Effects of the rove beetle, *Dalotia coriaria*, on western flower thrips, *Frankliniella occidentalis*, under laboratory conditions; and integrating the entomopathogenic fungus, *Beauveria bassiana*, with *D. coriaria* to suppress western flower thrips populations under greenhouse conditions

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

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Abstract

Western flower thrips, *Frankliniella occidentalis*, is one of the most destructive insect pests in greenhouse production systems due to direct and indirect plant damage resulting in substantial economic losses. In addition, western flower thrips has developed resistance to many insecticides. Therefore, alternative plant protection strategies are warranted, such as augmentative biological control. This research was designed to evaluate 1) the effect of different absolute numbers of predator (rove beetle, *Dalotia coriaria*) and prey (western flower thrips) on predation efficacy of rove beetle under laboratory conditions; 2) the effect of western flower thrips pupal stage, predator-prey ratio, predator-prey number, and searchable area on predation efficacy of rove beetle in the laboratory; and 3) the effectiveness and cost of integrating the entomopathogenic fungus, *Beauveria bassiana*, and the rove beetle, *D. coriaria*, in suppressing western flower thrips populations under greenhouse conditions.

Three laboratory experiments were conducted to assess predation efficacy of rove beetle adults on three western flower thrips pupal stages [prepupa, pupa, and prepupa-pupa combination (50%:50%)]. In each experiment, there were six numbers (0, 1, 2, 3, 4, and 5) of rove beetle adults and four initial numbers (15, 20, 25, and 30) of one western flower thrips pupal stage. This treatment configuration allowed for assessing the effect of predator-prey ratios (1:5, 1:10, and 1:15), accounting for different initial prey numbers, on predation efficacy of the rove beetle. Overall, for each pupal stage, the estimated mean probability of western flower thrips adults captured on yellow sticky cards decreased as the number of rove beetle adults released increased from 1 to 3, although the effect of additional rove beetle adult releases was not apparent. Furthermore, across the pupal stages considered in this study, in general, there was no evidence

of any differences due to predator-prey ratios or initial prey numbers within each predator-prey ratio.

Two laboratory experiments were conducted to assess the effects of western flower thrips pupal stage, predator-prey ratio, predator-prey number, and searchable area on predation efficacy of rove beetle adults. In experiment 1, there were two western flower thrips pupal stages (prepupa and pupa), three predator-prey ratios (rove beetle:western flower thrips—1:5, 1:10, and 1:15), and three predator-prey numbers (2, 3, and 4 times). Experiment 2 evaluated the latter two factors in combination with searchable area defined by container sizes [15.2 cm (1,834.82 cm^3) and 11.5 cm (701.79 cm^3)]. The estimated mean probability of western flower thrips adults captured on yellow sticky cards was significantly higher for the 1:5 predator-prey ratio [61.1% (48.5-72.4%)] than 1:10 [39% (28.1-51.2%)] and 1:15 predator-prey ratio [34.7% (24.7-46.3%)]. The estimated mean probability of western flower thrips adults captured on yellow sticky cards for 2 times the predator-prey number [57% (44.3-68.8%)] was significantly higher than 3 [37.2% (26.6-49.3%)] and 4 [40.6% (30-52.3%)] times the predator-prey number. In addition, a significantly higher estimated mean probability of western flower thrips adults was captured on the yellow sticky cards in the 15.2 cm than 11.5 cm containers.

Two greenhouse experiments were conducted that evaluated five treatments: combination of insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin), *B. bassiana*, *D. coriaria*, *B. bassiana* and *D. coriaria* combination, and a water control. Overall, the estimated mean number of western flower thrips adults captured on yellow sticky cards was significantly lower for the insecticide treatment (mean range: 0, 46) than for the *B. bassiana* and *D. coriaria* combination (mean range: 0.3, 105.1) over eight weeks. There were no significant differences in final foliage quality of chrysanthemum, *Dendranthema* x *grandiflorum*, plants among the five treatments in

experiment 1, but there were significant differences in experiment 2. However, in experiment 2, the chrysanthemum plants across all treatments were not marketable due to substantial feeding damage by western flower thrips. The cost of the insecticide treatment was nearly twice that of the *B. bassiana* and *D. coriaria* combination ($963.50 vs. $495.67) and was over twice that of the *B. bassiana* only treatment ($963.50 vs. $417.04). The *D. coriaria* only treatment was the least expensive at $78.63.

The results of the research provide insight into the predatory behavior of *D. coriaria* on western flower thrips pupal stages, which may have practical implications for greenhouse production systems. However, predation efficacy of rove beetle adults on western flower thrips is influenced by predator-prey ratio, predator-prey number, and searchable area. Finally, greenhouse producers must initiate insecticide applications or release rove beetle adults early in the production cycle when western flower thrips populations are low to minimize plant damage.

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Chapter 1 - Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest of horticultural crops worldwide (Robb and Parrella 1995, Lewis 1997, Kirk 2002, Cloyd 2009, Reitz 2009). Western flower thrips cause direct damage to greenhouse-grown horticultural crops by feeding on and ovipositing in leaves, flowers and fruits (Chisholm and Lewis 1984, Hunter and Ullman 1989, Harrewijn et al. 1996, Childers 1997, Lewis 1997). Moreover, western flower thrips cause indirect damage by vectoring the tospoviruses: *Tomato spotted wilt* and *Impatiens necrotic spot* viruses (Allen and Broadbent 1986, Pappu et al. 2009). The direct and indirect damage has led to significant economic losses. For instance, Goldbach and Peters (1994) estimated that, on an annual basis, *Tomato spotted wilt virus* accounts for an economic loss of >$1 billion (US dollars) in agricultural crops globally. Furthermore, in 2006, damage to ornamental crops in Georgia by western flower thrips resulted in an economic loss of >$15 million (US dollars) (Reitz and Funderburk 2012).

The tolerance for damage caused by western flower thrips is very low since any damage can reduce aesthetic quality and marketability of greenhouse-grown horticultural crops (Parrella and Jones 1987, Loughner et al. 2005). Therefore, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Kontsedalov et al. 1998, Loughner et al. 2005, Cloyd 2009, Reitz 2009). However, the intensive application of insecticides has led to the development of insecticide resistance in western flower thrips populations (Brødsgaard 1994, Jensen 2000, Bielza et al. 2007, Kay and Herron 2010). Furthermore, there is a limited number of effective insecticides labeled for use against western flower thrips (Loughner et al. 2005, Reitz and Funderburk 2012). Therefore, alternative plant protection strategies are warranted, such as

augmentative biological control that involves releasing or applying natural enemies (e.g., predators, parasitoids, or entomopathogenic fungi) to suppress pest populations (Cloyd 2009).

Biological control of western flower thrips should target as many life stages as possible to maintain western flower thrips populations below damaging levels (Mouden et al. 2017). Furthermore, entomopathogenic microorganisms, such as; bacteria and fungi, can be used along with arthropod natural enemies to target different pest life stages (Gonzalez et al. 2016, Mouden et al. 2017, Ullah and Lim 2017). Thus, in the following research, western flower thrips larvae and adults were the target aboveground life stages using a commercially available entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin. Simultaneously, the soil-dwelling predatory rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), was used against the soil-inhabiting life stages (prepupae and pupae) of western flower thrips. The strategy of integrating *B. bassiana* with *D. coriaria* needs to be effective and economical. Therefore, the greenhouse study focused on evaluating the effectiveness associated with integrating *B. bassiana* with *D. coriaria* to suppress western flower thrips populations, and assessing the direct cost related to products and application rates. Moreover, laboratory studies were conducted to determine predation of rove beetle adults on western flower thrips pupal stages. As such, the objective, rationale and hypotheses associated with each study are presented below:

Effect of different numbers of predator and prey on predation of western flower thrips, *Frankliniella occidentalis*, pupal stages by rove beetle, *Dalotia coriaria*, adults (Chapter 3)

Objective: determine the effects of 1) different absolute numbers of predator (rove beetle) and prey (western flower thrips), 2) different initial prey numbers, and 3) different predator-prey ratios (1:5, 1:10, and 1:15) on predation efficacy of *D. coriaria*.

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Rationale: Predation efficacy is a measurement designed to evaluate the effectiveness of predators in suppressing prey populations, which is important in selecting biological control agents (Farhadi et al. 2011) and in assessing the numbers of predators to release in augmentative biological control programs (Echegaray et al. 2015). Due to low pest tolerance and capacity of most greenhouse insect and mite pests to increase populations rapidly, determining an appropriate predator-prey ratio to effectively suppress pest populations is important. Consequently, studies have evaluated predator-prey ratios associated with different predators (Gaudchau 1982, Gilkeson and Hill 1987, Opit et al. 2004, Cheng et al. 2012, Echegaray et al. 2015). Furthermore, different initial prey numbers at a given predator-prey ratio may also affect predation efficacy (Echegaray et al. 2015).

Successful suppression of western flower thrips populations using the rove beetle, *D. coriaria,* is contingent on evaluating predation efficacy based on different numbers of predator and prey, as well as determining an appropriate predator-prey ratio. However, the effect of *D. coriaria* on western flower thrips is not well understood.

Hypotheses: First, at the same initial number of one western flower thrips pupal stage [prepupae, pupae, or prepupal-pupal combination (prepupae:pupae=50%:50%)], the estimated mean probability of western flower thrips adults captured on yellow sticky cards should decrease as the number of rove beetle adults increases because more rove beetle adults should consume higher numbers of prey. Second, at one western flower thrips pupal stage, the estimated mean probability of western flower thrips adults captured on yellow sticky cards should decrease as predator-prey ratio increases. The reason being is that as predator-prey ratio increases, the proportion of rove beetle adults to prey is greater. Therefore, more predators are present to feed on prey. Third, at each predator-prey ratio, the estimated mean probability of western flower

thrips adults captured on yellow sticky cards should increase as the initial number of prey increases, and correspondingly as more rove beetle adults are released. The reason for this is that when more rove beetle adults are released in the same container, mutual interference [competition occurs when access to resources is negatively affected by the presence of other individuals within a population (DeLong and Vasseur 2011)] among rove beetle adults should occur. Consequently, rove beetle adults should spend less time foraging for prey.

Effect of western flower thrips, *Frankliniella occidentalis*, pupal stage, predator-prey ratio, predator-prey number, and searchable area on predation efficacy of rove beetle, *Dalotia coriaria*, adults (Chapter 4)

Objective: evaluate the effects of 1) western flower thrips pupal stage, predator-prey ratio, and predator-prey number; and 2) predator-prey ratio, predator-prey number, and searchable area on the predation efficacy of rove beetle adults.

Rationale: Predation efficacy is a measurement designed to evaluate predator effectiveness in suppressing prey populations, which is important in selecting biological control agents (Farhadi et al. 2011). Low pest tolerance and capacity of most greenhouse insect and mite pests to increase populations rapidly, has increased the need to determine appropriate predator-prey ratios that sufficiently suppress pest populations. Consequently, studies have evaluated predator-prey ratios for different predators (Gaudchau 1982, Gilkeson and Hill 1987, Opit et al. 2004, Cheng et al. 2012, Echegaray et al. 2015). Furthermore, different predator-prey numbers, based on variations in predator-prey ratios, may also influence predation efficacy (Echegaray et al. 2015).

Although comparisons of predation efficacy among different predator-prey numbers within each predator-prey ratio (1:5, 1:10, or 1:15) were previously conducted in Chapter 3, there were different predator-prey numbers within each predator-prey ratio: 3 times, 4 times, and 5 times in 1:5 ratio; 2 times and 3 times in 1:10 ratio; as well as 1 time and 2 times in 1:15 ratio. As such, the interaction between predator-prey ratio and predator-prey number could not be determined. Therefore, in the study conducted in Chapter 4, predation efficacy was evaluated based on three predator-prey numbers (2 times, 3 times, and 4 times) within each predator-prey ratio (1:5, 1:10, or 1:15) to assess the interaction between predator-prey ratio and predator-prey number.

Western flower thrips late second instar larvae migrate or fall onto the growing medium surface to pupate (Helyer et al. 1995, Tommasini and Maini 1995, Kirk 1996, Wiethoff et al. 2004). Western flower thrips prepupae and pupae take two to three days and one to two days to develop into adults, respectively (Zhang et al. 2007). Since exposure time of prepupae to rove beetle adults, prior to western flower thrips adult eclosion, is longer than pupae, rove beetle adults may have more time (about one day) to forage for western flower thrips prepupae than pupae. As such, predation efficacy may be influenced by western flower thrips pupal stages (prepupa and pupa). Therefore, to account for the effect of different exposure time of western flower thrips pupal stages on rove beetle adult predation, pupal stage was included in this study as a factor that could potentially affect predation efficacy. Furthermore, the searchable area may also affect predation efficacy of *D. coriaria* on soil-dwelling prey, which has been proposed previously associated with the fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae), larvae (Echegaray et al. 2015, Herrick and Cloyd 2017). However, minimal information is available on the effect of western flower thrips pupal stage, searchable area,

predator-prey ratio, and predator-prey number on predation efficacy of the adult rove beetle, *D. coriaria*.

Hypotheses: First, at the same predator-prey ratio and same predator-prey number, the estimated mean probability of western flower thrips adults captured on yellow sticky cards from prepupae should be lower than the pupae. The reason being is that rove beetle adults have about one more day to forage on prepupae, before western flower thrips adults eclosion, than the pupae. Second, at the same predator-prey number and same pupal stage, as predator-prey ratio increases, the estimated mean probability of western flower thrips adults captured on yellow sticky cards should increase. The reason for this is that as the predator-prey ratio increases, there are more predators relative to prey; thus, mutual interference among rove beetle adults should occur. Consequently, rove beetle adults should spend less time foraging for western flower thrips prepupae or pupae. Third, at the same predator-prey ratio and same pupal stage, the estimated mean probability of western flower thrips adults captured on yellow sticky cards should increase as the predator-prey number increases and subsequently as more rove beetle adults are released. The reason being is that when more rove beetle adults are released in the same container, mutual interference among rove beetle adults should occur. Therefore, rove beetle adults should spend less time foraging for western flower thrips prepupae or pupae. Fourth, at the same predator-prey ratio and same predator-prey number, the estimated mean probability of western flower thrips adults captured on yellow sticky cards should increase as searchable area increases. The reason for this is that rove beetle adults should spend more time searching for prey as searchable area increases.

Effect of integrating the entomopathogenic fungus, *Beauveria bassiana*, and the rove beetle, *Dalotia coriaria*, in suppressing western flower thrips, *Frankliniella occidentalis*, populations under greenhouse conditions (Chapter 5)

Objective: 1) ascertain the effectiveness of using the entomopathogenic fungus, *B. bassiana* in conjunction with the rove beetle, *D. coriaria*, to suppress western flower thrips populations under greenhouse conditions; and 2) compare the direct costs associated with products and application rates.

Rationale: Previous studies have concentrated on combining different arthropod natural enemies or integrating arthropod natural enemies with entomopathogens or entomopathogenic nematodes to target one or more western flower thrips life stage (Ebssa et al. 2006, Manners et al. 2013, Messelink and Janssen 2014, Wu et al. 2016). However, foliar applications of the entomopathogenic fungus, *B. bassiana* along with the soil-dwelling rove beetle, *D. coriaria* targeting the aboveground and soil-dwelling life stages of western flower thrips have not been investigated. In this study, *B. bassiana* was used against western flower thrips larvae and adults on plants, and *D. coriaria* was used against the prepupae and pupae that reside in the growing medium.

Hypotheses: First, the final foliage damage rating for the *B. bassiana* and rove beetle combination should not be significantly different from the insecticide treatment. The reason for this is that the *B. bassiana* and rove beetle combination treatment, and insecticide treatment, should provide a similar level of suppression of western flower thrips populations. Second, the *B. bassiana* and rove beetle combination treatment should provide greater suppression of western flower thrips populations than *B. bassiana* and rove beetle individual treatments. Because synergistic (mortality greater than the sum of mortality that the individual provides) effects are

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expected to occur when using the *B. bassiana* and rove beetle combination. Third, the *B. bassiana* and rove beetle combination treatment should cost no more than the insecticide treatment.

Chapter 2 - Literature review

Biology of western flower thrips, *Frankliniella occidentalis*

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), adults are approximately 1-2 mm in length and possess piercing-sucking mouthparts (Moritz 1997, Jensen 2000). Males are generally smaller than females (Robb 1989, Jensen 2000). Western flower thrips prefers to aggregate within flowers or other enclosed, protected areas on plants, such as terminal buds (Kirk 1997a, Hansen et al. 2003). A female deposits eggs into plant tissues using a saw-like ovipositor (Reitz 2009).

Western flower thrips life cycle consists of an egg, two larval instars (first and second), two pupal instars (prepupae and pupae), and an adult (Tommasini and Maini 1995, Reitz 2009). Eggs are inserted into plant tissues and subsequently hatch into larvae. Eventually second instar larvae stop feeding and pupate (Gaum et al. 1994). Pupae develop into adults after approximately six days (Robb et al. 1988). Both larvae and adults feed on leaves, flowers and fruits (Hansen et al. 2003, Loughner et al. 2005). Therefore, compared to the egg and pupal stages, larvae and adults are more exposed to insecticides and natural enemies (e.g., predators, parasitoids, and entomopathogenic fungi).

Second instar larvae eventually migrate down the plant stem or fall onto the growing medium surface to pupate (Helyer et al. 1995, Tommasini and Maini 1995, Kirk 1996, Wiethoff et al. 2004). However, western flower thrips may also pupate in flowers (Lewis 1973, Jacobson 1997, Broadbent et al. 2003, Bennison et al. 2004, Buitenhuis and Shipp 2008). Pupation may be affected by plant species and plant growth stages. For example, Bennison et al. (2004) indicated that 98% of western flower thrips larvae on cucumber (*Cucumis sativus*) plants and

approximately 96% on flowering chrysanthemum (*Dendranthema* x *morifolium*) plants migrated

to the growing medium to pupate. However, Broadbent et al. (2003) found that more than 50%

of western flower thrips larvae pupated within the disc florets of potted chrysanthemum flowers.

Furthermore, Buitenhuis and Shipp (2008) demonstrated that about 93% of western flower thrips

pupated in the growing medium of non-flowering rose (*Rosa rugosa*) and chrysanthemum plants,

but only 87% (rose) and 60% (chrysanthemum) pupated in the growing medium when plants

were flowering. In addition, a study reported that approximately 78% of western flower thrips

pupated at a depth of 1 to 5 mm in the compost that was a freshly steam-sterilised mixture (50:50

by volume) of loam and medium grade sphagnum peat, about 20% pupated at a depth of 25 to 35

mm, and approximately 2% pupated at a depth of 6 to 24 mm (Helyer et al. 1995).

The development time of western flower thrips, from egg to adult, is two to three weeks

at 20 to 25ºC but varies depending on temperature and host plant (Tommasini and Maini 1995).

For instance, development time of the life cycle (egg to adult) was 19 days at 20°C and 14 days

at 25°C on peanut (*Arachis hypogaea*) (Lowry et al. 1992). Moreover, Zhang et al. (2007) found

that at 27 ± 1°C and a 16:8 (light:dark) hour photoperiod, developmental time of western flower

thrips, from egg to adult, was 9.2 days on cucumber, 10.1 days on cabbage (*Brassica oleracea*),

10.4 days on kidney bean (*Phaseolus vulgaris*), 12.1 days on capsicum (*Capsicum annuum*), and

12.9 days on tomato (*Lycopersicon esculentum*) leaves. Therefore, due to a relatively short life

cycle, multiple generations of western flower thrips may occur within a single cropping season

(Loughner et al. 2005, Cloyd 2009, Reitz 2009).

Western flower thrips females can live 26 days at 28°C under laboratory conditions

(Reitz 2008), with some females living up to 35 days (Trichilo and Leigh 1988, Hulshof et al.

2002). The average life span of female western flower thrips adults from a laboratory colony was

39.1 days under laboratory conditions at 20-24ºC, 50% to 60% relative humidity, and constant light (see Appendix D). Furthermore, western flower thrips females lay between 150 and 300 eggs during their lifetime (Cloyd 2009). In addition, sex-determination in western flower thrips populations is haplo-diploid, with unfertilized eggs developing into haploid males and fertilized eggs developing into diploid females (Moritz 1997).

Plant damage caused by western flower thrips, *Frankliniella occidentalis*

Western flower thrips feed on over 250 plant species affiliated with 60 plant families (Robb 1989, Tommasini and Maini 1995, Lewis 1997). Larvae and adults feed on plant fluids with their piercing-sucking mouthparts (Hunter and Ullman 1989, Harrewijn et al. 1996, Kirk 1997b). Direct damage caused by larval and adult feeding includes: leaf, flower and fruit scarring; distortion and discoloration of flowers; and fruit deformation (Chisholm and Lewis 1984, Hunter and Ullman 1989, Childers 1997, Lewis 1997). A female inserts eggs into leaves, flowers or developing fruits using a saw-like ovipositor. The wounds created by oviposition in plant tissues cause plant damage, which may reduce marketability of horticultural crops (Childers 1997, Cloyd 2009). However, the most important concern associated with western flower thrips is the indirect damage caused by vectoring plant viruses, including the tospoviruses: *Tomato spotted wilt* and *Impatiens necrotic spot* viruses (Whitfield et al. 2005). Western flower thrips larvae (first and second instar) acquire the viruses after feeding on infected plants, and then transmit the viruses as late second instar larvae and/or adults during feeding (Tsuda et al. 1996, van de Wetering et al. 1996).

Western flower thrips cause significant economic losses due to direct and indirect damage. Global losses associated with *Tomato spotted wilt virus* were >$1 billion annually

(Goldbach and Peters 1994). Murphy et al. (1998) reported that, in California, the cost of suppressing western flower thrips populations in cut flower production was 7.5% of the total production cost. Moreover, in 2006, western flower thrips damage resulted in an economic loss of >$15 million to the ornamental industry in Georgia (Reitz and Funderburk 2012). Overall, due to substantial economic losses, western flower thrips is one of the most destructive insect pests of the horticultural industry worldwide.

Insecticide resistance of western flower thrips, *Frankliniella occidentalis*

Since damage caused by western flower thrips reduces aesthetic quality and marketability of greenhouse-grown horticultural crops, the tolerance for this insect pest is very low (Parrella and Jones 1987). Therefore, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Cloyd 2009, Reitz 2009). However, many biological factors associated with western flower thrips may limit the effectiveness of insecticides. For example, the small body size (1-2 mm in length for adults) and cryptic behavior protect western flower thrips from exposure to many contact insecticides (Cloyd 2009). In addition, adults residing in the flowers escape exposure from systemic insecticides (Daughtrey et al. 1997, Cloyd and Sadof, 1998). Moreover, high female fecundity and short generation time result in multiple generations of western flower thrips populations occurring during a single growing season (Reitz 2009). Furthermore, a wide host range and haplo-diploid breeding system allow western flower thrips to avoid any issue associated with plant allelochemicals, which consequently contributes to larvae and adults being able to overcome secondary plant defenses (Feyereisen 1999, Jensen 2000, Espinosa et al. 2005).

Greenhouse producers apply insecticides to mitigate both direct and indirect damage caused by western flower thrips (Parrella and Jones 1987). However, continuous use of insecticides places intensive selection pressure on western flower thrips populations. Consequently, insecticide resistance has developed in a number of western flower thrips populations worldwide associated with many chemical classes (Brødsgaard 1994, Jensen 2000, Bielza et al. 2007, Kay and Herron 2010, Whalon et al. 2014).

To mitigate the development of insecticide resistance in western flower thrips populations, a common recommendation is to rotate insecticides with different modes of action (Cloyd 2009). However, greenhouse producers only have a limited selection of commercially available insecticides due to the high costs affiliated with developing and registering new insecticides (Reitz and Funderburk 2012). Therefore, alternative plant protection strategies are warranted, such as augmentative biological control that involves releasing or applying natural enemies (e.g., predators, parasitoids, and entomopathogenic fungi) to suppress pest populations (Cloyd 2009). A potential strategy for managing western flower thrips is to target the foliar-feeding life stages (larvae and adults) using an entomopathogenic fungus, and target the soil-inhabiting life stages (prepupae and pupae) using a soil-dwelling predator.

