INTRAGUILD INTERACTIONS BETWEEN TWO NATURAL ENEMIES OF *TRIALEURODES VAPORARIORUM* (HOMOPTERA: ALEYRODIDAE), THE PREDATOR, *DICYPHUS HESPERUS* (HETEROPTERA: MIRIDAE), AND THE ENTOMOPATHOGENIC FUNGUS, *PAECILOMYCES FUMOSOROSEUS* APOPKA-97 (DEUTEROMYCOTINA: HYPHOMYCETES) (PFR-97™)

by

Colleen Rosanne Alma
Bachelor of Science in Agriculture, University of Guelph, 2002

THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

Spring 2005

APPROVAL

Name: Colleen Rosanne Alma

Degree: Master of Science

Title of Thesis:

Intraguild interactions between two natural enemies of *Trialeurodes vaporariorum* (Homoptera:Aleyrodidae), the predator, *Dicyphus hesperus* (~~Hemip~~Heterop tera:Miridae), and the entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97 (Deuteromycotina: Hyphomycetes) (PFR-97™)

Examining Committee:

Chair: Dr. J. Mattsson, Assistant Professor

Dr. B.D. Roitberg, Professor
Department of Biological Sciences, S.F.U.

Dr. M.S. Goettel, Research Scientist
Agriculture and Agri-Food Canada and Adjunct Professor
Department of Biological Sciences, S.F.U.

Dr. D.R. Gillespie, Research Scientist
Agriculture and Agri-Food Canada and Adjunct Professor
Department of Biological Sciences, S.F.U.

Dr. Z.K. Punja, Professor
Department of Biological Sciences, S.F.U.

Dr. D.A. Raworth, Research Scientist
PAFRC, Agriculture and Agri-Food Canada
Public Examiner

Date Approved

SIMON FRASER UNIVERSITY

PARTIAL COPYRIGHT LICENCE

ABSTRACT

My focus was to investigate the intraguild interactions between two natural enemies of the greenhouse whitefly: *Dicyphus hesperus*, and *Paecilomyces fumosoroseus* Apopka-97 (PFR-97™). PFR-97™ spray applications made to whitefly nymphs resulted in a LC_{50} of 1.33×10^5 blastospores/ml. Lethal time estimates of whitefly nymphs decreased with increasing concentration of the PFR-97™ spray application. When *D. hesperus* females were exposed to leaf discs treated with PFR-97™ at a concentration of 100 x LC_{50} of whitefly nymphs, 38% of the females died. The surviving females experienced a 38% feeding reduction. Adult *D. hesperus* females were able to discriminate between healthy and PFR-97™-treated whitefly nymphs when the nymphs were offered to them five days after spray application. The simultaneous use of *D. hesperus* and PFR-97™ in tomato greenhouse microcosms resulted in additive whitefly mortality; together they reduced whitefly densities by 62% relative to the control plants in six weeks.

ACKNOWLEDGEMENTS

Foremost, I would like to thank my supervisory committee, Bernard Roitberg, Mark Goettel, David Gillespie and Zamir Punja, for their guidance and assistance during the past two years. This work was supported by NSERC, Biocontrol Network, Agriculture and Agri-Food Canada (AAFC), and the British Columbia Greenhouse Research Council. The commercial formulation PFR-97™ was kindly provided by Certis USA, Columbia MD. Plants were grown and maintained by Mark Gross (Pacific Agri-Food and Research Center, Agassiz). I acknowledge Ian Bercovitz for his assistance with the statistical analyses of the greenhouse trial. I am grateful to William deVane for his willingness to assist me with inquiries related to PFR-97™ and to Dr. Russell Nicholson for the use of his electrobalance. I would like to express my gratitude to Grant Duke for his guidance and assistance during the preparation and execution of the bioassays. I am very grateful to Angela Mah and Jasmin Gee for their assistance with the collection of experimental data. Lastly, I would like to thank my husband, Randy, for lending a hand whenever it was needed, my sister, Carolyn, and the rest of my family for their support during the last two years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

1.1 Abstract

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), continues to be a major priority for vegetable greenhouse growers in British Columbia (BC). Populations of *T. vaporariorum* are prevalent throughout the BC growing season, starting in January and extending into November (BC Greenhouse Grower's Association, pers. comm.[1]). The population threshold level for *T. vaporariorum* is low; densities of 1 adult *T. vaporariorum* per 100 tomato plants can lead to population outbreaks if control measures are not initiated (D. Elliot, pers. comm.[2]).

Exclusive control with chemical insecticides is no longer feasible due to the quick development of resistance by *T. vaporariorum* populations, and the associated health and environmental issues related to insecticide usage. These issues initiated a search for alternative control methods such as biological control. Biological control programs use natural enemies of pests, to reduce pest populations below the economic threshold level. The use of multiple natural enemies for control of a pest can enhance its biological control program if their simultaneous use does not lead to antagonistic intraguild interactions. Since intraguild interactions are widespread among communities of biological control agents, the occurrence of intraguild interactions between natural

[1] Author unknown. BC Greenhouse Grower's Association. Personal communication via e-mail. December 6, 2004.
[2] Don Elliot. Personal communication via e-mail. December 6, 2004.

enemies must be investigated prior to their combined release in biological control programs.

This chapter is a review of the major natural enemies that are being used or evaluated for the management of *T. vaporariorum*. These include parasitoids, predators and entomopathogenic fungi. Special emphasis is placed on the predatory bug, *Dicyphus hesperus* Knight (Hemiptera: Miridae), and the entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97 (Wize) Brown and Smith (Deuteromycotina: Hyphomycetes), as the primary focus of my thesis is to investigate their intraguild interactions for their joint control of *T. vaporariorum* populations. Implications of the simultaneous use of natural enemies in biological control programs are also discussed.

1.2 Literature Review

1.2.1 *Trialeurodes vaporariorum*

1.2.1.1 Life History

The life cycle of *T. vaporariorum* consists of six stages: the egg, four larval instars and the adult (Gill, 1990). The spindle-shaped eggs are laid by females in a circular pattern on the underside of young leaves, which is caused by simultaneous feeding and oviposition (van Lenteren and Noldus, 1990). A female will probe the underside of leaf surfaces for a suitable feeding site and as she inserts her proboscis into the leaf, she rotates around the point of insertion. As she rotates, she sucks the leaf contents and lays her eggs on the leaf surface (van Lenteren and Noldus, 1990). After hatching, the first-instar nymph crawls on the leaf surface for a short time looking for a suitable feeding site (Gill, 1990). First-instar nymphs are oval, pale green, and have well-developed legs and antennae (Osborne and Landa, 1992). The legs and antennae become

non-functional in the second-instar stage, and the remaining nymphal stages are sedentary (Osborne and Landa, 1992). Second and third-instar nymphs are transparent, flattened on the leaf and only differ from one another in size; second-instar nymphs are 0.3-0.4 mm long whereas third-instar nymphs are 0.5 mm long (Hulspas-Jordaan and van Lenteren, 1989 and Malais and Ravensberg, 2003). The developing fourth-instar nymphs progress in appearance from flattened and translucent to opaque-white and then to opaque, thickened and covered with wax (Osborne and Landa, 1992). When the red eyes of the adult *T. vaporariorum* are visible, the nymph is sometimes referred to as the "pupa" (Hulspas-Jordaan and van Lenteren, 1989). Adults emerge from a T-shaped opening in the pupal case; adults are 0.9-1.1 mm long with 2 pairs of wings, functional legs and antennae and soon after emergence, become covered in wax (Gill, 1990; Malais and Ravensberg, 2003).

Hulspas-Jordaan and van Lenteren (1989) reviewed the *T. vaporariorum* literature. The average developmental time from egg to adult is approximately 22 days at 25°C. The adult sex ratio is 1:1 (males: females). Adult longevity varies from a few days to two months; at 25°C, adult longevity is approximately 14 days. The oviposition rate for females changes over time. Females oviposit at a faster rate for a few days after emergence, then at a slower rate prior to death. At 22°C, the average oviposition rate on tomato plants is 5.5 eggs per day (Hulspas-Jordaan and van Lenteren, 1989). These statistics vary with temperature and host plant.

1.2.1.2 Host Plant Damage

A variety of greenhouse crops can be damaged by *T. vaporariorum* populations (Malais and Ravensberg, 2003). Crop damage is a result of phloem feeding and

honeydew secretion. Phloem feeding by large populations of *T. vaporariorum* retards plant vigor. The production of copious amounts of honeydew on leaves and fruits stimulates the growth of sooty mould fungi thereby reducing the photosynthetic capacity of the leaves and reduces fruit quality (Byrne *et al.*, 1990; Osborne and Landa, 1992; Fransen and van Lenteren, 1993; Poprawski *et al*, 1998). Furthermore, *T. vaporariorum* can transmit several plant viruses; such as the beet pseudo yellows virus and the tomato chlorosis virus (Malais and Ravensberg, 2003).

1.2.1.3 Resistance to Chemical Insecticides

Controlling *T. vaporariorum* with chemical insecticides had limited success because overuse of chemical insecticides has selected for resistant *T. vaporariorum* populations. Chemical resistance was first reported in 1972, when malathion and DDT provided inadequate control of *T. vaporariorum* populations (French *et al.*, 1973). During the next few years, marked resistance of *T. vaporariorum* populations to several organophosphorus and organochlorine insecticides was also reported (French *et al.*, 1973; Wardlow *et al.*, 1975; Elhag and Horn, 1984). Elhag and Horn (1983) demonstrated that insecticide resistance developed when there was a high selection pressure. The occurrence of resistance in *T. vaporariorum* populations was positively correlated with a history of insecticide exposure. Resistance was virtually inevitable when *T. vaporariorum* populations were exposed to frequent and prolonged exposure to insecticides (Elhag and Horn, 1983). The short generation time and high fecundity of *T. vaporariorum* contributed to the development of resistance (Elhag and Horn, 1983).

1.2.2 Natural Enemies of *Trialeurodes vaporariorum*

1.2.2.1 Parasitoids

The parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is the most commonly used, commercially produced biological control agent of *T. vaporariorum* (van Lenteren *et al.*, 1997). Adult females are 0.6 mm long and have a brown-black coloured head and thorax with a yellow abdomen (Malais and Ravensberg, 2003). Females of *E. formosa* actively search for and parasitize *T. vaporariorum* nymphs. Once a nymph is located, the female will lay an egg inside it. When the egg hatches, the developing *E. formosa* larva feeds on and eventually kills the *T. vaporariorum* nymph. Adult females also kill *T. vaporariorum* nymphs through host-feeding. Halfway through the development of the larva, the host nymph turns black in colour (Malais and Ravensberg, 2003). The *E. formosa* adult emerges from the nymph by chewing a hole through the cuticle (van Lenteren *et al.*, 1997).

Extensive research has shown that *E. formosa* is a highly successful parasitoid of *T. vaporariorum* on greenhouse vegetable crops (van Roermund and van Lenteren, 1995b; van Lenteren *et al.*, 1996; van Lenteren *et al.*, 1997; Hoddle *et al.*, 1998a). When conditions are suitable for *E. formosa*, it is possible to achieve a parasitism level between 90-95% (Onillon, 1990). There are, however, certain factors that can negatively affect the parasitism rate of *E. formosa*, such as low temperatures, presence of honeydew, high trichome densities, fluctuations in barometric pressure and host-parasitoid ratio (Kassis and Michelakis, 1993; Zchori-Fein *et al.*, 1994; van Roermund and van Lenteren, 1995a; van Roermund and van Lenteren, 1995b). Horticultural practices in greenhouse vegetable crops, such as the removal of lower leaves, reduce *E. formosa* populations

because most of the removed leaves contain non-emerged parasitoids (D. Gillespie, personal communication[3]). Furthermore, *E. formosa* is a specialist of whiteflies and its use is therefore limited. Even with different whitefly species, *E. formosa* is not equally successful; it is not as effective at parasitizing *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) compared to *T. vaporariorum* populations (Bosclair, 1990).

Another parasitic wasp which is native to the western United States is *Eretmocerus eremicus* Rose (Hymenoptera: Aphelinidae). It has been reported to be efficacious towards whitefly species and is being produced commercially. Females are 0.75 mm long and have a yellow-orange body with green eyes (Malais and Ravensberg, 2003). Unlike *E. formosa*, *E. eremicus* females lay their eggs beneath *T. vaporariorum* nymphs. When the egg hatches, the *E. eremicus* larva penetrates the *T. vaporariorum* nymph where it completes its development and emerges as an adult (Malais and Ravensberg, 2003). Females of *E. eremicus* performed similarly on both *T. vaporariorum* and *B. argentifolii* in terms of development, female progeny, and female progeny longevity; however, females parasitized more *B. argentifolii* nymphs than *T. vaporariorum* nymphs (Greensberg *et al.*, 2002). Hoddle *et al.* (1998b) compared the effectiveness of *E. eremicus* and *E. formosa* (strain adapted to *B. argentifolii*) on *B. argentifolii* on greenhouse grown poinsettias and found that *E. eremicus* was more effective in terms of searching ability, parasitization and persistence in the environment. Since *E. eremicus* is efficacious towards *B. argentifolii,* it is more commonly used as a natural enemy of *B. argentifolii* or when *T. vaporariorum* and *B. argentifolii* populations are both present on the crop.

[3] Pacific Agri-Food Research Center, Agriculture and Agri-Food Canada. Agassiz, B.C. November 27, 2002.

1.2.2.2 Predators Other Than *Dicyphus hesperus*

Many predaceous hemipterans have been reported to prey on *T. vaporariorum* under laboratory or field conditions. Species of *Campylomma* (Hemiptera: Miridae) collected from Fukuoka, Japan had high (70-100%) levels of success at attacking all stages of *T. vaporariorum* on tomato leaflets under laboratory conditions (Kajita, 1984). Adults of *Orius sauteri* Poppius (Hemiptera: Anthocoridae) successfully attacked (90%) all life stages, including eggs of *T. vaporariorum* under laboratory conditions (Kajita, 1982). In tomato glasshouses in Hungary, nymphs and adults of *Dicyphus hyalinipennis* Burmeister (Hemiptera: Miridae) were observed feeding on *T. vaporariorum*; they could each consume approximately 12 *T. vaporariorum* nymphs per day (Ceglarska, 1999).

The four most common and commercially produced predatory biological control agents of *T. vaporariorum* are: *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), *Dicyphus tamaninii* Wagner (Hemiptera: Miridae), *Delphastus pusillus* (LeConte) (Coleoptera: Coccinellidae) and *D. hesperus*. The predatory mirids, *M. caliginosus* and *D. tamaninii*, occur in the Mediterranean region where they have been found to naturally migrate into greenhouses (Alomar *et al.*, 1990). Adults and nymphs of *M. caliginosus* are mainly used to suppress whitefly populations; however, *M. caliginosus* also preys upon thrips (Jakobsen *et al.*, 2002), mites (Koskula *et al.*, 1999), leafminers, caterpillars and aphids (Nedstam and Johansson-Kron, 1999). When used in combination with other biological control agents, *M. caliginosus* is an efficacious predator. Adequate control of *T. vaporariorum* populations was achieved with the combined use of *M. caliginosus* and *E. formosa* on protected tomato crops (Sampson and King, 1996). When used in combination with *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae), *M.*

caliginosus enhanced the control of *Tetranychus urticae* Koch (Acarina: Tetranychidae) on tomato greenhouse crops (Koskula *et al.*, 1999).

Dicyphus tamaninii is also polyphagous; nymphs and adults feed on whitefly species, lepidopteran eggs, thrips, aphids, and spider mites (Salamero *et al.*, 1987; Albajes *et al.*, 1996; Castane *et al.*, 1996). A nymph can consume up to 59 *T. vaporariorum* nymphs during its developmental time (21 days) (Salamero *et al.*, 1987). Populations of *T. vaporariorum* are greatly affected by the presence of *D. tamaninii* on tomato crops in Spain (Gabarra *et al.*, 1988). Both *D. tamaninii* and *M. caliginosus* are phytophagous and have been known to cause direct damage to tomato fruit. Under low prey densities, *D. tamaninii* has been seen puncturing tomato fruit and causing cosmetic damage (Albajes *et al.*, 1996). In a United Kingdom survey, *M. caliginosus* damage resulted in a 20-22% fruit loss due to down-grading of fruit (Sampson and Jacobson, 1999).

The coccinellid predator, *D. pusillus*, preys on several whitefly species; however, it is considered the most efficacious biological control agent of *Bemisia* species (see review by Obrycki and Kring, 1998). Adult beetles are small, with a black body and a brown head. Females and males of *D. pusillus* consumed more *B. argentifolii* third-instar nymphs than eggs over a three-day period. Prey consumption and oviposition by *D. pusillus* was higher on plants with lower trichome densities (Heinz and Parrella, 1994). On tomentose cotton leaves, *D. pusillus* had a difficult time locating prey as evidenced by their lower predation rate on these leaves compared to glaborous leaves (Guershon and Gerling, 1999). This beetle located prey on tomentose leaves by dipping its head between the leaf hairs, while walking on the tops of the trichomes, to reach the leaf

surface. Predation on adult *B. tabaci* was not observed on tomentose leaves due to the inhibition of the trichomes (Guershon and Gerling, 1999). Reproduction ceases for *D. pusillus* at low prey densities; therefore, researchers recommend that this beetle be used for suppressing whitefly outbreaks, or that frequent releases be made during low whitefly densities (Heinz and Parrella, 1994).

Lastly, the mirid predator, *D. hesperus* is also an effective natural enemy of *T. vaporariorum* (McGregor *et al.*, 1999; Sanchez *et al.*, 2003). An in depth summary of its biology and its potential as a greenhouse biological control agent will follow in the respective section.

1.2.2.3 Entomopathogenic Fungi Other Than *Paecilomyces fumosoroseus*

Various entomopathogenic fungi suppress *T. vaporariorum* populations: *Aschersonia aleyrodis* Webber (Deuteromycotina: Coelomycetes) (Fransen *et al.*, 1987; Fransen and van Lenteren, 1994; Meekes *et al.*, 2002), *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (Poprawski *et al.*, 2000; Fargues *et al.*, 2003), *Lecanicillium lecanii* (Zimmerman) Gams and Zare (*Verticillium lecanii* (Zimmerman) Viegas) (Deuteromycotina: Hyphomycetes) (Osborne and Landa, 1992; Fargues *et al.*, 2003) and *P. fumosoroseus* Apopka-97 (Poprawski *et al.*, 2000; Osborne and Landa, 1992). Three of these entomopathogenic fungi have been developed into biopesticides for greenhouse use. Products based on the fungus strain *B. bassiana* GHA are available under the tradenames Mycotrol™ and BotaniGard™ (USA). Formulations of *L. lecanii* are being sold in European countries under the tradenames Vertalec™ and Mycotal™. The formulated products based on the fungus *P. fumosoroseus* Apopka-97 are PFR-97™ (USA) and PreFeRal™ (Europe). To date, only the specialist fungus, *A.*

aleyrodis, has not been developed into a biopesticide. None of the aforementioned biopesticides are currently registered in Canada.

1.2.3 *Dicyphus hesperus*

1.2.3.1 Life history

D. hesperus was first identified by Knight in 1941, on whiteflower leafcup, *Polymnia canadensis* L. (Compositae) in well developed woods, and then was subsequently found inhabiting a wide range of host plants (Knight, 1941). This species was re-described by Cassis (1986), as being mostly black in colour with yellow-testaceous markings and red to fuscous-red eyes. Males and females are approximately 3.5 mm long, and sparsely covered with pale-brown, erect, long setae (Cassis, 1986). There are four nymphal stages of *D. hesperus* that can only be differentiated by size and colour. All nymphs have red eyes and their bodies become increasingly green in colour and larger through development. Twenty-four hours prior to moulting into an adult, the tips of the wing buds become black. Adult females embed their eggs into thickened plant tissue, mainly plant stems, veins and stalks. Oviposition occurs very quickly; the visible, sword-like ovipositor is swung out from under the abdomen and inserted into the plant tissue.

Along with all predatory Heteroptera, *D. hesperus* uses intact non-refluxing extra-oral digestion as its mode of prey feeding. It is a process in which predators inject digestive enzymes into prey to liquefy solids and reduce viscosity of liquids so that the partially digested prey contents can be withdrawn from the body of the prey and ingested by the predator (Cohen, 1995; Cohen, 1998). Intact digestion refers to the fact that the cuticle of the prey essentially remains intact and the chemical liquefaction occurs entirely

within the prey (Cohen, 1995). Non-refluxing refers to the movement of digestive enzymes; non-refluxers inject a given amount of digestive enzymes only once into the prey. In contrast, predatory refluxers such as beetles and spiders, repeatedly pump and suck the enzymes to and from their prey (Cohen, 1998).

1.2.3.2 Potential as a Biological Control Agent

In 1998, *D. hesperus* was collected in Summerland (BC) on catnip, *Nepeta cataria* L. (Labiatae) and mullein, *Verbascum thapsus* L. (Scrophulariacae) plants (McGregor et al., 1999), with the intent to evaluate it as an omnivorous predator that could be used as a general biological control agent in the BC tomato industry. Since then, laboratory and field studies on *D. hesperus* have been conducted to learn more about its development, prey preference, response to prey populations in greenhouse situations, and phytophagous nature.

Females of *D. hesperus* that were collected from Summerland, BC (BC population) entered reproductive diapause at a daylength of 15.5 hours (D.R. Gillespie, unpublished data). Hence, the population would not remain reproductively active and biologically efficacious throughout the greenhouse vegetable growing season in BC. Therefore, a population of *D. hesperus* was collected in California, United States (CA population), which had a better suited reproductive diapause of 13.5 hours (D. R. Gillespie, unpublished data). In terms of temperature effects on the rate of egg and nymphal development, the differences detected between the two populations were biologically insignificant. Also, developmental thresholds did not differ between populations (Gillespie *et al.*, 2004).

Developmental time from hatch to adults at 20°C for *D. hesperus* males and females consuming *T. vaporariorum* nymphs was 25 days (McGregor et al., 1999). Increasing the temperature resulted in a decrease in the developmental time from oviposition to hatch (egg development) and from hatch to adult (nymph development) (Gillespie *et al.*, 2004). Adults of *D. hesperus* fed readily on *T. vaporariorum* nymphs and *Tetranychus urticae* Koch (Acarina: Tetranychidae) and *D. hesperus* nymphs were able to complete their development on either pest species (McGregor et al., 1999). Nymphs and adults also feed on a variety of small, soft-bodied insects and eggs (Gillespie *et al.*, 2004). VanLaerhoven *et al.* (2003) observed that *D. hesperus* females were more active at night than in the day. In whole plant laboratory studies, *D. hesperus* females consumed prey at a higher rate during the night than the day regardless of the diel cycle (either L16: D8 or L8: D16) (VanLaerhoven *et al.*, 2003).

Populations of *T. vaporariorum* were successfully controlled by *D. hesperus* in tomato greenhouses. As the density of *T. vaporariorum* increased, the proportion of *D. hesperus* foraging on tomato plants also increased until *T. vaporariorum* populations declined dramatically (Sanchez *et al.*, 2003). The presence of mullein plants, *V. thapsus*, interspersed in the greenhouse appeared to cause a quicker response of *D. hesperus* to changes in prey density and contributed to the maintenance of *D. hesperus* populations during prey scarcity. One explanation is that mullein acted as an alternate host plant for *D. hesperus* during prey scarcity (Sanchez *et al.*, 2003), as *D. hesperus* is also phytophagous (Gillespie and McGregor, 2000; McGregor *et al.*, 2000). Laboratory experiments revealed that *D. hesperus* females fed on tomato leaves and fruits; however, females showed a preference for the tomato leaves. As their plant feeding and resultant

economic damage makes them unfavourable as a biological control agent, the relationship between prey and plant feeding is being investigated. Feeding on leaves or having access to a supplementary water source was required for prey feeding, growth and development of *D. hesperus* (Gillespie and McGregor, 2000). Females that were deprived of water for 24 hours and then offered a tobacco seedling, fed on plants more frequently and longer than those that had access to water (Sinia *et al.*, 2003). Females that were provided with prey and deprived of a water source for 24 hours, fed on plants at a higher frequency than prey-deprived individuals when offered the tobacco seedling (Sinia *et al.*, 2003). Sinia *et al.* (2003) concluded that plant feeding assisted in prey feeding by providing water that could be used in extra-oral digestion or to replace the water that was used during previous predation bouts.

1.2.4 *Paecilomyces fumosoroseus*

1.2.4.1 Biology and Infection Cycle

The entomopathogenic fungus, *P. fumosoroseus,* produces two kinds of asexual spores: conidia and blastospores. On the cuticle of the insect, *P. fumosoroseus* produces single or multiple condiophores on which conidia are formed. The conidia are cylindrical to fusiform in shape, smooth, hyaline, and borne in chains up to 5 μm in length (Onions, 1979). Blastospores of *P. fumosoroseus* are produced during the infection cycle inside the insect and are formed by budding off of hyphae. The blastospores vary in shape from yeast-like cells to elongate structures resembling hypha fragments (Inch *et al.*, 1986).

The infection cycle of *P. fumosoroseus* begins with the dispersal of conidia by wind, air movement or by other infected hosts. Once the conidia are in contact with the cuticle, they germinate and a germ tube penetrates the cuticle via natural orifices and

body segments (Osborne and Landa, 1992). Mycelia invade the haemocoel and spread throughout the body, producing blastospores that develop into additional hyphae (Goettel and Inglis, 1997). The death of the host is usually from a combination of physical obstruction of blood circulation, nutrient depletion and disruption of organs (Goettel and Inglis, 1997). To date, there has been no evidence of the production of mycotoxins by *P. fumosoroseus* (Bolckmans *et al.*, 1995). Once the host is dead, hyphae emerge from the cadaver and form conidiophores, which in turn produce conidia. The developed conidia become airborne and spread the fungus to other susceptible hosts (Goettel and Inglis, 1997). The infection cycle of *P. fumosoroseus* is rapid compared to the infection cycle of *A. aleyrodis* and *L. lecanii*. Within 24 hours of contact with the cuticle of the host, hyphae are present in the haemocoel. Hyphae can also be present on the surface of the host and sporulation can occur as soon as 48-72 hours after initial germination (Osborne *et al.*, 1990; Osborne and Landa, 1992).

The host range of *P. fumosoroseus* is broad; *P. fumosoroseus* infects insects in the orders Homoptera (Puterka *et al.*, 1994; Vandenberg, 1996; Poprawski *et al.*, 1999; Wraight *et al.*, 2000), Coleoptera (James and Lighthart, 1994), Lepidoptera (Altre and Vandenberg, 2001), and Diptera (Castillo *et al.*, 2000). It has also shown slight virulence to non-insect hosts such as *Rhipicephalus sanguineus* Latreille (Acari: Ixodidae) (Samish *et al.*, 2001).

