EFFICACY OF ENTOMOPATHOGENIC ORGANISMS *BEAUVERIA BASSIANA*, *ISARIA FUMOSOROSEUS*, *METARHIZIUM ANISOPLIAE* AND *CHROMOBACTERIUM SUBTSUGAE* AGAINST THE WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS*, UNDER BOTH LABORATORY AND GREENHOUSE CONDITIONS

by

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Abstract

Western flower thrips (WFT), *Frankliniella occidentalis*, is one of the most destructive insect pests of greenhouse production systems because of the direct and indirect damage they cause and their ability to develop resistance to a wide variety of insecticides. A common method of avoiding resistance development is by rotating insecticides that have different modes of action. Entomopathogenic organisms (EPO), such as fungi and bacteria, have modes of action that are very different from standard insecticides. Thus, by incorporating EPO into rotation programs, greenhouse producers may preserve the efficacy of insecticides used for suppression of WFT populations. Therefore, the objectives of this study were to 1) determine the efficacy of entomopathogenic fungi on WFT adults and nymphs, and to assess product effectiveness when used beyond the expiration date; 2) evaluate the efficacy of entomopathogenic fungi against WFT nymphs when combined with the insect growth regulator, azadirachtin; and 3) evaluate different rotation programs that include EPO and standard insecticides commonly used to suppress WFT populations.

To satisfy objective one and two, a series of laboratory bioassays were conducted in which WFT nymphs and adults were exposed to three entomopathogenic fungi (*Beauveria bassiana*, *Isaria fumosoroseus*, and *Metarhizium anisopliae*) at two label rates (maximum and minimum), and two product conditions (fresh and expired). Furthermore, a bioassay in which each entomopathogenic fungi was tested with and without azadirachtin was conducted to determine if there was any synergistic effect on WFT nymphal mortality. Results indicated that adults are generally more susceptible to infection by entomopathogenic fungi than nymphs, fresh products resulted in higher mortality than expired products, and azadirachtin, when mixed with

the entomopathogenic fungi, did not increase mortality of WFT nymphs except when combined with *M. ansiopliae*.

Insecticide rotation programs that included EPO were evaluated by conducting a series of greenhouse experiments in which chrysanthemum, *Dendranthema x morifolium* plants were artificially infested with WFT adults. Eight-week rotation programs were applied to each plant and weekly counts of adults captured on yellow sticky cards were recorded. A final quality assessment of damage due to WFT feeding on foliage and flowers (1 to 5 in which 1 = no damage, and 5 = greater than 75% damage) was also recorded. In addition, a cost comparison of each rotation program was determined. Generally, insecticide rotations programs which incorporated EPO resulted in no significant difference in WFT populations compared to standard insecticide rotation programs without EPO. Furthermore, there were no significant differences between any of the rotation programs in regards to foliage and flower quality. Based on the results of the cost comparison, there may be a cost savings associated with using EPO. Therefore, by incorporating EPO into insecticide rotation programs, greenhouse producers may reduce costs as well as reduce selection pressure on WFT populations, which may avoid or delay resistance development.

Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate this work to my husband, Ryan Kivett. Thank you for your unconditional love, patience and support while I pursued my master's degree.

Chapter 1 - Introduction and Literature Review

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is an important insect pest capable of causing significant economic losses to greenhouse-grown horticultural crops (Robb and Parrella, 1995; Kirk, 2002; Reitz, 2009). There is concern associated with WFT populations in greenhouse production systems primarily because of the direct and indirect damage they cause, as well as their ability to develop resistance to insecticides (Seaton et al., 1997; Jensen, 2000; Kirk, 2002). Western flower thrips is extremely polyphagous, feeding on a wide variety of greenhouse-grown horticultural crops (Brødsgaard, 1989; Tommasini and Maini, 1995; Parrella and Murphy, 1996; Lewis, 1997a). Due to a broad host range and low tolerance for WFT damage, insecticides have been the primary means of suppressing WFT populations (Parrella and Jones, 1987; van Lenteren and Woets, 1988; Brødsgaard, 2004). Greenhouse producers frequently spray insecticides in order to target specific life stages such as the nymphs and adults that were not susceptible during previous applications (Cloyd, 2009). The continuous use of insecticides places extensive selection pressure on WFT populations, which, in combination with their short life cycle and haplo-diploid breeding system, enhances the prospect of resistance developing in WFT populations (Moritz et al., 2004). Therefore, it is important to avoid resistance by rotating insecticides with different modes of action (Robb and Parrella, 1995). However, due to the limited number of new insecticides that have been introduced to the marketplace, greenhouse producers have few options regarding insecticides with different modes of action (Brødsgaard, 2004). A plant protection strategy that utilizes both insecticides and materials with broad modes of activity such as entomopathogenic organisms (EPO) may reduce the selection pressure on WFT populations. However, there is little information available

regarding the efficacy of EPO when used in rotation programs for the suppression of WFT populations.

Entomopathogenic organisms may be a viable option for greenhouse producers because they can be mass-produced, formulated to promote efficacy and stability during storage, and applications can be made with conventional spray equipment (Bateman et al., 1993; Stathers et al., 1993; Jenkins and Lomer, 1994; Shah and Pell, 2003). In addition, the use of EPO may have minimal effects on non-target organisms, including natural enemies (parasitoids and predators), and be less toxic to greenhouse workers than most conventional insecticides (Goettle and Hajek, 2000; Pell et al., 2001). Despite the benefits associated with EPO, there are some disadvantages such as the relatively long delay (3 to 14 days) (Gillespie and Claydon, 1989) in reducing WFT populations and limited shelf-life. However, there is minimal information regarding their efficacy when used beyond expiration dates. Therefore, the objectives of this study were to 1) evaluate the efficacy of fresh and expired products containing entomopathogenic fungi on WFT adults and nymphs; 2) assess the efficacy of entomopathogenic fungi against WFT nymphs when mixed with the insect growth regulator, azadirachtin; and 3) determine the feasibility of incorporating the EPO *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosoroseus* (Wize), *Metarhizium anisopliae* (Metschnikoff) Sorokin, and *Chromobacterium subtsugae* into rotation programs designed to suppress WFT populations. This research will benefit the greenhouse industry by potentially minimizing the development of resistance and thus preserving the efficacy and longevity of existing insecticides by incorporating EPO into rotation programs designed to suppress WFT populations.

Literature Review

Western flower thrips

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an economically important insect pest of greenhouse-grown horticultural crops (Robb and Parrella, 1995). Western flower thrips was first discovered in California, USA, in 1895 (Pergande, 1895) and has spread world-wide primarily by the movement of infested plant material (Kirk and Terry, 2003).

Western flower thrips biology and development

Western flower thrips are small (2.0 mm in length) insects with piercing-sucking mouthparts (Moritz, 1997). The life cycle includes an egg, two nymphal stages, two pupal stages, and adult (Ananthakrishnan, 1993; Tommasini and Maini, 1995; Mound, 1996). At temperatures between 26 and 29°C, the life cycle may be completed in 7 to 13 days (Lublinkhof and Foster, 1977; Robb, 1989; Gaum et al., 1994; Tommasini and Maini, 1995). Western flower thrips possess a haplo-diploid breeding system (Mound, 1996; Reitz, 2009) in which the haploid, or unfertilized egg, develops into a male and the diploid, or fertilized egg, develops into a female (Mound, 1996; Moritz et al., 2004). This haplo-diploid breeding system may result in resistant genes being fully expressed in the haploid males, while diploid females may be associated with recessive or co-dominance for resistance (Havron et al., 1987; Denholm et al., 1998). Female WFT can lay between 150 and 300 eggs during their lifetime, which is approximately five weeks (Trichilo and Leigh, 1988). Eggs are laid into the leaf surface, where they are protected from insecticides and natural enemies (Lewis, 1997a; Brødsgaard, 2004). The first two nymphal instars feed on leaf tissue with their piercing-sucking mouthparts (Chisholm and Lewis, 1984;

Hunter and Ullman, 1989). Eventually, the second instar nymphs migrate to the growing medium (Thoeming et al., 2003) or within flower parts (Broadbent et al., 2003) to pupate. The pupae do not feed and are therefore, not susceptible to insecticides (Tommasini and Maini, 1995; Kirk, 1997; Seaton et al., 1997). After approximately six days, winged adults develop and migrate to the plant canopy (Robb, 1989).

Although adults do have wings, they are weak fliers, primarily relying on air currents to disperse within greenhouses (Mound, 1996; Daughtrey et al., 1997). Western flower thrips prefer tight, protected, or enclosed spaces within terminal buds and flowers due to their thigmotactic behavior, which protects them from insecticides and natural enemies (Tommasini and Maini, 1995; Mound, 1996; Jensen, 2000; Brødsgaard, 2004). Due to their small size, high female reproductive capacity, and thigmotactic behavior, WFT populations can quickly build-up to levels that result in significant damage to crops.

Western flower thrips damage and management

Western flower thrips feed on flowers, leaves and stems of over 250 plant species (Robb, 1989; Tommasini and Maini, 1995; Lewis, 1997b; Brødsgaard, 2004). They cause direct and indirect damage to plants (Kirk, 2002). Direct damage to plant leaves and flowers is due to feeding using their piercing-sucking mouthparts, which causes leaf scarring, distorted growth, sunken tissues on leaf undersides, and deformation of flowers and fruit (Lewis, 1997b). Leaves and flowers also exhibit a “silvery” appearance after WFT consume fluids within plant cells (Chisholm and Lewis, 1984; van Dijken et al., 1994; Lewis, 1997b). Direct damage may also be a result of females ovipositing into leaf tissue, just below the epidermis (Jensen, 2000). Indirect damage is associated with adults vectoring the tospoviruses tomato spotted wilt and impatiens necrotic spot virus (Allen and Broadbent, 1986; Cho et al., 1988; Stobbs et al., 1992; Daughtrey

et al., 1997). The first and second instar nymphs acquire the virus while feeding, and then adults transmit the virus from plant to plant during the feeding process (Ullman et al., 1992; Mound, 1996; de Assis Filho et al., 2004; Moritz et al., 2004). Both direct and indirect damage may result in an economic loss to greenhouse producers (Kirk, 2002; Cloyd, 2009). Consequently, greenhouse producers have a near zero tolerance for the presence of WFT in greenhouses (Parrella and Jones, 1987). Therefore, effective management of WFT populations is critical in order to produce a marketable crop.

Western flower thrips are difficult to manage because of their cryptic behavior, broad host range, high female reproductive capacity, short life cycle, and resistance to insecticides (Fery and Schalk, 1991; Seaton et al., 1997; Jensen, 2000; Thoeming et al., 2003). Plant protection strategies for WFT include scouting, sanitation, physical, and the use of insecticides and/or natural enemies. Currently, insecticides are still the primary means of suppressing WFT populations in greenhouse production systems (Cloyd, 2009; Reitz, 2009).

The key to effective suppression of WFT populations with insecticides is early detection and timing of spray applications. Most insecticides used against WFT have either contact or translaminar activity, in which the material penetrates the leaf tissue and forms a reservoir of active ingredient (Cloyd, 2011). Systemic insecticides are less effective, in general, because the active ingredient is not transported into the flower parts where adult WFT typically feed (Cresswell et al., 1994; Daughtrey et al., 1997; Lewis, 1997c; Cloyd and Sadof, 1998). Therefore, high volume sprays are necessary in order to contact WFT located within protected areas of plants (Lewis, 1997c). Ideally, efficient suppression of WFT populations occurs when populations are low and insecticide applications are conducted before nymphs or adults enter the terminal buds or flowers. If WFT populations are extensive and overlapping generations occur

simultaneously, then it may be necessary to spray every 3 to 5 days in order to kill WFT that were in life stages (eggs and pupae) not affected by prior applications (Seaton et al., 1997; Cloyd, 2009). Frequent insecticide applications, however, increase selection pressure, so it is important to rotate insecticides with different modes of action in order to mitigate resistance development (Cloyd, 2009). Moreover, greenhouse producers have a limited number of insecticide options, which will likely continue due to the cost of developing and registering new insecticides (Reitz and Funderburk, 2012). Therefore, greenhouse producers need to exercise proper stewardship of existing insecticides, and develop sound rotation programs to avoid the prospect of resistance developing in WFT populations during the same cropping cycle.

The first reported case of resistance in a WFT population was in 1990, and since then, WFT are documented to be resistant to 26 different active ingredients (Whalon et al., 2014) including those in various chemical classes such as organophosphates, carbamates, pyrethroids, macrocyclic lactones (Immaraju et al., 1992; Zhao et al., 1995; Broadbent and Pree, 1997) and spinosyns (Loughner et al., 2005; Herron and James, 2005). Due to resistance development and the limited number of effective insecticides for WFT suppression (Reitz and Funderburk, 2012), the use of biological control has been increasing (Vestergaard et al., 1995; Blaeser et al., 2004; Gao et al., 2012).

Biological control of WFT includes the use of natural enemies such as predatory mites *Neoseiulus cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese), *Amblyseius swirskii* (Athias-Henriot), *Stratiolaelaps scimitus* (Womersley), and *Hypoaspis* (*Geolaelaps) aculeifer* (Canestrini); the minute pirate bug, *Orius insidiosus* (Say); and an entomopathogenic nematode, *Steinernema feltiae* Filpjev (Brødsgaard, 2004; Cloyd, 2009). However, biological control alone may not provide sufficient suppression of WFT populations and many natural enemies may not

be feasible to use in conjunction with insecticides (Reitz and Funderburk, 2012). Another option for greenhouse producers, that may suppress WFT populations, and possibly reduce selection pressure associated with commonly-used insecticides, is the use of entomopathogenic organisms (EPO) such as fungi and bacteria. However, there is minimal information available on the use of EPO in rotation with standard insecticides against WFT.

Entomopathogenic fungi

Entomopathogenic fungi have been utilized for pest management for over 100 years (Zimmerman, 1994). The first known entomopathogenic fungus to be used was *Metarhizium anisopliae* (Metschn) Sorokin against the beet weevil, *Cleonus punctiventris* (Germar), and the wheat cockchafer, *Anisoplia austriaca* (Herbst) (Gillespie, 1988; Lord, 2005). This entomopathogenic fungus was mass produced on beer mash and scattered in the field (Lord, 2005). Since then, a number of entomopathogenic fungi have been developed and are commercially available including *Beauveria bassiana* (Balsamo) Vuillemin, and *Isaria fumosoroseus* (Wize).

Biology of entomopathogenic fungi

Entomopathogenic fungi are relatively ubiquitous world-wide (Kanzok and Jacobs-Lorena 2006; Starnes et al., 1993). Generally, entomopathogenic fungi infect the host cuticle through enzymatic degradation and mechanical pressure (Gillespie and Claydon, 1989; Clarkson and Charnley, 1996). Once inside the host, the entomopathogenic fungus may be distributed throughout the haemocoel as yeast-like blastospores, hyphal bodies or protoplasts (Clarkson and Charnley, 1996). The change in growth from mycelium to blastospores, hyphal bodies or protoplasts may aid in dispersal and colonization of the haemocoel, absorption of nutrients, and disruption of the host cellular immune system (Clarkson and Charnley, 1996). Typically, the first

change in the infected host is a reduction in feeding behavior (Hajek, 1989; Moore et al., 1992). The host usually dies 3 to 14 days after spores contact the host (Gillespie and Claydon, 1989). Mortality is likely caused by a combination of mechanical damage by spore penetration, resulting in water loss, nutrient exhaustion, and toxicosis (poisoning by toxins produced by the entomopathogenic fungus) (Gillespie and Claydon, 1989). Mortality is generally dose-dependent, with higher spore concentrations resulting in faster kill and higher mortality rates (Vestergaard et al., 1995; James et al., 1998). After host death, and given the appropriate environmental conditions, specifically high relative humidity (>70%) (Azaizeh et al., 2002; Shipp et al. 2003), the entomopathogenic fungus may revert back to a mycelial growth form and exit the host cadaver to sporulate and infect another host (Gillespie and Claydon, 1989). If the environmental conditions are not optimal for sporulation, the entomopathogenic fungus remains dormant inside the host for up to several months until the appropriate environmental conditions occur (Gillespie and Claydon, 1989).

Optimal environmental conditions for host infection by entomopathogenic fungi include a high relative humidity close to the saturation point (>70%) (Azaizeh et al., 2002; Shipp et al., 2003) and temperatures between 20° and 30°C (Ferron, 1978). The most common causes of spore mortality are temperatures >35°C, desiccation, and solar radiation (Ferron, 1978; Weiser, 1982; Zimmerman, 1982; Carruthers et al., 1988). For example, *M. anisopliae* conidia were rapidly inactivated after 24 minutes of exposure to simulated sunlight (Zimmerman, 1982). In this same study, Zimmerman (1982) determined that *M. anisopliae* resistance to high temperatures is correlated with relative humidity in which spores in an aqueous suspension exposed to 50°C for 30 minutes resulted in spore mortality; while dry spores exposed to 100, 76, and 33% relative humidity, died at 55°, 75°, and 85°C respectively. While ambient

environmental conditions may not be optimal for entomopathogenic fungi, it has been reported that the temperature and relative humidity at the leaf surface or plant canopy may be substantially different (Willmer, 1986). Furthermore, studies have found there is adequate moisture within the microhabitat of the host's body surface to support infection (Ferron, 1977; Ramoska, 1984; Marcandier and Khachatourians, 1987; Fargues et al., 1997). Therefore, the use of entomopathogenic fungi for WFT management may not require a substantial change in the environment.

Entomopathogenic fungi and western flower thrips management

Numerous studies have been conducted to evaluate the efficacy of entomopathogenic fungi against WFT. Maniania et al. (2001) found that weekly applications of *M. anisopliae* to chrysanthemum, *Dendranthema x morifolium* Ramat cuttings provided comparable control of WFT as the insecticide Lannate® (methomyl). Likewise, Azaizeh et al. (2002) obtained a significant reduction in WFT numbers under greenhouse conditions on cucumbers, *Cucumis sativus* L., sprayed with *M. anisopliae* compared to the water control. Under laboratory conditions, Ekesi and Maniania (2000) observed 64 to 100% mortality of the adult legume flower thrips, *Megalurothrips sjostedti* (Trybom), depending on the spore concentration of *M. anisopliae*. Wu et al. (2014) observed 96% mortality of WFT adults when exposed to *B. bassiana.*

Entomopathogenic fungi may be effective against both WFT nymphs and adults; however, nymphs are less susceptible because they shed their cuticle more frequently and have thicker cuticles than adults (Vestergaard et al., 1995; Shipp et al., 2003). Germinated and ungerminated conidia have been observed on the exuvia of WFT nymphs following infection (Vestergaard et al., 1995). Moreover, WFT adults are more susceptible to entomopathogenic

fungi because they spend more time feeding in flowers where the relative humidity is higher and optimal for fungal infection (Murphy et al., 1998; Cloyd, 2009). A number of studies have observed similar effects with entomopathogenic fungi on the immature stages of insects such as diamondback moth, *Plutella xylostella* (Linnaeus) (Vandenberg et al., 1998), melon aphid, *Aphis gossypii* Glover, green peach aphid, *Myzus persicae* (Sulzer) and WFT (Shipp et al., 2003; Ugine et al., 2005; Wu et al., 2014). Ekesi and Maniania (2000) and Maniania et al. (2001) observed significantly reduced mortality of WFT nymphs when using *M. anisopliae* compared to adults. However, Wright and Kennedy (1996) obtained similar results for both nymphs and adult WFT when exposed to *B. bassiana.*

In order to enhance control of WFT nymphs with entomopathogenic fungi, greenhouse producers may apply mixtures of azadirachtin and an entomopathogenic fungus (James, 2003; Islam et al., 2010). Azadirachtin is an insect growth regulator that inhibits the molting process of various insects including WFT (Schmutterer, 1990; Thoeming et al., 2003). By delaying the molting process, spores of the entomopathogenic fungus are able to penetrate the cuticle and successfully infect WFT nymphs (Akbar et al., 2005; Hernandez et al., 2012). However, there is limited information available on the efficacy of azadirachtin in combination with formulated products of *B. bassiana*, *I. fumosoroseus*, and *M. anisopliae* on WFT nymphs.

Entomopathogenic bacteria

In addition to entomopathogenic fungi, there are also entomopathogenic bacteria. The first entomopathogenic bacterium to be used against insect pests was *Paenibacillus* (former *Bacillus*) *popilliae* Dutky (Milky Spore) against the Japanese beetle, *Popillia japonica* Newman (Dutky, 1940). For years, the most commonly used entomopathogenic bacterium has been *Bacillus thuringiensis* Berliner (Lacey et al., 2001), which was identified in 1911 (Beegle and

Yamamoto, 1992). *Bacillus thuringiensis* has been used extensively for control of a variety of insect pests including caterpillars (Luttrell et al., 1998) and fungus gnats (Osborne et al., 1985, Cloyd and Dickinson, 2006). Due to the specificity of entomopathogenic bacteria, they are not typically used against WFT; however, a bacterium called *Chromobacterium subtsugae* sp. nov. was recently introduced and is labeled for control of thrips.

Chromobacterium subtsugae

Chromobacterium subtsugae is a new strain of entomopathogenic bacterium discovered in 2000 (Martin et al., 2007a). The strain PRAA4-1^{T} was isolated from soil in a central Maryland forest (Martin et al., 2007b). The bacterium has since been approved by the Environmental Protection Agency (EPA) in 2011 and marketed under the trade name Grandevo® (Marrone Bio Innovations; Davis, California). The bacterium is fermented to extract toxic compounds that are produced by the bacterium. The extracted compounds are toxic by means of ingestion to certain chewing and sucking arthropod pests such as thrips, twospotted spider mite (*Tetranychus urticae* Koch), whiteflies, caterpillars, psyllids and beetles (Grandevo, 2013). It has also demonstrated antifeedant activity against insect pests such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); southern corn rootworm, *Diabrotica undecimpunctata howardi* (Barber); gypsy moth, *Lymantria dispar* (Linnaeus); and tobacco hornworm, *Manduca sexta* (Linnaeus) (Martin et al., 2004; 2007b). Pests typically die 2 to 7 days after ingestion of the bacterial toxins (Marrone, 2012). According to a summary by Marrone Bio Innovations, the bacterium has efficacy on WFT similar to or better than either spinetoram or spirotetramat (Marrone, 2012). However, since it is a relatively new introduction, minimal research has been conducted evaluating efficacy against WFT.

Biology of *Chromobacterium subtsugae*

Chromobacterium subtsugae is a facultative aerobic, motile, Gram-negative betaproteobacterium with polar flagella that develops a characteristic violet pigment called voilecein (Martin et al., 2007b; Koivunen et al., 2009). Cultures of the strain PRAA4-1^T grow optimally at 25° to 30°C and a pH of 6.0 to 8.0 (Martin et al., 2007b). However, viable bacterial cells are not required to cause mortality of insect pests. *Chromobacterium subtsugae* produces toxins that are lethal when consumed even in the absence of viable cells. These toxins have been shown to be heat stable and have demonstrated protease resistance (Martin et al., 2004). For example, there was no decrease in percent mortality of Colorado potato beetles after heat treatment of the culture by autoclaving at 65°C for 10 minutes and only a slight decrease observed when treated with a protease-treated filtrate (Martin et al., 2004).

Objectives

Greenhouse producers are continually encountering challenges producing a quality, marketable crop while simultaneously dealing with WFT using insecticides and trying to avoid resistance. Therefore, greenhouse producers are seeking solutions to preserve the efficacy of existing insecticides, which led to the development of the current study, to evaluate the efficacy of EPO based on three objectives.