Entomopathogenic fungus, *Beauveria bassiana*

Entomopathogenic fungi are major components of plant protection programs in greenhouse and field production systems (Langewald et al. 1997, Maniania et al. 2003, Migiro et al. 2010, Wang et al. 2013). Entomopathogenic fungi are viable alternatives to conventional insecticides due to a number of advantages. For example, Mendonça (1992) modified previous methods to ensure mass production of conidia by inoculating sterile rice (*Oryza sativa*) in plastic

bags and tubs. Moreover, entomopathogenic fungi can be formulated to enhance efficacy and storage stability and can also be applied using conventional equipment (Shah and Pell 2003). Compared to conventional insecticides, entomopathogenic fungi have minimal direct effects on non-target organisms (Goettel et al. 1990, Goettel and Hajek 2000, Pell et al. 2001).

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has a very broad host range infecting over 700 insect species (Samish et al. 2004, Akbar et al. 2005, Rehner and Buckley 2005, Devi et al. 2006, Dara et al. 2008). Moreover, *B. bassiana* is an alternative to insecticides for many agricultural pests, including: Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae); European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae); pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae); and false-eye leafhopper, *Empoasca vitis* (Göthe) (Hemiptera: Cicadellidae) (James et al. 1995, Lewis et al. 1996, Poprawski et al. 1997, Feng et al. 2004). In addition, *B. bassiana* is used against several greenhouse pests, such as: western flower thrips, silverleaf whitefly, *Bemisia argentifolii* (Gennadius) (Hemiptera: Aleyrodidae), and shore fly, *Scatella stagnalis* (Fallén) (Diptera: Ephydridae) (Wraight et al. 2000, Ugine et al. 2005, Castrillo et al. 2008, Kivett et al. 2015). *Beauveria bassiana* has been evaluated to suppress western flower thrips populations. For example, Ugine et al. (2007) reported a 30% to 40% reduction in western flower thrips populations after spraying a wettable powder formulation of *B. bassiana* strain GHA on greenhouse-grown impatiens (*Impatiens walleriana*). Ansari et al. (2008) found that mortality of western flower thrips larvae and pupae ranged from 54% to 84% when exposed to growing medium mixed with *B. bassiana*. Skinner et al. (2012) observed a 90% reduction in the mean total number of western flower thrips per plant using mycotized millet grains containing *B.*

bassiana in the soil. Therefore, the entomopathogenic fungus, *B. bassiana* may be a viable microbial pesticide (biopesticide) in suppressing western flower thrips populations.

Rove beetle, *Dalotia coriaria*

Rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), adults are dark-brown to shiny-black and 3 to 4 mm in length (Miller and Williams 1983). The development time of *D. coriaria* from egg to adult is 17 days at $26 \pm 3°C$, 50% to 60% relative humidity, and a 12:12 (light:dark) hour photoperiod (Echegaray and Cloyd 2013). Larvae and adults are active and adults can fly, which allows adults to disperse within a greenhouse away from the original release site. *Dalotia coriaria* prefers constant darkness and resides in growing medium or soil (Helyer et al. 2003). Furthermore, *D. coriaria* is commercially available from most biological control suppliers (Jandricic et al. 2005, Warner and Getz 2008).

Dalotia coriaria has been shown to feed on a number of greenhouse insect pests, including: soil-dwelling life stages (prepupae and pupae) of western flower thrips, and the larval stages of shore flies (*Scatella* spp.) and fungus gnats (*Bradysia* spp.) (Gillespie et al. 2001, Carney et al. 2002, Cox et al. 2006, Jandricic et al. 2006, Echegaray et al. 2015). For instance, Carney et al. (2002) found that one *D. coriaria* adult consumed 95 western flower thrips late second instar larvae or 78 pupae in 24 hours in Petri dishes under laboratory conditions. Furthermore, integrating rove beetles with other biological control agents (e.g., entomopathogenic fungi, nematodes, and predatory mites), insect growth regulators, reduced-risk pesticides, and plant growth regulators, has been evaluated (Jandricic et al. 2005, Jandricic et al. 2006, Cloyd et al. 2009, Echegaray and Cloyd 2012, Saito and Brownbridge 2016). However, intraguild predation [the killing and consumption of species that use similar, often limiting

resources and are thus potential competitors (Polis et al. 1989)] or mutual interference

[competition occurs when access to resources is negatively affected by the presence of other

individuals within a population (DeLong and Vasseur 2011)] may occur when using multiple

biological control agents including rove beetles, which may negatively affect biological control

programs (Jandricic et al. 2005, Jandricic et al. 2006). Therefore, *D. coriaria* may be a feasible

biological control agent in greenhouse production systems when used alone.

Chapter 3 - Effect of different numbers of predator and prey on predation of western flower thrips, *Frankliniella occidentalis*, pupal stages by rove beetle, *Dalotia coriaria*, adults

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest of horticultural crops worldwide (Robb and Parrella 1995, Lewis 1997, Kirk 2002, Cloyd 2009). Western flower thrips cause both direct and indirect plant damage (Chisholm and Lewis 1984, Hunter and Ullman 1989, Harrewijn et al. 1996, Pappu et al. 2009), which has resulted in substantial economic losses (Goldbach and Peters 1994, Reitz and Funderburk 2012). Consequently, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Kontsedalov et al. 1998, Loughner et al. 2005, Cloyd 2009).

However, due to the intensive selection pressure associated with insecticide applications, western flower thrips populations have developed resistance to insecticides from many different chemical classes (Brødsgaard 1994, Jensen 2000, Bielza et al. 2007, Kay and Herron 2010). Therefore, alternative plant protection strategies are warranted, such as augmentative biological control that involves releasing or applying natural enemies (e.g., predators, parasitoids, or entomopathogenic fungi) to suppress pest populations (Cloyd 2009, Reitz 2009).

The predatory rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), may be a viable biological control agent of western flower thrips (Carney et al. 2002) and is commercially available from most biological control suppliers (Jandricic et al. 2005, Warner and Getz 2008). Furthermore, *D. coriaria* has been reported to prey upon several greenhouse insect

pests, including: soil-dwelling life stages (prepupae and pupae) of western flower thrips, and the larval stages of shore flies (*Scatella* spp.) and fungus gnats (*Bradysia* spp.) (Gillespie et al. 2001, Carney et al. 2002, Cox et al. 2006, Jandricic et al. 2006, Echegaray et al. 2015).

Predation efficacy is a measurement designed to evaluate the effectiveness of predators in suppressing prey populations, which is important in selecting biological control agents (Farhadi et al. 2011) and in assessing the numbers of predators to release in augmentative biological control programs (Echegaray et al. 2015). Due to low pest tolerance and the capacity of most greenhouse insect and mite pests to increase populations rapidly, determining an appropriate predator-prey ratio to effectively suppress pest populations is important. Consequently, studies have evaluated predator-prey ratios associated with various predators and prey (Gaudchau 1982, Gilkeson and Hill 1987, Opit et al. 2004, Cheng et al. 2012, Echegaray et al. 2015). Furthermore, different initial prey numbers at a given predator-prey ratio may also affect predation efficacy (Echegaray et al. 2015).

Successful suppression of western flower thrips populations using the rove beetle, *D. coriaria,* is contingent on evaluating predation efficacy based on different numbers of predator and prey, as well as determining an appropriate predator-prey ratio. However, the effect of *D. coriaria* on western flower thrips is not well understood.

Based on previous studies, three hypotheses were developed. First, at the same initial number of one western flower thrips pupal stage [prepupa, pupa, or prepupa-pupa combination (prepupa:pupa=50%:50%)], the estimated mean probability of western flower thrips adults captured on yellow sticky cards should decrease as the number of rove beetle adults increases because more rove beetle adults should consume higher numbers of prey. Second, at one western flower thrips pupal stage, the estimated mean probability of western flower thrips adults captured

on the yellow sticky cards should decrease as predator-prey ratio increases. The reason for this is that as predator-prey ratio increases, the proportion of rove beetle adults to prey is greater leading to greater predation. Therefore, relatively more predators are available to feed on prey. Third, at each predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should increase as the initial number of prey increases, and correspondingly as more rove beetle adults are released. The reason being is that when more rove beetle adults are released in the same container, mutual interference [competition occurs when access to resources is negatively affected by the presence of other individuals within a population (DeLong and Vasseur 2011)] among rove beetle adults should occur. Consequently, rove beetle adults should spend less time foraging for prey.

Therefore, the objectives of this study were to determine the effects of: 1) different absolute numbers of predator (rove beetle) and prey (western flower thrips), 2) different initial prey numbers, and 3) different predator-prey ratios (1:5, 1:10, and 1:15) on predation efficacy of *D. coriaria*.

Materials and Methods

a. Insect colonies

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20

minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days.

A rove beetle colony was maintained in growing medium in 7.6 L plastic rectangular containers [34.8 x 24.7 x 12.4 cm (length x width x height] (Rubbermaid Home Products; Wooster, OH) under laboratory conditions: 20-24ºC, 45% to 60% relative humidity, and constant darkness simulating conditions similar to the natural habitat of rove beetles (Helyer et al. 2003). Growing medium preparation was as follows: a 6.0 L plastic container [28.5 x 11.0 cm (diameter x height)] (Rubbermaid Home Products; Wooster, OH) was filled with Sunshine LC1 RSi Professional Growing Mix (SunGro Horticulture, Inc.; Bellevue, WA) growing medium composed of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone. The growing medium was moistened with approximately 200 mL of water. The 6.0 L plastic container with growing medium was then heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.8 L of water was applied to the growing medium, which was then thoroughly mixed. About 3.0 L of the sterilized growing medium was placed into each 7.6 L plastic rectangular container. Approximately 15 g of dry oats (*Avena sativa* L.) (The Quaker Oats Company; Chicago, IL) were placed, every four to five days, onto the growing medium surface in a line (lengthwise) in the center of the growing medium within each 7.6 L plastic rectangular container. About 15 mL of water was sprayed, every one to two days, onto the oats using a 946 mL plastic spray bottle (Delta Industries; King of Prussia, PA) to maintain constant moisture. Adult rove beetles from previous colonies were then used to establish new colonies.

Western flower thrips and rove beetle specimens used in this study are deposited as voucher numbers 237 and 220, respectively in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Preparation of newly eclosed rove beetle adults

Five rove beetle third instar larvae were placed into a Gladware® container [7.8 x 5.1 cm (diameter x height)] (The Glad Products Company; Oakland, CA) with 20 mL of moistened growing medium and three to four pieces of dry oats. A total of 20 Gladware® containers with rove beetle third instar larvae were prepared as described above, then placed into an environmental growth chamber (Conviron® Controlled Environments Inc.; Pembina, ND) set at 21-27ºC and constant darkness. Rove beetle adults were observed seven days later with most adults emerging after 11 days (Yinping Li; personal observation). Newly eclosed adults (one to three days old) were individually placed in 9 dram (33 mL) plastic vials with lids using a soft-bristled brush. All 9 dram plastic vials containing rove beetle adults were returned to the environmental growth chamber, and the adults were starved for 24 hours. The sex ratio of rove beetle adults was 1:1 (female:male).

c. Experimental procedures

Three independent laboratory experiments were conducted to assess predation efficacy of rove beetles on three western flower thrips pupal stages, namely prepupa (experiment 1), pupa (experiment 2), and 50%:50% prepupa-pupa combination (experiment 3). This treatment structure allowed for evaluating the effects of predator-prey ratios (1:5, 1:10, and 1:15), accounting for different initial prey numbers, on predation efficacy of rove beetle adults. Each experiment was set-up as a randomized complete block design, with experimental round (day) as

a blocking factor. Each experiment was completed in five experimental rounds, one per day. In each experiment, predation efficacy was evaluated using a two-way factorial treatment structure consisting of all combinations of six numbers (0, 1, 2, 3, 4, and 5) of newly eclosed rove beetle adults and four initial numbers (15, 20, 25, and 30) of one of the three western flower thrips pupal stages. By combining factor levels, this treatment structure further enabled evaluation of the effects of selected predator-prey ratios (1:5, 1:10, and 1:15) with increasing initial prey numbers on predation efficacy of rove beetles (Table 1).

For each experiment, approximately 1.2 L of sterilized Sunshine LC1 RSi Professional Growing Mix growing medium was placed into each 15.2 cm diameter plastic container [15.2 x 14.3 cm (diameter x height)] (Dillen Products Inc; Middlefield, OH). A green bean (5 cm in length) was placed on the growing medium surface beside the inside rim of each 15.2 cm container to provide a food source for western flower thrips adults that eclosed.

Then, a certain number (15, 20, 25 or 30) of one western flower thrips pupal stage were randomly positioned on the growing medium surface. For the treatments involving uneven initial numbers (15 and 25) of western flower thrips prepupa-pupa combination, the numbers of prepupae and pupae were alternated on each day.

Prepupae and pupae are generally located at a depth of 1 to 5 mm in the compost that was a freshly steam-sterilised mixture (50:50 by volume) of loam and medium grade sphagnum peat (Helyer et al. 1995). Furthermore, prepupae and pupae are distributed throughout the growing medium via cracks and crevices present on the growing medium surface (Yinping Li; personal observation). Therefore, no additional growing medium was needed to cover the prepupae or pupae. One to two hours after the western flower thrips pupal stage was placed on the growing medium surface, a certain number (0, 1, 2, 3, 4 or 5) of newly eclosed rove beetle adults were

released into each 15.2 cm container. Each container was covered with No-Thrips insect screening to prevent rove beetle adults and western flower thrips adults from escaping. For each experimental round (day), a total of twenty-four 15.2 cm containers, one container per treatment combination, were prepared and maintained in the laboratory at 19-25ºC, 50% to 60% relative humidity, and a 16:8 (light:dark) hour photoperiod.

To prevent rove beetle adults to be captured on the yellow sticky cards prior to western flower thrips adult eclosion, a yellow sticky card was affixed onto the inside center of the No-Thrips insect screening within each container five days after the experiment was initiated. Simultaneously, the green beans were removed from the containers, and approximately 15 g of oats were placed on the growing medium surface as a food source for rove beetle adults to ensure adult survival after completion of the experiment. To maintain moisture, about 15 mL of water was applied to the oats through the No-Thrips insect screening using a 946 mL plastic spray bottle every one to two days.

The numbers of western flower thrips adults for each experiment and rove beetle adults for the last three experimental rounds of experiment 3 captured on the yellow sticky cards were recorded 17 days following initiation of the experiment. The number of western flower thrips adults captured on the yellow sticky cards was used as an indirect assessment of predation efficacy, which was in turn quantified by the binomial probability of western flower thrips adults captured on the yellow sticky cards (refer to Data analysis).

To confirm rove beetle adult survival during the experiment, the growing medium in the 15.2 cm containers was used to determine the number of rove beetle adults recovered in the last three experimental rounds of experiment 3. After completing the experiment, the growing medium from each 15.2 cm container was placed into a 9.4 L plastic rectangular container [40.2

x 26.4 x 12.8 cm (length x width x height)] (Rubbermaid Home Products; Wooster, OH). About 0.8 L of water was added to each 9.4 L plastic rectangular container to saturate the growing medium, causing rove beetles to emerge from the growing medium (Yinping Li; personal observation). Then, approximately 15 g of oats were placed on the growing medium surface as a food source for rove beetle adults. Each 9.4 L plastic rectangular container was covered using a modified lid with different insect screening (mesh size: 0.2 x 0.8 mm) (Greentek®; Edgerton, WI). The number of rove beetle adults in each 9.4 L plastic rectangular container was recorded 24 hours after the growing medium was saturated. The number of rove beetle adults recovered after completing the experiment was assessed based on the number of rove beetle adults captured on the yellow sticky cards and the number of rove beetle adults recovered from the growing medium.

Data Analysis

For each experiment, a generalized linear mixed model assuming a binomial distribution was fitted to the response variable defined as number of western flower thrips adults captured on the yellow sticky cards out of the initial number of one western flower thrips pupal stage in the container. A logit link function was used to estimate the probability of western flower thrips adults captured on the yellow sticky cards to the linear predictor, which included the fixed effects of number of rove beetle adults, initial number of one western flower thrips pupal stage, and the two-way interaction. Random effects associated with the linear predictor included the blocking effect of experimental round (day) and the effect of container as the unit of observation identified by the cross product of experimental round (day) and treatment combination. This was needed to account for over-dispersion observed in preliminary analyses.

Over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion in the final statistical model for each experiment. In each experiment, the final statistical model used for inferences was fitted using Residual Pseudo-likelihood. Degrees of freedom were approximated and estimated standard errors were adjusted using Kenward-Roger's procedure. All statistical models were fitted using the PROC GLIMMIX procedure (SAS Institute 2012). Pairwise comparisons were implemented using Tukey-Kramer's adjustment at the marginal level to avoid inflation of type I error due to multiple comparisons. Within each final statistical model, tailored contrasts were conducted to make selected comparisons of treatment combinations that represented the predator-prey ratios (1:5, 1:10, and 1:15) with different initial prey numbers.

Results

a. Assessment of baseline conditions

When no rove beetle adults were released, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was 99% (97.3-99.6%) [mean (95% confidence interval)] in experiment 1, 96.1% (91.7-98.2%) in experiment 2, and 97.6% (95-98.8%) in experiment 3 (Figure 1). The high recovery of western flower thrips adults in the absence of rove beetle adults suggests that neither natural mortality of western flower thrips prepupae and pupae nor mortality caused by handling technique inhibited prepupae and pupae from developing into adults. Moreover, descriptive statistics of rove beetle adults recovered at the end of the experiment confirm their general presence and survival during the experimental period (Table 2).

b. Effect of different absolute numbers of predator and prey on predation efficacy

No significant interactions were identified between the number of rove beetle adults released and the initial number of prepupae (F=1.36; df=15, 95.99; P=0.19) in experiment 1, pupae (F=1.00; df=15, 96; P=0.46) in experiment 2, or prepupae-pupae combination (F=1.07; df=15, 96; P=0.39) in experiment 3 on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Therefore, results are presented as marginal mean estimates for each of the treatment factors.

Experiment 1 (prepupa). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was negatively affected when increasing numbers of rove beetle adults were released (F=31.11; df=5, 96; P<0.0001: Figure 1A). Specifically, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased significantly when the number of rove beetle adults released increased from 1 to 3, 4, or 5. However, when 2 rove beetle adults were released, results were intermediate and not significantly different from any of the other release numbers. Furthermore, there was no evidence of any effect of initial numbers of western flower thrips prepupae (F=0.15; df=3, 96; P=0.93) on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards.

Experiment 2 (pupa). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was negatively affected by the numbers of rove beetle adults released (F=56.95; df=5, 96; P<0.0001: Figure 1B), but there was no evidence of any effect of initial numbers of western flower thrips pupae (F=1.21; df=3, 96; P=0.31). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly higher when releasing 1 compared to 4 rove beetle adults. However, there was no evidence that

the estimated mean probability of western flower thrips adults captured on the yellow sticky cards differed after releasing 1, 2, 3, and 5 rove beetle adults, or after releasing 2, 3, 4, and 5 rove beetle adults (Figure 1B).

Experiment 3 (prepupa-pupa combination). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was negatively affected by the numbers of rove beetle adults released (F=41.45; df=5, 96; P<0.0001: Figure 1C). However, there was no evidence of any effect of initial numbers associated with the western flower thrips prepupae-pupae combination (F=1.30; df=3, 96; P=0.28). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly higher when releasing 1 compared to 3, 4, and 5 rove beetle adults. However, there was no evidence that the estimated mean probability of western flower thrips adults captured on the yellow sticky cards differed when either 1 or 2 rove beetle adults were released, or among releasing 3, 4, and 5 rove beetle adults (Figure 1C).

c. Effects of predator-prey ratios, accounting for different initial prey numbers, on predation efficacy

Experiment 1 (prepupa). At the 1:15 predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased significantly from 68.7% (50.7-82.4%) to 33.4% (20.4-49.4%) when the initial number of western flower thrips prepupae increased from 15 to 30. However, there was no evidence that the estimated mean probability of western flower thrips adults captured on the yellow sticky cards differed due to initial prey numbers within the 1:10 (t=1.00, df=59.96, P=0.32) or 1:5 predator-prey ratio (F=0.97; df=2, 93; P=0.38) (Table 3).

After adjusting for the initial number of western flower thrips prepupae, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards at the 1:5 [19.5% (12.9-28.3%)] was significantly lower than that at the 1:15 predator-prey ratio [51.2% (38-64.1%)]. However, the estimate at 1:10 ratio [30.2% (20.4-42.2%)] was not significantly different from the other predator-prey ratios (1:5 and 1:15) (Table 3).

Experiment 2 (pupa). There was no evidence of any differences in the estimated mean probability of western flower thrips adults captured on the yellow sticky cards among the predator-prey ratios (F=2.15; df=2, 96; P=0.12), or among different initial prey numbers within each predator-prey ratio (1:5 ratio: F=1.11; df=2, 96; P=0.33; 1:10 ratio: t=0.42, df=70.29, P=0.68; and 1:15 ratio: t=1.40, df=66.08, P=0.17) (Table 4).

Experiment 3 (prepupa-pupa combination). At the 1:10 predator-prey ratio, the estimated mean probability of western flower thrips captured on the yellow sticky cards decreased significantly from 38.7% (23.8-56.1%) to 7.5% (3.4-15.8%) when the initial number of western flower thrips prepupae-pupae combination increased from 20 to 30. However, there was no evidence of any significant differences due to initial prey number at the 1:5 (F=0.38; df=2, 86.43; P=0.69) or 1:15 predator-prey ratio (t=1.72, df=68.51, P=0.091). In addition, there was no evidence of any significant differences in the estimated mean probability of western flower thrips adults captured on the yellow sticky cards among the three predator-prey ratios (F=0.76; df=2, 77.63; P=0.47) (Table 5).

Discussion

In this study, we quantitatively and indirectly evaluated the predation efficacy of rove beetle adults on western flower thrips pupal stages. Overall, for each pupal stage, the estimated

mean probability of western flower thrips adults captured on the yellow sticky cards decreased as the number of rove beetle adults released increased from 1 to 3; however, the effect of additional rove beetle adults released was not apparent. Moreover, across the pupal stages evaluated in this study, overall, there was no evidence of any differential effects associated with predator-prey ratios or initial prey numbers. Therefore, the results from this study provide insights into the potential predatory behavior of *D. coriaria* adults on the western flower thrips pupal stages, which may have practical implications for successful implementation of biological control programs in greenhouse production systems. In addition, the pupal stages of western flower thrips are tolerant to insecticides (Seaton et al. 1997). So, *D. coriaria* may provide mortality on life stages that are difficult to manage with insecticides.

The first hypothesis indicated that at each initial number of each western flower thrips pupal stage, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should decrease as the number of rove beetle adults increases because more rove beetle adults should consume higher numbers of prey. The results of this study partially confirmed this hypothesis. There was a general trend that for each western flower thrips pupal stage, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased when the number of rove beetle adults increased from 1 to 3, however, the effect of releasing additional rove beetle adults was not apparent. It is possible that mutual interference occurred when the number of rove beetle adults increased from 3 to 5. Consequently, rove beetle adults spent less time foraging for western flower thrips prepupae or pupae.

In the second hypothesis, for each western flower thrips pupal stage, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should decrease as

the predator-prey ratio increases. The reason for this is that as predator-prey ratio increases, the proportion of rove beetle adults to prey is greater. Therefore, more predators are available to feed on prey. Results from experiment 1 (prepupa) partially supported this hypothesis as the estimated mean probability of western flower thrips adults captured on the yellow sticky cards at the 1:5 ratio was significantly lower than the 1:15 ratio, but was not significantly different from the 1:10 ratio. However, results from experiments 2 (pupa) and 3 (prepupa-pupa combination) did not support this hypothesis. In experiments 2 and 3, there were no significant differences affiliated with the estimated mean probability of western flower thrips adults captured on the yellow sticky cards among the three predator-prey ratios. It is possible that as the predator-prey ratio increased, with fewer prey available for each rove beetle adult, mutual interference caused predators to spend less time foraging for prey (Echegaray et al., 2015). However, if what occurred in experiments 2 and 3 is true, it is unclear why mutual interference did not occur in experiment 1.

For the third hypothesis, at a given predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should increase as the numbers of one western flower thrips pupal stage and rove beetle adults increase. This hypothesis assumed that 1) cannibalism does not occur among rove beetle adults (Miller and Williams, 1983); 2) when more rove beetle adults are released in the same container, mutual interference will occur; consequently, reducing predator foraging efficiency. However, the results did not support the hypothesis as at the 1:15 predator-prey ratio in experiment 1 (prepupae) and 1:10 ratio in experiment 3 (prepupal-pupal combination), the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased significantly when the initial number of prey increased. Furthermore, as for the other predator-prey ratios in the three experiments, the estimated mean probability of western flower thrips adults captured on the

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yellow sticky cards did not differ significantly among the different initial numbers of prey. It is likely that as the initial number of prey increased, the numbers of rove beetle adults also increased, but the number of prey available to each rove beetle adult did not change. Thus, mutual interference likely did not occur among the rove beetle adults.

