

ABSTRACT

WONG, SARAH KATHRYN. Optimizing the Black Pearl Pepper Banker Plant System for Biological Control of Thrips. (Under the direction of Dr. Steven D. Frank.)

Work for this thesis dealt with validating and optimizing a banker plant system for biological control of thrips. A review of the literature dealing with Western Flower Thrips, *Frankliniella occidentalis* Pergande, biological control agents of the pest, and the proposed banker plant system was conducted. Thrips are the most economically important pest of greenhouses worldwide and their management is increasingly difficult due to their ability to develop resistance to insecticides. Biological control of thrips is becoming increasingly popular due to decreased efficacy of insecticides, regulatory policies involving the use of chemicals, and consumer demand for sustainable production practices. Thrips are still controlled for the most part with chemicals; however, several biological control agents such as entomopathogenic nematodes, predatory mites, and generalist predators, such as the minute pirate bug, are now available around the world for thrips management.

Banker plants are intended to enhance biological control by sustaining populations of natural enemies. Banker plants do this by providing alternative sources of food for natural enemies, e.g. pollen for omnivorous predators, thus decreasing the likelihood of their starvation and emigration from a cropping system when pest populations are low or absent. A banker plant system consisting of the Black Pearl pepper, *Capsicum annuum* 'Black Pearl', and the omnivorous minute pirate bug, *Orius insidiosus* Say (Heteroptera: Anthocoridae) has recently been proposed to improve biological control methods for thrips management. There

is currently no research published about how or if the system works to suppress thrips populations. A review of literature relevant to this project is presented in Chapter 1.

Although the Black Pearl pepper banker plant system is already employed by many greenhouse and nursery growers, there is no formal research on if or how pollen from this plant might enhance life-history traits of *O. insidiosus*. Therefore, the study presented in Chapter 2 tested how pollen from the Black Pearl pepper plant affects *O. insidiosus* fitness and abundance through a series of laboratory and greenhouse experiments. Based on results from this study, a mixed diet of pollen and thrips increased *O. insidiosus* female longevity, decreased nymphal development time, and yielded larger females compared to a diet of thrips alone. Furthermore, *O. insidiosus* abundance was greater on flowering pepper plants than non-flowering pepper plants. We found that pollen from Black Pearl pepper banker plants could improve biological control of thrips in two ways: 1) reduce starvation and increase longevity of *O. insidiosus* when prey is absent; 2) enhance *O. insidiosus* fitness and fecundity when prey is present by mixing plant and prey diets. These results encourage future studies with the Black Pearl pepper as a banker plant for improving biological control of thrips in commercial greenhouses.

Lastly, there is no information in the literature as to how or if the Black Pearl pepper banker plant system can be effective within a complex growing system. In Chapter 3, we investigated the efficacy of the Black Pearl pepper banker plant system compared to augmentative releases of *O. insidiosus* for thrips management in a commercial nursery. We found that augmentative releases of *O. insidiosus* reduced thrips abundance in hoop houses compared to houses where no predators were released. However the presence of banker plants did not further reduce thrips abundance. Interestingly, we encountered spiders in 82%

of banker plant samples and hypothesized that spiders could reduce access to floral resources provided by banker plants, thus reducing their benefits for biological control. We found that spiders reduced *O. insidiosus* abundance on banker plants by reducing their survival. We conclude that this banker plant system may be more successful in enclosed growing systems where higher-order predators and emigration of *O. insidiosus* is restricted.

Optimizing the Black Pearl Pepper Banker Plant System for Biological Control of Thrips

by
Sarah Kathryn Wong

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APPROVED BY:

Steven Frank
Committee Chair

George Kennedy

David Orr

DEDICATION

I am truly blessed to have such a loving and accepting family. This thesis is dedicated to my wonderful parents who have given me an even better life than I could have dreamed of for myself. You have always supported my goals with an open mind and an open heart and for that I cannot say it enough, thank you.

BIOGRAPHY

Sarah Wong earned a B.S. degree in Biological Sciences from North Carolina State University in May, 2009. Prior to graduation she worked as a research technician in the Fred Gould entomology laboratory at North Carolina State University. During her time in the Gould lab she assisted Ph.D. student Jennifer Petzold in researching plant-herbivore interactions between *Physalis angulata* and *Heliothis subflexa*. This experiment focused on the plant response to eggs being laid on the foliage and how a variety of plant responses affected the survival of *H. subflexa* eggs. Sarah also assisted in other graduate student research with Ph.D. candidate Rachael Walsh. This research dealt with population dynamics of natural populations in larval *Aedes albopictus*, a mosquito that transmits Dengue fever. Throughout her time at the Gould lab, Sarah also assisted in maintaining various colonies of *H. subflexa* and *H. virescens*.

In August, 2009 Sarah started a Masters program in Entomology at North Carolina State University under adviser Steve Frank.

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CHAPTER 1

Western Flower Thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae),
biological control with the minute pirate bug, *Orius insidiosus* Say (Heteroptera:
Anthocoridae): a review

Coauthor Steven Frank*.

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

Abstract

Western Flower Thrips, *Frankliniella occidentalis* Pergande, is the most economically important greenhouse pest in the world. Biological control of this pest is increasingly popular due to thrips ability to develop resistance to insecticides. Thrips are managed with natural enemies such as entomopathogenic nematodes, predatory mites, and generalist predators including the minute pirate bug. This review describes thrips pest status, life-history, and management practices with emphasis on biological control agents of thrips and their life-histories. Finally, the review proposes a banker plant system to improve biological control of thrips in commercial greenhouses.

1. Western Flower Thrips Overview

1.1 Pest status

Western Flower Thrips (WFT) *Frankliniella occidentalis* Pergande is the most economically important greenhouse pest in the world. WFT feeds on over 240 plant species in over 60 plant families including most vegetable and ornamental crop plants (Brodsgaard 2004). Thrips feed on most plant parts including leaf tissue, stems, buds, flowers, nectar, and pollen (Trichilo and Leigh, 1986). Damage done by thrips is a function of its feeding and oviposition behavior. When a thrips feeds, it pierces the plant tissue with its mouthparts to consume the tissue contents (Heming, 1978). Thrips feeding causes aesthetic damage to fruit, for example tomato fruits and pepper pods (Frantz and Mellinger, 2009), buds, and leaves (Rahman et al., 2010) which can lead to lower quality products or yield loss (Frantz

and Mellinger, 2009). Thrips oviposit into leaf tissues using a saw-like ovipositor, which causes aesthetic damage to that tissue.

WFT also transmit the deadly Tomato Spotted Wilt Virus (TSWV) and Impatiens Necrotic Spot Virus. TSWV alone is responsible for over a billion dollars in various crop losses annually (Prins, 1998) and several million dollars in tobacco crop losses per year in North Carolina (Burrack, 2010). Many greenhouse vegetables and ornamentals are susceptible to TSWV and INSV. Symptoms of these viruses include brown or yellow rings on fruit, and necrotic spots on leaves (Zitter et al., 1989). Both TSWV and Impatiens Necrotic Spot Virus are incurable and cause damage, if not death, to plants and result in lower plant quality and or crop yield (Funderburk, 2009).

F. occidentalis is presently the most important thrips vector of TSWV and INSV in greenhouse crops. Larval thrips acquire the virus by feeding on infected plants, after an incubation period of a few days, the adults can then transmit the virus to other non infected host plants (Zitter et al. 1989). Once infected, thrips individuals can transmit the virus for up a month. Adult infected thrips do not pass on TSWV to their offspring; hence, the cycle is continued when uninfected larvae acquire the virus from feeding on infected plants.

1.2 Biology

WFT life cycle consists of an egg stage; two larval instars, which are active feeding stages; two soil-dwelling pupal stages; and a feeding adult stage (Reitz, 2009). WFT are haplodiploid in which unfertilized eggs hatch into males and fertilized eggs hatch into females. The sex ratio of WFT is approximately 1:1 at 23 C, but changes becomes more

male biased at low temperatures (15 C), or more female biased at high temperatures (32 C) (Kumm and Moritz, 2010). At 25 C, female *F. occidentalis* mean adult longevity is about 22 days and in that time females can lay about 80 eggs (Nielsen et al., 2010). Eggs develop in 4-5 days and larvae mature into adults in a little under 10 days at 25 C, >60 RH (Nielsen et al., 2010). Total development takes 17.2-22.4 days at 20 C (Ishida et al., 2003; Lublinkhof and Foster, 1977) but 44.1 and 15 days at 15 and 30 C respectively (Lublinkhof and Foster, 1977). Plant resources such as pollen can decrease development time and increase overall thrips abundance (Zhi et al., 2005; Nondillo et al., 2009; Riley et al., 2010).

1.3 Conventional Control

Growers apply broad-spectrum insecticides to prevent crop damage by thrips (van Lenteren, 2000). Insecticides can quickly reduce WFT abundance and subsequent feeding damage (Kay and Herron, 2010). However, thrips are able to evade insecticides multiple ways. Eggs are laid underneath leaf tissue and as such are protected and are unaffected by foliar insecticide sprays (Jensen, 2000). Larvae leave the plant to pupate in the soil thereby evading foliar sprays. Larval and adult feeding stages are concealed inside parts of leaves and flowers, such as between petals, within buds, or on the underside of leaves (Jensen, 2000). As a result of these behaviors, thrips can repopulate a greenhouse after insecticide applications. To provide adequate control, multiple insecticide applications, for example as many as 16 in tomato crops, are required throughout the growing season (Bauske et al., 1998).

Insecticide resistance makes thrips increasingly difficult to control with insecticides (Jensen, 2000). There are several chemical classes that can be used to manage thrips, which include: organophosphates (e.g. acephate), pyrethroids (e.g. bifenthrin), and Spinosyns (e.g. spinosad) (Frank, 2010b). However, isolated populations of WFT have developed some level of resistance to all of these chemicals in several locations: carbamates in Canada (Broadbent and Pree, 1997), bifenthrin in California (Immaraju et al., 1992), fipronil in Australia (Herron and James, 2005), and spinosad in the United States (Loughner et al., 2005) and parts of Europe (Bielza et al., 2007).

Spinosad is the most commonly used insecticide to manage thrips in greenhouses. Spinosad kills insects by causing involuntary muscle contractions (Salgado, 1998). The insect eventually becomes paralyzed by the overuse and fatigue of muscle neurons (Salgado, 1998). Photodegradation of the insecticide is reduced by greenhouse coverings therefore, the residuals tend to last longer inside greenhouses (Jones et al., 2005). A positive aspect of spinosad is that it is less toxic to natural enemies than pyrethroid or organophosphate insecticides (Kirst, 2010).

Although insecticides provide short-term results by reducing susceptible WFT populations, the use of insecticides as a sole management strategy is not sustainable. Once insecticides are applied, small populations of thrips that were not killed, either because individuals were resistant or concealed in plant parts, will quickly rebound. Achieving satisfactory plant protection requires repeated applications that lead to evolution of resistance and pose risks to workers, non-target organisms including natural enemies, and the environment. Therefore, new management strategies are required that will provide effective

thrips suppression and reduce insecticide applications. One sustainable option is the use of natural enemies for the biological control of thrips.

1.4 Biological Control

Interest in developing effective biological control strategies for thrips management has increased in response to decreasing effectiveness of insecticides, risks posed to workers and the environment, and consumer demand for sustainable farming practices. Several natural enemies are commercially available for use in biological control programs (Syngenta Bioline, 2011; Koppert Biologicals, 2012). Natural enemies that have been used for the biological control of thrips include parasitic wasps (Loomans et al., 2006), entomopathogenic nematodes, predatory mites, and omnivorous plant bugs (van Lenteren et al., 1997).

Parasitoids are used for biological control of arthropod pests in greenhouses including aphids, whitefly, scale insects, and leafminers (van Lenteren et al., 1997). Parasitoids are effective biological control agents because they are specialists meaning they oviposit and develop in a particular host species or taxonomic group. A benefit of using parasitoids as opposed to generalist predators is that parasitoid efficacy is not reduced in the presence of other arthropods that otherwise would be additional food sources.

The most prevalent thrips parasitoids found in the United States are *Ceranisus menes* (Walker) and *Ceranisus americensis* (Girault) (Hymenoptera: Eulophidae) (Loomans, 2006). Although these parasitoids are found worldwide in arid environments, there is limited literature on their ability to reproduce on *F. occidentalis* (Loomans, 2006). Parasitoids in commercial cucumber and sweet pepper crops were able to establish a population but

produced low rates of parasitism (<2%) and failed to suppress thrips populations (Loomans et al., 2006). Parasitoids are not widely used in the biological control of thrips due to the lack of literature demonstrating effective control and a lack of commercial availability.

Entomopathogenic nematodes (EPN) from the families Steinernematidae and Heterohabditidae are used as biological control agents of soil-dwelling life stages of pest insects, such as beetle larvae or thrips pupae (van Lenteren et al., 1997). EPN's infect their host with symbiotic bacteria found in the nematodes alimentary tract. The bacteria then infect the hemolymph of soil-dwelling life stages of the targeted pest. The bacteria convert host tissues into a form that can be used by the nematodes.

EPN's can be applied to soil or foliage with equipment that was initially intended for conventional pesticide applications, such as hose end and backpack sprayers, or through irrigation lines. However some methods are more effective than others (Shapiro-Ilan et al., 2006). EPN's are most commonly applied to the soil, as that is where they are found naturally; however, studies have investigated both soil and foliar application, for efficacy in controlling WFT. Foliar applications of EPN's are less successful for managing WFT due to the fact that EPN's are highly sensitive to sunlight and relative humidity. The soil-dwelling *Steinernema feltiae* and flower-dwelling *Thripinema nicklewoodi* are unable to provide adequate WFT suppression when applied to foliage regardless of rate applied (Arthurs and Heinz, 2006). While entomopathogenic nematodes can effectively control soil-dwelling life stages of thrips (Ebssa et al., 2001a; Ebssa et al., 2001b; Premachandra et al., 2003) their efficacy as a sole management tactic is limited due to thrips rapid development and overlapping of generations.

Entomopathogenic nematode efficacy can be limited by nematode concentration, temperature, and host density (Ebssa et al., 2004). Unlike the aforementioned foliar application study of EPN's, this study observed significant thrips suppression and increasing thrips mortality as EPN concentration increased. Depending on the native region of the nematode species, temperature may affect efficacy, where tropical species perform better in warmer temperatures and temperate species perform better at lower temperatures (Ebssa et al., 2004). Furthermore, different EPN species have varying mobility; therefore, efficacy can be affected by host density. For example, highly mobile EPN species may be underutilized when host density is low, and less mobile EPN species may reach a plateau in host suppression when host density exceeds their rate of infection resulting in failed pest suppression (Ebssa et al., 2004). This study observed significant thrips control, however, only at generally high concentrations of EPN's, which, given production costs of nematodes, is not economical. The authors suggested that, combined with future efforts to manipulate factors that affect EPN efficacy such as temperature, decreasing production costs may eventually lead to an economical practice of using high concentrations of EPN's for WFT management.

