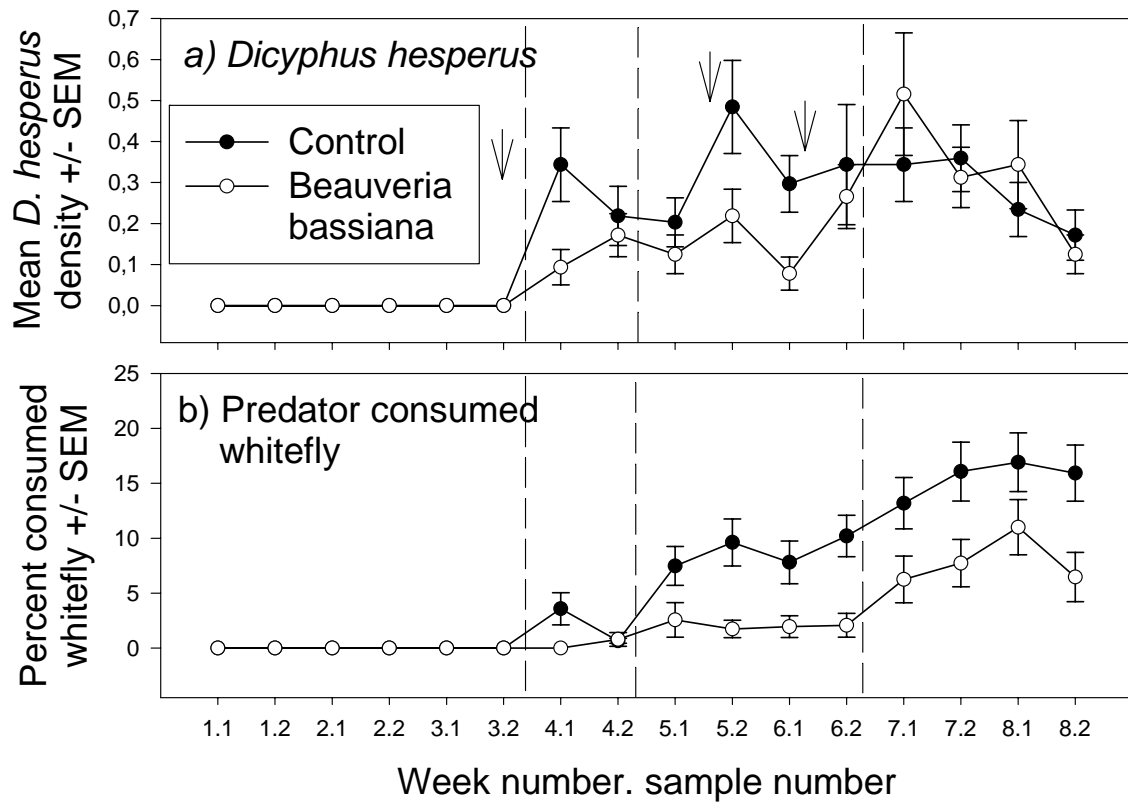


Figure 3-3 Impact of BotaniGard® treatment on a) the density per leaf of predator *Dicyphus hesperus* (\pm SEM) and b) on the density of predator consumed whitefly (\pm SEM). Values represent the mean (N=16) number of organisms on each of 4 canopy levels, in 2 compartments per house and 2 houses. Vertical lines indicate dates of BotaniGard® treatments. Arrows indicate dates of *D. hesperus* releases.