Selecting an appropriate predator-prey ratio may enhance the success of an augmentative biological control program (Hamlen and Lindquist 1981, Gaudchau 1982, Opit et al. 2004, Cheng et al. 2012, Echegaray et al. 2015, Amoah et al. 2016). For example, successful suppression has been achieved at ratios between 1:20 and 1:4 when using the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae), against the twospotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), on greenhouse ornamentals (Hamlen and Lindquist 1981). Moreover, Echegaray et al. (2015) recommended a 1:5 *D. coriaria* adult:fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae), larvae release ratio for greenhouse producers after evaluating the predation efficacy of *D. coriaria* adults on fungus gnat larvae under laboratory conditions. In this study, when predation efficacy was compared among predator-prey ratios in the experiments associated with pupa and the prepupa-pupa combination, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was not significantly different. Therefore, predation efficacy was not enhanced as the predator-prey ratio increased from 1:15 to 1:5. However, at the same initial prey number, fewer rove beetle adults need to be released at the predator-prey ratio of 1:15 compared to 1:10 and 1:5. Therefore, the 1:15 predator-prey ratio may be a practical recommendation for greenhouse producers when using *D. coriaria* adults against western flower thrips pupal stages in 15.2 cm containers.

Initial pest density may affect predation efficacy at a given predator-prey ratio (Echegaray et al. 2015). For instance, when the predatory mite, *P. persimilis* was released at a ratio of 1:10 (*P. persimilis*: twospotted spider mite, *T. urticae*), flower damage to potted *Impatiens walleriana* Hook. f. plants was greater when the initial pest population was higher (Alatawi 2006). When *D. coriaria* adults were released at a 1:5 (*D. coriaria* adult:fungus gnat larvae) predator-prey ratio to suppress fungus gnat populations under laboratory conditions, the percent fungus gnat adults captured on the yellow sticky cards decreased significantly from 59% to 37.5% as the initial number of fungus gnat larvae increased from 10 to 20 (Echegaray et al. 2015). Similarly, our results with prepupa indicated that, at a 1:15 predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased significantly from 68.7% to 33.4% when the initial number of western flower thrips prepupae increased from 15 to 30. Furthermore, for the prepupa-pupa combination, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards at 1:10 predator-prey ratio decreased significantly from 38.7% at an initial number of 20 western flower thrips prepupae-pupae combination to 7.5% at an initial number of 30 western flower thrips prepupae-pupae combination. It is likely that search time of rove beetle adults was reduced as prey number increased in the same searchable area.

This study is the first to evaluate predation of the rove beetle, *D. coriaria* on western flower thrips in 15.2 cm containers that are commonly used in greenhouse production systems (Reiley and Shry, Jr. 2001). Previous studies associated with *D. coriaria* feeding on western flower thrips were conducted in either Petri dishes or relatively small containers (such as 5.5 cm diameter cups). For instance, Carney et al. (2002) evaluated the potential predation (maximum numbers of prey consumed in 24 hours) of *D. coriaria* on western flower thrips late second instar

larvae or pupae in Petri dishes under laboratory conditions. Moreover, Saito and Brownbridge

(2016) assessed how integrating *D. coriaria* with entomopathogenic fungi or predatory mites,

would control the soil-dwelling stages (late second instar larvae, prepupae, and pupae) of

western flower thrips in 5.5 cm diameter plastic cups (120 mL in volume). However, searchable

area may affect the predation efficacy of *D. coriaria* on soil-dwelling prey (Echegaray et al.

2015, Herrick and Cloyd 2017). Therefore, this study was conducted in 15.2 cm containers

(1,834.8 cm^3 with 1.2 L of growing medium) typically used to grow greenhouse horticultural

plants (Reiley and Shry, Jr. 2001). So, the results of this study have practical value to greenhouse

producers who want to use rove beetles in biological control programs against the western flower

thrips. Furthermore, our findings indicate that regardless of the initial number of western flower

thrips prepupae, pupae, or prepupae-pupae combination, 3 rove beetle adults per 15.2 cm

container may be recommended within the range of the initial prey number tested here.

Figures and Tables

A (prepupa)
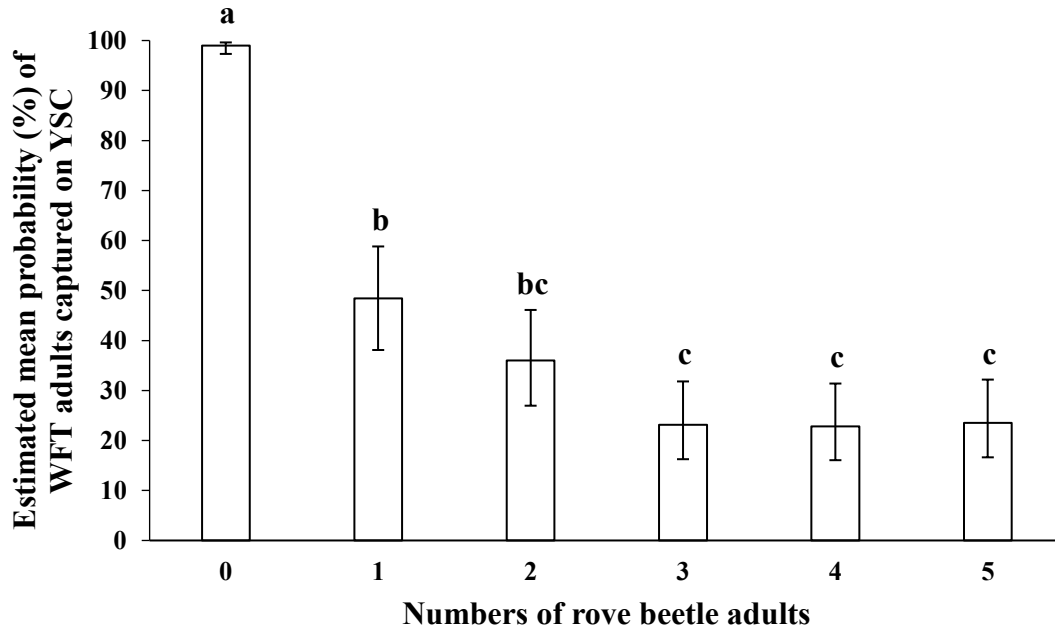
B (pupa)
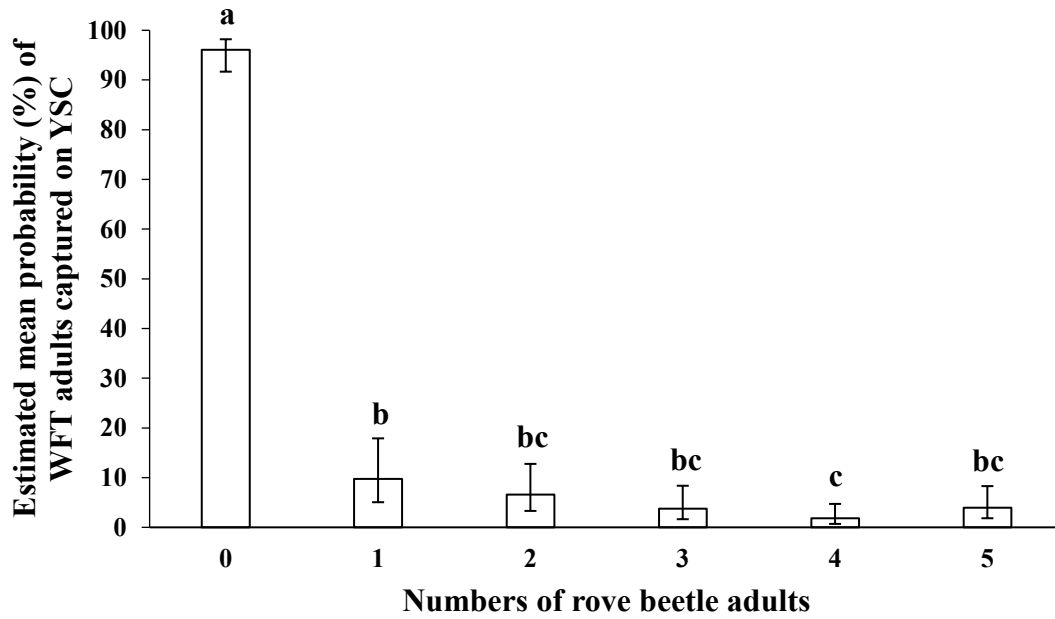
C (prepupa-pupa combination)
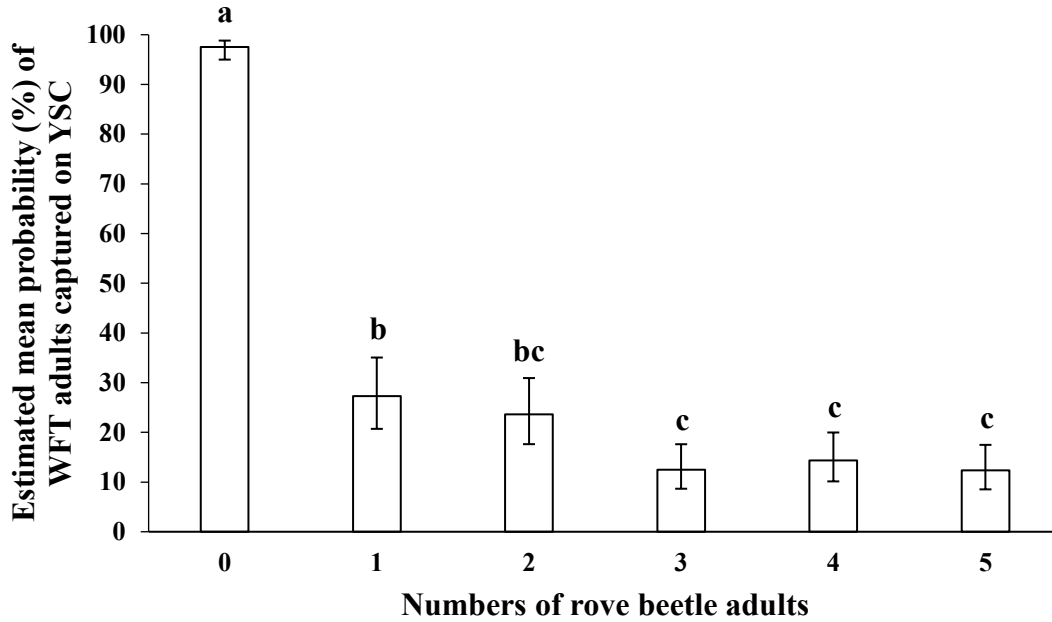
Figure 3-1 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) when 0, 1, 2, 3, 4, and 5 rove beetle, *Dalotia coriaria*, adults were released in experiment 1[A (prepupa)], experiment 2 [B (pupa)], and experiment 3 [C(prepupa-pupa combination)]. Estimated means followed by different letters within each experiment indicate significant differences (*P*<0.05) among different numbers of rove beetle adults released.

Table 3-1 Selected predator-prey ratios (1:5, 1:10, and 1:15) obtained by combining different predator numbers (1, 2, 3, 4, and 5) of rove beetle (RB), *Dalotia coriaria*, adults, and initial prey numbers (15, 20, 25, and 30) of western flower thrips (WFT), *Frankliniella occidentalis*, prepupa for experiment 1, pupa for experiment 2, and prepupa-pupa combination (50%:50%) for experiment 3.

Numbers of predators (rove beetle adults)	Predator-prey ratios					
	1:5		1:10		1:15	
	RB	WFT	RB	WFT	RB	WFT
1					1	15
2			2	20	2	30
3	3	15	3	30		
4	4	20				
5	5	25				

Table 3-2 Mean (minimum, maximum) number of rove beetle, *Dalotia coriaria*, adults recovered at the end of experiment 3 (prepupa-pupa combination) associated with treatment combinations consisting of initial numbers (15, 20, 25, and 30) of western flower thrips (WFT), *Frankliniella occidentalis*, prepupa-pupa combination (50%:50%) and five numbers (1, 2, 3, 4, and 5) of rove beetle adults initially released.

Numbers of rove beetle adults initially released	Initial numbers of WFT prepupa-pupa combination			
	15	20	25	30
1	1.0 (1, 1)	1.0 (1, 1)	1.0 (1, 1)	1.0 (1, 1)
2	0.7 (0, 1)	1.0 (0, 2)	1.7 (1, 2)	1.7 (1, 2)
3	1.7 (1, 2)	1.7 (1, 2)	1.7 (1, 2)	1.3 (1, 2)
4	1.7 (1, 2)	2.0 (1, 3)	1.7 (0, 3)	3.0 (3, 3)
5	2.7 (2, 3)	1.7 (1, 3)	2.7 (2, 4)	3.7 (2, 5)

Table 3-3 Comparisons associated with the estimated mean probability [95% confidence intervals (CI)] of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards among different initial prey numbers within each predator-prey ratio and among three predator-prey ratios (1:5, 1:10, and 1:15) in experiment 1.

Predator-prey ratios	Numbers of predator	Numbers of prey	Comparisons within each predator-prey ratio [Mean (95%CI), %]	Comparisons among three predator-prey ratios [Mean (95%CI), %]
1: 5	3	15	14.3 (6.6, 28.3) a	
1: 5	4	20	26.2 (14.6, 42.3) a	19.5 (12.9, 28.3) A
1: 5	5	25	19.3 (10.3, 33.2) a	
1: 10	2	20	35.3 (21.2, 52.6) a	
1: 10	3	30	25.5 (14.7, 40.6) a	30.2 (20.4, 42.2) AB
1: 15	1	15	68.7 (50.7, 82.4) b	
1: 15	2	30	33.4 (20.4, 49.4) a	51.2 (38.0, 64.1) B

Notes: Estimated means followed by different uppercase letters among the three predator-prey ratios are significantly different (*P*<0.05). Estimated means followed by the different lowercase letters within each predator-prey ratio are significantly different (*P*<0.05).

Table 3-4 Comparisons associated with the estimated mean probability [95% confidence intervals (CI)] of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards among different initial prey numbers within each predator-prey ratio and among three predator-prey ratios (1:5, 1:10, and 1:15) in experiment 2.

Predator-prey ratios	Numbers of predator	Numbers of prey	Comparisons within each predator-prey ratio [Mean (95%CI), %]	Comparisons among three predator-prey ratios [Mean (95%CI), %]
1: 5	3	15	5.4 (1.4, 17.9) a	
1: 5	4	20	0.9 (0.1, 7.6) a	2.8 (1.0, 7.3) A
1: 5	5	25	4.4 (1.3, 13.7) a	
1: 10	2	20	7.8 (2.6, 21.3) a	6.7 (2.9, 14.8) A
1: 10	3	30	5.8 (1.9, 16.2) a	
1: 15	1	15	14.2 (5.0, 34.1) a	8.9 (3.9, 18.9) A
1: 15	2	30	5.4 (1.8, 15.4) a	

Notes: Estimated means followed by different uppercase letters among the three predator-prey ratios are significantly different (*P*<0.05). Estimated means followed by the different lowercase letters within each predator-prey ratio are significantly different (*P*<0.05).

Table 3-5 Comparisons associated with the estimated mean probability [95% confidence intervals (CI)] of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards among different initial prey numbers within each predator-prey ratio and among three predator-prey ratios (1:5, 1:10, and 1:15) in experiment 3.

Predator-prey ratios	Numbers of predator	Numbers of prey	Comparisons within each predator-prey ratio [Mean (95%CI), %]	Comparisons among three predator-prey ratios [Mean (95%CI), %]
1: 5	3	15	16.2 (7.6, 31.1) a	
1: 5	4	20	20.6 (10.9, 35.5) a	16.8 (11.3, 24.1) A
1: 5	5	25	14.0 (7.0, 26.1) a	
1: 10	2	20	38.7 (23.8, 56.1) a	18.5 (11.6, 28.1) A
1: 10	3	30	7.5 (3.4, 15.8) b	
1: 15	1	15	32.5 (18.4, 50.7) a	23.5 (15.4, 34.2) A
1: 15	2	30	16.4 (8.7, 28.7) a	

Notes: Estimated means followed by different uppercase letters among the three predator-prey ratios are significantly different ($P<0.05$). Estimated means followed by the different lowercase letters within each predator-prey ratio are significantly different ($P<0.05$).

Chapter 4 - Effect of western flower thrips, *Frankliniella occidentalis*, pupal stage, predator-prey ratio, predator-prey number, and searchable area on predation efficacy of rove beetle, *Dalotia coriaria*, adults

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest associated with greenhouse-grown horticultural crops including ornamentals and vegetables (Robb and Parrella 1995, Lewis 1997, Kirk 2002, Cloyd 2009). Western flower thrips can cause direct and indirect plant damage (Chisholm and Lewis 1984, Hunter and Ullman 1989, Harrewijn et al. 1996, Pappu et al. 2009), resulting in substantial economic losses (Goldbach and Peters 1994, Reitz and Funderburk 2012). Consequently, greenhouse producers rely on regular applications of insecticides to suppress western flower thrips populations below damaging levels (Kontsedalov et al. 1998, Loughner et al. 2005, Cloyd 2009).

There is a concern that western flower thrips populations have developed resistance to insecticides associated with many chemical classes due to the intensive selection pressure from insecticide applications (Brødsgaard 1994, Jensen 2000, Bielza et al. 2007, Kay and Herron 2010). Therefore, biological control may be a viable alternative plant protection strategy to manage western flower thrips populations. (Cloyd 2009, Reitz 2009).

The predatory rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), is a potential biological control agent of western flower thrips (Carney et al. 2002) and is

commercially available from most biological control suppliers (Jandricic et al. 2005, Warner and

Getz 2008). Moreover, *D. coriaria* preys upon a number of greenhouse insect pests, including:

soil-dwelling life stages (prepupae and pupae) of western flower thrips, and the larval stages of

shore flies (*Scatella* spp.) and fungus gnats (*Bradysia* spp.) (Gillespie et al. 2001, Carney et al.

2002, Cox et al. 2006, Jandricic et al. 2006, Echegaray et al. 2015).

Predation efficacy is a measurement designed to evaluate predator effectiveness in

suppressing prey populations, which is important in selecting biological control agents (Farhadi

et al. 2011). Low pest tolerance and the capacity of most greenhouse insect and mite pests to

increase populations rapidly, has increased the need to determine appropriate predator-prey ratios

that sufficiently suppress pest populations. Consequently, studies have evaluated predator-prey

ratios for various predators and prey (Gaudchau 1982, Gilkeson and Hill 1987, Opit et al. 2004,

Cheng et al. 2012, Echegaray et al. 2015). Furthermore, different predator-prey numbers, based

on variations in predator-prey ratios, may also influence predation efficacy (Echegaray et al.

2015).

Although comparisons of predation efficacy among different predator-prey numbers

within each predator-prey ratio (1:5, 1:10, or 1:15) were previously conducted in Chapter 3, there

were different predator-prey numbers within each predator-prey ratio: 3 times, 4 times, and 5

times in 1:5 ratio; 2 times and 3 times in 1:10 ratio; as well as 1 time and 2 times in 1:15 ratio.

As such, the interaction between predator-prey ratio and predator-prey number could not be

determined. Therefore, in the study conducted in Chapter 4, predation efficacy was evaluated

based on three predator-prey numbers (2 times, 3 times, and 4 times) within each predator-prey

ratio (1:5, 1:10, or 1:15) to assess the interaction between predator-prey ratio and predator-prey

number.

Western flower thrips late second instar larvae migrate or fall onto the growing medium surface to pupate (Helyer et al. 1995, Tommasini and Maini 1995, Kirk 1996, Wiethoff et al. 2004). Western flower thrips prepupae and pupae take two to three days and one to two days to develop into adults, respectively (Zhang et al. 2007). Since exposure time of prepupae to rove beetle adults, prior to western flower thrips adult eclosion, is longer than pupae, rove beetle adults may have more time (about one day) to forage for western flower thrips prepupae than pupae. As such, predation efficacy may be influenced by duration of specific western flower thrips pupal stage (prepupa or pupa). Therefore, to account for the effect of different exposure time of western flower thrips pupal stages on rove beetle adult predation, pupal stage was included in this study as a factor that could potentially affect predation efficacy. Furthermore, the searchable area may also affect predation efficacy of *D. coriaria* on soil-dwelling prey, which has been proposed in previous associated with fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae), larvae (Echegaray et al. 2015, Herrick and Cloyd 2017). However, minimal information is available on how western flower thrips pupal stage, predator-prey ratio, predator-prey number, and searchable area influence predation efficacy of rove beetle, *D. coriaria*, adults.

Based on previous studies, four hypotheses were developed. First, at the same predator-prey ratio and same predator-prey number, the estimated mean probability of western flower thrips adults captured on yellow sticky cards from prepupae should be lower than the pupae. The reason being is that rove beetle adults have about one more day to forage on prepupae, before western flower thrips adults eclosion, than the pupae, potentially allowing greater predation on prepupae. Second, at the same predator-prey number and same pupal stage, as predator-prey ratio increases, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should increase. The reason for this is that as the predator-prey ratio increases, there

are more predators relative to prey; thus, mutual interference [competition occurs when access to resources is negatively affected by the presence of other individuals within a population (DeLong and Vasseur 2011)] among rove beetle adults should occur. Consequently, rove beetle adults should spend less time foraging for western flower thrips prepupae or pupae. Third, at the same predator-prey ratio and same pupal stage, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should increase as the predator-prey number increases and subsequently as more rove beetle adults are released. The reason being is that when more rove beetle adults are released in the same container, mutual interference among rove beetle adults should occur. Subsequently, rove beetle adults should spend less time foraging for western flower thrips prepupae or pupae. Fourth, at the same predator-prey ratio and same predator-prey number, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards should increase as searchable area increases because rove beetle adults should spend more time searching for prey as searchable area increases.

Therefore, the objectives of this study were to evaluate the effects of: 1) western flower thrips pupal stage, predator-prey ratio, and predator-prey number; and 2) predator-prey ratio, predator-prey number, and searchable area on the predation efficacy of rove beetle adults.

Materials and Methods

a. Insect colonies

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size:

0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 10 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days.

A rove beetle colony was maintained in growing medium in 7.6 L plastic rectangular containers [34.8 x 24.7 x 12.4 cm (length x width x height] (Rubbermaid Home Products; Wooster, OH) under laboratory conditions: 20-24ºC, 45% to 60% relative humidity, and constant darkness simulating conditions similar to the natural habitat of rove beetles (Helyer et al. 2003). Substrate preparation was as follows: a 6.0 L plastic container [28.5 x 11.0 cm (diameter x height)] (Rubbermaid Home Products; Wooster, OH) was filled with Sunshine LC1 RSi Professional Growing Mix (SunGro Horticulture, Inc.; Bellevue, WA) growing medium consisting of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone. The growing medium was moistened with approximately 200 mL of water. The 6.0 L plastic container with growing medium was then heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.8 L of water was applied to the growing medium, which was then thoroughly mixed. About 3.0 L of the sterilized growing medium was placed into each 7.6 L plastic rectangular container. About 15 g of dry oats (*Avena sativa* L.) (The Quaker Oats Company; Chicago, IL) were placed, every four to five days, onto the growing medium surface in a line (lengthwise) in the center of the growing medium within each 7.6 L plastic rectangular container. Approximately 15 mL of water was applied, every one to two days, onto the oats using a 946 mL plastic spray bottle

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(Delta Industries; King of Prussia, PA) to maintain constant moisture. Adult rove beetles from previous colonies were then used to establish new colonies.

Western flower thrips and rove beetle specimens used in this study are deposited as voucher numbers 237 and 220, respectively in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Preparation of newly eclosed rove beetle adults

Five rove beetle third instar larvae were placed into a Gladware® container [7.8 x 5.1 cm (diameter x height)] (The Glad Products Company; Oakland, CA) with 20 mL of moistened growing medium and three to four pieces of oats. A total of 20 Gladware® containers with rove beetle third instar larvae were prepared following the above method, and then placed into an environmental growth chamber (Conviron® Controlled Environments Inc.; Pembina, ND) set at 21-27ºC and constant darkness. Rove beetle adults were observed seven days later with most adults emerging after 11 days (Yinping Li; personal observation). Newly eclosed adults (one to three days old) were individually placed in 9 dram (33 mL) plastic vials with lids using a soft-bristled brush. All 9 dram plastic vials containing rove beetle adults were returned to the environmental growth chamber, and the adults were starved for 24 hours. The sex ratio of rove beetle adults was 1:1 (female:male).

c. Experimental procedures

Two independent experiments were conducted under laboratory conditions. Each experiment was set-up as a randomized complete block design, with experimental round (day) as a blocking factor. Each experiment was completed in five experimental rounds, one per day, with each experimental round involving 18 treatment combinations (described below).