1.2.4.2 Strain Apopka-97 (ATCC 20874)

In 1986, a strain of *P. fumosoroseus* was isolated from a mealybug, *Phenococcus solani* (Hemiptera: Pseudococcidae), on a velvet plant in a conservatory in Apopka, Florida (Copping, 2001). This strain, Apopka-97 (ATCC 20874), was discovered to be

highly virulent towards whitefly species. Vidal *et al.* (1997) reported a LC_{50} of 6.2 conidia/mm^2 on second-instar nymphs of *B. argentifolii* on ornamental sweetpotato leaf discs. Wraight *et al.* (1998) reported a considerably higher LC_{50} of approximately 150 conidia/mm^2 on third-instar nymphs of *B. argentifolii* on excised hibiscus leaves. Differences in pathogenicity of *P. fumosoroseus* Apopka-97 may be due to instar age at time of spray application, environmental conditions after spray application, leaf quality, and host plant species. Within 14 days after treatment, 77% of early second-instar nymphs of *B. argentifolii* on rooted cabbage leaves died when exposed to 38 blastospores/mm^2 (Lacey *et al.*, 1999). Landa *et al.* (1994) developed a novel bioassay method to test the pathogenicity of entomopathogenic fungi on whitefly species in which whitefly nymphs were removed from the leaf surface and placed in the middle of a conidial drop on a sterile microscope slide. Nymphs were then assessed daily using a rating system based on the degree of fungus development. Fourth-instar nymphs of *T. vaporariorum* placed in *P. fumosoroseus* Apopka-97 conidial drops (0.003 ml aliquots) at a concentration of 1.0×10^7 conidia/ml showed signs of fungus sporulation within 6 days (Landa *et al.*, 1994). Using a similar methodology, with the exception that the slides containing the nymphs were dipped into the suspension, a concentration of 1.0×10^6 conidia/ml caused 90% mortality of fourth-instar nymphs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) within 72 hours (Osborne *et al.*, 1990). This bioassay method has important limitations as pointed out by Vidal *et al.* (1997); the effect of host plant and the influence of nutrients cannot be considered. The stress associated with handling and relocation most likely weakens the immunity of nymphs; in addition, it can only be used on the non-feeding stage of whiteflies (fourth instar nymphs).

This strain, Apopka-97, was licensed to Certis USA, LLC (formerly Thermo Trilogy Corporation) and developed into a biopesticide that is registered in the United States and in Europe under the tradenames PFR-97™ and PreFeRal™, respectively (Faria and Wraight, 2001). PFR-97™ is sold as mainly desiccated blastospores on water dispersible granules containing 1 x 10^9 colony forming units per gram (Shah and Goettel, 1999). Compared to conidia, blastospores have a shorter shelf life but can be produced with a higher efficiency (Faria and Wraight, 2001). PFR-97™ has a wide host range; its target pests include whitefly species, aphids, thrips and spider mites (Shah and Goettel, 1999). The use of PFR-97™ in the United States is restricted to ornamental plants grown in greenhouses, nurseries and interiorscapes and it is not registered for use on food crops. PreFeRal™ is registered in Europe for use on protected tomato and cucumber plants.

Much of the efficacy data on PFR-97™ and PreFeRal™ are in the domain of the manufacturing companies; however, a few studies have been published. An application of PreFeRal™ at a concentration of 2 x 10^6 colony forming units/ml reduced *T. vaporariorum* densities by 38% in 3 weeks, whereas by 9 weeks, a second application reduced densities by 77% (Bolckmans *et al.*, 1995). Control of *T. vaporariorum* was achieved faster and required fewer spray applications on cucumber than on tomato plants (Bolckmans *et al.*, 1995). However, Vidal *et al.* (1998) reported no effect of host plant on the effectiveness of PreFeRal™ for control of *B. argentifolii* populations on cucumber, cabbage and three cultivars of tomato plants. Van de Veire and Degheele (1996) reported a 63% reduction of *T. vaporariorum* densities on greenhouse tomato plants three weeks after the first PFR-97™ application at a rate of 1 gram/1 liter water, and by four weeks, a second PFR-97™ application reduced densities by 79%.

Limited information is available on the effects of PFR-97™ on natural enemies used in biological control programs. Moderate mortality (26 % corrected mortality) of *M. caliginosus* was observed when *M. caliginosus* were placed on greenhouse tomato plants or bean plants that had been sprayed one hour previously with PreFeRal™ at a concentration of 5 x 10^6 colony forming units/ml (Sterk *et al.*, 1995a). Using the same experimental methods, PreFeRal™ caused low mortality (11 %) of *Orius* species and moderate mortality (0-25%) of *Amblyseius degenerans* (Berlese) (Acarina: Phytoseiidae) (Sterk *et al.*, 1995a). PreFeRal™ at a concentrations of 5 x 10^6 colony forming units per gram did not have any substantial lethal effects on *Bombus terrestris* (L) (Hymenoptera: Apidae) in terms of oral, vapour, and contact toxicity of adults, and oral toxicity of the brood (Sterk *et al.*, 1995b). PFR-97™ did not affect the developing parasitoid *E. formosa* inside *T. vaporariorum* nymphs on bean seedlings when sprayed at the recommended label rate under laboratory conditions (Van de Veire and Degheele, 1996). Osborne and Landa (1992) reported that PFR-97™ was very compatible with *Eretmocerus* species and *D. pusillus*.

1.2.5 Interaction between Natural Enemies

In some cases, one natural enemy is not sufficient to control whitefly populations and an additional control method is needed. For example, the combined use of *D. hesperus* and PFR-97™ may be more efficacious than using them separately and may result in complementary control of *T. vaporariorum*. However, their simultaneous use can also be detrimental to *T. vaporariorum* control if they negatively interact. Intraguild interactions occur when two species that are competing for the same prey also feed on or compete with each other (Polis and Holt, 1992). The three possible outcomes

to intraguild interactions in the context of biological control as defined by Ferguson and Stiling (1996) are: (1) the natural enemies interact synergistically and their combined effects on pest mortality is greater than the sum of their individual effects; (2) the natural enemies do not interact and their combined effects on pest mortality is equal to the sum of their individual effects and; (3) the natural enemies interact antagonistically and their combined effects on pest mortality are less than additive. Intraguild interactions are widespread among communities of biological control agents including between insect and pathogen natural enemies (Rosenheim *et al.*, 1995). Fungal pathogen control agents can affect insect natural enemies directly by infecting them, or indirectly, by depleting the prey population (Goettel *et al.*, 1990; Roy and Pell, 2000). In turn, insect natural enemies can affect fungal control agents by consuming prey that is infected with the fungus (Roy and Pell, 2000). PFR-97™ has a broad host range and can potentially infect and kill *D. hesperus*. Given that *D. hesperus* actively searches for prey and are phytophagous, it is likely that *D. hesperus* will come into contact with *P. fumosoroseus* spores while foraging and plant feeding. Furthermore, *D. hesperus* is a generalist predator that can reduce population densities of PFR-97™, if *P. fumosoroseus*-infected *T. vaporariorum* nymphs are suitable prey items. Consequently, it is critical to investigate the occurrence of intraguild interactions between PFR-97™ and *D. hesperus* prior to their combined usage for *T. vaporariorum* control.

The compatibility of multiple biological control agents for the control of whitefly populations has been previously studied. An application of PFR-97™ did not affect the developing parasitoid *E. formosa* inside *T. vaporariorum* nymphs on bean seedlings when sprayed at the recommended label rate under laboratory conditions (Van de Veire

and Degheele, 1996). Also in the laboratory, *E. formosa* were able to reject *T. vaporariorum* nymphs that had been treated seven days before with the fungus *A. aleyrodis*, thereby causing reductions in *T. vaporariorum* populations complementary to the fungus (Fransen and van Lenteren, 1993). Furthermore, an application of *A. aleyrodis* at a concentration of 2.2×10^7 spores/ml did not affect the developing *E. formosa* inside parasitized *T. vaporariorum* nymphs when treated at least 4 days after parasitization with no direct or indirect effects on the emerged parasitoid and their progeny (Fransen and van Lenteren, 1994). The combination of the parasitoids, *E. formosa* and *E. eremicus*, or *E. formosa* and *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) has been reported to provide adequate control of *B. tabaci* populations. Adding a third biological control agent such as *M. caliginosus* provided better control over a long period (in review by van Lenteren, 2000).

Intraguild interactions between arthropod and fungal pathogen natural enemies are usually investigated under laboratory conditions (see examples in Rosenheim *et al.*, 1995; Pell *et al,* 1997; Poprawski *et al*, 1998; Roy *et al,* 1998; Pell and Vandenberg, 2002). Only a small number of studies have been done outside the laboratory (James *et al.*, 1995; Mesquita *et al.*, 1997; Poprawski *et al.*, 1997; Jacobson *et al.*, 2001). Laboratory experiments can be poor predictors of what truly occurs in "nature" (Hajek *et al.*, 1996; Goettel *et al.*, 2001; Inglis *et al*, 2001). Artificial laboratory conditions can exclude or ignore important factors such alternate prey, and spatial and temporal heterogeneity that can affect the occurrence and severity of the interference between natural enemies (Rosenheim *et al.*, 1995; Roy and Pell, 2000).

In summary, both *D. hesperus* and PFR-97™ are efficient biological control agents of *T. vaporariorum* populations. In the past, the combination of fungal pathogen and arthropod natural enemies has enhanced the biological control of the target pest. However, it is critical to investigate the potential of intraguild interactions because they can have severe consequences on a biological control program. The simultaneous use of *D. hesperus* and PFR-97™ for *T. vaporariorum* control is worthwhile to investigate as they can provide BC greenhouse growers with an effective alternative to chemical insecticides.

1.3 Research Objectives

My primary focus was to investigate the intraguild interactions of *D. hesperus* and *P. fumosoroseus* Apopka-97 (PFR-97™) for control of *T. vaporariorum* populations on greenhouse tomato plants. My objectives were to:

(1) Determine the pathogenicity of PFR-97™ on *T. vaporariorum* nymphs under laboratory conditions. The basic measure of pathogenicity was the estimated concentration and time required to kill 50% of *T. vaporariorum* nymphs with PFR-97™ (LC_{50} and LT_{50} respectively). This laboratory assay enabled me to become more familiar with PFR-97™ and the infection cycle of *P. fumosoroseus* and provided me with a dose-time-mortality response of *T. vaporariorum* nymphs to applications of PFR-97™. These data were then used to test the effects of PFR-97™ on *D. hesperus*, as *T. vaporariorum* nymphs were used as a reference.

(2) Determine the potential of *D. hesperus* and PFR-97™ to antagonistically interact under laboratory conditions. In this study, three aspects of potential antagonistic

interactions between these natural enemies was investigated: (1) the effect of direct exposure of PFR-97™ on the mortality and predation rate of *D. hesperus* females; (2) the effect of indirect exposure to PFR-97™ on the mortality and predation rate of *D. hesperus* females and; (3) discrimination of *D. hesperus* females between healthy and PFR-97™-treated *T. vaporariorum* nymphs. These laboratory trials identified the antagonistic interactions that needed to be further investigated in a greenhouse situation.

(3) Determine the individual and combined effects of *D. hesperus* and PFR-97™ on *T. vaporariorum* on greenhouse tomato plants. Densities of *T. vaporariorum* nymphs on tomato plants were measured weekly in a factorial experiment with or without releases of *D. hesperus* adult and with applications of either PFR-97™ or water (control). Their effect on plant growth and on the proportion of flowers that set fruit was also investigated.

1.4 References

Albajes, R., O. Alomar, J. Riudavets, C. Castane, J. Arno, and R. Gabarra, 1996. The mirid bug *Dicyphus tamaninii*: an effective predator for vegetable crops OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996 19(1): 1-4.

Alomar, O., C. Castane, R. Gabarra, and R. Albajes, 1990. Mirid bugs - another strategy for IPM on Mediterranean vegetable crops? SROP/WPRS Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Copenhagen, Denmark, 5-8 June 8(5): 6-9.

Altre, J.A. and J.D. Vandenberg, 2001. Comparison of blastospores of two *Paecilomyces fumosoroseus* isolates: in vitro traits and virulence when injected into fall armyworm, *Spodoptera frugiperda* Journal of Invertebrate Pathology 78: 170-175.

Boisclair, J.E., 1990. A comparative study of the parasitization behaviour of the greenhouse whitefly, (*Trialeurodes vaporariorum*) Master of Pest Management Dissertation Simon Fraser University, Burnaby, British Columbia, Canada.

Bolckmans, K., G. Sterk, J. Eyal, B. Sels, and W. Stepman, 1995. PreFeRal, (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 707-711.

Byrne, D.N., T.S. Bellows, and M. P. Parrella, 1990. Whiteflies in Agricultural Systems Whiteflies: their bionomics, pest status and management (ed. D. Gerling) Intercept, Andover, pg. 227-261.

Cassis G. 1986. A systematic study of the subfamily Dicyphinae (Heteroptera: Miridae) PhD Dissertation Oregon State University, Corvallis, OR.

Castane, C., O. Alomar, and J. Riudavets, 1996. Management of western flower thrips on cucumber with *Dicyphus tamaninii* (Heteroptera: Miridae) Biological Control 7: 114-120.

Castillo, M-A., P. Moya, E. Hernandez, and E. Primo-Yufera, 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts Biological Control 19: 274-282.

Ceglarska, E.B., 1999. *Dicyphus hyalinipennis* Burm. (Heteroptera: Miridae): A potential biological control agent for glasshouse pests in Hungary IOBC Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Brest, France, 25-29 May 1999 22(1): 33-36.

Cohen, A.C., 1995. Extra-oral digestion in predaceous terrestrial arthropoda Annual Review of Entomology 40: 85-103.

Cohen, A.C., 1998. Solid-to-liquid feeding: the inside(s) story of extra-oral digestion in predaceous arthropoda American Entomologist 44: 103-117.

Copping, L.G., 2001. The BioPesticide Manual, British Crop Protection Council.

Elhag, E.A. and D. Horn, 1983. Resistance of greenhouse whitefly (Homoptera: Aleyrodidae) to insecticides in selected Ohio greenhouses Journal of Economical Entomology 76: 945-948.

Elhag, E.A. and D. Horn, 1984. Laboratory selection of greenhouse whitefly for resistance to malathion Entomologia Experimentalis et Applicata 35: 21-26.

Fargues, J., C. Vidal, N. Smits, M. Rougier, T. Boulard, M. Mermier, P. Nicot, P. Reich, B. Jeannequin, G. Ridray, and J. Lagier., 2003. Climatic factors on entomopathogenic hyphomycetes infection of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in Mediterranean tomato glasshouse Biological Control 28: 320-331.

Faria, M. and S.P. Wraight, 2001. Biological control of *Bemisia tabaci* with fungi Crop Protection 20: 767-778.

Ferguson, K.I. and P. Stiling, 1996. Non-additive effects of multiple natural enemies on aphid populations Oecologia 108: 375-379.

Fransen, J.J., K. Winkelman, and J.C. van Lenteren, 1987. The differential mortality various life stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), by infection of the fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes) Journal of Invertebrate Pathology 50: 158-165.

Fransen, J.J. and J.C. van Lenteren, 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 69: 239-249.

Fransen, J.J.and J.C. van Lenteren, 1994. Survival of the parasitoid *Encarsia formosa* after treatment of parasitized greenhouse whitefly larvae with fungal spores of *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 71: 235-243.

French, N., F.A.B.Ludlam, and L.R. Wardlow, 1973. Observations on the effects of insecticides on glasshouse whitefly (*Trialeurodes vaporariorum* Westw.) Plant Pathology 22: 99-107.

Gabarra, R., C. Castane, E. Bordas, and R. Albajes, 1988. *D. tamaninii* as a beneficial insect and pest in tomato crops in Catalonia, Spain Entomophaga 33: 219-228.

Gill, R.J., 1990. The morphology of whiteflies Whiteflies: their bionomics, pest status and management (ed. D. Gerling) Intercept, Andover, pg.13-46.

Gillespie, D.R. and R.R. McGregor, 2000. The functions of plant feeding in the omnivorous predator *Dicyphus hesperus*: water limits on predation Ecological Entomology 25: 380-386.

Gillespie, D.R., J.A. Sanchez and R.R. McGregor, 2004. Cumulative temperature requirements and development thresholds in two populations of *Dicyphus hesperus* (Hemiptera: Miridae) The Canadian Entomologist 136: 675-683.

Goettel, M.S., T.J. Poprawski, J.D. Vandenberg, Z. Li, and D.W. Roberts, 1990. Safety to nontarget invertebrates of fungal biocontrol agents, in Safety of Microbial Insecticides (eds. Lard, M., Lacey, L.A. and Davidson, E.W.) CRC Press, Boca Raton, Fl, USA, 209-231.

Goettel, M.S. and G.D. Inglis, 1997. Fungi: Hyphomycetes in Manual of techniques in insect pathology (ed. L.A. Lacey), Academic Press, San Diego, CA, 409.

Goettel, M.S., A.E. Hajek, J.P. Siegel and H.C. Evans, 2001. Safety of fungal biocontrol agents. Chapter 13 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 347-375.

Greensberg, S.M., W.A. Jones and T.X. Liu, 2002. Interactions among two species of *Eretmocerus* (Hymenoptera: Aphelinidae), two species of whiteflies (Homoptera: Aleyrodidae), and tomato Environmental Entomology 31(2): 397-402.

Guershon, M. and D. Gerling, 1999. Predatory behaviour of *Delphastus pusillus* in relation to the phenotypic plasticity of *Bemisia tabaci* nymphs Entomologia Experimentalis et Applicata 92: 239-248.

Hajek, A.E., L. Butler, S.R.A. Walsh, J.C. Silver, F.P. Hain, F.L. Hastings, T.M. Odell, and D.R. Smitley, 1996. Host range of the gypsy moth (Lepidoptera: Lymantridae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthales) in the field versus the laboratory Environmental Entomology 25: 709-721.

Heinz, K.M. and M. P. Parrella, 1994. Poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz) cultivar-mediated differences in performance of five natural enemies of *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) Biological Control 4: 305-318.

Hoddle, M.S., R.G. van Driesche and J.P. Sanderson, 1998a. Biology and use of the whitefly parasitoid *E. formosa* Annual Review of Entomology 43: 645-669.

Hoddle, M.S., R.G. van Driesche, J.S. Elkinton and J.P. Sanderson, 1998b. Discovery and utilization of *Bemisia argentifolii* patches by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville strain) in greenhouses Entomologia Experimentalis et Applicata 87: 15-28.

Hulspas-Jordaan, P.M. and J.C. van Lenteren, 1989. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) Modeling population growth of greenhouse whitefly on tomato Agricultural University Wageningen Papers 89(2): 2-18.

Inch, J.M.M., A.M. Humphreys, A.PJ. Trinci and A.T. Gillespie, 1986. Growth and blastospore formation by *Paecilomyces fumosoroseus,* a pathogen of brown planthopper, (*Nilaparvata lugens*) Transactions of the British Mycological Society 87: 215-222.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser, 2001. Use of hyphomycetous fungi for managing insect pests Chapter 3 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan) CAB International, pg. 23-69.

Jacobson, R.J., D. Chandler, J. Fenlon, and K.M. Russell, 2001. Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants Biocontrol Science and Technology 11(3): 391-400.

Jakobsen, L., A. Enkegaard, and H.F. Brodsgaard, 2002. Interactions between the two polyphagous predators *Orius majusculus* and *Macrolophus caliginosus* IOBC/WPRS Bulletin: Proceedings of the meeting, integrated control in protected crops, temperate climates, held in British Columbia, Canada, 25(1): 115-118.

James, R.R. and B. Lighthart, 1994. Susceptibility of the convergent lady beetle (Coleoptera: Coccinellidae) to four entomogenous fungi Environmental Entomology 23: 190-192.

James, R., B. Shaffer, B. Croft and B. Lighthart, 1995. Field evaluation of *Beauveria bassiana:* its persistence and effects on the pea aphid and a non-target coccinellid in alfalfa Biocontrol Science and Technology 5: 425-437.

Kajita, H., 1982. Predation of adult *Orius sauteri* Poppius (Hemiptera: Anthocoridae) on the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) Applied Entomology and Zoology 17: 424-425.

Kajita, H., 1984. Predation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) by *Campylomma* sp. (Hemiptera: Miridae) Applied Entomology and Zoology 19: 67-74.

Kassis G. and S. Michelakis, 1993. The effectiveness of biological control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom., Aleyrodidae) by *E. formosa* Gahan (Hym., Aphelinidae) Journal of Applied Entomology 116: 298-302.

Koskula, H., I. Vanninen and I. Lindqvist, 1999. The role of *Macrolophus caliginosus* (Het.: Miridae) in controlling the two-spotted spider mite in greenhouse tomato under North-European conditions IOBC Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Brest, France, 25-29 May 1999, 22(1): 129-132.

Lacey, L.A., A.A. Kirk, L. Millar, G. Mercadier and C. Vidal, 1999. Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects Biocontrol Science and Technology 9: 9-18.

Landa, Z., L. Osborne, F. Lopez and J. Eyal, 1994. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies Biological Control 4: 341-350.

Malais, M.H. and W.J. Ravensberg, 2003. Knowing and recognizing: the biology of glasshouse pests and their natural enemies Koppert B.V., the Netherlands.

McGregor, R.R., D.R. Gillespie, D.M.J. Quiring and M.R.J. Foisy, 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes Biological Control 16: 104-110.

McGregor, R.R., D.R. Gillespie, C.G. Park, D.M.J. Quiring and M.R.J. Foisy, 2000. Leaves or fruit? The potential of damage to tomato fruits by the omnivorous predator, *Dicyphus hesperus* Entomologia Experimentalis et Applicata 95: 325-328.

Meekes, E.T.M., J.J. Fransen and J.C. van Lenteren, 2002. Pathogenicity of *Aschersonia* spp. against whiteflies *Bemisia argentifolii* and *Trialeurodes vaporariorum* Journal of Invertebrate Pathology 81: 1-11.

Mesquita, A.L.M., L.A. Lacey, and F. Lecant, 1997. Individual and combined effects of the fungus, *Paecilomyces fumosoroseus* and parasitoid, *Aphelinus asychis* Walker (Hym., Aphelinidae) on confined populations of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hom.: Aphididae) under field conditions Journal of Applied Entomology 121: 151-163.

Nedstam, B. and M. Johansson-Kron. 1999. *Diglyphus isaea* (Walker) and *Macrolophus caliginosus* Wagner for biological control of *Liriomyza bryoniae* (Kaltenbach) on tomato. IOBC Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Brest, France, 25-29 May 1999, 22(1): 185-187.

Obrycki, J.J. and T.J. Kring, 1998. Predaceous coccinellidae in biological control Annual Review of Entomology 43: 295-321.

Onillon, J.C., 1990. The use of natural enemies for the biological control of whiteflies Whiteflies: their bionomics, pest status and management (ed. D. Gerling) Intercept, Andover, pg. 287-313.

Onions, A.H.S., 1979. CMI description of pathogenic fungi and bacteria: *Paecilomyces fumosoroseus*, No. 614. Issued by the Commonwealth Mycological Institute, The Cambrian News: Great Britain.

Osborne, L.S., G.K. Storey, C.W. McCoy, 1990. Potential for controlling the sweet potato whitefly, *Bemisia tabaci*, with the fungus, *Paecilomyces fumosoroseus* In: Proceedings and Abstracts Vth International Colloquium on Invertebrate Pathology and Microbial Control, Society of Invertebrate Pathology Adelaide, Australia, August 20-24, pg. 386-390.

Osborne, L.S. and Z. Landa, 1992. Biological control of whiteflies with entomopathogenic fungi Florida Entomologist 75(4): 456-471.

Pell, J.K., R. Pluke, S.J. Clark, M.G. Kenward, and P.G. Alderson, 1997. Interactions between two aphid natural enemies, the entomopathogenic fungus, *Erynia neoaphidis* Remaudiere and Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Journal of Invertebrate Pathology 69: 261-268.

Pell, J.K. and J.D. Vandenberg, 2002. Interactions among the aphid *Diuraphis noxia*, the entomopathogenic fungus *Paecilomyces fumosoroseus* and the coccinellid *Hippodamia convergens* Biocontrol Science and Technology 12: 217-224.

Polis, G.A., and R.D. Holt, 1992. Intra-guild predation: the dynamics of complex trophic interactions Trends in Ecology and Evolution 7: 151-154.

Poprawski, T.J., R.I. Carruthers, J.Speece III, D.C. Vadek, and L.E. Wendel, 1997. Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle Biological Control 10: 48-57.

Poprawski, T.J., J. C. Legaspi and P. Parker, 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Aleyrodidae) Biological Control 27(3): 785-795.

Poprawski, T.J., P.E. Parker, and J.H. Tsai, 1999. Laboratory and field evaluation of Hyphomycete insect pathogenic fungi for control of brown citrus aphid (Homoptera: Aphididae) Environmental Entomology 28: 315-321.

Poprawski, T.J., S.M. Greenberg, and M.A. Ciomperlik, 2000. Effects of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) Environmental Entomology 29: 1048-1053.

Puterka, G.J., R.A. Humber, and T.J. Poprawski, 1994. Virulence of fungal pathogens (imperfect fungi: Hyphomycetes) to pear psylla (Homoptera: Psyllidae) Environmental Entomology 23: 514-520.

Rosenheim, J.A., H.K. Kaya, L.E. Ehler, J.J. Marois and B.A. Jaffee, 1995. Intraguild predation among biological-control agents: theory and evidence Biological Control 5: 303-335.

Roy, H.E., J.K. Pell, S.J. Clark, and P.G. Alderson, 1998. Implications of predator foraging on aphid pathogen dynamics Journal of Invertebrate Pathology 71: 236-247.

Roy, H.E. and J.K. Pell, 2000. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control Biocontrol Science and Technology 10: 737-752.

Salamero, A., R. Gabarra and R. Albajes, 1987. Observations on the predatory and phytophagous habits of *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). SROP/WPRS Bulletin: Proceedings of the meeting, integrated control in glasshouses held in Budapest, Hungary 26-30 April 1987 10(2): 165-169.

Samish, M., G. Glindin, E. Alekseev, and I. Glazer, 2001. Pathogenicity of entomopathogenic fungi to different developmental stages of *Rhipicephalus sanguineus* (Acari: Ixodidae) Journal of Parasitology 87: 1355-1359.

Sampson C., and V.J. King, 1996. *Macrolophus caliginosus*, field establishment and pest control effect in protected tomatoes OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 143-146.

Sampson, C. and R.J. Jacobson, 1999. *Macrolophus caliginosus* Wagner (Heteroptera: Miridae): A predator causing damage to UK tomatoes IOBC Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Brest, France, 25-29 May 1999, 22(1): 213-216.

Sanchez, J.A., D.R. Gillespie and R.R. McGregor, 2003. The effects of mullein plants (*Verbascum thapsus*) on the population dynamics of *D. hesperus* (Heteroptera: Miridae) in tomato greenhouses Biological Control 28: 313-319.

Shah, P.A. and M.S. Goettel, 1999. Directory of microbial control products and services Microbial Control Division, Society of Invertebrate Pathology, 1-30

Sinia, A., B.D. Roitberg, R.R. McGregor and D.R. Gillespie, 2004. Prey feeding increases water stress in the omnivorous predator *Dicyphus hesperus* Entomologia Experimentalis et Applicata 110: 243-248.

Sterk, G., K. Bolckmans, M. Van de Veire, B. Sels, and W. Stepman, 1995a. Side-effects of the microbial insecticide PreFeRal (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97) on different species of beneficial arthropods Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 719-723.