At any given time, only 35% of the thrips population in a greenhouse is pupating in the soil (Premachandra et al., 2003) so while EPN's are effective at reducing the number of thrips emerging from the soil, they have no affect on existing eggs and larvae that reside on a plant. EPN's cannot be expected to provide adequate control when curative releases are necessary to control already dense populations of thrips. Therefore, to improve biological control, EPN's are used in combination with other biological control agents that target the

feeding life stages of thrips. From the limitations of parasitoids and nematodes provided above, it is apparent that the implementation of either of these biological control agents is in need of improvement or these organisms should be used only in addition with other biological control agents.

Generalist and omnivorous predators can survive and reproduce in crop habitats even in the absence of pests by feeding on alternative foods or exhibiting prey switching (Coll and Guershon, 2002). Predators are especially important biological control agents of thrips for reasons in which other agents fall short: generalists and omnivores can feed on a variety of food items, thus maintaining populations longer than host-specific parasitoids which cease to reproduce once all suitable hosts have been utilized, and can consume multiple life stages of thrips unlike EPN's.

Mites in the family Phytoseiidae are widely used commercially available predators for biological control of thrips. Two of the most commonly used mite species are *Amblyseius swirskii*, and *Neoseilus cucumeris* (Gerson and Weintraub, 2007) and both have demonstrated significant reduction of thrips populations (Calvo et al., 2011; Messelink et al., 2006). However, reduction of thrips to acceptable levels for management is not always achieved with predatory mites (Weintraub et al., 2011). *A. swirskii* is a Mediterranean species, which primarily eats first instar thrips larvae (Wimmer et al., 2008) and can survive and reproduce on pollen alone (Park et al., 2011). *N. cucumeris* consume first instar thrips larvae and reside on lower parts of plants (Messelink et al., 2006), and can survive and reproduce on various pollen sources in the absence of prey food (Van Rijn and Tanigoshi, 1999).

Mites are also used in combination with other biological control agents such as EPN's, and can significantly reduce the number of thrips emerging from the soil as adults (Ebssa et al., 2006; Premachandra et al., 2003). Premachandra et al. (2003) found that combined releases of predatory mites and EPN's from either family Steinernematidae or Heterohabditidae reduced the number of emerging thrips. Furthermore, Ebssa et al. (2006) found that applications of entomopathogenic nematodes combined with predatory mites decreased thrips densities better than either natural enemy alone. Although mites can successfully reduce thrips abundance, growers who wish to use predatory mites must take into consideration the cost of manually distributing the mites, which can be a time consuming process (Opit et al., 2005).

Predators in the family Miridae, often referred to as plant bugs or leaf bugs, feed on a variety of target pests such as aphids, whiteflies, leafhoppers, spider mites, and thrips (Shipp and Wang, 2006). *Dicyphus tamaninii* (Heteroptera: Miridae), has been shown to be a successful biological control agent of WFT in cucumber (Gabarra et al., 1995), although similar success has not been demonstrated with other Mirid species. For example, *Dicyphus hersperus* (Heteroptera: Miridae) is not able to suppress thrips populations in time to prevent fruit damage in tomatoes and the predator itself can cause noticeable damage to fruit resulting in fruit loss (Shipp and Wang, 2006). Despite the potential, of Mirids, drawbacks such as slow development and damage to crops hinder their widespread use in biological control of thrips. Furthermore, Mirids are not commercially available in the United States and are not frequently used for thrips biological control in Europe.

Predatory bugs in the genus *Orius* (Heteroptera: Anthrocoridae) are sold commercially for biological control of many thrips species including *F. occidentalis* (van Lenteren et al., 1997). This predator is an avid hunter and can disperse farther than other biological control agents, such as mites, and feeds on multiple life stages. *Orius spp.* available for purchase in the United States include: *O. majusculus*, *O. laevigatus*, and most commonly *O. insidiosus*. Predators in this genus are commonly referred to as “minute pirate bugs”. Minute pirate bugs can be mass produced and have many desirable traits that make them good candidates for augmentative biological control. These traits include: unique behavior in which the predators aggregate around and within flowers, omnivory with the ability to develop and reproduce on pollen, and all life stages are predacious and will consume adult and larval thrips.

2. *Orius insidiosus* Overview

2.1 Biology

Orius insidiosus is native to the nearctic region (Sabelis and van Rijn, 1997) and is available for purchase in the United States. It is advertised as a biological control agent for thrips in greenhouses and is distributed by a number of biological control companies, including Syngenta Bioline, Koppert Biological Systems, and Arbico Organics. *O. insidiosus* is a hemi-metabolous ‘true bug’ with five nymphal instars and belongs to the order Heteroptera, family Anthocoridae. As an omnivore, *O. insidiosus* feeds on a variety of small, soft bodied arthropods including thrips, mites and aphids but also feeds on pollen (McCaffrey and Horsburgh, 1986; Corey et al., 1998). *O. insidiosus* is commonly referred to as the

‘insidious flower bug’ because the species often reside inside flowers where they encounter thrips adults and larvae (Baez et al., 2004).

Total development time for *O. insidiosus* females and males is around 30, and 29 days respectively at 18 degrees Celsius (van den Meiracker, 1994). However, as with thrips, development time decreases as temperatures increase (Isenhour and Yeargan, 1981; Mendes et al., 2005). At 20 C, egg development time is 8.8 days and total nymphal development time is 24.8 days (Isenhour and Yeargan, 1981). At increased temperatures of 32 C, egg development time is 3.5 days and total nymphal development time is 8.6 days (Isenhour and Yeargan, 1981). Although longer than thrips, *O. insidiosus* development time when reared on a diet of thrips is shorter than other biological control agents, such as Mirids, which should improve efficacy (Blaeser et al., 2004).

2.2 Diapause

Diapause is the temporary halt in development and activity in response to environmental conditions such as daylength, temperature, or availability of resources. WFT do not exhibit diapause, however adult *O. insidiosus* exhibit reproductive diapause during seasons when day length is short and temperatures are low. Diapause is one concern about the efficacy of *O. insidiosus* as a biological control agent. As such, certain growing situations and climates will not be suitable for *Orius* spp. to establish populations and effectively perform and suppress thrips populations when conditions are favorable for diapause.

O. insidiosus diapause is affected by a combination of temperature and photoperiod. The critical daylength (the minimum hours of daylight required to avoid diapause) for *O. insidiosus* is 11 to 12 hours (van den Meiracker, 1994). The low temperature development threshold for *O. insidiosus* is 10.3 degrees Celsius (Kingsley and Harrington, 1981). Although *O. insidiosus*, does not usually diapause at temperatures of 20-25 degrees Celsius, it will do so at these temperatures if the photoperiod is less than 11 hours (van den Meiracker, 1994).

Diapause can be averted or reversed using supplemental light and maintaining warm temperatures. For example, diapause was avoided when otherwise diapausing females were exposed to a short daylength of 9L:15D supplemented with blue light at varying temperatures (Stack and Drummond, 1997). Diapause can be reversed in *O. insidiosus* adults by increasing temperatures to 25 degrees Celsius and photoperiod to 16 hours (van den Meiracker, 1994).

2.3 Uses in Biological Control

Various *Orius spp.* including *O. insidiosus* can reduce thrips populations and maintain economically acceptable pest densities in different types of crops such as chrysanthemums and African violets (Silveira et al., 2004; Blaeser et al., 2004), sweet peppers (Bosco et al., 2008; Weintraub et al., 2011), and beans (Xu et al., 2006). *O. insidiosus* can suppress thrips populations in field peppers (Ramachandran et al., 2001) even better than broad spectrum insecticides (Funderburk et al., 2000).

Other predators such as mites, are used in combination with *Orius* spp. in an effort to improve biological control of thrips, however, the combination of the two does not always further suppress thrips populations. For example, when comparing thrips suppression in cut roses using both *O. insidiosus* and a predatory mite, *Amblyseius (Iphiseius) degenerans* and *Amblyseius (Typhlodromips) swirskii*, there were fewer thrips on roses when predators were present but combining mites and *O. insidiosus* did not further decrease thrips abundance (Chow et al., 2008; Chow et al., 2010). Control of thrips by predatory mites can be enhanced by adding *Orius* spp. For example, the predatory mite *Neoseiulus cucumeris* has failed in some instances to perform as well as *Orius* spp. at consuming thrips (Brown et al., 1999; Skirvin et al., 2005) but when *N. cucumeris* is used in combination with *Orius laevigatus*, thrips control can be successful (Urbaneja et al., 2003). Combining *Orius* spp. and predatory mites can control multiple pests as seen when combining *O. insidiosus* and the predatory mite *Phytoseiulus persimilis* to suppress WFT and spider mites (Fejt and Jarošik, 2000). This information may be useful in systems which are better suited for biological control with predatory mites due to plant composition or economic and labor feasibility.

3. Factors affecting biological control by generalist predators and *Orius* spp.

*3.1 Effect of crop plants on *Orius* spp. efficacy*

O. insidiosus behavior and efficacy is affected by the crop in which it is used. When given a choice of different plants (morning glories, greenbeans, soybeans, pigweed, and velvetleaf), no *O. insidiosus* lay eggs on velvetleaf plants and prefer morning glories and green beans (Lundgren et al., 2008). In this same study, nymphs that hatched on morning

glories and green beans had the greatest longevity, which suggests that female *O. insidiosus* select plants for oviposition based on traits that favor nymphal survival. *O. insidiosus* failed to suppress thrips on tomatoes despite an abundance of 10 adults per plant, which suggested that the predators failed to reproduce or produce reproductive populations and did not establish in tomatoes (Shipp and Wang, 2003). Dead *O. insidiosus* nymphs were found stuck to the leaves of tomato plants in this study, which is consistent with other studies reporting that *Orius* searching ability is hindered on tomatoes due to the glandular hairs of the plant (Coll and Ridgeway, 1995). As such, *O. insidiosus* should not be expected to perform well on plants with similar structural traits.

3.2 Effect of floral resources on generalist predator and Orius spp. efficacy.

Pollen provides insects with a source of essential nutrients (Stanley and Linskins, 1974). Pollen as an alternative source of food can enhance survival, decrease development time, and improve fecundity among omnivores (Abdallah et al., 2001; De Clercq et al., 2005; Vanderkerkhove and De Clercq, 2010). Omnivores, such as ladybeetles will consume plant based foods even in the presence of abundant prey resources, indicating that plant foods complement a prey based diet (Berkvens et al., 2010; Davidson and Evans, 2010). In some instances, pollen alone is sufficiently nutritious to support predator reproduction and development (Carrillo et al., 2010; Beckman and Hurd, 2003; Berkvens et al., 2008; de Oliveira et al., 2010; Perdakis and Lykouressis, 1999). The effects of pollen and plant resources on life-history traits such as longevity, survival, development, fecundity, and pest consumption is important to understand when using natural enemies. These effects have been

investigated for several omnivores including some popular biological control agents such as parasitoid wasps, ladybeetles, predatory mites, and *Orius* spp.

Pollen and plant resources alone or in addition to prey can increase longevity of natural enemies. Providing floral resources such as nectar can increase parasitoid longevity 15-fold and improve fecundity (Wratten et al., 2003). Another study found that longevity of the aphid parasitoid, *Aphidius colemani*, was significantly increased when exposed to Yellow Giant Hyssop, but not so when exposed to Bee Balm, although the flowering plants are in the same family of Lamiaceae (Walton and Isaacs, 2011).

Pollen is considered most important when prey items are low-quality or scarce (Lundgren, 2009). For example, lacewing larvae (*Chrysoperla carnea*) fed pollen in suspension with nectar and *Drosophila melanogaster* developed faster and yielded larger adults than larvae reared on low-quality, prey-only diets (Patt et al., 2003). In another similar example, survival of big eyed bugs (*Geocoris punctipes*) fed a high-quality prey diet of moth eggs was unchanged given high- or low-quality plant based food; lima bean pods or leaves respectively (Eubanks and Denno, 1999). However, the same study found that when big eyed bugs were fed a low-quality prey diet of aphids, survival was significantly improved when mixed with high quality plant food, lima bean pods. Other benefits of mixing plant and prey foods include the ability of nymphs to develop solely on pollen, for example ladybeetles and corn pollen (Lundgren and Widenmann, 2004), or increased fecundity in ladybeetles (Kajita and Evans, 2010).

O. insidiosus is a generalist predator and may exhibit prey switching in the presence of multiple prey species. Baez et al. (2004) compared predation by *O. insidiosus* of *F.*

occidentalis to the predation of another thrips species, *Frankliniella tritici*, in petri dishes. *O. insidiosus* was more likely to prey upon *F. occidentalis* than *F. tritici* although it had more encounters with *F. tritici*, which the authors hypothesized was caused by a higher success rate of capture of the more sedentary *F. occidentalis* by *O. insidiosus*. Similarly, Reitz et al. (2006) observed that *O. insidiosus* preferred *F. occidentalis* over the thrips species *Frankliniella bispinosa*, which was able to escape *O. insidiosus* more often than the less active *F. occidentalis*. The same pattern is witnessed among less mobile thrips larvae compared to adults where *Orius* spp. is more likely to consume larvae (Isenhour and Yeargan, 1981; Baez et al., 2004). Predators often choose prey that are considered more vulnerable even if it is not the most nutritious as seen in predatory Carabid beetles and various prey options such as aphids, fruit flies, and grasshoppers (Lang and Gsodl, 2001). The tendency for *O. insidiosus* to switch to more abundant and vulnerable prey is an important consideration when using this predator in biological control.

As an omnivore, *O. insidiosus* feeds on a number of different arthropod and plant resources. Omnivory is just one trait that makes *O. insidiosus* a good candidate for biological control because omnivores can exploit various resources as they are available during the growing season in order to survive and maintain populations (Landis et al., 2000; Symondson et al., 2002 Griffiths et al., 2008). As a result, when offered multiple options for food and/or habitat, omnivorous predators like *O. insidiosus* have the potential to sustain populations throughout an entire growing season with fewer or even a single augmentative release (Coll and Guershon, 2002). Female *Orius* spp. that feed on only pollen can produce viable eggs and offspring (Cocuzza et al., 1997) and nymphs can complete development on

certain types of pollen alone or in combination with other plant material in the absence of prey (Kiman and Yeargan, 1985; Richards and Schmidt, 1996). When comparing various diets of plant and prey material alone or in combination, nymphs were able to complete development on honeybee pollen (maple pollen) alone, although development was significantly faster on a mixed diet of thrips and pollen (Kiman and Yeargan, 1985). In another study, nymphs were not able to complete development solely on corn pollen, yet were able to do so on green beans alone, and the combination of both pollen and green beans significantly reduced development time compared to green beans alone (Richards and Schmidt, 1996).

Lastly, *Orius* spp. are attracted to flowers where they feed on pollen (McCaffrey and Horsburgh, 1986; Corey et al., 1998) and are found in diverse, flowering weed field borders (Atakan, 2010). However, the addition of pollen may have negative effects for biological control. For example, overall predation of thrips by *O. laevigatus* is greater when pollen is absent (Shakya et al., 2009). Reasons for this may be that predators spend less time searching for prey when pollen is available or predators become satiated more easily when offered multiple types of food (Shakya et al., 2009). Furthermore predation of thrips by single *O. laevigatus* adults is decreased in the presence of pollen, but predators were also more likely to remain on plants when pollen was present (Skirvin et al., 2007). On the other hand, *Orius* spp. adults visit flowers more often than other plant parts (Atakan, 2006) and pollen shedding in corn correlates with peak population densities of *O. insidiosus* (Coll and Bottrell, 1991). *O. insidiosus* and thrips alike reside within flowers, which provide the two

with a common meeting place (Hansen et al., 2003) and may actually improve biological control by increasing encounters between predator and prey.