In experiment 1, predation efficacy of rove beetle adults was evaluated using a three-way factorial treatment structure consisting of all combinations of two western flower thrips pupal stages (prepupa and pupa), three predator-prey ratios (1:5, 1:10, and 1:15), and three predator-prey numbers (2, 3, and 4 times) (Table 1). In experiment 2, rove beetle adult predation was assessed based on a three-way factorial treatment structure including all combinations of two searchable areas [15.2 cm diameter containers (1,834.82 cm^3 with 1.2 L of growing medium) and 11.5 cm diameter containers (701.79 cm^3 with 0.4 L of growing medium)], three predator-prey ratios (1:5, 1:10, and 1:15), and three predator-prey numbers (2, 3, and 4 times) (Table 2).

In experiment 1, approximately 1.2 L of sterilized Sunshine LC1 RSi Professional Growing Mix growing medium was placed into each 15.2 cm container (Dillen Products Inc; Middlefield, OH). In experiment 2, to account for different searchable areas, two container sizes were used: 15.2 and 11.5 cm diameter. About 1.2 L and 0.4 L of sterilized Sunshine LC1 RSi Professional Growing Mix growing medium was placed into each 15.2 and 11.5 cm container, respectively. A section of green bean (5 cm in length) was placed on the growing medium surface beside the inside rim of each container to provide a food source for western flower thrips adults that eclosed. Then, a pre-determined number of western flower thrips prepupae or pupae were randomly positioned on the growing medium surface.

Prepupae and pupae are generally located at a depth of 1 to 5 mm in the compost that was a freshly steam-sterilised mixture (50:50 by volume) of loam and medium grade sphagnum peat (Helyer et al. 1995). Furthermore, prepupae and pupae are distributed throughout the growing medium via cracks and crevices present on the growing medium surface (Yinping Li; personal observation). Thus, no additional growing medium was needed to cover the prepupae or pupae. One to two hours after western flower thrips prepupae or pupae were positioned on the growing

medium surface, a pre-determined number of newly eclosed rove beetle adults, based on the designated treatments, were released into each container. Only western flower thrips prepupae were used in experiment 2 based on the results from experiment 1 where predation efficacy of rove beetle adults was not significantly different between western flower thrips prepupae and pupae.

Each container was covered with No-Thrips insect screening to prevent rove beetle adults and western flower thrips adults from escaping. For each experimental round (day), a total of eighteen 15.2 cm containers for experiment 1, and nine 15.2 cm and nine 11.5 cm containers for experiment 2 were prepared and maintained in the laboratory at 19-25ºC, 50% to 60% relative humidity, and a 16:8 (light:dark) hour photoperiod. To prevent rove beetle adults to be captured on the yellow sticky cards prior to western flower thrips adult eclosion, yellow sticky cards were affixed onto the inside center of the No-Thrips insect screening within the containers five days after the experiment was initiated. One yellow sticky card was used for each 15.2 cm container and a half section of a yellow sticky card for each 11.5 cm container. Simultaneously, green beans were removed from the containers, and approximately 15 g of oats were placed on the growing medium surface as a food source for rove beetle adults to ensure adult survival after completing experiment 1. To maintain moisture, about 15 mL of water was applied to the oats through the No-Thrips insect screening using a 946 mL plastic spray bottle every one to two days. In experiment 2, no oats were placed on the growing medium surface after removing the green beans.

The numbers of western flower thrips adults and rove beetle adults captured on the yellow sticky cards were recorded 17 days after initiating the experiments. The number of western flower thrips adults captured on the yellow sticky cards was used as an indirect

assessment of predation efficacy, which in turn was quantified by the binomial probability of western flower thrips adults captured on the yellow sticky cards (refer to Data analysis).

To confirm rove beetle adult survival during the experiment, the growing medium in the 15.2 cm containers was used to determine the number of rove beetle adults recovered in experiment 1. After experiment 1 was completed, the growing medium in each 15.2 cm container was placed into a 9.4 L plastic rectangular container [40.2 x 26.4 x 12.8 cm (length x width x height)] (Rubbermaid Home Products; Wooster, OH). About 0.8 L of water was added to each 9.4 L plastic rectangular container to saturate the growing medium, causing rove beetles to emerge from the growing medium (Yinping Li; personal observation). Then, approximately 15 g of oats were placed on the growing medium surface as a food source for rove beetle adults. The 9.4 L plastic rectangular containers were covered using modified lids with insect screening (mesh size: 0.2 x 0.8 mm) (Greentek®; Edgerton, WI). The number of rove beetle adults in each 9.4 L plastic rectangular container was checked 24 hours after the growing medium was saturated. Recovery of rove beetle adults at the end of the experiment was based on the number of rove beetle adults captured on yellow sticky cards and the number of rove beetle adults recovered from the growing medium. In experiment 2, the number of rove beetle adults recovered at the end of experiment was based on the number of rove beetle adults captured on yellow sticky cards.

Data Analysis

For each experiment, a generalized linear mixed model assuming a binomial distribution was fitted to the response variable defined as number of western flower thrips adults captured on the yellow sticky cards out of the initial number of one western flower thrips pupal stage in the

container. A logit link function was used to estimate the probability of western flower thrips adults captured on the yellow sticky cards, which was an indicator of predation efficacy.

In experiment 1, the linear predictor in the model included the fixed effects of western flower thrips pupal stage, predator-prey ratio, predator-prey number, as well as all two- and three-way interactions. In experiment 2, the fixed effects in the linear predictor consisted of predator-prey ratio, predator-prey number, searchable area, as well as all two- and three-way interactions. For both experiments, random effects associated with the linear predictor included the blocking effect of experimental round (day) and an effect of the container as the unit of observation, defined as the cross product of experimental round (day) and treatment combination. This specification was needed to account for over-dispersion in the data, as observed in preliminary analyses.

Over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. For the two experiments, the final model used for inference showed no evidence of over-dispersion. Estimation was conducted 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 PROC GLIMMIX procedure (SAS Institute 2012) implemented using Newton-Raphson with ridging as the optimization technique. Pairwise comparisons were conducted using Tukey-Kramer's or Bonferroni's adjustments, as appropriate in each case, to avoid inflation of type I error due to multiple comparisons.

Results

a. Assessment of baseline conditions for experiment 1

When no rove beetle adults were released, the mean percent western flower thrips adults captured on the yellow sticky cards ranged from 98.2% to 99.4% for prepupae and 89.2% to 97.9% for pupae (Appendix A). The high recovery of western flower thrips adults in the absence of rove beetle adults suggests that neither natural mortality of western flower thrips prepupae and pupae nor mortality caused by handling technique were contributing factors that inhibited prepupae and pupae from developing into adults. Furthermore, descriptive statistics of rove beetle adults recovered at the end of the experiment confirmed their general presence and survival during the experimental period (Table 3).

b. Effect of western flower thrips pupal stage, predator-prey ratio, and predator-prey number on predation efficacy of rove beetle adults (Experiment 1)

No significant two- or three-way interactions were detected among pupal stage, predator-prey ratio, and predator-prey number on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards (western flower thrips pupal stage x predator-prey ratio interaction: $F=0.73$; df=2, 66.02; $P=0.48$; western flower thrips pupal stage x predator-prey number interaction: $F=2.15$; df=2, 66.36; $P=0.12$; predator-prey ratio x predator-prey number interaction: $F=0.93$; df=4, 65.77; $P=0.45$; and western flower thrips pupal stage x predator-prey ratio x predator-prey number interaction: $F=0.23$; df=4, 65.75; $P=0.92$).

Furthermore, there was no evidence of any marginal differences between prepupae and pupae on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards ($F=0.00$; df=1, 66.65; $P=0.98$). However, a main effect of predator-prey ratio was

detected (F=7.25; df=2, 66.06; P=0.0014). Whereby, regardless of pupal stage, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly higher at the 1:5 predator-prey ratio [61.1% (48.5-72.4%)] [mean (95% confidence interval)] than the 1:10 [39% (28.1-51.2%)] (t=2.96, df=72, P=0.01) and 1:15 predator-prey ratio [34.7% (24.7-46.3%)] (t=3.62, df=69.07, P=0.002). However, the latter two predator-prey ratios were not significantly different from each other (t=0.65, df=59.06, P=0.80) (Figure 1).

A main effect of predator-prey number was also identified for the estimated mean probability of western flower thrips adults captured on the yellow sticky cards (F=3.99; df=2, 66.36; P=0.02). At 2 times the predator-prey number, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards [57% (44.3-68.8%)], was significantly higher than 3 [37.2% (26.6-49.3%)] (t=2.66, df=70.62, P=0.03) and 4 times [40.6% (30-52.3%)] (t=2.21, df=68.15, P=0.04). However, the latter two predator-prey numbers were not significantly different from each other (t=-0.49, df=61.39, P=0.88) (Figure 2).

c. Assessment of baseline conditions for experiment 2

When no rove beetle adults were released, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards ranged from 84.1% to 90.2% for the 11.5 cm containers and 87.3% to 94.2% for the 15.2 cm containers (For methodology refers to Appendix A). Moreover, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards without rove beetle adults suggests that neither natural mortality of western flower thrips prepupae and pupae nor mortality caused by handling technique were contributing factors inhibiting prepupae and pupae from developing into adults. Furthermore, descriptive statistics of rove beetle adults recovered at the end of the experiment confirmed their general presence and survival during the experimental period (Table 4).

d. Effect of predator-prey ratio, predator-prey number, and searchable area on predation efficacy of rove beetle adults (Experiment 2)

A significant three-way interaction was detected among predator-prey ratio, predator-prey number, and searchable area (F=3.21; df=4, 72; P=0.02). To explain this interaction, we evaluated the simple effects of searchable area on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards within combinations of predator-prey ratios and predator-prey numbers (Figure 3).

The estimated mean probability of western flower thrips adults captured on the yellow sticky cards, for the 1:5 predator-prey ratio under conditions of 4 times the predator-prey number, was significantly higher in the 15.2 cm containers than in 11.5 cm containers. However, this difference in searchable area was not siginificant when predator-prey numbers were 2 or 3 times (Figure 3A). In contrast, at the 1:10 predator-prey ratio, the effects of searchable area on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significant at 2 and 3 times, but not at 4 times the predator-prey number (Figure 3B). Finally, at the 1:15 predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards in the 15.2 cm containers was significantly higher than that in the 11.5 cm containers across all predator-prey numbers (Figure 3C).

Discussion

This is the first study to assess the effect of western flower thrips pupal stage, predator-prey ratio, predator-prey number, and searchable area on predation of western flower thrips by the rove beetle, *D. coriaria*. This study demonstrated that the estimated mean probability of western flower thrips captured on yellow sticky cards was higher for the 1:5 predator-prey ratio

than the 1:10 and 1:15 predator-prey ratios. In addition, the estimated mean probability of western flower thrips captured on yellow sticky cards was higher for 2 times the predator-prey number than 3 and 4 times the predator-prey numbers. Furthermore, a significantly higher estimated mean probability of western flower thrips adults was captured on the yellow sticky cards in the 15.2 cm than 11.5 cm containers.

Exposure time of prey to a predator may influence predation efficacy. The exposure time of western flower thrips prepupae to rove beetle adults is longer (about one day) than the pupae (Zhang et al., 2007). However, in this study, the results did not confirm the first hypothesis as predation efficacy of rove beetle adults on western flower thrips prepupae was not significantly different from the pupae. It is possible that western flower thrips prepupae are active, whereas pupae are inactive; consequently, the prepupae may avoid rove beetle adult predation by striking rove beetle adults with their abdomen (Yinping Li; personal observation). Thus, rove beetle adults may spend more time searching for and engaging western flower thrips prepupae than pupae. Therefore, exposure time associated with the two western flower thrips pupal stages may not affect the predation efficacy of rove beetle adults.

Initial pest density may influence predation efficacy of a predator at a given predator-prey ratio (Echegaray et al., 2015). For instance, when *D. coriaria* adults were released at a 1:5 (*D. coriaria* adults:fungus gnat larvae) predator-prey ratio to suppress fungus gnat larval populations, percent fungus gnat adults captured on yellow sticky cards decreased significantly from 59% to 37.5% as the initial number of fungus gnat larvae increased from 10 to 20 (Echegaray et al., 2015). Similarly, results from experiment 1 (prepupa) of Chapter 3 indicated that, at a 1:15 predator-prey ratio, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards decreased significantly from 68.7% to 33.4% when the initial

number of western flower thrips prepupae increased from 15 to 30. In experiment 3 (prepupa-pupa combination) of Chapter 3, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards at the 1:10 predator-prey ratio decreased significantly from 38.7% at an initial number of 20 western flower thrips pupal combination to 7.5% at an initial number of 30 western flower thrips pupal combination. In this study, the results did not support the third hypothesis as the estimated mean probability of western flower thrips adults captured on yellow sticky cards decreased significantly when the predator-prey number increased from 2 times to 3 times predator-prey number, but was not significantly different when the predator-prey number increased from 3 times to 4 times the predator-prey number. It is possible that searching ability or/and handling proficiency improved when the predator-prey number increased from 2 times to 3 times, but did not change when predator-prey number increased from 3 times to 4 times.

Searchable area may affect the predation efficacy of *D. coriaria* on soil-dwelling prey, such as fungus gnat larvae (Echegaray et al., 2015; Herrick and Cloyd, 2017). Herrick and Cloyd (2017) proposed that predation of *D. coriaria* adults on fungus gnat larvae may be negatively affected by a greater searchable area associated with the 15.2 cm containers (1,834.82 cm^3 with 2.0 L of growing medium) used in the greenhouse experiment compared to the smaller searchable area of 473 mL deli containers (616.14 cm^3 with 0.3 L of growing medium) used in the laboratory experiment. In this study, results supported the fourth hypothesis with predation efficacy of rove beetle adults on western flower thrips prepupae in the 15.2 cm containers being significantly lower than in the 11.5 cm containers.

Evaluating an appropriate predator-prey ratio based on predation efficacy may enhance the success of an augmentative biological control program (Hamlen and Lindquist, 1981;

Gaudchau, 1982; Opit et al., 2004; Cheng et al., 2012; Echegaray et al., 2015; Amoah et al., 2016). For example, Cheng et al. (2012) found that the predator, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) suppressed populations of both *Tetranychus kanzawai* (Kishida) (Acari: Tetranychidae) and *Panonychus citri* (McGregor) (Acari: Tetranychidae) on papaya (*Carica* papaya L.) at a predator:prey ratio of 1:15 or greater. Moreover, effective suppression was achieved at a predator:prey ratio of 1:10 when using the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) against the twospotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) on lima bean (*Phaseolus lunatus* L.) plants (Amoah et al., 2016). In experiments 2 (pupa) and 3 (prepupa-pupa combination) of Chapter 3, predation efficacy was not significantly different among the three predator-prey ratios (1:5, 1:10, and 1:15). Furthermore, in this study, results partially supported the second hypothesis as predation efficacy at the 1:15 predator-prey ratio was significantly higher than the 1:5 ratio, but not significantly different from the 1:10 ratio. It is likely that mutual interference may have taken place at the predator-prey ratios of 1:5, but not 1:10 or 1:15. Since compared to the 1:10 and 1:15 ratios, there were less prey available for each rove beetle adult at the 1:5 ratio and the competition may have been greater among rove beetle adults. The results from Chapters 3 and this study provide evidence that predation efficacy was not enhanced as the predator-prey ratio increased from 1:15 to 1:5. However, at the same initial prey number, fewer rove beetle adults need to be released at the predator-prey ratio of 1:15 compared to 1:10 and 1:5. Therefore, the 1:15 predator-prey ratio may be a viable recommendation for greenhouse producers.

Figures and Tables
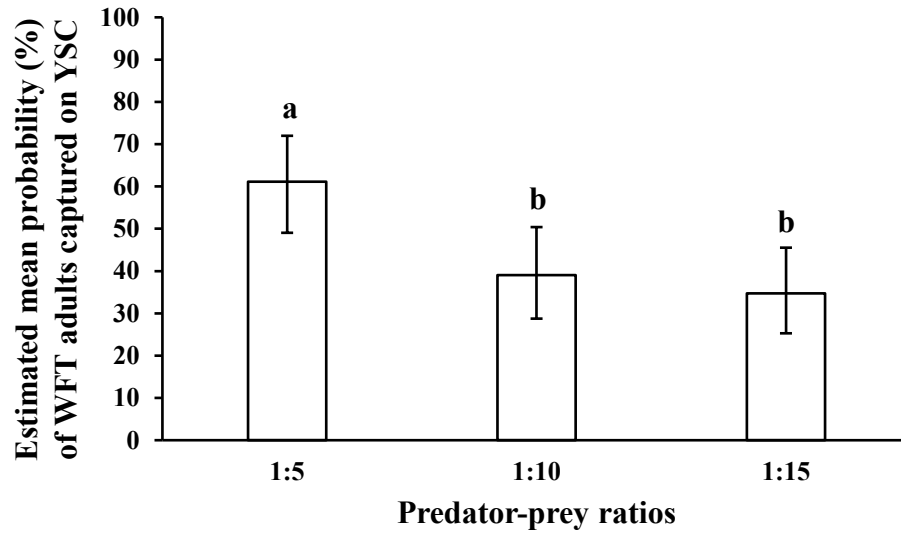
Figure 4-1 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis***, adults captured on yellow sticky cards (YSC) associated with three predator-prey ratios (1:5, 1:10, and 1:15) in experiment 1. Estimated means followed by different letters indicate significant differences (***P*<0.05) among the three predator-prey ratios.**
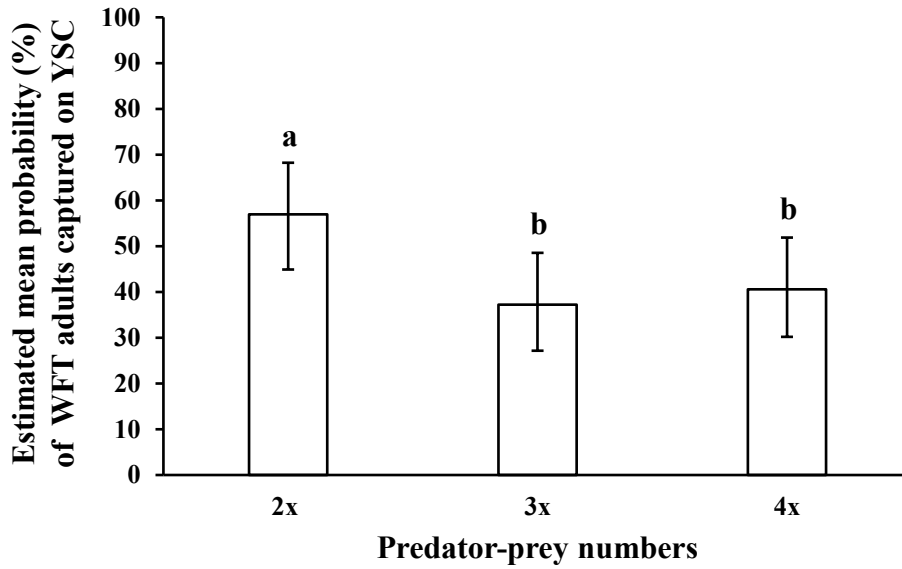
Figure 4-2 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, adults captured on yellow sticky cards (YSC) associated with three predator-prey numbers (2, 3, and 4 times or x) in experiment 1. Estimated means followed by different letters indicate significant differences (*P*<0.05) among the three predator-prey numbers.

A. 1:5 predator-prey ratio
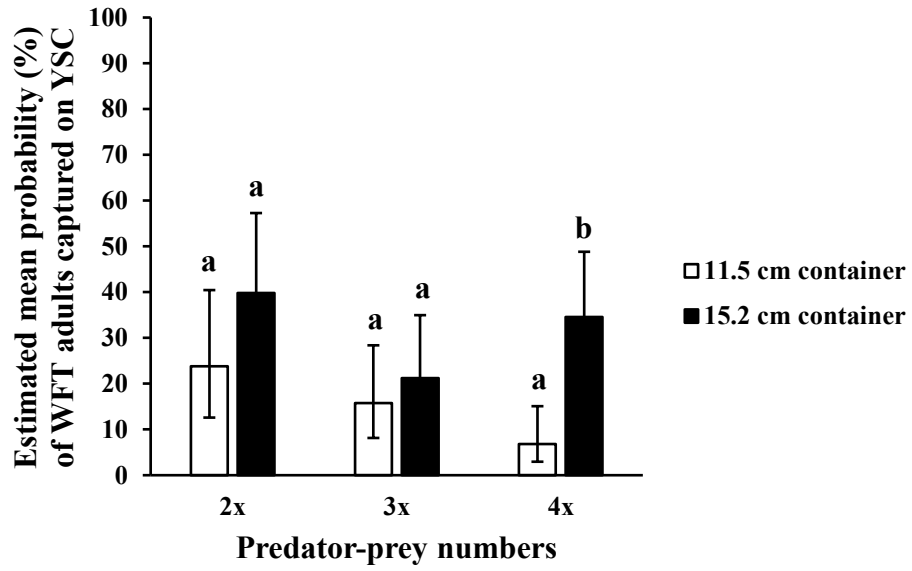
B. 1:10 predator-prey ratio
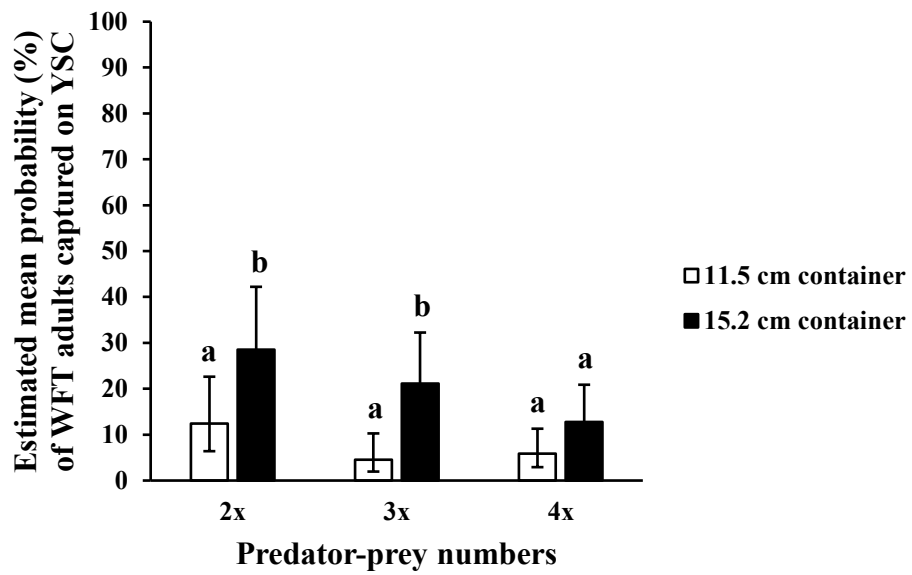
C. 1:15 predator-prey ratio
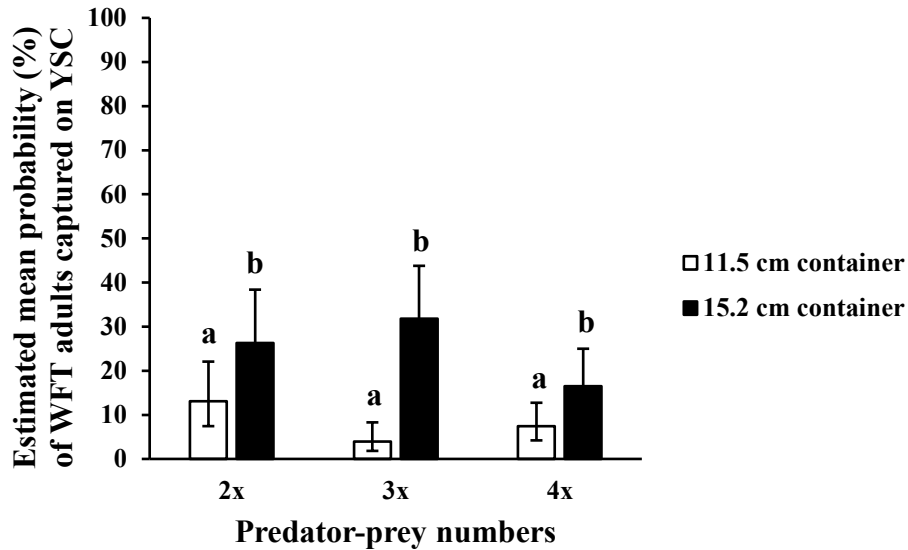
Figure 4-3 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis***, adults captured on yellow sticky cards (YSC) for two searchable areas [11.5 cm containers (701.79 cm^3 with 0.4 L of growing medium) and 15.2 cm containers (1,834.82 cm^3 with 1.2 L of growing medium)] within all combinations of three predator-prey ratios (1:5, 1:10, and 1:15) and three predator-prey numbers (2, 3, and 4 times or x) in experiment 2. Estimated means followed by different letters within the same predator-prey ratio and the same predator-prey number indicate significant differences (*P*<0.05) between the two searchable areas.**

Table 4-1 Number of rove beetle (RB), *Dalotia coriaria*, **adults released and initial number of western flower thrips (WFT),** *Frankliniella occidentalis*, **prepupae (PP) or pupae (P) in each 15.2 cm container on each day (1, 2, 3, 4, or 5) for experiment 1.**

Predator-prey numbers	Predator-prey ratios					
	1:5		1:10		1:15	
	RB	WFT PP/P	RB	WFT PP/P	RB	WFT PP/P
2	2	10	2	20	2	30
3	3	15	3	30	3	45
4	4	20	4	40	4	60

Table 4-2 Number of rove beetle (RB), *Dalotia coriaria*, adults released and initial number of western flower thrips (WFT), *Frankliniella occidentalis*, prepupae (PP) in each 15.2 cm container (1,834.82 cm^3 with 1.2 L of growing medium) or 11.5 cm container (701.79 cm^3 with 0.4 L of growing medium) on each day (1, 2, 3, 4, or 5) for experiment 2.

