EFFECTS OF COVER CROPS AND ORGANIC INSECTICIDES ON SQUASH BUG (*ANASA TRISTIS*) POPULATIONS

by

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(Under the Direction of David Berle)

ABSTRACT

Squash bugs (*Anasa tristis)* can be a serious insect pest for organic summer squash growers. The purpose of this research was to evaluate two methods to control *A. tristis* populations. The first experiment involved planting cover crops adjacent to summer squash in an effort to attract natural enemies to keep *A. tristis* populations in check. Natural enemies were attracted to the plots, but did not significantly reduce *A. tristis* populations. This may have been due to other food sources in the plots, such as pollen, nectar, and aphids. Also, summer squash yields were negatively affected by the cover crop treatments. The second experiment involved evaluating the efficacy of organic insecticides on *A. tristis* adults and nymphs. Results of this study showed pyrethrin-based sprays are best for controlling *A. tristis*.

INDEX WORDS: Summer squash, Diversified planting, Natural enemies, Pesticides, Organic agriculture, Sustainable agriculture, Biological control

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B.S., Indiana University, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2016

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DEDICATION

This thesis is dedicated to my friends, family, and fiancé. Thank you for pretending to care about squash bugs as much as I do.

ACKNOWLEDGEMENTS

First of all, I would like to thank my committee members for providing insight and supporting this project. Thank you to David Berle for his encouragement and guidance. Also thank you for teaching me so many practical and useful skills at UGArden that I will take with me when I leave. Thank you to Paul Guillebeau and Elizabeth Little for their expertise. I gained so much knowledge related to horticulture, entomology, and plant pathology during this project, and I contribute that to my supportive committee members.

Thank you to all UGArden staff and interns who helped during the field experiments. Thank you to Taylor Cotton for his positive attitude during harvests and bug counting days; I hope I haven't completely ruined summer squash for you. Special thank you to JoHannah Biang, for being the insightful eagle in my life, always ready to give advice and encouragement.

I appreciate all the statistical help I've received from Kim Love and Murilo Massaru Da Silva.

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INTRODUCTION AND LITERATURE REVIEW

Summer squash and organic agriculture

The genus *Cucurbita* includes many varieties of squash, pumpkins, and gourds. There are five domesticated *Cucurbita* species, with summer squash belonging to *Cucurbita pepo*. Cucurbit crops are important for growers in the United States. In 2013, the U.S. produced 778,000 metric tons, ranking 4th worldwide in *Cucurbita* production (Nations, 2012). The value of squash production in the United States totaled over $237 million in 2013 (Agriculture, 2013). Georgia is the 5th largest producer of squash in the United States, with production valued at $12 million (Agriculture, 2013).

Production of organic vegetables in the United States is increasing. From 1992-2011, over 2 million acres of USDA certified organic crops were added to production (Greene, 2013). Sales of organic food increased from $11 billion in 2004 to $25 billion in 2011, accounting for approximately 4% of total food sales (Greene et al., 2012). Nationally, organic squash sales totaled around $20 million in 2008, with Georgia organic production totaling approximately $85,000 (USDA, 2008)

Organic squash growers face many difficulties including a wide variety of insects and diseases that damage the crop during the spring, summer, and early fall. Conventional growers have adequate chemical tools to manage most squash insect pests, however organic growers have fewer insecticides from which to choose. With the market for organic produce growing, growers need better alternatives to keep insect populations under control.

Squash bug natural history

Various insects attack summer squash, including: squash vine borer (*Melittia cucurbitae*), spotted and striped cucumber beetle (*Diabrotica undecimpunctata, Acalymma vittatum*), pickleworm (*Diaphania nitidalis*), and squash bug (*Anasa tristis)* (Adam, 2006). Squash vine borer is a moth larva that bores into the stem of the plant to feed and causes damage that leads to decline of squash plants. Cucumber beetles and pickleworms feed on squash fruits, causing blemishes and holes, making fruits unmarketable. Squash bugs are the most notorious of the summer squash insect pests and inflict serious damage to squash crops. Squash bugs are in the order Hemiptera and family Coreidae. They are found throughout North America (Wadley, 1920).

Squash bugs overwinter as adults in a reproductive diapause stage near fields in the soil, leaf litter, old buildings, woodpiles, debris or any other location that will help keep them warm and dry. These bugs go through 1-2 generations in northern states and 2-3 generations in southern states (Adam, 2006). Photoperiod is the driving force that dictates when the insects enter diapause, with temperature and available food sources each playing a role as well. In laboratory studies of female reproductive organs, it was found that 100% entered diapause when photoperiod was shorter than 14:10, which corresponds to daylight present in late August-early September for southeastern states (Nechols, 1988). In the field, 50% of squash bug adults enter diapause during this photoperiod (Nechols, 1988). Oviposition stops about 30 days after the onset of diapause (Fielding, 1988). The bugs stay in diapause until late spring but end diapause before they disperse into the field from overwintering sites. This long diapause helps the bugs survive the winter and is timed to coincide with their cucurbit hosts (Nechols, 1987; Nechols, 1988).

After emerging from overwintering sites, adult squash bugs travel unknown distances to find newly planted squash and begin laying eggs. Eggs are 1/16 inch long, football shaped, and light brown when first laid, eventually becoming a more metallic, dark brown color (Wadley, 1920). The eggs are laid in clusters of approximately 20 on the underside or top of squash leaves near the leaf vein; females may lay an average of 250 eggs per season (Bauernfeind et al., 2005). The eggs hatch in approximately 7-14 days and nymphs spend 4-6 weeks going through five instars before reaching the adult stage (Adam, 2006). Between each instar, the nymphs molt and become larger; they begin with a green body and reddish appendages, and as they molt, they become more greyish (Wadley, 1920). When the adult stage is reached, the squash bug is ¾ inch long and brown-grey in appearance. The wings are held flat over the abdomen, with brownish stripes on the periphery and a dark brown diamond where the wings overlap on the abdomen. The last generation to emerge does not mate, but when they are triggered by shorter day lengths, they begin overwintering diapause and move to overwintering sites (Nechols, 1987).

Cucurbit yellow vine disease and Serratia marcescens

Squash bugs are true bugs and have piercing-sucking mouthparts. Adults and nymphs of squash bugs use their mouthparts to feed on the xylem of cucurbits (Adam, 2006). Squash bugs prefer yellow summer squash and pumpkin, but also feed on butternut and acorn squash, watermelon, muskmelon, and cucumber (Bonjour et al., 1990).