Objective 1: Efficacy of entomopathogenic fungi on western flower thrips

The first objective of this study was to evaluate the efficacy of entomopathogenic fungi against WFT adults and nymphs at different rates and assess product effectiveness when used beyond the designated expiration date. A series of laboratory bioassays were conducted to address this objective. The entomopathogenic fungi used for these bioassays were *Beauveria*

bassiana strain GHA (BotaniGard® 22WP: Bioworks, Inc.; Victor, NY), *Isaria fumosoroseus* strain FE 9901 (NoFly™: Novozymes Biologicals Inc.; Salem, VA), and *Metarhizium anisopliae* strain F52 (Met52® EC: Novozymes Biologicals Inc.; Salem, VA). Each of these products are labeled for use against thrips, however, there is minimal quantitative data available on their efficacy against WFT adults and nymphs, and when used beyond the expiration date.

Objective 2: Efficacy of entomopathogenic fungi in combination with azadirachtin

The second objective of this study was to determine whether azadirachtin could enhance the efficacy of entomopathogenic fungi against WFT nymphs. This objective was satisfied by conducting a laboratory bioassay in which the mortality of WFT nymphs by entomopathogenic fungi mixed with azadirachtin was compared to entomopathogenic fungi without azadirachtin. The same entomopathogenic fungi used for objective one were used for this study.

Objective 3: Effectiveness of entomopathogenic organisms in rotation programs

The third objective of this study was to assess the effectiveness of rotation programs that include EPO and insecticides commonly used to suppress WFT populations. This objective was satisfied by conducting two eight-week experiments in a greenhouse using chrysanthemum, *Dendranthema x morifolium* plants artificially infested with WFT adults. Six rotation programs were evaluated per experiment. The conventional insecticides used in this study were spinosad (Conserve® SC: Dow AgroSciences; Indianapolis, IN), pyridalyl (Overture® 35 WP: Valent U.S.A. Corp.; Walnut Creek, CA), abamectin (Avid® 0.15 EC: Syngenta Crop Protection; Greensboro, NC), and chlorfenapyr (Pylon®: BASF Corp.; Research Triangle Park, NC). The EPO used for this study were the same three entomopathogenic fungi used in objective one and two in addition to *Chromobacterium subtsugae* strain PRAA4-1^T (Grandevo®: Marrone Bio Innovations; Davis, CA). Although each of these products is labeled for use against thrips, there

is limited information available on the effectiveness of rotation programs that utilize these products against WFT populations in greenhouses.

Chapter 2 - Evaluation of Entomopathogenic Fungi against Western Flower Thrips, *Frankliniella occidentalis*, Under Laboratory Conditions

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an economically important insect pest of greenhouse-grown horticultural crops (Robb and Parrella, 1995). Western flower thrips is difficult to manage because of the high female reproductive capacity, short life cycle, and broad host range (Fery and Schalk, 1991; Seaton et al., 1997; Jensen, 2000; Thoeming et al., 2003). Female western flower thrips are capable of producing 150 to 300 eggs during their approximate five-week lifespan (Trichilo and Leigh, 1988). There are six life stages: egg, two nymphal stages, prepupa, pupa, and adult (Ananthakrishnan, 1993; Tommasini and Maini, 1995; Mound, 1996). The life cycle may be completed in 7 to 13 days at temperatures of 26 to 29°C (Lublinkhof and Foster, 1977; Robb, 1989; Gaum et al., 1994; Tommasini and Maini, 1995). Western flower thrips are extremely polyphagous, feeding on a wide variety of greenhouse-grown horticultural crops (Brødsgaard, 1989; Tommasini and Maini, 1995; Parrella and Murphy, 1996; Lewis, 1997a). Adults and nymphs feed on all above-ground plant parts (Robb, 1989; Tommasini and Maini, 1995; Lewis, 1997b; Brødsgaard, 2004), whereas, the pupal stages, which are located in the growing medium or sometimes in flowers, do not feed (Tommasini and Maini, 1995; Kirk, 1997; Seaton et al., 1997).

Western flower thrips are a concern to greenhouse producers because they cause both direct and indirect damage to plants (Seaton et al., 1997; Jensen, 2000; Kirk, 2002). Direct damage is associated with feeding using their piercing-sucking mouthparts, which results in

discoloration and deformation of flowers and foliage (Chisholm and Lewis, 1984; van Dijken et al., 1994). Indirect damage is a result of adult WFT vectoring tospoviruses such as tomato spotted wilt and impatiens necrotic spot virus (Allen and Broadbent, 1986; Cho et al., 1988; Stobbs et al., 1992; Daughtrey et al., 1997). As a result of the potential for both direct and indirect damage, greenhouse producers have a low tolerance for the presence of WFT populations (Parrella and Jones, 1987). Currently, insecticides are the primary means of suppressing WFT populations. However, the continuous use of insecticides during the growing season places extensive selection pressure on WFT populations (Cloyd, 2009). Western flower thrips have developed resistance to a variety of insecticides with different modes of action (Immaraju et al., 1992; Zhao et al., 1995; Broadbent and Pree, 1997; Loughner et al., 2005; Herron and James, 2005). Due to resistance development and the limited number of effective insecticides for WFT suppression (Reitz and Funderburk, 2012), the implementation of biological control programs has increased (Vestergaard et al., 1995; Blaeser et al., 2004). The use of entomopathogenic organisms such as fungi may be a viable option for suppression of WFT populations. By incorporating entomopathogenic fungi into a management strategy against WFT, greenhouse producers may reduce the selection pressure and possibly decrease the incidence of resistance development.

Entomopathogenic fungi may reduce the selection pressure due to the unique mode of action by which they cause mortality. Generally, entomopathogenic fungi infect the host cuticle by enzymatic degradation and mechanical pressure (Gillespie and Claydon, 1989; Clarkson and Charnley, 1996). Once inside a host, the entomopathogenic fungus colonizes the haemocoel, which maximizes nutrient absorption and compromises the integrity of the host cellular immune system (Clarkson and Charnley, 1996). A reduction in host feeding behavior is typically the first

symptom of infection (Hajek, 1989; Moore et al., 1992). The infected host generally dies 3 to 14 days after exposure to the fungus (Gillespie and Claydon, 1989). Mortality of the host is likely due to a combination of water loss as a result of mechanical damage to the cuticle by the entomopathogenic fungus, nutrient exhaustion, and toxins produced (Gillespie and Claydon, 1989). Mortality is generally dose-dependent, with higher spore concentrations resulting in faster kill and higher mortality rates (Vestergaard et al., 1995; James et al., 1998).

Although mortality is generally dose-dependent, entomopathogenic fungi may affect WFT nymphs and adults differently. Studies have shown that WFT nymphs are less susceptible than adults because nymphs molt more frequently and have thicker cuticles than adults (Vestergaard et al., 1995; Maniania et al., 2001; Shipp et al., 2003; Ugine et al., 2005; Wu et al., 2014). As such, spores may not have enough time to successfully penetrate the cuticle before ecdysis. For example, Vestergaard et al. (1995) observed germinated and ungerminated conidia on the exuvia of WFT nymphs following infection. Western flower thrips adults may be more susceptible because they spend more time feeding in flowers where the relative humidity is higher and ideal for fungal infection (Murphy et al., 1998; Cloyd, 2009). Azadirachtin, an insect growth regulator (ecdysone antagonist), may allow spore penetration through the cuticle by delaying the molting process of WFT nymphs (Schmutterer, 1990; Thoeming et al., 2003; Akbar et al., 2005; Hernandez et al., 2012). However, there is limited information available on the efficacy of entomopathogenic fungi when mixed with azadirachtin.

A disadvantage of entomopathogenic fungi is their relatively short shelf-life compared to conventional insecticides (Osborne and Oetting, 1989). According to the label for NoFly (*Isaria fumosoroseus*), the product performs best if used by three months of the manufacturing date. It is also recommended that Met52 (*Metarhizium anisopliae*) be used within one year and/or by the

expiration date noted on the label. The BotaniGard (*Beauveria bassiana*) label only provides an expiration date. Furthermore, there is no information available on the efficacy of these products when used beyond the manufacturer expiration date. It is important for greenhouse producers to understand how a product may perform if used beyond the expiration date as efficacy of the entomopathogenic fungi may decline over time. Therefore, the first objective of this study was to evaluate the efficacy of entomopathogenic fungi, under laboratory conditions, against WFT adults and nymphs at different rates, and to assess product effectiveness when used beyond the designated expiration date. The second objective was to determine the efficacy of entomopathogenic fungi against WFT nymphs when mixed with azadirachtin.

Materials and Methods

Rearing western flower thrips

Western flower thrips (WFT) colonies were maintained in the Department of Entomology at Kansas State University (Manhattan, KS). Both adults and nymphs were reared in ventilated plastic containers (11.5 x 17 x 9 cm) on a laboratory bench under 24:0 (L:D) hour photoperiod at 25 ± 3°C. Western flower thrips were maintained on green beans (*Phaseolus vulgaris* L.). After 2 to 3 days, beans were transferred to a fresh, ventilated plastic container where first instar nymphs began hatching after two days. Western flower thrips were transferred to a clean container every 2 to 3 days, and provided with 2 to 5 fresh beans as a food source and/or oviposition site for adult females. Two to 3 day old beans were either discarded or maintained for egg-hatching. Specimens used in this research are deposited as voucher number 237 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

Laboratory bioassays

Laboratory bioassays were conducted to evaluate the effect of rate and shelf-life of each entomopathogenic fungi: *Beauveria bassiana* strain GHA, *Isaria fumosoroseus* strain FE 9901, and *Metarhizium anisopliae* strain F52, on two life stages of WFT: nymphs and adults.

The first bioassay was conducted using a three-way factorial treatment structure consisting of three entomopathogenic fungi (*B. bassiana*, *I. fumosoroseus*, and *M. anisopliae*), two labeled rates (maximum and minimum), and two conditions (fresh and expired) plus a water control. Expired products were at least 10 months past the expiration date as indicated on the label. Each treatment combination was applied to WFT adults and nymphs. The *B. bassiana* and *I. fumosoroseus* mixtures were prepared by adding 0.24 g (1 lb/100 gal) and 0.48 g (2 lbs/100 gal) to 200 mL of water for the minimum and maximum labeled rates, respectively. The *M. anisopliae* mixture was prepared by adding 0.125 mL (0.5 pt/100 gal) and 0.5 mL (1 qt/100 gal) to 200 mL of water for the minimum and maximum labeled rates, respectively. Tween™ 20 Enzyme Grade (Fisher Scientific; Pittsburgh, PA) was added to *I. fumosoroseus* at a rate of 30 µL per 200 mL of water to help disperse the spores in the water mixture. For more information on the reason Tween™ 20 was added to *I. fumosoroseus*, refer to Appendix B. Each glass petri plate (100 x 15 mm) was lined with a 9 cm piece of P8 filter paper (Fisher Scientific; Pittsburgh, PA), and treated with 1 mL of each treatment mixture, which was dispensed uniformly around the entire filter paper with a 1 mL plastic syringe. Approximately 15 WFT adults or second instar nymphs were placed into each glass petri plate pre-treated with the appropriate entomopathogenic fungi mixture. A 2.5 cm piece of green bean, cut lengthwise into quarter sections, was provided as a food source. Every two days, the green bean was replaced with a fresh green bean. A foam circle (Darice, Inc.; Strongsville, OH) was fitted into the lid of each

petri plate and sealed with Parafilm® (Pechiney Plastic Packaging Company; Chicago, IL), which prevented WFT from escaping. For the duration of the bioassay, petri plates were maintained in an environmental growth chamber (Conviron; Winnipeg, Canada) under 0:24 (L:D) hour photoperiod and 24 ± 3°C. The number of live and dead WFT adults and nymphs were assessed after 24, 48, 72, 96 and 120 hours of incubation. To determine mortality, individuals were gently prodded with a single bristle paint brush. Any WFT that did not respond immediately were considered dead. This bioassay was set-up as a randomized complete block design with repeated measures in which a block was initiated every two days for a total of six blocks. There was one replication (petri plate) per treatment combination in each block (n=6).

The second bioassay was conducted using a three-way factorial treatment structure consisting of the three entomopathogenic fungi (*B. bassiana*, *I. fumosoroseus,* and *M. anisopliae*), two labeled rates (maximum and minimum), and two conditions (fresh and expired) plus a water control. Expired products were at least 10 months past the expiration date as indicated on the label. Due to the low mortality of WFT nymphs in the first bioassay, only adult WFT were used in this bioassay. The *B. bassiana* and *I. fumosoroseus* mixtures were prepared by adding 0.24 g (1 lb/100 gal) and 0.48 g (2 lbs/100 gal) to 200 mL of water for the minimum and maximum labeled rates, respectively. The *M. anisopliae* mixture was prepared by adding 0.125 mL (0.5 pt/100 gal) and 0.5 mL (1 qt/100 gal) to 200 mL of water for the minimum and maximum labeled rates, respectively. Tween™ 20 Enzyme Grade was added to *I. fumosoroseus* at a rate of 30 µL per 200 mL of water to aid in dispersing the spores in water. Each glass petri plate (100 x 15 mm) was lined with a 9 cm piece of P8 filter paper and treated with 1 mL of each treatment mixture, which was dispensed uniformly around the entire plate with a 1 mL plastic syringe. Approximately 15 WFT adults were placed into each glass petri plate pre-treated with

the appropriate entomopathogenic fungi mixture. A 2.5 cm piece of green bean, cut lengthwise into quarter sections, was provided as a food source. Every two days, the green bean was replaced with a fresh green bean. A foam circle was fitted into the lid of each petri plate and sealed with Parafilm®, which prevented WFT from escaping. For the duration of the bioassay, petri plates were maintained in an environmental growth chamber under 0:24 (L:D) hour photoperiod and 24 ± 3°C. The numbers of dead WFT adults were assessed every 24 hours up to 216 hours (9 days) post-application of the treatments. Based on the results of the first bioassay, the incubation time was extended due to the delayed activity observed for *M. anisopliae*. This bioassay was set-up as a randomized complete block design with repeated measures in which a block was initiated every two days for a total of six blocks. There was one replication (petri plate) per treatment combination in each block (n=6).

The third bioassay evaluated whether mixing azadirachtin with each of the entomopathogenic fungi products would increase mortality of WFT nymphs. Treatments included each of the entomopathogenic fungi products (*B. bassiana*, *I. fumosoroseus*, and *M. anisopliae*) with azadirachtin (AzaGuard™: Biosafe Systems, LLC; East Hartford, CT) and without azadirachtin, water, and azadirachtin alone. All products were fresh and the maximum labeled rate was used for each of the products. The *B. bassiana* and *I. fumosoroseus* mixtures were prepared by adding the labeled rate of 0.48 g (2 lbs/100 gal) to 200 mL of water. The *M. anisopliae* mixture was also prepared by adding the labeled rate of 0.5 mL (1 qt/100 gal) to 200 mL of water. Tween™ 20 Enzyme Grade was added to *I. fumosoroseus* at a rate of 30 µL per 200 mL of water to aid in dispersing the spores in the mixture. Treatments that included azadirachtin were prepared by adding 0.25 mL (16 oz/100 gal) to the 200 mL entomopathogenic fungi mixtures. Each glass petri plate (100 x 15 mm) was lined with a 9 cm piece of P8 filter paper and

treated with 1 mL of each treatment mixture, which was dispensed uniformly around the entire plate with a 1 mL plastic syringe. Approximately 15 WFT second instar nymphs were placed into petri plates with the appropriate treatment. A 2.5 cm piece of green bean, cut lengthwise into quarter sections, was provided as a food source. Every two days, the green bean was replaced with a fresh green bean. A foam circle was fitted into the lid of each petri plate and sealed with Parafilm®, which prevented WFT from escaping. For the duration of the bioassay, petri plates were maintained at room temperature (22 ± 1°C) under 9:15 (L:D) hour photoperiod. The numbers of dead WFT nymphs were assessed every 24 hours up to 144 hours post-application of the treatments. This bioassay was set-up as a randomized complete block design with repeated measures in which a block was initiated every two days for a total of six blocks. There was one replication (petri plate) per treatment combination in each block (n=6).

Statistical analysis

The bioassays were analyzed by fitting a generalized linear mixed model to a binomial response consisting of the number of dead WFT adults or nymphs at a given time in a petri plate. A separate analysis was done for adults and nymphs. A logit link was used to connect the binomial probability of WFT mortality with the linear predictors, which included the fixed effects of treatment (13 levels, consisting of combinations of three entomopathogenic fungi, two rates and two conditions, plus a water control) and time. The linear predictors for the third bioassay included the fixed effects of entomopathogenic fungi, azadirachtin and time. The 24-hour time point was removed from the analysis for WFT adult mortality and the 24, 48, and 72-hour time points were removed from the analysis for WFT nymphal mortality due to extreme category problems, which prevented convergence and model fitting. The two-way interaction associated with treatment and time was also fitted. Random effects in the linear predictor

included the blocking factor and its cross-product with treatment, which recognized the appropriate experimental unit of petri plate.

Over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion. The final statistical model for bioassay one and three was fitted using residual Pseudo-likelihood. Kenward Roger's procedure with manual fine-tuning was used to estimate degrees of freedom and the corresponding adjustments for the estimated standard errors. However, the second bioassay was fitted using a Laplace approximation to maximum likelihood due to convergence problems associated with quasi-complete separation of data points for some treatments at the later time points of the incubation period. Furthermore, the degrees of freedom for the second bioassay were approximated manually. Models for all three bioassays were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute, Cary, NC) implemented using Newton-Raphson with ridging as the optimization technique. All relevant pairwise comparisons were conducted using Bonferroni adjustments to avoid inflation of type I error rate due to multiple comparisons. Tailored contrasts were conducted to assess main effects and interactions of entomopathogenic fungi, rate, and product conditions.

Results

First bioassay

Results in which WFT adult mortality was evaluated over a 5-day (120-hour) incubation period are presented in Table 2.1. There was significant two-way interaction between treatment and time on adult WFT mortality (F=2.88; df=36, 260; P<0.001). Fresh *B. bassiana* and *I. fumosoroseus* (at both maximum and minimum rates) resulted in a significant increase in WFT

adult mortality from 48 to 120 hours of incubation (P<0.0001). However, fresh *M. anisopliae* did not result in any significant increase in adult WFT mortality from 48 to 120 hours for either the maximum (P=0.056) or minimum labeled rates (P=1.000).

After 96 and 120 hours, any treatment effects on adult WFT mortality were associated with the two-way interaction between entomopathogenic fungi and product freshness (96 hrs: F=6.48; df=2, 109.3; P=0.002 and 120 hrs: F=9.06; df=2, 89.22; P=0.0003). Fresh products resulted in higher adult mortality when *B. bassiana* and *I. fumosoroseus* was used but not for *M. anisopliae*. Fresh *B. bassiana* resulted in significantly higher adult mortality than expired at both 96 (P<0.0001) and 120 hours (P<0.0001). Furthermore, fresh *B. bassiana* resulted in significantly higher adult mortality than fresh *M. anisopliae* at 96 (P=0.00005) and 120 hours (P<0.0001) (Figure 2.1). Likewise, fresh *I. fumosoroseus* resulted in significantly higher adult mortality than expired at 120 hours (P=0.007) as well as significantly higher adult mortality than fresh *M. anisopliae* at 96 (P=0.00009) and 120 hours (P<0.0001). In addition, expired *I. fumosoroseus* resulted in significantly higher adult mortality than expired products of *B. bassiana* (P=0.029) and *M. anisopliae* (P=0.026) at 120 hours (Figure 2.1). However, for *M. anisopliae*, there was no significant increase in adult mortality when fresh product was used compared to expired at 96 (P=0.95) or 120 (P=0.98) hours of incubation. There was also a significant main effect of rate at both 96 (F=5.1; df=1, 113.7; P=0.026) and 120 hours (F=9.01; df=1, 91.53; P=0.004) in which adult WFT mortality was greater at the maximum rate than at the minimum rate after 96 (P=0.009) and 120 (P=0.049) hours of incubation regardless of entomopathogenic fungi or product condition.

Western flower thrips nymphal mortality was not evident until 96 and 120 hours of incubation. Therefore, only these two time points were included in the statistical analysis (Table

2.2). For WFT nymphal mortality, there was no significant treatment by time interaction after 96 hours of incubation (F=0.35; df=12, 130; P=0.98). However, there was a significant treatment effect (F=2.32; df=12, 54.42; P=0.018) on nymphal mortality, which was primarily due to the main effect of entomopathogenic fungi (F=8.14; df=2, 57.52; P=0.0008). Estimated probability of nymphal mortality by 120 hours of incubation was significantly higher for *I. fumosoroseus* (>11%) than *M. anisopliae* (<5.2%), regardless of rate or product freshness (P=0.012) (Table 2.2). However, there were no significant differences in mortality between any of the entomopathogenic fungi and the water control (*B. bassiana*, P=0.24; *I. fumosoroseus*, P=0.073; *M. anisopliae*, P=0.86). Furthermore, there was no significant effect of rate (F=0.07; df=1, 59.72; P=0.79) or product freshness (F=0.97; df=1, 59.54; P=0.33) on mortality of WFT nymphs. However, average mortality of WFT nymphs was significantly greater at 120 hours (8%) than 96 hours (5%) of incubation (P=0.035) across all entomopathogenic fungi.

There was a significant effect of treatment by life stage interaction on WFT mortality for 120 hours of incubation (F=2.07; df=12, 130; P=0.023). Fresh *B. bassiana* resulted in significantly greater adult mortality than nymphs at both the maximum and minimum labeled rates (P=0.007 and P<0.0001, respectively). Fresh *I. fumosoroseus* at the maximum labeled rate also resulted in significantly greater adult mortality than nymphs (P=0.0005) (Figure 2.2). However, there was no significant difference between adult and nymphal mortality when *M. anisopliae* or any expired product was used. Furthermore, a two-way interaction between product freshness and life stage was apparent for both *B. bassiana* and *I. fumosoroseus*. Both species of entomopathogenic fungi, when fresh, resulted in greater mortality of adult WFT than nymphs (P<0.0001). Fresh *B. bassiana* resulted in nearly 60% greater estimated probability of adult WFT mortality than nymphs (Figure 2.3). Fresh *I. fumosoroseus* resulted in 54% greater estimated

probability of adult WFT mortality than nymphs (Figure 2.4). Expired *I. fumosoroseus* also resulted in a 21% greater mortality of adults than nymphs (P=0.025) (Figure 2.4). A two-way interaction was also apparent between rate and life stage for *M. anisopliae*. Regardless of product freshness, *M. anisopliae* resulted in significantly greater mortality of adults than nymphs when the maximum labeled rate was used (P=0.002) (Figure 2.5). However, estimated probability of adult WFT mortality at the maximum labeled rate for *M. anisopliae* was only 19% (Figure 2.5).

Second Bioassay

Results from the second bioassay in which adult WFT mortality was assessed over a 9-day (216-hour) incubation period in a three-way factorial of entomopathogenic fungi, rate, and product freshness is presented in Table 2.3. There was a significant effect associated with the treatment by time interaction on adult WFT mortality (F=3.69; df=81, 455; P<0.0001), in which treatment effects were only apparent after 72 hours of incubation. These treatment effects on adult WFT mortality may be explained by the two-way interaction between entomopathogenic fungi and product freshness at 72 (P=0.005), 96 (P=0.0002), and 120 (P=0.047) hours of incubation. Fresh *B. bassiana* resulted in significantly higher estimated probability of adult mortality than expired at 72 (P=0.0003), 96 (P<0.0001), and 120 (P<0.0001) hours, where the greatest difference in estimated probability between fresh and expired (55%) occurred at 120 hours (Figure 2.6). Fresh *I. fumosoroseus* also resulted in significantly higher mortality than expired at 72 (P=0.007), 96 (P=0.0007), and 120 (P=0.01) hours, in which the greatest difference between fresh and expired (49%) occurred at 96 hours (Figure 2.7). However, fresh *M. anisopliae* was only significantly different than expired at 120 hours (P=0.004) where fresh product resulted in a 44% estimated probability of adult mortality compared to 15% probability associated with expired *M. anisopliae* (Figure 2.8).