Predator-prey numbers	Predator-prey ratios					
	1:5		1:10		1:15	
	RB	WFT PP	RB	WFT PP	RB	WFT PP
2	2	10	2	20	2	30
3	3	15	3	30	3	45
4	4	20	4	40	4	60

Table 4-3 Mean (minimum, maximum) number of rove beetle, *Dalotia coriaria*, adults recovered at the end of experiment 1 associated with all treatment combinations of two western flower thrips (WFT), *Frankliniella occidentalis*, pupal stages [prepupae (PP) and pupae (P)], three predator-prey ratios (1:5, 1:10, and 1:15), and three predator-prey numbers (2, 3, and 4 times).

| Predator-prey numbers | Predator-prey ratios | | | | | |
| | 1:5 | | 1:10 | | 1:15 | |
	WFT PP	WFT P	WFT PP	WFT P	WFT PP	WFT P
2	1.4 (1, 2)	1.6 (0, 2)	2.0 (2, 2)	1.6 (1, 2)	1.6 (1, 5)	1.4 (1, 2)
3	1.8 (1, 2)	2.0 (1, 3)	2.6 (2, 3)	1.8 (0, 3)	2.2 (2, 3)	2.4 (0, 3)
4	2.8 (0, 4)	3.6 (3, 4)	2.4 (1, 4)	3.0 (2, 4)	2.6 (0, 4)	3.4 (2, 4)

Table 4-4 Mean (minimum, maximum) number of rove beetle, *Dalotia coriaria*, adults captured on the yellow sticky cards at the end of experiment 2 associated with all treatment combinations of two searchable areas [11.5 cm containers (701.79 cm^3 with 0.4 L of growing medium) and 15.2 cm containers (1,834.82 cm^3 with 1.2 L of growing medium)], three predator-prey ratios (1:5, 1:10, and 1:15), and three predator-prey numbers (2, 3, and 4 times).

Predator-prey numbers	Predator-prey ratios					
	1:5		1:10		1:15	
	11.5 cm containers	15.2 cm containers	11.5 cm containers	15.2 cm containers	11.5 cm containers	15.2 cm containers
2	0.5 (0, 1)	1.2 (0, 2)	1.2 (0, 2)	1.2 (0, 2)	1.7 (1, 2)	1.0 (0, 2)
3	1.5 (0, 3)	2.5 (1, 3)	1.2 (0, 3)	1.7 (0, 2)	1.6 (1, 3)	1.2 (0, 3)
4	1.8 (0, 4)	1.3 (0, 2)	2.2 (1, 4)	1.8 (0, 4)	1.8 (1, 3)	1.7 (1, 3)

Chapter 5 - Effect of integrating the entomopathogenic fungus, *Beauveria bassiana*, and the rove beetle, *Dalotia coriaria*, in suppressing western flower thrips, *Frankliniella occidentalis*, populations under greenhouse conditions

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest of horticultural crops worldwide (Robb and Parrella 1995, Lewis 1997, Kirk 2002, Cloyd 2009). Western flower thrips cause direct and indirect plant damage (Chisholm and Lewis 1984, Hunter and Ullman 1989, Harrewijn et al. 1996, Pappu et al. 2009) that has resulted in substantial economic losses (Goldbach and Peters 1994, Reitz and Funderburk 2012). Therefore, greenhouse producers regularly apply insecticides to suppress western flower thrips populations (Kontsedalov et al. 1998, Loughner et al. 2005, Cloyd 2009).

However, due to the intensive selection pressure associated with insecticide applications, western flower thrips populations have developed resistance to a broad range of insecticides in different chemical classes (Brødsgaard 1994, Jensen 2000, Bielza et al. 2007, Kay and Herron 2010). Therefore, alternative plant protection strategies are warranted, such as augmentative biological control that involves releasing or applying natural enemies (e.g. predators, parasitoids, or entomopathogenic fungi) to suppress pest populations (Cloyd 2009, Reitz 2009). Furthermore, there is a general trend in combining entomopathogenic microorganisms, such as; bacteria and fungi, with arthropod natural enemies to target different pest life stages (Gonzalez et al. 2016, Mouden et al. 2017, Ullah and Lim 2017).

Entomopathogenic fungi, including a commercially available entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, are viable alternatives to conventional insecticides as entomopathogenic fungi can be mass-produced, formulated, and applied using conventional equipment (Mendonça 1992, Shah and Pell 2003). Entomopathogenic fungi tend to have minimal direct effects on non-target organisms (Goettel et al. 1990, Goettel and Hajek 2000, Pell et al. 2001). Previous studies have demonstrated that *B. bassiana* may be effective in managing western flower thrips populations (Ugine et al. 2007, Ansari et al. 2008, Skinner et al. 2012, Kivett et al. 2016).

The soil-dwelling predatory rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), has been shown, primarily under laboratory conditions, to prey upon soil-dwelling life stages (prepupae and pupae) of western flower thrips, and the larval stages of shore flies (*Scatella* spp.) and fungus gnats (*Bradysia* spp.) (Gillespie et al. 2001, Carney et al. 2002, Cox et al. 2006, Jandricic et al. 2006, Echegaray et al. 2015). *Dalotia coriaria* is commercially available from many biological control suppliers (Jandricic et al. 2005, Warner and Getz 2008). Therefore, *D. coriaria* could be a feasible biological control agent of western flower thrips prepupae and pupae in the growing medium.

Previous studies have concentrated on combining different arthropod natural enemies or integrating arthropod natural enemies with entomopathogens or entomopathogenic nematodes to target one or more western flower thrips life stage (Ebssa et al. 2006, Manners et al. 2013, Messelink and Janssen 2014, Wu et al. 2016). However, foliar applications of the entomopathogenic fungus, *B. bassiana* along with the soil-dwelling rove beetle, *D. coriaria* targeting aboveground and soil-dwelling life stages of western flower thrips have not been investigated. In this study, *B. bassiana* was used against western flower thrips larvae and adults

on plants, and *D. coriaria* was used against the prepupae and pupae that reside in the growing medium.

Based on previous studies, three hypotheses were developed. First, the final foliage damage rating for the *B. bassiana* and rove beetle combination should not be significantly different from the insecticide treatment. The reason for this is that the *B. bassiana* and rove beetle combination treatment, and insecticide treatment, should provide a similar level of suppression of western flower thrips populations. Second, the *B. bassiana* and rove beetle combination treatment should provide greater suppression of western flower thrips populations than *B. bassiana* and rove beetle individual treatments. Because synergistic (mortality greater than the sum of mortality that the individual provides) are expected to occur when using the *B. bassiana* and rove beetle combination. Third, the *B. bassiana* and rove beetle combination treatment should cost no more than the insecticide treatment.

Therefore, the objectives of this study were to 1) ascertain the effectiveness of using the entomopathogenic fungus, *B. bassiana* in conjunction with the rove beetle, *D. coriaria*, to suppress western flower thrips populations under greenhouse conditions; and 2) compare the direct costs associated with products and application rates.

Materials and Methods

a. Insect colonies

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size:

0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days.

A rove beetle colony was maintained using growing medium in 7.6 L plastic containers [34.8 x 24.7 x 12.4 cm (length x width x height] (Rubbermaid Home Products; Wooster, OH) under laboratory conditions: 20-24ºC, 45% to 60% relative humidity, and constant darkness simulating conditions similar to the natural habitat of rove beetles (Helyer et al. 2003). A rectangular section (22 x 11 cm: length x width) was cut in the lid and covered with No-Thrips insect screening.

Growing medium preparation was as follows: a 6.0 L plastic container [28.5 x 11 cm (diameter x height)] (Rubbermaid Home Products; Wooster, OH) was filled with Sunshine LC1 RSi Professional Growing Mix (SunGro Horticulture, Inc.; Bellevue, WA) growing medium consisting of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone. The growing medium was moistened with approximately 200 mL of water. The 6.0 L plastic container with growing medium was then heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.8 L of water was applied to the growing medium, which was then thoroughly mixed. About 3.0 L of the sterilized growing medium was placed into each 7.6 L plastic rectangular container.

Approximately 15 g of dry oats (*Avena sativa* L.) (The Quaker Oats Company; Chicago, IL) were placed, every four to five days, onto the growing medium surface in a line (lengthwise) in the center of the growing medium within each 7.6 L plastic rectangular container. About 15

mL of water was applied, every one to two days, onto the oats using a 946 mL plastic spray bottle (Delta Industries; King of Prussia, PA) to maintain constant moisture. Adult rove beetles from previous colonies were then used to establish new colonies.

Western flower thrips and rove beetle specimens used in this study are deposited as voucher numbers 237 and 220, respectively in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Preparation of newly eclosed rove beetle adults

Five rove beetle third instar larvae were placed into a Gladware® container [7.8 x 5.1 cm (diameter x height)] (The Glad Products Company; Oakland, CA) with 20 mL of moistened growing medium and three to four pieces of dry oats. A total of 20 Gladware® containers with rove beetle third instar larvae were prepared following the above method, then placed into an environmental growth chamber (Conviron® Controlled Environments Inc.; Pembina, ND) set at 21-27ºC and constant darkness. Rove beetle adults were observed seven days later with most adults eclosing from pupae after 11 days (Yinping Li; personal observation). Newly eclosed adults (one to three days old) were individually placed in 9 dram (33 mL) plastic vials with lids using a soft-bristled brush. All 9 dram plastic vials containing rove beetle adults were returned to the environmental growth chamber and the adults were starved for 24 hours. The sex ratio of rove beetle adults was 1:1 (female:male).

c. Experimental procedures

Two experiments were conducted in a glass-covered greenhouse at the Kansas State University Throckmorton Plant Sciences Center (Manhattan, KS). The difference between the two experiments was the number of western flower thrips adults initially released onto each

plant: 20 for experiment 1 and 40 for experiment 2. Each experiment was replicated twice to take into account any effect of season related to temperature, relative humidity, light intensity, and photoperiod (day length). Herein, each repetition of an experiment was designated a trial, with each trial conducted for eight weeks. For experiment 1, trial 1.1 was conducted from June 20 to August 15, 2016 (summer) and trial 1.2 from October 14 to December 9, 2016 (fall). For experiment 2, trial 2.1 was conducted from March 17 to May 12, 2017 (spring) and trial 2.2 from September 22 to November 17, 2017 (fall).

In each trial, 35 chrysanthemum, [*Dendranthema* x *grandiflorum* (Ramat.) Kitam.], plants were purchased from greenhouse producers in containers [9.4 x 8.7 cm (diameter x height)] (Dillen Products Inc; Middlefield, OH). Chrysanthemum was used for the experiments due to availability, length of flowering time (Post 1949), and susceptibility to western flower thrips (De Jager 1995a, De Jager 1995b). The chrysanthemum plants (cultivar: 'Paradiso Yellow') for the first two trials (1.1 and 1.2) were obtained from 5-H Greenhouse (Wamego, KS). For the last two trials (2.1 and 2.2), the plants (cultivars: 'Bloomfield Yellow' and 'Honeybush Yellow,' respectively) were obtained from Neosho Gardens (Council Grove, KS). The flower color of all the chrysanthemum plants was yellow. Each plant was repotted into a 15.2 cm diameter container [15.2 x 14.3 cm (diameter x height)] (Dillen Products Inc; Middlefield, OH) using Sunshine LC1 RSi Professional Growing Mix growing medium. For trials 1.1 and 1.2, initial plant height was 12.8 ± 0.4 cm (mean $\pm$ SEM) and 13.7 ± 0.2 cm, respectively. For trials 2.1 and 2.2, initial plant height was 17.9 ± 0.3 cm and 12.9 ± 0.2 cm, respectively.

Plants were randomized and individually placed into 35 clear plastic observation 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) covered with No-Thrips insect screening: one hole on the lid and two holes on opposite sides of the cage. These openings allowed for ventilation but prevented western flower thrips and rove beetle adults from escaping.

Each plant was checked every two days to confirm whether water was needed. Water was applied directly to the growing medium to avoid getting the foliage wet and dislodging western flower thrips. Old senescing flowers were removed and placed beside the plant inside the cage so that any western flower thrips larvae or adults residing within the flowers could return to the plant. On week four, all plants were fertilized with 200 mL of a 3.7 g/L fertilizer solution [Miracle-Gro 24-3.5-13.2 (N-P-K) Water Soluble All-Purpose Plant Food] (Scotts Miracle-Gro Products, Inc.; Marysville, OH).

Twenty (experiment 1) or 40 (experiment 2) western flower thrips adults [one to four days post-eclosion, mixture of 9:1 (female:male)] (Kivett et al., 2015), were collected from the laboratory colony into a 9 dram plastic vial with a lid using an aspirator, and then released onto the leaves of each of the 35 chrysanthemum plants. Afterward, each 9 dram plastic vial was placed horizontally on the growing medium surface in the event that some western flower thrips adults were still present in the 9 dram plastic vial. All the 9 dram plastic vials were collected the day after releasing western flower thrips adults. Western flower thrips populations were allowed to establish on the plants for two weeks prior to application of the treatments.

To account for potential temperature gradients and light intensity differences within the greenhouse, as well as to accommodate space constraints, each trial was set-up as a randomized block design. Observation cages, each containing an individual plant, were organized in eight location blocks within the greenhouse. Each of six blocks was comprised of five cages, one per

treatment within each block. The other two blocks consisted of three and two cages, respectively, one per each of the treatments. (Figure 1)

In both experiments, treatments consisted of: A) insecticides (described below), B) *B. bassiana*, C) *D. coriaria*, D) *B. bassiana* and *D. coriaria* combination, and E) water control. For treatment A, the insecticides and rates used, and application scheme were: spinosad (Conserve® SC: Dow AgroSciences; Indianapolis, IN) at 6.0 fl oz/100 gallons (0.44 mL/946 mL) for weeks one (week one: the week when the first treatment application was conducted) and two, pyridalyl (Overture® 35 WP: Valent U.S.A. Corp.; Walnut Creek, CA) at 8.0 oz/100 gallons (0.57 g/946 mL) for weeks three and four, chlorfenapyr (Pylon®: BASF Corp.; Research Triangle Park, NC) at 5.2 fl oz/100 gallons (0.38 mL/946 mL) for weeks five and six, and abamectin (Avid® 0.15 EC: Syngenta Crop Protection, Inc.; Greensboro, NC) at 8.0 fl oz/100 gallons (0.59 mL/946 mL) for weeks seven and eight. The entomopathogenic fungus used in treatments B and D was *B. bassiana* Strain GHA (BotaniGard® 22WP: BioWorks Inc.; Victor, NY) applied at 1 lb/100 gallons (1.13 g/946 mL) each week over the eight-week period.

Application rates of the insecticides and *B. bassiana* were all based on label rates for western flower thrips. For treatments C and D, five rove beetle adults (one to three days post-eclosion and starved for 24 hours) were released onto the growing medium surface of each 15.2 cm container when the first treatment application was made, with no additional releases afterward. Product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides and *B. bassiana* are presented in Table 1. The treatment application scheme over the eight-week period is presented in Table 2.

Each treatment solution was applied by removing one plant from a cage, placing on a concrete block, then spraying the plant using a 946 mL plastic spray bottle. Approximately 30

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mL of spray solution was applied to each plant, which was a sufficient volume to thoroughly cover all plant parts, including leaves (lower and upper sides) and flowers, with minimal run-off. To avoid any cross contamination, each plant was sprayed individually and returned to the cage before the next plant was removed from its cage for treatment. Latex gloves (Fisher Scientific; Pittsburgh, PA) were worn to avoid exposure to any insecticide or *B. bassiana* residues on the chrysanthemum plants. Due to photosensitivity of *B. bassiana*, the treatment applications were conducted one hour before sunset to minimize exposing *B. bassiana* conidia to sunlight (Braga et al. 2001, Braga et al. 2002, Cory and Hoover 2006). For treatment D in the first application, the *B. bassiana* spray solution was applied to the plants, then five rove beetle adults were released onto the growing medium surface to avoid exposing rove beetle adults to direct sprays of the *B. bassiana* solution. After completing each trial, all the clear plastic observation cages were thoroughly cleaned to avoid any carry-over effects associated with insecticide or *B. bassiana* residues.

A yellow sticky card [7.7 x 10.4 cm (length x width)] (Pestrap Phytotronics, Inc.; Earth City, MO) was held in place vertically by a wooden clothes pin attached to a bamboo stake. The bamboo stake was supported by inserting it into a circular plastic container (250 mL) filled with sand. One circular plastic container was placed into each clear plastic observation cage, next to the plant, with the yellow sticky card positioned 5 cm above the plant canopy (Gillespie and Vernon 1990, Heinz et al. 1992, Brødsgaard 1993) two hours before the first treatment application was conducted. Only the membranous film on one side of a yellow sticky card was removed, and the sticky side was positioned toward the plant. After one week, but before the next treatment was applied, the number of western flower thrips adults captured on the sticky side of the yellow sticky card was recorded. Then, the membranous film on the other side of the

same yellow sticky card was removed and evenly placed on the used side of the yellow sticky card. The new sticky side was positioned toward the plant the following week. Yellow sticky cards were replaced every two weeks during the eight-week period.

The number of western flower thrips adults captured on the yellow sticky cards was recorded the following week after each treatment application. On the final assessment day, all plants were rated for foliage and flower feeding damage, based on a 1 to 5 rating scale: 1=no visible damage, 2=1% to 25% damage, 3=26% to 50% damage, 4=51% to 75% damage, and 5>75% damage. The damage rating scale was modified from Cloyd and Cycholl (2002) and used to quantify plant damage due to western flower thrips feeding. Since all flowers had senesced, and were removed after completing each trial, final flower quality ratings were not determined.

After completing each trial, the number of rove beetle larvae and adults in the growing medium of each 15.2 cm container associated with treatments C (*D. coriaria*) and D (*B. bassiana* and *D. coriaria* combination) was recorded. In trial 1.1 (summer 2016), the aboveground plant parts (leaves and stems) in treatments C and D were excised at the growing medium level. A yellow sticky card was affixed onto the inside center of the No-Thrips insect screening. Then each 15.2 cm container was covered by the No-Thrips insect screening with a yellow sticky card inside to capture rove beetle adults. Furthermore, to capture rove beetle adults emerging from the drainage holes located in the bottom of the 15.2 cm containers, each 15.2 cm container was placed on one yellow sticky card, with the sticky surface facing upward. No rove beetle larvae or adults were recovered from trial 1.1 using the above method. Therefore, the numbers of rove beetle larvae and adults in the next three trials were determined using a different method (described below).

To confirm rove beetle adult survival during the experiment, the growing medium of each 15.2 cm container in treatments C and D involving rove beetle adults was used to determine the number of rove beetle adults recovered at the end of experiments. The growing medium from each 15.2 cm container in treatments C and D was placed into a 9.4 L plastic rectangular container [40.2 x 26.4 x 12.8 cm (length x width x height)] (Rubbermaid Home Products; Wooster, OH). About 0.8 L of water was added to each 9.4 L plastic rectangular container to saturate the growing medium, causing rove beetles to emerge from the growing medium (Yinping Li; personal observation). Then, approximately 15 g of oats were placed on the growing medium surface as a food source for rove beetle larvae and adults. Each 9.4 L plastic rectangular container had a rectangular section (22 x 11 cm: length x width) cut in the lid, which was covered with different No-Thrips insect screening (mesh size: 0.2 x 0.8 mm) (Greentek®; Edgerton, WI). The numbers of rove beetle larvae and adults in each 9.4 L plastic rectangular container were recorded 24 hours after the growing medium was saturated.

Environmental data, including: temperature, relative humidity, and light intensity, were recorded using HOBO® Data Loggers (Onset Computer Corporation; Bourne, MA). Eight data loggers were used in each trial. Each of two wire-screen benches was evenly divided into two sections widthwise with two data loggers per section. In each section, one data logger was positioned on the center half of the wire-screen bench, and the other data logger was placed inside the cage located in the central half of the wire-screen bench (Figure 1). A summary of the environmental conditions for each trial is presented in Table 3.

d. Cost comparisons

The cost of the eight-week treatment applications associated with the products and application rates was determined. The costs of the insecticides and BotaniGard® 22WP were

based on the 2016-2017 Hummert International Commercial Catalog (Hummert International; Earth City, MO) (Kivett et al. 2015). The labeled application rates of the insecticides and BotaniGard® 22WP for western flower thrips were utilized to calculate the cost of each associated treatment application per week based on 100 gallons of spray solution. The cost of *D. coriaria*, which were obtained from our colonies, would cost $0.75 per application for five adults (https://greenmethods.com/product/atheta-coriaria-rove-beetle-adults/; assessed on November 15, 2017). However, greenhouse producers, in general, can only purchase quantities of 500 or more from commercial suppliers with the cost of 500 rove beetles being $78.63 (excluding shipping and handling costs) (2018 Biobest Catalog: Biobest Canada Ltd.; Leamington, ON, Canada). Therefore, we used the cost per 500 to determine the total cost over eight weeks.

Data Analysis

For experiment 1, data from trials 1.1 (summer 2016) and 1.2 (fall 2016) were analyzed separately due to convergence problems. In both trials, the number of western flower thrips adults captured on the yellow sticky cards was analyzed using a generalized linear mixed model that assumed a negative binomial distribution of the response with a log link function. The linear predictor in the model included the fixed effects of treatment, week, and the two-way interaction. Random effects in the linear predictor included location block and the cross product of location block and treatment to recognize plant as the experimental unit for treatment. For the model fitted to data from trial 1.2, the variance component for the random effect of location block converged to zero and thus this random effect was removed from the model. Analysis of Variance skeleton regarding the response variable of the number of western flower thrips adults captured on the yellow sticky cards for trial 1.1 (summer 2016) and trial 1.2 (fall 2016) was the same and is presented in Table 4.

For experiment 2, data on the number of western flower thrips adults captured on yellow sticky cards for trials 2.1 (spring 2017) and 2.2 (fall 2017) was analyzed jointly using a generalized linear mixed model with a poisson distribution. The linear predictor in the model included the fixed effects of season, treatment, week, as well as all two- and three-way interactions. Random effects associated with the linear predictor included location block nested within each season, its cross product with treatment (to properly identify the experimental unit of treatment) and the cross product of block, treatment and week (to account for over-dispersion at the level of observation). Analysis of Variance skeleton regarding the response variable of the number of western flower thrips adults captured on the yellow sticky cards for experiment 2 is presented in Table 5.