Sterk, G., K. Bolckmans, R. De Jonghe, L. De Wael, and J. Vermeulen, 1995b. Side-effects of the microbial insecticide PreFeRal (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97) on *Bombus terrestris* (L.) Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 713-717.

Vandenberg, J.D., 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the Russian wheat aphid (Homoptera: Aphididae) Journal of Economic Entomology 89: 1418-1423.

Van de Veire, M., and D. Degheele, 1996. Toxicity of the fungal pathogen *Paecilomyces fumosoroseus* strain Apopka-97 to the greenhouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*, and the first results of a control experiment in glasshouse tomatoes OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 191-194.

VanLaerhoven, S.L., D.R. Gillespie and B.D. Roitberg, 2003. Diel activity pattern and predation rate of the generalist predator *Dicyphus hesperus* Entomologia Experimentalis et Applicata 107: 149-154.

van Lenteren, J.C., and J.P. Noldus, 1990. Whitefly-plant relationships: behavioral and ecological aspects Whiteflies: their bionomics, pest status and management (ed. D. Gerling) Intercept, Andover, 47-89.

van Lenteren, J.C., H.J.W. van Roermund and S. Sutterlin, 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum)* with the parasitoid *E. formosa*: How does it work? Biological Control 6: 1-10.

van Lenteren, J.C., Y.C. Drost, H.J.W. van Roermund and C.J.A.M. Posthuma-Doodeman, 1997. Aphelinid parasitoids as suitable biological control agents in greenhouses Journal of Applied Entomology 121: 473-485.

van Lenteren, J.C., 2000. A greenhouse without pesticides; fact or fantasy? Crop Protection 19: 375-384.

van Roermund, H.J.W. and J.C. van Lenteren, 1995a. Residence times of the greenhouse whitefly parasitoid *E. formosa* Gahan (Hym., Aphelinidae) on tomato leaflets Journal of Applied Entomology 119: 465-471.

van Roermund, H.J.W., and J.C. van Lenteren, 1995b. Foraging behaviour of the whitefly parasitoid *E. formosa* on tomato leaflets Entomologia Experimentalis et Applicata 76: 313-324.

Vidal, C., L.A. Lacey and J. Fargues, 1997. Pathogenicity of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay method Journal of Economic Entomology 90(3): 765-772.

Vidal, C., L.S. Osborne, L.A Lacey, and J. Fargues, 1998. Effect of host plant on the potential of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for controlling the silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae) in greenhouses Biological Control 12: 191-199.

Wardlow, L.R., F.A.B. Ludlam and P. Hammon, 1975. A comparison of the effectiveness of insecticides against glasshouse whitefly (*Trialeurodes vaporariorum*) Annals of Applied Biology 81: 433-435.

Wraight, S.P., R.I. Carruthers, C.A. Bradley, S.T. Jaronski, L.A. Lacey, P. Wood, and S. Galaini-Wraight, 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii* Journal of Invertebrate Pathology 71: 217-226.

Wraight, S.P., R.I. Carruthers, S.T. Jaronski, C.A. Bradley, C.J. Garza, and S. Galaini-Wraight, 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii* Biological Control 17: 203-217.

Zchori-Fein, E., R.T. Roush and J.P. Sanderson, 1994. Potential for integration of biological and chemical control of greenhouse whitefly (Homoptera: Aleyrodidae) using *E. formosa* (Hymenoptera: Aphelinidae) and abamectin Biological Control 23(5): 1277-1282.

CHAPTER 2 PATHOGENICITY OF PFR-97™ AGAINST *TRIALEURODES VAPORARIORUM*

2.1 Abstract

The pathogenicity of the biopesticide, PFR-97™ against *Trialeurodes vaporariorum* was investigated under laboratory conditions. The active ingredient in PFR-97™ is the entomopathogenic fungus *Paecilomyces fumosoroseus* Apopka-97. The basic measure of pathogenicity was the estimated concentration and time required to kill 50% and 90% of *T. vaporariorum* nymphs ($LC_{50, 90}$ and $LT_{50, 90}$ respectively). Bioassays were conducted on tobacco leaf discs containing fourth-instar nymphs of *T. vaporariorum* and placed into Petri dishes half-filled with 2.6% water agar. PFR-97™ was sprayed on nymphs using an artist's airbrush (Aztek, Model 4702). Five spray concentrations were used, ranging from 1×10^5 to 1.5×10^7 blastospores/ml. Immediately after spraying, the dishes were incubated at 23°C and 90-100% relative humidity for 24 hours, followed by 27°C and 77% relative humidity for nine days. Nymphs were monitored daily for mortality. PFR-97™ resulted in an estimated LC_{50} of 1.33×10^5 blastospores/ml and a LC_{90} of 4.5×10^6 blastospores/ml which equated to an actual blastospore deposition of 6.84 blastospores/mm^2 and 224.38 blastospores/mm^2, respectively. Lethal time estimates were negatively correlated with the concentration of the PFR-97™ spray application. Increasing the concentration from 3×10^6 to 9×10^6 blastospores/ml, reduced the LT_{90} by one day (9 – 8.1 days), however, increasing the spray concentration from 9×10^6 to 1.5×10^7 blastospores/ml did not further reduce the

estimated LT_{90}. Lethal time estimates were highly variable when spray applications were made at concentrations of 1 x 10^5 and 1 x 10^6 blastospores/ml and LT_{90}s ranged from 2-30 weeks and 1-3 weeks, respectively. The pathogenicity of PFR-97™ against *T. vaporariorum* nymphs was similar to previous studies that investigated the pathogenicity of unformulated suspensions of *P. fumosoroseus* Apopka-97 against nymphs of whitefly species.

2.2 Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is an important pest that causes damage to greenhouse vegetable crops (Malais and Ravensberg, 2003). Crop damage results from phloem feeding and honeydew secretion. Phloem feeding by large populations of *T. vaporariorum* retards plant vigor. The production of copious amounts of honeydew on leaves and fruits stimulates the growth of sooty mould fungi thereby reducing the photosynthetic capacity of the leaves and reduces fruit quality (Osborne and Landa, 1992; Fransen and van Lenteren, 1993; Poprawski *et al*, 1998). Excellent biological control of *T. vaporariorum* populations has been achieved with the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (van Lenteren *et al.*, 1996; van Lenteren *et al.*, 1997). Because *E. formosa* is a specialist of whiteflies and its use in biological control programs is not always successful (van Roermund and van Lenteren, 1995a; van Roermund and Lenteren, 1995b; Kassis and Michelakis, 1993), the efficacy of alternative generalist biological control agents has been researched.

In 1986, a strain of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Deuteromycotina: Hyphomycetes) was isolated from a

mealybug, *Phenococcus solani* (Hemiptera: Pseudococcidae) on a velvet plant in a conservatory in Apopka, Florida (Copping, 2001). This strain, Apopka-97, was discovered to be highly virulent towards *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and other greenhouse pests (Osborne *et al.*, 1990). It was then licensed to Certis USA, LLC (formerly Thermo Trilogy Corporation) and developed into a biopesticide that was registered in the United States of America and in Europe under the tradenames, PFR-97™ and PreFeRal™, respectively (Faria and Wraight, 2001). PFR-97™ has a wide host range; its target pests include whitefly species, aphids, thrips and spider mites (Shah and Goettel, 1999). Much of the efficacy data on PFR-97™ and PreFeRal™ are in the domain of the manufacturing companies. Bolckmans *et al.* (1995) reported a 77% reduction in *T. vaporariorum* populations on glasshouse tomato plants after 9 weeks with two PreFeRal™ applications. Van de Veire and Degheele (1996) reported a 79% reduction of *T. vaporariorum* populations on glasshouse tomato plants after 4 weeks with two PFR-97™ applications. The quicker efficacy achieved by Van de Veire and Degheele (1996) may be due to the use of a surfactant (Citowett), a different formulation (PFR-97™ versus PreFeRal™), environmental conditions after spray applications or the initial density of *T. vaporariorum* populations.

The pathogenicity of unformulated *P. fumosoroseus* Apopka-97 against whitefly species has been studied extensively (Osborne *et al.*, 1990; Landa *et al.*, 1994; Vidal *et al.*, 1997; Wraight *et al.*, 1998). However, the dose-time-mortality response of *T. vaporariorum* to PFR-97™ has not been investigated. As the performance of unformulated products tends to differ from that of formulated products, due to the addition of materials such as oils, humectants, UV-protectants, and nutrients in the

formulation process (Faria and Wraight, 2001), the purpose of this laboratory assay was to determine the pathogenicity of PFR-97™ against fourth-instar nymphs of *T. vaporariorum* on tobacco leaf discs. The basic measure of pathogenicity was the estimated concentration and time required to kill 50% and 90% of *T. vaporariorum* nymphs with PFR-97™ ($LC_{50, 90}$ and $LT_{50, 90}$ respectively). Data from this study can be used to test the effects of PFR-97™ on non-target organisms wherein fourth-instar nymphs can be used as a reference.

2.3 Materials and Methods

2.3.1 Insect Cultures and Host Plants

Excised tobacco leaves, *Nicotiana tabacum* L. (Solanaceae), containing nymphs of *T. vaporariorum* were obtained from Applied Bionomics, Victoria, British Columbia and were used to produce a whitefly colony on potted tobacco plants. This colony was maintained in an environment-controlled rearing room at a constant temperature of 26°C and a 24 hour light photoperiod.

Tobacco plants were grown under greenhouse conditions at the Pacific Agri-Food Research Center (Agassiz, British Columbia). When the plants were 25-30 cm tall, groups of four plants were placed separately into a rearing cage (90 x 60 x 60 cm). Adults of *T. vaporariorum* were introduced into this cage and females were allowed to oviposit for 48 hours. The plants were routinely shaken to disturb the adults in order to obtain a homogenous *T. vaporariorum* population on the underside of the leaves. Adults were then removed, and the plants with eggs were kept in a greenhouse at 24 ± 1.1 °C and 52 ± 3 % relative humidity until the nymphs reached the fourth-instar stage (16-18 days).

2.3.2 Fungal Preparations

The commercial formulation, PFR-97™ 20% WDG, was manufactured by and obtained from Certis USA, Columbia MD. PFR-97™ is sold as desiccated blastospores of the fungus *Paecilomyces fumosoroseus* Apopka Strain 97 (ATCC 20874), on water dispersible granules containing 1 x 10^9 colony forming units per gram (CFU/g). This product was stored in a sealed container at approximately 4°C.

A blastospore stock suspension was prepared by suspending PFR-97™ granules into a phosphate buffer (pH = 7.15) amended with Tween 80 (0.01%). The blastospore stock suspension was further diluted with phosphate buffer to obtain a range of application dosages. Spray applications were made using an artist's airbrush (Aztek, Model 4702) equipped with a siphon-fed fine-spray mist nozzle (0.3 mm orifice aperture). The average droplet size delivered by the airbrush was determined by spraying krome-cote paper in a similar fashion to the test insects. The diameter of forty randomly chosen droplets per card, for 5 cards, was measured and used to calculate the area of the droplets (mm^2).

Blastospore quantification and viability of the spray applications were determined using the guidelines outlined by Goettel and Inglis (1997). Blastospores were quantified using a standard (improved Neubauer) hemacytometer at 400x magnification. A 0.01 ml aliquot of the suspension was loaded into six hemacytometer chambers and the number of blastospores per five cells for each chamber was counted. The estimation of blastospores per ml was then obtained by multiplying the average blastospores/cell by the volume conversion factor. Viability of the blastospores was determined by plating a 0.1 ml aliquot of the stock suspension onto three potato dextrose agar (PDA) plates amended

with 0.005% benomyl (Goettel and Inglis, 1997). The inoculated plates were then incubated at 25°C for 24 hours in the dark. After the incubation period, three microscopic fields (400 x magnification) were scanned for each plate. A total of 500 spores were examined and scored as either viable or non-viable. A blastospore was deemed viable if a germ tube was present.

Blastospore deposition was determined for each spray application from two cylinders of 2.6% water agar (10 mm diameter) placed inside the spray arena. Immediately after spraying, the agar cylinders were collected and stored in sealed Petri dishes at 4°C until they were processed 4 hours later. The number of blastospores in three microscope fields (400 x magnification) per agar cylinder was averaged, adjusted for viability, and expressed as viable blastospores per square millimeter.

2.3.3 Bioassay Protocol

The activity of PFR-97™ against nymphs of *T. vaporariorum* was assessed using five application dosages plus the control (Table 2.7-1). Prior to the spray applications, the leaves of the tobacco plants containing *T. vaporariorum* were cut using a 10 mm diameter cork borer to produce leaf discs. Each leaf disc contained approximately 15 nymphs on the abaxial surface. In order to minimize contamination by saprophytic organisms, the leaf discs were disinfected by exposing the adaxial surfaces to 0.2% sodium hypochlorite for 1 minute, and then rinsed with sterile water for 3 minutes. The discs were then placed into sterile Petri dishes (60 mm diameter) half filled with 2.6% water agar. Spray applications were made to *T. vaporariorum* leaf discs as 0.60 ml aliquots of blastospore suspension using the artist's airbrush at a pressure of 20 psi, and at a fixed height of 30 cm. Leaf discs that served as the carrier control group were sprayed

with phosphate buffer. Each dose application was replicated 8 times and the entire bioassay was repeated once on a different day for a total of 16 replicates per dose application.

After spray applications, each Petri dish containing a *T. vaporariorum* leaf disc was sealed with a strip of Parafilm® and maintained for 24 hours under saturated humidity conditions at 23°C. Saturated conditions were used to increase the rate of fungal infection so that the blastospores had sufficient time to penetrate the nymphs before the nymphs emerged as adults and the leaf discs desiccated. Afterwards, the Petri dish lids were replaced with ventilated lids having a 17 mm diameter hole covered with a fine-screened mesh. The Petri dishes were randomly placed into a growth chamber and further incubated at 27.02 ± 0.06 °C and a photoperiod of 16:8 (L:D) hours. Because the growth chamber did not regulate relative humidity, the Petri dishes were placed in sealed tubs that contained a saturated salt solution (360 g of NaCl/ 1 L water), that maintained a relative humidity of 76.90 ± 0.20 % inside the tubs (Richardson and Malthus, 1955).

2.3.4 Sampling Protocol

Nymphs of *T. vaporariorum* were monitored daily for 10 days under a compound microscope for signs of mortality. Nymph mortality in the carrier control group was evidenced by concaving of the integument which quickly led to body desiccation. Infection and death of nymphs by *P. fumosoroseus* was diagnosed by the presence of orange discoloration, which was typically displayed near the death of nymphs and was indicative of extensive fungal colonization of the haemolymph (Wraight *et al.*, 1998). Not all infected nymphs showed the visible discoloration; however, in such cases, death was evidenced by the same characteristics displayed in the carrier control group.

Infection of nymphs in the treated group was verified by surface sterilizing the nymphs for two minutes in 0.2% sodium hypochlorite, rinsing twice in sterile water, and plating on 2% water agar at 25°C and 100% relative humidity to encourage sporulation of *P. fumosoroseus*. Sporulating cadavers (overt mycosis) were used to make slide cultures, following the guidelines outlined by Goettel and Inglis (1997), to verify that the spores were indeed produced by the hyphomycete, *Paecilomyces fumosoroseus*. For each dose application, the percent mortality was calculated and then corrected according to the Abbott formula using mortality of the nymphs in the carrier control group as the correcting factor.

2.3.5 Statistical Analysis

Each *T. vaporariorum* leaf disc, which served as a replicate, contained 15 *T. vaporariorum* nymphs in order to minimize application time of the fungus, reduce pathogenicity variation among nymphs caused by amount of honeydew and wax secretion, and minimize mortality of nymphs due to handling. Replications corresponded to a continuous response (percent mortality) instead of a binomial response. Consequently, a probit analysis could not be used to determine the lethal time and lethal dose estimates since it is limited to quantal data. Therefore, the lethal dose estimates were calculated using a non-linear regression and the lethal time estimates were calculated using mortality versus time linear regressions.

To determine the lethal dose estimates, blastospore deposition (viable blastospores/mm^2) of the spray applications was used as the independent variable and the total nymph mortality was the independent variable in a non-linear regression model. For the lethal time estimates, the natural logarithm of time (hours) was used as the

independent variable and the cumulative nymph mortality data were entered as the independent variable in a linear regression model for each dose. Cumulative nymph mortality data were constrained by excluding mortality observed in the first 48 hours from the analysis because death by *P. fumosoroseus* infection was not possible within that time frame. Data were pooled across trials when the trial slopes were not significantly different as determined by *t*-tests ($P < 0.05$). All statistical analyses were performed using the JMP 5: Statistical Discovery software (SAS Institute, 2002) except the lethal dose estimates which were determined using Systat version 10.2 (Statistical Software Inc.).

2.4 Results

The actual percent mortality of nymphs in the carrier control group was 6.27 ± 1.74 % (mean ● SEM) after 10 days (including mortality in the first 48 hours). The viability of *P. fumosoroseus* blastospores was 73.27 ● 1.9 %. Mean (± SEM) droplet size delivered by the airbrush (spray applicator) was 4.04 ● 0.412 mm^2, which is likely an overestimation due to coalescing of the droplets. Slide cultures confirmed that sporulating cadavers were infected with the hyphomycete, *P. fumosoroseus*. The phialids were swollen at the base and tapered at the top; conidiophores were usually branched bearing three phialids with the conidia cylindrical in shape, and borne in chains (Onions, 1979). The total Abbott-corrected nymph mortality was not different from the observed nymph mortality caused by mycosis ($t_{96} = 0.80$; $P = 0.4257$; Mean difference ● SEM = 0.012 ± 0.015).

There were no blocking or trial effects on total nymph mortality (Blocking: 1-ANOVA: $F_{3, 39} = 0.435$; $P = 0.63$; *t*-test: $t_{92} = 0.41$; $P = 0.6802$). Spray applications of

PFR-97™ were highly pathogenic towards fourth-instar nymphs of *T. vaporariorum*; even at the lowest concentration (1 x 10^5 blastospores/ml) nymph mortality was 48%, 10 days after application (Table 2.7-1). Nymphal mortality increased with increasing concentration of the PFR-97™ spray applications (Table 2.7-1). Using the non-linear regression model (illustrated in Figure 2.6-1), the estimated LC_{50} of PFR-97™ on fourth-instar nymphs of *T. vaporariorum* was 1.33 x 10^5 blastospores/ml which corresponded to an actual blastospore deposition of 6.84 blastospores/mm^2 (95% CI: 5.76 – 8.39 blastospores/mm^2). The estimated LC_{90} was 4.5 x 10^6 blastospores/ml which corresponded to a blastospore deposition of 224.38 blastospores/mm^2 (95% CI: 196.77 – 262.83 blastospores/mm^2).

Generally, the stages of infection by *P. fumosoroseus* blastospores on nymphs of *T. vaporariorum* followed the same trend. Regardless of spray concentration, fungus-infected nymphs were not observed during the first 2 days after spray application. Infection first became apparent as an orange discoloration or by the presence of mycelium on the integument of the nymph. Soon after, the integument of the nymph became concave. The body then either desiccated under low relative humidity or the fungus sporulated on the cadaver when exposed to a saturated atmosphere.

Determining the lethal time estimates for the two lowest spray concentrations was difficult since there was an effect of trial date on cumulative mortality for both concentrations (1 x 10^5 blastospores/ml: $t_{160} = 6.67$; $P < 0.0001$; 1 x 10^6 blastospores/ml: $t_{160} = 6.21$; $P < 0.0001$). Nymph mortality did increase over time as indicated by the positive slopes; however, trial 2 reported a consistently lower nymph mortality compared to trial 1 (Figure 2.6-2). Spray applications made at concentrations of 1 x 10^5 and 1 x 10^6

blastospores/ml reported lethal time estimates that were beyond the number of days that the nymphs were monitored, as well as being past the maturation period of the fourth-instar nymphs. Fourth-instar nymphs will emerge as adults within 10 days and at these concentrations, the vast majority of the nymphs would have emerged, thereby escaping fungal infection and resultant death. An exception is the estimated LT_{50} of nymphs at the spray concentration of 1 x 10^6 blastospores/ml; 50% of the nymphs died within 8 days (Table 2.7-2).

There was a positive correlation between nymph mortality and time as indicated by the steep positive slopes for the 3 x 10^6, 9 x 10^6, and 1 x 10^7 blastospores/ml spray concentrations (Figure 2.6-3). Increasing the concentration of the spray application from 3 x 10^6 to 9 x 10^6 blastospores/ml, reduced both the LT_{50} and LT_{90} by one day (5.1 – 4.1 days and 9 – 8.1 days, respectively); however, increasing the spray concentration from 9 x 10^6 to 1.5 x 10^7 blastospores/ml did not further reduce either of the estimates (Table 2.7-2).

2.5 Discussion

The commercial formulation of the fungal strain, *P. fumosoroseus* Apopka-97 (PFR-97™) was highly pathogenic towards fourth-instar nymphs of *T. vaporariorum* in the laboratory. PFR-97™ resulted in an estimated LC_{50} of 1.33 x 10^5 blastospores/ml and a LC_{90} of 4.5 x 10^6 blastospores/ml which equated to an actual blastospore deposition of 6.84 blastospores/mm^2 and 224.38 blastospores/mm^2, respectively. Lethal time estimates were negatively correlated with the concentration of the PFR-97™ application. Increasing the concentration from 3 x 10^6 to 9 x 10^6 blastospores/ml, reduced the LT_{90} by

one day (9 – 8.1 days); however, increasing the spray concentration from 9 x 10^6 to 1.5 x 10^7 blastospores/ml did not further reduce the estimated LT_{90}.

My results are similar to previous laboratory dose-mortality bioassays that investigated the pathogenicity of unformulated suspensions of *P. fumosoroseus* Apopka-97. Vidal *et al.* (1997) reported a LC_{50} of 6.2 conidia/mm^2 on second-instar nymphs of *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) on ornamental sweetpotato leaf discs. Wraight *et al.* (1998) reported a considerably higher LC_{50} of approximately 150 conidia/mm^2 on third-instar nymphs of *B. argentifolii* on excised hibiscus leaves. Differences in pathogenicity may be due to instar age at time of spray application, environmental conditions after spray application, leaf quality, and host plant species. Within 14 days after treatment, 77% of early second-instar nymphs of *B. argentifolii* on rooted cabbage leaves died when they were exposed to 38 blastospores/mm^2 (Lacey *et al.*, 1999).

Landa *et al.* (1994) developed a novel bioassay method to test the pathogenicity of entomopathogenic fungi on whitefly species in which whitefly nymphs were removed from the leaf surface and placed in the middle of a conidial drop on a sterile microscope slide. Nymphs were then assessed daily using a rating system based on the degree of fungal development. Fourth-instar nymphs of *T. vaporariorum* placed in conidial drops at a concentration of 1.0 x 10^7 conidia/ml showed signs of fungal sporulation within 6 days (Landa *et al.*, 1994). Using a similar methodology, with the exception that the slides containing the nymphs were dipped into the suspension, a concentration of 1.0 x 10^6 conidia/ml caused 90% mortality of fourth-instar nymphs of *B. tabaci* within 72 hours (Osborne *et al.*, 1990). This bioassay method has important limitations as pointed

out by Vidal *et al.* (1997); the effect of host plant and the influence of nutrients cannot be considered. The stress associated with handling and relocation most likely affects the immunity of nymphs. As well, it can only be used on the non-feeding stage of whiteflies (fourth instar nymphs).

Caution must be exercised when making inferences from laboratory results to greenhouse situations as they can be poor predictors of what truly occurs in "nature" (Hajek *et al.*, 1996; Goettel *et al.*, 2001; Inglis *et al.*, 2001). Laboratory bioassays tend to provide environmental conditions that favor fungal infection of the host (moderate temperatures and high, stable relative humidities) but rarely are these conditions met in the greenhouse. Although laboratory assays are important for screening fungal species and isolates, these assays do not incorporate all the aspects of greenhouse conditions that can affect the degree of pathogenicity of entomopathogenic fungi.

The commercial formulation of *P. fumosoroseus* Apopka-97 is highly pathogenic towards *T. vaporariorum* nymphs in greenhouse situations. An application of PreFeRal™ at a concentration of 2×10^6 colony forming units/ml reduced *T. vaporariorum* densities on tomato plants by 38% in 3 weeks, whereas by 9 weeks, the second application reduced densities by 77% (Bolckmans *et al.*, 1995). Van de Veire and Degheele (1996) reported a 63% reduction of *T. vaporariorum* densities three weeks after the first PFR-97™ application at a rate of 1 gram product/liter water, and by four weeks, a second application reduced densities by 79%. The higher *T. vaporariorum* reductions reported by Van de Veire and Degheele (1996) may be due to the use of a surfactant (Citowett), commercial formulation, concentration of the spray applications,

environmental conditions after applications and the initial density of *T. vaporariorum* populations.

This study provides a time-dose-mortality response of fourth-instar nymphs of *T. vaporariorum* exposed to spray applications of PFR-97™. The pathogenicity of PFR-97™ was similar to previous studies investigating the pathogenicity of unformulated suspensions of *P. fumosoroseus* Apopka-97. Data from this study can be used to test the effects of PFR-97™ on non-target organisms using fourth-instar nymphs as a reference.

2.6 Figures

Figure 2.6-1: The non-linear regression of blastospore deposition of PFR-97™ spray applications (viable blastospores/mm^2) by the Abbott-corrected nymph mortality of fourth-instar nymphs of *T. vaporariorum*. The fitted slope and intercept values were used to predict the lethal time and lethal dose estimates of *T. vaporariorum* nymphs exposed to PFR-97™ spray applications. Control mortality never exceeded 10%.