3.3 Effect of alternative food on intraguild predation among natural enemies and Orius spp.

In addition to the above mentioned benefits of mixing plant and prey diets, the availability of pollen and plant resources may reduce intraguild predation and cannibalism. Natural enemy abundance is often positively affected by plant diversity. For example: aphid predator abundance generally increases as vegetational diversity increases (Elliott et al., 1998) and diamondback moth parasitoids are recruited by buckwheat plants in broccoli fields (Lavandero et al., 2005). Corn pollen in addition to prey, the Cassava green mite, increased survival and longevity of two predatory mites and significantly decreased intraguild predation (Onzo et al., 2005). Intraguild predation can even be reduced by various plant structures, such as domatia (an example would be tufts of hair near leaf veins, which provide structural complexity). Floral resources that are structurally complex may have unforeseen benefits to reducing intraguild predation by providing refuges for natural enemies (Ferreira et al., 2011).

Conversely, increasing natural enemy abundance and diversity with floral resources may sometimes allow for intraguild predation and reduce efficacy of natural enemies (Vance-Chalcraft et al., 2007). Providing plant resources could reduce predator efficacy by providing alternative food, thus reducing the number of pests consumed by an omnivore (Frank et al., 2011). In addition, providing plant resources to recruit or conserve generalist predators may interrupt biological control when one recruited predator eats the biological

control agent as seen in carabid species (Prasad and Snyder, 2004). In the above example with the Cassava green mite, suppression of the pest was not enhanced when both predators were present regardless of the presence of corn pollen, and pest suppression was greatest with a single predator (Onzo et al., 2005). Pollen and floral resources may recruit and increase generalist predator abundance, which is desired in many agricultural settings, however the effects of this may not translate into increased pest suppression in all cases.

When released in addition to other biological control agents, *O. insidiosus* has the potential to switch prey and become an intraguild predator; however, this has mixed impacts on biological control. When combining predators, *O. insidiosus* is most commonly released with predatory mites. Chow et. al. (2008, 2010) found that *O. insidiosus* began to preferentially feed on predatory mites in cut roses when the mites were more abundant than the target prey, thrips. Although *O. insidiosus* acted as an intraguild predator in these studies, it did not affect thrips management as there were similar numbers of harvestable flowers when *O. insidiosus* was the sole predator and in combination with the predatory mite (Chow et al., 2008). Similar results were found in pepper when *O. insidiosus* was released with the predatory mite *Amblyseius swirskii*: biological control of thrips was not improved by combining the two predators, however the effects of intraguild predation were not great enough to discourage using the predators in combination (Dogramaci et al., 2011). Combined releases of *O. insidiosus* and predatory mites did not significantly reduce Flower Thrips in blueberries, suggesting intraguild predation may have been occurring (Arevalo et al., 2009).

Orius spp. will also interact with other predators commonly found in agricultural and natural systems but this also has inconsistent or insignificant effects on pest suppression. *Orius majusculus* fed on Mirid predators even in the presence of primary prey such as thrips, but this did not affect *O. majusculus* predation rate of thrips and the same study found that the pirate bugs preferred thrips over Mirids (Jakobsen et al., 2004). Hemipteran predators such as *Orius tristicolor* and big-eyed bugs disrupt the ability of predatory mites to establish, but improve spider mite control in annual agroecosystems (Colfer et al., 2003). *Orius spp.* are also intermediate predators in some instances, for example, *O. tristicolor* is suppressed by big-eyed bugs in cotton when targeting spider mites, however, the effect that this intraguild interaction had on spider mite suppression by *O. tristicolor* was not tested (Rosenheim, 2005).

3.4 Effect of pesticides on *Orius spp.* efficacy

Growers rarely rely solely on biological control and often combine biological control with standing practices of chemical control; however, natural enemies can be negatively affected by pesticides, the use of which becomes increasingly important when timing applications with natural enemy release. On greenhouse and field cotton, chemicals including fipronil, abamectin, and emamectin benzoate caused significant mortality (>50%-100%) of *O. insidiosus*, whereas spinosad had the lowest overall mortality rate of 11.3-33.8% (Studebaker and Kring, 2003). These findings are consistent with other greenhouse trials where the mortality rate of *O. insidiosus* exposed to spinosad was less than 25% eight days after application (Jones et al., 2005). In one study, augmentative releases of *O. insidiosus*

effectively suppressed thrips throughout a season in chrysanthemums (Silveira et al., 2004). However, an application of deltamethrin at the end of the growing season killed *O. insidiosus* eliminating predation of thrips, and as a result, thrips populations rebounded (Silveira et al., 2004). Similar results were seen in field-grown eggplant where thrips populations remained low in fields that were not treated, however thrips populations eventually increased in fields treated with fenthion due to *Orius* sp. mortality (Nagai, 1990). Growers can promote survival and efficacy of natural enemies by separating natural enemies and pesticides in time and/or space. In addition, growers trying to integrate pesticides with biological control should use pesticides and application techniques that are compatible with natural enemies, and reduce the frequency of insecticide applications.

4. Proposal of a banker plant system for thrips management with *O. insidiosus*

Banker plants are a technique in biological control that blends methods from augmentation and conservation biological control. Banker plants have been defined by Frank (2010) as “A plant that directly or indirectly provides resources, such as food, prey, or hosts, to natural enemies that are deliberately released within a cropping system.” The first author to use the term “banker plant” investigated control of greenhouse whiteflies in tomato using the parasitoid *Encarsia formosa* (Stacey, 1977). In this example, the author used tomato plants which were infested with parasitized whitefly scale as ‘banker plants’ and then placed them into glasshouses with a tomato crop. The goal of a banker plant system is to decrease pest abundance by sustaining populations of natural enemies. Although the tomato crop was

not heavily infested with whiteflies, natural enemy populations of parasitoids were sustained on banker plants, which were providing alternative whitefly hosts.

The most well studied banker plant system uses hymenopteran parasitoids to control pest aphids in greenhouses (Goh et al., 2001; Van Driesche et al., 2008; reviewed in Frank, 2010). In this system, a banker plant consists of a cereal plant infested with non-pest aphids, often the bird cherry oat aphid that feeds strictly on grasses. The non-pest aphids are then parasitized by the wasps and the banker plant placed inside the growing system. The emerging second generation of parasitoids will move throughout the greenhouse to parasitize pest aphids that are feeding on the crop. In this parasitoid-aphid system, the banker plant is supporting natural enemies by providing an alternative prey host. Other banker plant systems could directly support omnivorous predators by providing plant resources like pollen and nectar.

The Black Pearl Pepper is an ornamental pepper plant that has generated interest among commercial growers as a banker plant to be used in a system for managing thrips with *O. insidiosus* (Rodda, 2011; Valentin, 2011). The goal of this banker plant system is to provide enough pollen, to sustain populations of *O. insidiosus* whether or not prey are present. The Black Pearl pepper is a worthwhile candidate for investigating as a banker plant for several reasons that deal with logistics of implementation and high output of floral resources, not to mention the fact that several growers are already using the system without confirmation of its efficacy. The pepper plant is already sold commercially as an ornamental pepper and as such can be purchased readily as small plugs or plants or even grown in-house from seeds. The pepper also has a woody stem to keep it from drooping or falling over onto crop plants. Most

importantly, this pepper exhibits indeterminate growth in which it continuously flowers year-round, and is encouraged to increase flower production by manually picking off fruit.

As the greenhouse ornamental industry in the United States grows and consumers demand the use of sustainable growing practices, the need for comprehensive banker plant systems will increase for growers trying to incorporate biological control in their Integrated Pest Management programs. Currently there is not enough literature to design such a system for the use of *O.insidiosus* to control WFT; however, commercial growers are currently using this system without any evidence of its efficacy. There is a clear need for sustainable thrips management due to their development of resistance to insecticides and unique characteristics which make them difficult to control. There are well documented benefits of providing plant resources like pollen to omnivores, however not all pollen and plant resources are equivalent. Therefore if the Black Pearl pepper is to be developed as a banker plant for managing thrips with *O. insidiosus* in commercial greenhouses, research is needed to elucidate how pollen from this plant may or may not benefit this predator. If pollen from this plant improves life-history traits of *O. insidiosus*, then developing a banker plant system with Black Pearl Pepper would be a novel and avenue to explore for sustainable thrips management.

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CHAPTER 2

Pollen increases fitness and abundance of *Orius insidiosus* Say (Heteroptera: Anthocoridae)
on banker plants

Coauthor Steven Frank*.

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

Abstract

Banker plants are intended to enhance biological control by sustaining populations of natural enemies. Banker plants do this by providing alternative sources of food for natural enemies, such as pollen for omnivorous predators, thus decreasing the likelihood of their starvation and emigration from a cropping system when pest populations are low or absent. A banker plant system consisting of the Black Pearl pepper, *Capsicum annuum* 'Black Pearl', and the omnivorous minute pirate bug, *Orius insidiosus* Say (Heteroptera: Anthocoridae) has recently been proposed to improve biological control methods for thrips management. There is currently no research published about how or if the system works to suppress thrips populations. Therefore, this study tested how pollen from the Black Pearl pepper plant affects *O. insidiosus* fitness and abundance through a series of laboratory and greenhouse experiments. Based on results from this study, a mixed diet of pollen and thrips increased *O. insidiosus* female longevity, decreased nymphal development time, and yielded larger females compared to a diet of thrips alone. Furthermore, *O. insidiosus* abundance was greater on flowering pepper plants than non-flowering pepper plants. From these results, we see that pollen from Black Pearl pepper banker plants could increase *O. insidiosus* abundance for the purpose of biological control in two ways: 1) reduce starvation and increase longevity of *O. insidiosus* when prey is absent; 2) enhance *O. insidiosus* fitness and fecundity when prey is present by mixing plant and prey diets. These results encourage future studies with the Black Pearl pepper as a banker plant for improving biological control of thrips in commercial greenhouses.

1. Introduction

Banker plant systems are intended to improve fitness, abundance, and efficacy of biological control agents in greenhouses (Frank 2010). Banker plant systems consist of non-crop plants that provide alternative hosts for parasitoids, prey for predators, or plant-based resources such as nectar and pollen for omnivores (Frank, 2010). Therefore, banker plant systems are intended to decouple natural enemy-pest population dynamics in order to sustain natural enemy survival and reproduction within a growing system when pest populations are low or absent (Frank, 2010). Interest in banker plant systems is increasing among researchers and growers due to their potential to improve biological control by sustaining populations of natural enemies (Huang et al., 2011; Frank, 2010; Rodda, 2011; Valentin, 2011). However, research is needed to demonstrate and improve the efficacy of this biological control strategy.

Recently, the Black Pearl pepper (*Capsicum annuum* 'Black pearl') has been proposed as a possible banker plant intended to improve biological control of thrips (Valentin, 2011). Growers use the ornamental Black Pearl pepper as a banker plant instead of other peppers because they grow and flower indeterminately thus producing pollen continuously. A typical Black Pearl pepper banker plant system consists of placing pepper plants throughout a greenhouse and releasing omnivorous Minute Pirate Bugs, *Orius insidiosus* Say (Hemiptera: Anthocoridae) with the goal of preventing a thrips outbreak.

The first proposed benefit of this system is that *O. insidiosus* can avoid starvation by feeding on pollen provided by Black Pearl pepper flowers if thrips populations are low or absent. This is especially important in crops without suitable pollen sources. In addition, it

has been proposed that pepper flowers may increase encounters between the predator, *O. insidiosus* and prey, *Frankliniella* spp. (Thysanoptera: Thripidae) by providing a common meeting place for the two since both reside in and around flowers (Hansen et al., 2003). The second proposed benefit of this system is to enhance *O. insidiosus* fitness and fecundity traits when prey is present by mixing plant and prey diets. Despite growers' present use of this system and claims that it works as part of a biological control program, there is no published research documenting how or if Black Pearl pepper pollen benefits *O. insidiosus* fitness for the purpose of suppressing thrips populations in commercial greenhouses.

Omnivores often benefit from or even require pollen as part of their diet (Coll and Guershon, 2002). Availability of pollen as an alternative source of food can enhance survival, decrease development time, and improve fecundity of omnivorous predators (Abdallah et al., 2001; De Clercq et al., 2005; Vanderkerkhove and De Clercq, 2010). *O. insidiosus* is an omnivore that feeds on a variety of small, soft bodied arthropods including thrips, mites and aphids but also feeds on pollen (McCaffrey and Horsburgh, 1986; Corey et al., 1998). The effect of pollen on *Orius* fitness and development is the subject of conflicting reports. For example, longevity of *O. insidiosus* adult females is significantly increased when corn pollen is added to a diet of green beans and/or *Ephestia kuehniella* eggs compared to either diet alone or in combination (Richards and Schmidt, 1996). In contrast, *O. sauteri* adult female survival is not significantly increased when a diet of aphids, *Aphis gossypii*, is supplemented with corn pollen (Funao and Yoshiyasu, 1995). Likewise, *O. insidiosus* nymphs have been reported to develop solely on pollen from Common mullein, *Verbascum thapsus* L. (McCaffrey and Horsburgh, 1986) and honeybee pollen (Kiman and Yeargan,

1985). However, *O. insidiosus* nymphs fed corn pollen are not able to complete development (Richards and Schmidt, 1996). Black Pearl pepper banker plants could conserve or increase abundance of *O. insidiosus* populations in the absence of prey if pollen alone improves *O. insidiosus* survival and development.

In the presence of prey, Black Pearl pepper banker plants could increase *O. insidiosus* abundance and efficacy if pollen supports *O. insidiosus* fitness more than prey or if a mixed diet of pollen and prey increases *O. insidiosus* fitness more than prey alone. Based on a preliminary greenhouse experiment, our hypothesis is that Black Pearl pepper pollen will increase *O. insidiosus* abundance through the benefits of diet mixing (Wong and Frank, 2011). We test this hypothesis and the underlying mechanisms with a series of factorial greenhouse and laboratory experiments designed to determine how pollen from Black Pearl pepper plants affects *O. insidiosus* fitness and abundance. This study specifically looked at how pollen affects survival and development time, female size, and abundance on individual banker plants. Results from this study will provide a foundation of information as to how Black Pearl pepper pollen affects *O. insidiosus* fitness and abundance and whether the pepper plants merit further investigation as banker plants for improving biological control of thrips in commercial greenhouses.

2. Methods

2.1 Study Organisms

All *O. insidiosus* were purchased from Koppert Biologicals (Howell, MI), and used for experiments within one week of arrival. When not being used, *O. insidiosus* were kept in

a refrigerator set on its least cool setting. Black Pearl pepper plants (128 plugs/flat; plants <7cm in height) were supplied by Van Wingerden International (Mills River, NC) and C. Raker and Sons Inc (Litchfield, MI). Plants were transplanted to 10.2 cm pots and allowed to grow for eight weeks until they were transplanted to 11.36 liter pots. Fafard 2 light weight soil mix was used for all plants with 473 ml of Osmocote/soil bag (14-14-14; 2.8cu ft or 0.85cu meters). Western flower thrips, *Frankliniella occidentalis* Pergande, were obtained from a colony started from field collected adults and maintained in the laboratory on green beans and cabbage.