For each experiment, final foliage quality ratings were analyzed using a generalized linear mixed model that recognized the multinomial distribution of the response fitted with a cumulative logit link function. The linear predictor of the statistical model included the fixed effects of season, treatment, and the two-way interaction. The random effect in the linear predictor included location block nested in each season. Analysis of Variance skeleton reagarding the response variable of the final foliage quality ratings for experiments 1 and 2 was the same and is presented in Table 6.

In both experiments, over-dispersion was evaluated using the maximum-likelihood based fit statistic Pearson Chi-Square/DF and there was no evidence that over-dispersion was apparent in the models used for final inference. All statistical models used for inferences were fitted using the GLIMMIX procedure (SAS Institute 2012). Pairwise comparisons were conducted using Bonferroni adjustment to avoid inflation of type I error due to multiple comparisons.

Results

Experiment 1. The environmental conditions during trials 1.1 (summer) and 1.2 (fall) in 2016 are presented in Table 3. In trial 1.1, there was a significant interaction between treatment and week (F=2.34; df=28, 210; P=0.0004). The estimated mean number of western flower thrips adults captured on the yellow sticky cards for treatment A was significantly lower than those of treatments B and D at weeks five and six, as well as that of treatments D and E at week seven. However, after eight-weeks, there were no significant differences among the treatments (Table 7). In trial 1.2, there was no evidence of a significant interaction between treatment and week (F=1.43; df=28, 210; P=0.083), nor was there any main effect of treatment (F=2.56; df=4, 30; P=0.059). However, a main effect of week was significant (F=4.33; df=7, 210; P=0.0002) (Table 8).

For the final foliage quality ratings in experiment 1, there was no significant interaction between season and treatment (F=1.09; df=4, 33; P=0.38), nor any evidence of season differences (F=0.00; df=1, 14; P=0.97) or treatment effects (F=0.68; df=4, 33; P=0.61). Overall, the final foliage quality rating for 92% (n=70) of the plants in experiment 1 was 1 (=no visible damage) or 2 (=1% to 25% damage).

Experiment 2. The environmental conditions associated with trials 2.1 (spring) and 2.2 (fall) in 2017 for experiment 2 are presented in Table 3. There was no evidence of significant interactions among season, treatment, and week (F=1.18; df=28, 420; P=0.24); between treatment and week (F=1.30; df=28, 420; P=0.14); or between season and treatment (F=1.98; df=4, 46; P=0.11). A significant season x week interaction (F=29.83; df=7, 420; P<0.0001) was

identified, along with a main effect of treatment (F=4.45; df=4, 46; P=0.004). Therefore, the treatment effects over the eight-week period were evaluated within each season.

In trial 2.1 (spring 2017), the estimated mean number of western flower thrips adults captured on yellow sticky cards at week two was significantly lower in treatment D than that of treatments B and C. There were significantly fewer western flower thrips adults captured on the yellow sticky cards for treatment A than treatments B and E at week four; than treatments B, C, and E at week five; than treatments B and C at week six; as well as than treatments B, C, D, and E at week eight (Table 9). In trial 2.2 (fall 2017), the estimated mean number of western flower thrips adults captured on the yellow sticky cards was significantly lower for treatment A compared to: treatment D at week four; treatments B and D at week five; treatments B, C, D, and E at week six; and treatment B at weeks seven and eight (Table 10).

For the final foliage quality ratings in experiment 2, there was no significant treatment x season interaction (F=0.06; df=3, 43; P=0.98). The final foliage quality ratings were significantly different among the five treatments (F=3.60; df=4, 43; P=0.013), but not between the two seasons (F=0.00; df=1, 14; P=0.95). However, the final foliage quality ratings were significantly different only between treatments D and E. Overall, the final foliage quality rating for 69% (n=70) of the plants was 5 (>75% damage).

Rove beetle recovery. Descriptive statistics for the number of rove beetle larvae and adults recovered from the growing medium in each 15.2 cm container for treatments C (*D. coriaria*) and D (*B. bassiana* and *D. coriaria* combination) after completing each trial are presented in Table 11. Rove beetle larvae and adults were recovered from the growing medium for treatments C and D in trials 1.2, 2.1, and 2.2. The mean number of rove beetle adults recovered from the growing medium in treatments C (n=24) and D (n=23) in trial 1.2 was almost

five-fold the number of rove beetle adults initially released (n=5), which confirms that rove beetle adults released not only survived, but also reproduced and established populations during the experimental period (Table 11).

Cost comparisons. The total application cost for each eight-week treatment is presented in Table 12. The insecticide treatment would cost greenhouse producers about $963.50 to spray 100 gallons of insecticide solution once a week for eight weeks. In contrast, using *B. bassiana* (BotaniGard®) in conjunction with *D. coriaria* adults would save about $468 compared to the insecticide treatment ($495.67 vs. $936.50, respectively). There was a $78.63 cost difference between the individual *B. bassiana* treatment, and *B. bassiana* and *D. coriaria* combination ($417.04 vs. $495.67, respectively). The cost of the *D. coriaria* only treatment would be $78.63 (excluding shipping and handling) (Table 12).

Discussion

This is the first study to evaluate the efficacy of integrating foliar applications of an entomopathogenic fungus with a soil-dwelling arthropod natural enemy to suppress western flower thrips populations under greenhouse conditions. Combined applications of arthropod natural enemies with entomopathogenic fungi are considered an alternative plant protection strategy to deal with western flower thrips populations in greenhouse production systems (Mouden et al. 2017). In the current study, the *B. bassiana* and *D. coriaria* combination, and *B. bassiana* and *D. coriaria* individual treatments provided a level of suppression that was not significantly different from that of the insecticide treatments.

Drench applications of *B. bassiana* did not negatively affect *D. coriaria* development (Echegaray and Cloyd 2012). Thus, any antagonistic effects (mortality less than the sum of

mortality that the individual provides) when using the *B. bassiana* and *D. coriaria* combination were not expected. Results from the current study indicated that rove beetle adults not only survived, but also reproduced during the experimental period. However, there was lack of any additive (mortality equal to the sum of mortality that the individual provides) or synergistic (mortality greater than the sum of mortality that the individual provides) effects when combining *B. bassiana* and *D. coriaria*, which did not support the second hypothesis. This could be due to resource competition between *B. bassiana* and *D. coriaria*. Resource competition occurs when two biological control agents compete for a shared host/prey (Janssen et al. 1998). Foliar applications of *B. bassiana* target western flower thrips larvae and adults residing within the plant canopy (Ugine et al. 2007, Wu et al. 2016). Therefore, fewer second instar larvae would migrate from plants down to the growing medium to pupate. Consequently, available prey for the soil-dwelling rove beetle, *D. coriaria,* would be reduced. In contrast, predation of rove beetles on the western flower thrips soil-inhabiting life stages (prepupae and pupae) would reduce the number of newly eclosed western flower thrips adults, and larvae that would be produced by the newly eclosed female adults for *B. bassiana* infection.

Temperature, relative humidity, and light intensity are important environmental factors that can influence growth and development of western flower thrips, as well as infectivity of entomopathogenic fungi (Gaum et al. 1994, Butt and Brownbridge 1997). In the current study, temperature, relative humidity, and light intensity inside and outside the cages in each trial were monitored and recorded (Table 3). The mean temperature inside the cages in each trial was higher (0.2 to 0.8ºC) than outside the cages. The optimal temperature for western flower thrips development and reproduction is 25 to 30ºC, and the temperature for entomopathogenic fungal infection ranges from 15 to 30ºC (Gaum et al. 1994, Inglis et al. 2001). Therefore, the relative

higher temperatures (22 to 29ºC) inside the cages were conducive for the growth and development of western flower thrips populations, as well as infection by *B. bassiana*. Furthermore, relative humidity can affect the ability of entomopathogenic fungi to infect hosts (Ferron 1977, Fargues et al. 1997, Mukawa et al. 2011). The mean relative humidity inside the cages for each trial was higher (2.2 to 8.3%) than outside the cages. The higher relative humidity inside the cages favored host infection by *B. bassiana*. Ultraviolet light can negatively affect entomopathogenic fungi (Inglis et al. 2001). In this study, the mean light intensity inside the cages for each trial was lower (7 to 16 µmol/m²/s of PAR) than outside the cages, which likely enhanced the survival of *B. bassiana* conidia.

The use of colored sticky cards has limitations and may not provide a sufficient means of assessing the efficacy of treatments since the colored sticky cards only capture western flower thrips adults; not eggs, larvae or pupae (Kivett et al. 2015). Furthermore, western flower thrips adults may be more attracted to flowers (depending on colors) than colored sticky cards because they prefer to aggregate in flowers (Blumthal et al. 2005, Cloyd 2009, Reitz 2009). As such, the colored sticky cards may only capture a certain number of western flower thrips adults, which may not represent the actual number present. Nonetheless, colored sticky cards are the most cost effective and least time-consuming method in determining trends in western flower thrips adult populations (Heinz et al. 1992).

The final foliage quality ratings were essential in assessing the effectiveness of the five treatments in the study because this is associated with marketability and salability of the crop. The final foliage quality ratings were not significantly different among the five treatments in experiment 1. Even plants treated with the water control had a final foliage quality rating of 1 (=no visible damage) or 2 (=1% to 25% damage), which would be considered marketable by

greenhouse producers (Cloyd and Cycholl 2002). Results from experiment 1 indicated that the western flower thrips population was low enough so that even the water control provided sufficient suppression. In fact, studies have found that overhead irrigation and water sprays may provide a certain level of pest suppression (Chandler et al. 1979, Brunner and Burts 1981, McHugh and Foster 1995, Opit et al. 2006, Kivett et al. 2015). However, in experiment 2, although the final foliage quality ratings among all the treatments were significantly different, 69% of the plants were not marketable due to substantial feeding damage by western flower thrips. Therefore, water sprays are more effective when western flower thrips adult populations are low.

The number of western flower thrips adults initially released onto each plant was increased from 20 in experiment 1 to 40 in experiment 2. The different numbers of western flower thrips initially released onto each plant between experiments 1 and 2 may be responsible for the differences observed in the final foliage quality ratings of most plants. Therefore, experiment 1 demonstrated that the key to managing western flower thrips populations, when using either insecticides or biological control agents, in greenhouse production systems is to make applications or releases early before extensive western flower thrips populations become established.

The foliage quality ratings were evaluated weekly although only the final foliage quality ratings were analyzed in the current study. Differences in weekly foliage quality ratings of plants among the five treatments were observed before most flowers senesced and were removed in experiment 2. Western flower thrips larvae and adults prefer to reside inside flowers, and flower pollen has been shown to enhance the development and reproduction of western flower thrips (Trichilo and Leigh 1988, Higgins 1992). For experiment 2, 63 to 100% (n=35) of the plants

were flowering on week two after initiation of each trial. Most western flower thrips larvae and adults were present inside the flowers (Yinping Li; personal observation). All senescing flowers were removed before each trial was completed. Consequently, western flower thrips larvae and adults emerged from the senesced flowers and fed on plant leaves, resulting in substantial foliage damage in less than one week for most of the plants associated with the five treatments (Yinping Li; personal observation). As such, the effect of the five treatments on western flower thrips could not be determined. Therefore, future experiments should be conducted on younger plants so that the eight-week program is completed before flowers have senesced and western flower thrips populations are extensive.

Overall, the estimated mean number of western flower thrips adults captured on the yellow sticky cards was significantly lower in the insecticide treatment than the *B. bassiana* and *D. coriaria* combination. However, the final foliage quality ratings were not significantly different between the insecticides and the *B. bassiana* and *D. coriaria* combination, which supported the first hypothesis. Moreover, using the *B. bassiana* and *D. coriaria* combination could reduce selection pressure affiliated with insecticide applications, consequently delaying the development of insecticide resistance in western flower thrips populations, and preserving the effectiveness of existing insecticides. The *B. bassiana* and *D. coriaria* combination, and *B. bassiana* and *D. coriaria* individual treatments resulted in a substantial cost savings compared to the insecticides, which supported the third hypothesis. Therefore, the differential results obtained from both experiments indicate that greenhouse producers consider using *B. bassiana* and *D. coriaria*, early in the production cycle, which will help to effectively deal with the foliar-feeding and soil-dwelling life stages of the western flower thrips simultaneously.

Figure and Tables
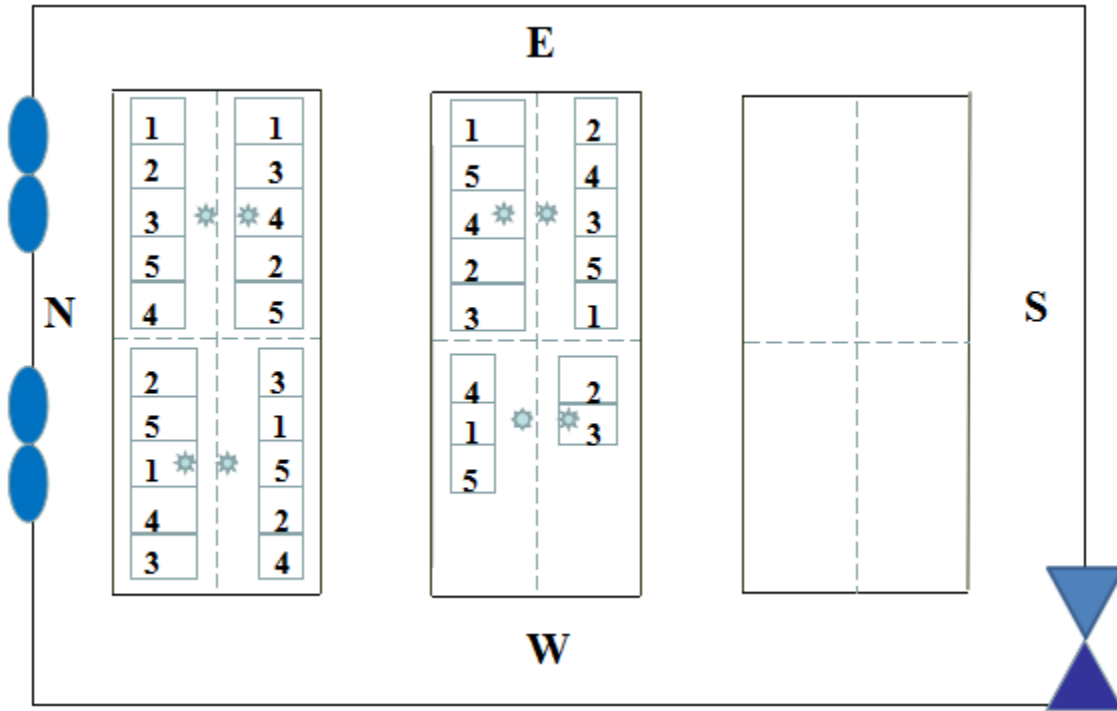
Figure 5-1 Greenhouse layout for the four trials (summer and fall 2016, and spring and fall 2017) associated with experiments 1 and 2.

Notes:

"N, S, E, W" represent direction of greenhouse: North, South, East, and West.

☐ : clear plastic observation cage.

1, 2, 3, 4, 5: treatment per plant.

⬤ : fan on the north wall.

▽▲ : greenhouse door on the south side.

✺ : Hobo data loggers.

Table 5-1 Product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* used in experiments 1 and 2.

Common names	Trade names	Manufacturers	Label rates (per 100 gallons)	Application rates (per 946 mL)
spinosad	Conserve® SC	Dow AgroSciences; Indianapolis, IN	6.0 fl oz	0.44 mL
pyridalyl	Overture® 35 WP	Valent U.S.A. Corp.; Walnut Greek, CA	8.0 oz	0.57 g
chlorfenapyr	Pylon®	BASF Corp.; Research Triangle Park, NC	5.2 fl oz	0.38 mL
abamectin	Avid® 0.15 EC	Syngenta Crop Protection; Greensboro, NC	8.0 fl oz	0.59 mL
Beauveria bassiana	BotaniGard® 22 WP	BioWorks Inc.; Victor, NY	1.0 lb	1.13 g

Table 5-2 Treatment application scheme over an eight-week period to suppress western flower thrips, *Frankliniella occidentalis*, populations under greenhouse conditions for each trial [1.1 (summer 2016), 1.2 (fall 2016), 2.1 (spring 2017), and 2.2 (fall 2017)] associated with experiments 1 and 2.

Treatments[a]	Weeks			
	1 and 2	3 and 4	5 and 6	7 and 8
A	spinosad	pyridalyl	chlorfenapyr	abamectin
B	*Beauveria bassiana*	*B. bassiana*	*B. bassiana*	*B. bassiana*
C	*Dalotia coriaria*	---	---	---
D	*B. bassiana + D. coriaria*	*B. bassiana*	*B. bassiana*	*B. bassiana*
E	water	water	water	water

[a] For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released on week one, with no additional releases afterward.

Table 5-3 Mean (± SEM) environmental parameters including: light intensity (µmol/m²/s of PAR for 400-700 nm); high, low, and mean temperature (°C); and relative humidity inside and outside cages in the greenhouse for four trials [trial 1.1 (summer) and trial 1.2 (fall) in 2016; trial 2.1 (spring) and trial 2.2 (fall) in 2017)] associated with experiments 1 and 2.

Outside cages

Experiment: Trial	Light intensity[a] µmol/m²/s of PAR	Temperature °C High	Low	Mean	Relative Humidity (%)
Experiment 1					
Trial 1.1: summer 2016	224 (± 5.6)	36.2	20.7	27.8 (± 0.1)	70.8 (± 0.2)
Trial 1.2: fall 2016	135 (± 4.0)	29.1	18.6	21.0 (± 0.1)	47.2 (± 0.4)
Experiment 2					
Trial 2.1: spring 2017	188 (± 5.9)	32.8	15.2	21.6 (± 0.1)	50.9 (± 0.4)
Trial 2.2: fall 2017	177 (± 6.0)	40.5	8.7	23.6 (± 0.1)	45.6 (± 0.5)

Inside cages

Experiment: Trial	Light intensity µmol/m²/s of PAR	Temperature °C High	Low	Mean	Relative Humidity (%)
Experiment 1					
Trial 1.1: summer 2016	217 (± 5.5)	39.1	20.4	28.6 (± 0.1)	73.0 (± 0.2)
Trial 1.2: fall 2016	119 (± 3.6)	31.1	18.1	21.2 (± 0.1)	55.5 (± 0.3)
Experiment 2					
Trial 2.1: spring 2017	179 (± 5.7)	38.6	16.6	22.3 (± 0.1)	54.5 (± 0.4)
Trial 2.2: fall 2017	163 (± 5.7)	41.1	8.7	24.0 (± 0.1)	52.4 (± 0.5)

[a] To exclude periods of darkness from the light intensity averages, only readings >15 lumens/ft² were included. To convert these units to µmol/m²/s of PAR (Photosynthetically Active Radiation) for 400-700 nm, light intensity measured in lumens/ft² was multiplied by 0.20 (Thimijan and Heins 1983).

Table 5-4 Analysis of Variance skeleton regarding the response variable of the number of western flower thrips adults captured on the yellow sticky cards for trials 1.1 (summer 2016) and 1.2 (fall 2016) of experiment 1.

Topographical effects[a]		Treatment effects		Combined effects	
Source	**df**	**Source**	**df**	**Source**	**df**
Block	7			Block	7
		Treatment	4	Treatment	4
Cage (block)	27			Block*Treatment	23
		Week	7	Week	7
		Treatment*Week	28	Treatment*Week	28
Observation	245			Block*Treatment*Week	210
Total	279			Total	279

[a]Topographical effects include the factors associated with the experiment design, such as the blocking factor.

Table 5-5 Analysis of Variance skeleton regarding the response variable of the number of western flower thrips adults captured on the yellow sticky cards for experiment 2 (spring and fall 2017).

Topographical effects[a]		Treatment effects		Combined effects	
Source	df	Source	df	Source	df
		Season	1	Season	1
Block	15			Block (Season)	14
		Treatment	4	Treatment	4
		Season*Treatment	4	Season*Treatment	4
Cage (block)	54			Cage	46
		Week	7	Week	7
		Treatment*Week	28	Treatment*Week	28
		Season*Week	7	Season*Week	7
		Season*Treatment*Week	28	Season*Treatment* Week	28
Observation	490			Block (Season)*Treatment*Week	420
Total	559			Total	559

[a]Topographical effects include the factors associated with the experiment design, such as the blocking factor.

Table 5-6 Analysis of Variance skeleton reagarding the response variable of the final foliage quality ratings for experiments 1 (summer and fall 2016) and 2 (spring and fall 2017).

Topographical effects[a]		Treatment effects		Combined effects	
Source	df	Source	df	Source	df
		Season	1	Season	1
Block	15			Block (Season)	14
		Treatment	4	Treatment	4
		Season*Treatment	4	Season*Treatment	4
Cage (block)	54			Treatment*Block(Season)	46
Total	69			Total	69

[a]Topographical effects include the factors associated with the experiment design, such as the blocking factor.

Table 5-7 Estimated mean number (95% confidence intervals) of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards each week over an eight-week period for treatments (Trt) A through E in trial 1.1 (summer 2016) of experiment 1.

Trt	Weeks							
	1	2	3	4	5	6	7	8
A	23.0 (11.0-48.4) a	12.6 (6.0-26.5) a	19.7 (9.2-41.9) a	3.6 (1.6-8.2) a	0.4 (0.1-1.6) a	0.9 (0.3-2.5) a	0.9 (0.3-2.5) a	0.7 (0.2-2.0) a
B	13.2 (6.2-28.2) a	10.2 (4.8-21.6) a	11.5 (5.5-24.0) a	4.1 (1.9-9.1) a	3.4 (1.5-7.8) b	5.1 (2.3-11.1) b	4.7 (2.1-10.3) ab	3.6 (1.6-8.1) a
C	44.4 (21.5-91.7) a	14.6 (7.0-30.4) a	20.9 (10.1-43.3) a	14.1 (6.6-29.8) a	2.6 (1.1-6.1) ab	2.3 (1.0-5.6) ab	4.3 (1.9-9.6) ab	2.3 (1.0-5.5) a
D	20.7 (9.7-44.0) a	8.3 (3.9-17.6) a	21.1 (10.2-43.7) a	14.4 (6.8-30.2) a	7.4 (3.4-15.9) b	8.4 (3.9-18.1) b	9.9 (4.7-21.0) b	5.1 (2.3-11.4) a
E	17.3 (8.2-36.3) a	9.2 (4.3-19.6) a	10.4 (4.9-21.8) a	3.9 (1.8-8.7) a	1.7 (0.7-4.3) ab	3.8 (1.7-8.5) ab	6.6 (3.0-14.4) b	2.6 (1.1-6.1) a

Notes: For treatments (A, B, C, D, and E) and treatment application scheme refer to Table 2. For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released in the first treatment application, with no additional releases afterward. Estimated means followed by different letters within each week indicate significant differences ($P<0.05$) among the five treatments.

Table 5-8 Estimated mean number (95% confidence intervals) of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards each week over an eight-week period for treatments (Trt) A through E in trial 1.2 (fall 2016) of experiment 1.

Trt	Weeks							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	1.0 (0.4-2.4)	0.7 (0.2-1.9)	0.3 (0.1-1.2)	0.7 (0.2-2.0)	1.1 (0.4-2.6)	1.0 (0.4-2.5)	0.6 (0.2-1.7)	0.0 (0, 0)
B	0.8 (0.3-2.2)	1.6 (0.7-3.6)	3.8 (1.9-7.5)	2.8 (1.4-5.7)	5.3 (2.8-10.1)	0.8 (0.3-2.1)	1.6 (0.7-3.6)	0.5 (0.2-1.6)
C	1.6 (0.7-3.5)	1.4 (0.6-3.1)	2.4 (1.2-5.0)	6.6 (3.5-12.1)	2.2 (1.1-4.6)	5.5 (2.9-10.4)	2.7 (1.3-5.4)	1.1 (0.5-2.7)
D	0.4 (0.1-1.4)	1.1 (0.4-2.7)	1.4 (0.6-3.2)	3.8 (1.9-7.4)	1.2 (0.5-2.8)	3.0 (1.5-6.0)	1.6 (0.7-3.5)	1.1 (0.4-2.6)
E	0.5 (0.2-1.7)	1.2 (0.5-2.8)	1.0 (0.4-2.5)	2.9 (1.4-5.8)	2.6 (1.2-5.2)	1.6 (0.7-3.6)	1.1 (0.4-2.6)	0.7 (0.2-2.0)

Notes: For treatments (A, B, C, D, and E) and treatment application scheme refer to Table 2. For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released in the first treatment application, with no additional releases afterward.