Although squash bug feeding causes physical damage to the squash plant, a decline also occurs due to the role squash bugs play as vectors of *Serratia marcescens*, the bacteria responsible for cucurbit yellow vine disease (CYVD) (Bruton et al., 2003). This disease can cause variable yield losses of 5-100%, depending on the squash bug populations (Bruton et al., 2003). It was first discovered in 1988 on squash and pumpkin growing in Oklahoma and Texas.

It was then noted again in Oklahoma watermelon fields in 1991 (Bruton et al., 1995). The disease has since been noted in several other states, including Georgia (Besler, 2014). Cucurbit yellow vine disease has mostly been reported in squash because squash bugs prefer to colonize on squash, but CYVD has also been noted in other cucurbits. Plants affected by CYVD turn yellow, wilt and eventually die; the most characteristic trait of CYVD is a honey-brown coloring of the phloem and is seen in all cucurbits affected (Bruton et al., 1998). Symptoms of CYVD are similar to other diseases and when first detected, it was thought to be an already described disease, such as bacterial wilt (*Erwinia tracheiphila)* or Fusarium wilt (*Fusarium oxysporum*). However, Bruton discovered that the phloem-colonizing bacterium, *S. marcescens*, was responsible for causing the disease (Bruton et al., 2003)

S. marcescens is a rod shaped, gram-negative, spore forming bacterium in the family Enterobacteriaceae. The bacterium is resistant to many antibiotics and grows in a wide range of environmental conditions. *Serratia marcescens* is an ecologically and genetically diverse bacterial species. Different strains survive as water or soil saprophytes, rhizobacteria, insect pathogens, plant endophytes, and opportunistic human pathogens. Bruton's research showed that insecticides helped decrease the incidence of CYVD, while soil fumigation had no effect, suggesting insects vectored the disease (2003). It was also shown that, when squash plants were covered with row covers, disease prevalence was lower than those not covered. In 2003 Bruton showed that squash bugs harbor *S. marcescens* and pass it to cucurbits (Bruton et al., 2003). Entomologists were surprised at this discovery because the bacterium was known to be a pathogen for more than 70 insect species and that no other Coreids have been shown to transmit this pathogen (Heppler, 2007). The exact mechanism of survival of *S. marcescens* within the insect is unknown, however squash bugs have been shown to transmit the bacterium after molting, suggesting the bacterium is present in the hemocoel and not in the foregut (Wayadande et al., 2005). Squash bugs do not appear to be negatively affected by the bacterium. Heppler exposed squash bug nymphs to four different strains, including ZO1-A, which is responsible for CYVD. The strains not associated with CYVD did shorten the nymphal life span, while ZO1-A did not, suggesting ZO1-A is not pathogenic for squash bugs. It is possible the ZO1-A strain and squash bug may have adapted together (Heppler, 2007).

Squash bugs obtain the bacterium when probing the plant for feeding. The bugs feed on the xylem of plants with their stylet, but when probing for the xylem they can probe the phloem and obtain the bacterium (Bonjour et al., 1990). The bugs harbor the bacterium while overwintering and transmit it to cucurbits when they begin feeding the next year (Pair et al., 2004). Overwintering squash bug adults were found to have varying rates of infection, ranging from 10% to 50% (Besler, 2014; Bruton et al., 2003). It was originally thought that nymphs were not able to maintain the bacterium due to molting, but Wayadande found that some nymphs were able to transmit the disease, although much less often than adults (Wayadande et al., 2005). These findings suggest that control of squash bug nymphs is just as critical as adults.

Integrated pest management

Integrated pest management (IPM) strategies can help control the squash bug. IPM uses appropriate pest control by considering the economic, ecological, and social consequences of the strategies (Hajek, 2004). These strategies include biological control, pheromones, genetic manipulation, plant resistance, cultural practices, and pesticides as a last resort. Previous IPM strategies for squash bug have focused on cultural practices and pesticides, with more recent interest in biological control.

Cultural practices can decrease squash bug populations, but these methods are not totally effective and may not reduce insect populations below the economic injury level. Row covers act as a physical barrier, decreasing contact between squash plants and squash bugs, but do little to control the insect itself. In Kentucky field plots, fewer squash bugs were found on summer squash plants when covered with row covers (Schmidt et al., 2014). Also, Besler showed that zucchini plants covered with row covers for at least three weeks had no CYVD symptoms (Besler, 2014). However, squash bugs may move to other cucurbits such as pumpkin or watermelon if they are unable to feed on squash.

Many organic growers utilize crop rotation to help manage insects and diseases, but squash bugs are very strong fliers and can travel from overwintering sites to find newly planted squash. Sanitation methods require removing dead squash plants from fields and hand removal of adults and eggs and may help lower populations, but may be inefficient for many growers and may be impossible for medium to large-scale organic farms. Growers could shift their production to squash types that squash bugs do not prefer, such as winter squash, but strong consumer demand for organic summer squash provides a strong incentive to grow summer squash in spite of difficulties presented by squash bugs (Nations, 2012).

Organic insecticides

Conventional growers do not have similar issues with squash bugs because neonicotinoid insecticides provide an acceptable degree of control of these insects. There are several conventional insecticides available for squash bug control that are not used in organic agriculture. In field studies, carbaryl has shown decreased squash bug populations when applied at the base of pumpkins and watermelons (Cranshaw et al., 2001; Dogramaci et al., 2004). In the field, Palumbo found that plots sprayed with cypermethrin had lower squash bug populations than control, and that also the timing of the insecticide spraying greatly plays a role in the success of the insecticide (Palumbo et al., 1993).

While previous insecticide studies on squash bugs have focused mostly on conventional insecticides, there are several insecticides labeled for use on squash that may provide adequate control of squash bug adults and nymphs. Insecticides approved for use on squash by the Organic Materials Review Institute (OMRI) include azadirachtin (Monterey neem oil), mineral oil (Monterey horticultural oil), spinosyn A and D (Monterey garden insect spray), pyrethrin (PyGanic), azadirachtin + pyrethrin (Azera), and K salts of fatty acids (Safer Soap). These insecticides vary in their active ingredients and effect on insects.

Azadirachtin is derived from the seeds of the neem tree, *Azadirachta indica*. Azadirachtin kills insects by inhibiting molting (Buss et al., 2002). Mineral oil works by suffocating insects when spray is directly applied (Buss et al., 2002). Spinosyn A and D is derived from bacteria and affects the nervous system of the insect, causing paralysis and death (Bunch, 2014). Pyrethrins are derived from *Chrysanthemum cinerariaefolium.* Contact with pyrethrins causes immediate paralysis to many insects, but some are able to metabolize the pyrethrins and recover (Buss et al., 2002). Potassium salts of fatty acids disrupt the insect cuticle, although the soap may affect other aspects of the insect as well (Buss et al., 2002).