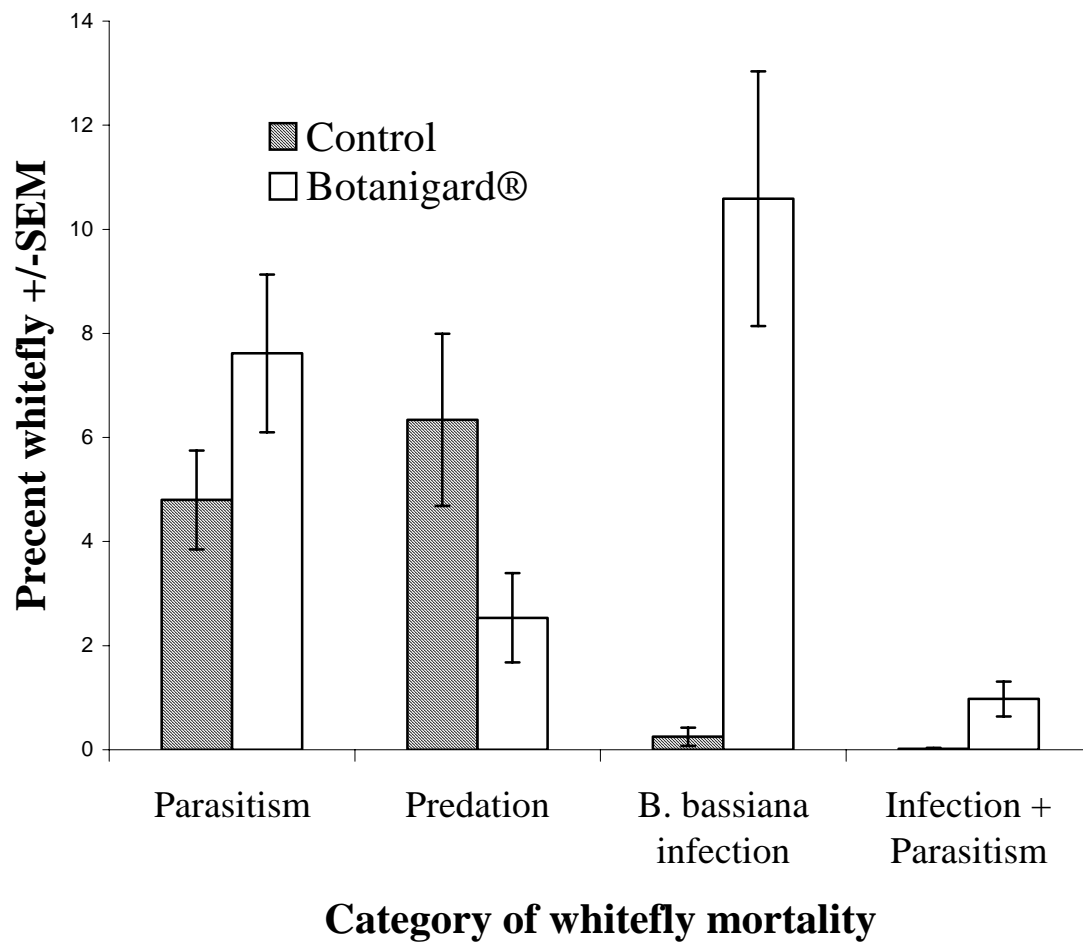


Figure 3-4 Effect of BotaniGard® treatment over the course of the experiment on the mean percentage of immature whitefly infected by *Beauveria bassiana*, parasitized by *Encarsia formosa*, *Dicyphus hesperus* predator fed, or both parasitized and infected.

3.5.4. Partitioning whitefly mortality

The mortality of all immature whitefly sampled during this experiment was categorized for each source identified (Figure 3-4). In the treated compartments, *B. bassiana* was the most effective biological control agent and caused infection in 10.6% of all immature whitefly over the course of this experiment (Figure 3-4). Parasitism was a greater source of whitefly mortality in treated versus control compartments. Predation by *D. hesperus* was lower in the presence of *B. bassiana* (Figure 3-4).

3.6. Discussion

In this study, whitefly mortality resulted not only from infection by *Beauveria bassiana* but also through parasitism by *Encarsia formosa* and through predation by *Dicyphus hesperus*. However, *B. bassiana* was the most important cause of whitefly mortality with 10.6 ± 0.9 % of examined whitefly in treated compartments infected by this fungus alone. Parasitism by *E. formosa* was the second leading cause of whitefly mortality (7.61 ± 1.5 %). Finally, *D. hesperus* consumed 6.3 ± 1.7 % of immature whitefly in control compartments.

3.6.1. Whitefly – pathogen interaction

The mortality response of whitefly from infection by *B. bassiana* varied according to host developmental stage. However, we did not identify factors that lead to fluctuations in each of the individual whitefly life stages. In order to determine the susceptibility of the different whitefly stages, laboratory bioassays should be performed. While it appeared that adult whitefly were little affected by the pathogen early during the course of the experiment, N1-2 whitefly populations were reduced almost immediately following the first pathogen application (Figure 3-1). The observed reduction of early whitefly instar density may be explained in part by a reduction in the number of eggs laid by adults or to the infection and death of early instars. Immature whiteflies are immobile, small and have limited wax coating, which may make them susceptible to germinating conidia. Siongers and Coosemans (2003) studied BotaniGard® effects on developmental stages of *T.*

vaporariorum on the greenhouse cucumber. They showed that the first larval stage was the most sensitive, and that the pathogen did not influence the number of eggs hatched. A treatment shortly before hatching had a residual effect on new nymphs. When considering the response of whitefly populations to *B. bassiana*, it is evident that major differences between treated and control compartments appeared after the second pathogen application. Significant differences appeared almost immediately after the first treatment for N1-2 whitefly instars and by the second treatment, all whitefly classes were significantly affected (Figure 3-2). The remarkable decrease of adult whitefly over time may reduce the number of individuals of all other whitefly classes (Figure 3-2).