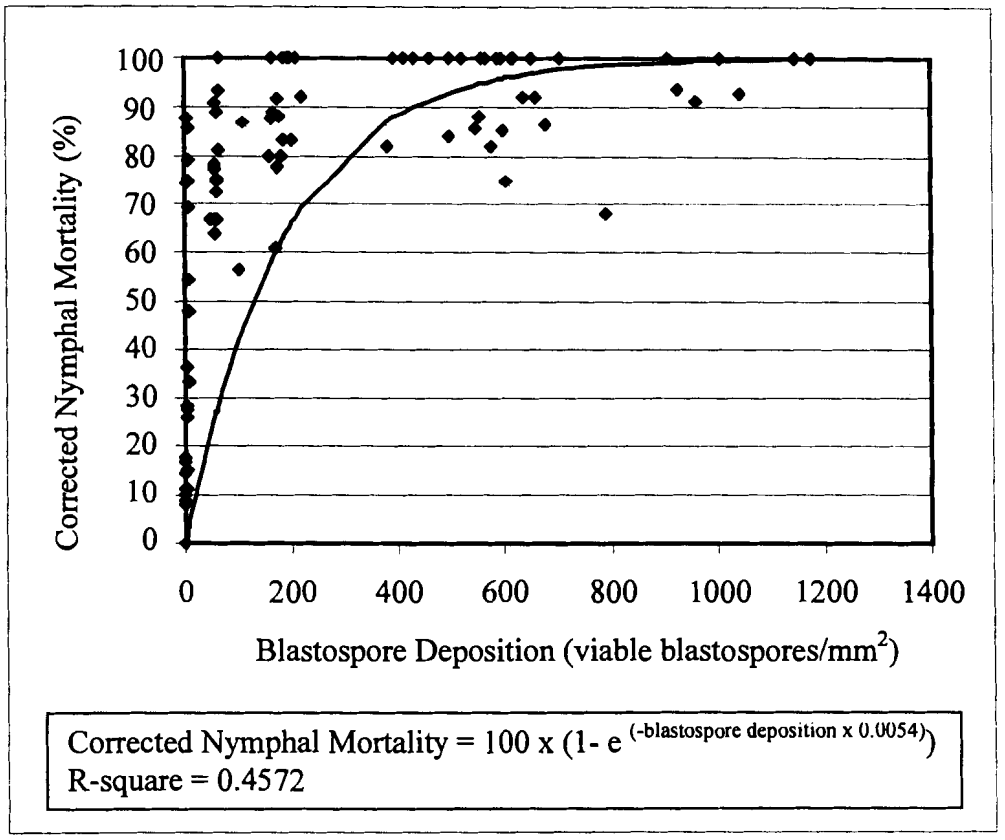
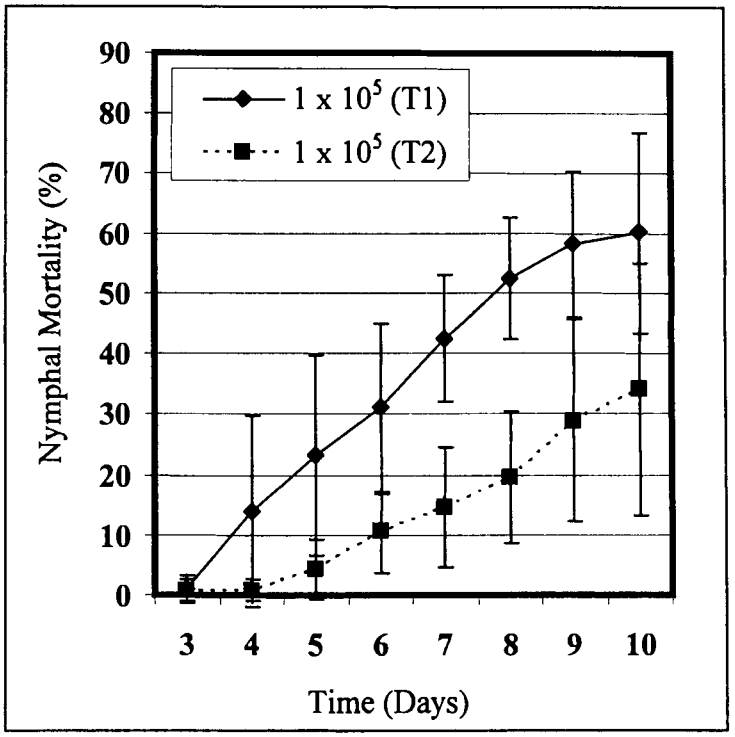

Corrected Nymphal Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
1200
1400
Blastospore Deposition (viable blastospores/mm2)
Corrected Nymphal Mortality = 100 x (1- e (-blastospore deposition x 0.0054))
R-square = 0.4572

Figure 2.6-2: Cumulative Abbott-corrected mortality (mean ± 95% CI) of fourth-instar nymphs of *Trialeurodes vaporariorum* exposed to a single application of PFR-97™ blastospore suspension at the noted concentration (blastospores/ml). The dotted lines represent trial 1 data (T1) and solid lines represent trial 2 data (T2). Mortality occurring in the first 48 hours was excluded from the analysis. Control mortality never exceeded 10%.


90
80
70
60
50
40
30
20
10
0
Nymphal Mortality (%)
1 x 10^5 (T1)
1 x 10^5 (T2)
3
4
5
6
7
8
9
10
Time (Days)

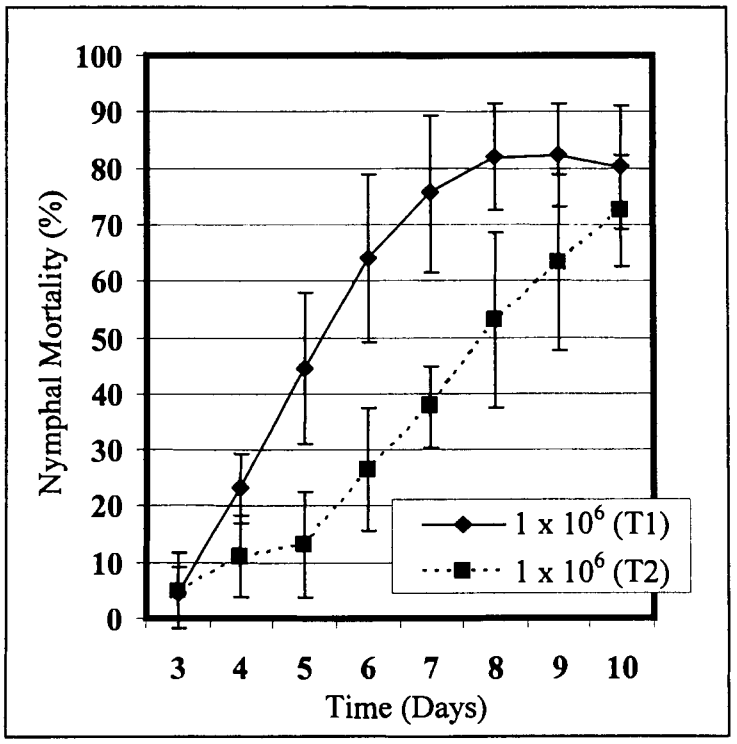
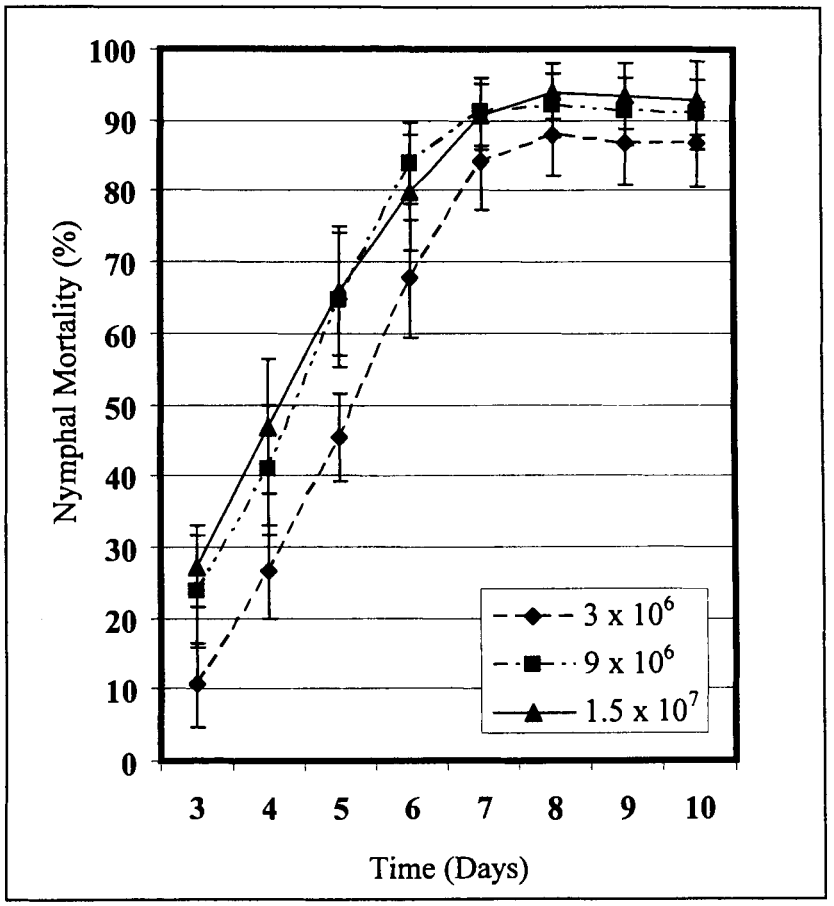

100
90
80
70
60
50
40
30
20
10
0
Nymphal Mortality (%)
1 x 10^6 (T1)
1 x 10^6 (T2)
3
4
5
6
7
8
9
10
Time (Days)

Figure 2.6-3: Cumulative Abbott-corrected mortality (mean ± 95% CI) of fourth-instar nymphs of *Trialeurodes vaporariorum* exposed to a single spray application of PFR-97™ blastospore suspension at different spray concentrations (blastospores/ml). Mortality occurring in the first 48 hours was excluded from the analysis.


100
90
80
70
60
50
40
30
20
10
0
Nymphal Mortality (%)
3
4
5
6
7
8
9
10
Time (Days)
3 x 10^6
9 x 10^6
1.5 x 10^7

2.7 Tables

Table 2.7-1: Mean (± SEM) mortality of fourth-instar nymphs of *T. vaporariorum* on tobacco leaf discs 10 days after being exposed to different concentrations of PFR-97™ spray applications. Actual spore deposition in the spray arena was the mean (± SEM) number of viable blastospores per mm^2. There were 16 replicates per concentration with15 nymphs per replicate. The entire bioassay was repeated once.

Blastospore concentration (spores/ml)	Blastospore deposition (viable blastospores/mm^2)	Abbott-correct nymphal mortality (%)
0 (carrier control)	0	6.27 ± 1.7
1×10^5	4.84 ± 0.190	47.6 ± 6.9
1×10^6	54.6 ± 2.83	77.5 ± 3.1
3×10^6	155 ± 2.32	88.4 ± 2.7
9×10^6	430 ± 11.3	92.7 ± 2.3
1.5×10^7	667 ± 18.7	94.7 ± 2.1

Table 2.7-2: Estimated mean LT_{50} and LT_{90} of fourth-instar nymphs of *Trialeurodes vaporariorum* exposed to a single application of PFR-97™ at the various blastospore concentrations. The associated 95% confidence intervals for each estimate are recorded below the mean LT_{50} and LT_{90}. Data could not be pooled between trials for the first 2 concentrations but were pooled for the last 3 concentrations.

Blastospore Concentration (spores/ml)	Estimated LT_{50}, 95% CI (days)		Estimated LT_{90}, 95%CI (days)	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
1×10^5	**8.1** 7.6 – 8.8	**>10**	**> 10**	
1×10^6	**5.2** 5.0 – 5.5	**7.6** 7.2 – 8.2	**9.2** 8.7 – 9.8	**> 10**
3×10^6	**5.1** 4.9 – 5.3		**9.0** 8.6 – 9.4	
9×10^6	**4.1** 3.9 – 4.4		**8.1** 7.7 – 8.5	
1.5×10^7	**4.0** 3.8 – 4.3		**8.0** 7.6 – 8.4	

2.8 References

Bolckmans, K., G. Sterk, J. Eyal, B. Sels, and W. Stepman, 1995. PreFeRal, (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 707-711.

Copping, L.G. 2001. The BioPesticide Manual. British Crop Protection Council.

Faria, M. and S.P. Wraight, 2001. Biological control of *Bemisia tabaci* with fungi Crop Protection 20: 767-778.

Fransen, J.J. and J.C. van Lenteren, 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 69: 239-249.

Goettel, M.S., and G.D. Inglis, 1997. Fungi: Hyphomycetes. Chapter 5 of Manual of techniques in insect pathology (ed L.A. Lacey) Academic Press, San Diego California, pg. 213-249.

Goettel, M.S., A.E. Hajek, J.P. Siegel and H.C. Evans, 2001. Safety of fungal biocontrol agents. Chapter 13 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 347-375.

Hajek, A.E., L. Butler, S.R.A. Walsh, J.C. Silver, F.P. Hain, F.L. Hastings, T.M. Odell, and D.R. Smitley, 1996. Host range of the gypsy moth (Lepidoptera: Lymantridae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthales) in the field versus the laboratory Environmental Entomology 25: 709-721.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser, 2001. Use of hyphomycetous fungi for managing insect pests. Chapter 3 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 23-69.

Kassis G. and S. Michelakis, 1993. The effectiveness of biological control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom., Aleyrodidae) by *E. formosa* Gahan (Hym., Aphelinidae) Journal of Applied Entomology 116: 298-302.

Lacey, L.A., A.A. Kirk, L. Millar, G. Mercadier and C. Vidal, 1999. Ovicidal and larvicidal activity of conidia and blastospores of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects Biocontrol Science and Technology 9: 9-18.

Landa, Z., L. Osborne, F. Lopez and J. Eyal, 1994. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies Biological Control 4: 341-350.

Malais, M.H. and W.J. Ravensberg, 2003. Knowing and recognizing: the biology of glasshouse pests and their natural enemies Koppert B.V., the Netherlands.

Onions, A.H.S. 1979. CMI description of pathogenic fungi and bacteria: *Paecilomyces fumosoroseus*, No. 614. Issued by the Commonwealth Mycological Institute, The Cambrian News: Great Britain.

Osborne, L.S., G.K. Storey, and C.W. McCoy, 1990. Potential for controlling the sweet potato whitefly, *Bemisia tabaci*, with the fungus, *Paecilomyces fumosoroseus*. Proceedings and abstracts fifth international colloquium on invertebrate pathology and microbial control Society of Invertebrate Pathology, Adelaide, Australia, August 20-24, 386-390.

Osborne, L.S. and Z. Landa, 1992. Biological control of whiteflies with entomopathogenic fungi Florida Entomologist 75(4): 456-471.

Poprawski, T.J., J. C. Legaspi and P. Parker, 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Aleyrodidae) Biological Control 27(3): 785-795.

Richardson, G.M., and R.S. Malthus, 1955. Salts for static control of humidity at relatively low levels Journal of Applied Chemistry 5: 557-567.

SAS Institute Inc., 2002. Version JMP 5.0. Cary, NC. USA.

Shah, P.A. and M.S. Goettel, 1999. Directory of microbial control products and services. Microbial Control Division, Society for Invertebrate Pathology, 1-30.

Van de Veire, M., and D. Degheele, 1996. Toxicity of the fungal pathogen *Paecilomyces fumosoroseus* strain Apopka 97 to the greenhouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*, and the first results of a control experiment in glasshouse tomatoes OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 191-194.

van Lenteren, J.C., H.J.W. van Roermund and S. Sutterlin, 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum)* with the parasitoid *E. formosa*: How does it work? Biological Control 6: 1-10

van Lenteren, J.C., Y.C. Drost, H.J.W. van Roermund and C.J.A.M. Posthuma-Doodeman, 1997. Aphelinid parasitoids as suitable biological control agents in greenhouses Journal of Applied Entomology 121: 473-485.

van Roermund, H.J.W., and J.C. van Lenteren, 1995a. Residence times of the greenhouse whitefly parasitoid *E. formosa* Gahan (Hym., Aphelinidae) on tomato leaflets Journal of Applied Entomology 119: 465-471.

van Roermund, H.J.W., and J.C. van Lenteren, 1995b. Foraging behaviour of the whitefly parasitoid *E. formosa* on tomato leaflets Entomologia Experimentalis et Applicata 76: 313-324.

Vidal, C., L.A. Lacey and J. Fargues, 1997. Pathogenicity of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay method Journal of Economic Entomology 90(3): 765-772.

Wraight, S.P., R.I. Carruthers, C.A. Bradley, S.T. Jaronski, L.A. Lacey, P. Wood, and S. Galaini-Wraight, 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii* Journal of Invertebrate Pathology 71: 217-226.

CHAPTER 3 INFLUENCE OF PFR-97™ ON *DICYPHUS HESPERUS*

3.1 Abstract

The effect of *Paecilomyces fumosoroseus* Apopka-97 (Wize) Brown and Smith (PFR-97™) on the mortality and predation rate of *Dicyphus hesperus* Knight was investigated in the laboratory. Prey selection by *D. hesperus* females was tested by offering females one of the following *T. vaporariorum* feeding choices for a 24 hour period: (1) 2 healthy nymphs; (2) a healthy and a treated nymph; and (3) 2 treated nymphs. Fourth-instar nymphs of *T. vaporariorum* were offered to *D. hesperus* females either three or five days following spray application, in order to test different stages of prey infection. Adult *D. hesperus* females were not infected with *P. fumosoroseus* when PFR-97™ was applied directly to the ventral thorax at a concentration of 1.20×10^7 viable blastospores/ml. Moderate mortality (28%) of adult *D. hesperus* females occurred when females were indirectly exposed to *P. fumosoroseus* by being confined with leaf discs that had been sprayed with PFR-97™ at a concentration of 1.26×10^7 viable blastospores/ml. Predation rate of *D. hesperus* females was unaffected by the one-time direct exposure to *P. fumosoroseus.* Females that received a one-time indirect exposure and survived treatment, however, consumed prey at a lower rate (38%) than the control group during a 6-day evaluation period. Nymphs of *T. vaporariorum* sprayed with PFR-97™ at a concentration of 5.87×10^6 viable blastospores/ml, three days before being offered to *D. hesperus* females were accepted as prey and consumed readily by *D.*

hesperus. However, *D. hesperus* females only rarely consumed treated *T. vaporariorum* nymphs that had been sprayed 5 days earlier. When offered the choice between a healthy nymph and a treated nymph (sprayed with PFR-97™ 5 days earlier), *D. hesperus* consumed the healthy nymph 75% of the time. Complete consumption of a treated nymph by *D. hesperus* was not observed. The time to reject a healthy nymph and a treated nymph was not significantly different; rejection time was approximately 7 seconds. The implications of these results for the use of *D. hesperus* and PFR-97™ in biological control programs are discussed.

3.2 Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is an important pest that causes damage to greenhouse vegetable crops (Malais and Ravensberg, 2003). Crop damage results from phloem feeding and honeydew secretion. Phloem feeding by large populations of *T. vaporariorum* retards plant vigor. The production of copious amounts of honeydew on leaves and fruits stimulates the growth of sooty mould fungi thereby reducing the photosynthetic capacity of the leaves and reducing fruit quality (Osborne and Landa, 1992; Fransen and van Lenteren, 1993; Poprawski *et al*, 1998). Excellent biological control of *T. vaporariorum* populations has been achieved with the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aleyrodidae) (van Lenteren *et al.*, 1996; van Lenteren *et al.*, 1997). Because *E. formosa* is a specialist of whiteflies and its use in biological control programs is not always successful (van Roermund and van Lenteren, 1995a; van Roermund and van Lenteren, 1995b; Kassis and Michelakis, 1993), the efficacy of alternative or complementary generalist biological control agents has been explored.

Both the entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97 (Wize) Brown and Smith (Deuteromycotina: Hyphomycetes) and the generalist predator, *Dicyphus hesperus* Knight (Hemiptera: Miridae) is currently being investigated as an alternative to *E. formosa* (Bolckmans *et al.*, 1995; Van de Veire and Degheele, 1996; McGregor *et al.*, 1999; Sanchez *et al.*, 2003). Their simultaneous use could greatly enhance the control of *T. vaporariorum* populations if the interference between *P. fumosoroseus* Apopka-97 and *D. hesperus* is minimal. Interference between insect and fungal pathogen natural enemies is usually caused by infection, predation, or competition, or a combination of these antagonistic interactions (Roy and Pell, 2000). There are many opportunities for antagonistic interactions between *P. fumosoroseus* Apopka-97 and *D. hesperus* due to their individual biology. The fungal pathogen has a broad host range (Osborne and Landa, 1992) and could potentially reduce *D. hesperus* populations by directly infecting them. Host infection and ensuing death is caused by the penetration of germ tubes of germinated spores through the cuticle of the host that then invades the haemoceol and generates hyphal bodies that proliferate throughout the body (Osborne and Landa, 1992; Goettel and Inglis, 1997). Given that *D. hesperus* actively searches for prey and is phytophagous (McGregor *et al.*, 2000; Gillespie and McGregor, 2000), it is likely that *D. hesperus* will come into contact with *P. fumosoroseus* spores while foraging and plant-feeding. Furthermore, *D. hesperus* is a generalist predator and could actually reduce population densities of *P. fumosoroseus,* if *P. fumosoroseus*-infected *T. vaporariorum* nymphs are acceptable prey items.

The detrimental effects of interference among predator and fungal pathogen natural enemies in biological control programs has lead to laboratory studies examining

the potential for these natural enemies to interact (Pell *et al,* 1997; Poprawski *et al*, 1998; Roy *et al,* 1998; Pell and Vandenberg, 2002). Prior to the combined use of *D. hesperus* and *P. fumosoroseus* Apopka-97 for biological control of *T. vaporariorum*, their potential antagonistic interactions need to be quantified. Three aspects of antagonistic interactions between these two species were investigated: (1) the effect of direct exposure of *P. fumosoroseus* Apopka-97 on the mortality and predation rate of *D. hesperus* females; (2) the effect of indirect exposure of *P. fumosoroseus* Apopka-97 on the mortality and predation rate of *D. hesperus* females and; (3) discrimination of *D. hesperus* females between healthy and *P. fumosoroseus*-treated *T. vaporariorum* nymphs.

3.3 Materials and Methods

3.3.1 Insect Cultures and Host Plants

Colonies of *D. hesperus* were initially established from collections made near Woody, California USA, at 35° 43' N, 116° 49' W, elevation 300 m. on white stem hedgenettle, *Stachys albens* Gray (Lamiaceae) and were maintained indoors on tobacco plants, *Nicotiana tabacum* L. (Solanaceae), with eggs of *Ephestia kuehniella* Zeller (Lepidopotera: Pyralidae) as a prey source. The rearing room was maintained at a temperature of 22°C, with 70% relative humidity, and a photoperiod of 16:8 (L: D). The colony was reared in 36 x 42 x 30 cm screened Plexiglass insect cages. Five-week-old females were selected for all three experiments.

Nymphs of *T. vaporariorum* on excised tobacco leaves were obtained from Applied Bionomics, Victoria, British Columbia and were used as prey sources for *D. hesperus*. Eggs of *E. kuehniella*, obtained from Koppert Canada, Scarborough, Ontario, were also used as a prey source for *D. hesperus*. Leaves containing *T. vaporariorum*

nymphs were stored in the fridge for up to a week at 4°C until needed. The *E. kuehniella* eggs were stored in sealed containers in the freezer. Leaf discs used in these experiments were cut from two-month-old tomato plants, *Lycopersicon esculentum* Mill. cv. Rhapsodie (Enza Seeds), grown in the Pacific Agri-Food Research Centre greenhouses (Agassiz).

3.3.2 Fungal Preparations

The commercial formulation, PFR-97™ 20% WDG was manufactured by and obtained from Certis USA, Columbia MD. PFR-97™ is sold as desiccated blastospores of the fungus *P. fumosoroseus* strain Apopka-97 (ATCC 20874), on water dispersible granules containing 1 x 10^9 colony forming units per gram (CFU/g). This product was stored in a sealed container at approximately 4°C. PFR-97™ suspensions were prepared using a phosphate buffer (pH = 7.11) amended with Tween 80 (0.01%).

Blastospore quantification and viability of the PFR-97™ suspensions were determined using the guidelines outlined by Goettel and Inglis (1997). Blastospores were quantified using a standard (improved Neubauer) hemacytometer at 400x magnification. A 0.01 ml aliquot of the suspension was loaded into six hemacytometer chambers and the number of blastospores per five cells for each chamber was counted. The estimation of blastospores per ml was then obtained by multiplying the average blastospores/cell by the volume conversion factor. Viability of the blastospores was determined by plating a 0.1 ml aliquot of the stock suspension onto three potato dextrose agar (PDA) plates amended with 0.005% benomyl (Goettel and Inglis, 1997). The inoculated plates were then incubated at 25°C for 24 hours in the dark. After the incubation period, three microscopic fields (400 x magnification) were scanned for each plate; a total of 500 spores examined

and scored as either viable or non-viable. A blastospore was deemed viable if a germ tube was present. The percent viability was then used to adjust the concentration of the PFR-97™ suspensions to be expressed as the number of viable blastospores per milliliter.

For experiments 2 and 3, spore deposition was determined for each application of PFR-97™ suspension from cylinders of 2.6% water agar (10 mm diameter) placed inside the spray arena. Immediately after spraying, the agar cylinders were collected and stored in sealed Petri dishes at 4°C until they were processed 4 hours later. The number of blastospores in three microscope fields (400 x magnification) per agar cylinder was averaged, adjusted for viability, and expressed as viable blastospores per square millimeter. Spray applications were made using an artist's airbrush (Aztek, Model 4702) equipped with a siphon-fed fine-spray mist nozzle (0.3 mm orifice aperture) at a pressure of 20 pounds per square inch (psi).

3.3.3 Description and Statistical Analysis of Experiments

3.3.3.1 Experiment 1: Effect of Direct Exposure of PFR-97™ on Mortality and Predation Rate of *D. hesperus* Females

Females of *D. hesperus* were released individually into 35 x 10 mm Petri dishes containing two pieces of moistened Whatman filter paper (10 mm diameter) on the bottom of each dish. A tomato leaf disc was placed on top of the moistened filter paper. The lids of the dishes were ventilated with a 10 mm diameter hole covered by a fine screened mesh. For 24 hours, *D. hesperus* females were not given a prey source but were able to feed on the tomato leaf disc. This allowed females to acclimate to their surroundings prior to treatment and provided an equivalent level of hunger among females. The temperature and relative humidity (mean ± SEM) for the duration of the

evaluation period, as measured outside the dishes, was 21.29 ± 0.26°C and 41.18 ± 0.77%.

After 24 hours, *D. hesperus* females were treated with either PFR-97™ suspension or phosphate buffer. Females treated with phosphate buffer served as the carrier control group. Each female was first immobilized with carbon dioxide, then removed from the Petri dish and placed on filter paper dorsal side down. Treatments were administered with a micropipette by applying a 15 µl drop of blastospore suspension or phosphate buffer onto the ventral thorax. The concentration of the blastospore suspension was $1.20 \times 10^7 \pm 2.5 \times 10^4$ viable blastospores/ml which was equivalent to 100 x LC_{50} of fourth-instar nymphs of *T. vaporariorum* (*Chapter 2*). Immediately following treatment application, *D. hesperus* females were replaced into their original Petri dishes. Thawed eggs of *E. kuehniella* were provided as a prey source. The eggs were placed onto the sticky end of a 3.15 cm^2 strip of yellow adhesive Post-it note pad (3M, St. Paul, MN) using a fine point paintbrush. Each strip contained approximately 120 eggs. The individual egg strips were initially weighed using an electrobalance (model CaHN 21, Vetron Corporation Cerritos, CA). Five egg strips were assigned to individual Petri dishes containing moistened filter paper and a tomato leaf disc but without a *D. hesperus* female in order to determine the weight loss from egg strips in the absence of predators.

Non-feeding *T. vaporariorum* puparia were used as a positive control group. Puparia were considered to be late fourth-instar nymphs that showed the red eyes of the forming *T. vaporariorum* adult (Malais and Ravensberg, 2003). Individual puparia were removed from the tobacco leaf using a fine-point needle and placed onto a leaf disc inside a Petri dish containing moistened filter paper. Puparia were indirectly or directly exposed

to either PFR-97™ suspension or phosphate buffer in the same manner as *D. hesperus* females. Including the positive control group, each treatment was replicated 15 times and the experiment was repeated once on a different day for a total of 30 replicates per treatment.