Watkins looked at how several different conventional insecticides affected eggs, young nymphs, old nymphs, and adults and found that pyrethrin based sprays were best of those tested at killing young nymphs and were the most economically feasible for growers; other sprays did kill significant numbers of bugs, but required very high levels in order to do so (Watkins, 1946). None of the sprays were very effective at killing eggs or older life stages of squash bugs (Watkins, 1946).

There are few studies analyzing the effects of the other organic insecticides on squash bug mortality, but work has been done with the brown-marmorated stink bug (*Halyomorpha halys*), also a Hemipteran insect pest but in the Pentatomidae family. Stink bugs exposed to spinosyn A and D, K salts of fatty acids, mineral oil, azadirachtin, and pyrethrin showed higher mortality rates than control (Bergmann et al., 2014). Pyrethrin killed approximately 95% of nymphs and adults 48 hr after exposure, while the other insecticides killed at least 40% of the nymphs and adults (Bergmann et al., 2014). In another study analyzing efficacy of organic insecticides on stink bugs, pyrethrins with kaolin showed highest mortality rates 7 days after exposure, with mortality rates higher than 80% (Lee et al., 2014). Azadirachtin, K salts, and spinosyn A and D showed similar results after 7 days, with death rates around 60% (Lee et al., 2014).

Each of these insecticides may be useful in decreasing insect populations, but there is little information about which is best for squash bugs and at what stage. With the exception of pyrethrin, most of the research on squash bug insecticides targets conventional growers.

Conservation biological control: Natural enemies of squash bugs

Biological control practices have been utilized to manage pest populations in many crops and may be useful in lowering squash bug populations. Biological control happens naturally or as the result of human intervention. Applied biological control involves the introduction of predators, parasites, and/or pathogens by humans to decrease the pest population (De Bach, 1964). There are several different types of applied biological control, including classical, augmentation, neoclassical, and conservation (Hajek, 2004). Classical control is the importation of natural enemies to decrease exotic insect pests, while neoclassical would be the introduction of exotic insects to decrease native insect pests. Augmentation control relies on the release of natural enemies at certain times and levels. Conservation control focuses on enhancing natural enemy populations to decrease natural insect pest populations.

Ideally, natural insect enemies must possess certain characteristics to be useful in biological control of squash bugs. Natural enemies must prey on the squash bug either as a generalist or specialist. Generalist natural enemies eat other species from other genera as well as the squash bug, while specialists will eat a few other species within the *Anasa* genus. The natural enemies must affect the density-dependent mortality of the squash bug (i.e. as squash bug populations rise, the enemy is able to grow its population as well). Natural enemies must also have a strong reproductive rate, be able to find squash bugs easily, have a lifecycle that is similar to the squash bug, and be able to survive when squash bug numbers are low or have an alternate food source (Hajek, 2004).

Squash bug adults secrete a foul odor when handled, so predation of adults is rare, however, nymphs and eggs have several natural enemies. (Adam, 2006). In a study by Schmidt et al, gut contents of 640 insect predators were analyzed for squash bug DNA to study which insects are top squash bug predators. Overall, 11% of the predators contained squash bug DNA. Lady beetles (Coccinellidae*)*, big eyed bugs (Geocoridae), damsel bugs (Nabidae), web building and hunting spiders (Arenae) were found to be top predators, respectively (Schmidt et al., 2014). Predators were also monitored in the field, with top predators varying depending on time of year and density of squash bugs. Hunting spiders were the top predator when squash bug densities were low in late May, while lady beetles were top when squash bug densities were higher in mid-summer (Schmidt et al., 2014). Female big-eyed bugs have been shown to prey upon squash bug eggs and first and second instar nymphs, suggesting this natural enemy is an important squash bug predator (Fair, 2015). There are several other predators noted to prey on squash bugs including: ground beetles (Carabidae), rove beetles (Staphylinidae), spined soldier bugs (*Podisus maculiventris*), and lace wings (Chrysopidae) (Beard, 1940; Decker et al., 2008; Snyder, 2014; Snyder et al., 1999).

Parasitic wasps lay eggs in the squash bug eggs, effectively killing the bugs by stopping egg development of the squash bug. Several species of parasitic wasps target squash bug eggs, the most prevalent being *Gryon pennsylvanicum* and three *Ooencytrus* species (Bauernfeind et al., 2005; Nechols et al., 1989). In one study, these four parasitic wasps were compared to determine the best for an augmentative or conservation biological control program. *G. pennsylvanicum* was found to have the highest fecundity and parasitism rates (Nechols et al., 1989). Adult squash bugs can be parasitized by a tachinid fly (*Trichopoda pennipes).* The fly lays its eggs on the underside of the adults; when the larvae emerge, they eat the squash bugs (Decker et al., 2008).

Natural enemies hypothesis versus resource concentration hypothesis

Conservation biological control encourages natural populations of natural enemies to populate an area and prey on insect pest populations. In this scenario, the farm is viewed as an ecosystem. When one insect is dominant, the ecosystem is out of balance. By viewing the farm this way, natural enemies can be encouraged to move in and help balance the farm ecosystem.

One method to increase farm diversity is to intercrop cash crops with plants that will attract natural enemies. Cover crops can attract natural enemies and also provide other benefits to the farm, such as erosion control, increased soil fertility and weed control (Wang, 2012). To be effective, a cover crop should attract natural enemies as a food source or as a habitat and not attract insect pests (Wang, 2012). For example, to attract lady beetles, the cover crop must include pollen because lady beetles need pollen in their diet, and if trying to attract predaceous spiders, a low growing habitat that allows them to hide is preferred. The flowering period and ability to out-compete weeds are also characteristics of cover crops to consider (Wang, 2012).

Insect pest pressure can be greater in monoculture plantings. One of the earliest experiments to claim this was Pimental's broccoli research. He showed that broccoli grown in a mixed planting had significantly fewer insect pest issues than broccoli grown alone (Pimentel, 1961). Although insect pest populations have been shown to be greater in monoculture, the mechanisms behind this phenomenon are still debated. Some argue monoculture fields have a lack of natural enemies, which allow more insect pests to thrive. The natural enemies hypothesis states that a more diverse planting system creates a greater diversity of habitats and attracts more prey species, allowing a greater diversity of predators and parasitoids to thrive on a varied diet (Root, 1973).

However, factors other than natural enemies may affect insect populations. Root studied collards grown in pure stands and collards grown between strips of meadow vegetation and monitored the insect populations. He found the biomass of herbivores to be higher in the pure stands, but found similar biomass of natural enemies in both habitats and higher diversity of predator and parasitoids in the pure stands. Root proposed a new hypothesis called the resource concentration hypothesis, which does not claim that natural enemies significantly decrease herbivore populations (Root, 1973). He argued that herbivores are more likely to find and stay in a monoculture habitat and the most specialized herbivores have the highest survival rates. Thus, in monocultures, a few herbivore species become concentrated (Root, 1973). Based on these hypotheses, many studies have been conducted in which researchers analyzed different methods to increase agricultural ecosystem diversity, including intercropping, living mulch, uncultivated corridors, and weedy culture.