Table 5-9 Estimated mean number (95% confidence intervals) of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards each week over an eight-week period for treatments (Trt) A through E in trial 2.1 (spring 2017) of experiment 2.

Trt	Weeks							
	1	2	3	4	5	6	7	8
A	0.4 (0.1-1.4) a	0.0 (0-0) ab	1.6 (0.8-3.5) a	1.8 (0.9-3.8) a	1.9 (0.9-4.0) a	1.9 (0.9-4.0) a	2.8 (1.5-5.6) a	0.9 (0.4-2.3) a
B	0.8 (0.3-2.1) a	6.5 (3.6-11.8) b	3.1 (1.6-6.1) a	8.2 (4.6-14.7) b	8.4 (4.7-15.1) b	10.4 (5.9-18.4) b	4.4 (2.3-8.2) a	4.4 (2.3-8.3) b
C	1.0 (0.4-2.4) a	4.6 (2.5-8.6) b	3.8 (2.0-7.1) a	7.0 (3.9-12.6) ab	8.3 (4.6-14.8) b	8.4 (4.7-15.0) b	8.9 (5.0-15.8) a	9.1 (5.1-16.3) b
D	0.3 (0.1-1.2) a	0.7 (0.3-1.8) a	1.5 (0.7-3.3) a	4.2 (2.2-7.9) ab	5.3 (2.8-9.8) ab	4.3 (2.3-8.1) ab	6.0 (3.3-10.9) a	7.0 (3.8-12.7) b
E	0.4 (0.1-1.4) a	3.0 (1.5-5.8) ab	3.1 (1.6-6.0) a	7.2 (4.0-13.0) b	9.6 (5.4-17.1) b	6.8 (3.8-12.4) ab	6.7 (3.7-12.1) a	5.4 (2.9-10.1) b

Notes: For treatments (A, B, C, D, and E) and treatment application scheme refer to Table 2. For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released in the first treatment application, with no additional releases afterward. Estimated means followed by different letters within each week indicate significant differences ($P<0.05$) among the five treatments.

Table 5-10 Estimated mean number (95% confidence intervals) of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards each week over an eight-week period for treatments (Trt) A through E in trial 2.2 (fall 2017) of experiment 2.

Trt	Weeks							
	1	2	3	4	5	6	7	8
A	3.4 (1.8-6.5) a	2.6 (1.3-5.1) a	2.4 (1.2-4.8) a	2.6 (1.3-5.2) a	7.9 (4.4-14.3) a	9.2 (5.1-16.4) a	46.6 (27.4-79.2) a	37 (21.7-63.1) a
B	2.4 (1.2-4.9) a	3.3 (1.7-6.4) a	4.5 (2.4-8.3) a	6.2 (3.4-11.3) ab	27.2 (15.8-46.8) b	51.3 (30.2-87.2) b	128.5 (76.2-216.6) b	112.8 (66.8-190.3) b
C	7.3 (4.0-13.1) a	7.6 (4.2-13.7) a	4.0 (2.1-7.6) a	8.8 (4.9-15.8) ab	21.6 (12.5-37.3) ab	31.6 (18.5-54.1) b	87.7 (51.9-148.3) ab	89.7 (53.1-151.7) ab
D	4.4 (2.3-8.2) a	5.2 (2.8-9.7) a	3.4 (1.8-6.6) a	10.3 (5.8-18.3) b	26.5 (15.4-45.6) b	41.7 (24.5-71.0) b	99.2 (58.5-167.4) ab	105.1 (62.3-177.4) ab
E	6.2 (3.4-11.3) a	7 (3.8-12.8) a	4.9 (2.6-9.1) a	9.2 (5.1-16.6) ab	25.2 (14.7-43.3) ab	45 (26.5-76.5) b	81 (47.9-137.0) ab	104.2 (61.8-175.8) ab

Notes: For treatments (A, B, C, D, and E) and treatment application scheme refer to Table 2. For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released in the first treatment application, with no additional releases afterward. Estimated means followed by different letters within each week indicate significant differences (*P*<0.05) among the five treatments.

Table 5-11 Mean (± SEM) number of rove beetle, *Dalotia coriaria*, larvae and adults recovered from growing medium in each 15.2 cm container for treatments C (five rove beetle adults released on week one) and D [five rove beetle adults released on week one and *Beauveria bassiana* (BotaniGard®) applied each week for eight weeks] following completion of four trials [trial 1.1 (summer) and 1.2 (fall) in 2016; trial 2.1 (spring) and trial 2.2 (fall) in 2017] associated with experiments 1 and 2.

Experiment: Trial	Treatment C		Treatment D	
	Larvae	**Adults**	**Larvae**	**Adults**
Experiment 1				
Trial 1.1: summer 2016	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)
Trial 1.2: fall 2016	0.9 (± 0.3)	24.0 (± 10.5)	2.3 (± 1.1)	22.6 (± 6.9)
Experiment 2				
Trial 2.1: spring 2017	0.1 (± 0.1)	4.0 (± 1.3)	0.0 (± 0.0)	2.1 (± 0.9)
Trial 2.2: fall 2017	0.0 (± 0.0)	2.6 (± 1.3)	4.1 (± 3.8)	3.9 (± 1.5)

Table 5-12 Total cost of the five treatments over the eight-week period associated with experiments 1 and 2.

Treatments[a]	Weeks				Total cost
	1 and 2	3 and 4	5 and 6	7 and 8	
A	spinosad	pyridalyl	chlorfenapyr	abamectin	
	($90.00)[b]	($164.00)	($539.50)	($170.00)	$963.50
B	*Beauveria bassiana*	*B. bassiana*	*B. bassiana*	*B. bassiana*	
	($104.26)	($104.26)	($104.26)	($104.26)	$417.04
C	*Dalotia coriaria*	---	---	---	
	($78.63/500)	($0)	($0)	($0)	$78.63
D	*B. bassiana* + *D. coriaria*	*B. bassiana*	*B. bassiana*	*B. bassiana*	
	($182.89)	($104.26)	($104.26)	($104.26)	$495.67
E	water	water	water	water	
	($0)	($0)	($0)	($0)	$0

[a]For treatments (A, B, C, D, and E) and treatment application scheme refer to Table 2. For product information (common name, trade name, and manufacturer), label rates, and application rates associated with the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* refer to Table 1. For treatments involving *Dalotia coriaria*, five *D. coriaria* adults were released in the first treatment application, with no additional releases afterward. The costs of the treatments including solution applications were based on 100 gallons of spray solutions per week at the label rates for western flower thrips, *Frankliniella occidentalis*.

[b]The product costs of the insecticides (spinosad, pyridalyl, chlorfenapyr, and abamectin) and *Beauveria bassiana* were based on the 2016-2017 Hummert International Commercial Catalog (Hummert International; Earth City, MO). The cost of the treatments involving *D. coriaria* adults was based on the cost of 500 rove beetle adults at $78.63 (2018 Biobest Catalog: Biobest Canada Ltd.; Leamington, ON, Canada).

Chapter 6 - Summary

Three studies were involved in this research to determine: 1) effect of different numbers of predator and prey on predation of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), pupal stages by rove beetle, *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae), adults; 2) effect of western flower thrips pupal stage, searchable area, predator-prey ratio, and predator-prey number on predation efficacy of rove beetle adults; and 3) effectiveness of integrating the entomopathogenic fungus, *Beauveria bassiana*, and the rove beetle, *D. coriaria* in suppressing western flower thrips populations under greenhouse conditions.

Results from the first study showed that predation of rove beetle adults on western flower thrips pupal stages was influenced by different numbers of rove beetle adults released, and that there was no evidence of any differences due to predator-prey ratios or initial prey numbers within each predator-prey ratio. In the second study, predation efficacy of rove beetle adults on western flower thrips pupal stages was influenced by searchable area, predator-prey ratio, and predator-prey number. Thus, these factors should be considered when using *D. coriaria* adults against western flower thrips pupal stages. Results from the third study demonstrated that greenhouse producers must initiate insecticide applications or release rove beetle adults early in the production cycle when western flower thrips populations are low to minimize plant damage.

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Appendixes

Appendix A - Recovery of western flower thrips, *Frankliniella occidentalis*, adults from prepupae and pupae

Materials and Methods

a. Insect colony

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days. Western flower thrips specimens used in this study are deposited as voucher numbers 237 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Experimental procedures

Two independent experiments were conducted in two container sizes to determine recovery of western flower thrips adults that were initially prepupae and pupae in the growing medium. Each experiment was set-up as a completely randomized design.

Growing medium preparation was as follows: a 6.0 L plastic container [28.5 x 11 cm (diameter x height)] (Rubbermaid Home Products; Wooster, OH) was filled with Sunshine LC1 RSi Professional Growing Mix (SunGro Horticulture, Inc.; Bellevue, WA) growing medium

consisting of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone. The growing medium was moistened with approximately 200 mL of water. The 6.0 L plastic container with growing medium was then heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.8 L of water was applied to the growing medium, which was then thoroughly mixed.

Deli containers. Approximately 300 mL of the sterilized growing medium was placed into a 473 mL deli container. Twenty prepupae or pupae were randomly positioned on the growing medium surface in each 473 mL deli container. Prepupae and pupae are generally located at a depth of 1 to 5 mm in the compost that was a freshly steam-sterilised mixture (50:50 by volume) of loam and medium grade sphagnum peat (Helyer et al., 1995). Furthermore, the prepupae and pupae are distributed throughout the growing medium via cracks and crevices present on the growing medium surface (Yinping Li; personal observation). Therefore, no additional growing medium was needed to cover the prepupae and pupae. A lid was modified with No-Thrips insect screening for ventilation and prevented western flower thrips adults from escaping. A half-section of a yellow sticky card [7.7 x 10.4 cm (length x width)] (Pestrap Phytotronics, Inc.; Earth City, MO] was affixed to the inside center of the lid to capture western flower thrips adults. There were ten replications per pupal stage (prepupae or pupae). Thus, a total of ten deli containers per pupal stage were prepared and exposed to laboratory conditions of 20-24ºC, 50% to 60% relative humidity, and constant light. The numbers of western flower thrips adults captured on the yellow sticky cards were recorded 17 days for prepupae and 15 days for pupae after the experiment was initiated.

15.2 cm containers. The procedures were similar to those described above except for the following. Approximately 1,200 mL of the growing medium was placed into a 15.2 cm

container. The drainage holes in the bottom were covered with No-Thrips insect screening to prevent western flower thrips adults from escaping. Each 15.2 cm container was covered with No-Thrips insect screening, and a yellow sticky card was affixed onto the inside center of the screening to capture eclosing western flower thrips adults.

Results

For the 473 mL deli containers, mean percent western flower thrips adults recovered from prepupae and pupae was 84.5% (± 13.6%) [mean (± SEM)] (n=200) and 93.0% (± 11.7%) (n=200). For the 15.2 cm containers, the mean percent western flower thrips adults recovered from prepupae and pupae was 90.0% (± 18.3%) (n=200) and 90.5% (± 2.8%) (n=200). The respective recovery of western flower thrips adults ensured that the results associated with the subsequent predation experiments were directly due to predator predation, and not other confounding factors, such as: handling technique or natural mortality of western flower thrips prepupae and pupae.

The recovery of western flower thrips was determined regularly twice a month. In the experiment 1 of Chapter 4, the mean percent western flower thrips adults captured on the yellow sticky cards ranged from 98.2% to 99.4% for prepupae and 89.2% to 97.9% for pupae.

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Appendix B - Rove beetle, *Dalotia coriaria*, adult feeding preference on western flower thrips, *Frankliniella occidentalis*, prepupae and pupae

Materials and Methods

a. Insect colonies

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days.

A rove beetle colony was maintained using growing medium in 7.6 L plastic containers [34.8 x 24.7 x 12.4 cm (length x width x height] (Rubbermaid Home Products; Wooster, OH) under laboratory conditions: 20-24ºC, 45% to 60% relative humidity, and constant darkness simulating conditions similar to the natural habitat of rove beetles (Helyer et al. 2003). A rectangular section (22 x 11 cm: length x width) was cut in the lid and covered with No-Thrips insect screening.

Growing medium preparation was as follows: a 6.0 L plastic container [28.5 x 11 cm (diameter x height)] (Rubbermaid Home Products; Wooster, OH) was filled with Sunshine LC1 RSi Professional Growing Mix (SunGro Horticulture, Inc.; Bellevue, WA) growing medium consisting of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone. The growing medium was moistened with approximately 200 mL of water. The 6.0 L plastic container with growing medium was then heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.8 L of water was applied to the growing medium, which was then thoroughly mixed. About 3.0 L of the sterilized growing medium was placed into each 7.6 L plastic rectangular container.

Approximately 15 g of dry oats (*Avena sativa* L.) (The Quaker Oats Company; Chicago, IL) were placed, every four to five days, onto the growing medium surface in a line (lengthwise) in the center of the growing medium within each 7.6 L plastic rectangular container. About 15 mL of water was applied, every one to two days, onto the oats using a 946 mL plastic spray bottle (Delta Industries; King of Prussia, PA) to maintain constant moisture. Adult rove beetles from previous colonies was then used to establish new colonies.

Western flower thrips and rove beetle specimens used in this study are deposited as voucher numbers 237 and 220, respectively in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Preparation of newly eclosed rove beetle adults

Five rove beetle third instar larvae were placed into a Gladware® container [7.8 x 5.1 cm (diameter x height)] (The Glad Products Company; Oakland, CA) with 20 mL of moistened growing medium and three to four pieces of dry oats. A total of 20 Gladware® containers with rove beetle third instar larvae were prepared following the above method, then placed into an

environmental growth chamber (Conviron® Controlled Environments Inc.; Pembina, ND) set at 21-27ºC and constant darkness. Rove beetle adults were observed seven days later with most adults eclosing from pupae after 11 days (Yinping Li; personal observation). Newly eclosed adults (one to three days old) were individually placed in 9 dram (33 mL) plastic vials with lids using a soft-bristled brush. All 9 dram plastic vials containing rove beetle adults were returned to the environmental growth chamber and the adults were starved for 24 hours. The sex ratio of rove beetle adults was 1:1 (female:male).

c. Experimental procedures

Filter paper (150 mm diameter) (Whatman® International Ltd.; Maidstone, England) was placed in the bottom of a Petri dish and moistened with water using a 946 mL plastic spray bottle. A foam disk was inserted inside the lid of the Petri dish to prevent rove beetle adults, as well as western flower thrips prepupae and pupae from escaping. Choice and no-choice tests were conducted sequentially.

Choice test. The choice test was set-up as a randomized complete block design, with one Petri dish serving as a block. Prepupae and pupae were collected from the western flower thrips laboratory colony using a soft-bristled brush under a stereomicroscope (SMZ1000; Nikon Instruments Inc.; Japan). Prepupae are identified by their short wing sheaths and erect antennae; whereas pupae have long wing sheaths that nearly extend to the end of the abdomen, and the antennae are bent backwards along the head (Zhang et al. 2007). Based on preliminary experiments, four prepupae and four pupae were positioned alternately into one Petri dish, equidistant from each other, in a circular pattern. Then, one newly eclosed rove beetle adult (two to three days old, starved for 24 hours) was placed into the center of the Petri dish. A total of ten

Petri dishes were prepared following the above procedure and maintained in the environmental growth chamber set at 21-27ºC and constant darkness. The exposure period was two hours.

No-choice test. The no-choice test was set-up as a randomized complete block design, with a pair of Petri dishes serving as a block. One pair of Petri dishes involved placing eight prepupae into one Petri dish and eight pupae into the other Petri dish. The procedures were the same as those described above in the choice test. The no-choice test was completed using 10 pairs of Petri dishes.

After a two-hour exposure period, a soft-bristled brush was used to contact the prepupae and pupae, and determine the number fed upon by rove beetle adults under a stereomicroscope. If prepupae or pupae moved after being prodded with the soft-bristled brush, they were considered alive. However, if prepupae or pupae were not present or were damaged (exuding body fluids), they were considered to have been fed upon by rove beetle adults.

Data Analysis

In each test, a generalized linear mixed model assuming a binomial distribution was fitted to the response variable defined as the number of western flower thrips prepupae or pupae fed upon out of the initial number of western flower thrips prepupae or pupae in the same Petri dish. A logit link function was used to estimate the probability of western flower thrips prepupae or pupae fed upon by rove beetle adults. In the choice test, the linear predictor in the model included the fixed effect of western flower thrips pupal stage. Random effect associated with the linear predictor included one Petri dish as a blocking factor. In the no-choice test, the fixed effect in the linear predictor included western flower thrips pupal stage. Random effects included the blocking effect of one pair of Petri dishes.

Over-dispersion was determined using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion for the two tests. 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 PROC GLIMMIX procedure (SAS Institute 2012) implemented Newton-Raphson with ridging as the optimization technique. Pairwise comparisons were conducted using Tukey-Kramer's or Bonferroni's procedure to adjust for multiple comparisons in order to avoid inflation of type I error due to multiple comparisons.

Results

There were no significant differences associated with the estimated mean probability of prey (western flower thrips prepupae or pupae) fed upon by rove beetle adults between western flower thrips prepupae and pupae in either the choice test ($F = 2.43$; df=1, 9; P=0.36), or the no-choice test ($F = 0.03$; df=1, 9; P=0.90).

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Appendix C - Effects of insecticide drench applications on western flower thrips, *Frankliniella occidentalis*, pupae in growing medium

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important insect pest of greenhouse-grown horticultural crops worldwide (Robb and Parrella 1995, Lewis 1997a, Kirk 2002, Cloyd 2009, Reitz 2009). Western flower thrips causes direct damage by feeding on and ovipositing in leaves, flowers, and fruits (Chisholm and Lewis 1984, Hunter and Ullman 1989, Harrewijn et al. 1996, Childers 1997, Lewis 1997a). Moreover, western flower thrips cause indirect damage by vectoring the tospoviruses: *Tomato spotted wilt* and *Impatiens necrotic spot* viruses (Allen and Broadbent 1986, Pappu et al. 2009). The direct and indirect damage has led to significant economic losses. For instance, Goldbach and Peters (1994) estimated that, on an annual basis, *Tomato spotted wilt virus* accounts for an economic loss of >$1 billion (US dollars) in agricultural crops globally. Furthermore, in 2006, damage to ornamental crops in Georgia by western flower thrips resulted in an economic loss of >$15 million (US dollars) (Reitz and Funderburk 2012).

The tolerance for damage caused by western flower thrips is very low since damage reduces the aesthetic quality and marketability of greenhouse-grown horticultural crops (Parrella and Jones 1987, Loughner et al. 2005). The primary management strategy used against western flower thrips is applications of insecticides (Parrella and Murphy 1996, Lewis 1997b, Herron and James 2005). Therefore, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Kontsedalov et al. 1998, Loughner et al. 2005, Cloyd 2009, Reitz 2009).

Larvae and adults of western flower thrips feed on the foliage whereas prepupae and pupae reside in the growing medium (Tommasini and Maini 1995, Reitz 2009). Most late second instar larvae migrate down the plant stem and pupate in the growing medium or soil (Helyer et al. 1995, Tommasini and Maini 1995, Kirk 1996, Wiethoff et al. 2004). Foliar insecticide applications are the primary means of suppressing western flower thrips larvae and adult populations; whereas less attention has been directed at insecticide use against the soil-dwelling life stages (Berndt 2003, Belay et al. 2005, Ansari et al. 2008). The reason being is that soil-dwelling life stages are supposedly less susceptible to insecticides (Seaton et al. 1997). Although insecticides have been evaluated against western flower thrips pupae (Helyer and Brobyn 1992, Ludwig and Oetting 2001), none are actually labeled for use as drenches against western flower thrips pupae (Ansari et al. 2008, Cloyd 2009). In addition, some entomopathogenic fungi have been used against the soil-dwelling life stages of western flower thrips, including *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (*brunneum*) (Metchnikoff) Sorokin (Helyer et al. 1995, Ansari et al. 2007, Ansari et al. 2008, Skinner et al. 2012). Another candidate entomopathogenic fungus, *Isaria fumosorosea* (Wize) (Hypocreales: Cordycipitaceae), infects a wide-range of citrus pests and is less harmful to non-target arthropod natural enemies than conventional insecticides (Sterk et al. 1995a, b; Avery 2002, Avery et al. 2008). Furthermore, *I. fumosorosea* Apopka Strain 97 is commercially available as a microbial insecticide (Vidal et al. 1998, Faria and Wraight 2001).

Pyriproxyfen, (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine), is a juvenile hormone analog and a relatively stable aromatic compound (Sullivan and Goh 2008). Pyriproxyfen mimics juvenile hormone activities by competing for binding sites on juvenile hormone receptors; consequently, larvae do not develop into adults (Sullivan and Goh 2008).

Pyriproxyfen is registered for household, agricultural, and horticultural applications to control many insect pests, including: the fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae); the common housefly, *Musca domestica* (Lintner) (Diptera: Muscidae); mosquitoes; the red imported fire ant, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae); and the silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring) (Hemiptera: Aleyrodidae) (Miyamoto et al. 1993, Cloyd and Dickinson 2006, Sullivan and Goh 2008).

Anecdotal information from greenhouse producers has suggested that applications of an entomopathogenic fungus or pyriproxyfen in the growing medium, lead to fewer problems with western flower thrips adults. The inclination is that the insecticides are active on the pupal stages. However, there is no quantitative data to confirm these claims. In fact, no scientific studies have assessed the efficacy of *I. fumosorosea* or pyriproxyfen on the soil-dwelling life stages of western flower thrips when applied as a drench. Therefore, the objective of the study was to assess the effect of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae. Four experiments were conducted to determine: 1) the efficacy of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae in growing media, 2) the effect of pyriproxyfen in two growing media on western flower thrips pupae, and 3) the residual activity of pyriproxyfen in growing medium on western flower thrips pupae 3, 5, 7, and 14 days after treatments were applied.

Materials and Methods

a. Insect colony

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50% to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole

(9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days. 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).

b. Experimental procedures

Design structure. Four independent laboratory experiments were conducted to evaluate the effect of drench applications of *I. fumosorosea* and pyriproxyfen on western flower thrips pupae in growing medium. Each experiment was set-up as a completely randomized design. A single 473 mL deli container constituted a unit of replication. Five deli containers were associated with each treatment-combination in each experiment.

Treatment structure of experiment 1. The efficacy of *I. fumosorosea* and pyriproxyfen applied as drenches to the growing medium on western flower thrips pupae was evaluated using five treatments: 1.0 g Ancora [*I. fumosorosea* Apopka Strain 97 (Ancora™: OHP, Inc.; Mainland, PA) at 1.0 g/946 mL], 2.0 g Ancora [*I. fumosorosea* Apopka Strain 97 at 2.0 g/946 mL], Fulcrum [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL], an untreated check, and a water control. The rates for *I. fumosorosea* and pyriproxyfen were the label rates associated with drench applications to the growing medium.

Treatment structure of experiment 2. The effect of pyriproxyfen applied as drenches to two growing media on western flower thrips pupae was assessed using a two-way factorial

treatment structure consisting of all combinations of three treatments and two growing media. The three treatments were: pyriproxyfen at 0.14 mL/946 mL, an untreated check, and a water control. The two growing media were: LC1 (Sunshine LC1 RSi Professional Growing Mix: SunGro Horticulture Canada Ltd.; Seba Beach, Alberta, Canada) composed of 70% to 80% Canadian sphagnum peat moss, perlite, 0.0001% silicone dioxide, and dolomitic limestone; and Berger (Berger BM1 All-Purpose Mix: Berger; Saint-Modeste, Quebec, Canada) composed of 75% to 85% sphagnum peat moss, perlite, and vermiculite.

Treatment structure of experiments 3 and 4. The residual activity of pyriproxyfen applied as drenches to the growing medium on western flower thrips pupae was determined using a two-way factorial treatment structure with all combinations of three treatments and two western flower thrips pupal inoculation days after application of the treatments. The three treatments in each experiment were: pyriproxyfen at 0.14 mL/946 mL, an untreated check, and a water control. Furthermore, there were two western flower thrips pupal inoculation days (7 and 14 days for experiment 3, and 3 and 5 days for experiment 4) after the treatments were applied.