2.2 Effect of flowers and thrips on female *O. insidiosus* longevity

To determine if Black Pearl pollen, thrips prey, or a mixed diet of both increase *O. insidiosus* adult longevity, we conducted a 2x2 factorial laboratory experiment that crossed two pollen treatments (absent or present) with two thrips treatments (absent or present). The experiment was conducted in arenas made from plastic 50mL Corning vials (Corning, Corning, NY). One hole (2 cm diameter) was made in the top and side of each vial for ventilation. Holes were covered with thrips screen and secured with hot glue to prevent escape of experimental organisms. A third smaller hole (0.7 cm diameter) was made through the bottom tip of the vial. A schematic of the vials is presented in Figure 1.

Black Pearl pepper stems were collected from greenhouse pepper plants. Each stem was 6-8 cm in length and had three leaves. All buds and flowers were picked from ‘flower absent’ treatment stems. Buds and 1-2 open flowers were left on stems in ‘flower present’ treatments. The bottom-half of each stem was wrapped in cotton then pulled through the

small hole in the bottom of vials so that the stem fit snugly in the hole. The cut end was inserted into a #55 (12.7cm, 10 ml) floral-pick (Syndicate Sales Inc., Kokomo, Indiana) filled with tap water. Floral-picks were stuck into a Styrofoam board and a wire-grid was made from craft wire in order to support the experimental vials in an up-right position. An example of this set-up is presented in Figure 1. To establish ‘thrips present’ and ‘thrips absent’ treatments, 20-30 adult thrips were added to ‘thrips present’ treatment vials. Then one female *O. insidiosus* was added to each vial. 10 replicates of each treatment were conducted simultaneously. The entire procedure was repeated yielding 20 replicates of each treatment.

O. insidiosus survival was determined daily by gently tapping and turning each vial until the female was found alive or dead. Living females were transferred to a new vial, set up as described, twice a week so *O. insidiosus* would have fresh plant and insect material for refuge and food. Vials were placed in an incubator at 27-28° C and 55-60% RH for the entirety of the experiment.

Statistical Analysis. ANOVA was used to test the effect of pollen, thrips, and their interaction on total number of days females survived (SAS, 2008).

2.3 Effect of pollen and thrips on development of *O. insidiosus* nymphs

To determine how pollen and thrips prey affect development of *O. insidiosus* nymphs, we conducted a factorial laboratory experiment that crossed two pollen levels (absent or present) with two thrips levels (absent or present). Experiments were conducted using *O. insidiosus* nymphs within 24 hours of hatching. *O. insidiosus* nymphs were reared

similarly to Kiman and Yeargan (1985) procedures. To obtain eggs, adult *O. insidiosus* were placed in a plastic container with two moist cotton balls for water, *Heliothis subflexa* Guenée (Lepidoptera: Noctuidae) eggs (obtained from NCSU colony) for food, and pole beans or green beans as an oviposition substrate. The adult colony container was placed in an incubator at 27-28° C and 55-60% RH so adult *O. insidiosus* could lay eggs. Every 2-3 days, beans were inspected under a stereo dissecting scope for eggs. Sections of bean that contained eggs were removed and placed on filter paper in 5 cm petri dishes and placed back in the incubator. Petri dishes were inspected for newly emerged nymphs daily.

New nymphs were placed into an experimental arena. Experimental arenas were 5 cm petri dishes that contained a piece of filter paper moistened with 1-2 drops of tap water. A disc of Black Pearl pepper leaf was cut out using a 2 cm cork borer, soaked in tap-water to regain turgidity, and placed on the filter paper. To establish ‘thrips present’ treatments, 10 second instar thrips were placed in each petri dish. To establish ‘pollen present’ treatments, eight anthers from Black Pearl pepper flowers were added to each petri dish. Anthers were obtained by picking open Black Pearl pepper flowers that had pollen producing anthers and cutting the anthers out with dissecting scissors. This was done once a week or as needed and anthers were stored in a sealed plastic container in a refrigerator. As a precaution, the plastic container was filled with CO₂ and sealed for 20 minutes in order to kill any small arthropods, such as mites, in or on the anthers. Depending on the treatment, dishes were replenished with 4-5 anthers and 5-10 thrips (depending on how many were still alive from the previous day) so nymphs did not run out of food. Every two-three days nymphs were placed in new arenas with a new leaf disc and filter paper. Since nymphs were emerging daily, a few

nymphs at a time were assigned to each treatment until a minimum total of 20 nymphs had been assigned to each treatment. All petri dishes were placed in an incubator at 27-28° C and 55-60% RH for the entirety of the experiment. Arenas were inspected daily with a dissecting scope to record nymphal survival. Observation of nymphal development lasted until all nymphs had died or matured into adults.

Statistical Analysis. A chi-square test was conducted to compare the total number of *O. insidiosus* completing development among the treatments. ANOVA was used to test for significant effects of pollen, thrips, and their interaction on the number of days nymphs took to complete development from egg hatch to adult eclosion (SAS, 2008).

2.4 Effect of pollen and thrips on O. insidiosus adult size

To determine the effect of pollen and thrips on adult *O. insidiosus* size we conducted a laboratory experiment with two treatments: ‘thrips’ and ‘thrips + pollen’. These were the only treatments that enabled *O. insidiosus* to complete development in the previous experiment. *O. insidiosus* was reared from first instar to adult according to the procedures described in Section 2.3. Once nymphs had matured into adults, they were sexed, and the length of their hind tibia measured.

Statistical Analysis. A t-test was used to compare the length of adult female and male hind tibias among treatments (SAS, 2008).

2.5 Effect of flowers and thrips on *O. insidiosus* abundance

To determine how flowers affect *O. insidiosus* abundance on Black Pearl pepper banker plants, we conducted a greenhouse experiment with individually bagged peppers as experimental units. Two treatments were created by allowing plants to flower versus continuously picking buds and flowers from other plants. There were a total of 40 plants, or 20 replicates per treatment.

Plants were dipped in insecticidal soap (Safer Brand, Lititz, PA) every two days for one week to remove thrips and other arthropods prior to establishing either ‘flower present’ or ‘flower absent’ treatments. One week before the experiment began, individual plants were placed in a bag made from organdy fabric (Jo-Anns Fabric, Raleigh, NC; 60cm wide x 121.9cm tall). Two 91.4 cm bamboo stakes were inserted on the outer edge of the pot to support the bag. The top of the bag was sealed by twisting the fabric and securing it with a large binder clip. Plants remained in bags for the entirety of the experiment.

Plants in the ‘flower present’ treatment were encouraged to flower by picking all peppers and dying flowers from each plant. To establish ‘flower absent’ treatment plants, open flowers and buds were removed from each plant. This procedure was repeated the day before the experiment began and twice per week for the duration of the experiment to maintain the ‘flower present’ and ‘flower absent’ treatments. After both flower treatments were established, 20 adult thrips were also added to each plant so that thrips could lay eggs on the plants and build populations in the absence of predators. Plant material removed before the experiment began was discarded. On week zero, one week after treatments were

established and thrips were added to plants, 20 adult *O. insidiosus* (male:female ratio of 1:1) were added to each plant.

Plants were sampled for arthropods once a week. Each plant was beaten a total of four times for four seconds each time into a 33 x 40.5cm white tray. *O. insidiosus* adults and nymphs, and thrips were counted and collected using an aspirator. Plants were then visually inspected to count open flowers from ‘flowers present’ treatment plants and remove buds or open flowers from ‘flowers absent’ treatment plants. All trapping materials on the beat tray, picked peppers, flowers, buds, and aspirated insects were placed back on their respective plant. This was done so that any insects that were not aspirated, or eggs that had been laid on foliage, would be put back onto plants. This experiment ran for a total of six weeks from 6 June 2011 – 19 July 2011.

Statistical Analysis. A repeated measures ANOVA was used to test the effect of flowering and non-flowering plants on *O. insidiosus* adult and nymphal abundance and thrips abundance over a course of six weeks. Data for both nymphs and thrips was $\log(x+1)$ transformed to correct for non-normal distribution (SAS, 2008).

3. Results

3.1 Effect of flowers and thrips on female O. insidiosus longevity

The presence of flowers significantly increased female *O. insidiosus* longevity ($F = 10.46$; $df = 1, 32$; $P = 0.003$) such that females in either ‘flower present’ treatment lived on average 1.67 - 2.09 days longer than females in ‘flower absent’ treatments (Fig. 3). Thrips did not significantly affect *O. insidiosus* female longevity ($F = 1.42$; $df = 1, 32$; $P = 0.242$)

nor was there a significant interaction between flowers and thrips ($F = 2.58$; $df = 1, 32$; $P = 0.118$).

3.2 Effect of pollen and thrips on development of *O. insidiosus* nymphs

A Chi-square test confirmed that there was a significant difference among the four treatments ($X^2_3 = 55.5$; $P < 0.0001$) in number of nymphs that completed development. The frequency of nymphs completing development in either of the ‘thrips absent’ treatments was 0% regardless of the presence of pollen. The Chi-square analysis showed that significantly more nymphs in the ‘thrips present’ treatments completed development in the presence of thrips and pollen (74%) compared to thrips only (42%) ($X^2_3 = 6.08$; $P = 0.014$).

Furthermore, in the presence of thrips, flowers significantly decreased total development time by 1.18 days ($t = 5.78$; $df = 31$; $P < 0.0001$; Fig. 4).

3.3 Effect of pollen and thrips on *O. insidiosus* adult size

Overall hind tibia lengths were greater for females (36.94 ± 0.39) than males (35.15 ± 0.63) ($t = 2.54$; $df = 46$; $P = 0.0147$). Females with a mixed diet of thrips and pollen had longer hind tibiae than females fed only thrips ($t = 2.17$; $df = 25$; $P = 0.0398$) but males did not ($t = 0.70$; $df = 19$; $P = 0.4894$) (Fig. 4). The interaction of diet and sex was not significant ($F = 0.25$; $df: 1, 47$; $P = 0.6171$).

3.4 Effect of flowers and thrips on *O. insidiosus* abundance

Although some flowers were able to open in the ‘flowers absent’ treatment, the overall mean abundance of flowers was significantly greater on ‘flowers present’ plants (15.23 ± 0.99) than ‘flowers absent’ plants (2.07 ± 0.57) ($F = 408.12$; $df = 1, 215$; $P = <0.0001$). For abundance of *O. insidiosus* adults, there was a significant interaction of sampling date and treatment ($F = 6.98$; $df = 5, 224$; $P = <0.0001$; Fig. 7) as demonstrated by oscillating adult abundance due to population cycles. Similarly, there was a significant interaction of sampling date and treatment for abundance of *O. insidiosus* nymphs ($F = 12.53$; $df = 5, 223$; $P = <0.0001$; Fig. 7) due to population cycles where nymphal abundance was decreased after nymphs had matured to adults. Adult and nymphal abundance was greater in the presence of flowers ($F = 251.57$; $df = 1, 224$; $P = <0.0001$ and $F = 446.64$; $df = 1, 223$; $P = <0.0001$ respectively). Abundance for adults and nymphs changed significantly by sampling date ($F = 6.53$; $df = 5, 224$; $P = <0.0001$ and $F = 14.65$; $df = 5, 223$; $P = <0.0001$), respectively. During observation, nymphs could be seen in and around Black Pearl pepper flowers probing for pollen.

Total thrips (adults + nymphs) abundance also showed a significant interaction of sampling date and treatment as *O. insidiosus* adults and nymphs ($F = 16.64$; $df = 5, 223$; $P = <0.0001$). Thrips abundance was greater when flowers were absent ($F = 8.49$; $df = 1, 223$; $P = 0.0039$; Fig. 6), and here was a significant difference in thrips abundance among sampling dates ($F = 4.7$; $df = 5, 223$; $P = 0.0004$).

4. Discussion

The goal of banker plant systems is to improve biological control by supporting populations of natural enemies when pest populations are low or absent (Frank, 2010). This study found that Black Pearl pepper pollen can benefit *O.insidiosus* fitness and development resulting in larger populations of the natural enemy on Black Pearl pepper plants when flowers are present. This is the first evidence that Black Pearl pepper banker plants can increase longevity when thrips prey are absent and reduce development time and increase likelihood of survival to adult when prey are present. These results support the claim that Black Pearl pepper banker plants can sustain populations of *O. insidiosus* when prey are scarce or absent and is a valuable step in validating the benefit of Black Pearl pepper banker plant systems that growers are already implementing.

The first goal of this banker plant system is to reduce starvation and increase longevity of *O. insidiosus* when prey is absent by providing pollen, which allows them to survive long enough to reproduce and consume incoming or growing pest populations. Providing plant resources can improve biological control by sustaining populations of omnivorous natural enemies rather than letting them starve or emigrate from the cropping system in search of food (Landis et al., 2000; Coll and Guershon 2002; Griffiths et al., 2008). Sometimes providing pollen in the total absence of prey is enough to sustain omnivores for a period of time (Beckman and Hurd, 2003; Carrillo et al. 2010). This study found that *O. insidiosus* females survived longer when flowers were present regardless of thrips presence. These results are consistent with other studies in which *O. insidiosus* have similar if not

increased longevity when pollen is present regardless of prey availability (Kiman and Yeargan, 1985; Cocuzza et al., 1997).

Secondly, this banker plant may provide nutrition that is lacking in prey and as a result, enhance *O. insidiosus* fitness and fecundity traits when prey is present by mixing plant and prey diets. Several taxa of omnivorous predators are represented in the literature as being able to complete development on pollen alone, such as mites (Carrillo et al., 2010), mantids (Beckman and Hurd, 2003), ladybird beetles (Berkvens et al., 2008), green lacewings (de Oliveira et al., 2010), and mirids (Perdikis and Lykouressis, 1999). While no nymphs were able to complete development when pollen was the only source of food, a mixed diet of pollen and thrips allowed nymphs to complete development in significantly less time than nymphs fed only thrips. These results are similar to other studies supporting the fact that mixed diets of prey and plant food can enhance various life history traits including decreasing development time (Patt et al., 2003; De Clercq et al., 2005; Lundgren, 2009). This study also found that a mixed diet of thrips and pollen as opposed to a prey only diet yielded significantly larger females. This may have positive consequences and increase female fecundity as demonstrated by other insect taxa such as Coleoptera and Hemiptera (Kajita and Evans, 2010; Moya-Raygoza and Garcia-Medina, 2010) and the generalized theory that larger individuals are more fecund (Honek, 1993). Furthermore, mixed diets of plant and prey food may provide benefits not covered in the present study, for example female ladybird beetles fed a mixed diet of pollen and *E. kuehniella* eggs had shorter pre-oviposition periods than females fed a prey-only diet (Berkvens et al., 2008).