3.6.2. Parasitoid-pathogen interaction

Over the course of this experiment, the abundance of *E. formosa* parasitoids in treated compartments was similar to that in control compartments. In addition to consistent parasitoid levels, the abundance of parasitized whitefly was significantly higher in treated compartments compared to control compartments, with a small but significant fraction of these infected by *B. bassiana*.

Though the impact of infection on *E. formosa* during development in the whitefly host was not determined in our study, other parasitoid-pathogen interactions have been studied and should be considered (Rombach and Gillespie, 1988; Fransen and van Lenteren, 1993; 1994; Mesquita *et al.*, 1997; Lacey *et al.*, 1997; Furlong, 2004). In these studies factors such as the temporal separation of species and intraguild interactions seem to contribute to the outcome of parasitoid-pathogen interactions. A study of the interaction between the aphelinid parasitoid, *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) and the entomopathogenic fungus *Paecilomyces fumosoroseus* Wize (Deuteromycotina: Hyphomycetes) exploiting the Russian wheat aphid in barley fields showed that these natural enemies are compatible as they work additively to enhance aphid control (Mesquita *et al.* 1997). In this study, no difference in the number of mummies recovered per plant was found between the *A. asychis* parasitism treatment alone and the combined effect of parasitism and infection by *P. fumosoroseus* together. Also, the comparable *A. asychis* emergence in both of these treatments observed suggests the absence of interference

between pathogen and parasitoid. In contrast, laboratory tests showed that adult parasitoids of *A. asychis* were susceptible to fungal infection by *P. fumosoroseus* (Lacey *et al.*, 1997). These contradictory results highlight the importance of evaluating interactions between fungus and parasitoid under both laboratory and field conditions.

Timing the application of a pathogen during the use of a parasitoid is often an important factor contributing to the success of biological control. Furlong (2004) showed that *B. bassiana* was detrimental to the endolarval parasitoid *Plutella xylostella* L. (Lepidoptera: Plutellidae) developing within the immature stages of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae). Emergence of parasitoids was reduced and infection of parasitoid larvae by *B. bassiana* increased when the concentration of the pathogen increased (Furlong, 2004). However, parasitoid mortality was avoided through the careful timing of pathogen applications. *Beauveria bassiana* may be effectively applied at least one day before the predicted parasitoid cocoon formation, thus avoiding infection to parasitoids. Temporal separation was also an important factor in the interaction of *E. formosa* and the entomopathogen *Aschersonia aleyrodis* (Fransen and van Lenteren, 1993; 1994). Infection of parasitoids diminished as developing parasitoids became older. Fungal spores of *A. aleyrodis* did not infect parasitized whiteflies with immature parasitoids older than three days (Fransen and van Lenteren, 1993). When appropriate measures of temporal separation are applied, the potential use of *E. formosa* fungi such as *Verticillium lecanii* Zimm. (Deuteromycotina: Hyphomycetes) (Rombach and Gillespie, 1988) or *P. fumosoroseus* (van de Veire and Degheele, 1996), may also be effective.

Host discrimination by *E. formosa* adults may also have played a role in population dynamics of the developing parasitoid. The higher abundance of parasitized whitefly observed in treated compartments (Figure 3-2) may be influenced by the selective oviposition of females (Fransen and van Lenteren, 1993; 1994). *Encarsia formosa* rarely chooses to oviposit whitefly pupae infected by *Aschersonia aleyrodis* (Fransen and van Lenteren, 1993; 1994). In this light, it is believed that oviposition of pathogen infected hosts is generally avoided by *E. formosa* adults. Jazzar and Hammad (2004) demonstrated that ovipositing females of *E. formosa* discriminated between healthy and *Verticillium*

lecanii infected *Bemisia argentifolii* whitefly and therefore reduced interference between these natural enemies. The combined use of *E. formosa* and *V. lecanii* resulted in a 70.7% whitefly mortality. Of this amount, 10% of whitefly mortality was due exclusively to parasitism and 58.3% due to infection. Lacey *et al.*, (1996) reported that *E. formosa* under field conditions resulted in a synergistic interaction with the naturally occurring *V. lecanii*. In our study, the absence of interference between the pathogen and the parasitoid suggested by the greater abundance of parasitized whitefly in treated compartments signals the compatibility of the parasitoid and the pathogen (Figure 3-4). Parasitoids were occasionally found dead on leaves following the application of both water or pathogen treatments (Labbe, personal observation). It was proposed that these parasitoids would have died of drowning, a factor that may be considered equally important in all compartments.