Intercropping vegetable crops with cover crops has been shown to have positive effects on insect pests and natural enemy populations. When Amirault and Caldwell grew cover crop strips between cucurbit plantings, the cover crops positively affected insect ecology. Buckwheat interplanted with pumpkin and cucumber reduced cucumber beetles by 60% and counts of ground beetle predators were twice as high in the buckwheat than in the control (Amirault et al., 1998). In this study, they also found the Pennsylvania leatherwing predator population to be 2.7 to 10 times greater. McNeil et al grew velvet beans, sunn hemp, sorghum sudan grass, and pearl millet, flail mowed the cover crops and disked them into the soil, and then yellow squash was then sown into the plots. Natural enemy populations were monitored and sorghum-sudangrass treatments had the highest parasitoid populations but also the highest whiteflies. Treatments with sorghum-sudangrass and velvet bean had high levels of hover flies (Syrphidae) which helped keep aphid populations low (McNeill et al., 2012). Hooks and Johnson found that broccoli under-sown with living clover mulches had higher spider populations later in the season compared with bare ground treatments (2004). Also, Lepidopteran pests were more abundant in bare control treatments (Hooks et al., 2004). Zucchini inter-planted with sunn hemp helped to significantly reduce cucumber beetle populations with predaceous spiders more abundant in the control treatments (Hinds et al., 2013).

Some studies did not monitor natural enemy populations as part of their research, but focused on the effects of agricultural plant diversity on insect pests. Manandhar et al intercropped buckwheat, white clover, sunn hemp, and okra with zucchini in an attempt to decrease whiteflies that transmit squash silver leaf disorder. Cover crops and okra were found to help keep insect pest populations low some years, but each year different results were found (Manandhar et al., 2009). In the first year buckwheat treatments had a lower incidence of disease, but in the second year, okra treatments had the lowest populations of whiteflies and disease, and buckwheat treatments had the highest incidence of disease. During the last year of the study, sunn hemp significantly reduced aphid numbers and incidence of disease (Manandhar et al., 2009). Hooks performed a similar study to reduce aphids, whiteflies, and disease but found much more consistent results. Zucchini was interplanted with buckwheat and yellow mustard. These cover crop treatments significantly decreased aphid populations on zucchini for two years of trials (Hooks et al., 1998).

Sometimes intercropping cash crops with cover crops does not help the ecosystem. For example, ground beetle activity was shown to be greater in refuge strips, but the ground beetle populations were not higher in surrounding crops (Carmona et al., 1999). The predators may be attracted to the refuge and populate there, but if they do not move into the cash crop then they are not able to eat the insect pests that are damaging the cash crop. In another study, researchers planted floral resources near summer squash in an effort to increase natural enemies to prey upon squash bugs. The floral resources had varying results in regards to natural enemies populations, overall there was not a consistent benefit to having the floral resources nearby (Fair, 2015). Also, the floral resources may have been attracting squash vine borer and other insect pests to the area (Fair, 2015).

There has been a great deal of research looking at how these diversified plantings affect natural enemies and insect pests. In a review of 219 of these studies, 51.9% of them saw lower herbivore populations in polyculture plantings compared to monoculture, while 20% of the studies had variable results, and 12.5% saw no change in herbivore populations. Natural enemy predator populations were found highest in polyculture in 42.7% of studies (Andow, 1991). Also, 30.3% of studies had variable results in terms of predator populations between the two planting strategies and 15.7% saw no change (Andow, 1991). In another meta-analysis of 26 papers, it was found that polyculture systems can be a win-win in terms of yield and biological control, but only if crop density is similar among plots (Iverson et al., 2014).

The overall benefits of diversified plantings are still debated. However, some studies support the natural enemies hypothesis while others support the resource concentration hypothesis. A variety of vegetables and insect pests have been targeted in these studies, but summer squash and squash bug populations have not been analyzed in diverse cropping scenarios with cover crops in great detail.

Research objectives

The goal of this project is to evaluate practices to reduce squash bug populations in organic summer squash plantings. Project objectives are to:

1) Evaluate cover crops as strategy to decrease squash bug populations in summer squash
    - a) Evaluate cover crops as method of attracting natural enemies
    - b) Evaluate cover crops as method to discourage squash bug populations
    - c) Evaluate the effect of cover crops on summer squash yield
2) Evaluate OMRI-approved insecticides for squash bug adult and nymph control

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CHAPTER 2

EFFECTS OF COVER CROPS ON SQUASH BUG (*ANASA TRISTIS*) POPULATIONS[1]

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Abstract

Organic growers struggle with growing squash due to insects and diseases that negatively impact yield. One important insect pest is the squash bug, *Anasa tristis* (DeGeer), which feeds on the xylem of cucurbits and is responsible for transmitting a bacterium, *Serratia marcescens*. This bacterium causes cucurbit yellow vine disease, which can kill an entire cucurbit planting (Bruton et al., 2003). Organic growers utilize row covers, organic insecticides, and field sanitation techniques to control this insect, however, none are completely effective. Intercropping cash crops with cover crops could help keep insect pest populations in balance by attracting natural enemies. For this project, field studies were conducted to analyze the effect of *Anasa tristis* populations when cover crops were grown adjacent to summer squash. Buckwheat (*Fagopyrum esculentum*), cowpeas (*Vigna unguiculata)*, and sunn hemp (*Crotalaria juncea)* were planted adjacent to summer squash during summers of 2014 and 2015. Overall, cover crop treatments did not provide a reliably positive benefit. However, natural enemy populations were generally higher in cover crop plots. The natural enemies may have been preoccupied with other food sources, which could explain why they did not strongly impact *A. tristis* populations. Timing and spacing of cover crop plantings, as well as the relationship between natural enemies and *A. tristis,* requires further study.

Introduction

Cucurbit crops are important for United States growers. The United States produced 778,000 metric tons of *Cucurbita* crops in 2012, ranking fourth worldwide in production (Nations, 2012). In 2013, squash production in the United States totaled over $237 million (Agriculture, 2013). Georgia is the fifth largest producer of squash in the United States, with production valued at $12 million (Agriculture, 2013). In addition, production of organic vegetables in the United States is increasing. Each year more land is put into organic production. From 1992-2011, over 2 million acres of USDA certified organic crops were added (Greene, 2013).