Survivorship was measured daily for seven days by recording the number of live and dead *D. hesperus* females and *T. vaporariorum* puparia in each treatment group. Dead *D. hesperus* adults were surface sterilized for two minutes in 0.2% sodium hypochlorite, rinsed twice in sterile water, and plated on 2% water agar at 25°C and 100% relative humidity to encourage sporulation of *P. fumosoroseus* on infected cadavers. Prey consumption by *D. hesperus* was measured every other day for six days by reweighing the egg strips. Predation rate expressed as milligrams/48 hours was determined by: initial weight – (final weight + evaporation weight). Fresh, pre-weighed egg strips replaced the removed egg strips.

A chi-square test was used to determine if the proportion of dead *D. hesperus* females on day 7 was the same for the two treatment groups. The analysis incorporated the Cochran-Mantel-Haenszel test which accounted for blocking as the experiment was replicated once on a different day. A chi-square test was performed in similar fashion for *T. vaporariorum* puparia. Test insects that died within 24 hours were excluded from the chi-square tests as the mortality could have been due to experimental procedures rather than treatment effects.

A two-factor repeated measures analysis of variance (2-MANOVA) was used to determine the effects of treatment on the predation rate of *D. hesperus* over the 6 day period. The analysis incorporated a second factor which accounted for blocking to ensure

that no interaction between block and treatment was influencing the statistical outcome. Females of *D. hesperus* that died within 6 days were excluded from the 2-MANOVA as only the predation rate for those that survived treatment was of interest. All statistical analyses were performed using the JMP 5: SAS Institute, 2002, Toronto Canada.

3.3.3.2 Experiment 2: Effect of Indirect Exposure of PFR-97™ on Mortality and Predation Rate of *D. hesperus* Females

A similar protocol to *Experiment 1* was used with the following exceptions. Treatment applications were administered to the leaf discs in the Petri dishes instead of directly on the test insects. Leaf discs were individually sprayed with either 200 µl of phosphate buffer or 200 µl of PFR-97™ suspension containing $1.26 \times 10^7 \pm 1.8 \times 10^4$ viable blastospores/ml which corresponded to coverage of 1234.64 ± 110.92 viable blastospores/mm^2. Immediately after treatment application, *D. hesperus* females and *T. vaporariorum* puparia were returned to their respective Petri dishes while the leaf discs were still wet. Once the leaf discs were dry, which was approximately 5 hours after treatment application, an *E. kuehniella* egg strip was placed into each Petri dish containing *D. hesperus*. Data were recorded and analyzed as in *Experiment 1*.

3.3.3.3 Experiment 3: Prey Selection Made by *D. hesperus* between Control-Treated and PFR-97™-Treated *T. vaporariorum* Nymphs

Pieces of tobacco leaves containing fourth-instar nymphs of *T. vaporariorum* were sprayed with 700 µl of PFR-97™ suspension containing $5.87 \times 10^6 \pm 1.46 \times 10^3$ viable blastospores/ml which corresponded to coverage of 325.71 ± 10.87 viable blastospores/mm^2. This rate was equivalent to the LC_{90} of fourth-instar nymphs of *T. vaporariorum* (*Chapter 2*). The carrier control group consisted of fourth-instar nymphs

of *T. vaporariorum* on tobacco leaf pieces sprayed with 700 µl of phosphate buffer. Leaf pieces were kept hydrated by being placed in Petri dishes containing 2.6% water agar. They were allowed to air dry before incubation for 24 hours at 25°C and 90-100% relative humidity under a photoperiod of 16:8 (L:D) hours. Leaves were then maintained at 25°C and 75% relative humidity for either 3 or 5 days. Females of *D. hesperus* were prepared and maintained during the experiment in the same way as in *Experiments 1* and *2.*

Nymphs of *T. vaporariorum* were offered to *D. hesperus* females at two different times after the females were starved for 24 hours: (1) three days following spray application, and (2) five days following spray application. The carrier control group, consisting of nymphs sprayed with buffer, will be henceforth referred to as healthy nymphs. Nymphs sprayed with PFR-97™ blastospores will be henceforth referred to as treated nymphs. For each interval, *T. vaporariorum* nymphs were offered to *D. hesperus* females as one of the following simultaneous two-prey feeding choices: (1) two healthy nymphs (H, H); (2) one healthy nymph and one treated nymph (H, T); (3) two treated nymphs. (T, T). Nymphs were placed 2 to 3 mm apart in the middle of the Petri dish containing a *D. hesperus* female. For feeding choices involving a healthy and a treated nymph, placement of nymphs was randomized to ensure that the feeding outcome made by *D. hesperus* was not biased by the position of the nymphs. For each time, feeding choices were replicated 16 times. The experiment was repeated once, making a total of 32 replicates per feeding choice.

Nymphs were offered to *D. hesperus* at two separate time intervals in order to obtain different stages of prey infection. Treated nymphs that were offered to *D.*

hesperus females three days after being sprayed with PFR-97™ blastospores showed no outwardly detectable signs of infection; however, nymphs did have a cloudy haemolymph upon dissection which was indicative of the first stages of fungal infection. Treated nymphs that were offered to *D. hesperus* females five days after being sprayed with PFR-97™ blastospores showed an orange discoloration which is typically displayed near death and indicates extensive fungal colonization of the haemolymph (Wraight *et al.*, 1998). Fungal colonization was verified by dissection.

Prey feeding choices were offered to *D. hesperus* females for 24 consecutive hours. After 24 hours, the number and type of nymphs consumed, either healthy or infected, was recorded for each feeding choice. Fully consumed nymphs were indicated by emptied pupal capsules. Behavioural observations of *D. hesperus* were made during the first hour of the 24 hour period to determine the number and duration of complete prey consumption, partial prey consumption, and prey rejection of healthy and treated nymphs. Distinctions between these feeding activities were based on a preliminary trial in which the 95% confidence limit for the mean complete consumption of *T. vaporariorum* fourth-instar nymphs by *D. hesperus* females was 14.43 – 22.26 minutes (n = 12). Consequently, complete prey consumption by *D. hesperus* was defined as insertion of the stylet into the *T. vaporariorum* nymph for more than 14 minutes. Partial prey consumption was defined as stylet insertion for more than 1 minute but less than 14 minutes and prey rejection was defined as stylet insertion for less than 1 minute. These observations were based on the first nymph that each *D. hesperus* encountered during the hour. Three *D. hesperus* females were observed simultaneously. A white screen with a height of 45 mm was placed around each Petri dish to minimize distraction for *D.*

hesperus by external movements during observation. The feeding activities were each timed with stopwatches.

For each time interval, the influence of feeding choice on the number and type of nymphs consumed after the 24 hour time period was determined using chi-square tests. The analysis incorporated the Cochran-Mantel-Haenszel test which accounted for blocking as the experiment was replicated once. The influence of feeding choice on the number and duration of feeding activities during the one hour observation was analyzed using chi-square tests and one factor analysis of variance (ANOVA), respectively. Differences in the number and duration of feeding activities exhibited by *D. hesperus* females between healthy nymphs and treated nymphs were also determined using chi square tests and one factor analysis of variance (ANOVA), respectively. All statistical analyses were performed using the JMP 5: SAS Institute, 2002, Toronto Canada.

3.4 Results

3.4.1 Experiment 1: Effect of Direct Exposure of PFR-97™ on Mortality and Predation Rate of *D. hesperus* Females

The proportion of dead females between treatments was not different seven days after treatment application, indicating that PFR-97™ did not affect the survival of *D. hesperus* females when applied to their ventral thorax (Table 3.7-1: Chi-square: $\chi^2(1, 58) = 0$, $P = 1.00$). No sporulating cadavers were observed in the PFR-97™ suspension treatment or in the carrier control treatment. Differences in the proportion of dead *T. vaporariorum* puparia between treatments (Table 3.7-1: Chi-square: $\chi^2(1, 53) = 24.34$, $P < 0.0001$) confirmed that PFR-97™ was able to cause infection.

The predation rate of *D. hesperus* females was not affected by treatment, as measured every 48 hours for 6 consecutive days (Figure 3.6-1). There was a non-significant effect of treatment on predation rate through time and a non-significant interaction between block and treatment (2-MANOVA: Treatment: $F_{1, 50} = 1.67$, P = 0.202; Block*Treatment: $F_{1, 50} = 0.997$, P = 0.323). Females that survived a one-time exposure to PFR-97™ blastospores consumed prey at a similar rate to *D. hesperus* females exposed to the carrier control.

3.4.2 Experiment 2: Effect of Indirect Exposure of PFR-97™ on Mortality and Predation Rate of *D. hesperus* Females

The proportion of dead *D. hesperus* females was higher in the PFR-97™ treatment group than the carrier control group, seven days after treatment application to the leaf discs (Table 3.7-1: Chi-square: $\chi^2(1, 58) = 4.22$, P = 0.04). Taking into account the observed mortality in the standard control group, the control-corrected mortality in the PFR-97™ treatment group was 28%. The control corrected mortality was determined using a variation of the Abbott's formula in which calculations were based on dead rather than live individuals (in Aregger, 1992):

$$\% \text{ mortality} = \frac{\% \text{ dead in treated} - \% \text{ dead in control}}{100 - \% \text{ dead in control}} \times 100$$

Of the 11 *D. hesperus* cadavers recovered in the PFR-97™ treatment group, 9 of those sporulated during the incubation period. No sporulating cadavers were observed in the carrier control group. Differences in the proportion of dead *T. vaporariorum* puparia between treatments (Table 3.7-1: Chi-square: $\chi^2(1, 50) = 21.44$, P < 0.0001) confirmed that PFR-97™ suspensions were able to cause infection under the environmental conditions of the experiment.

Treatment affected the predation rate of *D. hesperus* females, as measured every 48 hours for 6 consecutive days (Figure 3.6-1: 2-MANOVA: Treatment: $F_{1, 41} = 10.76$, P = 0.0021; Block*Treatment: $F_{1, 41} = 0.2.21$, P = 0.146). *D. hesperus* females that survived a one-time exposure to PFR-97™ blastospores consumed prey at a lower rate than *D. hesperus* females exposed to the carrier control. When averaged over the 6-day period, PFR-97™ blastospores contributed to a reduction in *D. hesperus* feeding by 38% relative to the control group.

3.4.3 Experiment 3: Prey Selection Made by *D. hesperus* between Control-Treated and PFR-97™-Treated *T. vaporariorum* Nymphs

Feeding choice did not affect the number or type of nymphs consumed by *D. hesperus* females when *T. vaporariorum* nymphs were offered to *D. hesperus* three days after spray application with PFR-97™ blastospores or buffer. The percentage of *D. hesperus* females that consumed one, both or no nymphs in a 24 hour period was not significantly different between the three feeding choices (Table 3.7-2: Chi-square: $\chi^2(4, 89) = 2.453$, P = 0.653). When offered the option between a healthy and a treated nymph, *D. hesperus* consumed the treated nymph 72% of the time. Moreover, when offered two treated nymphs, *D. hesperus* consumed one or both of them 90% of the time. These data indicate that *D. hesperus* consumed the nymphs irrespective of the spray application made to the nymphs three days previously.

Feeding choice had a considerable impact on the number and type of nymphs consumed by *D. hesperus* females when the nymphs were offered to them five days after spray application (Table 3.7-2: Chi-square: $\chi^2(4, 90) = 81.848$, P < 0.0001). When offered the option between a healthy and a treated nymph, *D. hesperus* consumed the

treated nymph only 25% of the time. Healthy nymphs were consumed readily; *D. hesperus* females always consumed one or both healthy nymphs. Treated nymphs were seldom consumed. When two treated nymphs were presented, females consumed one of the two treated nymphs 34% of the time and both treated nymphs were never consumed. Female *D. hesperus* always consumed at least one nymph during the 24 hour period when offered one or two healthy nymphs. However, 66% of the time, *D. hesperus* did not consume either of the treated nymphs that were offered. These data indicate that *D. hesperus* preferred to consume healthy nymphs than treated nymphs when the nymphs were offered to them five days after spray application.

One-hour observations were made on the behaviour of *D. hesperus* females to determine if there were differences in *D. hesperus* behaviour towards healthy nymphs versus treated nymphs. Only the five day data were analyzed. There was no difference in the proportion of feeding activities exhibited by *D. hesperus* between feeding choices, regardless of whether a healthy nymph or a treated nymph was encountered first (Chi-square: Healthy: $\chi^2(2, 24) = 0.994$, $P = 0.61$; Treated: $\chi^2(1, 24) = 0.551$, $P = 0.46$). The proportion of full and partial prey consumption and prey rejection on the first nymphs encountered was irrespective of the state (healthy or treated) of the second nymph. The proportions of feeding activities were pooled across feeding choices to determine if the feeding activities exhibited by *D. hesperus* females on the first nymph encountered was effected by the state of that nymph. The proportion of feeding activities exhibited by *D. hesperus* females on the encountered nymph was affected by the state of that nymph (Table 3.7-3: Chi-square: $\chi^2(2, 50) = 19.80$, $P < 0.0001$). Treated nymphs were rejected by *D. hesperus* females 96% of the time, compared to 39% of the time with healthy

nymphs. Complete or partial consumption of healthy nymphs by *D. hesperus* was frequently observed however, complete consumption of a treated nymph did not occur and partial consumption was only observed once.

The time for *D. hesperus* females to reject a nymph was not affected by the state of that nymph (Table 3.7-3: 1-ANOVA: $F_{1, 34} = 1.630$, $P = 0.13$). Rejection of either a healthy or a treated nymph took approximately 7 seconds. The average time to completely consume a healthy nymph was 27 minutes and partial consumption took 5 minutes.

3.5 Discussion

Adult *D. hesperus* females were not susceptible to infection by PFR-97™ when it was directly applied to the ventral thorax as a 15 µl aliquot at a concentration of 1.20×10^7 viable blastospores/ml. Moderate mortality (28%) of adult *D. hesperus* occurred when females were indirectly exposed to PFR-97™ by being confined with leaf discs that had been sprayed with a concentration of 1.26×10^7 viable blastospores/ml. Predation rate of *D. hesperus* females was unaffected by the one-time direct exposure to PFR-97™; however, females that received an indirect exposure and survived treatment, consumed prey at a lower rate (38%) than the control group during a 6-day evaluation period. Nymphs of *T. vaporariorum* exposed to PFR-97™ at a concentration of 5.87×10^6 viable blastospores/ml three days before being offered to *D. hesperus* females were acceptable prey and were consumed readily by *D. hesperus*. However, when offered the choice between a healthy nymph and a treated nymph (sprayed with PFR-97™ five days earlier), *D. hesperus* consumed the healthy nymph 75% of the time. Complete consumption of a treated nymph by *D. hesperus* was not observed.

Attachment of blastospores to various body parts of *D. hesperus* may have played a role in fungal infection and subsequent mortality of *D. hesperus* females that were indirectly exposed to PFR-97™. Blastospores may have attached to tarsi when *D. hesperus* walked on the treated leaf disc or may have adhered to their mouthparts when *D. hesperus* probed the PFR-97™ suspension droplets. These areas may have been more favorable to spore germination and penetration than the ventral thorax as not all areas of the body are equally vulnerable (Butt and Goettel, 2000). On *Nezara viridula* L. (Hemiptera: Pentatomidae), spores attached to areas with abundant setae, such as the antennal tips and apical portions of the tarsi and tibia (Sosa-Gomez, *et al.*, 1997). Feeding on wheat-bran formulation containing spores of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes), allowed the spores to attach and germinate on the internal mouthparts of *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) (Jeffs *et al.*, 1997). Spore attachment and germination was facilitated by the presence of hairs and cavities on the mouthparts (Jeffs *et al.*, 1997). Spore germination also occurred frequently in intersegmental areas (Hatzipapas *et al.*, 2002; Hajek and Eastburn, 2003).

Limited information is available on the effect of PFR-97™ or PreFeRal™ on natural enemies used in biological control programs. PreFeRal™ is a commercial formulation of *P. fumosoroseus* Apopka-97 that is available in Europe. Moderate mortality (26 % corrected mortality) of *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) was observed when *M. caliginosus* were placed on greenhouse tomato plants or bean plants that had been sprayed one hour previously with PreFeRal™ at a concentration of 5×10^6 colony forming units/ml (Sterk *et al.*, 1995). Under the same experimental methods, PreFeRal™ resulted in negligible mortality (11 %) of *Orius*

species (Hemiptera: Anthocoridae) (Sterk *et al.*, 1995). PFR-97™ did not affect the developing parasitoid *E. formosa* inside *T. vaporariorum* nymphs on bean seedlings when sprayed at the recommended label rate under laboratory conditions (Van de Veire and Degheele, 1996). Osborne and Landa (1992) reported that PFR-97™ was compatible with *Eretmocerus* species (Hymenoptera: Aphelinidae) and *Delphastus pusillus* (LeConte) (Coleoptera: Coccinellidae).

Previous studies have also noted a reduction in feeding of insects exposed to an entomopathogenic fungus. The fungal pathogen, *Metarhizium anisopliae* var. *acridum* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) caused significant feeding reductions in *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) and *Zonocerus variegatus* L. (Orthoptera: Acrididae) within four days after treatment (Moore *et al.*, 1992; Thomas *et al.*, 1997). The fungus also caused a 65% feeding reduction of *Locustana pardalina* (Walker) (Orthoptera: Acrididae) (Arthurs and Thomas, 2000), and feeding reductions of *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), that were detected 2-4 days after treatment (Ekesi and Maniania, 2000). Ekesi and Maniania (2000) agreed with previous authors that feeding reductions were attributable to the production of toxins and disturbance of tissues by the developing fungus. In contrast, greater feeding damage by *Lygus hesperus* Knight (Hemiptera; Miridae) was noted when the bugs were exposed to *B. bassiana* (Noma and Strickler, 2000). The authors hypothesized that increased feeding may have been the response of the insect to depletion of nutrient reserves by the fungus (Noma and Strickler, 2000).

The absence of a difference between the numbers of healthy versus treated nymphs encountered by *D. hesperus* indicates that *D. hesperus* relies on stylet probing to

determine prey suitability. Consequently, *D. hesperus* might increase transmission of *P. fumosoroseus* Apopka-97 from infected to uninfected *T. vaporariorum* nymphs by foraging in treated *T. vaporariorum* populations. Although pathogen transmission by *D. hesperus* was not investigated, it is a possibility. Predatory ladybird beetles have been shown to cause an increase in pathogen transmission by foraging on fungus-infected aphids (Pell *et al*, 1997; Pell and Vandenberg, 2002). Studies investigating fungal transmission by *D. hesperus* should be considered because the joint use of *D. hesperus* with PFR-97™ would cause synergistic mortality of *T. vaporariorum* if *D. hesperus* could transfer infection to healthy *T. vaporariorum* populations.

The ability of *D. hesperus* to discriminate between healthy nymphs and treated nymphs through stylet insertion into the prey and subsequent contact with fungal structures might enable *D. hesperus* to cause prey mortality that is complementary to the mortality caused by PFR-97™. Discrimination of infected hosts or prey is a common feature of parasitoids and predators when used in combination with fungal pathogens. The parasitoid, *E. formosa* was able to detect and reject *T. vaporariorum* nymphs treated with the fungal pathogen, *Aschersonia aleyrodis* Webber (Deuteromycotina: Coelomycetes), seven days after treatment application (Fransen and van Lenteren, 1993). The female parasitoid, *Aphelinus asychis* (Walker) (Hymenoptera: Aphelinidae) was able to detect the unsuitability of *P. fumosoroseus*-treated aphids as an oviposition site when the aphids were infected for three days (Mesquita and Lacey, 2001). Fourth-instar larvae of the seven-spotted ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) were able to discriminate between healthy and sporulating aphids (Roy *et al*, 1998). Furthermore, the absence of a difference between the time it took *D. hesperus* to reject a

healthy versus a treated nymph, indicates that *D. hesperus* should be efficient foragers in PFR-97™-treated *T. vaporariorum* populations.

In summary, a number of potentially antagonistic interactions have been demonstrated among *D. hesperus* and PFR-97™ in the laboratory. These antagonistic interactions appear to be biologically insignificant and would not have a substantial impact in a greenhouse system. Furthermore, these antagonistic interactions could be avoided if releases of *D. hesperus* were made five days after PFR-97™ application. With proper timing, the simultaneous use of both natural enemies could greatly enhance the biological control of *T. vaporariorum*. Moreover, the observed prey discrimination and efficient rejection of treated nymphs by *D. hesperus* will reduce the severity of the antagonistic interactions between *D. hesperus* and PFR-97™.

Caution must be exercised when making inferences from laboratory results to greenhouse situations as they can be poor predictors of what truly occurs in "nature" (Hajek *et al*, 1996; Goettel *et al*, 2001; Inglis *et al*, 2001). Although laboratory studies are important in identifying the potential for interference to occur between natural enemies, these studies do not incorporate all aspects of the greenhouse situation that can affect the presence and severity of the interactions (Roy and Pell, 2000). A greenhouse trial assessing the potential interactions between *D. hesperus,* PFR-97™ and *T. vaporariorum*, should be conducted prior to their joint usage in *T. vaporariorum* biological control programs.

3.6 Figures

Figure 3.6-1: Effect of treatment on the mean predation rate (± 95% CI) of 5-week-old *D. hesperus* females during the 6-day experiments. Treatments were applied as a 15 µl aliquot to the ventral thorax of *D. hesperus*. The concentration of the PFR-97™ aliquot was $1.20 \times 10^7 \pm 2.5 \times 10^4$ viable blastospores/ml. Test insects that died within 6 days were excluded from the analyses.

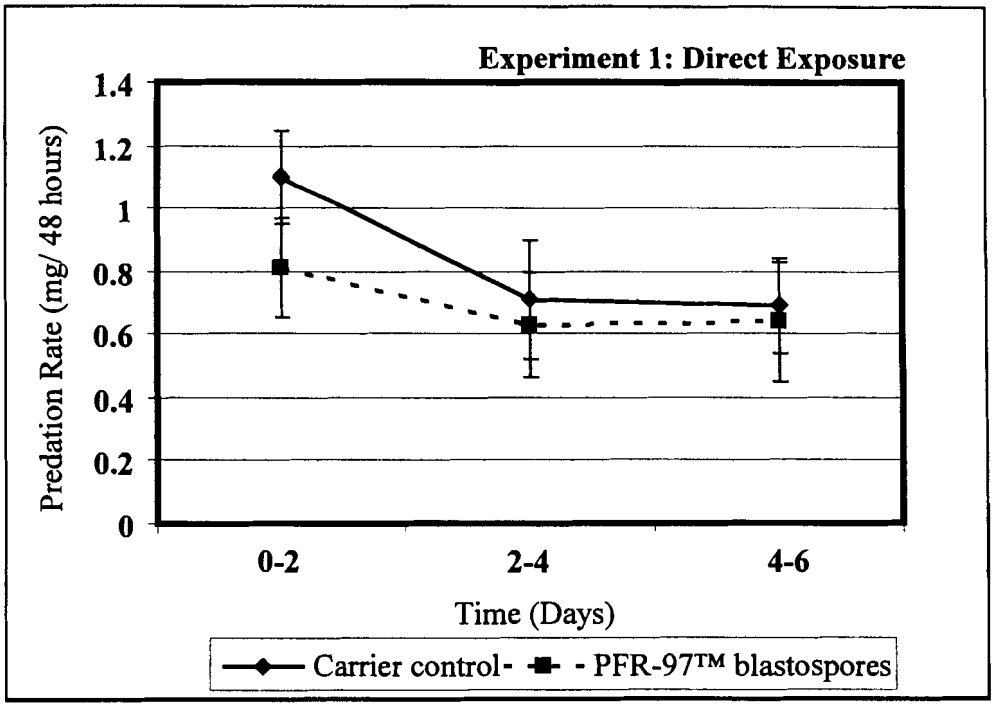
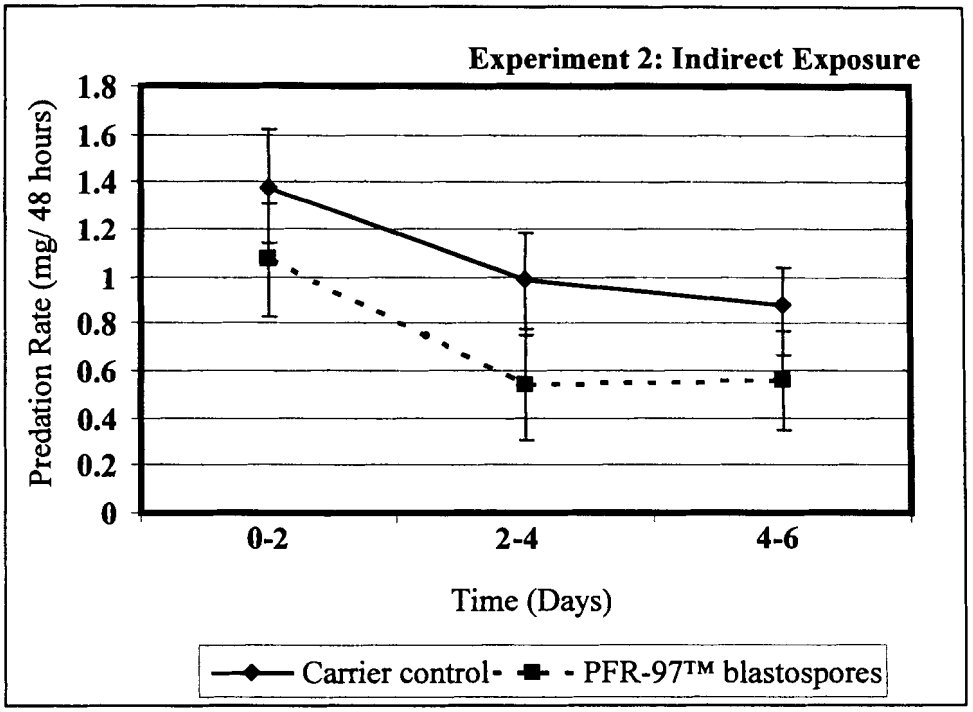

Experiment 1: Direct Exposure
Predation Rate (mg/ 48 hours)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0-2
2-4
4-6
Time (Days)
Carrier control- - PFR-97™ blastospores

Figure 3.6-2: Effect of treatment on the mean predation rate (± 95% CI) of 5-week-old *D. hesperus* females during the 6-day experiments. Treatments were sprayed onto the surface of the leaf discs that were exposed to *D. hesperus* females. Leaf discs treated with PFR-97 were sprayed at a concentration of $1.26 \times 10^7 \pm 1.8 \times 10^4$ viable blastospores/ml. Test insects that died within 6 days were excluded from the analyses.


Experiment 2: Indirect Exposure
Predation Rate (mg/ 48 hours)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0-2
2-4
4-6
Time (Days)
Carrier control
PFR-97™ blastospores

3.7 Tables

Table 3.7-1: Mortality of 5-week old *D. hesperus* females expressed as a percentage that were treated with a 1-time exposure to PFR-97™ blastospores or phosphate buffer (carrier control). Treatments were either applied as a 15 µl aliquot to the ventral thorax of *D. hesperus* (Experiment 1) or sprayed onto the surface of leaf discs that were exposed to *D. hesperus* (Experiment 2). Puparia of *T. vaporariorum* served as a positive control group. Test insects that died within 24 hours were excluded from the analysis.