In the greenhouse experiment, *O. insidiosus* abundance was significantly higher on flowering plants than non-flowering plants, which is consistent with laboratory results from this study that demonstrated that pollen increases female longevity. Survival is the first benefit of banker plants in which the goal is to increase the likelihood that adults will survive long enough to reproduce. On the first sampling date, less than five adults survived per non-flowering plant, whereas flowering plants had more than twice as many adults per plant. Thus nymphal abundance on flowering plants increased between week one and two. The lack of nymphs on non-flowering plants indicates adults that survived on these plants did not reproduce, perhaps due to decreased fecundity or increased nymphal mortality on non-flowering plants. Although not tested directly, nymphal mortality could have increased if nymphs had difficulty finding food. In addition, alternative plant resources can reduce cannibalism among juvenile insects (Frank et al. 2010).

There seem to be some compounding factors affecting *O. insidiosus* abundance on flowering plants, as there was an extreme difference in abundance between the two treatments. Few of the initial 20 adult *O. insidiosus* survived from week zero to week one (Fig. 7) on non-flowering plants, whereas nearly half survived on flowering plants. These results are consistent with our laboratory results (Fig. 3) in which females fed a prey only diet survived less than a week but females fed a mixed diet survived a little more than a week. Adult populations continued to decrease from week one to week two in flowering treatments (Fig. 7), which was not surprising as female longevity was less than two weeks according to our laboratory results and there were only a handful of potential adults (nymphs) found on average in samples on week one. However, nymphal abundance increased from

week one to week two, and translated into increased adult abundance on week three, which is consistent with our laboratory results in which nymphs fed a mixed diet of prey and pollen completed development in about a week (Fig. 4). In the absence of pollen, not only were there fewer adults to reproduce and lay eggs after the first week, it is likely that fewer nymphs completed development, and did so more slowly than nymphs on flowering plants. This same trend was seen in our laboratory results (Section 3.2) when significantly fewer nymphs completed development and developed slower on a prey only diet compared to a mixed diet of thrips and pollen. Overall, the greenhouse abundance experiment mimicked our laboratory experiments in adult longevity and nymphal survival and development time, which, in combination, led to a dramatic difference in *O. insidiosus* abundance between flowering and non flowering plants.

One factor not examined in the laboratory was the within-plant distribution of *O. insidiosus* and prey, which may have changed in the absence of flowers. One study noted that 90% of *F. occidentalis*' time was spent within pepper flowers and almost 100% of thrips feeding occurred inside flowers (Reitz et al., 2006). It is known that *O. insidiosus* aggregate within flowers with *F. occidentalis* (Shipp et al., 1992; Hansen et al., 2003); without flowers, *O. insidiosus* and thrips have lost a common meeting place. Thus *O. insidiosus* may have had difficulty finding or capturing thrips, which would have negative fitness consequences. As a consequence, the predator must spend more time in search of prey, which could impair nymphal development or decrease the total number of eggs laid.

Another phenomenon observed in the greenhouse experiment was that thrips abundance was significantly lower on flowering plants compared to plants without flowers.

This is inconsistent with studies demonstrating that pollen increases thrips abundance (Nondillo et al., 2009; Riley et al., 2010). However in this case, thrips populations were higher on non-flowering plants despite an overall decrease in nutrition for thrips and the presence of predators that would be highly dependent on prey food in the absence of pollen. The fact that there were still a number of thrips, although fewer, on flowering plants provides useful insight into this system. First, the presence of flowers did not result in a significant increase in thrips abundance such that would damage a crop. Second, flowering Black Pearl pepper banker plants may be providing small sentinel populations of thrips, which would help sustain populations of *O. insidiosus*.

This study found that Black Pearl pepper pollen can enhance life-history traits of *O. insidiosus* in a number of ways: increase female longevity, decrease nymphal development time, increase female size, and increase predator abundance on flowering peppers. The first potential benefit to enhancing the fitness and abundance of *O. insidiosus* would be a reduced need for repeated releases of natural enemies. This benefit comes as a consequence of sustaining the initially released population of *O. insidiosus* and supporting subsequent offspring by increasing their rate of survival, decreasing their development time so that they reach reproductive maturity faster, and improving their fecundity. A second potential benefit would be increased predator efficacy through increased *O. insidiosus* abundance. These results indicate that pollen from the Black Pearl pepper banker plant is a potentially valuable tool for enhancing biological control, future studies should investigate implementation of this system.

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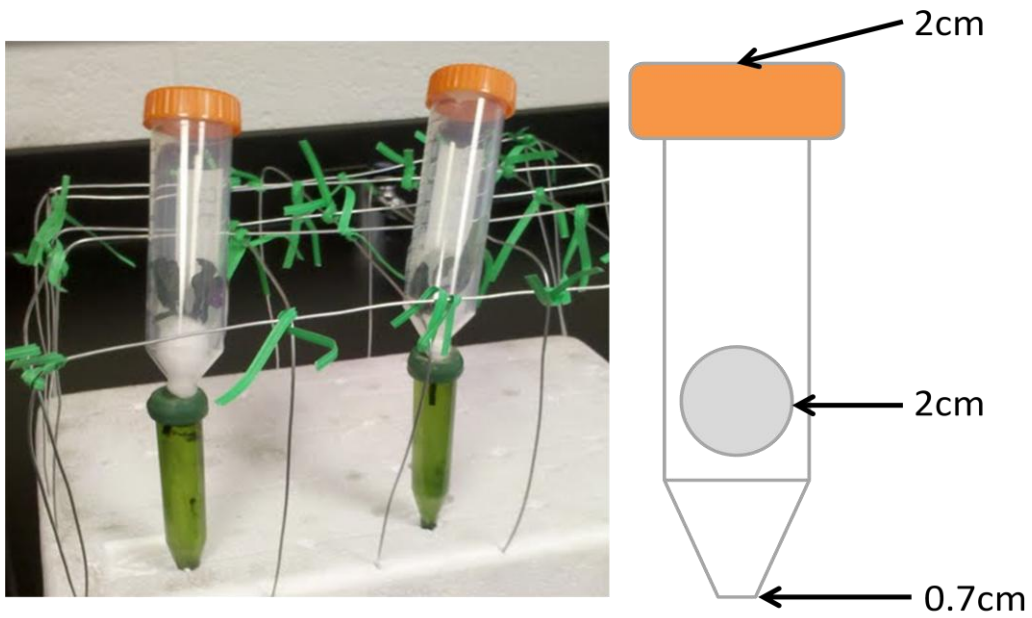


Figure 1. Experimental arenas made from 50ml Corning Vial held up by a wire grid with Black Pearl pepper stems in water picks (left), and schematic of vial with hole sizes (right).

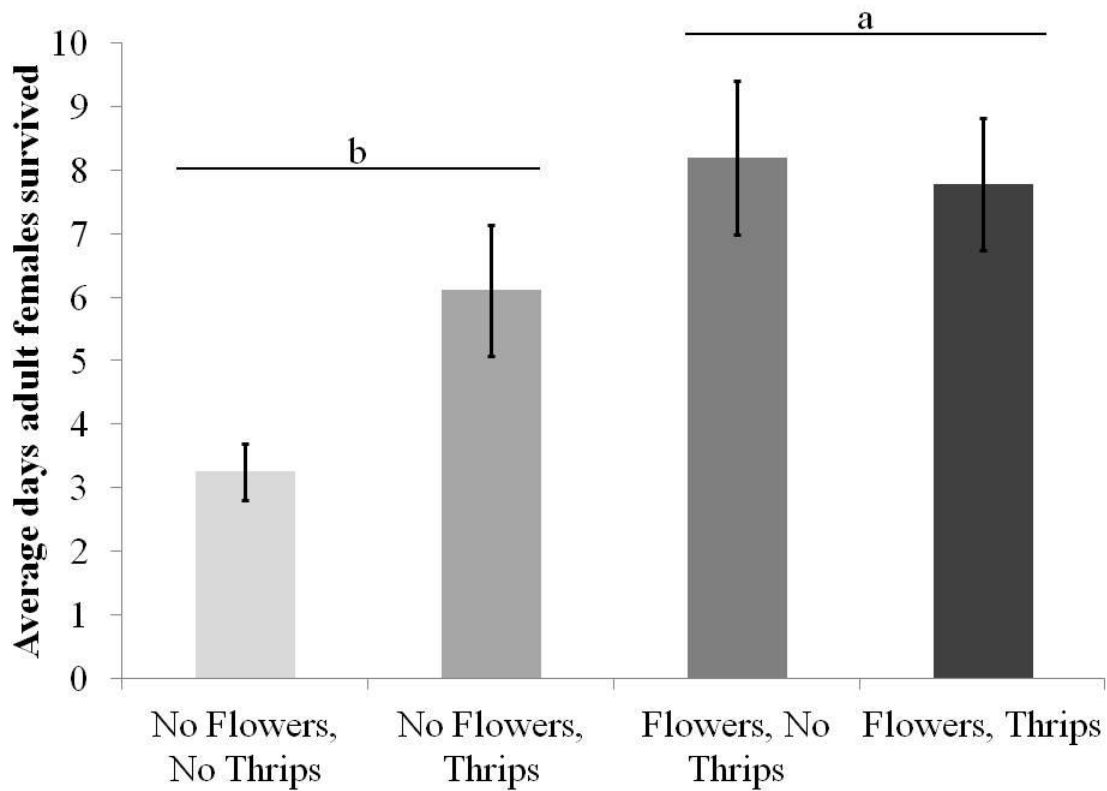


Figure 2. Mean (\pm SEM) days adult female *O. insidiosus* survived when fed diets of Black Pearl pepper flowers, *F. occidentalis*, both, or neither. Females survived on average (from left to right in the figure) 3.5 (\pm 0.45), 6.11 (\pm 1.03), 8.2 (\pm 1.21), and 7.78 (\pm 1.05) days. All experimental arenas contained a Black Pearl pepper leaf disk and moist filter paper. Different letters above horizontal bars indicate significant ($P < 0.05$) main effect of flowers on *O. insidiosus* survival.

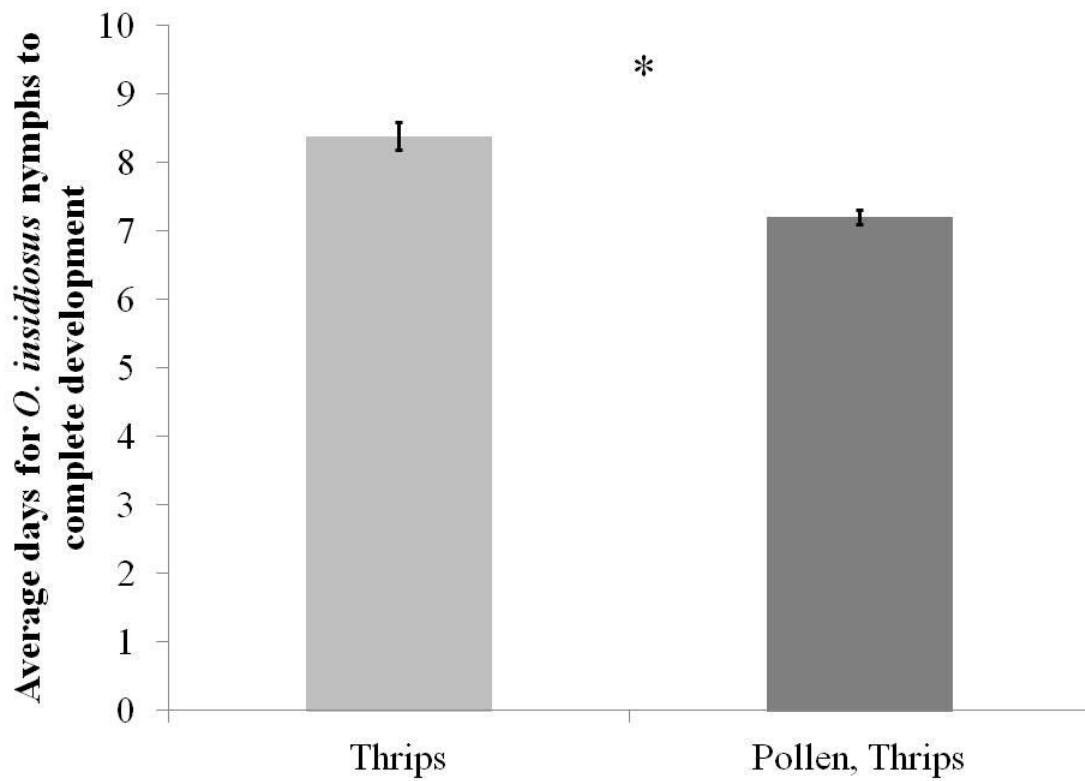


Figure 3. Mean (\pm SEM) days required for *O. insidiosus* nymphs to complete development from egg-hatch to adult eclosion on a diet of *F. occidentalis* alone or in combination with Black Pearl pepper pollen. * indicates significant difference between treatments ($P < 0.05$).

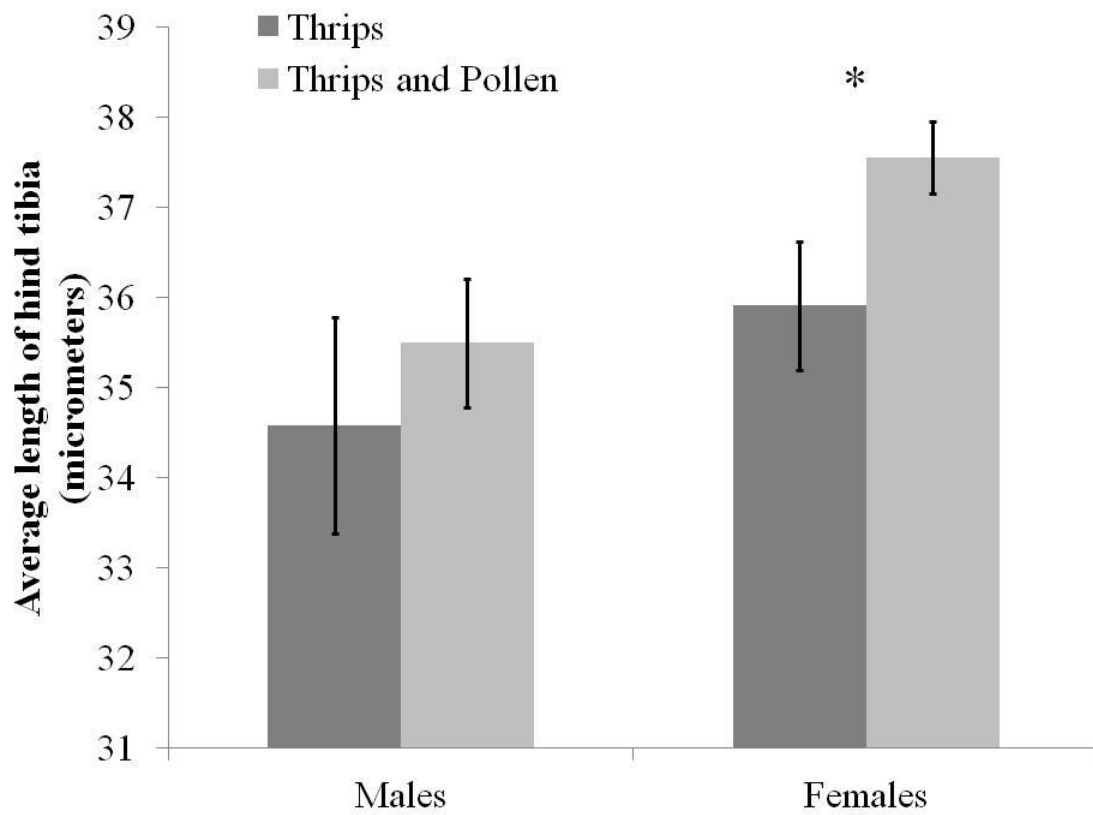


Figure 4. Average length of hind tibia (\pm SEM) among males and females fed thrips only or a mixed diet of thrips and pollen. * indicates significant difference between treatments ($P < 0.05$).