Organic squash growers face many challenges including a wide variety of insects and diseases that damage the crop during the spring, summer, and early fall. *Anasa tristis* are the most notorious of the summer squash insect pests and inflict serious damage to squash crops. They cause damage while feeding and can transmit a bacterium, *Serratia marcescens*, which causes cucurbit yellow vine disease (CYVD) (Bruton et al., 2003). Plants affected by CYVD turn yellow, wilt, and eventually die. The most characteristic trait of CYVD is a honey-brown discoloration of the phloem and is seen in all cucurbits affected by CYVD (Bruton et al., 1998) (Figure 1).

Yield losses due to CYVD are variable and depend on *A. tristis* populations, with growers losing anywhere from 5-100% of their plantings (Bruton et al., 2003). The disease has been noted in several states, including Georgia (Besler, 2014; Bruton et al., 1995).

Anasa tristis obtain the bacterium while probing the plant for feeding. The bugs feed on the xylem of plants with their stylet, but while probing for the xylem, they can probe the phloem and obtain the bacterium (Bonjour et al., 1990). *Anasa tristis* harbor the bacterium while overwintering and transmit it to cucurbits when they begin feeding the following year (Pair et al., 2004). In field studies, overwintering adults were found to have varying rates of infection, ranging from 10% to 50% (Besler, 2014; Bruton et al., 2003). Wayadande et al demonstrated that both adults and nymphs of *A. tristis* have the ability to transmit the bacterium multiple times (Wayadande et al., 2005).

Organic growers employ several different methods to control this insect. Row covers help decrease contact between squash plants and *A. tristis*, but do little to control the bug itself. In a Kentucky study, fewer *A. tristis* were found on summer squash when covered with row covers (Schmidt et al., 2014). Many organic growers utilize crop rotation to manage insects and diseases, but *A. tristis* are very strong fliers and can travel from overwintering sites to find newly planted squash. Sanitation methods require hand removal of adults and eggs and removing infected squash plants from the field. This can help lower populations, but may be an ineffective use of time for a small grower and may be impossible for medium to large-scale operations. Growers could simply grow cucurbits that *A. tristis* do not prefer, such as melons. While these various methods help to reduce *A. tristis* populations, many organic growers still face yield losses due to these insects. With strong consumer demand for organic summer squash, there is incentive for growers to produce summer squash in spite of difficulties presented by *A. tristis* (Nations, 2012).

Biological control practices have been utilized to manage insect pest populations in many crops and may be useful in lowering *A. tristis* populations. Conservation control focuses on enhancing natural enemy populations to decrease natural insect pest populations. One way to increase natural enemy populations is to diversify the farm planting to attract natural enemies to the farm.

Anasa tristis adults secrete a foul odor when handled, so predation of adults is rare, however, nymphs and eggs have several natural enemies. (Adam, 2006). In a study by Schmidt et al, gut contents of 640 insect predators were analyzed for *A. tristis* DNA to determine which insects are top predators. Overall, 11% of the predators contained *A. tristis* DNA. Lady beetles (Coccinellidae), big-eyed bugs (Geocoridae), damsel bugs (Nabidae), and web building and predatory spiders (Arenae) were found to be top predators (Schmidt et al., 2014). There are several other predators known to prey on *A. tristis* including: ground beetles (Carabidae), rove beetles (Staphylinidae), green lacewings (Chrysopidae), and spined-soldier bugs, (*Podisus maculiventris)* (Beard, 1940; Decker et al., 2008; Snyder, 2014; Snyder et al., 1999).

There has been a great deal of research investigating how diversified plantings, polyculture, or intercropping affects natural enemies and insect pests. A review of 219 studies found that 51.9% of the studies saw lower herbivore populations in polyculture plantings compared to monoculture and natural enemy predator populations were found highest in polyculture in 42.7% of studies (Andow, 1991). In another meta-analysis, it was determined that polyculture systems can be a win-win in terms of yield and biological control, but only if crop density is similar among plots (Iverson et al., 2014).

There are many ways a polyculture can be created. One method utilizes cover crops that attract natural enemies and provides other benefits to the farm, such as erosion control, increased soil fertility, and weed control (Wang, 2012). Intercropping vegetable crops with cover crops has been shown to positively affect insect pests and natural enemies. *Fagopyrum esculentum* interplanted with pumpkin and cucumber was shown to reduce cucumber beetle, *Acalymma vittatum,* populations by 60% and counts of Carabids were twice as high in the *F. esculentum* than in the control (Amirault et al., 1998). Squash grown with sorghum-sudan grass, *Sorghum* *bicolor x S. bicolor var. Sudanese,* and velvet beans, *Mucuna pruriens,* had high levels of hover flies, Syrphidae, which may have helped to keep aphid populations low (McNeill et al., 2012). Zucchini inter-planted with *C. juncea* helped significantly reduce *A. vittatum* with predaceous spiders more abundant in the treatments (Hinds et al., 2013).

These studies focus on the natural enemies hypothesis, stating that diverse systems keep insect pest populations low because natural enemies help reduce insect pests (Pimentel, 1961). There is also a resource concentration argument that may have a stronger effect (Root, 1973). Root showed that collards grown in a mixed planting had significantly fewer insect pests, and he hypothesized this was due to the fact that insect pests are more likely to locate and stay in a monoculture habitat and the most specialized herbivores have the best survival rates (Root, 1973).

While there can be benefits to a polyculture system, there are also several examples where increased diversity has negative, neutral, or variable effects. For example, Carabid activity was shown to be greater in refuge strips of a diverse system, but the populations were not higher in surrounding crops (Carmona et al., 1999). The predators may be attracted to the refuge and populate there, but if they do not move into the cash crop then they are not able to eat the insect pests that are damaging the cash crop. In a different study, floral resources were planted near summer squash in an effort to increase natural enemies to decrease *A. tristis* populations (Fair, 2015). The floral resources provided inconsistent benefits, in terms of their effect on natural enemy populations and *A. tristis* populations (Fair, 2015). In a review of diversified plantings, 20.2% of the studies had variable results in terms of whether polyculture or monoculture had higher insect pest populations and 12.5% had no change in herbivore populations (Andow, 1991). Also, 30.3% of studies had variable results in terms of predator populations between the two planting strategies and 15.7% saw no change (Andow, 1991).

The goal of this study was to determine the effect of cover crops planted adjacent to summer squash *A. tristis* populations, natural enemies, yield, and CYVD.