Third Bioassay

Results in which mortality of WFT nymphs was assessed based on combinations of azadirachtin and entomopathogenic fungi is presented in Figure 2.9. There was a significant two-way interaction between entomopathogenic fungi and azadirachtin by 144 hours of incubation (F=9.34; df=3, 37.84; P<0.0001). Azadirachtin significantly enhanced the efficacy of *M. anisopliae* (P=0.0004) in which *M. anisopliae* without azadirachtin resulted in a 1.5% estimated probability and 30% probability of nymphal mortality with azadirachtin (Figure 2.9). However, there was no evidence that azadirachtin enhanced the efficacy of either *B. bassiana* (P=0.38) or *I. fumosoroseus* (P=0.071). Furthermore, the individual treatments of *B. bassiana* and *I. fumosoroseus*, without azadirachtin, resulted in greater estimated probability of nymphal mortality (17% and 19%, respectively) than *M. anisopliae* (1.5%) without azadirachtin or the water control (1.4%).

Discussion

This study evaluated the efficacy of entomopathogenic fungi against WFT adults and nymphs at maximum and minimum labeled rates using fresh and expired products. Furthermore, the addition of azadirachtin to each entomopathogenic fungi was also assessed. Results from this laboratory study are based on environmental conditions (temperature and relative humidity) that are ideal for entomopathogenic fungi to cause infection. The relative humidity inside the petri plates were likely higher than the relative humidity present in a greenhouse environment, resulting in higher WFT mortality. Future research is necessary to determine the efficacy of entomopathogenic fungi against WFT under greenhouse conditions. Regardless, the laboratory results clearly indicate that: 1) products containing fresh entomopathogenic fungi result in greater adult mortality than expired products especially for *B. bassiana* and *I. fumosoroseus*; 2)

mortality by *M. anisopliae* is substantially delayed compared to *B. bassiana* or *I. fumosoroseus*; 3) WFT adults are more susceptible than nymphs to fresh products of *B. bassiana* and *I. fumosoroseus*; and 4) azadirachtin only enhanced the activity of *M. anisopliae* on nymphal mortality by 144 hours of incubation.

Overall, fresh products resulted in higher WFT adult mortality than expired products. This is the first study to compare the efficacy of entomopathogenic fungi when used before and after the expiration date. Ansari and Butt (2011) found that percent conidial germination of *M. anisopliae* strain F52 declined nearly 50% after four months stored at 4°C and nearly 70% after four months stored at 20°C. Expired products used in this study were at least ten months past the expiration date as indicated on the label, and stored at approximately 4°C. In the first bioassay, fresh products of *B. bassiana* and *I. fumosoroseus* resulted in significantly higher adult WFT mortality than the expired products. In the second bioassay, fresh product for all three entomopathogenic fungi resulted in higher adult WFT mortality than expired products by 120 hours of incubation. Therefore, it is important that greenhouse producers use entomopathogenic fungi products before the expiration date and properly store them as indicated on the label. If used after the expiration date, a decrease in efficacy against WFT is likely.

In addition to fresh products performing better than expired products, it was also observed that *M. anisopliae* exhibited a delayed effect on WFT mortality compared to the other two species of fungi. In the first bioassay, fresh *B. bassiana* and *I. fumosoroseus* resulted in >70% estimated probability of adult WFT mortality by 120 hours of incubation; whereas, fresh *M. anisopliae* resulted in approximately 17% estimated probability of adult WFT mortality. As a result, we extended the incubation time for the second bioassay to 216 hours, in which fresh *M. anisopliae* did not reach 100% probability of adult WFT mortality until 168 hours (7 days) of

incubation; whereas, *I. fumosoroseus* was nearly 100% by 120 hours (5 days) of incubation. However, *B. bassiana* did not reach maximum mortality (99%) until 192 hours (8 days) of incubation. Likewise, Vestergaard et al. (1995) observed 94% mortality of WFT adults for *M. anisopliae* after 7 days at 20 ± 1°C. Based on the results of the current study, *M. anisopliae* appears to have a delayed effect initially, while the other entomopathogenic fungi steadily increased in mortality over time. This may be due to a formulation difference associated with Met52 (*M. anisopliae*), which is an emulsifiable concentrate; whereas, BotaniGard (*B. bassiana*) and NoFly (*I. fumosoroseus*) are both wettable powders. Certain inert ingredients such as surfactants found in formulated products may be toxic to pests in addition to the active ingredient (Cowles et al., 2000). If this is the case, BotaniGard and NoFly, may contain inert ingredients that cause WFT mortality in addition to the entomopathogenic fungi. Furthermore, *M. anisopliae* may be a slower acting entomopathogenic fungus than *B. bassiana* or *I. fumosoroseus*. Additional research is necessary to compare the speed of activity for each fungal species.

It was also determined that WFT adults are more susceptible than nymphs to fresh *B. bassiana* and *I. fumosoroseus*. Western flower thrips adults were approximately 60% more likely to become infected by fresh *B. bassiana* and *I. fumosoroseus* than nymphs. Although other treatment combinations were not significantly different, there was a general trend in which adult WFT mortality was greater than nymphs, which is similar to the findings of other studies (Shipp et al., 2003; Ugine et al., 2005; Wu et al., 2014). It has been suggested that nymphs are less susceptible to infection because they molt more frequently and have thicker cuticles than adults (Vestergaard et al., 1995; Shipp et al., 2003). Due to the difference in susceptibility between adults and nymphs, it is important that greenhouse producers apply entomopathogenic fungi early in the production cycle before the occurrence of overlapping generations. This will

alleviate problems associated with different life stages (eggs, nymphs, pupae, and adults) present simultaneously thus resulting in more effective suppression of WFT populations.

If it is not possible to apply entomopathogenic fungi early in the production cycle, one option to potentially increase the effectiveness of entomopathogenic fungi against WFT nymphs is by the addition of azadirachtin, an insect growth regulator that inhibits or delays the molting process (Schmutterer, 1990; Thoeming et al., 2003; James, 2003; Islam et al., 2010). By delaying the molting process, the spores of the entomopathogenic fungi may be able to penetrate the cuticle and infect WFT nymphs before ecdysis (Akbar et al., 2005; Hernandez et al., 2012). However, in this study, the addition of azadirachtin to *B. bassiana* or *I. fumosoroseus* did not produce an additive or synergistic effect when applied to WFT nymphs. While the addition of azadirachtin to *M. anisopliae* did result in significantly higher nymphal mortality, it only resulted in a 30% estimated probability of mortality. Adult WFT were not used in the bioassay because azadirachtin, an insect growth regulator, is less effective against adults because they do not molt (Vestergaard et al., 1995; Shipp et al., 2003). Akbar et al. (2005) obtained similar results in which adding azadirachtin (Neemix 4.5: Certis USA; Columbia, MD) to *B. bassiana* resulted in lower mortality of the red flour beetle, *Tribolium castaneum* (Herbst) larvae than *B. bassiana* without azadirachtin. Furthermore, an initial greenhouse study in which azadirachtin (Ornazin 3% EC: SePRO; Carmel, IN) was added to two different formulations of *B. bassiana* (BotaniGard ES and BotaniGard WP) showed no difference in WFT nymphal mortality with or without azadirachtin (Cloyd, unpublished data). Additionally, James (2003) demonstrated that azadirachtin (Neemix 4.5) had an inhibitory effect on the growth and germination of *I. fumosoroseus*. Moreover, Shah et al. (2008) reported enhanced efficacy of *M. anisopliae* against the black vine weevil, *Otiorhynuchs sulcatus* Fabricius, when combined with neem seed cake, a

bi-product of neem oil production. A reason for lack of effectiveness with *B. bassiana* and *I. fumosoroseus* in the current study may be that azadirachtin is toxic or inhibits certain fungal spores (James, 2003). Studies have demonstrated that azadirachtin can be effective against certain plant pathogenic fungi (Singh et al., 1980; Pasini et al., 1997; Singh and Prithiviraj, 1997). Furthermore, the rate for AzaGuard used in this study was the maximum labeled rate (16 oz/100 gal). A lower rate should be used to reduce any inhibitory effect that azadirachtin may have on the entomopathogenic fungi (James, 2003). Therefore, based on the results of this study, there appears to be no benefit of adding azadirachtin to the entomopathogenic fungi, *B. bassiana* and *I. fumosoroseus* and only a marginal benefit of adding it to *M. anisopliae*.

In conclusion, this study had demonstrated that adult WFT are more susceptible to entomopathogenic fungi than nymphs. Furthermore, entomopathogenic fungi may have a delayed effect associated with mortality, especially *M. anisopliae*. This should be taken into consideration when applying entomopathogenic fungi. In addition, greenhouse producers may achieve better results when entomopathogenic fungi are applied early in the cropping cycle. This will allow the slower acting entomopathogenic fungi to kill adult WFT before multiple generations overlap in which adults and nymphs occur simultaneously. Moreover, there was no evidence that azadirachtin increased mortality of WFT nymphs except when added to *M. anisopliae*. Finally, fresh entomopathogenic fungal products should be used before the designated expiration date in order to maximize WFT mortality.

Table 2.1 Mean (± SEM) percent probability of mortality associated with adult western flower thrips, *Frankliniella occidentalis* after 48, 72, 96, and 120 hours of exposure to three entomopathogenic fungi, *Beauveria bassiana, Isaria fumosoroseus*, and *Metarhizium anisopliae* at maximum and minimum labeled rates, and expired and fresh products.

			Hours of Exposure			
Treatment	**Rate[z]**	**Condition**	**48**	**72**	**96**	**120**
Beauveria bassiana	Max	Expired	2.2 (± 1.7)a[y]	4.4 (± 2.8)a[y]	10.1 (± 4.9)ab[y]	19.3 (± 7.6)ac[y]
	Max	Fresh	3.2 (± 2.2)a	18.0 (± 7.2)a	36.7 (± 10.7)ab	66.5 (± 10.3)ab
	Min	Expired	3.9 (± 2.5)a	3.9 (± 2.5)a	3.9 (± 2.5)a	7.9 (± 4.0)c
	Min	Fresh	3.2 (± 2.2)a	19.3 (± 7.5)a	47.1 (± 11.3)b	71.8 (± 9.4)b
Isaria fumosoroseus	Max	Expired	1.0 (± 0.8)a	2.1 (± 1.4)a	19.5 (± 7.8)ab	41.6 (± 11.6)ab
	Max	Fresh	0.9 (± 1.0)a	4.8 (± 2.8)a	52.8 (± 11.3)a	85.0 (± 6.4)a
	Min	Expired	1.3 (± 1.1)a	1.3 (± 1.1)a	14.2 (± 6.2)ab	33.8 (± 10.5)b
	Min	Fresh	2.5 (± 1.6)a	4.6 (± 2.6)a	29.5 (± 9.9)ab	61.7 (± 11.1)ab
Metarhizium anisopliae	Max	Expired	3.6 (± 2.3)a	6.4 (± 3.4)a	13.4 (± 5.8)a	20.8 (± 7.9)a
	Max	Fresh	4.7 (± 2.8)a	8.7 (± 4.3)a	9.7 (± 4.6)a	16.9 (± 6.9)a
	Min	Expired	2.1 (± 1.7)a	3.2 (± 2.3)a	5.4 (± 3.3)a	6.6 (± 3.7)a
	Min	Fresh	0.9 (± 1.0)a	1.9 (± 1.5)a	2.8 (± 2.0)a	3.8 (± 2.4)a
Water	--	Control	1.5 (± 1.3)a	2.3 (± 1.7)a	5.7 (± 3.1)a	8.4 (± 4.2)a

[z] Maximum and minimum labeled rates (per 200 mL): *Beauveria bassiana* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); *Isaria fumosoroseus* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); and *Metarhizium anisopliae* = 0.5 pt and 1 qt/100 gal (0.125 and 0.5 mL/200 mL).
[y] Means followed by a common lowercase letter within a column and within an entomopathogenic fungi treatment are not significantly different from each other ($P>0.05$) based on Bonferroni adjusted P-values.

Table 2.2 Mean (± SEM) percent probability of mortality associated with western flower thrips, *Frankliniella occidentalis* nymphs after 96 and 120 hours of exposure to the entomopathogenic fungi, *Beauveria bassiana*, *Isaria fumosoroseus*, and *Metarhizium anisopliae* at maximum and minimum labeled rates, and expired and fresh products.

			Hours of Exposure	
Treatment	**Rate[z]**	**Condition**	**96**	**120**
Beauveria bassiana	Max	Expired	3.2 (± 2.4)	4.6 (± 3.0)
	Max	Fresh	13.1 (± 6.3)	15.5 (± 7.1)
	Min	Expired	2.9 (± 2.1)	5.9 (± 3.5)
	Min	Fresh	5.8 (± 3.5)	6.8 (± 3.9)
Isaria fumosoroseus	Max	Expired	4.4 (± 2.8)	11.4 (± 5.8)
	Max	Fresh	21.3 (± 8.8)	23.8 (± 9.5)
	Min	Expired	13.1 (± 6.3)	22.7 (± 9.3)
	Min	Fresh	10.9 (± 5.5)	19.6 (± 8.4)
Metarhizium anisopliae	Max	Expired	4.1 (± 2.7)	5.2 (± 3.2)
	Max	Fresh	1.0 (± 1.1)	2.0 (± 1.7)
	Min	Expired	3.2 (± 2.2)	4.1 (± 2.6)
	Min	Fresh	4.3 (± 2.8)	4.3 (± 2.8)
Water	--	Control	2.8 (± 2.1)	2.8 (± 2.1)

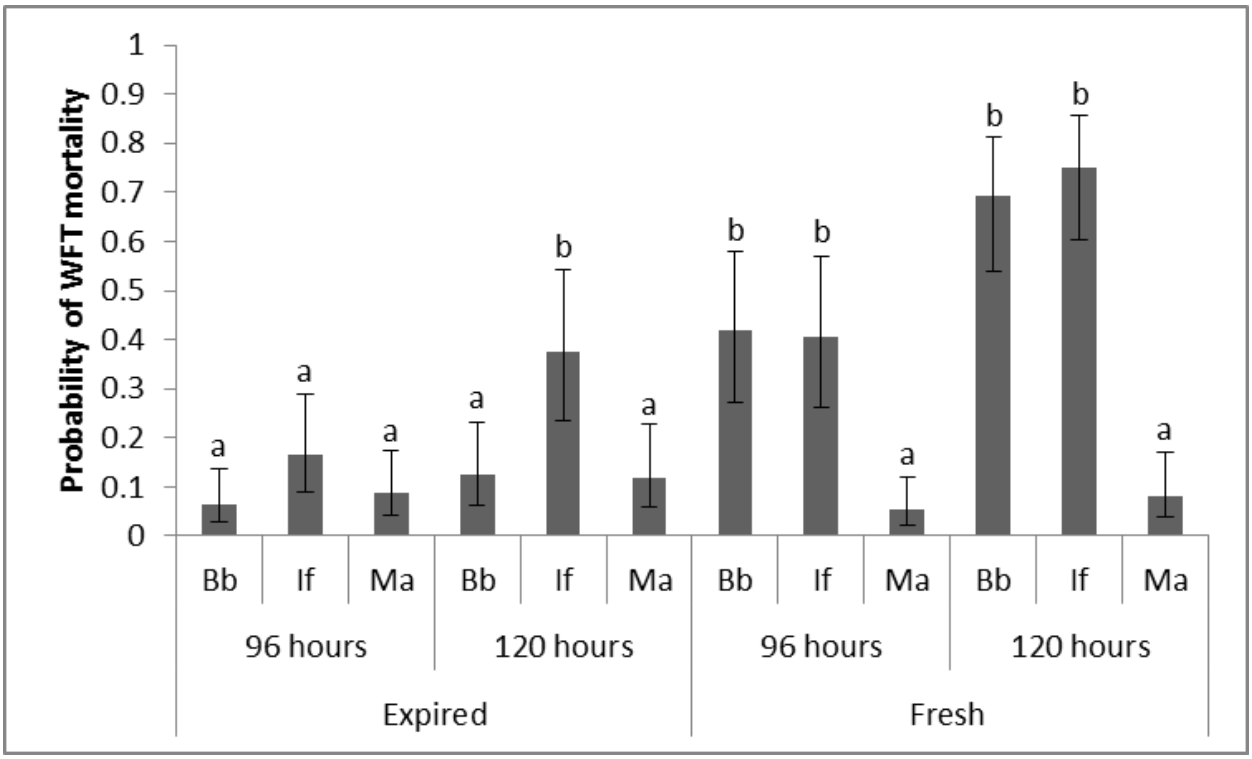
[z] Maximum and minimum labeled rates (per 200 mL): *Beauveria bassiana* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); *Isaria fumosoroseus* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); and *Metarhizium anisopliae* = 0.5 pt and 1 qt/100 gal (0.125 and 0.5 mL/200 mL).

Table 2.3 Mean (± SEM) percent probability of mortality associated with adult western flower thrips, *Frankliniella occidentalis* after 48, 72, 96, 120, 144, 168, 192, and 216 hours of exposure to three entomopathogenic fungi, *Beauveria bassiana*, *Isaria fumosoroseus*, and *Metarhizium anisopliae* at maximum and minimum labeled rates, and expired and fresh products.

			Hours of Exposure							
Treatment	**Rate[z]**	**Condition**	**48**	**72**	**96**	**120**	**144**	**168**	**192**	**216**
Beauveria bassiana	Max	Expired	1.6 ± 1.1a[y]	3.6 ± 1.8ad[y]	3.6 ± 1.8acd[y]	10.7 ± 3.9a[y]	32.8 ± 7.4af[y]	50.2 ± 8.1ad[y]	65.3 ± 7.3adg[y]	75.2 ± 8.6ac[y]
	Max	Fresh	3.0 ± 1.9a	23.6 ± 6.0bc	60.2 ± 7.4be	71.8 ± 6.5bcd	85.3 ± 4.6bd	92.8 ± 3.0bcf	99.0 ± 1.0bce	99.0 ± 1.1ab
	Min	Expired	0.0 ± 0.0a	1.3 ± 1.0a	1.9 ± 1.2ad	9.5 ± 3.5a	28.2 ± 6.8af	40.1 ± 7.7a	52.0 ± 7.9ag	70.6 ± 9.4ac
	Min	Fresh	0.8 ± 0.8a	12.8 ± 4.2ac	37.6 ± 7.3bf	58.3 ± 7.6bc	83.5 ± 4.9bd	92.6 ± 3.0bcf	98.2 ± 1.3bc	99.1 ± 1.0ab
Isaria fumosoroseus	Max	Expired	0.0 ± 0.0a	2.3 ± 1.3ad	45.2 ± 8.2beg	87.4 ± 4.4ce	97.3 ± 1.4be	97.3 ± 1.4bc	97.8 ± 1.2bc	99.7 ± 0.3bd
	Max	Fresh	8.3 ± 4.7a	25.3 ± 10.5cd	86.9 ± 6.7e	98.4 ± 1.4de	100.0 ± 0.0cdef	100.0 ± 0.0ace	100.0 ± 0.0cfg	100.0 ± 0.0acd
	Min	Expired	0.0 ± 0.0a	0.5 ± 0.5a	19.7 ± 5.6cfgi	52.7 ± 8.6bfg	82.3 ± 5.5bed	88.1 ± 4.2bce	93.3 ± 2.7bf	96.0 ± 2.2ad
	Min	Fresh	1.3 ± 1.1a	7.4 ± 4.1ac	70.6 ± 10.8be	91.8 ± 4.4cdf	99.3 ± 0.8be	100.0 ± 0.0ace	100.0 ± 0.0cfg	100.0 ± 0.0acd
Metarhizium anisopliae	Max	Expired	2.8 ± 1.7a	7.6 ± 3.1ac	10.7 ± 3.8afh	20.2 ± 5.5ag	41.8 ± 7.5fg	61.8 ± 7.3ade	76.6 ± 5.9aef	86.3 ± 5.8ad
	Max	Fresh	0.9 ± 1.1a	6.7 ± 4.2ac	22.5 ± 10.4abgh	67.3 ± 12.8cg	96.1 ± 2.8bed	100.0 ± 0.0ace	100.0 ± 0.0cfg	100.0 ± 0.0acd
	Min	Expired	1.0 ± 1.0a	2.9 ± 1.8ac	4.8 ± 2.4dhi	10.9 ± 3.8a	17.3 ± 5.0f	40.5 ± 7.4a	60.3 ± 7.4ag	73.3 ± 8.9a
	Min	Fresh	3.5 ± 1.9a	5.5 ± 2.5ac	10.5 ± 3.8dfh	23.3 ± 6.0ag	62.6 ± 7.6acdg	77.3 ± 6.1def	90.5 ± 3.6cdf	97.5 ± 1.8ad
Water	--	Control	1.0 ± 1.1a	3.9 ± 2.7ac	4.8 ± 3.1dfh	5.8 ± 3.6a	8.8 ± 4.9f	16.0 ± 7.7a	26.9 ± 10.9g	31.2 ± 9.6c

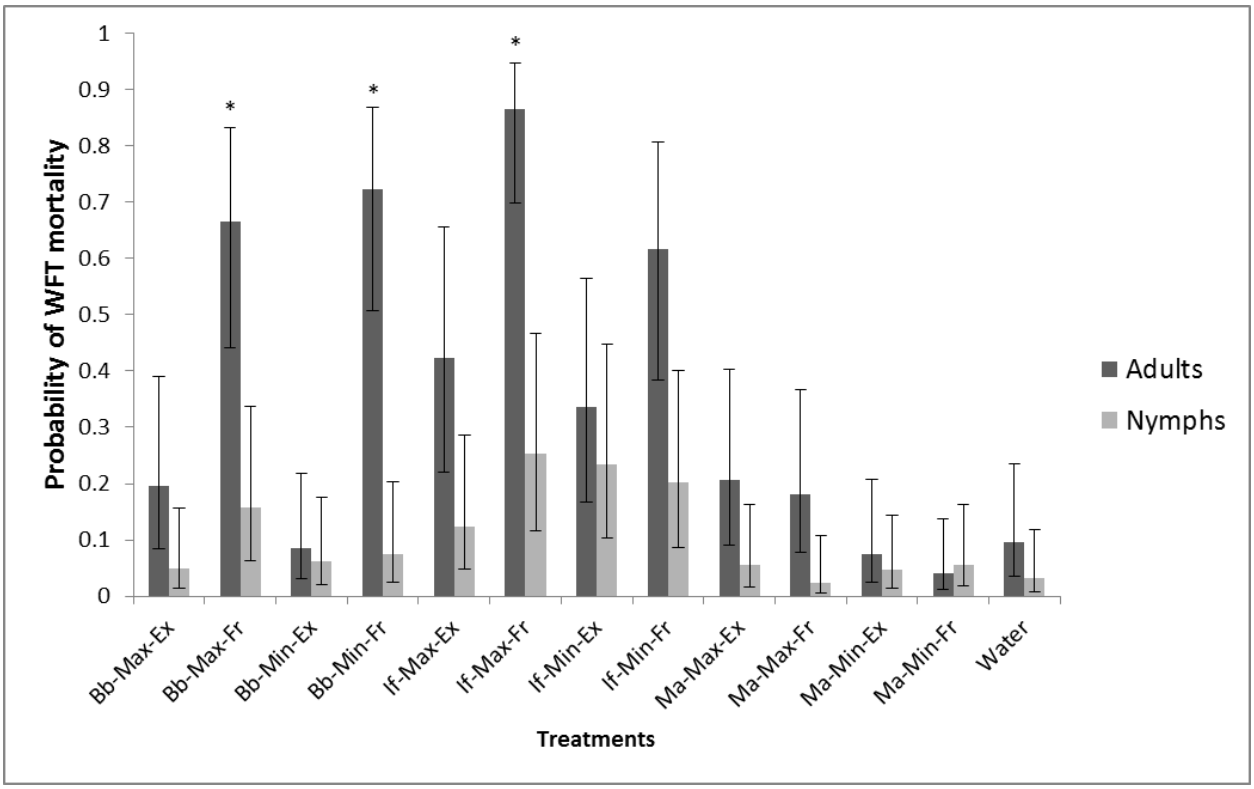
[z] Maximum and minimum labeled rates (per 200 mL): *Beauveria bassiana* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); *Isaria fumosoroseus* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); and *Metarhizium anisopliae* = 0.5 pt and 1 qt/100 gal (0.125 and 0.5 mL/200 mL).
[y] Means followed by a common lowercase letter within a column are not significantly different from each other ($P>0.05$) based on Bonferroni adjusted *P*-values.