Experiment 1: Direct Exposure	Percent live	Percent dead
Test insects: *D. hesperus* females		
treated with PFR-97™	93	7
treated with buffer	93	7
	$\chi^2(1,58) = 0$, P = 1.00	
Positive Control Group: *T. vaporariorum* puparia		
treated with PFR-97™	18	82
treated with buffer	85	15
	$\chi^2(1,53) = 24.34$, P < 0.0001	

Experiment 2: Indirect Exposure		
Test insects: *D. hesperus* females		
Leaves treated with PFR-97™	63	37
Leaves treated with buffer	87	13
	$\chi^2(1,58) = 4.22$, P = 0.04	
Positive Control Group: *T. vaporariorum* puparia		
Leaves treated with PFR-97™	18	82
Leaves treated with buffer	88	12
	$\chi^2(1,50) = 21.44$, P < 0.0001	

Table 3.7-2: Effect of feeding choice on the feeding outcome made by 5-week old *D. hesperus* females. Feeding outcome was expressed as the percentage of *D. hesperus* that consumed no, one, or both prey items per feeding choice. Prey items were fourth-instar nymphs of *T. vaporariorum*. Healthy *T. vaporariorum* nymphs (H) were sprayed topically with phosphate buffer (carrier control). Treated *T. vaporariorum* nymphs (T) were sprayed topically with PFR-97™ blastospores at a concentration of $5.87 \times 10^6 \pm 1.46 \times 10^3$ viable blastospores/ml. Nymphs sprayed with PFR-97™ or buffer were offered to *D. hesperus* females either 3 days after spray application or 5 days after spray application.

Feeding Choices	Percentage of *D. hesperus* that consumed:		
	0 prey	**1 prey**	**2 prey**
Nymphs offered to *D. hesperus* 3 days after spray application			
(H, H)	9	25	66
(H, T)	16	34: 12 H, 22 T	50
(T, T)	10	39	51
Nymphs offered to *D. hesperus* 5 days after spray application			
(H, H)	0	28	72
(H, T)	0	81: 75 H, 6 T	19
(T, T)	66	34	0

Table 3.7-3: One-hour behavioural observations on the feeding of 5-week old *D. hesperus* females on fourth-instar nymphs of *T. vaporariorum*. Results are expressed as the percentage of *D. hesperus* females that fully consumed, partially consumed, or rejected, either a healthy or visibly infected *T. vaporariorum* nymph. Healthy nymphs were those treated with phosphate buffer (carrier) and treated nymphs were those treated topically with PFR-97™ blastospores 5 days before they were offered to *D. hesperus*.

Observed feeding activities by *D. hesperus* females	**Percent of *D. hesperus* females exhibiting feeding activities on 4th instar nymphs of *T. vaporariorum*** **healthy**	**treated**	**Time (Mean ± SEM)**
complete consumption	29	0	27 ± 2.55 min
partial consumption	32	4	5.1 ± 1.32 min
rejection	39	96	6.6 ± 2 sec
Total encounters	*28*	*27*	
	$\chi^2(2,51) = 19.80, P < 0.0001$		

3.8 References

Aregger, E., 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2°C Journal of Invertebrate Pathology 59: 2-10.

Arthurs, S and M.B. Thomas, 2000. Effects of a mycoinsecticide on feeding and fecundity of the brown locust *Locustana pardalina* Biocontrol Science and Technology 10 (3): 321-329.

Bolckmans, K., G. Sterk, J. Eyal, B. Sels, and W. Stepman, 1995. PreFeRal, (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses. Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 707-711.

Butt, T.M. and M.S. Goettel, 2000. Bioassays of entomogenous fungi Chapter 4 of Bioassays of Entomopathogenic Microbes and Nematodes (eds A. Navon and K.R.S. Ascher), pg.141- 191.

Ekesi, S. and N.K. Maniania, 2000. Susceptibility of *Megalurothrips sjostedti* developmental stages to *Metarhizium anisopliae* and the effects of infection on feeding, adult fecundity, egg fertility and longevity Entomologia Experimentalis et Applicata 94: 229-236.

Fransen, J.J. and J.C. van Lenteren, 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 69: 239-249.

Gillespie, D.R. and R.R. McGregor, 2000. The functions of plant feeding in the omnivorous predator *Dicyphus hesperus*: water places limits on predation Ecological Entomology 25: 380-386.

Goettel, M.S. and G.D. Inglis, 1997. Fungi: Hyphomycetes Chapter 5 of Manual of techniques in insect pathology (ed L.A. Lacey), Academic Press, San Diego California, pg. 213-249.

Goettel, M.S., A.E. Hajek, J.P. Siegel, and H.C. Evans, 2001. Safety of fungal biocontrol agents. Chapter 13 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 347-375.

Hajek, A.E., L. Butler, S.R.A. Walsh, J.C. Silver, F.P. Hain, F.L. Hastings, T.M. Odell, and D.R. Smitley, 1996. Host range of the gypsy moth (Lepidoptera: Lymantridae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthales) in the field versus the laboratory Environmental Entomology 25: 709-721.

Hajek, A.E. and C.C. Eastburn, 2003. Attachment and germination of *Entomophaga maimaiga* conidia on host and non-host larval cuticle Journal of Invertebrate Pathology 82 (1): 12-22.

Hatzipapas, P., K. Kalosaka, A. Dara, and C. Christias, 2002. Spore germination and appressorium formation in the entomopathogenic *Alternaria alternata* Mycological Research 106 (11): 1349-1359.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser, 2001. Use of hyphomycetous fungi for managing insect pests Chapter 3 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 23-69.

Jeffs, L.B., M-G Feng, J.E. Falkowsky, and G.G. Khachatourians, 1997. Infection of the migratory grasshopper (Orthoptera: Acrididae) by ingestion of the entomopathogenic fungus *Beauveria bassiana* Journal of Economic Entomology 90 (2): 383-390.

Kassis G. and S. Michelakis. 1993. The effectiveness of biological control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom., Aleyrodidae) by *E. formosa* Gahan (Hym., Aphelinidae) Journal of Applied Entomology 116: 298-302.

Malais, M.H. and W.J. Ravensberg, 2003. Knowing and recognizing: the biology of glasshouse pests and their natural enemies. Koppert B.V., the Netherlands.

McGregor, R.M, D.R. Gillespie, D.M.J. Quiring, and M.R.J. Foisy, 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes Biological Control 16: 104-110.

McGregor, R.M, D.R. Gillespie, C.J. Park, D.M.J. Quiring, and M.R.J. Foisy, 2000. Leaves or fruit? The potential for damage to tomato fruits by the omnivorous predator, *Dicyphus hesperus* Entomologia Experimentalis et Applicata 95: 325-328.

Mesquita, A.L.M., and L.A. Lacey, 2001. Interactions among the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes), the parasitoid, *Aphelinus asychis* (Hymenoptera: Aphelinidae), and their aphid host Biological Control 22: 51-59.

Moore, D., M. Reed, G. Le Patourel, Y.J. Abraham, and C. Prior, 1992. Reduction in feeding by the desert locust, *Schistocerca gregaria,* after infection with *Metarhizium flavoviride* Journal of Invertebrate Pathology 60: 304-307.

Noma, T. and K. Strickler, 2000. Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition Environmental Entomology 29 (2): 394-402.

Osborne, L.S. and Z. Landa, 1992. Biological control of whiteflies with entomopathogenic fungi Florida Entomologist 75(4): 456-471.

Pell, J.K., R. Pluke, S.J. Clark, M.G. Kenward, and P.G. Alderson, 1997. Interactions between two aphid natural enemies, the entomopathogenic fungus, *Erynia neoaphidis* Remaudiere & Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Journal of Invertebrate Pathology 69: 261-268.

Pell, J.K. and J.D. Vandenberg, 2002. Interactions among the aphid *Diuraphis noxia*, the entomopathogenic fungus *Paecilomyces fumosoroseus* and the coccinellid *Hippodamia convergens* Biocontrol Science and Technology 12: 217-224.

Poprawski, T.J., J. C. Legaspi and P. Parker, 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Aleyrodidae) Biological Control 27(3): 785-795.

Roy, H.E., J.K. Pell, S.J. Clark, and P.G. Alderson, 1998. Implications of predator foraging on aphid pathogen dynamics Journal of Invertebrate Pathology 71: 236-247.

Roy, H.E. and J.K. Pell, 2000. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control Biocontrol Science and Technology 10: 737-752.

Sanchez, J.A., D.R. Gillespie and R.R. McGregor, 2003. The effects of mullein plants (*Verbascum thapsus*) on the population dynamics of *Dicyphus hesperus* (Heteroptera: Miridae) in tomato greenhouses Biological Control 28: 313-319.

SAS Institute Inc., 2002. Version JMP 5.0 Cary, NC. USA.

Sosa-Gomez, D.R., J.L. Nation, and D.G. Boucias, 1997. Attachment of *Metarhizium anisopliae* to the southern green stink bug *Nezara viridula* cuticle and fungistatic effect of cuticular lipids and aldehydes Journal of Invertebrate Pathology 69 (1): 31-39.

Sterk, G., K. Bolckmans, M. Van de Veire, B. Sels, and W. Stepman, 1995. Side-effects of PreFeRal WG (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97) on different species of beneficial insects Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 719-723.

Thomas, M.B., S. Blandford, and C.J. Lomer, 1997. Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection by the fungal pathogen, *Metarhizium flavoviride* Biocontrol Science and Technology 7: 327-334.

Van de Veire, M. and D. Degheele, 1996. Toxicity of the fungal pathogen *Paecilomyces fumosoroseus* strain Apopka 97 to the greenhouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*, and the first results of a control experiment in glasshouse tomatoes. OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 191-194.

van Lenteren, J.C., H.J.W. van Roermund and S. Sutterlin, 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum)* with the parasitoid *E. formosa*: How does it work? Biological Control 6: 1-10

van Lenteren, J.C., Y.C. Drost, H.J.W. van Roermund and C.J.A.M. Posthuma-Doodeman, 1997. Aphelinid parasitoids as suitable biological control agents in greenhouses Journal of Applied Entomology 121: 473-485.

van Roermund, H.J.W. and J.C. van Lenteren, 1995a. Residence times of the greenhouse whitefly parasitoid *E. formosa* Gahan (Hym., Aphelinidae) on tomato leaflets Journal of Applied Entomology 119: 465-471.

van Roermund, H.J.W. and J.C. van Lenteren, 1995b. Foraging behaviour of the whitefly parasitoid *E. formosa* on tomato leaflets Entomologia Experimentalis et Applicata 76: 313-324.

Wraight, S.P., R.I. Carruthers, C.A. Bradley, S.T. Jaronski, L.A. Lacey, P. Wood, and S. Galaini-Wraight, 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii* Journal of Invertebrate Pathology 71: 217-226.

CHAPTER 4 INTRAGUILD INTERACTIONS BETWEEN *DICYPHUS HESPERUS* AND PFR-97™

4.1 Abstract

A 6 week greenhouse trial investigated the individual and combined effects of an entomopathogenic fungus, *Paecilomyces fumosoroseus* Apopka-97 (PFR-97™) and the predatory mirid, *Dicyphus hesperus,* on populations of the greenhouse whitefly, *Trialeurodes vaporariorum* on caged tomato plants (*Lycopersicon esculentum* cv. Rhapsodie). Densities of *T. vaporariorum* nymphs were measured weekly in a factorial experiment on tomato plants with or without releases of *D. hesperus* adults (20 per plant), and with three applications of either PFR-97™ or water (control). The first application of PFR-97™ was made at 100 times the recommended label rate to expose *D. hesperus* adults to a maximum challenge concentration. The second and third applications were made at 10 times the recommended label rate. This design allowed me to determine if the natural enemies would engage in intraguild interactions that could potentially disrupt or enhance the biological control of *T. vaporariorum*. Independently, *D. hesperus* and PFR-97™ caused a 35% and a 48% respective reduction in *T. vaporariorum* populations relative to the controls in the last week of the 6-week trial. Acting in concert, the two biological control agents reduced *T. vaporariorum* densities by 62% relative to the control plants. Adults of *D. hesperus* did not feed on visibly fungus-infected *T. vaporariorum* nymphs. Foliar applications of PFR-97™ did not affect the predation rate of *D. hesperus* adults. Furthermore, the survival and position within the plant of *D.*

hesperus adults were unaffected by PFR-97™. In addition, when the two biological control agents were used alone or in combination, they did not affect the growth of plants or the proportion of flowers that set fruit. My results indicate that the simultaneous use of *D. hesperus* and PFR-97™ had an additive effect on *T. vaporariorum* densities and enhanced the biological control of *T. vaporariorum*.

4.2 Introduction

The greenhouse whitefly, *Trialeurodes* va*porariorum* (Westwood) (Hemiptera: Aleyrodidae), is an important pest that causes damage to greenhouse vegetable crops (Malais and Ravensberg, 2003). Crop damage results from phloem feeding and honeydew secretion. Phloem feeding by large populations of *T. vaporariorum* retards plant vigor. The production of copious amounts of honeydew on leaves and fruits stimulates the growth of sooty mould fungi thereby reducing the photosynthetic processes of the leaves and fruit quality (Osborne and Landa, 1992; Fransen and van Lenteren, 1993; Poprawski *et al*, 1998). Moderate to excellent control of *T. vaporariorum* populations has been achieved with various types of natural enemies including the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (van Lenteren *et al.*, 1996; van Lenteren *et al.*, 1997), and the predators *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) (Salamero and Gabarra, 1987; Gabarra *et al.*, 1988; Albajaes *et al.*, 1996) *Dicyphus hesperus* Knight (Hemiptera: Miridae) (McGregor *et al.*, 1999; Sanchez *et al.*, 2003) and *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) (Sampson and King, 1996; Jakobsen *et al.*, 2002). Various entomopathogenic fungi have also been shown to be efficacious in suppressing *T. vaporariorum* populations: *Aschersonia aleyrodis* Webber (Deuteromycotina: Coelomycetes) (Fransen *et al.*, 1987; Fransen and

van Lenteren, 1994; Meekes *et al.*, 2002), *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (Poprawski *et al.*, 2000; Fargues *et al.*, 2003), *Lecanicillium lecanii* (Zimmerman) Gams and Zare [*Verticillium lecanii* (Zimmerman) Viegas] (Deuteromycotina: Hyphomycetes) (Osborne and Landa, 1992; Fargues *et al.*, 2003) and *Paecilomyces fumosoroseus* Apopka-97 (Wize) Brown and Smith (Deuteromycotina: Hyphomycetes) (Poprawski *et al.*, 2000; Osborne and Landa, 1992).

D. hesperus is a high-order predator that actively searches and preys upon *T. vaporariorum* and *Tetranychus urticae* Koch (Acarina: Tetranychidae) (McGregor *et al.*, 1999). Like most higher-order predators, *D. hesperus* is a generalist feeder, and has a long generation time (McGregor *et al.*, 1999; Brodeur *et al.*, 2002). Adults of *D. hesperus* are also omnivorous and have been observed to feed on plant leaves and fruit (McGregor *et al.*, 2000). Current research is focusing on their spatial distribution within the greenhouse, the effects of alternate host plants, and the functional relationship between plant and prey feeding as related to their biocontrol efficacy within the greenhouse (Sanchez *et al.*, 2002; Sanchez *et al.*, 2003; Sinia *et al.*, 2004). The fungus, *P. fumosoroseus* Apopka-97 has been commercially formulated into a biopesticide under the tradenames PFR-97™ (USA) and PreFeRal™ (Europe) (Faria and Wraight, 2001). PFR-97™ has a wide host range; its target pests include whitefly species, aphids, thrips and spider mites (Shah and Goettel, 1999). PFR-97™ and PreFeRal™ are highly efficacious against *T. vaporariorum* populations (Bolckmans *et al.*, 1995; Van de Veire and Degheele, 1996).

The combined effects of *D. hesperus* and PFR-97™ on *T. vaporariorum* populations have not been investigated. As both are generalists, their simultaneous use

for control of *T. vaporariorum* populations in greenhouses may lead to intraguild interactions that could either be beneficial or detrimental to the biological control program (Roy and Pell, 2000; Muller and Brodeur, 2002). Intraguild interactions occur when two species which are competing for a shared prey also engage in trophic interactions with each other (competition and predation) (Polis and Holt, 1992). The three possible outcomes to intraguild interactions in the context of biological control as defined by Ferguson and Stiling (1996) are: (1) the natural enemies interact synergistically and their combined effects on pest mortality is greater than the sum of their individual effects; (2) the natural enemies do not interact and their combined effects on pest mortality is equal to the sum of their individual effects and; (3) the natural enemies interact antagonistically and their combined effects on pest mortality are less than additive. Intraguild interactions are widespread among communities of biological control agents including interactions between insect and fungal natural enemies (Rosenheim *et al.*, 1995). Fungal control agents can affect insect natural enemies directly by infecting them, or indirectly by depleting the prey population (Goettel *et al.*, 1990 and Roy and Pell, 2000). In turn, insect natural enemies can affect fungal control agents by consuming prey that are infected with the fungus (Roy and Pell, 2000). Therefore, it is critical to consider the occurrence of intraguild interactions between PFR-97™ and *D. hesperus* prior to their combined release in biological control programs.

This greenhouse trial investigated the individual and combined effects of *D. hesperus* and PFR-97™ on *T. vaporariorum* populations on confined tomato plants. Their effects were tested using a factorial design experiment in which the two factors were *D. hesperus* and PFR-97™ each with two levels (presence or absence) on the plants.

Their effects on prey populations and each other were quantified by sampling the plants weekly and counting the number of *T. vaporariorum* nymphs on selected leaflets. Their effects on plant growth and on the proportion of flowers that set fruit were also investigated.

4.3 Materials and Methods

The greenhouse trial was performed in August, September, and October 2003, in experimental greenhouses at the Pacific Agri-Food Research Center in Agassiz, British Columbia. A research permit (50-RP-03) was issued by the Pest Management Regulatory Agency (PMRA) under the Pest Control Products Act for the experimental use of the microbial insecticide, PFR-97™ 20% WDG.

4.3.1 Insect Cultures

Excised tobacco leaves, *Nicotiana tabacum* L. (Solanaceae), containing nymphs of *T. vaporariorum* were obtained from Applied Bionomics, Victoria, British Columbia and were used to produce a whitefly colony on potted tobacco plants. This colony was maintained in an environment-controlled rearing room at a temperature of 26°C and a 24 hour light photoperiod due to technical reasons.

Colonies of *D. hesperus* were established from collections made near Woody, California USA, at 35° 43' N, 116° 49' W, elevation 300 meters on white stem hedgenettle, *Stachys albens* Gray (Lamiaceae) and maintained on tobacco plants with eggs of *Ephestia kuehniella* Zeller (Lepidopotera: Pyralidae) as a prey source. The rearing room was maintained at a temperature of 22°C, with 70% relative humidity, and a

photoperiod of 16:8 (light: dark). The colony was reared in 36 x 42 x 30 cm screened Plexiglass insect cages. Five-week-old females and males were selected for this trial.

4.3.2 Fungal Preparations

The commercial formulation, PFR-97™ 20% WDG was manufactured by and obtained from Certis USA, Columbia MD. PFR-97™ is sold as desiccated blastospores of the fungus *P. fumosoroseus* Strain Apopka-97 (ATCC 20874), on water dispersible granules containing 1 x 10^9 colony forming units per gram (CFU/g). Blastospore suspensions were prepared in accordance with the product label; PFR-97™ granules were placed in a container with tap water and continuously agitated for thirty minutes until the granules had dispersed. The sediment in the suspension was then allowed to settle and the upper layer of the suspension, which contained the fungus spores, was decanted into the spray tank for application. Decanting prevented clogging of the sprayer with sediment. Spray applications were made at 6 pm, by evenly spraying the underside of leaves until run-off, using a single nozzle seven-liter backpack sprayer (Hudson Industrial Sprayer # 65010). Plants that were not assigned an application of PFR-97™ suspension were sprayed with water using a separate, identical sprayer that had not been used for fungus preparations.

Blastospore quantification and viability of the PFR-97™ suspensions were determined using the guidelines outlined by Goettel and Inglis (1997). Blastospores were quantified using a standard (improved Neubauer) hemacytometer at 400x magnification. A 0.01 ml aliquot of the final suspension was loaded into six hemacytometer chambers and the number of blastospores per five cells for each chamber was counted. The estimation of blastospores per ml was then obtained by multiplying the average

blastospores/cell by the volume conversion factor. Viability of the blastospores was determined by plating a 0.1 ml aliquot of the final suspension onto three potato dextrose agar (PDA) plates amended with 0.005% benomyl. The inoculated plates were then incubated at 25°C for 24 hours in the dark. After the incubation period, three microscope fields (400 x magnification) were scanned for each plate; a total of 500 spores were examined and scored as either viable or non-viable. A blastospore was deemed viable if a germ tube was present. The percent viability was then used to adjust the concentration of the PFR-97™ suspensions to be expressed as the number of viable blastospores per milliliter.

Spore deposition was determined for each application of PFR-97™ suspension from cylinders of 2.6% water agar pinned to the underside of the leaves. The cylinders of agar were cut using a 10 mm diameter cork borer and were 6 mm. long Immediately after spraying, the agar cylinders were collected and stored in sealed Petri dishes at 4°C until they were processed 6 hours later. The number of blastospores in three microscope fields (400 x magnification) per agar cylinder, having three agar cylinders per plant, was averaged, adjusted for viability, and expressed as viable blastospores per square millimeter.

4.3.3 Climate Data

The temperature and relative humidity were recorded daily, every six hours, for the duration of the trial using portable dataloggers (HOBO™, 2 channel; Onset Incorporated). The dataloggers were placed unprotected in the mid-canopy of the plant. For twelve hours after each application of PFR-97™ suspensions, the relative humidity in the compartments was increased by closing the greenhouse vents, and by placing three

containers full of water in the centre aisle, to encourage fungal germination and penetration of insect cuticles. During this time, the temperature and relative humidity readings were taken hourly.

4.3.4 Preparation, Infestation and Spraying of Plants

The trial was performed in two greenhouse compartments, each containing 24 tomato plants (*Lycopersicon esculentum* cv. Rhapsodie (Enza Seeds)) approximately 1.5 m tall and grown in rockwool. Plants were enclosed in cages made of 4 x 4 m polyvinyl chloride (PVC) pipe frames, and a spunbond rowcover fabric (Agryl P17 cloth). The frames were centered above each plant and were attached to wires strung across the ceiling of the compartments. Fabric was then hung from each frame to the compartment floor with a flap secured with clothespins on the front face of each cage to provide access to the enclosed plant. The plants were singly caged in order to treat them as individual replicates in the statistical analyses. Tomato plants inside cages were infested with 60 *T. vaporariorum* adults each and left untouched for three weeks to allow for the production and establishment of nymphs.

Within each greenhouse compartment, plants were randomly assigned to one of four treatment combinations: (1) application of PFR-97™ suspension and no release of *D. hesperus* adults (+P, -D); (2) release of *D. hesperus* adults and no application of PFR-97™ suspension (+D, -P); (3) application of PFR-97™ suspension plus release of *D. hesperus* adults (+P, +D); (4) no application of PFR-97™ suspension and no release of *D. hesperus* adults (control) (-P, -D). Every treatment combination was replicated six times within each compartment for a total of 12 replicates.

The trial was conducted over six weeks. During this time, plants assigned (+P, -D) and (+P, +D) received three applications of PFR-97™ suspension and plants assigned (+D, -P) and (+P, +D) received two releases of *D. hesperus* adults (Figure 4.6-1). The first PFR-97™ application was at 100 times the recommended label rate with the intent to expose *D. hesperus* adults to the maximum challenge concentration set out by the Pest Management Regulatory Agency (PMRA) to determine their susceptibility to *P. fumosoroseus* blastospores (Regulatory Directive DIR2001-02, 2004). The second and third applications were made at concentrations equal to 10 times the maximum label rate. For each PFR-97™ application, one leaflet was excised from every plant 12 hours after treatment and incubated under laboratory conditions (28°C and 50% RH). These leaflets were used to detect cross contamination during spraying which would be indicated by sporulating *T. vaporariorum* nymphs and also served as positive controls. Adults of *D. hesperus* were released twice each at a rate of 5 females and 5 males per plant in the appropriate treatment groups.

4.3.5 Sampling Protocols and Statistical Analyses

4.3.5.1 Impact of Treatments on *T. vaporariorum* Population Density

Each plant was sampled *in situ* by counting the number of *T. vaporariorum* nymphs (1^{st}, 2^{nd}, 3^{rd} and 4^{th} instars including puparia), visibly fungus-infected *T. vaporariorum* fourth-instar nymphs, and *T. vaporariorum* fourth-instar nymphs fed on by *D. hesperus*. Twelve leaflets at an approximate area of 40 cm^2 were chosen to be sampled for each plant. To ensure uniform sampling and to guarantee that the sampling represented the overall density of *T. vaporariorum*, the twelve leaflets were chosen with some restriction. The plant was divided into four strata from each of which three leaflets

were randomly sampled; level 1 consisted of leaves 1-4 from the top of the plant, level 2 was leaves 5-8, level 3 was leaves 9-12 and level 4 was leaves 13-18. Plants were sampled twice weekly for seven weeks starting in week zero.

Nymphs of *T. vaporariorum* infected with *P. fumosoroseus* quite often exhibited an orange discoloration prior to death (also observed by Wraight *et al*, 1998). This discolouration is indicative of colonization of the nymph by the fungus and was used to discriminate between healthy and infected *T. vaporariorum* fourth-instar nymphs during sampling. Nymphs exhibiting the orange discoloration were classed as visibly-infected. Nymphs that died as a result of fungal infection remained on the plant leaves and could be easily sampled. Fourth-instar nymphs of *T. vaporariorum* that were fed upon by *D. hesperus* were identified during sampling by an emptied pupal capsule. Other life stages of *T. vaporariorum* did not show discernible signs of infection and were too small to observe signs of *D. hesperus* feeding to be included and consequently the efficacy of both PFR-97™ and *D. hesperus* was severely underestimated in these samples.

Two-factor repeated measures analysis of variance with initial *T. vaporariorum* densities used as a covariate (2-MANOVA-COVARIATE) was used to determine the influence of *D. hesperus* and PFR-97™ on *T. vaporariorum* nymph densities through time. Two-factor analysis of variance (2-ANOVA) was performed to determine the effects of *D. hesperus* and PFR-97™ on *T. vaporariorum* nymph densities in the final week of the trial (Week 6). Two-factor repeated measures analysis of variance (2-MANOVA) was performed to determine the effects of *D. hesperus* on the density of visibly fungus-infected *T. vaporariorum* fourth-instar nymphs through time. A similar analysis was performed (2-MANOVA) to determine the effects of PFR-97™ on the

density of *T. vaporariorum* fourth-instar nymphs fed on by *D. hesperus* adults through time. Such analyses could indicate the occurrence of intraguild predation and identify the intraguild predator(s) by detecting significant interactions between *D. hesperus* and PFR-97™.