3.6.3. Predator-pathogen interaction

It is apparent from our research results that some interference may result from the combined application of *B. bassiana* with the predator *D. hesperus*. Though the abundance of *D. hesperus* was not reduced, a significant decrease in the predation of immature whitefly in treated compartments was observed. This reduction in predation observed early during the experiment may represent a non-lethal infection of the predator or to changes in predator behaviour.

Competition for healthy prey may be an important factor contributing to changes in the distribution of predator *D. hesperus*. In many ecosystems, a change in distribution may occur when an organism inhabits an area or a time where its optimal food resource is limited (Polis *et al.*, 1989). In this study the distribution of *D. hesperus* was positively correlated to that of whitefly so that predators were likely aggregated in regions where a high density of immature whitefly were found, regardless of whether these were infected (Labbé, unpublished data). This suggests that when the whitefly resource is limited, predators may have little option but to prey upon infected whitefly. In a study by Dixon (1998), a similar distribution was observed when examining the interactions between different species of aphid parasitoids. Notably, the patchiness of aphid hosts favoured the

aggregation of aphid natural enemies around them, resulting in intra- and interspecific competition for this same limited resource. While it appears normal that a predator or parasitoid would aggregate around its primary resource, results presented in the previous chapter of this thesis suggest that it should not be the case for predator *D. hesperus* feeding upon infected whitefly. In particular, I reported that during laboratory experiments, *D. hesperus* avoided feeding on *B. bassiana* infected prey showing the presence of hyphae or of oosporein, a red pigment produced by the fungus.

A number of field studies in invertebrate communities have shown that though most herbivores are attacked by large guilds of natural enemies, complex and unexpected interactions between predators, parasitoids and pathogens may significantly reduce their impact on herbivore populations (Brodeur and Rosenheim, 2000). Predators, parasitoids and pathogens have been previously considered to have parallel and largely independent effects on herbivore populations, and potentially significant interactions between these natural enemies were neglected (Brodeur and Rosenheim, 2000). Recent attempts to extend current population models by adding interactions between natural enemies have contributed to the emergence of a more dynamic and reliable understanding of the factors that regulate the density of animal populations (see Polis *et al.*, 1989; Polis, 1991; Strong, 1992; Kareiva, 1994; Polis and Strong, 1996; Losey and Denno, 1998; Rosenheim, 1998).

Multispecies assemblages of biological control organisms may provide a balance between organisms in greenhouse agroecosystems that is necessary for consistent pest suppression. When species with known biological control properties are chosen to interact together, the shortcomings and limitations of individual species may be compensated for within the same guild. For example, whitefly suppression by polyphagous predators are rarely effective to lower pest populations below an acceptable level (Gerling, 1990), but they may be useful in containing their exponential growth by conservation, augmentation or even inoculation in the environment (Gerling, 1990). In the case of the specific parasitoid, *E. formosa*, during some periods of the year when peak high or low temperatures are observed, whitefly populations may become difficult to control solely by this species. In such situations,

biological control practitioners resort to the use of alternate control measures such as provided by the use of generalist pathogens (Perdikis and Lykouressis, 2002). Our study showed that while the application of the entomopathogen *B. bassiana* caused an important reduction in whitefly populations, other natural enemy populations were maintained. These findings promote the use of multispecies complexes in greenhouse biological control that may serve to recreate a complete trophic web. Such a web is composed of a number of interactions each having an important role in community dynamics.