Figure 2.1 Mean (± 95% confidence interval) probability of adult western flower thrips (WFT), *Frankliniella occidentalis* mortality after 96 and 120 hours of exposure to the entomopathogenic fungi *Beauveria bassiana* (Bb), *Isaria fumosoroseus* (If), and *Metarhizium anisopliae* (Ma) for fresh and expired products.



Bars within the same time point and product condition with the same letter are not significantly different from each other (P>0.05) based on Bonferroni adjusted P-values.

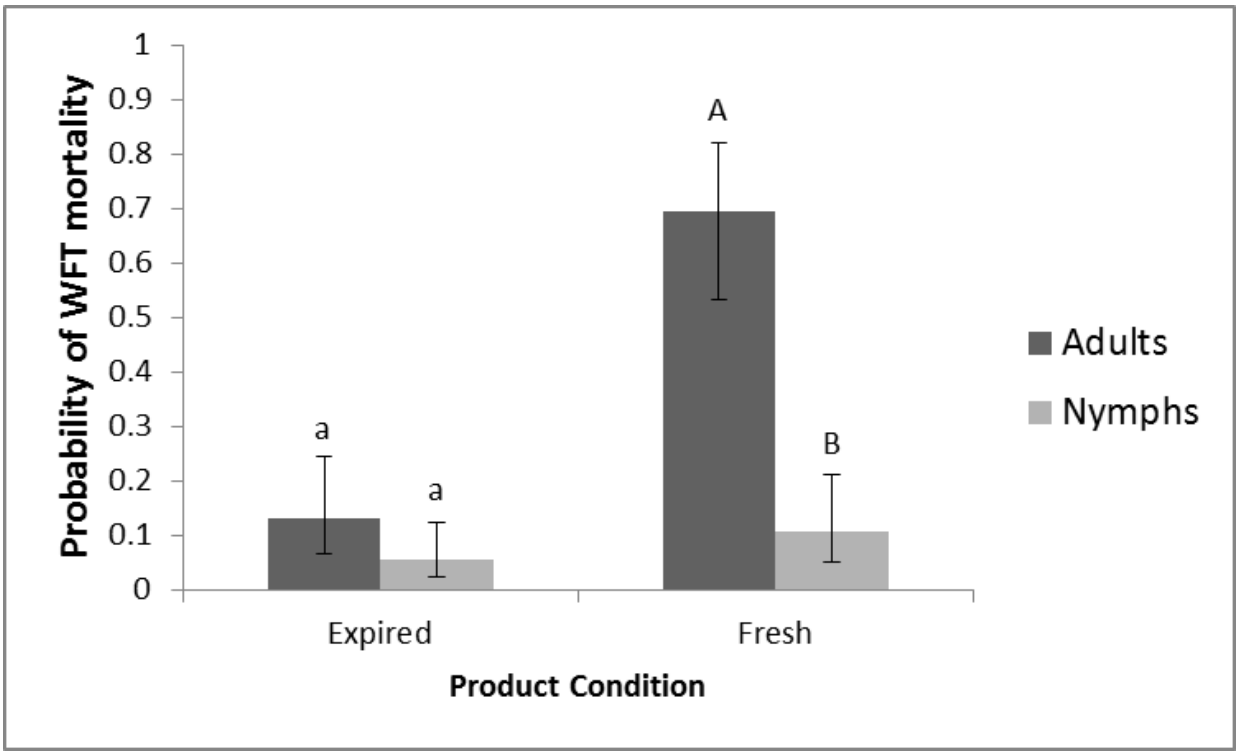
Figure 2.2 Mean (± 95% confidence intervals) probability of mortality associated with western flower thrips (WFT), *Frankliniella occidentalis* adults and nymphs when exposed to the entomopathogenic fungi *Beauveria bassiana* (Bb), *Isaria fumosoroseus* (If), and *Metarhizium anisopliae* (Ma) at the maximum (Max) and minimum (Min) labeled rates[1] of expired (Ex) and fresh (Fr) products.



* Indicates treatments with significant differences between adult and nymphal mortality ($P \leq 0.05$) based on Bonferroni adjusted *P*-values.

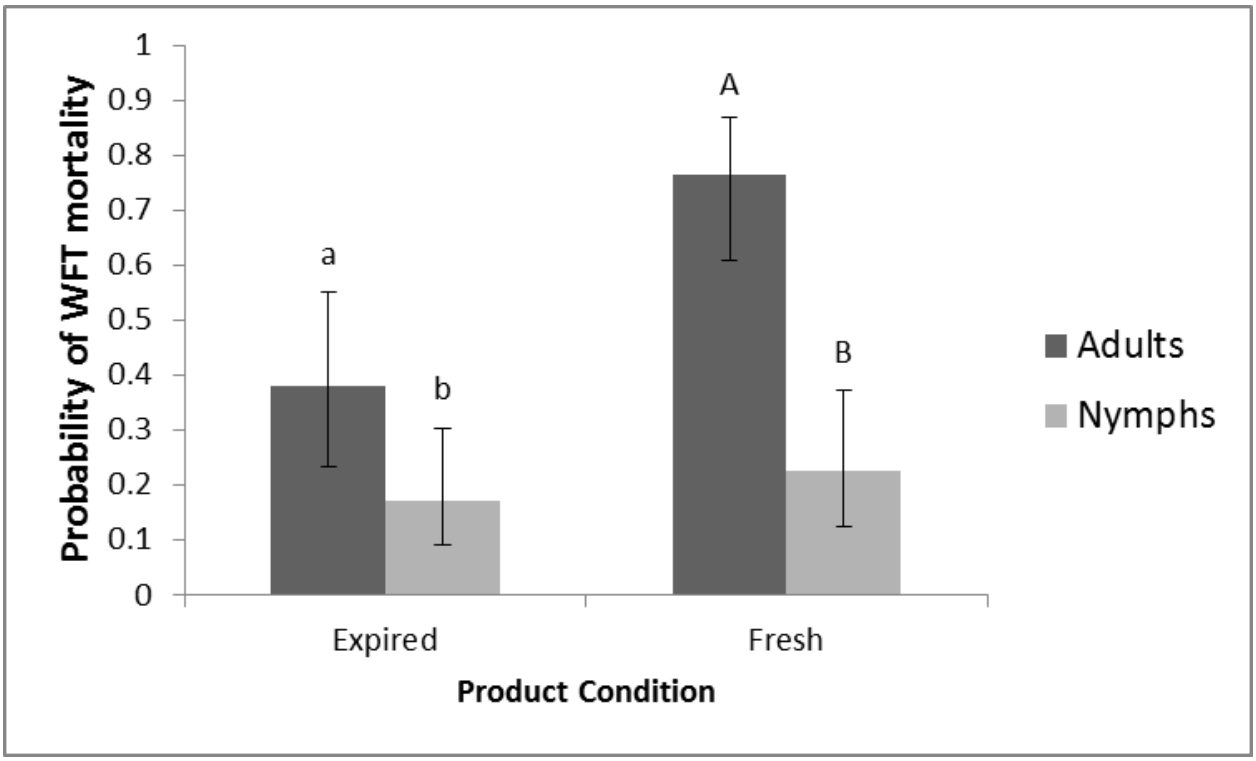
[1] Maximum and minimum labeled rates (per 200 mL): *Beauveria bassiana* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); *Isaria fumosoroseus* = 1 and 2 lbs/100 gal (0.24 g and 0.48 g/200 mL); and *Metarhizium anisopliae* = 0.5 pt and 1 qt/100 gal (0.125 and 0.5 mL/200 mL).

Figure 2.3 Mean (± 95% confidence intervals) probability of mortality associated with western flower thrips (WFT), *Frankliniella occidentalis* adults and nymphs after exposure to expired and fresh *Beauveria bassiana*.



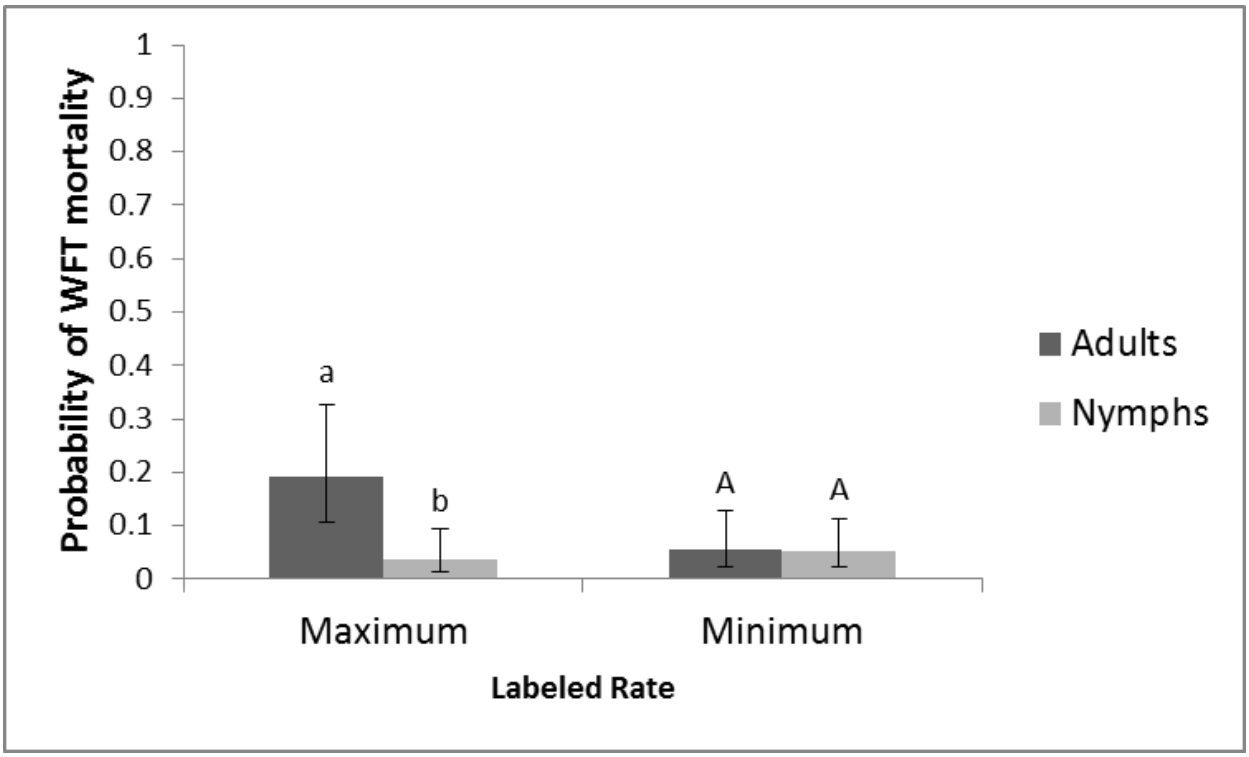
Bars with the same upper or lowercase letter are not significantly different from each other (P>0.05) based on Bonferroni adjusted P-values.

Figure 2.4 Mean (± 95% confidence intervals) probability of mortality associated with western flower thrips (WFT), *Frankliniella occidentalis* adults and nymphs after exposure to expired and fresh *Isaria fumosoroseus*.



Bars with the same upper or lowercase letter are not significantly different from each other (P>0.05) based on Bonferroni adjusted P-values.

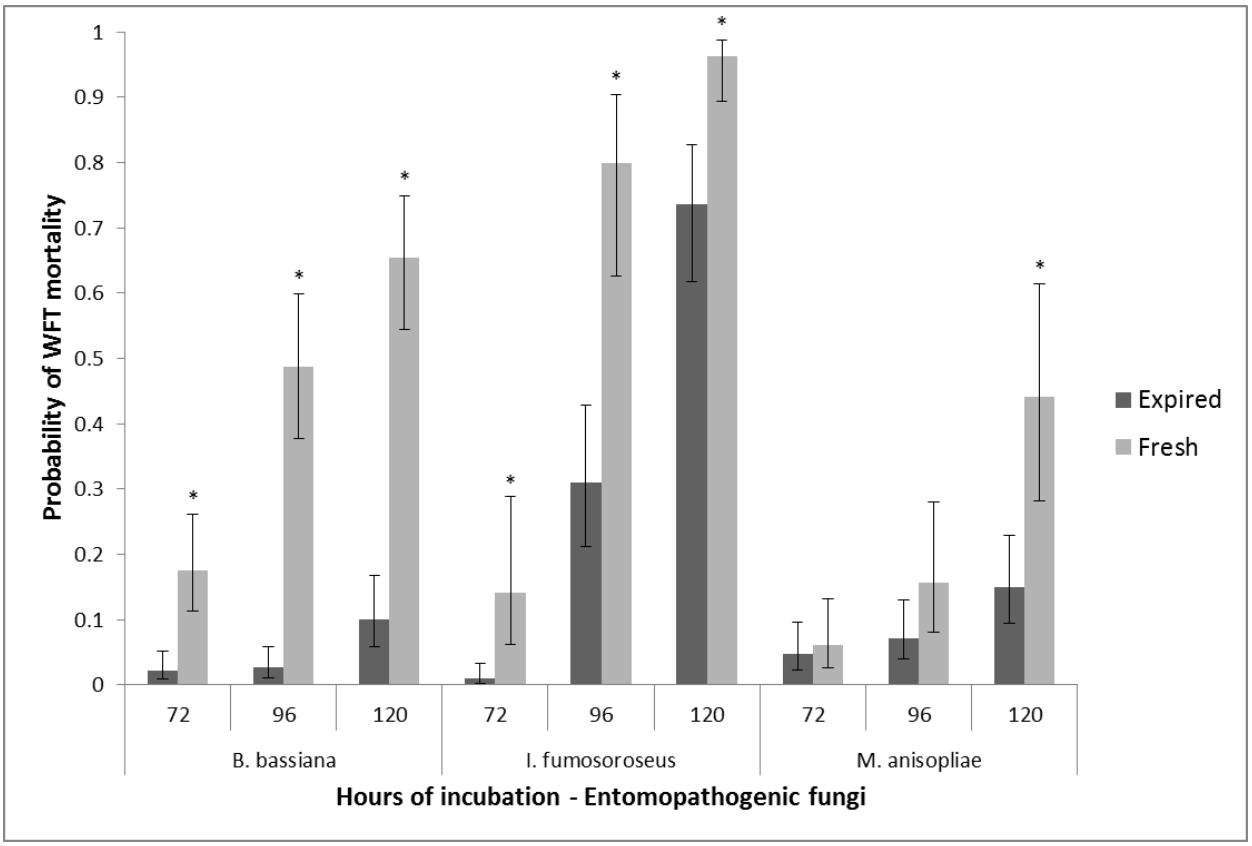
Figure 2.5 Mean (± 95% confidence intervals) probability of mortality associated with western flower thrips (WFT), *Frankliniella occidentalis* adults and nymphs when exposed to maximum and minimum labeled rates* of *Metarhizium anisopliae*.



Bars with the same upper or lowercase letter are not significantly different from each other (P>0.05) based on Bonferroni adjusted P-values.

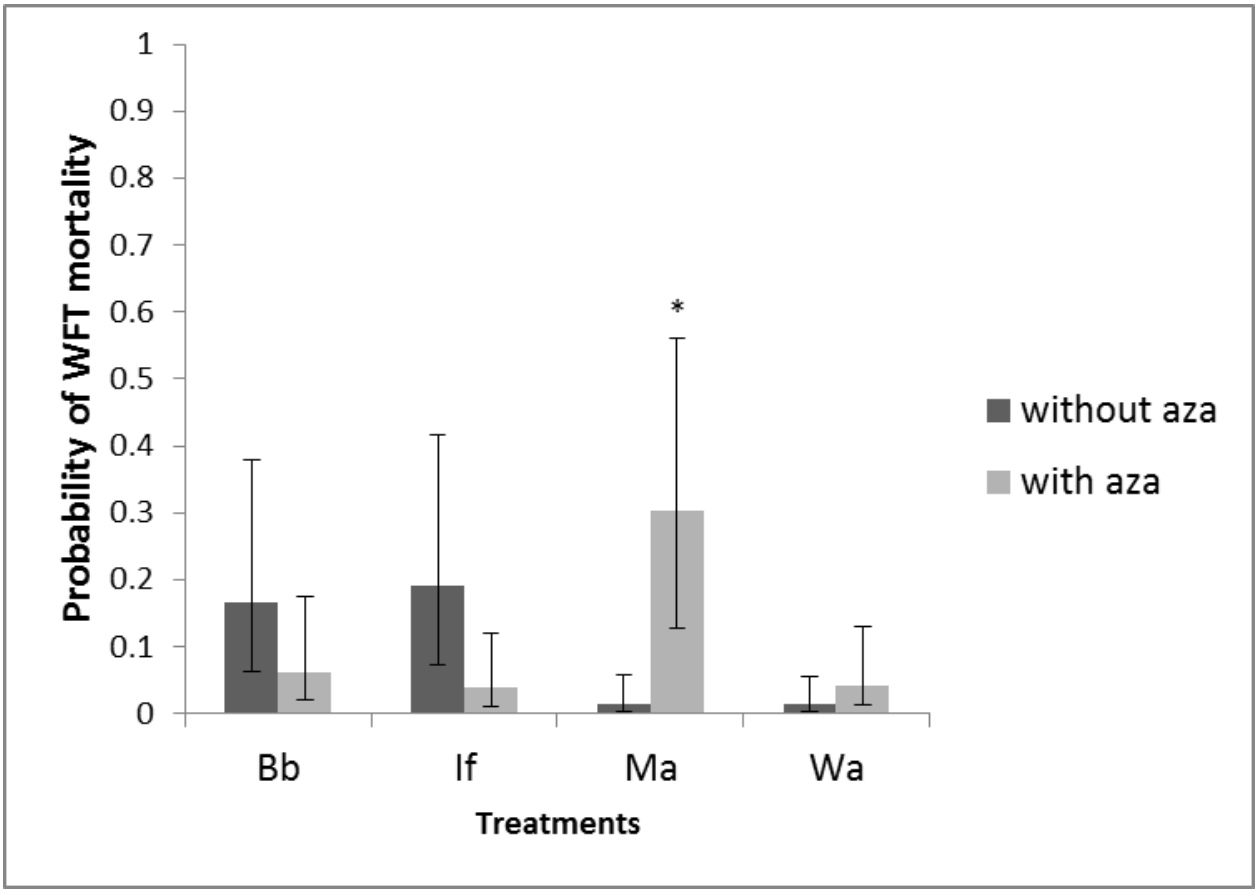
* Minimum and maximum labeled rates: 0.5 pt and 1 qt/100 gal (0.125 and 0.5 mL/200 mL).

Figure 2.6 Mean (± 95% confidence intervals) probability of adult western flower thrips (WFT), *Frankliniella occidentalis* mortality after 72, 96, and 120 hours of exposure to expired and fresh *Beauveria bassiana*, *Isaria fumosoroseus*, and *Metarhizium anisopliae*.



* Indicates treatments with significant differences between expired and fresh products within entomopathogenic fungal species ($P \leq 0.05$) based on Bonferroni adjusted *P*-values.

Figure 2.7 Mean (± 95% confidence intervals) probability of western flower thrips (WFT), *Frankliniella occidentalis* nymphal mortality when exposed to the entomopathogenic fungi, *Beauveria bassiana* (Bb), *Isaria fumosoroseus* (If), and *Metarhizium anisopliae* (Ma), and water (Wa) combined with and without azadirachtin (aza).



* Indicates treatments with significant differences between products with and without azadirachtin ($P \leq 0.05$) based on Bonferroni adjusted *P*-values.

Chapter 3 - Comparison of Insecticide Rotation Programs with Entomopathogenic Organisms for Suppression of Western Flower Thrips, *Frankliniella occidentalis* Populations under Greenhouse Conditions

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest causing significant economic losses in horticultural cropping systems (Robb and Parrella, 1995; Kirk, 2002; Reitz, 2009). Western flower thrips are difficult to manage due to their short life cycle, high reproductive capacity, and cryptic behavior (Jensen, 2000; Thoeming et al., 2003). Their life cycle, which comprises six life stages (egg, two nymphal stages, prepupa, pupa, and adult) (Ananthakrishnan, 1993; Tommasini and Maini, 1995; Mound, 1996), may be completed in one to two weeks at temperatures between 26° and 29°C (Lublinkhof and Foster, 1977; Robb, 1989; Gaum et al., 1994; Tommasini and Maini, 1995). Females lay between 150 and 300 eggs during their approximate five-week life span (Trichilo and Leigh, 1988). A major portion of their life cycle, is spent embedded in plant tissue (eggs), inside buds and open flowers (adults and nymphs), or in the growing medium (prepupa and pupa) (Tommasini and Maini, 1995; Mound, 1996; Jensen, 2000; Thoeming et al., 2003). This thigmotactic behavior, preferring to reside in tight enclosed areas, helps protects WFT from insecticides and natural enemies (Tommasini and Maini, 1995; Mound, 1996; Jensen, 2000; Brødsgaard, 2004).

Damage affiliated with WFT can be direct and indirect. Direct damage occurs when WFT adults and nymphs feed on plant fluids with their piercing-sucking mouthparts, causing distortion and discoloration of flowers and foliage (Chisholm and Lewis, 1984; Hunter and Ullman, 1989;

Lewis, 1997b). Indirect damage is associated with adults vectoring two tospoviruses: impatiens necrotic spot and tomato spotted wilt virus (Allen and Broadbent, 1986). Due to the aesthetic value of greenhouse-grown horticultural crops and the ability of WFT to transmit plant viruses, greenhouse producers have a very low tolerance for the presence of WFT populations (Parrella and Jones, 1987). Therefore, in order to suppress WFT populations, greenhouse producers rely on the use of insecticides (Cloyd, 2009; Reitz, 2009). However, the frequent use of insecticides results in intense selection pressure. Consequently, WFT have developed resistance to many different active ingredients (Whalon et al., 2014), including those in various chemical classes such as organophosphates, carbamates, pyrethroids, macrocyclic lactones (Immaraju et al., 1992; Zhao et al., 1995; Broadbent and Pree, 1997), and spinosyns (Loughner et al., 2005; Herron and James, 2005). In order to avoid resistance developing in WFT populations, it is important to rotate insecticides with different modes of action (Cloyd, 2009). However, greenhouse producers have a limited number of insecticide options, which will likely continue due to the cost of developing and registering new insecticides (Reitz and Funderburk, 2012). Therefore, by including entomopathogenic organisms (EPO) that have broad modes of activity, greenhouse producers may be able to effectively suppress WFT populations as well as reduce selection pressure.