Materials and Methods

Cover Crop Treatments

Three field trials were conducted at UGArden demonstration farm in Athens, GA (long. 33.898133, lat. -83.375523). Soil samples indicated that pH, P, and K levels were adequate (Extension, 2007). The cover crops selected included: *Fagopyrum esculentum* (buckwheat), *Vigna unguiculata* (cowpeas), and *Crotalaria juncea* (sunn hemp). These three cover crops were chosen based on their known history of attracting natural enemies that prey on *A. tristis*. *Fagopyrum esculentum* encourages lady beetles, predatory wasps, beetles and parasitic flies and wasps (Clarke, 2008). *Vigna unguiculata* have been shown to attract lady beetles, predatory wasps and ants (Clarke, 2008; Valenzuela et al., 2002). *Crotalaria juncea* has been useful in attracting spiders (Wang, 2012).

Experimental Design

Each treatment, plus control, had four replicates for a total of 16 plots. Plots were arranged in a complete randomized block design with 4 blocks. For Trial 1, each replicated plot was 4.3 x 6.7 m with 0.91 m space between treatments and 1.5 m between replicated plots (Fig. 2.1). Cover crops were planted using a hand-held broadcast seeder (*F. esculentum* 0.38 kg/29 m^2, *V. unguiculata* 0.33 kg/29 m^2, and *C. juncea* 0.25 kg/29 m^2,) 46 days before planting squash and then rolled with a cultipacker. These seeding rates were on the high end of the recommended seeding rate to ensure a good stand (Clarke, 2008).

Three days before planting squash, three 0.24 m strips were tilled in each plot with a BCS tiller (Model 853, Portland, Oregon, United States) to provide a planting row for the summer squash. Strips were tilled once, and then 1.1 kg of feather meal (13N-0P-0K) was added to each summer squash row. This rate approximates average organic N fertilization rates for growing yellow squash in soil with adequate levels of P and K. Half of the *F. esculentum* was tilled under and resown (using same seeding rate as used previously) 16 days after squash planting because some plants had already started going to seed. Only half was resown to keep some *F. esculentum* growing while new plants were being established.

On 2 June 2014, three-week old squash greenhouse-grown transplants (*C. pepo* 'Multi Pik') were planted 0.61 m on center within the row and 0.91 m between rows, with three rows per treatment. In total, there were 30 squash plants per treatment and four replications of each treatment for a total of 480 plants in the trial. Drip irrigation was installed in a single line per squash row using Agrifilm (SF7412) 1.3 cm tube with 3.8 L/h emitters 0.3 m apart. Water was applied 3 days a week for 1 h, or less depending on rainfall. Cover crops were watered by hand or by sprinklers as needed. One day after planting, 57 g of $NaNO_3$ (Sodium Nitrate, 16N-0P-0K) was dissolved in 3.8 L water; 0.11 L of this mix was applied to each squash plant.

Changes were made to the experimental design for Trial 2 due to competition between squash and cover crops (Table 2.1). Each replicated plot was 4.6 x 5.8 m with 0.91 m space between treatments and 1.5 m between replicated plots. Cover crops were sown in two 5.3 m^2 blocks adjacent to squash instead of the spacing done in Trial 1 (Figure 2.2). Squash were planted in two rows in between the cover crop blocks, with 0.91 m between the two rows of squash and 0.91 m between cover crop and squash rows. To increase germination and density rof cover crops, *Crotalaria juncea* and *V. unguiculata* were planted with a Yang seeder 14 days before planting squash since these cover crops took longer to establish than *F. esculentum.* *Crotalaria juncea* was planted in rows at a rate of 9-15 seeds/0.30 m with 0.30 m between rows. *V. unguiculata* were planted at a rate of 8-10 seeds/0.30 m with 0.15 m between rows. On 11 Aug., 10 squash/row were planted for a total of 20 per plot and 320 plants in the trial. Also on 11 Aug., 0.27 kg/5.3 m^2 of *F. esculentum* was broadcasted into both blocks of the treatment but it did not germinate well by broadcasting. One week after squash planting, an Earthway seeder was utilized to sow *F. esculentum* at the rate of 13-16 seeds/0.30 m.

Trial 3 was carried out similar to Trial 2, with a few additional modifications (Table 2.1). Each replicated plot was 4.6 x 6.7 m with 0.91 m space between treatments and 1.8 m between replicated plots (Fig. 2.2). *Vigna unguiculata* and *C. juncea* were planted with a Yang seeder 28 days before squash planting. On 5 May, squash transplants were planted. *Fagopyrum esculentum* was seeded the day after squash planting using the Earthway seeder. *Crotalaria juncea* was cut back twice to reduce shading effects on squash.

Squash Harvest

For all trials, squash fruit were harvested on a Monday-Wednesday-Friday schedule. For Trial 1, harvest began 19 days after squash planting and lasted 3 weeks. For Trial 2, harvest began 21 days after squash planting and lasted 4 weeks. For Trial 3, harvest began 22 days after squash planting and lasted 5 weeks. Marketable fruit was free from blemishes and within the diameter of 3.8 – 6.4 cm (Boyhan et al., 2004).

Insect Collection

Insects were monitored and counted using yellow pan traps, pitfall traps, sticky traps, sweep nets, and visual observation, similar to methods done in other experiments (Hinds et al., 2013; Hooks et al., 1998; McNeill et al., 2012). One of each trap was placed in the middle squash row of each plot and one in the adjacent cover crop or control strip, in order to compare counts within the squash row and within the cover or control row. For Trial 1, traps were placed in all plots on squash planting day and collected for five weeks. For Trial 2, insect traps were placed in plots 2 weeks after squash planting and collected for 4 weeks. For Trial 3, traps were placed on in plots 2 weeks after squash planting and collected for 5 weeks.

Insects from pan traps were collected every 3 days. The contents were poured into a sieve and insects placed in a plastic bag. Pitfall traps, sticky traps, and sweep nets were collected weekly on Mondays and the contents put into plastic bags. Sweep nets samples consisted of 10 sweeps taken in the middle cover crop strip; one back and forth motion equaled one sweep. All bags with insects were placed in a freezer and later identified. Visual observation for *A. tristis* took place once a week on Wednesdays for the amount of time insect traps were collected. For the first 2 weeks, all plant leaves and stems were examined for *A. tristis* adults, nymphs, and eggs. For the last 2 or 3 weeks, squash plants were significantly larger so approximately 50% of leaves and stems were examined, as outlined in previous studies (Frank et al., 2005; Hooks et al., 1998).

Anasa tristis capture and releases

As an added measure, for Trial 3, *A. tristis* were collected from nearby farms and released into plots to insure higher population levels. Twenty days after squash planting, 88 adults were collected from Farm A and 22 released into the middle of each of the larger plots so bugs would have equal access to the treatments and control. Twenty-eight days after squash planting, 56 adults released from Farm B and 38 days after squash planting, 26 adults released from Farm C.