4.3.5.2 Effect of PFR-97™ on Population Density and within-Plant Distribution of *D. hesperus*

Whole plant counts of the number of live and dead *D. hesperus* adults per plant, and number of *D. hesperus* adults in each of the four levels of the plant were made weekly, *in situ*. Nymphs of *D. hesperus* could not be counted because they were cryptic and moved quickly within the plant canopy. Adults of *D. hesperus* were also cryptic and consequently the number observed per plant was most likely underestimated. Recovered, dead *D. hesperus* adults were surface sterilized for two minutes in 0.2% sodium hypochlorite, rinsed twice in sterile water, and plated on 2% water agar at 25°C and 100% relative humidity to encourage sporulation of *P. fumosoroseus* on infected cadavers.

The effects of PFR-97™ on the density of live *D. hesperus* adults per plant and the effects of PFR-97™ on the mean proportion of *D. hesperus* adults observed in each of the four plant levels through time were determined using 2-MANOVAs. The latter analyses (one for each level) were specifically designed to identify the effects of PFR-97™ on the position of *D. hesperus* within the plant.

4.3.5.3 Effects of Treatments on Plant Growth and Fruit Set

At the end of week 0, the initial height of each plant was measured. At the end of week 6, the final height of each plant was measured and the growth of each plant over the six week trial was calculated. Following a similar protocol, the second flower truss from

the top of each plant was marked in week 0 with a plastic flag and the number of flower buds present was recorded. At the end of week 6, the marked trusses were examined for the number of developing fruit and the proportion of flowers that set fruit was determined. The influence of PFR-97™ and *D. hesperus* on plant growth and the proportion of flowers that set fruit during the 6-week trial were determined by 2-ANOVAs.

Each data set was analyzed as a balanced design with two fixed factors (*D. hesperus* and PFR-97™) each at two levels (presence or absence) including a compartment blocking factor. Prior to each analysis, residuals were checked for non-uniformity. Data were analyzed using JMP 5: SAS Institute, 2002, Toronto Canada.

4.4 Results

The concentration of the three sequential applications of PFR-97™ suspension were not homogenous; the first foliar application was 100 times the recommended label rate in order to expose *D. hesperus* to the maximum challenge concentration, and the second and third foliar application was 10 times the maximum concentration recommended by the PFR-97™ label (Table 4.7-1). During the six week trial, the mean temperature and relative humidity (± SEM) within plant canopy was 20.8 ± 0.14°C and 59.0 ± 0.48%. During the twelve hours after PFR-97™ applications, the mean temperature and relative humidity (± SEM) was 21.2 ± 0.21°C and 72.3 ± 1.1%. For each PFR-97™ application, leaflets sampled from each plant 12 hours after application and incubated under laboratory conditions revealed that there was no cross contamination of PFR-97™ blastospores as *T. vaporariorum* nymphs on untreated sampled leaflets did not

show signs of fungus infection. As a positive control, the leaflets revealed that the three applications of PFR-97™ suspension resulted in a corrected mortality for *T. vaporariorum* nymphs ranging from 45-65%, while the mortality observed on leaflets from plants treated with water never exceeded 15%.

4.4.1 Impact of Treatments on *T. vaporariorum* Population Density

Densities of *T. vaporariorum* were relatively high throughout the trial; densities nearly doubled each week until the carrying capacity of the plants was reached at approximately 100 nymphs/ 40 cm^2. Foliar applications of PFR-97™ effectively decreased *T. vaporariorum* densities by an average of 45% relative to the controls, during the last three weeks of the trial (Figure 4.6-2: 2-MANOVA-COVARIATE: $F_{1, 35} = 9.97$, $P = 0.0033$). Populations of *D. hesperus* did not cause an overall effect on *T. vaporariorum* densities (Figure 4.6-2: 2-MANOVA-COVARIATE: $F_{1, 35} = 1.81$, $P = 0.19$) and treatments caused a non-significant interaction effect on *T. vaporariorum* densities through time (2-MANOVA-COVARIATE: $F_{1, 35} = 0.28$, $P = 0.60$).

The greatest effect of *D. hesperus* alone was observed in the last week of the trial (week 6) when *T. vaporariorum* densities were reduced by 35% relative to the controls (2-ANOVA: $F_{1, 35} = 4.67$, $P = 0.038$). The sole presence of PFR-97™ had a similar effect, causing a 48% reduction in *T. vaporariorum* densities relative to the control plants (2-ANOVA: $F_{1, 35} = 10.63$, $P = 0.0025$). The combination of *D. hesperus* and PFR-97™ together in week 6 produced an additive effect on *T. vaporariorum* densities as detected by a non-significant interaction effect (Figure 4.6-3: 2-ANOVA: $F_{1, 35} = 0.69$, $P = 0.41$). Together, the treatments caused a reduction in *T. vaporariorum* densities which was greater than that caused by either treatment alone; the combination

of *D. hesperus* and PFR-97™ reduced *T. vaporariorum* densities by 62% relative to the controls.

The density of visibly fungus-infected *T. vaporariorum* fourth-instar nymphs was not affected by the presence of *D. hesperus* (Figure 4.6-4: 2-MANOVA: $F_{1, 36} = 1.89$, $P = 0.18$). The predation rate of *D. hesperus* on fourth *T. vaporariorum* instar nymphs was not affected by foliar applications of PFR-97™ (Figure 4.6-5: 2-MANOVA: $F_{1, 36} = 0.68$, $P = 0.41$). Visibly infected *T. vaporariorum* nymphs were only observed on plants sprayed with PFR-97™ suspensions and started to appear a week after the first application of PFR-97™. Sporulating nymphs were not observed on plants in the greenhouse. Only on one occasion, *T. vaporariorum* nymphs fed on by *D. hesperus* were observed on one control plant (-P, -D) at very low densities due to contamination by a *D. hesperus* adult in week 4.

4.4.2 Effect of PFR-97™ on Population Density and within-Plant Distribution of *D. hesperus*

Fifteen *D. hesperus* cadavers were recovered from plants throughout the 6-week trial; of those, eight were from (+D, -P) treatment plants and seven were from (+P, +D) treatment plants. None showed visible symptoms of *P. fumosoroseus* infection in the greenhouse or after incubation in the laboratory to encourage fungal sporulation. Foliar applications of PFR-97™ did not significantly affect the density of live *D. hesperus* adults per plant (Figure 4.6-6: 2-MANOVA: $F_{1, 20} = 0.73$, $P = 0.40$). The proportion of *D. hesperus* adults within each plant level was not influenced by foliar applications of PFR-97™ indicating that the position of *D. hesperus* within the plant was irrespective of

the PFR-97™ treatment (2-MANOVAs: Level 1: $F_{1,20} = 0.80$, $P = 0.78$; Level 2: $F_{1,20} = 0.39$, $P = 0.54$; Level 3: $F_{1,20} = 2.28$, $P = 0.15$; Level 4: $F_{1,20} = 0.033$, $P = 0.86$).

4.4.3 Effects of Treatments on Plant Growth and Fruit Set

The combined addition of *D. hesperus* adults and PFR-97™ foliar applications did not produce a significant interactive effect on plant growth throughout the trial (2-ANOVA: $F_{1,35} = 3.15$, $P = 0.09$). Individually, the addition of treatments did not affect plant growth (Table 4.7-2: 2-ANOVA: PFR-97™: $F_{1,35} = 3.47$, $P = 0.07$; *D. hesperus*: $F_{1,35} = 0.82$, $P = 0.37$). The proportion of flowers that set fruit was unaffected by: releases of *D. hesperus* adults (Table 4.7-3: 2-ANOVA: $F_{1,36} = 2.69$, $P = 0.11$), foliar applications of PFR-97™ (2-ANOVA: $F_{1,36} = 0.022$, $P = 0.9632$) and a combination of the treatments (2-ANOVA: $F_{1,36} = 0.039$, $P = 0.8450$). The exclusion of pollinators by the plant cages was most likely the cause for the low proportion of flowers that set fruit (< 60%).

4.5 Discussion

When used separately, *D. hesperus* and PFR-97™ caused a 35% and a 48% reduction in *T. vaporariorum* populations relative to the controls in the last week of the 6-week trial, respectively. Acting in concert, the two biological control agents reduced *T. vaporariorum* densities by 62% relative to the control plants. Considering the three possible outcomes of intraguild interactions, my results support additivity. The simultaneous use of *D. hesperus* and PFR-97™ did not cause an interactive effect on *T. vaporariorum* densities and their combined effect was comparable to the sum of their individual effects. Adults of *D. hesperus* did not feed upon visibly infected *T. vaporariorum* nymphs thereby improving the efficacy of *P. fumosoroseus*. Foliar

applications of PFR-97™ did not affect the predation rate of *D. hesperus* adults on fourth-instar nymphs of *T. vaporariorum*. Furthermore, the survival and position within the plant of *D. hesperus* adults were unaffected by PFR-97™. In addition, when the two biological control agents were used alone or in combination, they did not affect the growth of plants or the proportion of flowers that set fruit.

The efficacy of PFR-97™ was relatively quick. Infected *T. vaporariorum* nymphs were detected a week following the first PFR-97™ application and densities of *T. vaporariorum* were significantly reduced by 43%, four weeks after the second application. Previous studies demonstrated similar reduction rates of *T. vaporariorum* populations on greenhouse tomato plants by one or few applications of PFR-97™ (PreFeRal™). An application of PreFeRal™ at a concentration of 2×10^6 colony forming units/ml reduced *T. vaporariorum* densities by 38% in 3 weeks, whereas by 9 weeks, the second application reduced densities by 77% (Bolckmans *et al.*, 1995). Van de Veire and Degheele (1996) reported a 63% reduction of *T. vaporariorum* densities three weeks after the first PFR-97™ application at a rate of 1 gram product/liter water, and by four weeks, a second PFR-97™ application reduced densities by 79%. The greater efficacy reported by Van de Veire and Degheele (1996) may be due to the use of a surfactant (Citowett), different formulations, spray concentrations, environmental conditions after applications and the initial density of *T. vaporariorum* populations.

Conversely, *D. hesperus* adults did not significantly reduce *T. vaporariorum* densities until the sixth and final week of the trial. A number of factors may have been individually or collectively responsible for this delay. Firstly, the number of *D. hesperus* adults released may have been insufficient to control *T. vaporariorum* populations. In the

last two weeks, *D. hesperus* efficacy may have been enhanced by the presence of *D. hesperus* nymphs and newly emerged adults thereby increasing the density of *D. hesperus* feeding on *T. vaporariorum* populations. The presence of *D. hesperus* nymphs and newly emerged adults indicates the tolerance of *D. hesperus* to applications of PFR-97™ as female adults laid eggs in treated plants and these eggs hatched and produced a new generation. This indicates that *D. hesperus* can likely establish populations in commercial greenhouse tomato crops that are being treated with PFR-97™. Secondly, *D. hesperus* nymphs may have consumed more *T. vaporariorum* nymphs than *D. hesperus* adults consequently boosting the overall reduction of *T. vaporariorum* densities. Nymphs of *Dicyphus hyalinipennis* Burmeister (Hemiptera: Miridae) consumed more *Aphis gossypii* Glover (Hemiptera: Aphididae) and *T. urticae*, than *D. hyalinipennis* females although they had similar predation rates on *T. vaporariorum* and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Ceglarska, 1999). However, it is unlikely as nymphs and adults of *D. tamaninii* and *M. caliginosus* exhibited similar predation rates on all life stages of *B. tabaci* (Barnadas *et al.*, 1998). Thirdly, significant reductions of *T. vaporariorum* densities by *D. hesperus* may have been delayed because of their extensive establishment period. McGregor *et al.* (1999) reported that it took 5-6 weeks for *D. hesperus* adults to establish on greenhouse tomato plants containing *T. vaporariorum*. However, the use of alternate host plants such as mullein, *Verbascum thapsus* L. (Scrophulariaceae), has been shown to contribute to an earlier establishment of *D. hesperus* on greenhouse tomato crops thereby increasing their biocontrol efficacy of *T. vaporariorum*. During the six weeks after release, densities of *D. hesperus* increased by

fourteen times when mullein plants were interspersed within the tomato crop whereas the density only doubled on tomato plants in the absence of mullein (Sanchez *et al.*, 2003).

The compatibility of multiple biological control agents for the control of whitefly populations has been previously studied. An application of PFR-97™ did not affect the developing parasitoid, *E. formosa* inside *T. vaporariorum* nymphs on bean seedlings when sprayed at the recommended label rate under laboratory conditions (Van de Veire and Degheele, 1996). Also in the laboratory, *E. formosa* were able to reject *T. vaporariorum* nymphs that had been treated seven days before with the fungus *A. aleyrodis* thereby causing reductions in *T. vaporariorum* populations complementary to the fungus (Fransen and van Lenteren, 1993). Furthermore, an application of *A. aleyrodis* at a concentration of 2.2×10^7 spores/ml, did not affect the developing *E. formosa* inside parasitized *T. vaporariorum* nymphs when treated at least 4 days after parasitization with no direct or indirect effects on the emerged parasitoids and their progeny (Fransen and van Lenteren, 1994). The combination of the parasitoids, *E. formosa* and *Eretmocerus eremicus* Rose (Hymenoptera: Aphelinidae) or *E. formosa* and *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) has been reported to provide adequate control of *B. tabaci* populations. Adding a third biological control agent such as *M. caliginosus* provided better control over a long period (see review by van Lenteren, 2000).

Intraguild interactions between arthropod and fungal pathogen natural enemies are usually investigated under laboratory conditions (see examples in Rosenheim *et al.*, 1995; Pell *et al,* 1997; Poprawski *et al*, 1998; Roy *et al,* 1998; Pell and Vandenberg, 2002). Only a small number of studies have been done outside the laboratory (James *et*

al., 1995; Mesquita *et al.*, 1997; Poprawski *et al.*, 1997; Jacobson *et al.*, 2001). Laboratory experiments can be poor predictors of what truly occurs in "nature" (Hajek *et al*, 1996; Goettel *et al*, 2001; Inglis *et al*, 2001). Artificial laboratory conditions could exclude or wrongly manipulate important factors such alternate prey, and spatial and temporal heterogeneity that could affect the occurrence and severity of the interference between natural enemies (Rosenheim *et al.*, 1995 and Roy and Pell, 2000).

The use of multiple natural enemies for the control of *T. vaporariorum* populations is feasible but requires extensive knowledge about the biology of each of the natural enemies, the host and the potential occurrence and severity of intraguild interactions. I found that the predatory mirid, *D. hesperus* and the microbial insecticide, PFR-97™, are each efficacious natural enemies of *T. vaporariorum* populations and when used together improved *T. vaporariorum* control. In a biological control program combining both natural enemies, I recommend that *D. hesperus* should be used as a preventative control measure in which they are released early in the growing season while *T. vaporariorum* populations are low. Throughout the season, *T. vaporariorum* outbreaks could be controlled quickly by applications of PFR-97™.

4.6 Figures

Figure 4.6-1: Timing of *T. vaporariorum* release and establishment, and treatment addition to confined tomato plants during a greenhouse trial which investigated the individual and combined effects of PFR-97™ treatment and *D. hesperus* adults treatment on *T. vaporariorum* populations. Adult *D. hesperus* females were released (10/plant) at the times indicated by the vertical black arrows. Foliar applications of PFR-97™ suspensions were made to plants, using a single nozzle 7-liter backpack sprayer targeting the underside of the leaves, at the times indicated by the vertical grey arrows.

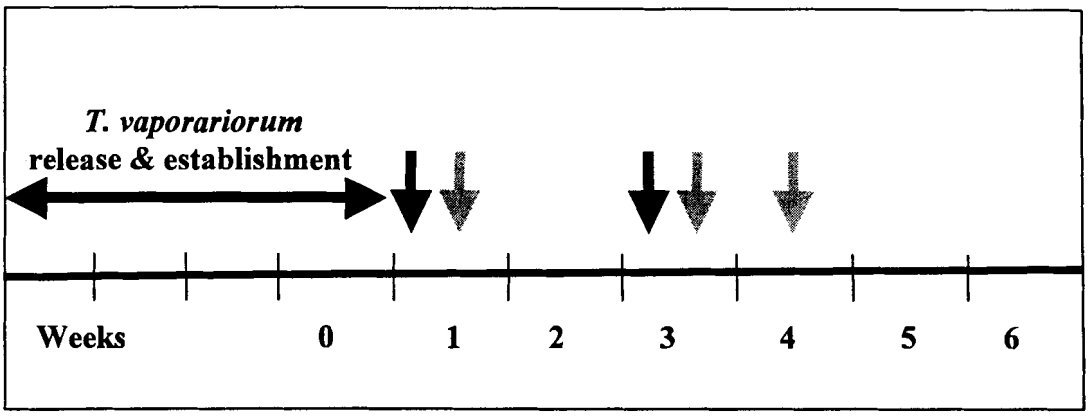

T. vaporariorum
release & establishment
Weeks
0
1
2
3
4
5
6

Figure 4.6-2: Mean densities of *T. vaporariorum* nymphs per leaflet (± SEM) throughout the 6-week greenhouse trial, as functions of PFR-97™ (P) and *D. hesperus* adult (D) treatment combinations. Black arrows signify releases of *D. hesperus* adults; grey arrows signify foliar applications of PFR-97™ suspensions. Sample size per bar was 12 plants.

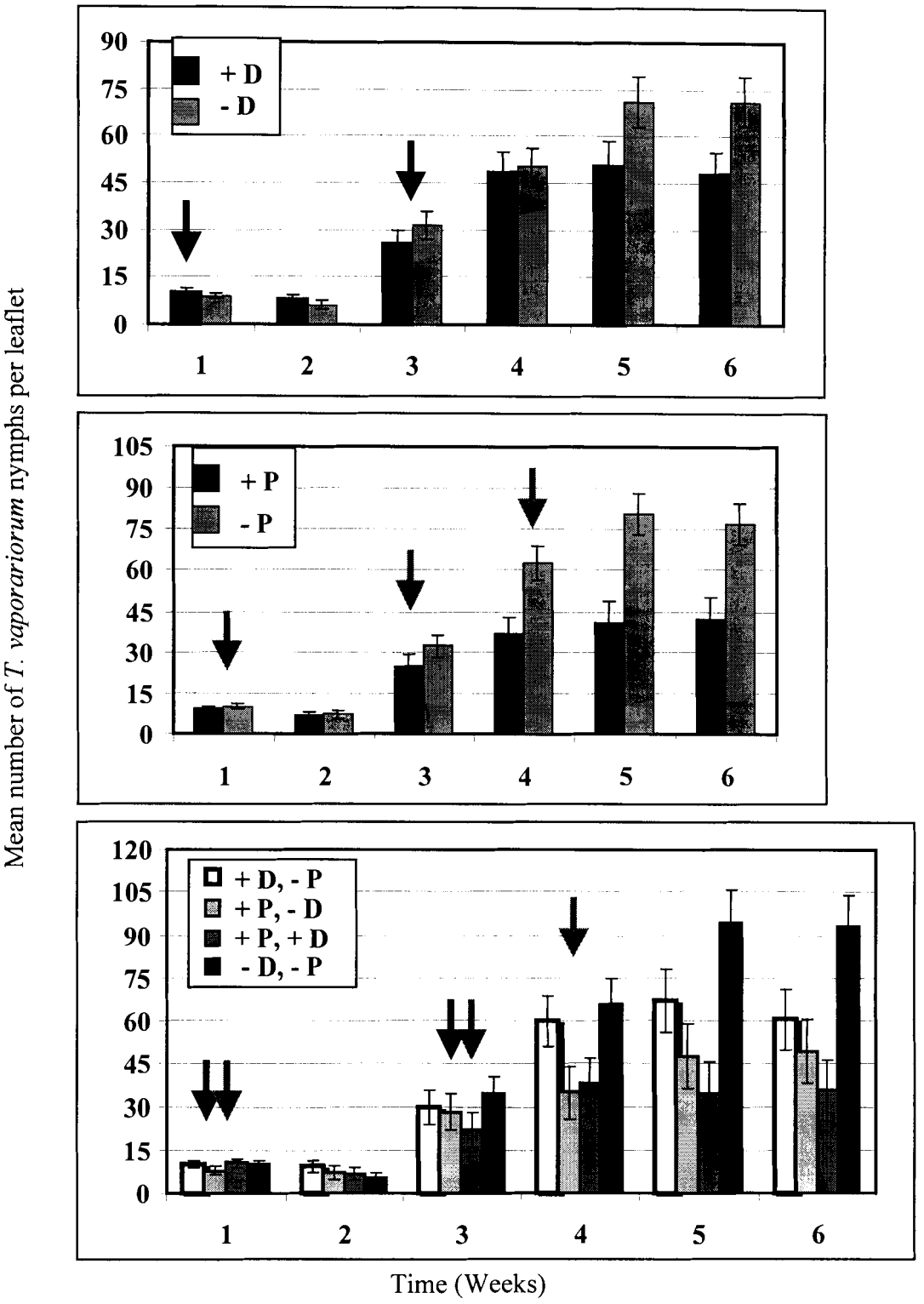


Figure 4.6-2: Mean densities of *T. vaporariorum* nymphs per leaflet (± SEM) throughout the 6-week greenhouse trial, as functions of PFR-97™ (P) and *D. hesperus* adults (D) treatment combinations. Black arrows signify releases of *D. hesperus* adults; grey arrows signify foliar applications of PFR-97™ suspensions. Sample size per bar was 12 plants.

Figure 4.6-3: Interaction plot for mean densities of *T. vaporariorum* nymphs (± SEM) during the last week of the trial (Week 6) showing the additive effects of *D. hesperus* (D) and PFR-97™ (P) on *T. vaporariorum* densities. The presence and absence of either treatment was indicated by "+" and "-" symbol, respectively. Sample size for each point was 12 plants.

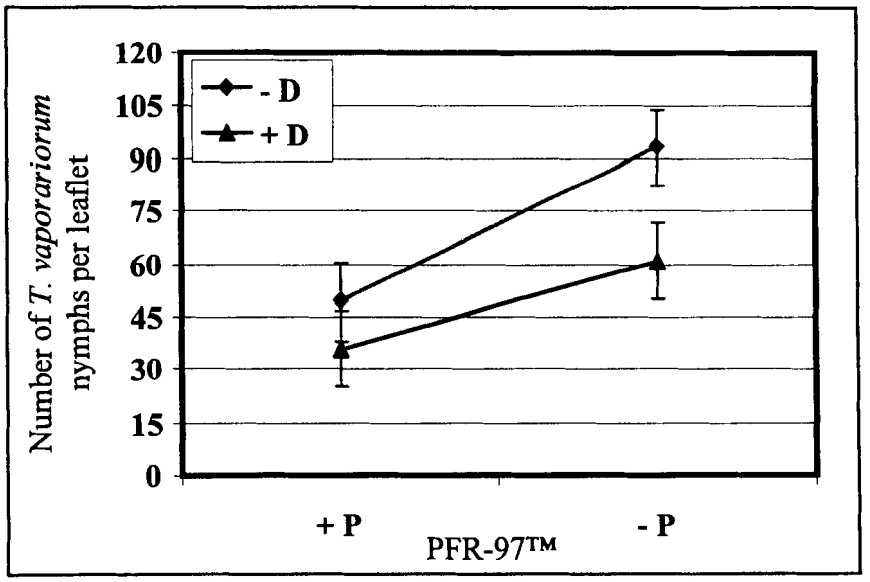

120
105
90
75
60
45
30
15
0
- D
+ D
Number of T. vaporariorum nymphs per leaflet
+ P
- P
PFR-97™

Figure 4.6-4: Effects of the presence (+D) and absence (-D) of *D. hesperus* adults on the mean density (± SEM) of fungus-infected fourth-instar nymphs of *T. vaporariorum* through time on caged greenhouse tomato plants, which received foliar applications of PFR-97™ (+P). Adult *D. hesperus* were released (10 per plant) at the times indicated by the black arrows. Sample size for each point was 12 plants.

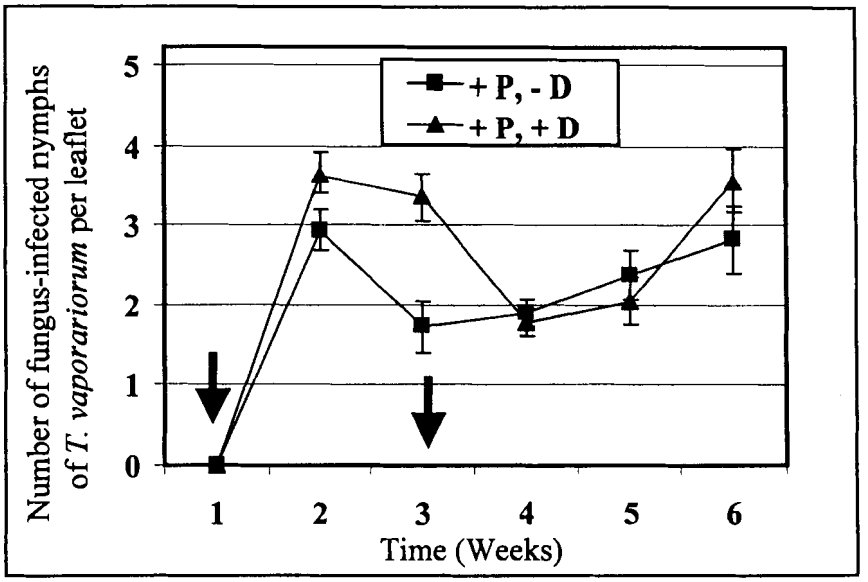

Number of fungus-infected nymphs of *T. vaporariorum* per leaflet
5
4
3
2
1
0
+ P, - D
+ P, + D
1
2
3
4
5
6
Time (Weeks)

Figure 4.6-5: Effects of the presence (+P) and absence (-P) of PFR-97™ applications on the mean density (± SEM) of fourth-instar nymphs of *T. vaporariorum* fed on by *D. hesperus* through time on caged greenhouse tomato plants, which received 2 releases of 10 *D. hesperus* adults per plant (+D). Grey arrows signify PFR-97™ applications made with a single nozzle backpack sprayer. Sample size for each point was 12 plants.