Entomopathogenic organisms such as fungi and bacteria may provide a viable option in managing WFT populations because they can be mass-produced, formulated to promote efficacy and stability during storage, and applications can be made with conventional spray equipment (Bateman et al., 1993; Stathers et al., 1993; Jenkins and Lomer, 1994; Shah and Pell, 2003). Additional benefits of EPO include safety to greenhouse workers, and minimal effects on non-target organisms including parasitoids and predators (Steinhaus, 1958; Goettle and Hajek, 2000;

Pell et al., 2001). A number of studies have demonstrated that EPO can be used in combination with insecticides (Neves et al., 2001; Oliveira et al., 2003; Er and Gokce, 2004; Cuthbertson et al., 2005, 2008; Islam et al., 2010; Ambethgar et al., 2009; Hernandez et al., 2012). However, no information is available regarding the use of EPO in insecticide rotation programs. Therefore, the objective of this study was to determine the effectiveness of rotation programs that include both standard insecticides and EPO in suppressing WFT populations.

Materials and Methods

This study consisted of an initial phytotoxicity test and two greenhouse experiments to assess the use of *Beauveria bassiana* (BotaniGard® 22WP: Bioworks, Inc.; Victor, NY), *Isaria fumosoroseus* (NoFly™: Novozymes Biologicals Inc.; Salem, VA), *Metarhizium anisopliae* (Met52® EC: Novozymes Biologicals Inc.; Salem, VA) and *Chromobacterium subtsugae* (Grandevo®: Marrone Bio Innovations; Davis, CA) in rotation with four standard insecticides: spinosad (Conserve® SC: Dow AgroSciences; Indianapolis, IN), chlorfenapyr (Pylon®: BASF Corp.; Research Triangle Park, NC), abamectin (Avid® 0.15 EC: Syngenta Crop Protection; Greensboro, NC), and pyridalyl (Overture® 35 WP: Valent U.S.A., Corp.; Walnut Creek, CA). These insecticides are commonly used to suppress WFT populations in greenhouse production systems (Cloyd, unpublished data). The rotation treatments for each experiment are presented in Tables 3.2 and 3.3. Environmental conditions including light intensity, temperature, and relative humidity were recorded every hour using HOBO® Data Loggers (Onset Computer Corporation; Bourne, MA). A summary of the environmental conditions are presented in Table 3.1. At the start of the experiment, plant height was 23 to 31 cm. Over the course of the experiment, plants grew approximately 15 cm. Western flower thrips specimens used in this study are deposited as

voucher number 237 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

Phytotoxicity test on transvaal daisy, *Gerbera jamesonii* plants

Sixteen transvaal daisy, *Gerbera jamesonii* (H. Bolus ex Hook.f) plants in 10.1 cm containers were obtained from Masson's Greenhouse (Linwood, KS). Plants were in flower, and repotted into 15.2 cm containers using Fafard® 2 Mix growing medium (SunGro Horticulture; Agawam, MA). Each plant was randomly assigned to be sprayed with one of the seven insecticides listed in Table 3.2 or a water control. Each plant was treated twice, allowing for one week between applications. Plants were randomized on a wire-mesh bench in a greenhouse at the Kansas State University Throckmorton Plant Sciences Center (Manhattan, KS). Observations associated with phytotoxicity were recorded seven days after the insecticide applications. Phytotoxicity was determined based on a 1 to 5 rating scale with 1 = no visible damage, 2 = 1 to 25% damage, 3 = 26 to 50% damage, 4 = 51 to 75% damage, and 5 = >75% damage. This damage rating was modified from Cloyd and Cycholl (2002). The data was used as baseline information for evaluating plant damage due to WFT feeding in subsequent experiments. Therefore, any damage observed would be strictly attributed to WFT feeding.

Caged experiments in the greenhouse

Two experiments were conducted in a glass-covered greenhouse at the Throckmorton Plant Sciences Center. Each experiment was repeated twice over time using flowering chrysanthemum, *Dendranthema x morifolium* Ramat, plants. Chrysanthemum was used for this study because of their length of flowering time (Post, 1949). Preliminary studies with transvaal

daisy plants had to be terminated after six weeks due to rapid decline in plant quality. All plants for the following experiments were obtained from Masson's Greenhouse (Linwood, KS).

Experiment 1: Eight-week rotation programs with entomopathogenic fungi

Thirty-six flowering chrysanthemum plants in 10.1 cm containers were repotted into 15.2 cm containers using Fafard® 2 Mix growing medium. Plants consisted of a variety of unknown cultivars with different flower colors. Plants were randomly placed into thirty-six individual clear plastic cages [45.7 x 45.7 x 60.9 cm (length x width x height)] arranged in four rows of nine cages located on two wire-mesh greenhouse benches (4.3 x 1.1 m). Each cage had a lid and three holes (12.7 cm diameter) covered with thrips screening (Greentek; Edgerton, WI); one on the lid, and two on each side of the cage. These openings allowed for ventilation but prevented WFT adults from escaping. Each plant was artificially infested with 25 WFT adults (7 to 10 days old) obtained from laboratory-reared colonies in the Department of Entomology at Kansas State University (Manhattan, KS). Western flower thrips were allowed to establish on the plants for one week before the first insecticide application was made. Six plants were randomly assigned to one of six rotation treatments (n=6) (Table 3.2). Treatments were applied at one week intervals for eight weeks. The standard insecticides used in this experiment were spinosad (Conserve® SC) at 6 fl oz/100 gal, chlorfenapyr (Pylon®) at 5.2 fl oz/100 gal, abamectin (Avid® 0.15 EC) at 8 fl oz/100 gal, and pyridalyl (Overture® 35 WP) at 8 oz/100 gal. Entomopathogenic fungi used in the experiment were *Beauveria bassiana* strain GHA (BotaniGard® 22WP) at 1 lb/100 gal, *Isaria fumosoroseus* strain FE 9901 (NoFly™) at 28 oz/100 gal, and *Metarhizium anisopliae* Strain F52 (Met52® EC) at 0.5 pt/100 gal. Treatment one represented a standard insecticide rotation program that greenhouse producers might use to suppress WFT populations (Cloyd, unpublished data). For treatments two, three, and four, one of the three entomopathogenic fungi were used in

weeks five and six. Treatment five was a rotation that included all three entomopathogenic fungi across all eight weeks and treatment six was a water control.

Treatments were applied by removing each plant from the cage, placing on a concrete floor, and spraying the plants using a 946 mL plastic spray bottle (The Home Depot; Manhattan, KS). Approximately 30 mL of each treatment was applied to each plant, which was a sufficient volume to thoroughly cover all plant parts, including leaves and flowers, with minimal run-off. To avoid any cross contamination among treatments, all plants associated with any given treatment were sprayed and returned to their cages before the next treatment was applied. Furthermore, latex gloves (Fisher Scientific; Pittsburgh, PA) that were worn during treatment application were also replaced after each treatment to avoid cross contamination associated with handling plants. All treatment applications were made in the late afternoon or early evening to avoid exposing fungal spores to mid-day sunlight. Old senescing flowers were removed, as needed, and placed beside the plant inside the cage so that any WFT adults and nymphs residing within the flowers could return to the plant. To irrigate, each plant was carefully removed from the cage, placed on a concrete floor, and then watered as needed with a plastic 7.6 L watering can. Water was applied directly to the growing medium in order to avoid wetting the foliage. Plants were returned to the cages immediately afterward. All plants were fertilized at week 4 using Miracle-Gro® 24:8:16 (N:P:K) Water Soluble All-Purpose Plant Food (Scotts Miracle-Gro Products, Inc.; Marysville, OH) at a rate of 3.9 mL/L.

A yellow sticky card (12.7 x 7.6 cm) (Pestrap™ Phytotronics, Inc.; Earth City, MO) was placed into each cage, approximately 8 cm from the plant, and held in position by a wooden clothes pin attached to a bamboo stake. Each bamboo stake was placed into a plastic container filled with sand in order to hold the stake upright. Western flower thrips adults were counted on

yellow sticky cards seven days post-application each week. Additionally, the presence or absence of flowers per plant was recorded. On the final assessment day, flowers and foliage of each plant was evaluated for quality based on a 1 to 5 rating scale with 1 = no visible damage, 2 = 1 to 25% damage, 3 = 26 to 50% damage, 4 = 51 to 75% damage, and 5 = >75% damage. This damage rating was modified from Cloyd and Cycholl (2002) and was used to quantify plant damage due to WFT feeding. The experiment was conducted twice over time: trial one [September 27 to November 29, 2013 (fall)] and trial two [December 9, 2013 to February 17, 2014 (winter)]. All cages were thoroughly cleaned before starting the next trial.

Experiment 2: Eight-week rotation programs with entomopathogenic fungi and bacterium

Thirty-six flowering chrysanthemum plants in 10.1 cm containers were repotted into 15.2 cm containers using Fafard® 2 Mix growing medium. Plants consisted of a variety of unknown cultivars with different flower colors. Plants were randomly placed into thirty-six individual clear plastic cages [45.7 x 45.7 x 60.9 cm (length x width x height)] arranged in four rows of nine cages located on two wire-mesh greenhouse benches (4.3 x 1.1 m). Each cage had a lid and three holes (12.7 cm diameter) covered with thrips screening; one on the lid, and two on each side of the cage. These openings allowed for ventilation but prevented WFT adults from escaping. Each plant was artificially infested with 25 WFT adults (7 to 10 days old) obtained from laboratory-reared colonies in the Department of Entomology at Kansas State University (Manhattan, KS). Western flower thrips were allowed to establish on the plants for one week before the first insecticide application was made. Six plants were randomly assigned to one of six rotation treatments (n=6) (Table 3.3). Treatments were applied at one week intervals for eight weeks. The standard insecticides and rates used in this experiment were the same as those used in experiment one. The entomopathogenic organisms and rates used in this experiment were

Beauveria bassiana strain GHA (BotaniGard® 22WP) at 1 lb/100 gal, *Metarhizium anisopliae* strain F52 (Met52® EC) at 0.5 pt/100 gal and *Chromobacterium subtsugae* strain PRAA4-1^{T} (Grandevo®) at 2 lbs/100 gal. For more information on why *C. subtsugae* was used instead of *I. fumosoroseus*, refer to Appendix B. Treatment one represented a standard insecticide rotation program that greenhouse producers might use to suppress WFT populations (Cloyd, unpublished data). For treatments two, three, and four, one of three entomopathogenic organisms were used in weeks three and four. Treatment five was a rotation that included all three entomopathogenic organisms across all eight weeks and treatment six was a water control. Treatments applications were made in the same manner as the first experiment taking care to avoid cross contamination between treatments. Old senescing flowers were removed as needed, and plants were irrigated and fertilized similar to experiment one.

Western flower thrips adults were counted on yellow sticky cards placed inside each cage seven days post-application each week. Additionally, the presence or absence of flowers per plant was recorded. On the final assessment day, flowers and foliage of each plant were evaluated for quality using the same rating scale as experiment one. The experiment was conducted twice over time: trial one [February 25 to May 1, 2014 (spring)] and trial two [May 5 to July 7, 2014 (summer)]. All cages were thoroughly cleaned before starting the next trial.

Cost comparison of insecticide rotation programs

To determine the cost of each rotation program in experiment two, the price of each product was determined from the 2014-2015 Hummert International Commercial Catalog (Hummert International; Earth City, MO). However, Grandevo (*C. subtsugae*) was not available in the Hummert catalog so the cost was determined from the Marrone Bio Innovations website (http://www.marronebioinnovations.com). A cost comparison for a rotation treatment including

I. fumosoroseus was not possible because the product NoFly is no longer available (refer to Appendix B). The labeled rates for WFT used in this study were used to calculate the cost of each application per week based on 100 gallon applications.

Statistical analysis

Both experiments were analyzed by fitting a generalized linear mixed model to the number of WFT adults captured on yellow sticky cards. This response was modeled using the negative binomial distribution with a canonical log link function. The linear predictors in the model included the fixed effects of season (fall and winter for experiment one, and spring and summer for experiment two), treatment (6 levels, consisting of specified rotations of EPO and insecticides, as well as a water control), and time (weeks 1 through 8), as well as all two- and three-way interactions. Random effects associated with the linear predictors for experiment one included row-bench combinations in the greenhouse nested within each cultivar and season; this allowed for recognition of the experimental unit for season. Random effects in the linear predictors for experiment two included cultivar nested within season in order to recognize the experimental unit for season, as well as row-bench combinations in the greenhouse for a given season and assigned to individual treatments. This made it possible to recognize cage as the experimental unit for treatment, as well as the unit of repeated measures over weeks. For experiment one, convergence problems prevented fitting a separate variance component to isolate variability between cultivars across seasons or between row-bench combinations as an overall blocking factor.

Over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion for either experiment one or two. The final statistical models used for inference were fitted using Residual Pseudo-likelihood. Degrees

of freedom were approximated and estimated standard errors were adjusted using Kenward-Roger's procedure. The statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute, Cary, NC) implemented using Newton-Raphson with ridging as the optimization technique. Relevant pairwise comparisons were conducted using Bonferroni adjustments to avoid inflation of type I error due to multiple comparisons.

Foliage and flower quality ratings were fitted as a generalized linear mixed model using a multinomial distribution of the conditional response fitted with a cumulative logit link function. The linear predictor of the statistical model used for analysis included the fixed effects of season, treatment, and their two-way interaction. Random effects in the linear predictor included cultivar nested within season. For experiment two, random effects in the linear predictor included row-bench combination as an overall blocking factor and also its effect nested within season and cultivar. Convergence problems from experiment two prevented fitting a separate variance component to isolate variability between cultivars across seasons. The statistical models for experiments one and two were fitted using the GLIMMIX procedure of SAS implemented using Newton-Raphson with ridging as the optimization technique. The method of estimation was Residual Pseudo-likelihood. Pairwise comparisons were conducted using Tukey-Kramer's or Bonferroni's procedure, as appropriate in each case, to adjust for multiple comparisons.

Results

Phytotoxicity test on transvaal daisy, *Gerbera jamesonii* plants

No phytotoxicity was observed based on visual inspection for any of the treatments associated with the initial experiment. Transvaal daisy plants were used to test for phytotoxic effects because of their sensitivity to chemical sprays (Cloyd and Raudenbush, 2014).

Experiment 1: Eight-week rotation programs with entomopathogenic fungi

Results associated with WFT counts from the rotation treatments in experiment one are presented in Figures 3.1 and 3.2. Furthermore, the environmental conditions recorded during experiment one and two over seasons are presented in Table 3.1. The average number of WFT adults captured on yellow sticky cards was significantly lower in the winter than the fall ($P<0.0001$). Therefore, treatment effects were evaluated over the eight weeks within each season. There was no significant difference in WFT counts among rotation treatments based on the two-way interaction between rotation treatments and week affiliated with the suppression of WFT populations ($F=1.41$; df=35, 480; $P=0.063$). In the fall, there was no evidence of any treatment differences within each week (Figure 3.1). However, in the winter, there was one treatment difference between rotation treatment one (standard insecticides) and treatment five (all three entomopathogenic fungi) during week two ($P=0.012$).

Final foliage quality ratings from experiment one are presented in Figures 3.3 and 3.4. The two-way interaction between rotation treatments and season was not significantly different in regards to foliage quality among the rotation treatments ($F=1.75$; df=5, 39; $P=0.15$). Results for the final flower quality ratings are presented in Figures 3.5 and 3.6. There was a significant difference associated with season in regards to flower quality rating ($F=5.63$; df=1, 9.974; $P=0.039$) in which lower ratings (representing less WFT feeding damage) appeared more frequently in the winter. However, there were no significant differences among rotation treatments in regards to flower quality rating ($F=1.15$; df=5, 35; $P=0.35$).

Experiment 2: Eight-week rotation programs with entomopathogenic fungi and bacterium

Results associated with WFT counts for experiment two are presented in Figures 3.7 and 3.8. Unlike experiment one, there was no significant difference between seasons (F=0.18; df=1, 4.6; P=0.69). Furthermore, the three-way interaction between the rotation treatments, seasons, and weeks in suppressing WFT populations was not significant (F=0.95; df=35, 480; P=0.56). However, the two-way interaction between rotation treatment and week was significant (F=3.12; df=35, 480; P<0.0001). In the spring, significant differences among treatments were observed in weeks six, seven, and eight (Figure 3.7). In week six, treatment four (*C. subtsugae*) had significantly fewer adult WFT captured on yellow sticky cards than treatment six (water) (P=0.023). In week seven, treatment one, comprised of the standard insecticides, had significantly fewer WFT adults captured on yellow sticky cards than treatment five, comprised of all three entomopathogenic fungi, and treatment six (water) (P=0.0002 and P=0.025, respectively). Treatment two (*M. anisopliae*) had significantly fewer WFT adults captured on yellow sticky cards than treatment five (all three entomopathogenic fungi) (P=0.006); and treatment four (*C. subtsugae*) had significantly fewer WFT adults captured on yellow sticky cards than both treatment five (all three entomopathogenic fungi) and treatment six (water) (P<0.0001 and P=0.014, respectively). By week eight, treatments one (standard insecticides), two (*M. anisopliae*), and four (*C. subtsugae*) had significantly fewer WFT captured on yellow sticky cards than treatment five (all three entomopathogenic fungi) and treatment six (water). Furthermore, treatment three (*B. bassiana*) had significantly fewer WFT adults than treatment five (all three entomopathogenic fungi) (P=0.004). In trial two (summer 2014), the only significant differences observed occurred in week six in which treatments one (standard

insecticides), two (*M. anisopliae*), three (*B. bassiana*), and four (*C. subtsugae*) had significantly fewer WFT captured on yellow sticky cards than treatment 6 (water) (P=0.0008, P=0.006, P=0.0005, and P=0.037, respectively) (Figure 3.8).

Results for the final foliage and flower quality ratings associated with the rotation treatments in experiment two are presented in Figures 3.9 through 3.11. No flower quality rating results were available in summer because all the flowers had senesced and were removed. In trial one (spring 2014), there were no significant differences among the rotation treatments in regards to flower quality (F=0.70; df=5, 27; P=0.63). Furthermore, there were no significant differences in foliage quality ratings among the rotation treatments regardless of season (F=1.62; df=5, 57; P=0.17).

Cost Comparison

The total cost for each eight-week rotation treatment from greenhouse experiment two is presented in Table 3.4. The standard insecticide program would cost greenhouse producers about $684.00 to spray 100 gallons of insecticide solution once a week for eight weeks. Using *B. bassiana* in place of abamectin would cost about $24.50 more than the standard insecticide program. However, if *M. anisopliae* or *C. subtsugae* were used instead of abamectin, there would be a savings of $73.50 or $55.50, respectively. This savings could be more depending on which standard insecticide was substituted out for an EPO. For example, chlorfenapyr cost $357.50 for two applications; whereas, two applications of EPO only cost $60 to $160, depending on the EPO. If all of the EPO are used for all eight weeks such as rotation treatment five, the cost would be $450, which is a savings of $234.

Discussion

This study compared various rotation programs that included EPO and commonly used standard insecticides for suppression of WFT populations in greenhouses. The standard insecticides used in this study were spinosad, pyridalyl, abamectin, and chlorfenapyr, which have been shown to be effective against WFT (Jones et al., 2002; Isayama et al., 2005; Broughton and Herron, 2009; Willmott et al., 2013). However, WFT populations have developed resistance to several of these compounds (Broadbent and Pree, 1997; Loughner et al., 2005; Whalon et al., 2014). A common method of avoiding insecticide resistance development is by rotating compounds with different modes of action (Cloyd, 2009). The EPO used in this study, *Beauveria bassiana*, *Isaria fumosoroseus*, *Metarhizium anisopliae*, and *Chromobacterium subtsugae* have also been shown to be effective in suppressing WFT populations (Murphy et al., 1998; Labanowski and Soika, 1999; Maniania et al., 2001) and should be considered by greenhouse producers for use in rotation programs with other insecticides having different modes of action.

Furthermore, this is the first study to evaluate the effectiveness of rotations programs against WFT that include EPO. McKenzie et al. (2014) compared different rotation programs that included entomopathogenic fungi on the effect of two cryptic species of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), on poinsettia, *Euphorbia pulcherrima* Willd. ex Klotzsch. The rotation programs that included entomopathogenic fungi were the least effective against sweetpotato whitefly. However, this was attributed to reduced efficacy due to the slow activity of entomopathogenic fungi compared to standard insecticides and the high whitefly populations present during the experiment. It was recommended to use entomopathogenic fungi early when populations are low.

Matheron and Porchas (2012) determined the effect of various fungicide rotation programs in suppressing powdery mildew, *Podosphaera xanthii* Castag., on cantaloupe, *Cucumis melo* L., that included standard fungicides and biofungicides in order to avoid resistance development. Rotation programs composed of only biofungicides resulted in significantly lower disease suppression than rotations programs involving standard fungicides. Nevertheless, when biofungicides and standard fungicides were used in the same rotation program, disease suppression was comparable to most rotation programs that included only standard fungicides (Matheron and Porchas, 2012). In other studies, the entomopathogenic fungus, *Lecanicillium muscarium* (Petch) Zare and Gams, has been used in sequential applications with insecticides commonly used for the suppression of the sweetpotato whitefly (Cuthbertson et al., 2005; 2008). Results indicated that there was no significant difference in whitefly larval mortality between treatments that included standard insecticides and *L. muscarium*, and treatments that included only the standard insecticides. In addition, all treatments with and without *L. muscarium* resulted in significantly higher whitefly larval mortality than the water control. However, there has been no quantitative evaluation to determine the effectiveness of insecticide rotation programs that include EPO for suppression of WFT populations in greenhouses.

Although it was not possible to compare the efficacy of the individual compounds within the rotations, our objective was to compare the effectiveness of various rotation programs as a whole. All of the compounds used have been shown to be effective against WFT populations. For example, past studies have demonstrated nearly 100% mortality of WFT with abamectin (Willmott et al., 2013; Kay and Herron, 2010) and spinosad (Jones et al., 2002; Cloyd and Sadof, 2000). Chlorfenapyr has provided > 90% mortality and pyridalyl has provided 80% mortality (Cloyd, unpublished data; 2007 and 2008). Vestergaard et al. (1995) observed 94% mortality of

WFT adults after seven days of incubation using *M. anisopliae* and Wu et al. (2014) obtained 96% mortality of WFT adults after four days of exposure to *B. bassiana*. There are limited data available on the efficacy of *I. fumosoroseus* on WFT mortality; however, fresh *I. fumosoroseus* used in the laboratory bioassays of this study (Chapter 2) resulted in 100% mortality of WFT adults after six days.

Due to the proven efficacy of the standard insecticides and the relatively slow activity of EPO, it was expected that the standard insecticide rotation treatment would provide the greatest suppression of WFT. However, in both greenhouse experiments there were no significant differences between the standard insecticide rotation program (treatment one) and those that included EPO (treatments two, three, and four) in reducing WFT populations. The only significant differences observed were in experiment two in which treatments five (only EPO) and six (water) had higher numbers of WFT captured on yellow sticky cards than the standard insecticide rotation (treatment one) and those that included both insecticides and EPO (treatments two, three, and four).