Experimental procedure. All treatment solutions were prepared in water using 946 mL plastic spray bottles (Delta Industries; King of Prussia, PA). Growing medium was prepared as follows: a 6.0 L plastic container (Rubbermaid Home Products; Wooster, OH) was filled with growing medium, which was moistened with approximately 200 mL of water. Then, the plastic container with growing medium was heated for 25 minutes in a microwave set at full-power (1,200 W output). After the growing medium was allowed to cool, 1.2 L of water was applied to the growing medium, which was then thoroughly mixed. Approximately 0.4 L of the sterilized growing medium was placed into a 473 mL deli container. The deli container was tapped five times to reduce the amount of air-space within the growing medium. The growing medium used

in experiments 1, 3, and 4 was LC1. Both LC1 and Berger growing media were used in experiment 2.

In experiments 1 and 2, 20 western flower thrips pupae, obtained from the laboratory colony, were randomly positioned on the growing medium surface of each 473 mL deli container. Pupae are generally located at a depth of 1 to 5 mm in the compost that was a freshly steam-sterilised mixture (50:50 by volume) of loam and medium grade sphagnum peat (Helyer et al. 1995). Furthermore, pupae are distributed throughout the growing medium via cracks and crevices present on the growing medium surface (Yinping Li; personal observation). Therefore, no additional growing medium was needed to cover the pupae. Then, 80 mL of each treatment solution was uniformly applied as a drench to the growing medium surface. In experiments 3 and 4, 80 mL of each treatment solution was initially uniformly applied to the growing medium surface of each 473 mL deli container; then, 20 western flower thrips pupae were randomly positioned on the growing medium surface after 7 or 14 days for experiment 3, or after 3 or 5 days for experiment 4.

A modified lid with No-Thrips insect screening was used for ventilation and to prevent western flower thrips adults from escaping upon eclosion. A half-section of a yellow sticky card [7.7 x 10.4 cm (length x width)] (Pestrap Phytotronics, Inc.; Earth City, MO) was hot-glued to the inside center of the lid to capture eclosing western flower thrips adults. The 473 mL deli containers had 12 holes perforated on the bottom to allow for drainage. Each 473 mL deli container was placed into a larger Petri dish (14 cm diameter) to collect any leachate from the bottom of the deli container. All deli containers for each experiment were prepared using the above procedure and placed in the laboratory at 20-24ºC, 50% to 60% relative humidity, and a 16:8 (light:dark) photoperiod. The numbers of western flower thrips adults captured on the

yellow sticky cards were recorded 15 days after the growing medium was inoculated with western flower thrips pupae. The number of western flower thrips adults captured on the yellow sticky cards was an indirect assessment of pupal mortality (Helyer et al. 1995).

Data Analysis

For each experiment, a generalized linear mixed model assuming a binomial distribution was fitted to the response variable defined as the number of western flower thrips adults captured on the yellow sticky cards out of the number of pupae initially positioned in the same container. A logit link function was used to estimate the probability of western flower thrips adults captured on the yellow sticky cards. For experiment 1, the fixed effect in the linear predictor was treatment. For experiment 2, the linear predictor in the model included the fixed effects of treatment, growing medium, and the two-way interaction. For experiments 3 and 4, the fixed effects in the linear predictor included treatment, western flower thrips pupal inoculation day (7 or 14 days in experiment 3, and 3 or 5 days in experiment 4) after the treatments were applied, and the two-way interaction. For experiment 4, to account for over-dispersion, a random effect was fitted for the level of observation defined as the cross product of replication, treatment, and western flower thrips pupae inoculation day after application of the treatments.

In each experiment, over-dispersion was determined using the maximum-likelihood based fit statistic Pearson Chi-Square/DF. There was no evidence of over-dispersion. In each experiment, 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 PROC GLIMMIX procedure (SAS Institute 2012) implemented using Newton–Raphson with ridging as the optimization technique. Pairwise comparisons were conducted using Tukey-Kramer's or

Bonferroni's adjustments, as appropriate for main effect or simple effect comparisons, to avoid inflation of type I error due to multiple comparisons.

Results

Experiment 1. There was a significant treatment effect associated with the estimated mean probability of western flower thrips adults captured on the yellow sticky cards ($F = 21.04$; df $= 4, 20$; $P < 0.0001$). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly lower for the pyriproxyfen treatment {17% (10.5-26.3%) [mean (95% confidence interval)]} compared to the other four treatments (Figure 1). The estimated mean probability of western flower thrips adults captured on the yellow sticky cards for the two treatments affiliated with *I. fumosorosea* Apopka Strain 97 (1.0 g Ancora and 2.0 g Ancora) was significantly lower than the untreated check, but there was no evidence of a significant difference from the water control (Figure 1).

Experiment 2. In both LC1 and Berger growing media, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly lower for the pyriproxyfen treatment [LC1: 20% (6.4-48.4%), Berger: 14% (3.9-40.5%)] than the untreated check, but not significantly different from the water control (Figure 2). For the water control, the estimated mean probability of western flower thrips adults captured on the yellow sticky cards was significantly higher in the LC1 [41% (31.4-51.4%)] than the Berger [24% (16.3-33.9%)] growing medium ($t = -2.54$, df $= 24$, $P = 0.018$) (Figure 3). However, for either the pyriproxyfen treatment or the untreated check, there was no evidence of any significant differences in the estimated mean probability of western flower thrips adults captured on the yellow sticky cards between the two growing media (Figure 3).

Experiment 3. When the growing medium was inoculated with western flower thrips pupae 7 days after application of the treatments, there was no significant treatment effect associated with the estimated mean probability of western flower thrips adults captured on yellow sticky cards (Figure 4). When the growing medium was inoculated with western flower thrips pupae 14 days after the treatments were applied, a significantly higher estimated mean probability of western flower thrips adults was captured on the yellow sticky cards in the untreated check than the water control, but there was no evidence of any significant differences between pyriproxyfen and the untreated check, or between pyriproxyfen and the water control (Figure 4).

Experiment 4. There was a significant interaction between treatment and western flower thrips pupal inoculation day after the treatments were applied ($F = 3.86$; df $= 2, 24$; $P = 0.035$). However, when the growing medium was inoculated with western flower thrips pupae 5 days after application of the treatments, there was no significant treatment effect based on the estimated mean probability of western flower thrips adults captured on the yellow sticky cards (Figure 5). When the growing medium was inoculated with western flower thrips pupae 3 days after application of the treatments, a significantly higher estimated mean probability of western flower thrips adults was captured on the yellow sticky cards in the untreated check than the pyriproxyfen treatment; however, there was no evidence of any significant differences between pyriproxyfen and the water control, or between the untreated check and the water control (Figure 5).

Discussion

This is the first study to evaluate the effects of pyriproxyfen and *I. fumosorosea* when applied as drench applications to the growing medium on western flower thrips pupae. Otieno et

al. (2016) found that azadirachtin, an insect growth regulator that inhibits metabolism of the molting hormone, ecdysone (Ware and Whitacre 2003), when applied as drenches against the soil-dwelling life stages of western flower thrips resulted in a 60% reduction in adult eclosion. Helyer et al. (1995) reported that soil applications of chlorpyrifos and malathion, which are both contact insecticides, provided 96.5% and 97.5%, respectively, mortality of western flower thrips pupae. In the current study, drench applications of pyriproxyfen to the growing medium resulted 17% (n=100) of western flower thrips adults captured on the yellow sticky cards in the first experiment. However, the estimated mean probability of western flower thrips captured on the yellow sticky cards was not significantly different between the pyriproxyfen treatment and the water control for the two growing media in the second experiment. Therefore, it appears that pyriproxyfen is inconsistent in affecting the pupal stage of the western flower thrips.

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* (*brunneum*), are reported to control the soil-dwelling life stages of western flower thrips in the growing medium or soil (Helyer et al. 1995, Ansari et al. 2007, Ansari et al. 2008, Skinner et al. 2012). For instance, Ansari et al. (2007) found that western flower thrips pupal mortality ranged from 70% to 90% when exposed to growing medium mixed with *M. anisopliae* (*brunneum*). Skinner et al. (2012) observed a 90% reduction in the mean total number of western flower thrips per plant using mycotized millet grains containing *B. bassiana* in the soil. However, in the current study, there was no evidence that drench applications of *I. fumosorosea* provided a significant difference from the water control in the estimated mean probability of western flower thrips adults captured on the yellow sticky cards (Figure 1). Therefore, *I. fumosorosea* may not be an effective microbial insecticide against western flower thrips pupae when applied as a drench. The reason

for the discrepancy among the entomopathogenic fungi against western flower thrips soil-dwelling life stages is not known at this time; consequently, further investigation is warranted.

Studies have demonstrated that growing medium or soil type can affect survival of soil-dwelling life stages of insect pests (Varatharajan and Daniel 1984, Hulthen and Clarke 2006, Chen and Shelton 2007, Holmes et al. 2013, Pietrantuono et al. 2015). Varatharajan and Daniel (1984) reported that the highest number of *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) pupae were found in loose soil with a layer of organic debris on the surface, rather than in clay soil. However, in the current study, there was no evidence that growing medium type in the untreated check treatment affected the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Furthermore, growing medium or soil type may also influence the effect of drench-applied insecticides on soil-dwelling pests. For instance, Cowles and Villani (1994) reported that soil pH and organic matter affected the efficacy of the insecticides: carbaryl, bendiocarb, and chlorpyrifos against Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), larvae. Nevertheless, in the current study, there was no evidence that growing medium type associated with the pyriproxyfen treatment affected the estimated mean probability of western flower thrips adults captured on the yellow sticky cards. Therefore, the results demonstrated that the two growing media may not influence the survival of western flower thrips pupae or the efficacy of pyriproxyfen on western flower thrips pupae.

Pyriproxyfen degrades rapidly in soil under aerobic conditions (Fathulla 1994), which is supported by the results from the third and fourth experiments as the estimated mean probability of western flower thrips adults captured on the yellow sticky cards for the pyriproxyfen treatment was not significantly different from the water control 7 and 14 days post-treatment for the third experiment, or 3 and 5 days post-treatment for the fourth experiment. Therefore, there was no

evidence that drench applications of pyriproxyfen had any residual activity on western flower thrips pupae in the growing medium 3 days after application of treatments.

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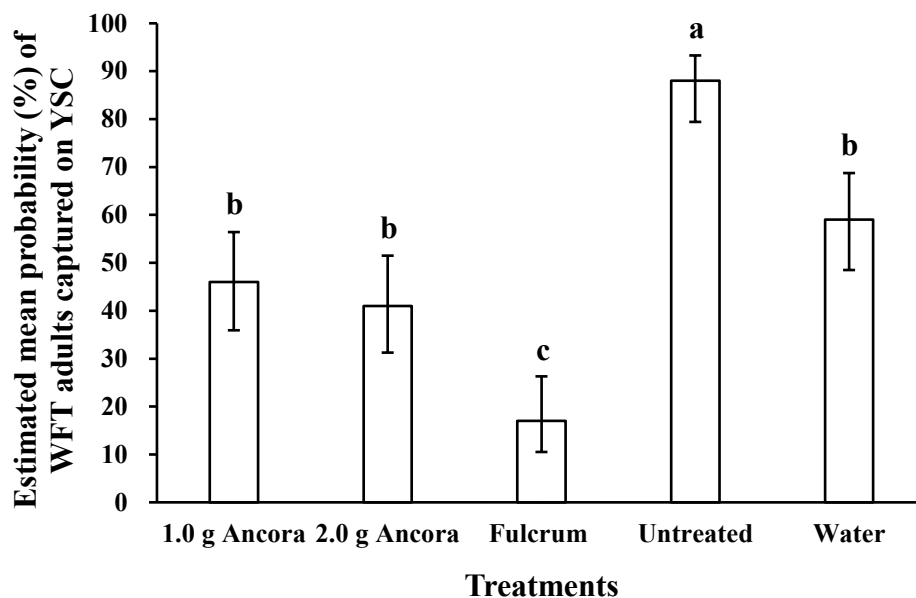
Figures

Figure C-1 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis***, adults captured on yellow sticky cards (YSC) for each treatment {1.0 g Ancora [***Isaria fumosorosea*** Apopka Strain 97 (Ancora™: OHP, Inc.; Mainland, PA) at 1.0 g/946 mL], 2.0 g Ancora [***I. fumosorosea*** Apopka Strain 97 at 2.0 g/946 mL], Fulcrum [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL], an untreated check, and a water control}. Estimated means followed by different letters indicate significant differences (***P*<0.05) among the treatments.**
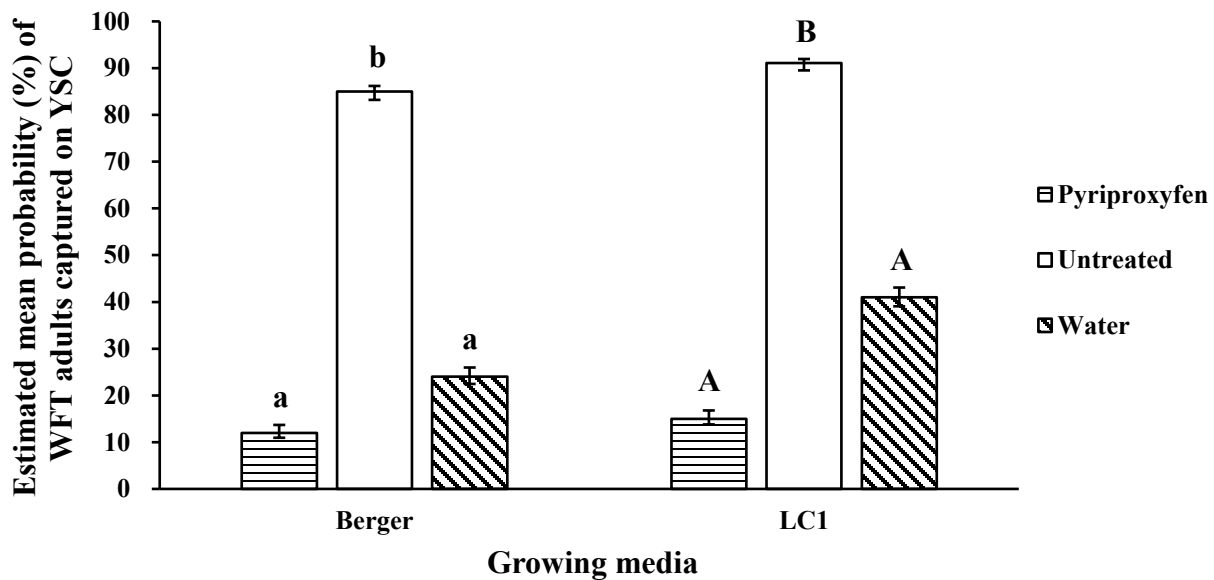
Figure C-2 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis***, adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] within each growing medium [Berger (Berger BM1 All-Purpose Mix: Berger; Saint-Modeste, Quebec, Canada) and LC1 (Sunshine LC1 RSi Professional Growing Mix: SunGro Horticulture Canada Ltd.; Seba Beach, Alberta, Canada)]. Estimated means followed by different lowercase or uppercase letters within each growing medium indicate significant differences (*P*<0.05) among the treatments.**
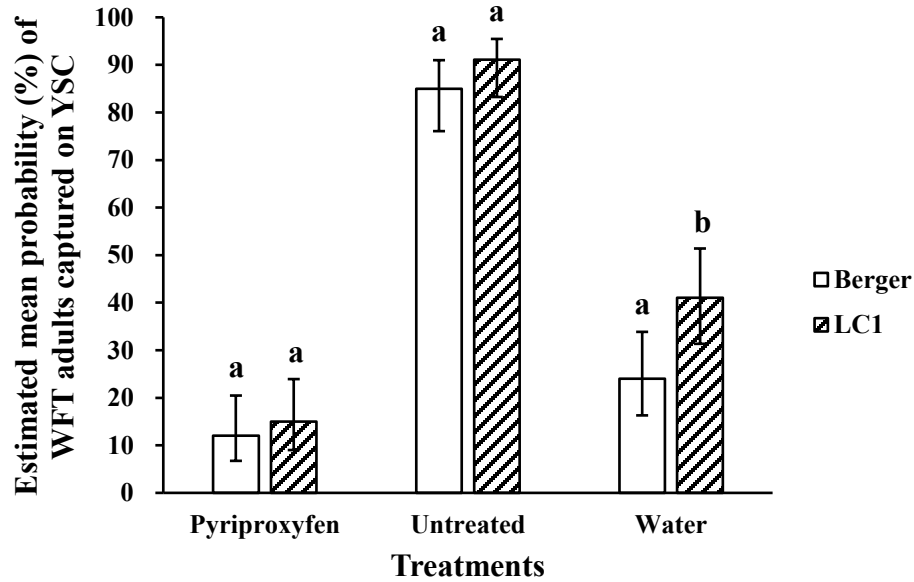
Figure C-3 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, **adults captured on yellow sticky cards (YSC) for each growing medium [Berger (Berger BM1 All-Purpose Mix: Berger; Saint-Modeste, Quebec, Canada) and LC1 (Sunshine LC1 RSi Professional Growing Mix: SunGro Horticulture Canada Ltd.; Seba Beach, Alberta, Canada)] within each treatment [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control]. Estimated means followed by different letters within each treatment indicate significant differences (*P*<0.05) between the two growing media.**
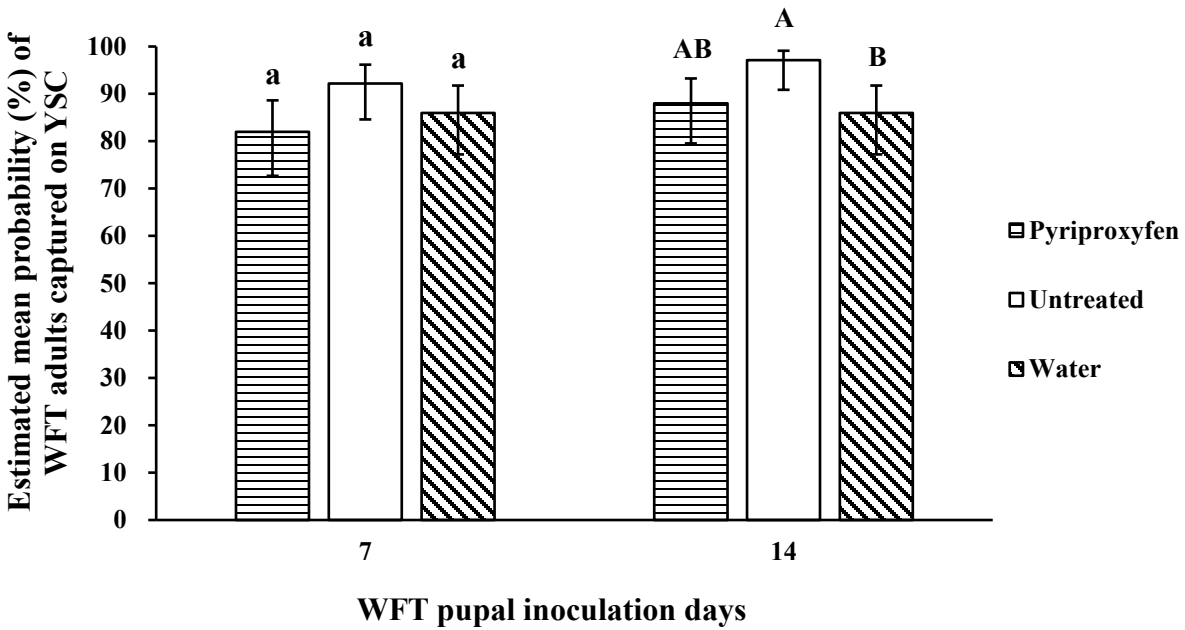
Figure C-4 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis*, **adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] on each WFT pupal inoculation day (7 and 14) after treatment application. Estimated means followed by different lowercase or uppercase letters within each WFT pupal inoculation day indicate significant differences ($P<0.05$) among the treatments.**
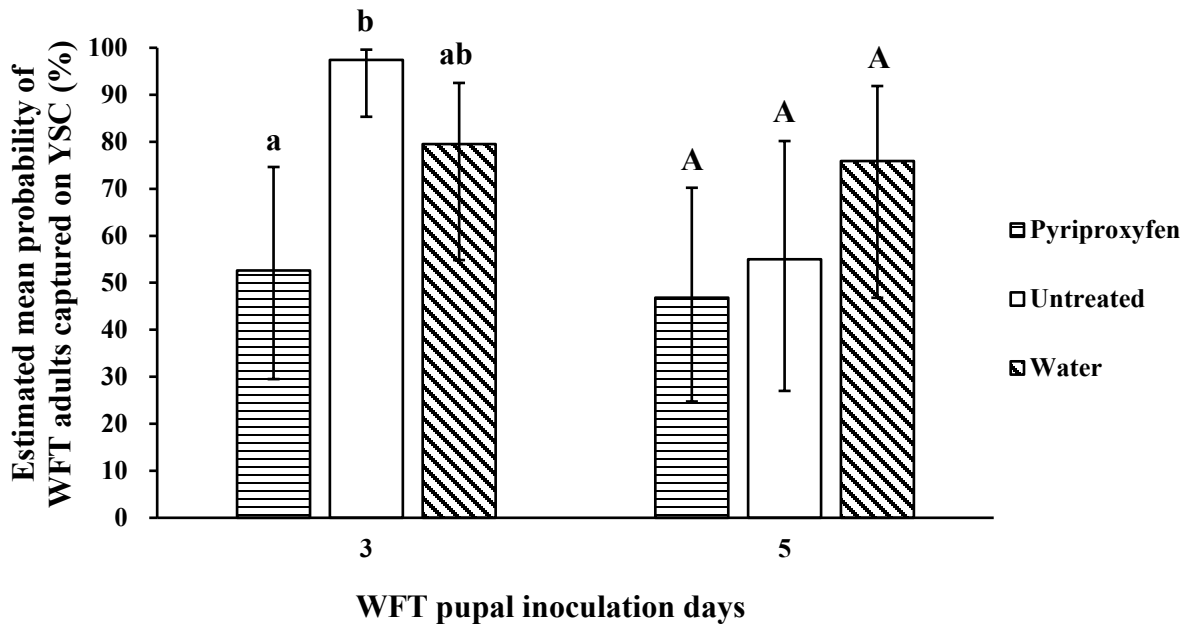
Figure C-5 Estimated mean probability (95% confidence intervals) of western flower thrips (WFT), *Frankliniella occidentalis***, adults captured on yellow sticky cards (YSC) for each treatment [pyriproxyfen (Fulcrum®: OHP, Inc.; Mainland, PA) at 0.14 mL/946 mL, an untreated check, and a water control] on each WFT pupal inoculation day (3 and 5) after treatment application. Estimated means followed by different lowercase or uppercase letters within each WFT pupal inoculation day indicate significant differences (*P*<0.05) among the treatments.**

Appendix D - Life span of female western flower thrips,

Frankliniella occidentalis, adults under laboratory conditions

Materials and Methods

a. Insect colony

A western flower thrips colony was maintained under laboratory conditions (20-24ºC, 50 to 60% relative humidity, and constant light) in Glad® plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] (The Glad Products Company; Oakland, CA) with a round hole (9.5 cm diameter) cut in the lid that was covered with No-Thrips insect screening (mesh size: 0.15 x 0.15 mm) (Greentek®; Edgerton, WI). Green beans (*Phaseolus vulgaris* L.) were purchased from a local supermarket (Dillons; Manhattan, KS), soaked in soapy water for 20 minutes, then rinsed with distilled water, and allowed to dry. The green beans were provided as food and oviposition sites for adults, as well as a food source for larvae. Green beans were changed every two to three days. Western flower thrips specimens used in this study are deposited as voucher numbers 237 in the Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

b. Experimental procedures

Twenty female western flower thrips pupae and 20 male pupae were removed from the laboratory colony using a soft-bristled brush and placed onto filter paper (55 mm diameter) (Whatman International Ltd.; Maidstone, England) in a Gladware® container [7.8 x 5.1 cm (diameter x height)] (The Glad Products Company; Oakland, CA) with a piece of green bean (2 cm in length). Female pupae were distinguished from male pupae based on size (Yinping Li; preliminary data). Two to three days later, western flower thrips pupae developed into adults.

One female adult and one male adult were removed from the Gladware® container and placed onto filter paper in a new Gladware® container with a piece of green bean. Fifteen Gladware® containers with one female and one male per container were prepared following the above procedure and exposed to the same laboratory conditions as the western flower thrips colony. Female western flower thrips adults were checked every two to three days to determine whether they were alive. If females died, the date was recorded. If females were alive, the green bean was changed.

Results

The mean (± SEM) life span of female western flower thrips adults under laboratory conditions was 39.1 (± 5.4) days.