In contrast, a large number of recent field studies in arthropod communities have shown that while most herbivores are attacked by large guilds of natural enemies, some unexpected and complex interactions between predators, parasitoids and pathogens may significantly reduce their impact on herbivore populations (Brodeur and Rosenheim, 2000). Community dynamics are affected by factors such as foraging behaviour, indirect mutualism or intraguild predation and may be the cause of trophic cascades that appear over time. However, intraguild predation may be effectively used to offset competition during prey shortage (Hochberg and Lawton, 1990). In our study, *B. bassiana* controlled an imminent pest outbreak with a minor disruption on biological control. It is clear that further research on the role of intraguild interactions in the greenhouse environment is warranted, and particularly so for the most currently applied biological control organisms. This research has identified the compatibility of whitefly natural enemies, and would be further enriched by studies of simple and paired interactions between natural enemies with the whitefly, as well as between other natural enemy species.

3.7. References

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Chapter 4. General Conclusion

4.1. General Conclusion

4.1.1. The role of entomopathogens in natural enemy guilds

Entomopathogens play an important role in biological control as they may quickly cause significant reductions in pest populations (Lacey and Goettel, 1995). The use of entomopathogens in greenhouses may effectively allow the reestablishment of equilibrium between pest populations and other arthropod natural enemies. Entomopathogens are therefore truly important components of biological integrated pest management.

However, reinstating equilibrium between arthropod natural enemies and their prey should require that entomopathogens have little or no impact on populations of beneficials. Predators and parasitoids may become infected through direct contact with pathogens applied onto a crop, and are indirectly affected by the reduction in prey availability following pathogen applications. This reduction may increase the competition between natural enemies for a limited prey resource, leading to an increased probability of intraguild predation or other negative interactions.

When arthropod natural enemies are susceptible to intraguild interactions, three strategies may be adopted to reduce negative outcomes between them (Brodeur *et al.*, 2002). First, their protection from the entomopathogen may be achieved by the spatial separation of natural enemies by applying *B. bassiana* in regions of highest predator or parasitoid density. It may also be achieved by applying the entomopathogen a few days after the application of parasitoids. Finally, in many greenhouse agroecosystems, the regular release of natural enemies such as the parasitoid *Encarsia formosa* may allow for a quick rehabilitation of its populations. In the case of generalist predators such as *Dicyphus hesperus*, regions of a crop where a deficit may be incurred may be replenished through the relocation of predators from other regions where the predator is abundant.

In this study, direct and indirect interactions between organisms were identified as mediators of biological control success. I have shown that the entomopathogenic fungus *B. bassiana* has a direct impact on populations of the greenhouse whitefly. Furthermore, *B. bassiana* had minimal negative effect on other biological control agents such as the

parasitoid, *Encarsia formosa* and the generalist predator, *Dicyphus hesperus*. We have also demonstrated that the predator *D. hesperus* indiscriminately fed upon *E. formosa* parasitised whitefly prey in the laboratory. The many factors influencing interactions between biological control organisms include arthropod behaviour, the dose of the applied entomopathogen, specificity, spatial and temporal separation, as well as ontogenetic factors. In the case of prey ontogeny, immature prey insects eventually develop into inedible or inaccessible adult stages. The later factor is considered important in nature whereby the vulnerability of certain parasitoid or predator life stages may vary, altering the impact of other intraguild members, such as pathogens or predators (Polis, 1988).

4.1.2. Potential cascade effects and alternate long term outcomes

In greenhouse experiments, we observed that application of *B. bassiana* caused a significant infection of whitefly, and a significant reduction in predation by *D. hesperus*. This result is most likely due to sub-lethal effects such as the reduction of predator fitness in the presence of the entomopathogen. While infection may not immediately kill a predator, it may in some way reduce activity and readiness to feed. Had the term of this study been prolonged, pathogen effects may have shown an eventual reduction in predator abundance. Lower predation could also result in decreased competition for whitefly hosts or may indirectly benefit parasitoids through reduced predation of developing parasitoids within whitefly hosts. This cascade may lead to increased whitefly suppression. In our laboratory experiments, *D. hesperus* equally preyed upon parasitized and unparasitized whiteflies.

With *D. hesperus*, predator density also has important implications on the quality of fruit produced. The tendency of *D. hesperus* to feed on tomato fruits causing undesirable blemishes (McGregor *et al.* 2000) is most evident in a crop where predator density is high (Jocelyne Moreau, personal communication, Savoura, Les Serres du Saint-Laurent). In such a circumstance, an overabundance of *D. hesperus* resulting from high whitefly densities may become a nuisance when the whitefly resource is depleted. Through the use of *B. bassiana*, an immediate reduction of whitefly densities may avoid a quick increase in the number of *D. hesperus* predators. By doing so, an equilibrium between predator and

prey can be maintained through avoiding an overpopulation of predators for a limited prey resource. In turn, keeping predator numbers low will minimize fruit injury.