Furthermore, foliage and flower quality ratings were not significantly different among any of the rotation treatments for both experiments. However, there appeared to be numerical differences in foliage and flower quality among some of the treatments (Figures 3.3, 3.5, and 3.11). This variability may be due to the subjective nature of the quality ratings based on visual observations. In addition, there may be a lack of statistical power to detect differences among treatments, especially in situations where some plants could not receive a flower quality rating because all the flowers had senesced and been removed. This reduced the number of replications per treatment to less than six for the flower quality ratings. However, all six plants within each treatment received a foliage quality rating. A plant with a foliage quality rating of one or two

may be considered by a greenhouse producer to be a marketable product. Based on the foliage quality ratings from rotation treatments one through four, more than 80% of the plants from winter and spring and greater than 60% of the plants from summer maintained a quality rating of one or two. This suggests that it may be possible to maintain foliage quality even in the presence of WFT populations for crops that are not susceptible to tospoviruses.

In addition to WFT counts and quality ratings, environmental conditions were recorded. Temperature, relative humidity and light intensity are important factors to consider because they affect the growth and development of WFT as well as the infectivity of entomopathogenic fungi. The lowest temperatures were observed in the winter (20°C) and the highest temperatures were observed in the summer (27°C). The lower temperatures associated with winter may have been responsible for the low number of WFT adults captured on yellow sticky cards. For example, the optimal temperature for most entomopathogenic fungi is between 20° and 25°C; however, infection can occur at temperatures between 15° and 30°C (Inglis et al., 2001). The optimal temperature for WFT development and reproduction is 30°C (Gaum et al., 1994).

Other environmental factors such as relative humidity and light intensity may have an effect on entomopathogenic fungi. Relative humidity was highest during summer (64%) and lowest in winter (32%). Relative humidity is an important factor in the success of entomopathogenic fungi infecting a host; however, this may be associated with the microhabitat environment at the leaf surface or host body (Ferron, 1977; Fargues et al., 1997; Inglis et al., 2001). While the relative humidity may have been lower in the winter, the relative humidity in the plant canopy and at the leaf surface may have been higher. Furthermore, light intensity was higher in the spring and summer (281 and 304 PAR, respectively) and lower in the fall and winter (170 and 177 PAR, respectively). Exposure to ultra-violet (UV) light can cause mortality

of entomopathogenic fungi (Inglis et al., 2001). For this reason, applications for all rotation treatments were applied in the late afternoon or evening to avoid exposure of the fungal spores to mid-day sunlight. Also, spores within the plant canopy may be protected from exposure to UV light (Inglis et al., 1993).

Finally, a cost comparison was done to determine if there would be any financial incentivies to incorporating EPO into rotation programs. Based on the cost comparison, greenhouse producers could save anywhere from $55 to $230, depending on the rotation program. The cost savings in combination with preserving the efficacy of existing insecticides could provide long term benefits to greenhouse producers.

In conclusion, this study has demonstrated that EPO may be used in rotation programs along with standard insecticides commonly used against WFT. By incorporating EPO into insecticide rotation programs, greenhouse producers may reduce selection pressure on WFT populations, thus, possibly reducing resistance as well as resulting in savings for pest management costs. Therefore, greenhouse producers should consider utilizing rotation programs that include both EPO and standard insecticides for the suppression of WFT populations. However, future research is warranted to compare other rotation programs including various combinations and sequences. Also, this study was conducted with a baseline population of WFT that has never been exposed to insecticides. Therefore, it is justified to determine how effective the rotation programs evaluated in this study would be against field populations of WFT with known resistance to one or more of the standard insecticides.

Table 3.1 Mean (± SEM) environmental parameters including light intensity (µmol/m^2/s of PAR for 400-700 nm); high, low, and mean temperatures (°C); and relative humidity for the eight-week periods of October 4 to November 29, 2013 (Fall 2013); December 23, 2013 to February 17, 2014 (Winter 2013-2014); March 6 to May 1, 2014 (Spring 2014); and May 12 to July 7, 2014 (Summer 2014).

Experiment-Season	Light intensity[a]	Temperature °C			Relative Humidity
	µmol/m²/s of PAR	High	Low	Mean	(%)
Experiment 1					
Fall 2013	170 (± 3.2)	33.9	17.2	20.8 (± 0.1)	45.4 (± 0.4)
Winter 2013-2014	177 (± 3.4)	31.1	11.3	19.7 (± 0.1)	31.6 (± 0.2)
Experiment 2					
Spring 2014	281 (± 5.5)	41.2	15.6	23.2 (± 0.2)	36.1 (± 0.4)
Summer 2014	304 (± 5.7)	45.1	11.7	27.1 (± 0.2)	63.8 (± 0.4)

[a] To exclude periods of darkness from the light intensity averages, only readings >15 lumens/ft^2 were included. To convert these units to µmol/m²/s of PAR (Photosynthetically Active Radiation) for 400-700 nm, light intensity measured in lumens/ft^2 was multiplied by 0.20 (Thimijan and Heins, 1983).

Table 3.2 Experiment one rotation treatments incorporating the entomopathogenic fungi *Beauveria bassiana, Isaria fumosoroseus,* and *Metarhizium anisopliae* into an eight-week insecticide rotation program designed to suppress western flower thrips, *Frankliniella occidentalis* populations.

	Weeks			
Treatments[z]	**1 and 2**	**3 and 4**	**5 and 6**	**7 and 8**
1	spinosad	pyridalyl	abamectin	chlorfenapyr
2	spinosad	pyridalyl	*M. anisopliae*	chlorfenapyr
3	spinosad	pyridalyl	*B. bassiana*	chlorfenapyr
4	spinosad	pyridalyl	*I. fumosoroseus*	chlorfenapyr
5	*B. bassiana*	*M. anisopliae*	*I. fumosoroseus*	*B. bassiana*
6	water	water	water	water

[z] Product Information: spinosad (Conserve® SC: Dow AgroSciences, Indianapolis, IN), chlorfenapyr (Pylon®: BASF Corp., Research Triangle Park, NC), abamectin (Avid® 0.15 EC: Syngenta Crop Protection, Greensboro, NC), pyridalyl (Overture® 35 WP: Valent U.S.A. Corp., Walnut Creek, CA), *Isaria fumosoroseus* strain FE 9901 (NoFly™: Novozymes Biologicals Inc., Salem, VA), *Metarhizium anisopliae* Strain F52 (Met52® EC: Novozymes Biologicals Inc., Salem, VA), and *Beauveria bassiana* strain GHA (BotaniGard® 22WP: Bioworks, Inc.; Victor, NY).

Table 3.3 Experiment two rotation treatments incorporating the entomopathogenic organisms *Beauveria bassiana*, *Metarhizium anisopliae* (fungi) and *Chromobacterium subtsugae* (bacterium) into an eight-week insecticide rotation program designed to suppress western flower thrips, *Frankliniella occidentalis* populations.

	Weeks			
Treatments[z]	**1 and 2**	**3 and 4**	**5 and 6**	**7 and 8**
1	spinosad	pyridalyl	abamectin	chlorfenapyr
2	spinosad	*M. anisopliae*	pyridalyl	chlorfenapyr
3	spinosad	*B. bassiana*	pyridalyl	chlorfenapyr
4	spinosad	*C. subtsugae*	pyridalyl	chlorfenapyr
5	*B. bassiana*	*M. anisopliae*	*C. subtsugae*	*B. bassiana*
6	water	water	water	water

[z] Product Information: spinosad (Conserve® SC: Dow AgroSciences, Indianapolis, IN), chlorfenapyr (Pylon®: BASF Corp., Research Triangle Park, NC), abamectin (Avid® 0.15 EC: Syngenta Crop Protection, Greensboro, NC), pyridalyl (Overture® 35 WP: Valent U.S.A. Corp., Walnut Creek, CA), *Metarhizium anisopliae* Strain F52 (Met52® EC: Novozymes Biologicals Inc., Salem, VA), *Beauveria bassiana* strain GHA (BotaniGard® 22WP: Bioworks, Inc., Victor, NY), and *Chromobacterium subtsugae* (Grandevo®: Marrone Bio Innovations, Davis, CA).

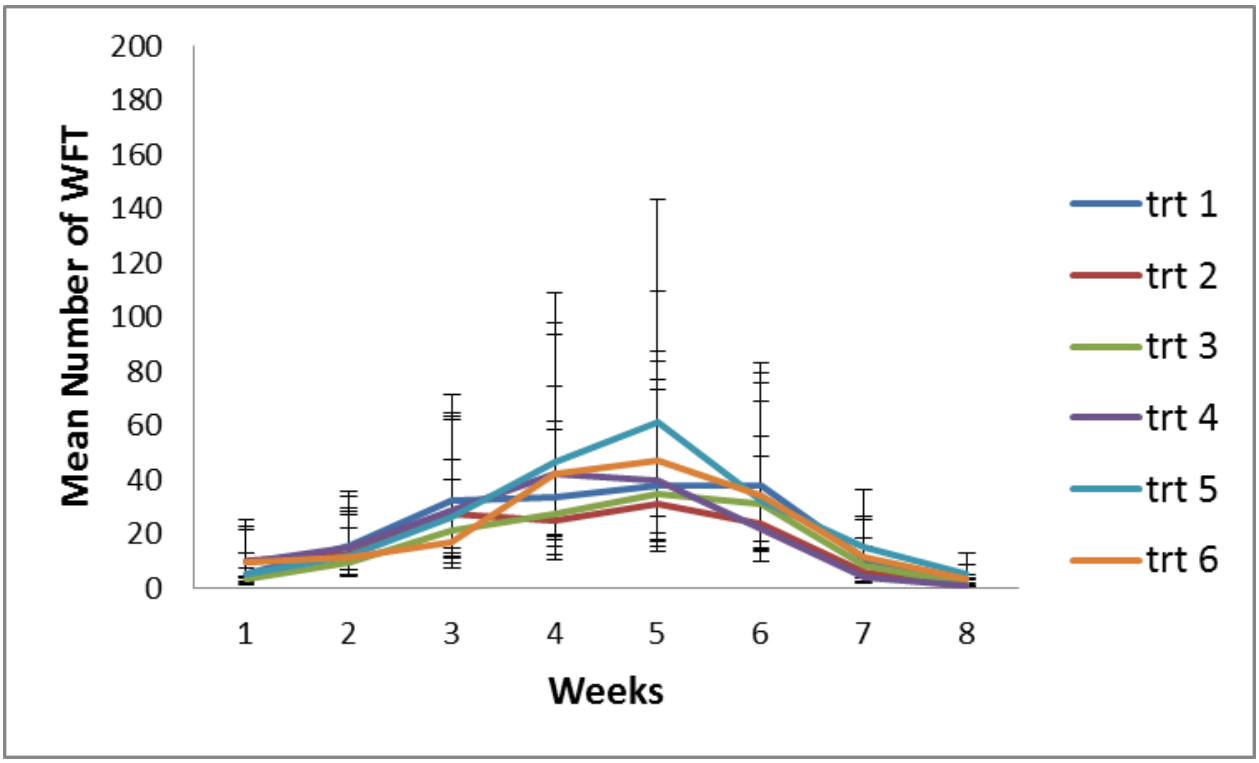
Table 3.4 Cost comparison of eight-week rotation treatments that include standard insecticides and the entomopathogenic organisms, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Chromobacterium subtsugae* based on 100 gallon applications each week at labeled rates for western flower thrips, *Frankliniella occidentalis*.

	Weeks				
Treatments[y]	**1 and 2**	**3 and 4**	**5 and 6**	**7 and 8**	**Total cost (100 gal applications)**
1	spinosad ($71.00)[z]	pyridalyl ($123.00)	abamectin ($132.50)	chlorfenapyr ($357.50)	$684.00
2	spinosad ($71.00)	*M. anisopliae* ($59.00)	Pyridalyl ($123.00)	chlorfenapyr ($357.50)	$610.50
3	spinosad ($71.00)	*B. bassiana* ($157.00)	Pyridalyl ($123.00)	chlorfenapyr ($357.50)	$708.50
4	spinosad ($71.00)	*C. subtsugae* ($77.00)	Pyridalyl ($123.00)	chlorfenapyr ($357.50)	$628.50
5	*B. bassiana* ($157.00)	*M. anisopliae* ($59.00)	*C. subtsugae* ($77.00)	*B. bassiana* ($157.00)	$450.00

[y] Product information and rates: spinosad at 6 fl oz/100 gal (Conserve® SC: Dow AgroSciences, Indianapolis, IN), chlorfenapyr at 5.2 fl oz/100 gal (Pylon®: BASF Corp., Research Triangle Park, NC), abamectin at 8 fl oz/100 gal (Avid® 0.15 EC: Syngenta Crop Protection, Greensboro, NC), pyridalyl at 8 oz/100 gal (Overture® 35 WP: Valent U.S.A. Corp., Walnut Creek, CA), *Metarhizium anisopliae* Strain F52 at 0.5 pt/100 gal (Met52® EC: Novozymes Biologicals Inc., Salem, VA), *Beauveria bassiana* strain GHA at 1 lb/100 gal (BotaniGard® 22WP: Bioworks, Inc., Victor, NY), and *Chromobacterium subtsugae* at 2 lbs/100 gal (Grandevo®: Marrone Bio Innovations, Davis, CA).

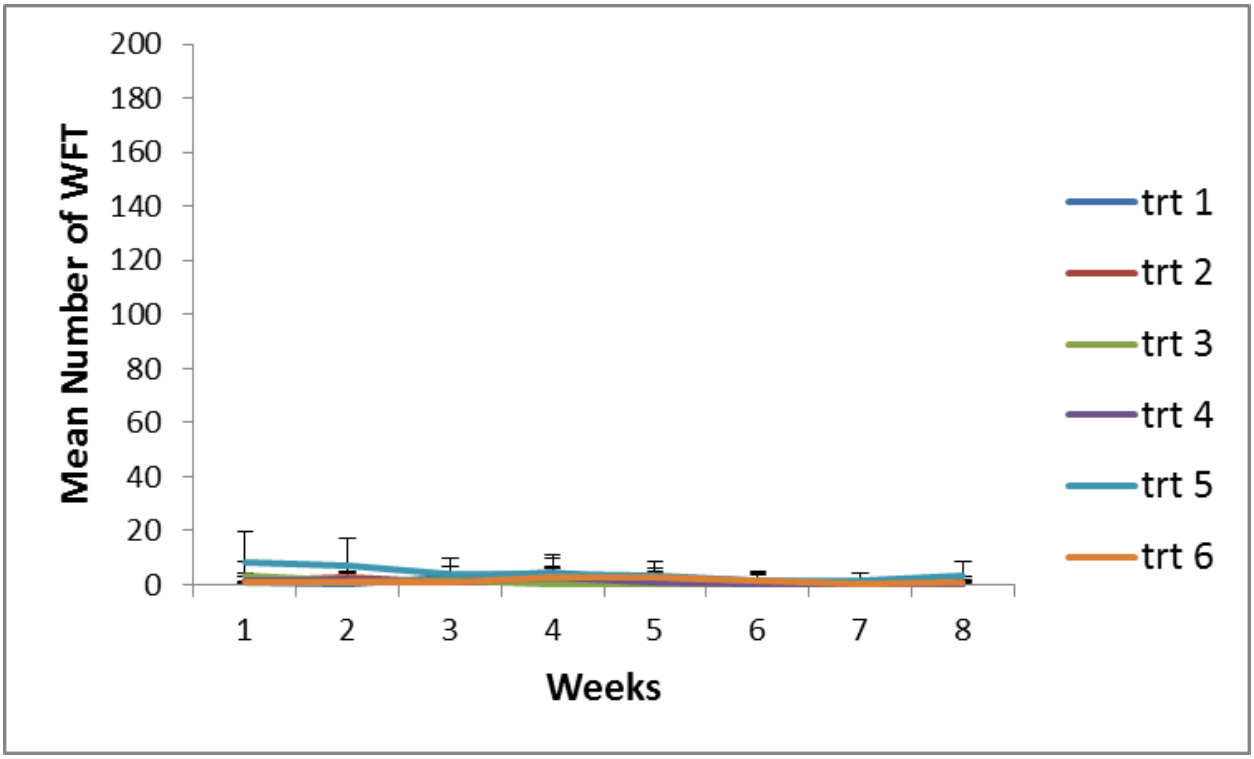
[z] Prices are based on the 2014-2015 Hummert International Commercial Catalog (Hummert International; Earth City, MO). Price of *C. subtsugae* is taken from the Marrone Bio Innovations website (http://www.marronebioinnovations.com).

Figure 3.1 Mean (± 95% confidence intervals) number of western flower thrips (WFT), *Frankliniella occidentalis* adults captured on yellow sticky cards per week for rotation treatments (trt) 1 through 6 associated with experiment 1 (October 4 to November 29, 2013).



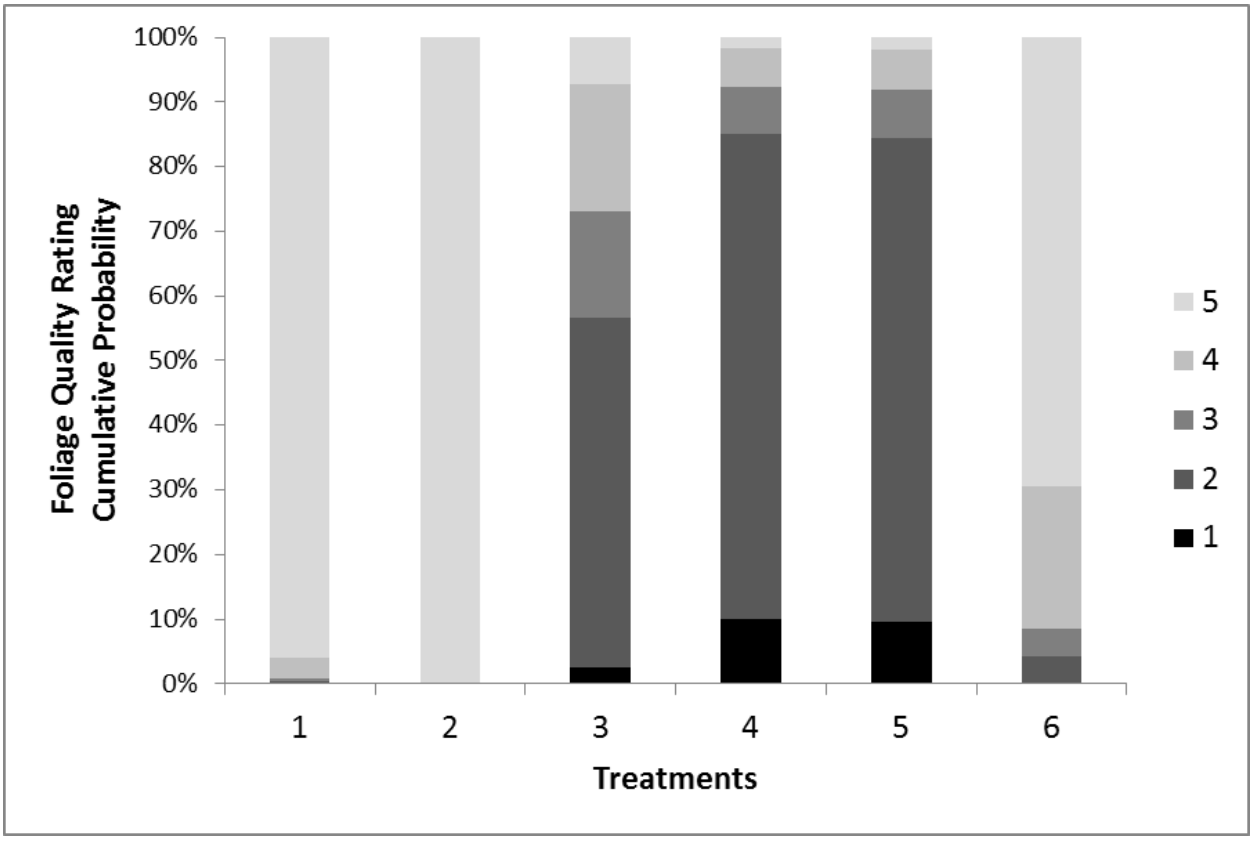
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.2 Mean (± 95% confidence intervals) number of western flower thrips (WFT), *Frankliniella occidentalis* adults captured on yellow sticky cards per week for rotation treatments (trt) 1 through 6 associated with experiment 1 (December 23, 2013 to February 17, 2014).