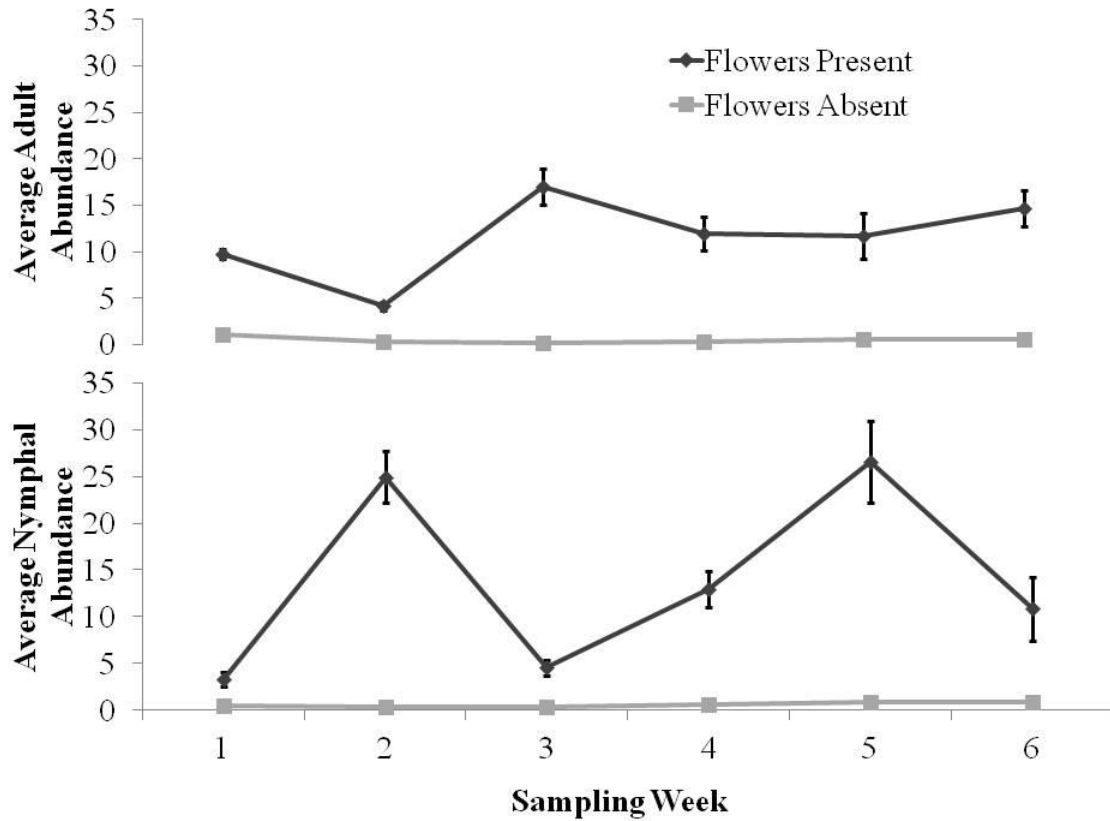


Figure 5. Weekly mean (\pm SEM) abundance of *O. insidiosus* adults (top) and nymphs (bottom) found in beat samples of caged Black Pearl pepper plants with and without flowers. Overall mean abundance for adults was 11.54 ± 1.80 /plant and 0.51 ± 0.13 /plant for flowers present and absent respectively. Overall treatment mean abundance for nymphs was 13.83 ± 4.06 /plant and 0.58 ± 0.09 /plant for flowers present and absent respectively.

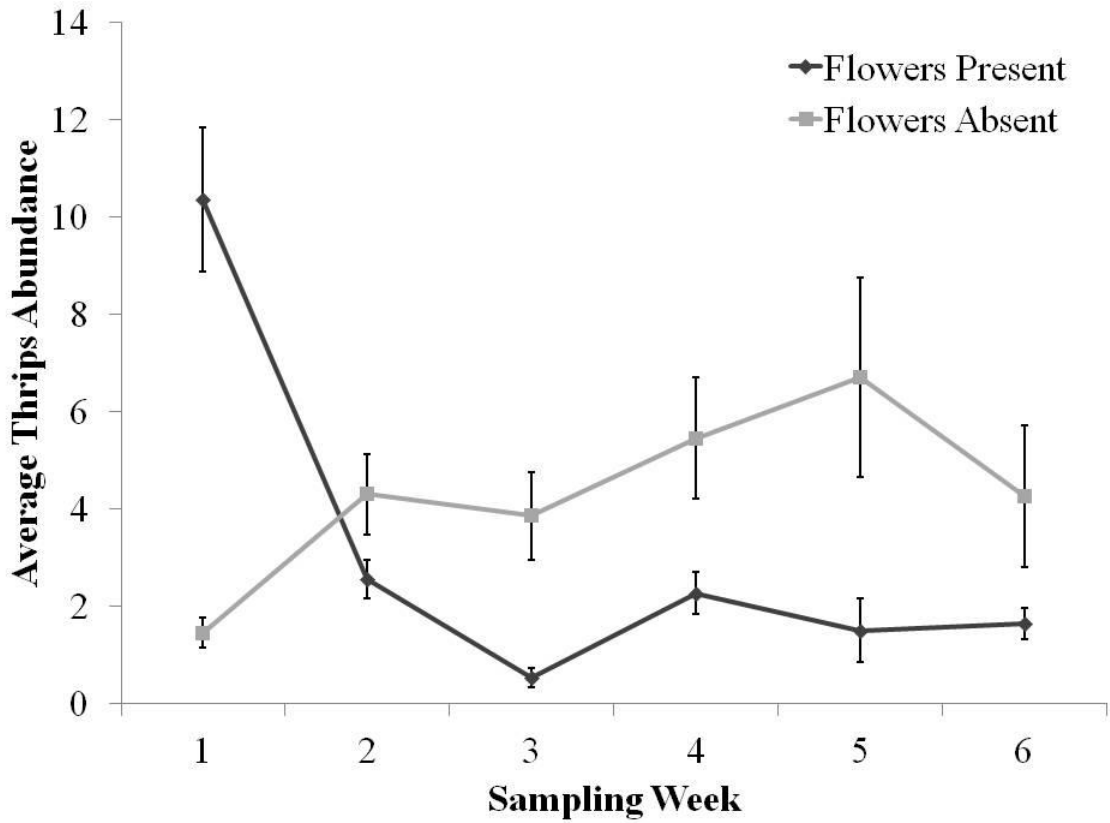


Figure 6. Weekly mean (\pm SEM) abundance of thrips found in beat samples. Overall treatment mean abundance for thrips was 3.14 ± 1.47 /plant and 4.33 ± 0.72 /plant for flowers present and absent respectively.

CHAPTER 3

Spiders disrupt conservation biological control by *Orius insidiosus* (Heteroptera:
Anthocoridae)

Coauthor Steven Frank*.

Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613

Abstract

Banker plant systems are a form of conservation biological control intended to enhance natural enemy efficacy by providing an alternative source of food when prey items are scarce or absent. The Black Pearl pepper, *Capsicum annuum* ‘Black Pearl’, banker plant system provides pollen to sustain populations of the omnivorous predator *Orius insidiosus* Say (Heteroptera: Anthocoridae). Black Pearl pepper pollen has been shown in previous studies to increase *O. insidiosus* longevity, survival to adult, female size, and abundance, and decrease nymphal development time. However, there is no research demonstrating the efficacy of this banker plant system in commercial crop production. We investigated the efficacy of the Black Pearl pepper banker plant system compared to augmentative releases of *O. insidiosus* for thrips management at a commercial nursery that produces native and ornamental grasses. We found that augmentative releases of *O. insidiosus* effectively reduced thrips abundance in hoop houses compared to houses where no predators were released. However the presence of banker plants did not further reduce thrips abundance. Interestingly, we found spiders in 82% of banker plant samples during this experiment and hypothesized that spiders could reduce access to floral resources provided by banker plants, thus reducing their benefits for biological control. We found that spiders reduced *O. insidiosus* abundance on banker plants by increasing the rate at which *O. insidiosus* emigrate and reducing their survival. We conclude that this banker plant system may be more successful in enclosed growing systems where higher-order predators and emigration of *O. insidiosus* is restricted.

1. Introduction

Banker plant systems are a form of conservation biological control intended to increase natural enemy longevity and reproduction within a cropping system (Frank 2010). Banker plant systems consist of non-crop plants that provide alternative hosts for parasitoids, prey for predators, or plant based resources such as nectar and pollen for omnivores (Frank 2010). For example, a banker plant system targeting aphids uses potted grain plants infested with non-pest aphids to provide hosts for parasitoid wasps (Goh et al., 2001; Van Driesche et al., 2008). This allows parasitoids to reproduce in greenhouses even when pest aphids are absent. Interest in banker plant systems is increasing among researchers and growers due to their potential to improve biological control by sustaining natural enemy populations (Frank, 2010; Huang 2011). However, there is uncertainty as to how banker plant systems will perform across a variety of growing systems with different crops and management practices.

Recently, the Black Pearl Pepper (*Capsicum annuum* 'Black pearl') banker plant system has been used by growers to improve biological control of thrips by the minute pirate bug, *Orius insidiosus* Say (Wong, unpublished results; Valentin, 2011). Biological control of thrips generally consists of augmentative releases of predatory mites (Acari: Phytoseiidae) or *Orius* spp. (Heteroptera: Anthocoridae) (van Lenteren, 2000; Reitz, 2009). However, thrips suppression has been mixed when using either predator alone or in combination (Shipp and Wang, 2003; Silveira, 2004; Messelink et al., 2006; Chow et al., 2010). Unreliable efficacy stems in part from predator mortality or emigration when pollen or prey is unavailable as food within the crop. Pollen from Black Pearl peppers has been shown to decrease *O. insidiosus* nymphal development time, and increase survival to adult, longevity, female size,

and overall abundance on flowering peppers (Wong, unpublished results). Although Black Pearl pepper pollen improves *O. insidiosus* fitness and abundance in laboratory experiments, the efficacy of this banker plant system has never been tested empirically.

Providing plant resources, such as pollen, as a conservation biological control tactic, can enhance natural enemy abundance (Lavandero et al., 2005; Gardiner et al., 2009; Atakan, 2010), which sometimes results in increased pest suppression (Root, 1973; Lavandero et al., 2005; Irvin et al., 2006), however sometimes pest suppression is not enhanced (Bugg and Wilson, 1989; Chang and Snyder, 2004). Providing plant resources may reduce predator efficacy by providing alternative food and reducing a predator's consumption of pests (Frank et al., 2011). However, since previous studies have shown that Black Pearl pepper pollen increases *O. insidiosus* fitness and abundance on Black Pearl pepper plants, we predict that any reduction in per-capita consumption of pests will be compensated by increased predator abundance. In addition, since *O. insidiosus* and thrips are both found feeding within flowers (Shipp, 1992), adding Black Pearl pepper banker plants to a cropping system may increase the likelihood that the two will meet (Hansen et al., 2003) and possibly provide even greater pest suppression.

Increasing plant diversity can increase natural enemy abundance and diversity (Elliott et al., 1998); therefore, conservation biological control can sometimes lead to predation of biological control agents by higher-order predators (Vance-Chalcraft et al., 2007). For example, predatory lacewing larvae survival was decreased with the addition of generalist hemipteran predators, which resulted in loss of control of aphid populations (Rosenheim et al., 1993). In another example, immigrating larger carabid beetles disrupted consumption of

fly eggs by smaller carabid and staphylinid beetles either by directly consuming the intermediate predators or disrupting their consumption of fly eggs (Prasad and Snyder, 2004). However, providing predators with alternative food can reduce negative effects on biological control agents (Rosenheim et al., 1995) as seen when cannibalism among carabid larvae was decreased due to addition of fly pupae as alternative food (Frank et al., 2010). Since intraguild predation is common among biological control agents released to control thrips (Chow et al., 2008; Chow et al., 2010), the potential for banker plant systems to increase or decrease negative interactions between predators will be an important factor affecting their efficacy (Frank, 2010).

As nursery and greenhouse growers are already interested in implementing banker plant systems, and Black Pearl Pepper pollen has been shown to improve *O. insidiosus* fitness and abundance, the next important step in developing this banker plant system is to implement it in a commercial setting and observe interactions that affect its efficacy. The first objective of this study was to determine how a Black Pearl Pepper banker plant system affects pest and natural enemy abundance in a commercial nursery. Secondly, we tested whether the presence of pollen affects *O. insidiosus* predation of thrips. Lastly, we examined how the presence of higher-order predators, endemic spiders, affects retention of *O. insidiosus* released on banker plants. By observing the Black Pearl Pepper banker plant system where it is currently being used, and testing hypotheses that might affect its efficacy, we hope to provide useful insight to improve efficacy and adoption of the Black Pearl Pepper banker plant system.

2. Methods

2.1 Effect of banker plants on pest abundance

To determine how Black Pearl pepper banker plants affect pest and natural enemy abundance compared to augmentative releases, we conducted an experiment at Hoffman Nursery in Rougemont, NC, which produces native and ornamental grasses in plastic covered hoop houses. Hoffman Nursery was selected for these experiments because they had been implementing the Black Pearl pepper banker plant system for two growing seasons and presently manages several of their hoop houses each season with biological control, using purchased natural enemies and compatible insecticides. The hoop houses used in these experiments were all 6.7m wide but had different lengths ranging from: smallest (25.6m) to largest (38.4m). Sides of the houses were rolled up in summer to maintain a cooler temperature. Each house had four rows of grasses in 30.48cm x 60.96cm flats with 32 grass plugs per flat. Each row was two flats wide (1.22 m) and as long as the houses.

We set up two treatments (Augmentation and Banker Plant) and one control replicated four times in 12 hoop houses. Augmentation and Banker Plant houses were treated the same as Control houses except that we released one bottle of 500 count *O. insidiosus* (Koppert Biologicals, Howell, MI) per house every three weeks (May 25th, June 15th, and July 7th, 2010). *O. insidiosus* were released by sprinkling them evenly on plant flats moving lengthwise from one end of the house to the other. Banker Plant houses had Black Pearl Pepper banker plants (2-3 feet tall; 9" pot) spaced six per row at alternating lengths within the house (Fig. 1) resulting in 24 banker plants per banker plant treatment house, with the exception of the smallest house which only had five banker plants per row for a total of

20 banker plants. To encourage continuous flowering, we picked peppers from each banker plant in banker plant houses for 1-2 minutes each week. Houses were not treated with synthetic insecticides but were spot-treated occasionally with insecticidal soap for aphids, spittle bugs, and leafhoppers which are not pests targeted by *O. insidiosus*. All houses were treated weekly with a rotation of fungicides (azoxystrobin, triazazole, iprodione, *Trichoderma harzianum* strain T-22, thiophanate-methyl, myclobutanil, polyoxin, phenylamide, strobilurine, carboxylic acid amide, mancozeb).