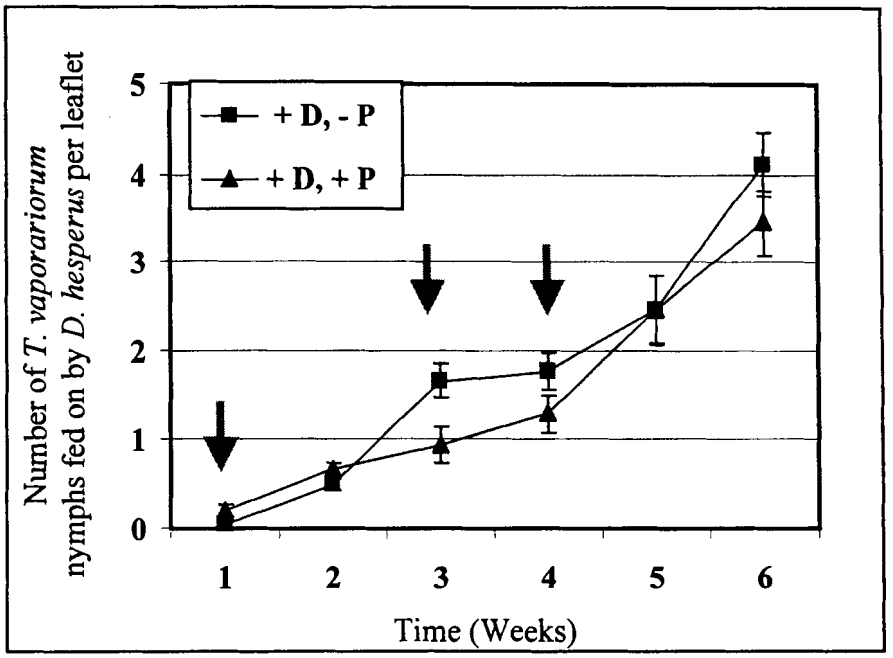
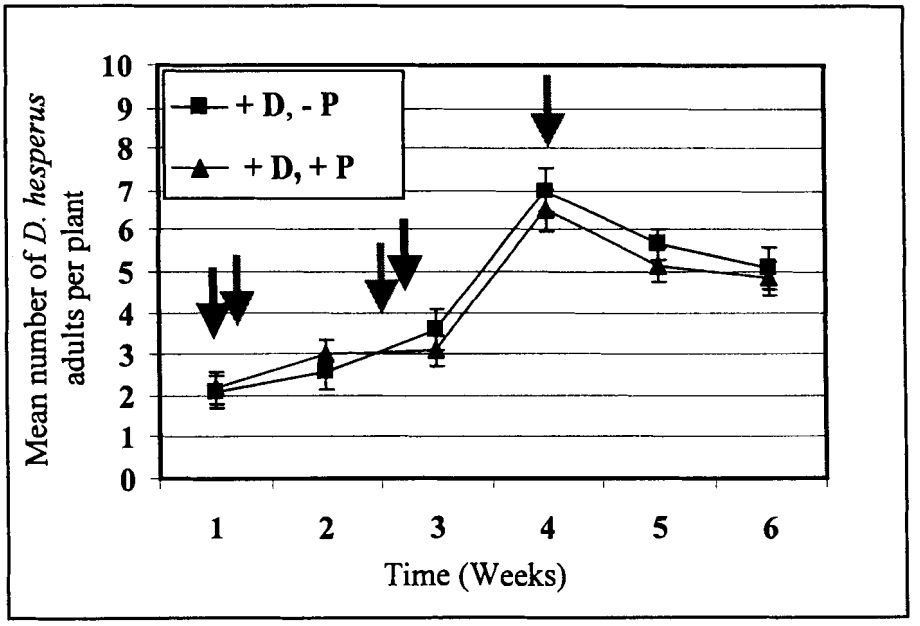

Number of T. vaporariorum nymphs fed on by D. hesperus per leaflet
5
4
3
2
1
0
+ D, - P
+ D, + P
1
2
3
4
5
6
Time (Weeks)

Figure 4.6-6: Effects of the presence (+P) and absence (-P) of PFR-97™ applications on the mean density (± SEM) of *D. hesperus* adults through time on caged greenhouse tomato plants. Adult *D. hesperus* were released (10/plant) at the times indicated by the black arrows and the grey arrows signify PFR-97™ applications made with a single nozzle backpack sprayer. Sample size for each point was 12 plants.


Mean number of D. hesperus adults per plant
10
9
8
7
6
5
4
3
2
1
0
+ D, - P
+ D, + P
1
2
3
4
5
6
Time (Weeks)

4.7 Tables

Table 4.7-1: Blastospore viability, concentration in suspension, and blastospores deposited per mm^2 (mean ± SEM) for 3 sequential applications of PFR-97™ suspension that were made during a 6-week greenhouse trial investigating the effects of PFR-97™ and *D. hesperus* adults on *T. vaporariorum* populations established on confined tomato plants.

Application Date (Week)	Blastospore Viability (%)	Concentration viable blastospores (x 10^7) / ml	Spore Deposition viable blastospores ($x10^3$) / mm^2
1	79 ± 1.90	18.50 ± 0.8[1]	7.66 ± 0.573
3	82.73 ± 1.41	1.27 ± .037[2]	1.59 ± 0.156
4	81.95 ± 0.69	1.23 ± 0.164[2]	1.502 ± 0.1401

[1] The first foliar application of PFR-97™ was at 100 times the recommended label rate in order to expose the non-target arthropod, *D. hesperus* to the maximum challenge concentration set out by the Pest Management Regulatory Agency (PMRA)

[2] The concentration of the second and third foliar application of PFR-97™ were 10 times the maximum recommended label rate

Table 4.7-2: The effect of releases of *D. hesperus* adults (+D) and applications of PFR-97™ (+P), or a combination of neither ((-D) nor (-P)) or both ((+D) and (+P)) on the mean growth (± SEM) of plants infested with *T. vaporariorum* during the 6-week trial. Sample size for each treatment was 12 plants.

Plant Growth (m)

Treatments	Mean ± SEM
+ D, - P	0.78 ± 0.02
+ P, - D	0.71 ± 0.03
+ P, + D	0.70 ± 0.02
- P, - D	0.71 ± 0.02

Means are not significantly different ($P < 0.05$) as determined with Tukey's HSD test

Table 4.7-3: The effects of releases of *D. hesperus* adults (+D) and applications of PFR-97™ (+P), or a combination of neither ((-D) nor (-P)) or both ((+D) and (+P)) on the mean proportion (± SEM) of flowers that set fruit during the 6-week trial. Sample size for each treatment was 12 plants.

Percent of Flowers that Set Fruit

Treatments	Mean ± SEM
+ D, - P	0.46 ± 0.065
+ P, - D	0.56 ± 0.068
+ P, + D	0.44 ± 0.062
- P, - D	0.55 ± 0.065

Means are not significantly different ($P < 0.05$) as determined with Tukey's HSD test for mean separation

4.8 References

Albajes, R., O. Alomar, J. Riudavets, C. Castane, J. Arno, and Gabarra, 1996. The mirid bug *Dicyphus tamaninii*: an effective predator for vegetable crops. OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 1-4.

Barnadas, I., R. Gabarra, and R. Albajes, 1998. Predatory capability of two mirid bugs preying on *Bemisia tabaci* Entomologia Experimentalis et Applicata 86: 215-219.

Bolckmans, K., G. Sterk, J. Eyal, B. Sels, and Stepman, 1995. PreFeRal, (*Paecilomyces fumosoroseus* (Wize) Brown and Smith, strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses Medische Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 707-711.

Brodeur, J., C. Cloutier and D. Gillespie, 2002. High order predators in greenhouse systems. IOBC/WPRS Bulletin: Integrated control in protected crops, temperate climate 25(1): 33-36.

Ceglarska, E.B., 1999. *Dicyphus hyalinipennis* Burm. (Heteroptera: Miridae): a potential biological control agent for glasshouse pests in Hungary IOBC Bulletin: Integrated Control in Glasshouses 22 (1): 33-36

Fargues, J., C. Vidal, N. Smits, M. Rougier, T. Boulard, M. Mermier, P. Nicot, P. Reich, B. Jeannequin, G. Ridray, and J. Lagier, 2003. Climatic factors on entomopathogenic hyphomycetes infection of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in Mediterranean glasshouse tomato Biological Control 28: 320-331.

Faria, M. and S.P. Wraight, 2001. Review Article: Biological control of *Bemisia tabaci* with fungi Crop Protection 20: 767-778.

Ferguson, K.I. and P. Stiling, 1996. Non-additive effects of multiple natural enemies on aphid populations Oecologia 108: 375-379.

Fransen, J.J., K. Winkelman, and J.C. van Lenteren, 1987. The differential mortality at various life stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), by infection of the fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomycetes) Journal of Invertebrate Pathology 50: 158-165.

Fransen, J.J. and J.C. van Lenteren, 1993. Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 69: 239-249.

Fransen, J.J. and J.C. van Lenteren, 1994. Survival of the parasitoid *Encarsia formosa* after treatment of parasitized greenhouse whitefly larva with fungal spores of *Aschersonia aleyrodis* Entomologia Experimentalis et Applicata 71: 235-243.

Gabarra, R., C. Castane, E. Bordas, and Albajes, 1988. *D. tamaninii* as a beneficial insect and pest in tomato crops in Catalonia, Spain Entomophaga 33: 219-228.

Goettel, M.S., T.J. Poprawski, J.D. Vandenberg, Z. Li, and D.W. Roberts, 1990. Safety to nontarget invertebrates of fungal biocontrol agents: in Safety of Microbial Insecticides (ed. M. Lard, L.A. Lacey, E.W. Davidson), pg. 209-231.

Goettel, M.S. and G.D. Inglis, 1997. Fungi: Hyphomycetes Chapter 5 of Manual of techniques in insect pathology (ed L.A. Lacey) Academic Press, San Diego California, pg. 213-249.

Goettel, M.S. A.E. Hajek, J.P. Siegel, and H.C. Evans, 2001. Safety of fungal biocontrol agents. Chapter 13 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan) CAB International, pg. 347-375.

Hajek, A.E., L. Butler, S.R.A. Walsh, J.C. Silver, F.P. Hain, F.L. Hastings, T.M. Odell, and D.R. Smitley, 1996. Host range of the gypsy moth (Lepidoptera: Lymantridae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomopthorales) in the field versus the laboratory Environmental Entomology 25: 709-721.

Inglis, G.D., M.S. Goettel, T.M. Butt, and H. Strasser, 2001. Use of hyphomycetous fungi for managing insect pests Chapter 3 of Fungi as biocontrol agents (eds T.M. Butt, C. Jackson, and N. Magan), CAB International, pg. 23-69.

Jacobson, R.J., D. Chandler, J. Fenlon, and K.M. Russell, 2001. Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants Biocontrol Science and Technology 11(3): 391-400.

Jakobsen, L., A. Enkegaard, and H.F. Brodsgaard. 2002. Interactions between the two polyphagous predators *Orius majusculus* and *Macrolophus caliginosus.* IOBC/WPRS Bulletin: Proceedings of the meeting, integrated control in protected crops, temperate climates, held in British Columbia, Canada, 6-9 May 2002, 25 (1): 115-118.

James, R.J., B.T. Shaffer, B. Croft, and B. Lighthart, 1995. Field evaluations of *Beauveria bassiana:* its persistence and effects on the pea aphid and a non-target coccinellid in alfalfa Biocontrol Science and Technology 5: 425-437.

Malais, M.H. and W.J. Ravensberg, 2003. Knowing and recognizing: the biology of glasshouse pests and their natural enemies. Koppert B.V., the Netherlands.

McGregor, R.M, D.R. Gillespie, D.M.J. Quiring, and M.R.J. Foisy, 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes Biological Control 16: 104-110.

McGregor, R.M., Gillespie, D.R., C.G. Park, D.M.J. Quiring, and M.R.J. Foisy, 2000. Leaves or fruit? The potential for damage to tomato fruits by the omnivorous predator, *Dicyphus hesperus* Entomologia Experimentalis et Applicata 95: 325-328.

Meekes, E.T.M., J.J. Fransen and J.C. van Lenteren, 2002. Pathogenicity of *Aschersonia* spp. against whiteflies *Bemisia argentifolii* and *Trialeurodes vaporariorum* Journal of Invertebrate Pathology 81: 1-11.

Mesquita, A.L.M., L.A. Lacey, and F. Lecant, 1997. Individual and combined effects of the fungus, *Paecilomyces fumosoroseus* and parasitoid, *Aphelinus asychis* Walker (Hym., Aphelinidae) on confined populations of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hom.: Aphididae) under field conditions Journal of Applied Entomology 121: 151-163.

Muller, C.B. and J. Brodeur, 2002. Intraguild predation in biological control and conservation biology Biological Control 25: 216-223.

Osborne, L.S. and Z. Landa, 1992. Biological control of whiteflies with entomopathogenic fungi Florida Entomologist 75(4): 456-471.

Pell, J.K., R. Pluke, S.J. Clark, M.G. Kenward, and P.G. Alderson, 1997. Interactions between two aphid natural enemies, the entomopathogenic fungus, *Erynia neoaphidis* Remaudiere and Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Journal of Invertebrate Pathology 69: 261-268.

Pell, J.K. and J.D. Vandenberg, 2002. Interactions among the aphid *Diuraphis noxia*, the entomopathogenic fungus *Paecilomyces fumosoroseus* and the coccinellid *Hippodamia convergens* Biocontrol Science and Technology 12: 217-224.

Polis, G.A. and R.D. Holt, 1992. Intra-guild predation: the dynamics of complex trophic interactions Trends in Ecology and Evolution 7: 151-154.

Poprawski, T.J., R.I. Carruthers, J.Speece III, D.C. Vadek, and L.E. Wendel, 1997. Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle Biological Control 10: 48-57.

Poprawski, T.J., J. C. Legaspi and P. Parker, 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Aleyrodidae) Biological Control 27(3): 785-795.

Poprawski, T.J., S.M. Greenberg, and M.A. Ciomperlik, 2000. Effect of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*- induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) Environmental Entomology 29(5): 1048-1053.

Regulatory Directive DIR2001-02, 2004. Guideline for the registration of microbial pest control agents and products. http://www.hc-sc-gc.ca/pmra-arla/english/pdf/dir/dir2001-02-e.pdf .

Rosenheim, J.A., H.K. Kaya, L.E. Ehler, J.J. Marois and B.A. Jaffee, 1995. Intraguild predation among biological-control agents: theory and evidence Biological Control 5: 303-335.

Roy, H.E., J.K. Pell, S.J. Clark, and P.G. Alderson, 1998. Implications of predator foraging on aphid pathogen dynamics Journal of Invertebrate Pathology 71: 236-247.

Roy, H.E. and J.K. Pell, 2000. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control Biocontrol Science and Technology 10: 737-752.

Salamero, A. and R. Gabarra, 1987. Observations on the predatory and phytophagous habits of *Dicyphus tamaninii* Wagner (Heteroptera: Miridae). SROP/WPRS Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Budapest, Hungary. 26-30 April 1987, 10(2): 165-169.

Sampson C. and V.J. King, 1996. *Macrolophus caliginosus*, field establishment and pest control effect in protected tomatoes. OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 143-146.

Sanchez, J.A., R.R. McGregor, and D.R. Gillespie, 2002. Sampling plan for *Dicyphus hesperus* (Heteroptera: Miridae) on greenhouse tomatoes Environmental Entomology 31(2): 331-338.

Sanchez, J.A., D.R. Gillespie and R.R. McGregor, 2003. The effects of mullein plants (*Verbascum thapsus*) on the population dynamics of *Dicyphus hesperus* (Heteroptera: Miridae) in tomato greenhouses Biological Control 28: 313-319.

SAS Institute Inc., 2002. Version JMP 5.0 Cary, NC. USA.

Shah, P.A. and M.S. Goettel, 1999. Directory of microbial control products and services. Microbial Control Division, Society for Invertebrate Pathology 1-30.

Sinia, A., B. Roitberg, R.R. McGregor, and D.R. Gillespie, 2004. Prey feeding increases water stress in the omnivorous predator *Dicyphus hesperus* Entomologia Experimentalis et Applicata 110: 243-248.

Van de Veire, M. and D. Degheele, 1996. Toxicity of the fungal pathogen *Paecilomyces fumosoroseus* strain Apopka 97 to the greenhouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*, and the first results of a control experiment in glasshouse tomatoes OILB/SROP Bulletin: Proceedings of the meeting, integrated control in glasshouses, held in Vienna, Austria, 20-25 May 1996, 19(1): 191-194.

van Lenteren, J.C., H.J.W. van Roermund and S. Sutterlin, 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum)* with the parasitoid *E. formosa*: How does it work? Biological Control 6: 1-10

van Lenteren, J.C., Y.C. Drost, H.J.W. van Roermund and C.J.A.M. Posthuma-Doodeman, 1997. Aphelinid parasitoids as suitable biological control agents in greenhouses Journal of Applied Entomology 121: 473-485.

van Lenteren, J.C., 2000. A greenhouse without pesticides; fact or fantasy? Crop Protection 19: 375-384.

Wraight, S.P., R.I. Carruthers, C.A. Bradley, S.T. Jaronski, L.A. Lacey, P. Wood, and S. Galaini-Wraight, 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii* Journal of Invertebrate Pathology 71: 217-226.

CHAPTER 5 CONCLUSION

5.1 Combination of Entomopathogenic Fungi and Insect Predators

The simultaneous use of an entomopathogenic fungus with an insect predator for the suppression of a pest species can be detrimental to the biological control program. Hyphomycete fungi tend to have broad host ranges and could potentially reduce the population of the complementary predator by directly infecting it (Goettel and Inglis, 1997). In turn, predators can pose a threat to the entomopathogenic fungus by consuming fungus-infected prey items, thereby reducing the population of the fungus. However, the feeding behaviour of the predator can play an important role in minimizing or eliminating the antagonistic interactions between itself and the entomopathogenic fungus. For example, the availability of feeding choices within the target pest population could reduce prey/host competition between the predator and the fungal pathogen (Pell *et al.*, 1997). When feeding choices are available, the predator will most likely consume the more suitable uninfected prey item rather than the nutrient deprived fungus-infected prey item. For example, when *D. hesperus* were offered the choice between a healthy prey item and an infected prey item, *D. hesperus* consumed the uninfected prey item 75% of the time (Chapter 3). Furthermore, the ability of the predator to discriminate between healthy and fungus-infected prey items minimizes the contact between the predator and the fungus. The predator is then less likely to become infected by the fungus and the fungal population is less likely to be reduced by the predator. Features defining the system in which the entomopathogenic fungus and the predator are co-existing can also

reduce or prevent antagonistic interaction between fungal pathogen and the insect predator such as environmental conditions, type of host plant, and spatial and temporal allocation (Roy and Pell, 2000).

The majority of studies investigating intraguild interactions between arthropod and fungal pathogen natural enemies are usually investigated under laboratory conditions (see examples in Rosenheim *et al.*, 1995; Pell *et al,* 1997; Poprawski *et al*, 1998; Roy *et al.,* 1998; Pell and Vandenberg, 2002). Only a small number of studies have been done under natural conditions such as in the greenhouse or field (James *et al.*, 1995; Mesquita *et al.*, 1997; Poprawski *et al.*, 1997; Jacobson *et al.*, 2001). Laboratory studies can be useful when attempting to identify the potential for intraguild interactions between natural enemies. However, they cannot be realistically used to make inferences to natural conditions because they do not incorporate all of the possible factors that can influence the presence, outcome and severity of intraguild interactions (Roy and Pell, 2000).

In summary, there are a number of factors that need to be considered along with the host range of the entomopathogenic fungus and the insect predator when investigating the potential of intraguild interactions. The behaviour of the predator, and the features of the system they co-exist in, can influence the outcome of the intraguild interactions which in turn impacts the biological control program of the target pest. Thus, intraguild interactions should be studied in natural conditions as these factors and others can be incorporated.

5.2 Proposed Recommendations

The simultaneous use of PFR-97™ and *D. hesperus* enhanced the biological control of *T. vaporariorum* populations. Both, PFR-97™ and *D. hesperus* independently reduced high population densities of *T. vaporariorum.* PFR-97™ did not cause substantial mortality of *D. hesperus* adults under laboratory or greenhouse conditions (Chapter 2 and Chapter 3). Interference between PFR-97™ and *D. hesperus* was minimized by the ability of *D. hesperus* to discriminate between infected and uninfected *T. vaporariorum* nymphs.

I recommend that *D. hesperus* should be used preventatively. Early release would provide *D. hesperus* time to establish on the crop prior to pest pressures; establishment takes approximately 6 weeks. Due to the generalist nature of *D. hesperus*, populations should be able to survive during low *T. vaporariorum* densities by preying on other greenhouse pests such as aphids, thrips and spider mites. In addition, the use of banker plants, such as mullein, could help sustain *D. hesperus* populations by serving as an alternate host plant and possibly, as an alternate food source. PFR-97™ should be used curatively, in which spray applications are made during *T. vaporariorum* outbreaks that are not sufficiently controlled by *D. hesperus*. PFR-97™ is a control product that is relatively quick acting and can reduce high *T. vaporariorum* densities. In these areas of overlapping control measures, minimum interference between *D. hesperus* and PFR-97™ would occur as they do not significantly affect each other's biological control efficacy. PFR-97™ could also be used effectively at the end of the growing season, when developing fruit is susceptible to feeding damage by *D. hesperus*. As with any biological control program, monitoring of the pests and the biological control agents is critical.

Even though PFR-97™ can reduce high *T. vaporariorum* densities, substantial reductions can take as long as two weeks depending on the dose. To ensure that *T. vaporariorum* outbreaks remain controlled, they need to be detected early.

5.3 Future Research Directions

5.3.1 Expansion of Chapter 3

In Chapter 3, I concentrated on identifying potential antagonistic intraguild interactions between *D. hesperus* and PFR-97™ in the laboratory. I propose that future research focus on: (1) effect of alternative prey on the feeding preferences of *D. hesperus*; (2) the impact of *D. hesperus* consuming infected prey and; (3) the ability of *D. hesperus* to vector *P. fumosoroseus* from infected to healthy prey. In my discrimination trials, I offered *D. hesperus* females a choice between healthy and PFR-97™-treated *T. vaporariorum* nymphs. However, the availability of alternative prey items could have an impact on their feeding decision. Being a generalist predator, *D. hesperus* feeds on multiple prey items and can survive and complete its nymphal development on spidermites, *Tetranychus urticae* (McGregor *et al.*, 1999). It would be interesting to look at the feeding decisions of *D. hesperus* when offered the choice between a PFR-97™-treated *T. vaporariorum* nymph and an alternative prey item, such as a healthy spidermite. Although spidermites are considered to be a lower food quality compared to *T. vaporariorum* (McGregor *et al.*, 1999), *D. hesperus* may regard them as a more favorable prey item when compared to an infected *T. vaporariorum* nymph.

Results indicated that *D. hesperus* will consume PFR-97™-treated *T. vaporariorum* nymphs when the nymphs are in the early stages of fungal infection.

Therefore, it is worthwhile examining the impact of *D. hesperus* feeding on infected prey. Although it is unlikely that the process of fungal infection would take place in the alimentary canal of *D. hesperus*, feeding and subsequent grooming could facilitate the penetration and germination of the spores on their head and body, resulting in mortality. From a behavioral aspect, it would be interesting to note if *D. hesperus* "learned" to avoid infected nymphs based on previous feeding bouts.

Lastly, the ability of *D. hesperus* to act as a vector of *P. fumosoroseus* inoculum would provide synergistic mortality of *T. vaporariorum*. Coccinellid beetles have been shown to act as vectors of fungal inoculum; *Hippodamia convergens* contaminated with spores transferred the spores to healthy aphid populations and initiated infection (Pell and Vandenberg, 2002). Fungal transmission by *D. hesperus* would cause syngeristic mortality of *T. vaporariorum* that would compensate for their antagonistic interactions with PFR-97™.

5.3.2 Expansion of Chapter 4

In Chapter 4, my primary objective was to determine if the simultaneous use of *D. hesperus* and PFR-97™ would lead to intraguild interactions. I propose that future research should focus on: (1) the potential of *D. hesperus* to interact with other biocontrol agents in the greenhouse; (2) the impact of PFR-97™ on *D. hesperus* nymphs and; (3) the ability of *D. hesperus* and PFR-97™ to reduce *T. vaporariorum* densities below the economic threshold. Being a high-order predator, it is likely that *D. hesperus* will consume biological control agents used in the greenhouse. For example, *D. hesperus* readily consumes spidermites and consequently, may also feed on beneficial predatory mites, such as *Amblyseius cucumeris* and *A. degenerans*, which are natural enemies of

thrips (Malais and Ravensberg, 2003). Intraguild interactions between biocontrol agents could have a significant impact on the overall success of biological control in the greenhouse.

Due to the cryptic nature of *D. hesperus* nymphs, I was unable to monitor their population density throughout the greenhouse trial. Consequently, I do not know how they were affected by PFR-97™ spray applications. I propose that the impact of PFR-97™ on *D. hesperus* nymphs should be investigated on potted tomato plants that are caged in the greenhouse. The size of the plant would help locate the nymphs and the potted plants will provide conditions that are similar to greenhouse grown plants. Because *D. hesperus* are used as a preventative control measure, it is important that they can establish themselves on the crop and increase their population in response to *T. vaporariorum* populations.

Lastly, it is important to test whether *D. hesperus* and PFR-97™ can reduce whitefly densities below the population threshold level (1 adult/100 plants). In my greenhouse microcosms, the plants were infested with a high density of *T. vaporariorum* to ensure that a treatment effect would be detected. Greenhouse growers would never let *T. vaporariorum* reach such high population levels. Potentially, *T. vaporariorum* density could have an impact on the severity of the intraguild interactions between *D. hesperus* and PFR-97™ as interference could be more severe under low prey densities.

5.4 References

Goettel, M.S. and G.D. Inglis, 1997. Fungi: Hyphomycetes Chapter 5 of Manual of techniques in insect pathology (ed L.A. Lacey) Academic Press, San Diego California, pg. 213-249.

Jacobson, R.J., D. Chandler, J. Fenlon, and K.M. Russell, 2001. Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants Biocontrol Science and Technology 11(3): 391-400.

James, R.J., B.T. Shaffer, B. Croft, and B. Lighthart, 1995. Field evaluations of *Beauveria bassiana:* its persistence and effects on the pea aphid and a non-target coccinellid in alfalfa Biocontrol Science and Technology 5: 425-437.

Malais, M.H. and W.J. Ravensberg, 2003. Knowing and recognizing: the biology of glasshouse pests and their natural enemies Koppert B.V., the Netherlands.

McGregor, R.R., D.R. Gillespie, D.M.J. Quiring and M.R.J. Foisy, 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes Biological Control 16: 104-110.

Mesquita, A.L.M., L.A. Lacey, and F. Lecant, 1997. Individual and combined effects of the fungus, *Paecilomyces fumosoroseus* and parasitoid, *Aphelinus asychis* Walker (Hym., Aphelinidae) on confined populations of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hom.: Aphididae) under field conditions Journal of Applied Entomology 121: 151-163.

Pell, J.K., R. Pluke, S.J. Clark, M.G. Kenward, and P.G. Alderson, 1997. Interactions between two aphid natural enemies, the entomopathogenic fungus, *Erynia neoaphidis* Remaudiere and Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Journal of Invertebrate Pathology 69: 261-268.

Pell, J.K. and J.D. Vandenberg, 2002. Interactions among the aphid *Diuraphis noxia*, the entomopathogenic fungus *Paecilomyces fumosoroseus* and the coccinellid *Hippodamia convergens* Biocontrol Science and Technology 12: 217-224.

Poprawski, T.J., R.I. Carruthers, J.Speece III, D.C. Vadek, and L.E. Wendel, 1997. Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle Biological Control 10: 48-57.

Poprawski, T.J., J. C. Legaspi and P. Parker, 1998. Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of whiteflies (Homoptera: Aleyrodidae) Biological Control 27(3): 785-795.

Rosenheim, J.A., H.K. Kaya, L.E. Ehler, J.J. Marois and B.A. Jaffee, 1995. Intraguild predation among biological-control agents: theory and evidence Biological Control 5: 303-335.

Roy, H.E., J.K. Pell, S.J. Clark, and P.G. Alderson, 1998. Implications of predator foraging on aphid pathogen dynamics Journal of Invertebrate Pathology 71: 236-247.

Roy, H.E. and J.K. Pell. 2000. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control Biocontrol Science and Technology 10: 737-752.