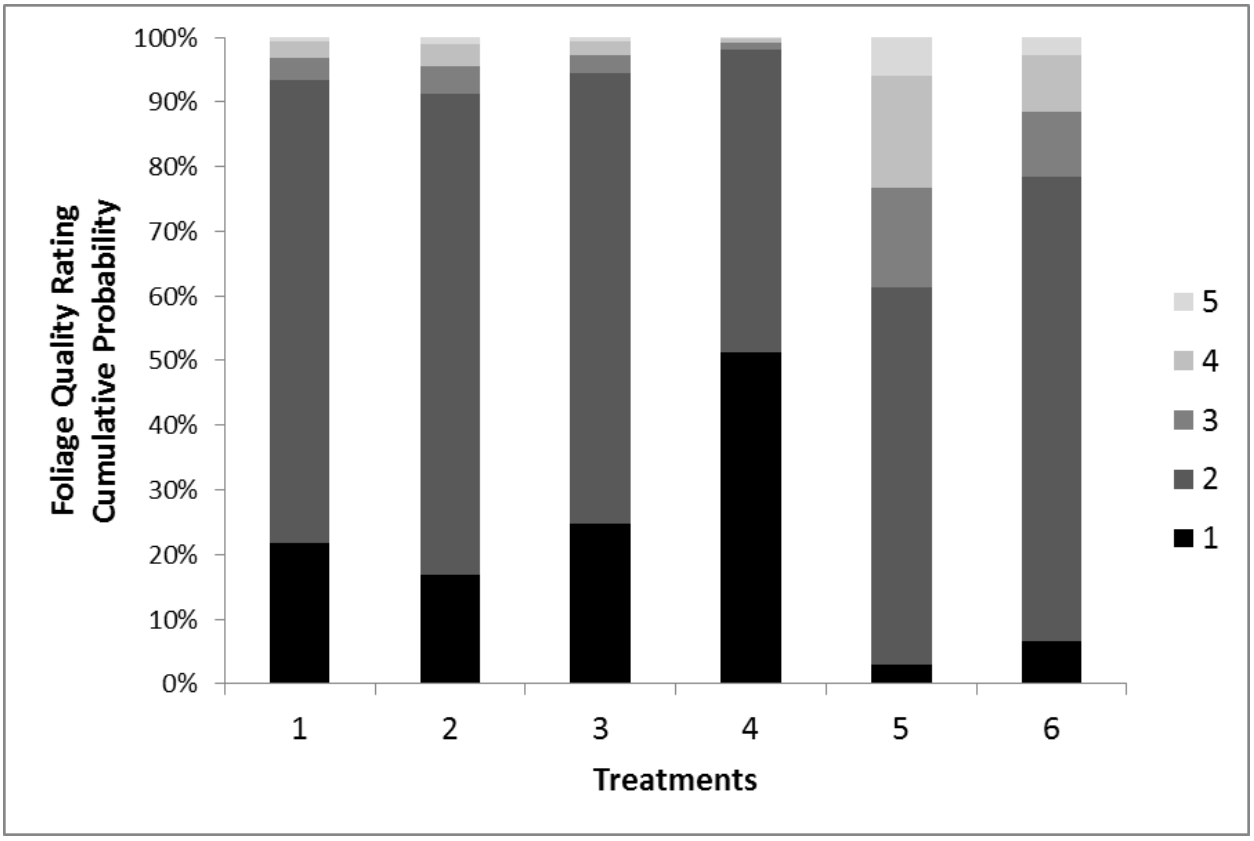
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.3 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the foliage of chrysanthemum, *Dendranthema x morifolium* plants for experiment 1 (October 4 to November 29, 2013). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



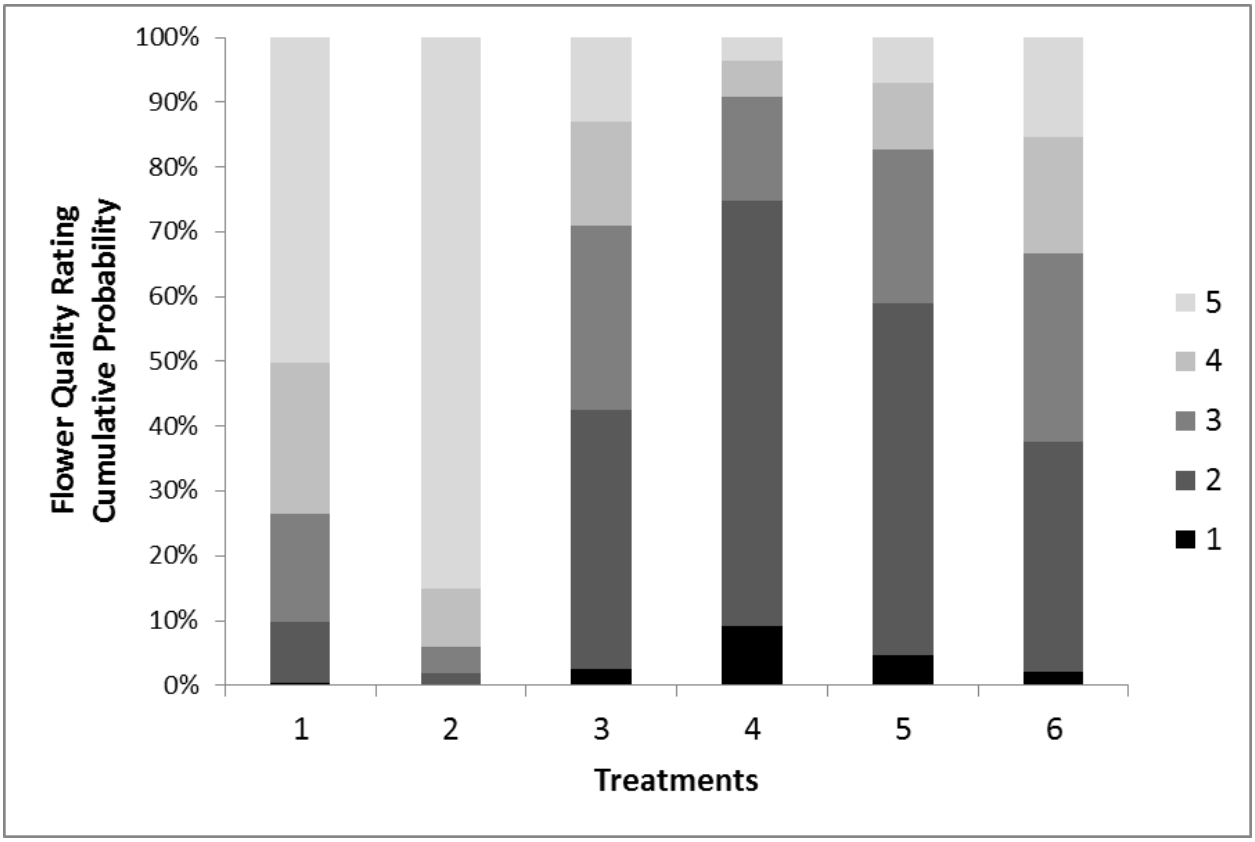
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.4 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the foliage of chrysanthemum, *Dendranthema x morifolium* plants for experiment 1 (December 23, 2013 to February 17, 2014). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



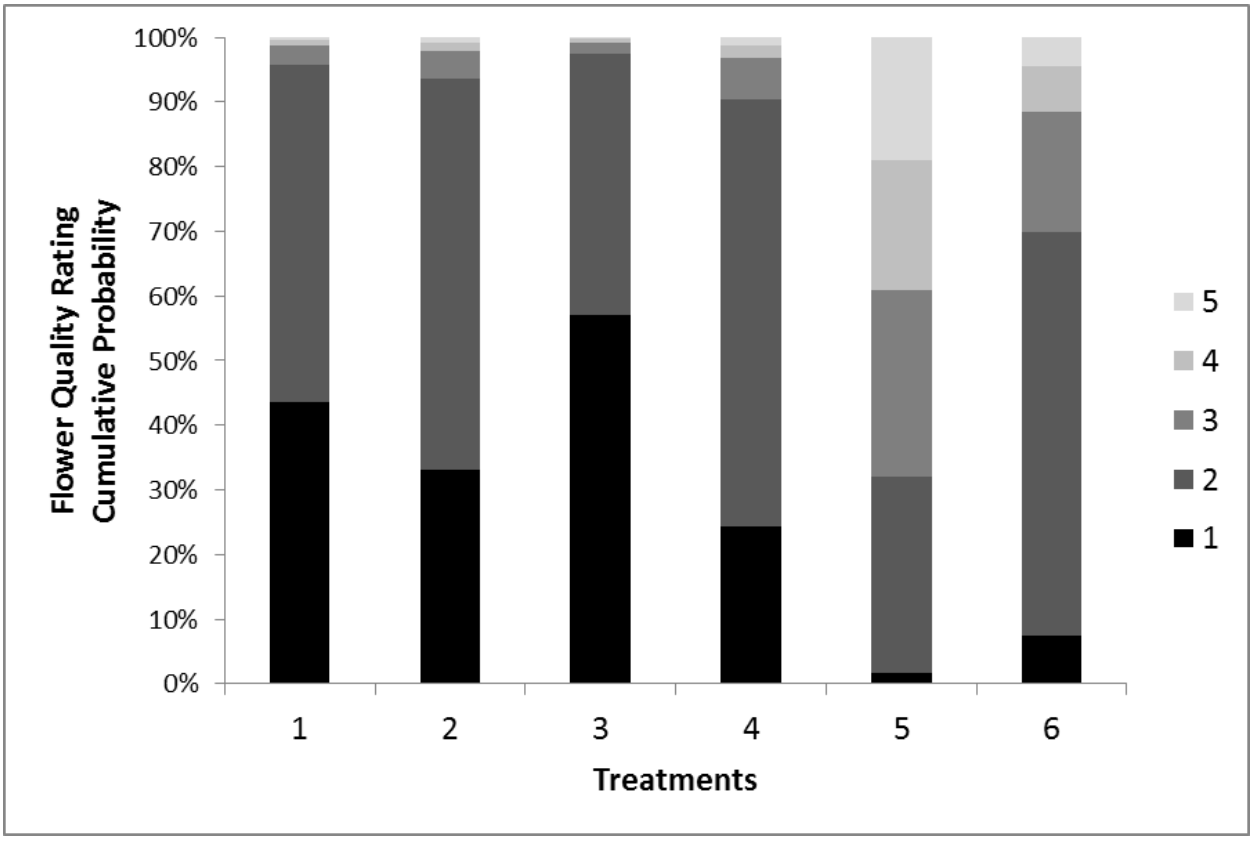
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.5 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the flowers of chrysanthemum, *Dendranthema x morifolium* plants for experiment 1 (October 4 to November 29, 2013). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



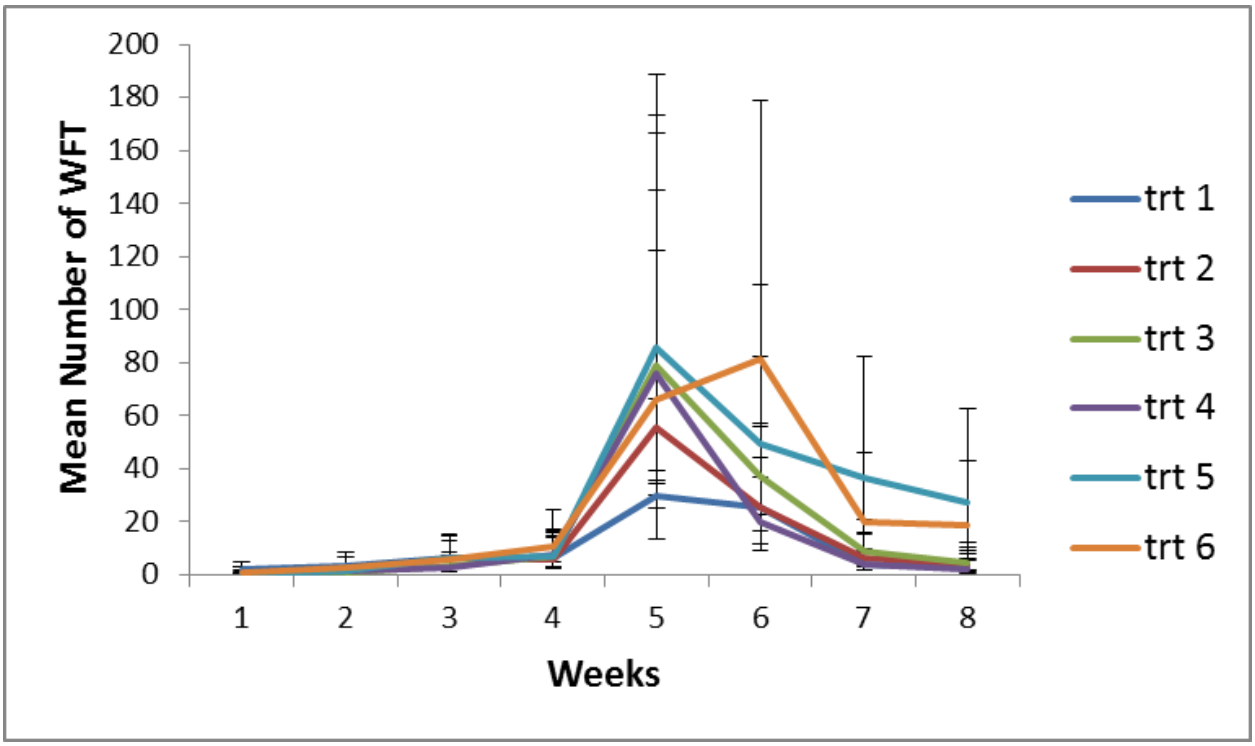
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.6 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the flowers of chrysanthemum, *Dendranthema x morifolium* plants for experiment 1 (December 23, 2013 to February 17, 2014). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



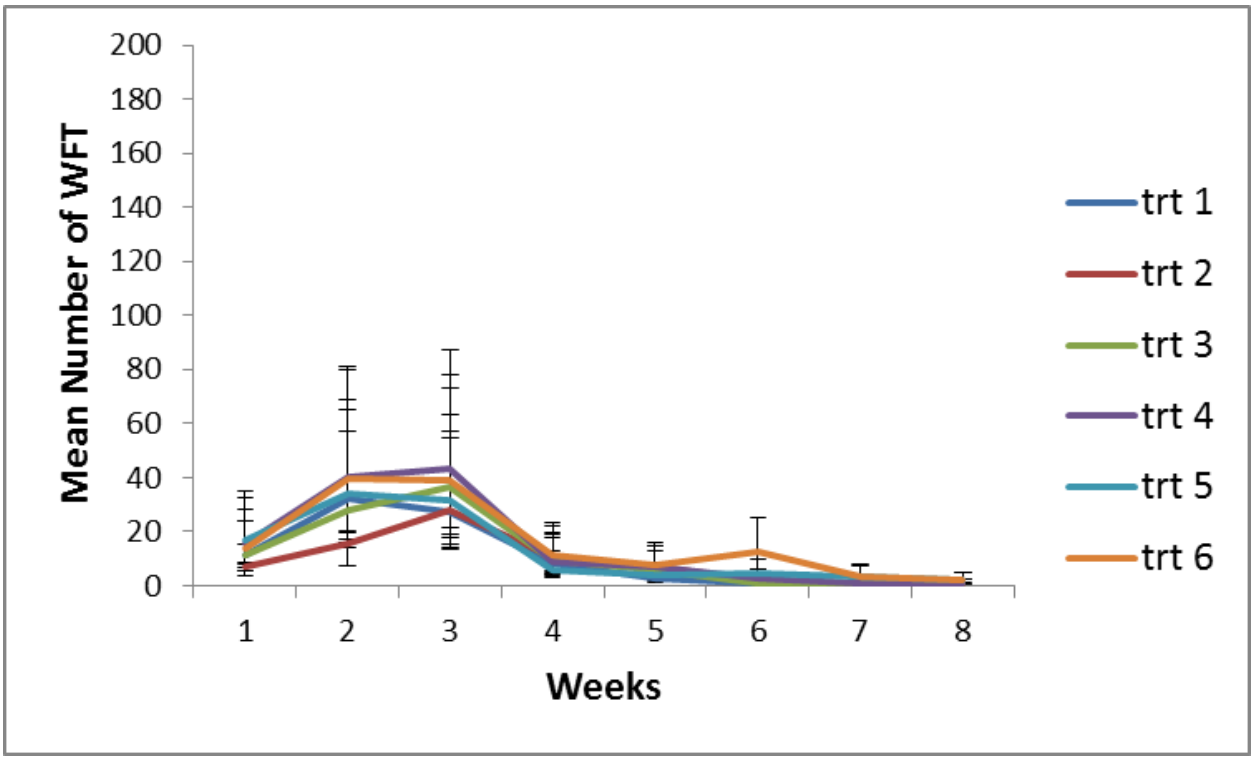
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr; **treatment 2**: spinosad, pyridalyl, *Metarhizium anisopliae*, and chlorfenapyr; **treatment 3**: spinosad, pyridalyl, *Beauveria bassiana*, and chlorfenapyr; **treatment 4**: spinosad, pyridalyl, *Isaria fumosoroseus*, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosoroseus*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.7 Mean (± 95% confidence intervals) number of western flower thrips (WFT), *Frankliniella occidentalis* adults captured on yellow sticky cards per week for rotation treatments (trt) 1 through 6 associated with experiment 2 (March 6 to May 1, 2014).



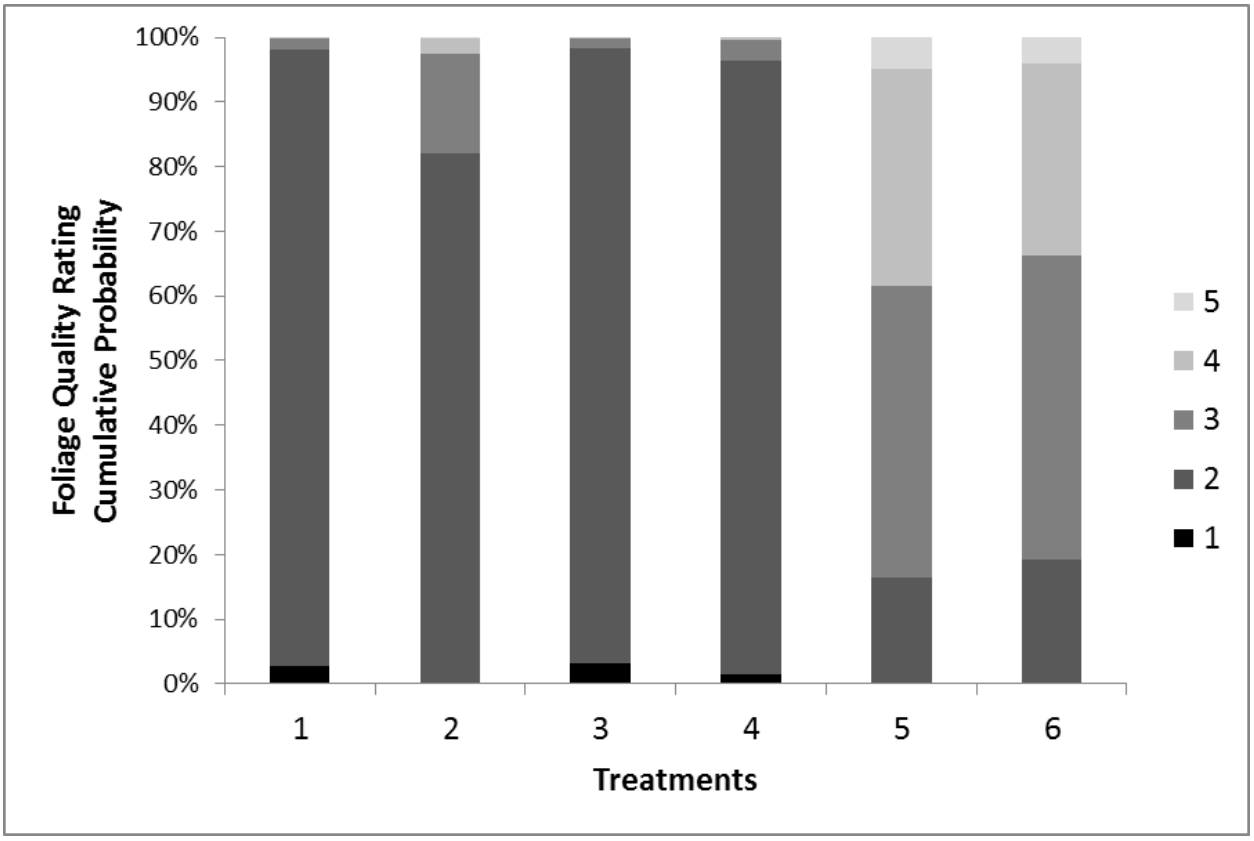
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr**; treatment 2**: spinosad, *Metarhizium anisopliae*, pyridalyl, and chlorfenapyr; **treatment 3**: spinosad, *Beauveria bassiana*, pyridalyl, and chlorfenapyr; **treatment 4**: spinosad, *Chromobacterium subtsugae*, pyridalyl, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Chromobacterium subtsugae*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.8 Mean (± 95% confidence intervals) number of western flower thrips (WFT), *Frankliniella occidentalis* adults captured on yellow sticky cards per week for rotation treatments (trt) 1 through 6 associated with experiment 2 (May 12 to July 7, 2014).



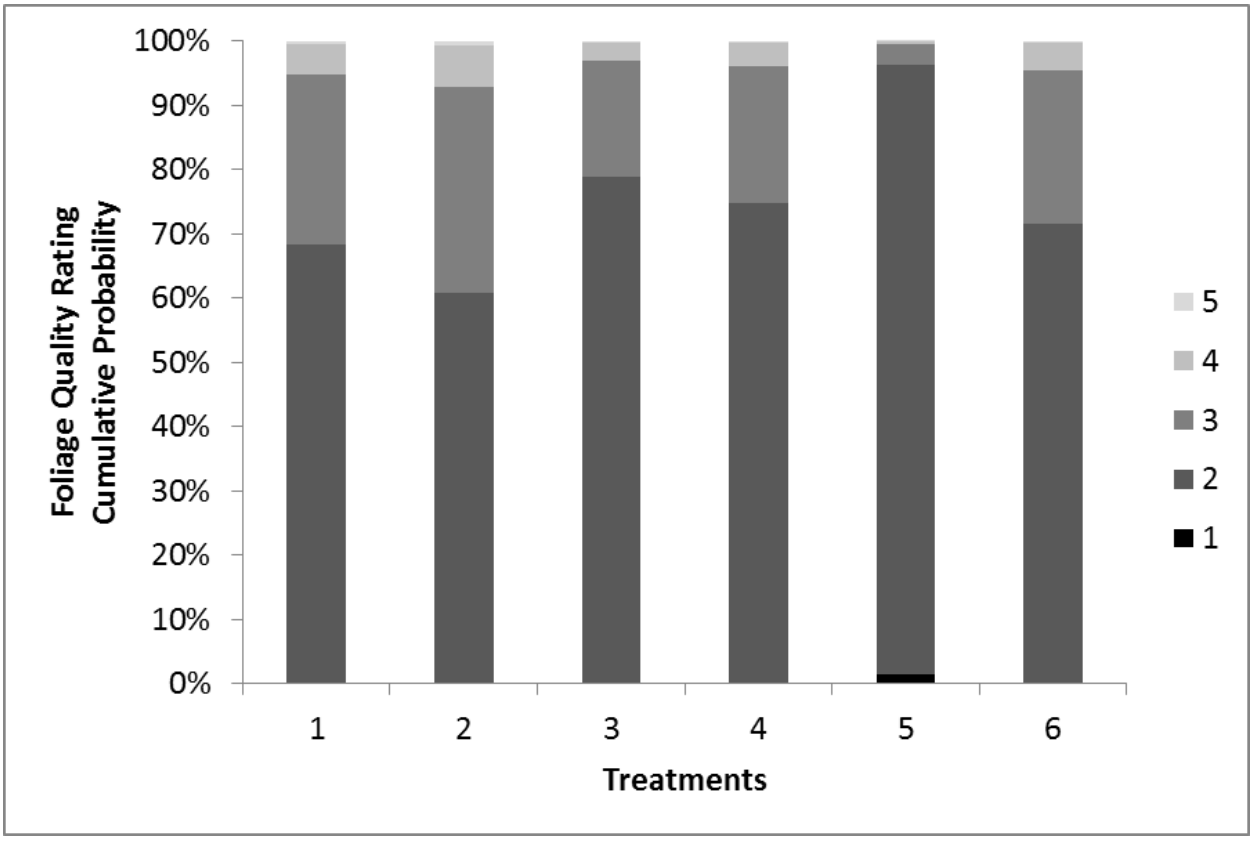
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr**; treatment 2**: spinosad, *Metarhizium anisopliae*, pyridalyl, and chlorfenapyr; **treatment 3**: spinosad, *Beauveria bassiana*, pyridalyl, and chlorfenapyr; **treatment 4**: spinosad, *Chromobacterium subtsugae*, pyridalyl, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Chromobacterium subtsugae*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.9 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the foliage of chrysanthemum, *Dendranthema x morifolium* plants for experiment 2 (March 6 to May 1, 2014). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



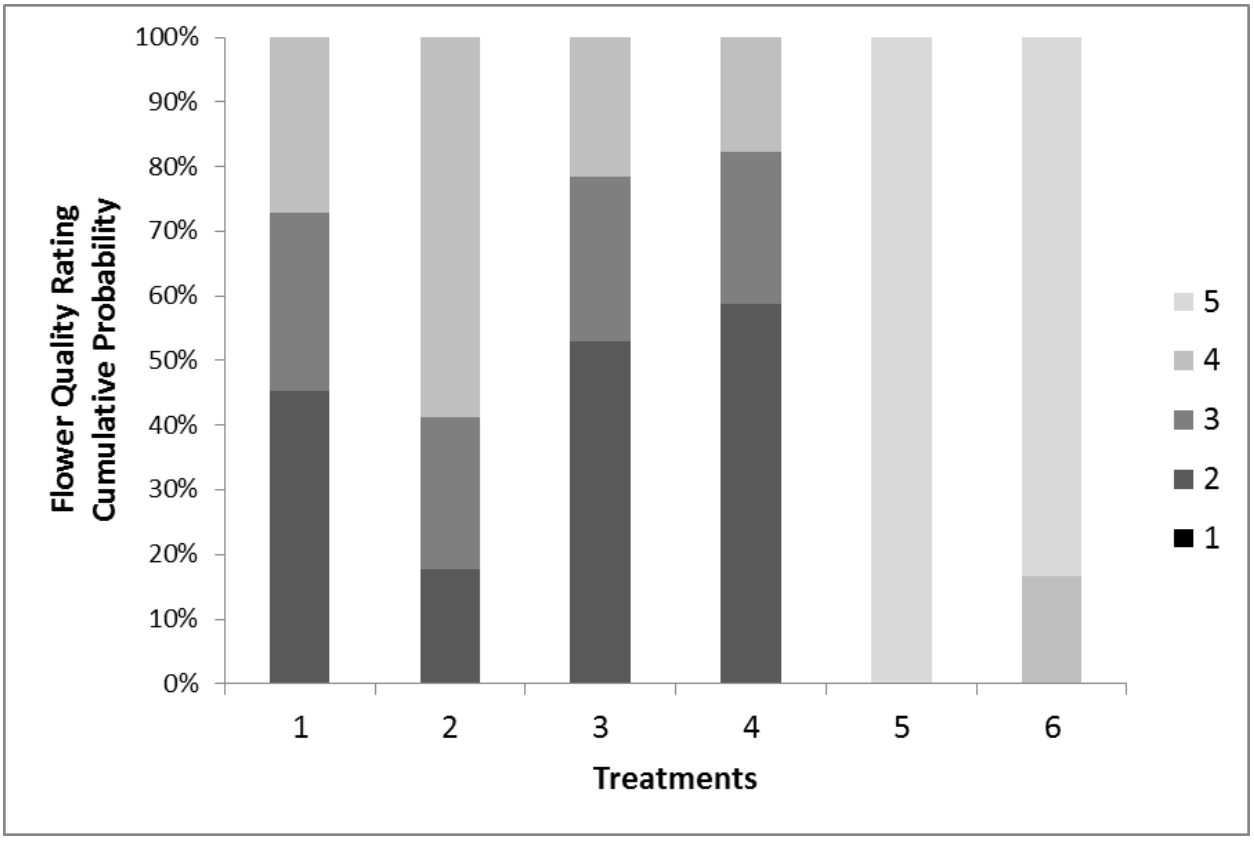
Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr**; treatment 2**: spinosad, *Metarhizium anisopliae*, pyridalyl, and chlorfenapyr; **treatment 3**: spinosad, *Beauveria bassiana*, pyridalyl, and chlorfenapyr; **treatment 4**: spinosad, *Chromobacterium subtsugae*, pyridalyl, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Chromobacterium subtsugae*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.10 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the foliage of chrysanthemum, *Dendranthema x morifolium* plants for experiment 2 (May 12 to July 7, 2014). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr**; treatment 2**: spinosad, *Metarhizium anisopliae*, pyridalyl, and chlorfenapyr; **treatment 3**: spinosad, *Beauveria bassiana*, pyridalyl, and chlorfenapyr; **treatment 4**: spinosad, *Chromobacterium subtsugae*, pyridalyl, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Chromobacterium subtsugae*, *Beauveria bassiana*; and **treatment 6**: water.