Arthropod sampling was conducted once a week for nine weeks (26 May – 20 July, 2010) by vacuuming plant flats using a modified Husky Blower Vac (Husqvarna, 125 BVX Series) fitted with organdy (Jo-Anns Fabric, Raleigh, NC) bags to catch insects. One randomly selected 1.22m x 1.83m section of grasses was vacuumed in each row (four per house) for a total of 8.93 m² per house. The four samples from each house were vacuumed into a single organdy bag. Bags were placed in a sealed jar in the freezer for 24 h to kill arthropods. Then the contents of each bag were emptied into a jar of 70% ETOH for later observation. Samples were viewed under a dissecting scope to count thrips and spider mites. On weeks when natural enemies were released, sampling took place the day after release.

We used a Maximum Likelihood repeated measures ANOVA to determine how banker plants and augmentative release affected the seasonal abundance of thrips and spider mites. All data were log (x+1) transformed to correct for non-normal distribution (SAS, 2008).

2.2 Banker plant sampling

Individual banker plants were sampled to monitor for *O. insidiosus*, spiders, and thrips. Four plants from each banker plant treatment house were sampled every week from June 8th – July 23rd by randomly selecting one plant from each of the four rows in a house and beating the plants over a 33 x 40.5cm white tray. Organisms were counted then returned to the plant.

2.3 *O. insidiosus* predation in the presence and absence of Black Pearl pepper pollen

To determine if the presence of pollen affected *O. insidiosus* predation rate of Western Flower Thrips, we conducted a factorial experiment with two flower treatments (present or absent) crossed with two predator treatments (*O. insidiosus* present or absent). Experimental arenas were made from 50ml plastic Corning vials according to Wong, unpublished methods.

Each arena had a 6-8 cm Black Pearl pepper stem. All buds and flowers were removed from ‘flowers absent’ treatment stems; buds and 1-2 open flowers were left on stems in ‘flowers present’ treatments. We placed 30 adult thrips in each container and then added a single female *O. insidiosus* to ‘predator present’ treatment vials. After 48 hours all contents of the vials were emptied and thrips were counted as alive or dead.

Statistical Analysis. ANOVA was used to test the effect of pollen, predators, and their interaction on total number of thrips remaining alive (SAS, 2008).

2.4 How spiders affect *O. insidiosus* retention on banker plants

To determine how spiders affected *O. insidiosus* abundance and retention on Black Pearl pepper banker plants, we manipulated spider abundance on banker plants at Hoffman Nursery. In follow-up experiments we measured the consumptive effects of spiders on *O. insidiosus* abundance and retention by restricting *O. insidiosus* emigration with cages. Then we measured the non-consumptive (behavioral) effects of spiders on *O. insidiosus* by creating ‘risk only’ spiders that had glued mouth parts and could scare but not consume *O. insidiosus*.

In the first experiment, we tested the abundance and retention rate of *O. insidiosus* on banker plants at Hoffman Nursery by conducting factorial experiment with two spider treatments (present or absent) crossed with two sampling time treatments (1 hour or 3 hours). Eight banker plants from each Banker Plant treatment house were prepared by beating them to remove spiders and *O. insidiosus*. Spiders were then collected from the surrounding landscape by beating grasses and banker plants. Salticidae spiders were used because they were the most prevalent spider family on banker plants at Hoffman Nursery. Twenty *O. insidiosus* (Koppert Biologicals, Howell, MI) were added to banker plants, followed by 4 spiders. After 1 or 3 hours, plants were sampled by beating them over a white tray (33 x 40.5cm) to count *O. insidiosus* and spiders. This experiment was repeated 30 June and 14 July 2010 for a total of 16 replicates per treatment.

To test the consumptive effects of spiders on *O. insidiosus* abundance and retention rate, we conducted another factorial experiment in a research greenhouse at North Carolina State University on July 21st, 2010. Treatments were the same as in the first experiment at

Hoffman Nursery. Plants were individually caged by placing them into 60 cm wide x 121.9 cm tall organdy bags (Jo-Anns Fabric, Raleigh, NC) so that *O. insidiosus* could not emigrate from plants. Approximately 120 spiders were captured from natural areas on campus, 75% of which were Oxyopidae, the third most prevalent spider family on banker plants at Hoffman Nursery, and 25% were Salticidae. After adding 20 *O. insidiosus* to each plant, 4-6 randomly selected spiders were added to 'spiders present' treatment plants. Plants were sampled at 1 or 3 hours as described above to count *O. insidiosus* and spiders. Banker plants that were similar in size, age, and maintenance to banker plants at Hoffman Nursery were used in this experiment for a total of 10 replicates for each treatment.

To test behavioral effects of spiders on *O. insidiosus* abundance and retention rate on banker plants, we conducted another factorial experiment at North Carolina State University on August 21st, 2010. The treatments and sampling methods were as described above in the previous two experiments. However, plants were not in cages so *O. insidiosus* could emigrate from plants. 'Risk only' spiders with non-functioning mouthparts were used to measure *O. insidiosus* that were emigrating from banker plants instead of being consumed by spiders. To do this, spider mouthparts were glued prior to the experiment with 'Liquid Bandage' (Rite Aid Corporation, Harrisburg, PA) surgical glue that is non-toxic and dries quickly. To apply glue, spiders were rendered unconscious with CO₂ and then held upside down with soft forceps to expose their mouthparts so that a small drop of glue could be applied with a camel-hair paintbrush. Once the glue was dry, spiders were placed in individual petri dishes in a refrigerator and used within 24 hours for the experiment.

For each of the above experiments, we conducted control experiments in the lab to determine if the spiders in each experiment were able to capture and consume *O. insidiosus*. To do this, we placed individual spiders in each of five petri dishes with five *O. insidiosus* per dish and checked the petri dishes at 1 and 3 hours to count eaten or dead *O. insidiosus*.

Statistical Analysis. Two-way ANOVA was used to test the effect of spiders, time, and their interaction on number of *O. insidiosus* recovered on banker plants (SAS, 2008).

3. Results

3.1 Effect of banker plants on pest abundance

Overall, there were more than twice as many thrips in the control treatment houses than either the banker plant or augmentation treatment houses ($F = 7.56$; $df = 2, 107$; $P = 0.0008$; Fig. 2). The overall effect of week on thrips abundance was significant ($F = 7.35$; $df = 8, 107$; $P = <0.0001$) but the interaction of treatment and week was not ($F = 0.41$; $df = 16, 107$; $P = 0.9768$). There were more than six times as many spider mites in the control houses than the augmentation treatment and the overall treatment effect was significant ($F = 7.74$; $df = 2, 12.4$; $P = 0.0066$; Fig. 3). The overall effect of week ($F = 4.71$; $df = 8, 95.6$; $P = <0.0001$) on spider mite abundance was significant, but the interaction of treatment and week was not ($F = 0.94$; $df = 16, 95.6$; $P = 0.5237$).

3.2 Banker plant sampling

Spiders were found in 82% of total banker plant samples. The most prevalent spider family on banker plants was Salticidae, followed by Lycosidae and Oxyopidae. *O. insidiosus*

adults and nymphs were only found in 8 (7%) banker plant samples over the course of the entire experiment. Other organisms found in banker plant samples but in low abundance were thrips (52%), mites (18%), aphids (22%), and all other predators such as ladybeetle larvae, lacewings, and preying mantids (7%).

3.2 *O. insidiosus* predation in the presence and absence of Black Pearl pepper pollen

23% fewer thrips survived when predators were present ($F = 10.48$; $df = 1, 48$; $P = 0.0022$; Fig. 4). However, the presence of flowers did not have an effect on the number of thrips that survived ($F = 0.39$; $df = 1, 48$; $P = 0.5344$). There was not a significant interaction between flowers and predators ($F = 0.74$; $df = 1, 48$; $P = 0.3940$).

3.3 How spiders affect *O. insidiosus* retention on banker plants

At Hoffman Nursery, an average of 1.5 fewer *O. insidiosus* remained on plants with spiders compared to plants without spiders ($F = 13.84$; $df = 1, 57$; $P = 0.0005$) (Fig. 5). There were fewer *O. insidiosus* recovered after 3 hours than 1 hour in either spider treatment ($F = 22.10$; $df = 1, 57$; $P = <0.0001$). However, the interaction between spider presence and time ($F = 0.01$; $df = 1, 57$; $P = 0.9372$) was not significant. In the Petri dish control experiment, 80% of spiders consumed *O. insidiosus* within one hour indicating Salticids are potential predators of *O. insidiosus*.

In the consumptive effects experiment, 3.8 fewer *O. insidiosus* were recovered on plants with spiders ($F = 15.68$; $df = 1, 36$ $P = 0.0003$; Fig. 6). There were 2.2 fewer *O. insidiosus* recovered after 3 hours than 1 hour ($F = 5.26$; $df = 1, 36$; $P = 0.0278$; Fig. 6).

There was no significant interaction of spider presence and time ($F = 0.00$; $df = 1, 36$; $P = 0.9996$). In Petri dishes, 100% of spiders consumed *O. insidiosus* within one hour.

In the behavioral effects experiment, an average 0.85 fewer *O. insidiosus* were recovered on plants with spiders ($F = 3.85$; $df = 1, 35$; $P = 0.0578$; Fig. 7). There was no significant effect of time ($F = 2.88$; $df = 1, 35$; $P = 0.0987$) or an interaction of spider presence and time ($F = 2.25$; $df = 1, 35$; $P = 0.1425$) on *O. insidiosus* retention on banker plants. After 3 hours, none of the spiders in Petri dishes had consumed any *O. insidiosus* confirming that glue effectively disabled spider mouthparts.

4. Discussion

One goal of banker plant systems is to improve augmentative biological control by sustaining populations of released natural enemies within a growing system (Frank, 2010). This study found that augmentation with released *O. insidiosus* was effective within the complex growing system at Hoffman Nursery; however, the addition of banker plants in this unique nursery system did not improve pest suppression. In fact, *O. insidiosus* were present in only 7% of total banker plant samples. Whereas spiders were found in 82% of total banker plant samples, which disrupted biological control by restricting access to floral resources. This is the first study to implement a Black Pearl pepper banker plant system to test pest suppression in a commercial nursery.

Black Pearl pepper banker plant pollen can increase female *O. insidiosus* longevity and size, nymphal survival to adult, and abundance, as well as decrease nymphal development time (Wong, unpublished results). These positive effects on *O. insidiosus* life history could

collectively contribute to sustaining or increase *O. insidiosus* abundance in certain types of growing systems. In other cropping systems, floral resources can increase natural enemy abundance (White et al., 1995; Pineda and Marcos-Garcia, 2008; Jacometti et al., 2010) and reduce emigration (Eubanks and Denno, 1999). However, at Hoffman Nursery there was no evidence that banker plants had any effect on *O. insidiosus* abundance or persistence in hoop houses. As such, we found no difference in pest suppression between augmentation and banker plant houses.

One concern when adding floral or other food resources to a cropping system is that they will distract or satiate predators thereby reducing consumption of pests. For example, additional plant resources decrease the number of pests killed by big-eyed bugs (Eubanks and Denno, 2000), lady beetles (Spellman et al., 2006), and carabids (Frank et al. 2011). However, predation rate is affected by several factors such as predator preference (Xu and Enkegaard, 2009), prey vulnerability (Lang and Gsodl, 2001), mobility (Baez et al., 2004), and density (Chow et al., 2008; Chow et al., 2010). Our study found that pollen did not reduce consumption of thrips by *O. insidiosus* adults. This may be because *O. insidiosus* and thrips both reside inside flowers and feed on pollen, so flowers should increase encounters between the two (Hansen et al., 2003). Thus we do not feel that the presence of pollen accounts for why banker plants did not improve thrips suppression by *O. insidiosus* compared to augmentative release.

Higher-order predators can decrease natural enemy effectiveness by decreasing natural enemy foraging time (Wilder and Rypstra, 2004; Martinou et al., 2009), and decreasing fecundity (Meisner et al., 2010). Since spiders were found in 82% of banker plant

samples, our second hypothesis as to why banker plants did not increase *O. insidiosus* efficacy at Hoffman Nursery was that spiders on banker plants were disrupting biological control by restricting access to floral resources by consuming or scaring away *O. insidiosus*. Spiders, including Lycosid and Oxyopid families, consume a wide variety of prey, which includes generalist predators (Nyffeler et al., 1987; Nyffeler, 1999). Specifically, spiders restrict floral resources to other beneficial insects like honeybees, which are less likely to visit flowers where crab spiders are present (Reader et al., 2006). Furthermore, wolf spiders can disrupt biological control by negatively affecting mirid predator density, which results in population growth of planthoppers (Finke and Denno, 2003).

In our study, spiders significantly decreased *O. insidiosus* retention on Black Pearl pepper banker plants. The presence of spiders reduced *O. insidiosus* abundance on banker plants by 46% and 36% in the Hoffman Nursery and consumptive effects experiments respectively. From these results, we can infer that spiders on banker plants restricted access to floral resources by consuming *O. insidiosus*. Thus, banker plants in this system could have been a sink for *O. insidiosus* populations. Sit-and-wait predators, like Oxyopids and other top-predators, can decrease biological control efficacy by consuming intermediate-predators, such as *O. insidiosus*, and relieving pests from pressure of intermediate predators (Rosenheim et al., 2004). This may help account for spider mite suppression, which was indistinguishable from control houses when banker plants were present. Lastly, the fact that less than a quarter of released *O. insidiosus* remained on banker plants in spider experiments where the natural enemy was free to disperse is an important observation for this system.

After *O. insidiosus* were released and free to disperse within and from houses, what few were left in houses were likely eaten or scared away by spiders.

Although we did not test this hypothesis, another reason why banker plants did not improve augmentation biological control may have been that *O. insidiosus* emigrated from hoop houses at Hoffman Nursery. Sides of the hoop-houses are rolled up in the summer to decrease temperatures within houses. Also, Hoffman has planted flower beds to attract natural enemies and maintains grass cropping beds outside of hoop-houses. *Orius spp.* are naturally found in diverse field borders (Atakan, 2010) and, as a generalist, benefit from a diverse set of prey and plant material (Root, 1973). *O. insidiosus* may have left houses to search for other plant or prey food in the surrounding vegetation. Although attraction to surrounding vegetation should have been similar in banker plant and augmentation houses, the presence of spiders on banker plants in combination with dense flower beds may have created a ‘push-pull’ effect (Cook et al., 2007) that increased *O. insidiosus* emigration from banker plant houses.