CYVD analysis

Suspected CYVD infected plants were initially confirmed with PCR as described in previous work (Besler, 2014). Later, infected squash were diagnosed based on visual symptoms (yellowing, collapse, honey colored phloem) (Fig. 3). Plots were monitored for disease until the end of harvest.

Statistical Analysis

Analysis was run using R version 3.2.2 statistical software. Count data was analyzed using Poisson regression followed by Tukey HSD (MASS package), if needed. Parametric bootstraps were run to analyze if data was over dispersed and if noted, negative binomials were used to analyze results. Yield results were analyzed using one-way analysis of variance with blocking and further analyzed with Tukey HSD. To analyze comparisons between counts in squash row and cover row, difference was taken between the rows and log transformed. Linear regression was then run on the log-transformed data. Disease incidence was analyzed with binomial regression. In all analyses, significance was determined if $P < 0.05$.

Results

Anasa tristis populations

Overall, *A. tristis* populations were low in Trials 1 and 2, with increased populations in Trial 3, in part, due to supplementation from outside farms. Total *A. tristis* counts were 36, 17, and 123, respectively, for each of the trials. *Anasa tristis* numbers did not differ between cover crop plots compared to the control for Trial 1 (Poisson, p=0.670, p=1.00, p=0.121) (Fig. 2.4A). For Trial 2, *A. tristis* were significantly less abundant in *F. esculentum* plots compared to control (Poisson, p=0.0371) (Fig. 2.4A). In Trial 3, even with an increase in the general population, *A.*

tristis adults did not differ among plots (Poisson, p=0.769, p=0.633, p=0.077) (Fig. 2.4A). Overall, *A. tristis* were found on squash plants, not in traps located within the cover crops.

Eggs and nymphs

As with the adult numbers, egg and nymph counts were highest in Trial 3. Only 14 egg masses were counted in Trial 1, and 19 in Trial 2, while Trial 3 had 430. Nymphal data was only analyzed for Trial 3 because 6 nymphs were counted in Trial 1, 0 in Trial 2, and 88 in Trial 3. Egg masses were counted, as opposed to individual eggs. For Trial 1, there was no difference among egg counts for the plots (Poisson, p=1.00, p=0.0971, p=0.0971) (Fig. 2.4B). In Trial 2, all cover crop plots had significantly lower counts of egg masses and nymphs than control (Poisson, p<0.01) (Fig. 2.4B). Egg masses for Trial 3 were found to have no significant differences among the different plots (Negative binomial, p=0.9865, p=0.8838, p=0.8947) (Fig. 4B). Nymphs for Trial 3 were highest in *C. juncea* plots compared to control (Tukey, p=0.0224) (Fig. 2.4C).

Anasa tristis natural enemies

Only those *A. tristis* natural enemies with adequate numbers were counted. Among those counted were: Arenae, Carabidae, Geocoridae, Coccinellidae, Solenopsis, and Staphylinidae. Overall, total counts within cover crop and squash rows of the *A. tristis* predators present for Trial 1 showed that *V. unguiculata* and *C. juncea* plots had significantly higher levels than control plots (Negative binomial, p=0.042, p=0.02) (Fig. 2.5). Total counts for Trial 2 showed *V. unguiculata* plots with significantly higher counts than control (Negative binomial, p<0.01). For Trial 3, all cover crops plots were shown to have significantly higher counts of natural enemies compared to control (Negative binomial, p<0.01). *Vigna unguiculata* plots had higher counts than *F. esculentum* and *C. juncea* (Tukey, p<0.01).

Counts were compared within the cover crop, or blank space for control, and within the squash rows to determine where insects were spending most of their time. For Trials 1 and 2 there were no significant differences between squash and cover crop rows ($p>0.05$) (Fig. 2.6A & B). For Trial 3, *V. unguiculata* and *C. juncea* plots had significant differences between squash and cover crop rows ($p=0.0140$, $p=0.0302$) (Fig. 2.6C).

Geocorids, Arenae, and Coccinellids are top *A. tristis* predators. For Trials 1 and 3, Geocorids were significantly higher in all cover crop plots (Trial 1: Negative binomial, $p<0.01$ and Trial 3: Negative binomial, $p<0.01$) (Fig. 2.7). For Trial 2, Geocoridae populations were higher in *F. esculentum* and *V. unguiculata* plots (Negative binomial, $p<0.01$ for both) (Fig. 2.7). For Trial 1, Arenae populations were higher in *V. unguiculata* and *C. juncea* plots and Coccinellidae populations were higher in *F. esculentum* and *C. juncea* plots, but overall none were significantly higher (Poisson, Fig. 2.8 & 2.9). In Trial 2, Arenae populations were higher in *V. unguiculata* plots while in Trial 3, Arenae populations were highest in *C. juncea* (Trial 2: Poisson, $p=0.0317$, Trial 3: Negative binomial, $p=0.0137$) (Fig. 2.8). In Trial 2, *Coccinellids* were highest in *F. esculentum* and *V. unguiculata* plots, but were not significantly higher than control (Fig. 2.9). For Trial 3, Coccinellids were significantly higher in all cover crop plots compared to control (Negative binomial, $p=0.0103$, $p<0.01$, $p=0.0245$) (Fig. 2.9).

When Geocorid counts were compared within squash and cover crop row, Geocorids were usually highest within the cover crop row (Fig. 2.10). For Trial 1 and 2, the difference in count numbers between squash and cover row were significantly higher for all cover crops compared to the control (Trial 1: $p<0.01$, $p<0.01$, $p=0.0146$, Trial 2: $p<0.01$, $p<0.01$, $p=0.0217$). For Trial 3, *F. esculentum* and *C. juncea* counts were significantly higher than control ($p<0.01$).

Arenae and Coccinellid counts were overall lower than Geocorid counts and few differences were noticed between cover and squash rows. For Arenae counts in Trial 1, *F. esculentum* and *C. juncea* plots had significantly lower difference compared to control ($p<0.01$, $p=0.0231$) (Fig. 2.11). In Trial 2 and 3 there were no differences ($p>0.05$) (Fig. 2.11). Coccinellid counts were too low in Trial 1 to compare between squash and cover plots. Trial 2 showed no differences ($p>0.05$) (Fig. 2.12). Trial 3 showed *V. unguiculata* plots having significantly greater difference between squash and treatment row compared to control ($p<0.01$) (Fig. 2.12).