Figure 3.11 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the flowers of chrysanthemum, *Dendranthema x morifolium* plants for experiment 2 (March 6 to May 1, 2014). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



Treatment 1: spinosad, pyridalyl, abamectin, and chlorfenapyr**; treatment 2**: spinosad, *Metarhizium anisopliae*, pyridalyl, and chlorfenapyr; **treatment 3**: spinosad, *Beauveria bassiana*, pyridalyl, and chlorfenapyr; **treatment 4**: spinosad, *Chromobacterium subtsugae*, pyridalyl, and chlorfenapyr; **treatment 5**: *Beauveria bassiana*, *Metarhizium anisopliae*, *Chromobacterium subtsugae*, *Beauveria bassiana*; and **treatment 6**: water.

Chapter 4 - Summary and Conclusions

The objectives of this study were to 1) determine the efficacy of three entomopathogenic fungi species on western flower thrips (WFT) adults and nymphs and compare efficacy of fresh and expired products, 2) assess the efficacy of entomopathogenic fungi when combined with azadirachtin against WFT nymphs, and 3) evaluate different rotation programs that include entomopathogenic organisms and standard insecticides commonly used to suppress WFT populations. In order to address these objectives, a series of laboratory bioassays and greenhouse experiments were conducted.

The laboratory study consisted of three bioassays. There were two bioassays in which WFT adults and nymphs were exposed to three entomopathogenic fungi *Beauveria bassiana* (BotaniGard® 22WP: Bioworks, Inc.; Victor, NY), *Isaria fumosoroseus* (NoFly™: Novozymes Biologicals Inc.; Salem, VA), and *Metarhizium anisopliae* (Met52® EC: Novozymes Biologicals Inc.; Salem, VA) at two labeled rates (maximum and minimum), and two conditions (fresh and expired) for 120 hours (first bioassay) and 216 hours (second bioassay). Results indicated that adults are more susceptible to infection than nymphs when fresh *B. bassiana* was used at the maximum (2 lbs/100 gal) and the minimum (1 lb/100 gal) labeled rates, and fresh *I. fumosoroseus* was used at the maximum rate (2 lbs/100 gal). Furthermore, fresh products resulted in higher mortality than expired products especially for fresh *B. bassiana* and *I. fumosoroseus*. In addition, *B. bassiana* and *I. fumosoroseus* resulted in greater WFT adult mortality (66% and 85%, respectively) than fresh *M. anisopliae* (17%) by 120 hours in the first bioassay. However, in the second bioassay, which was conducted for 216 hours (9 days), *M. anisopliae* eventually reached 100% mortality by 168 hours. This indicates that *M. anisopliae* is slower acting than *B. bassiana* or *I. fumosoroseus*. Finally, a third bioassay was conducted to

determine if azadirachtin, an insect growth regulator (ecdysone antagonist), would increase mortality of WFT nymphs if combined with the three entomopathogenic fungi. It was found that azadirachtin did not significantly enhance efficacy against WFT nymphs except when added to *M. anisopliae*. Although there was enhanced efficacy of *M. anisopliae* when combined with azadirachtin, it only resulted in a 30% estimated probability of nymphal mortality.

Since entomopathogenic fungi are generally more effective against WFT adults, it is important that they be applied early in the crop production cycle, before WFT generations overlap, resulting in the simultaneous presence of different life stages (egg, nymphs, pupae, and adults). Furthermore, entomopathogenic fungi have a shorter shelf-life than most insecticides. This study has demonstrated that greenhouse producers need to use entomopathogenic fungi products before the expiration date as indicated on the label in order to obtain sufficient mortality. Also, combining azadirachtin with entomopathogenic fungi may not increase mortality of WFT nymphs except when added to *M. anisopliae*.

The greenhouse study evaluated different eight-week rotation programs that included entomopathogenic organisms and standard insecticides through a series of two greenhouse experiments using chrysanthemum, *Dendranthema x morifolium* plants artificially infested with WFT adults. In addition, a cost comparison was conducted to determine whether incorporating entomopathogenic organisms into rotation programs could reduce costs. Results from the two experiments indicated that insecticide rotation programs that incorporated entomopathogenic organisms were not significantly different than standard insecticide rotation programs in suppressing WFT populations. Furthermore, there were no significant treatment differences regarding flower and foliage quality ratings. The cost comparison indicated that insecticide

rotation programs that include entomopathogenic organisms could save $55 to $230 depending on the rotation program.

Based on these results, greenhouse producers should consider incorporating entomopathogenic organisms into rotation programs. Although this study did not evaluate the impact of rotation programs on the ability of WFT populations to develop resistance, it is possible that by using entomopathogenic organisms with broad modes of action in rotation programs, the potential of WFT populations to develop resistance may be reduced or delayed. In addition, by incorporating entomopathogenic organisms into rotation programs, greenhouse producers could reduce application costs. Future research with entomopathogenic organisms should evaluate similar rotation programs against field populations of WFT with known resistance to one or more of the standard insecticides used. Furthermore, rotation programs that use different compounds or sequences need to be evaluated to determine additional effective rotation programs against WFT, which would greatly benefit greenhouse producers.

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Appendix A - Effect of Water Sprays on Western Flower Thrips, *Frankliniella occidentalis* Populations on Flowering Chrysanthemum, *Dendranthema x morifolium* plants

Results from the greenhouse experiments in study 2 (Chapter 3) indicated that the water treatment may reduce WFT populations. Several studies have evaluated the direct effect of water sprays or overhead irrigation on pest populations (Chandler et al., 1979; Brunner and Burts, 1981; McHugh and Foster, 1995; Opit et al., 2006). For example, Opit et al. (2006) observed a 68-fold reduction in twospotted spider mite, *Tetranychus urticae* Koch, populations when exposed to an overhead irrigation treatment three times every 2 to 4 days. As a result, garden impatiens, *Impatiens walleriana* Hook f., plants that received overhead irrigation sustained 4-fold less damage by twospotted spider mites compared to the drip irrigation treatment. Therefore, an additional experiment was conducted to address whether spraying chrysanthemum plants with water reduces WFT populations. The following experiment compared the level of WFT suppression on chrysanthemum plants sprayed with water to plants that were not sprayed.

Materials and Methods

Flowering chrysanthemum, *Dendranthema x morifolium* Ramat, plants in 10.1 cm containers were obtained from Masson's Greenhouse (Linwood, KS) and repotted into 15.2 cm containers using Fafard® 2 Mix growing medium (SunGro Horticulture; Agawam, MA). Plants consisted of a variety of unknown cultivars with different flower colors. Each plant was placed into individual clear plastic cages [45.7 x 45.7 x 60.9 cm (length x width x height)]. Each cage had a lid and three holes (12.7 cm diameter); one on the lid, and two on each side of the cage, covered with thrips screening (Greentek; Edgerton, WI). These openings allowed for ventilation,

but prevented WFT adults from escaping. There were ten cages per treatment for a total of 20 cages. The cages were randomized on two wire-mesh benches (4.3 x 1.1-m). Each plant was carefully watered by removing from the cage, placing on a concrete floor, and then irrigating with a sufficient volume as needed using a plastic 7.6-L watering can. Water was applied directly to the growing medium in order to avoid wetting the foliage. Plants were returned to the cages immediately afterward.

Each plant was artificially infested with 25 WFT adults (7 to 10 days old) obtained from laboratory-reared colonies in the Department of Entomology at Kansas State University (Manhattan, KS). Western flower thrips adults were allowed to establish on the plants for one week. There were two treatments: water and untreated control. There were ten plants (replications) per treatment for a total of 20 plants. Water treatments were applied by removing each plant from the cage, placing on a concrete floor, and spraying the plants using a 946 mL plastic spray bottle (The Home Depot; Manhattan, KS). Approximately 30 mL of water was applied to each plant for the water treatment, which was a sufficient volume to thoroughly cover all plant parts, including leaves and flowers, with minimal run-off. The untreated control plants were removed from the cage similar to the water treatment and immediately returned to the cage. This was done to account for any WFT that may have fallen off the plant due to handling. Treatments were applied at one week intervals for four weeks. A yellow sticky card (12.7 x 7.6 cm) (Pestrap™ Phytotronics, Inc.; Earth City, MO) was placed into each plastic cage, approximately 8 cm from the plant, and held in position by a wooden clothes pin attached to a bamboo stake. Each bamboo stake was placed into a plastic container filled with sand in order to hold the stake upright. Old or senescing flowers were removed, as needed, and placed beside the

plant inside the cage to allow any WFT adults and nymphs that may be residing in the flowers to return to the plant.

Western flower thrips adults were counted on the yellow sticky cards seven days post-application each week. Additionally, the presence or absence of flowers per plant was recorded. On the final assessment day, each plant was evaluated for quality based on a 1 to 5 rating scale with 1 = no visible damage, 2 = 1 to 25% damage, 3 = 26 to 50% damage, 4 = 51 to 75% damage, and 5 = >75% damage. This damage rating was modified from Cloyd and Cycholl (2002) and quantified plant damage due to WFT feeding. Environmental conditions inside of the cages including light intensity, temperature, and relative humidity were recorded every hour using HOBO® Data Loggers (Onset Computer Corporation; Bourne, MA). At the start of the experiment, plant height was 23 to 31 cm. Over the course of the experiment, plants grew approximately 10 cm. The experiment was conducted from July 30 to September 3, 2014.

Data were analyzed by fitting a generalized linear mixed model to the number of WFT adults captured on yellow sticky cards. This response was modeled using the negative binomial distribution with a canonical log link function. The linear predictors in the model included the fixed effects of treatment (two levels, consisting of water and untreated control), and time (weeks 1 through 4), as well as any two-way interaction. Random effects in the linear predictor included plant nested within treatment to recognize the experimental unit for the treatments, which were the individual plants. In addition, the random effects of row-bench combination and that of cultivar were considered as random blocking factors. However, the corresponding variance component estimates converged to zero and thus these effects were not included in the final model.

Over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion. The final statistical model, used for inference, was fitted using Residual Pseudo-likelihood. Degrees of freedom were approximated and estimated standard errors were adjusted using Kenward-Roger's procedure. The statistical model was fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute, Cary, NC) and utilizing Newton-Raphson with ridging as the optimization technique. Relevant pairwise comparisons were conducted using Bonferroni adjustments to avoid inflation of Type I error rate due to multiple comparisons.

Results and Discussion

Results associated with WFT adult counts on yellow sticky cards are presented in Figure A.1. There was no significant difference between chrysanthemums sprayed with water and the untreated control based on the two-way interaction of treatment and week (F=0.85; df=3, 55.26; P=0.472). There appeared to be a time effect on WFT adults captured on yellow sticky cards (F=57.05; df=3, 55.26; P<0.0001), which was apparent for both the water treatment and the untreated control. Western flower thrips adults captured on yellow sticky cards were significantly lower in week four than previous weeks for both treatments (P<0.0001). The significant decline in the number of WFT adults observed on yellow sticky cards may be associated with the decline in plant quality. Moreover, there was no treatment effect on foliage quality ratings (Figure A.2). Flower quality ratings were not recorded because all the flowers had senesced and were removed. This may have also been a factor in the decline of WFT adults captured on yellow sticky cards.

There was no significant difference between the water spray and the untreated control in regards to WFT suppression or quality ratings for both foliage and flowers. These results differ

from similar studies with water in which overhead irrigation physically removed pests from the host plants (Opit et al., 2006). A reason why there was no significant difference between the treatments may be due to the frequency of applying water. A once-a-week water spray may not have been frequent enough to negatively impact the WFT population. In addition to frequency of application, the volume of water associated with a spray application may not have been enough to have an impact on WFT populations. Furthermore, it is likely that multiple life stages of WFT were present, some of which occupy cryptic microhabitats, which would protect them from direct contact with water sprays. For example, eggs are embedded in plant tissue, and pupae reside in the growing medium (Thoeming et al., 2003). In addition, nymphs and adults often infest protected areas of plants such as floral buds. Another possibility may be associated with the level of infestation by WFT at the start of the experiment. There was a bench of chrysanthemum plants in the greenhouse at the time the experiment started and a natural population of WFT on these plants may have migrated onto the yellow sticky cards in the nearest row of test plants via air currents. These extra plants were eventually removed from the greenhouse (one week after the experiment began). The presence of additional WFT (>100 adults) on the first row of test plants may have increased the population such that the water sprays had minimal effect in suppressing WFT populations. Therefore, it is recommended to repeat this experiment under optimal conditions.

Figure A.1 Mean (± 95% confidence interval) number of western flower thrips (WFT), *Frankliniella occidentalis* adults captured on yellow sticky cards per week in a greenhouse experiment comparing water spray vs. untreated control (UTC).

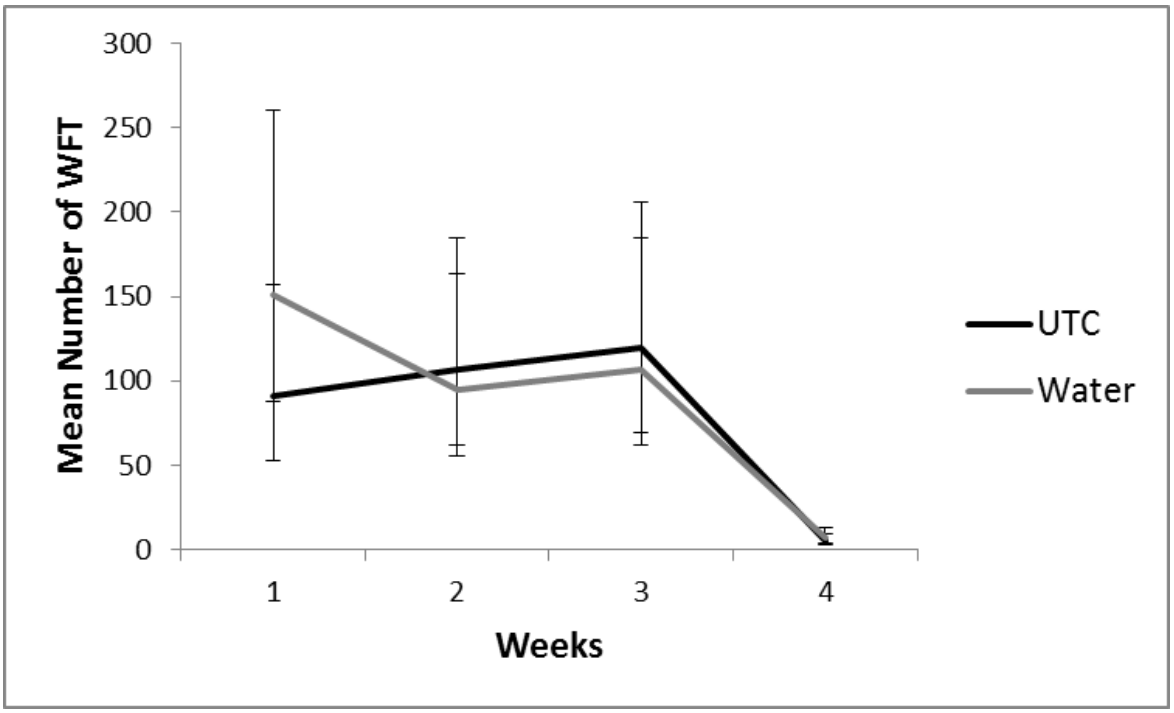
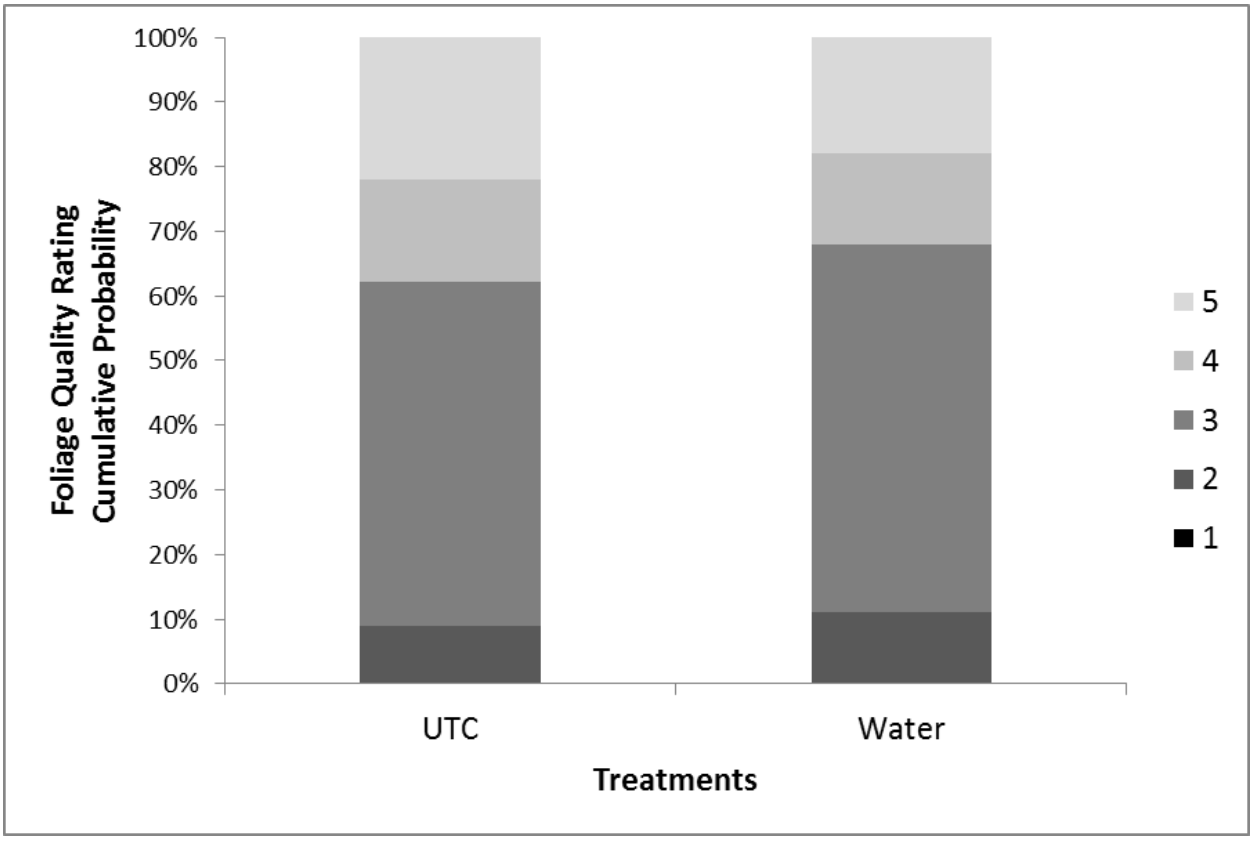


Figure A.2 Estimated cumulative probability of a plant displaying damage equivalent to or less than the rating administered to the foliage of chrysanthemum, *Dendranthema x morifolium* plants sprayed with water and untreated control (UTC). The rating scale is based on damage caused by western flower thrips, *Frankliniella occidentalis* feeding where 5 = > 75% damage, 4 = 51 to 75% damage, 3 = 26 to 50% damage, 2 = 1 to 25% damage, and 1 = no damage (modified from Cloyd and Cycholl, 2002).



Appendix B - Formulation Issues Associated with *Isaria fumosoroseus* (NoFly)

Entomopathogenic organisms (EPO) such as *Beauveria bassiana*, *Isaria fumosoroseus*, *Metarhizium anisopliae* and *Chromobacterium subtsugae* may be used in rotation programs along with insecticides as a means to mitigate the potential for resistance. However, there are disadvantages of using EPO such as relatively slow activity and limited shelf-life. Another disadvantage may be inconsistency associated with the formulation. While conducting the current study, problems were encountered with the formulation of NoFly™ (*Isaria fumosoroseus* strain FE 9901: Novozymes Biologicals Inc.; Salem, VA).

We first noticed the problem in January 2014. It was discovered that the dry formulation of NoFly was not mixing with water to form a homogenous mixture. The spores would clump together and float on the water surface. Even vigorous agitation and stirring would not suspend the spores in water. After contacting the manufacturer, they suggested adding a surfactant such as Tween™ 20 (Fisher Scientific; Waltham, MA). This reduced any clumping, however, the spores continued to float on the water surface. After the manufacturer was made aware of the problem, all NoFly products were removed from distribution and there was a suspension on sales. As a result, we could not acquire NoFly for use in the second greenhouse experiment. The product Grandevo®, which contains the entomopathogenic bacterium *Chromobacterium subtsugae* as the active ingredient, was used instead of NoFly.

This problem could impact the use of EPO by greenhouse producers. Lord (2005) stated that any mixing issue associated with the formulation may cause the end user to lose interest in the product. This lack of consistency may be a disadvantage when using EPO. Greenhouse producers will not invest in a product that fails to provide sufficient suppression of pest

populations. Furthermore, a product must be easy to mix and apply. Therefore, it is important that products containing EPO be viable, consistent and dependable. This is a challenge to manufacturers of EPO products since living organisms are involved. However, despite the formulation issue encountered, it is recommended that greenhouse producers consider incorporating EPO into insecticide rotation programs, in order to preserve the efficacy and longevity of commercially available insecticides.