This is the first study to test the Black Pearl pepper banker plant and *O. insidiosus* system for biological control. The first goal of this study was to determine how or if Black Pearl pepper banker plants were contributing to pest suppression by *O. insidiosus* at Hoffman Nursery. We found that augmentation biological control by *O. insidiosus* does work in a complex growing system like Hoffman Nursery, but banker plants do not further improve pest suppression compared to augmentative releases alone. The Black Pearl pepper banker plant system is popular among greenhouse and nursery growers alike, however this system was complicated by spiders and *O. insidiosus* emigration in the open environment at

Hoffman Nursery. As such we cannot recommend it to growers with open systems and would encourage future research to be conducted in a more controlled growing system. Enclosed greenhouses would reduce immigration by spiders and emigration by *O. insidiosus*, forcing the pirate bugs to utilize floral resources provided by banker plants and allowing them to access these resources by excluding immigrating top predators such as spiders.

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Figure 1. Banker plants at Hoffman Nursery (left, picture by Steven Frank) and banker plant layout schematic displaying four rows of grasses with alternatively spaced banker plants (right).

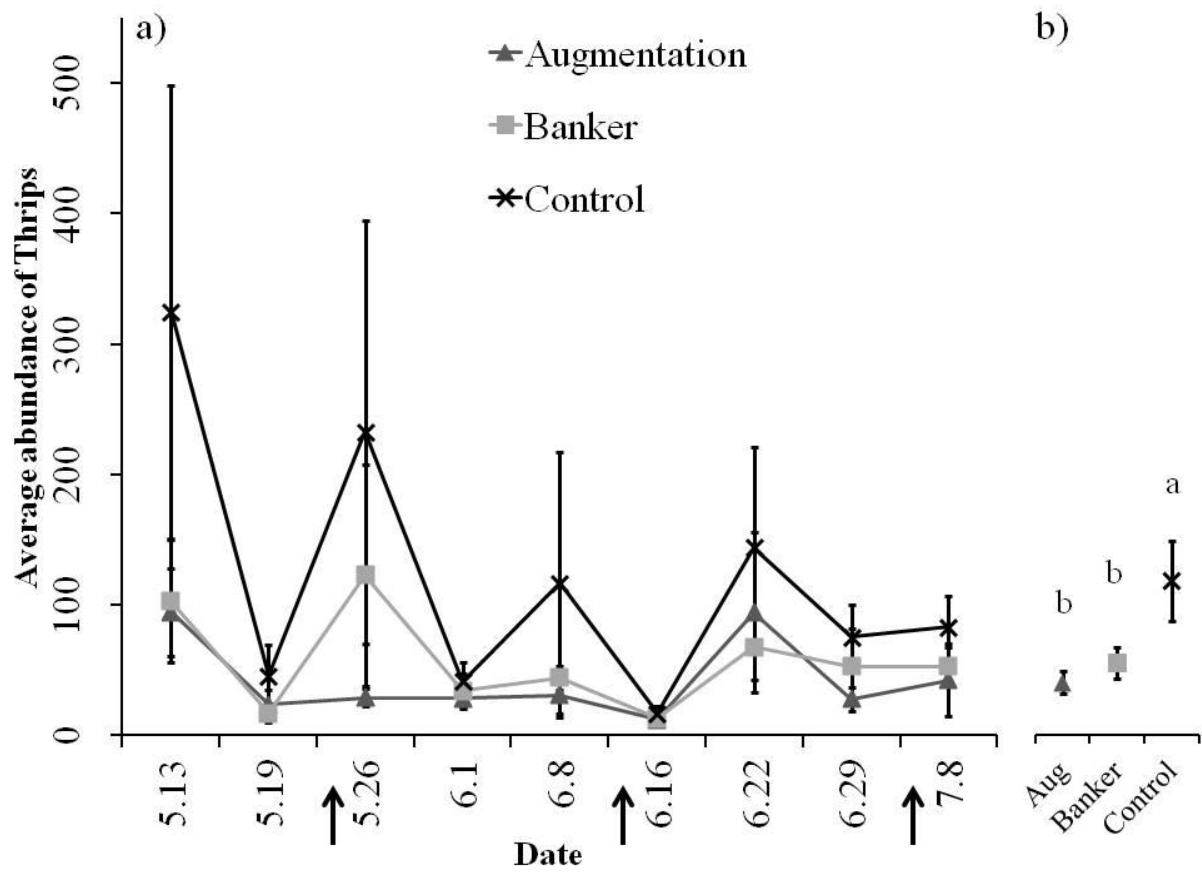


Figure 2. a) Weekly mean (\pm SEM) of thrips abundance over the course of nine weeks. b) Overall seasons mean of thrips abundance in Augmentation (Aug), Banker Plant (Banker), and Control houses. Arrows indicate when *O. insidiosus* releases were made. A significant difference between treatments is indicated by different lower case letters (P < 0.05).

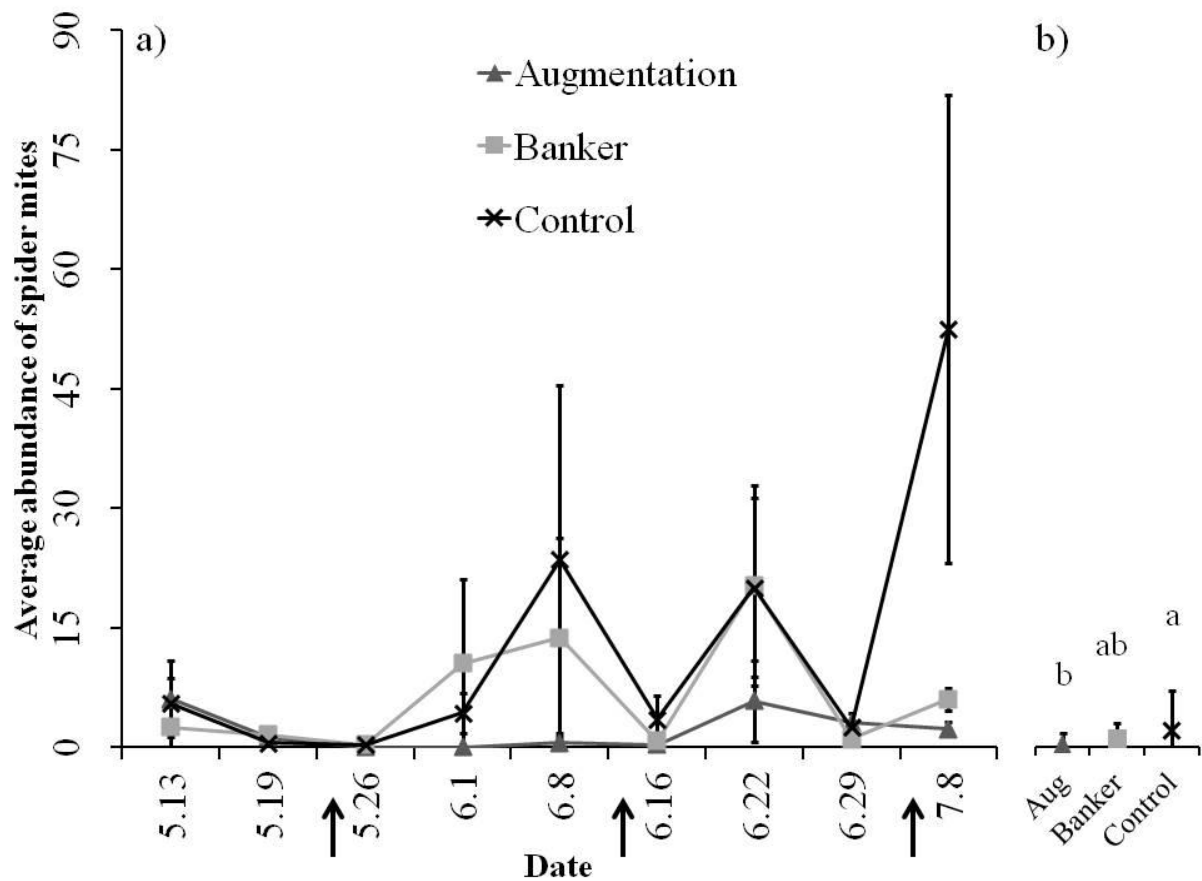


Figure 3. a) Weekly mean (\pm SEM) of spider mite abundance over the course of nine weeks. b) Overall seasons mean of spider mite abundance in Augmentation (Aug), Banker Plant (Banker), and Control treatments. Arrows indicate when *O. insidiosus* releases were made. A significant difference between treatments is indicated by different lower case letters ($P < 0.05$).

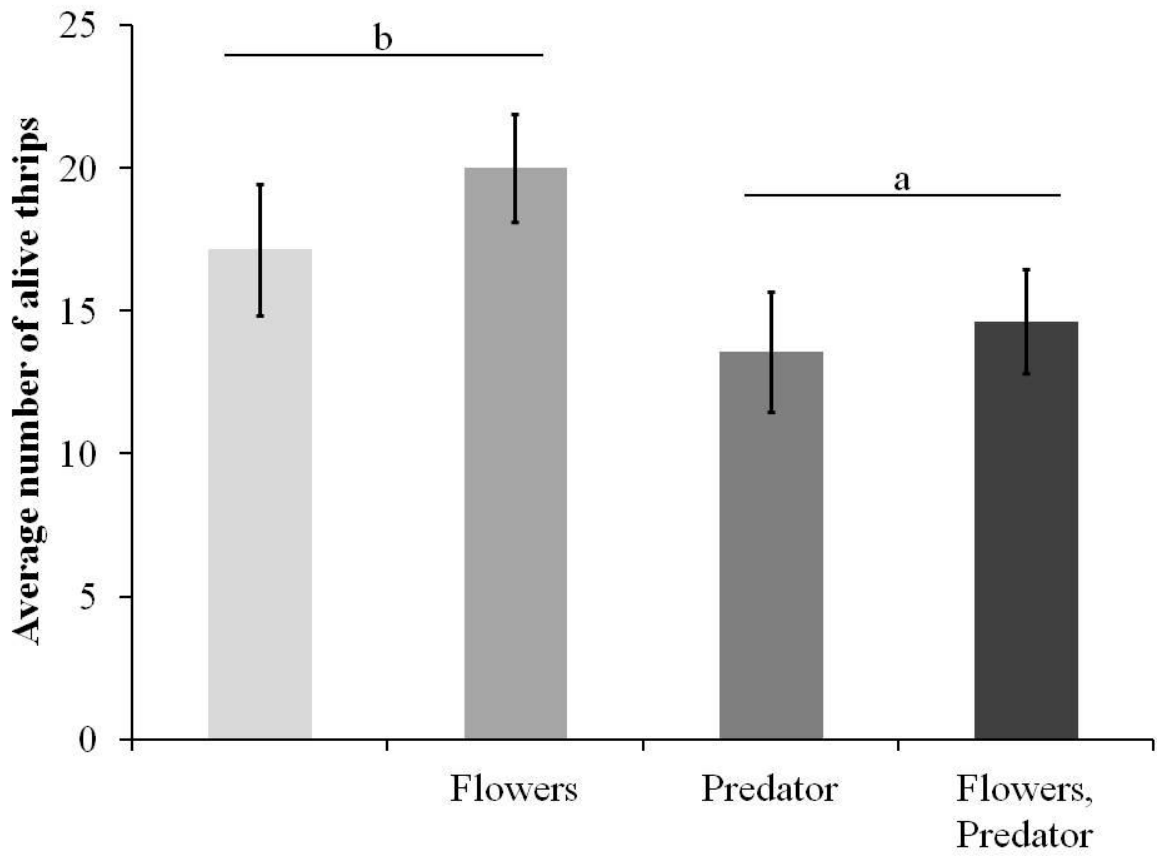


Figure 4. Average number of thrips surviving when flowers and predators were present or absent. Different letters above horizontal bars indicate significant between groups ($P < 0.05$).

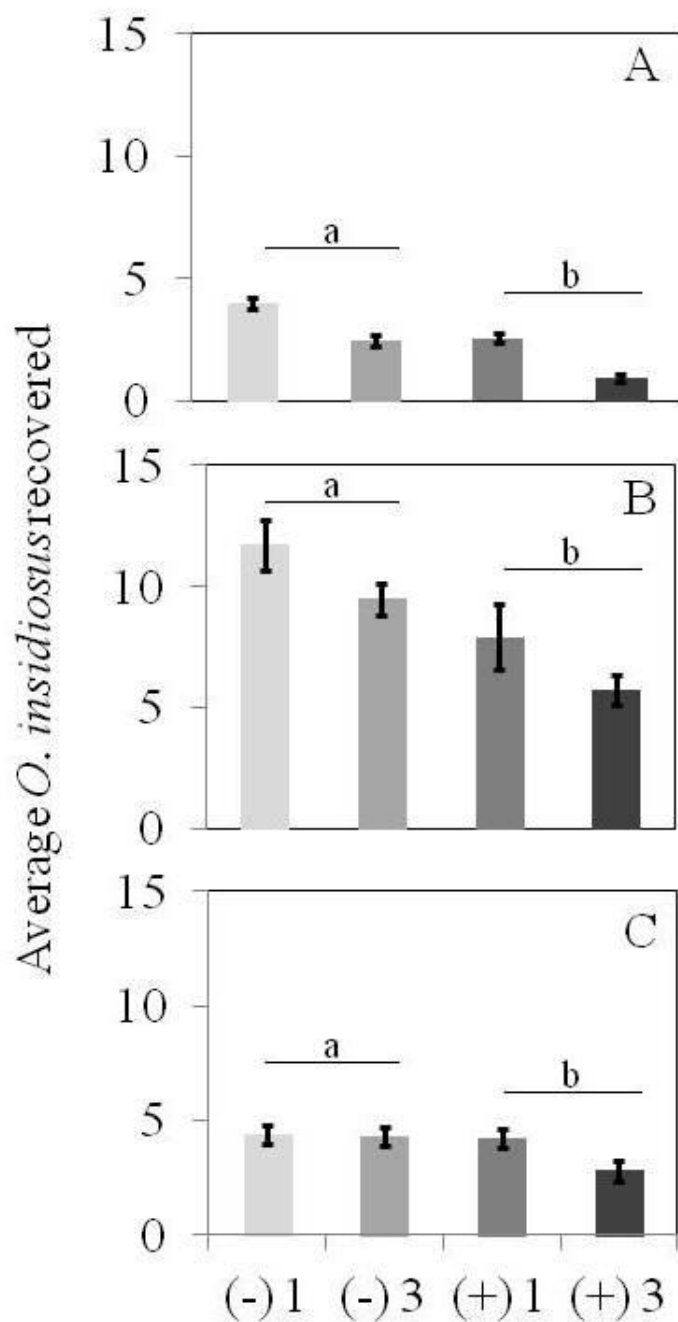


Figure 5. A: Average number of *O. insidiosus* recovered on banker plants at Hoffman Nursery. B: Average number of *O. insidiosus* recovered on banker plants enclosed in a cage. C: Average number of *O. insidiosus* recovered on banker plants with spiders with non-functioning mouthparts. Treatments are as follows: Spiders absent, 1 hour: (-)1; Spiders absent, 3 hours: (-)3; Spiders present, 1 hour: (+)1; Spiders present, 3 hours: (+)3. Different letters above horizontal bars indicate significant main effect of treatment ($P < 0.05$).