4.1.3. Specialists versus Generalists

Community structure has important implications for the success of biological control. Just as no one biological control organism may solve all problems incurred in a given agroecosystem, the members of a natural enemy guild must also be chosen with care, as interactions of members may lead to unexpected results. Biological control has generally favoured specificity of natural enemies, notably through the use of parasitoids for the reason that non-native generalist predators introduced for biological control are responsible for decimating indigenous fauna (Follett and Duan, 2000; Hokkanen and Lynch, 1995; Howarth, 1991). However, higher-order predators effectively mediate community structure in natural arthropod populations (Symondson *et al.*, 2002). Native generalist natural enemies have also played an important role in biological control (Rosenheim, 1998; Symondson *et al.*, 2002). Guilds of generalist predators may effectively suppress populations of both indigenous and exotic pests (Sunderland *et al.*, 1997; Van Driesche and Bellows, 1996), and may even be more effective than specialists. DeBach (1946) showed that generalist predators may be more effective at controlling pests in citrus trees than are specific parasitoids. In our study, it appeared that generalist biological control agents such as the entomopathogen *B. bassiana* could effectively suppress whitefly populations while having minimal impact on non-target species such as the parasitoid *E. formosa*.

Through experimentation, our understanding of inter-species population dynamics continues to grow. We no longer seek merely specialist biological control organisms, but now include generalists and even zoophytophagous organisms to contribute to sustainable pest management programs. A review of manipulative field studies by Symondson *et al.* (2002), showed that in 75% of studies surveyed, single species or species assemblages of generalist predators reduced pest numbers significantly. To understand the role of top predators on community dynamics, biological control researchers have applied ecological concepts of predator-prey relationships such as that of Tetranychid mite populations controlled by their natural predators (Huffaker 1970).

4.1.4. Importance of guilds of organisms

Greenhouse ecosystems are characterized by stochastic fluctuations in arthropod populations resulting from the absence of natural enemies (Brodeur *et al.*, 2002). However, as many natural enemies are applied, these may have complex interactions with one another that may dampen herbivore population fluctuations and stabilize the given system (McCann *et al.*, 1998). In theory, this condition greatly reduces the need to replenish a biological control system through the regular inundative application of specialist natural enemies, a process that is costly and short term. In our study, the use of the pathogen, *B. bassiana* and the predator, *D. hesperus* may complement the regular release of the parasitoid *E. formosa* to provide consistent whitefly suppression.

Maximizing whitefly biological control also requires consideration of greenhouse environmental variability that may affect the outcome of multispecies releases. Jazzar and Hammad (2004) proposed a strategy that would avoid an antagonistic interaction between *E. formosa* and the entomopathogen *Verticillium lecanii* (Mycotal®). Applications were timed so that only third to fourth instar nymphs of *Bemisia tabaci* were treated (Jazzar and Hammad, 2004). Environmental conditions may favour the use of only one of these biocontrol agents. On overcast days, when relative humidity is high and whitefly density is high, Mycotal® may be applied. However, the use of *E. formosa* is best in hot, dry conditions such as those occurring during peak periods of summertime. Management practices based on a consideration of such ecological parameters will contribute to the success of biological control. In this study, we determined that the use of the entomopathogen *B. bassiana* should be carefully monitored in order to avoid treating areas where *D. hesperus* is abundant. Through this strategy, the persistence of *D. hesperus* will allow persistent control of whitefly populations.

Biological control is the primary pest control strategy in the greenhouse vegetable industry in Canada (Murphy *et al.*, 2002), and an annual growth of about 10% in the use of biological control has been witnessed since 1983 (Gillespie, 2002). This growth may be in part due to our better understanding of the nature and interactions of natural enemies. Yet there is still much room for improving biological control. Growers using biological control

as their sole means of pest suppression may be discouraged by the variability of results, the cost of organisms or the time consumed in careful monitoring of crops for potential pest outbreaks. Furthermore, it has become increasingly obvious that there is no single agent that will resolve the problems associated with development of pest populations. In the contrary, the use of multiple biological control organisms that play a complementary role to one another may achieve pest suppression through their positive interaction. In particular, the greenhouse whitefly that is currently known as one of the most important greenhouse pests (Byrne *et al.*, 1990) may be effectively suppressed given the right tools and information. When used at the appropriate time, parasitoids, predators and entomopathogens may play a role in an effective integrated pest management strategy.

4.2. References

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