Carabids were higher in cover crop plots in all three trials than control plots, but only in Trial 1 was there significant difference; *C. juncea* plots had higher populations than control (Poisson, $p<0.01$) (Table 2.2). Solenopsis were significantly higher than control plots in *V. unguiculata* plots for Trial 2 and *F. esculentum* and *V. unguiculata* plots for Trial 3 (Trial 2: Negative binomial, $p=0.0148$, Trial 3: Negative binomial, $p<0.01$ both) (Table 2.2). *Fagopyrum esculentum* plots had significantly lower Solenopsis populations in Trial 1 (Poisson, $p<0.01$). Staphylinids were highest in *V. unguiculata* and *C. juncea* plots for Trial 1 (Poisson, $p<0.01$ both) (Table 2.2). For Trial 3, they were highest in *F. esculentum* and *V. unguiculata* plots (Poisson, $p=0.0101$, $p=0.0143$) (Table 2.2). *Fagopyrum esculentum* had significantly lower Staphylinid populations in Trial 1 (Poisson, $p=0.0322$).

Aphid Populations

Aphids were noted in all three of the trials, and had population levels that exceeded *A. tristis*. Aphid populations were highest in Trial 3, with each cover crop plot having an average of at least 100 (Fig. 2.13).

Squash Yields

In general, yields were higher in Trials 1 and 3 than Trial 2. Since Trial 1 involved 3 rows of squash in every treatment plot, the total yields were reduced by 1/3 to make the data comparable among trials. Also, the trials had different timelines for harvesting, based on growth of squash. For Trial 1, all cover crop plots had significantly lower squash yield than control ($p<0.01$) (Fig. 2.14). *Fagopyrum esculentum* plots had the highest yield of the cover crop treatments ($p<0.01$). In Trial 2, which took place later in the summer, yields were overall lower than Trial 1 and 3 (Fig. 2.14). *Fagopyrum esculentum* plots had comparable yields with control plots ($p=0.216$), while *V. unguiculata* and *C. juncea* plots were again lower than control ($p=0.049$, $p=0.003$). There was no difference among the cover crop plot yields ($p=0.749$, $p=0.067$, $p=0.289$). Trial 3 had highest yields of all the trials. All cover crop plots had significantly lower yield compared to control ($p<0.01$) (Fig. 2.14). Also, no differences were noted among the cover crop plots ($p=1.0$, $p=0.216$, $p=0.212$).

CYVD Incidence

CYVD was only noted during Trial 3, starting on 12 June. The rate of disease was highest in *F. esculentum* plots, with an average of 25% of plants becoming infected (Fig. 2.15). Both *F. esculentum* and *C. juncea* plots had a significantly higher incidence of disease ($p=0.009$ and $p=0.031$) compared to the *V.* unguiculata and control plots. Lowest incidence of disease was in control plots, with only 6% of plants infected.

Discussion

Overall, *A. tristis* populations did not proliferate within control plots; there may have even been slightly higher *A. tristis* populations within the cover crop plots based on egg and nymph data. However, Trial 2 had lower numbers of adults and eggs within some of the cover crops. These mixed findings were similar to Fair's research with planting floral resources adjacent to summer squash in order to increase natural enemy activity. He found inconsistent results amongst the trials in terms of natural enemies and how the *A. tristis* populations were affected (Fair, 2015).

These mixed findings seem unusual because counts of *A. tristis* natural enemies tended to be higher in cover crop plots. With higher natural enemy counts, a lower *A. tristis* population is expected, however, this was not the case. Though impossible to know the frequency of natural enemies traveling between cover crop and squash row, some natural enemies may have spent most of their time within the cover crop row, eating very few *A. tristis* eggs or nymphs within the squash row. Similar findings were found when flowering plants were planted adjacent to cash crops and Carabids were present within the flowering plant strip but levels of Carabids were not higher within the cash crop (Carmona et al., 1999). Of the top *A. tristis* predators in this study, Geocorids were generally highest in cover crop rows, in particular *F. esculentum*, for each trial. The other top predators did not show consistent trends among the three trials in terms of their preference for cover crop or squash row.

The diet of the natural enemies should be considered in analyzing the results. The natural enemies counted in the study are generalist predators that feed on a wide variety of different insects. The cover crops could have been attracting other insects that the natural enemies were preying upon. Aphids were present within cover crops and squash rows, and the natural enemies could have taken advantage of this more abundant food source. Other studies have shown that diversified plantings can increase presence of other insect pests. In a study done with floral resources planted adjacent to summer squash, the floral resources attracted squash vine borer, *Melitta curcurbitae,* to the area (Fair, 2015).

Many natural enemies, such as Coccinellids and Geocorids, rely on pollen and/or nectar as an important part of their diet. In a study done with Chrysopidae, Aphididae, and *F. esculentum*, the presence of the *F. esculentum* flowers reduced predation by the Chrysopids on aphids, probably due to Chrysopids feeding of pollen and nectar of *F. esculentum* (Robinson et al., 2008).

Overall, yield was highest in control plots. This is likely due to shading and crowding effects of the cover crops on the squash, dramatically reducing yield. In a similar study with collards planted with different weed species, the weeds did help to increase natural enemies, such as Coccinellids and Arenae, but overall negatively affected the yield (Schellhorn et al., 1997). This study involved additive density of cover crop plants as opposed to substitutive, where plots all had the same density of plants. In an analysis of 26 studies of additive and substitutive density, additive density studies demonstrated a tradeoff between yield and natural enemy activity, due to competition between the main crop and other crop (Iverson et al., 2014).

In this experiment, cover crop treatments provided a few environmental benefits, however, cover crops contributed negatively to squash growth. There were more natural enemies in cover crop plots, most notably Geocorids. The composition and numbers of natural enemies varied greatly from trial to trial, suggesting environmental conditions and season plays a great role in the numbers of these insect populations. Yields in the cover crop treatments were lower and the prevalence of CYVD greater, which could be due to the importation of squash bugs from other farms.

Future studies should focus on adjusting spacing of cover crops and squash plants to maintain adequate yields. Different planting arrangements may help to decrease competition between the squash and cover crops. Also, the cover crops may provide more benefit if they are planted earlier to allow natural enemy populations to become established before planting summer squash. It may be important to establish natural enemies very early; Besler showed that timing is important in terms of how CYVD is passed on to the plants. If squash bugs do not have contact with the plants during the first three weeks of growth, the squash plants do not acquire the CYVD bacterium (Besler, 2014).

Also, more work is needed to determine how to attract natural enemies to the squash plants, and not just to the cover crop areas near the squash. Studies should focus on the movement of squash bugs and natural enemies to learn more about their preferences and how far they can travel.

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Fig. 2.1. Research plots from Trial 1. Upper left-Control, Upper right-*F. esculentum*, Lower left-*V. unguiculata*, Lower right-*C. juncea*.

Fig. 2.2. Research plots from Trial 2 and 3. Top left-Control, Top Right-*F. esculentum*, Bottom Left-*V. unguiculata*, Bottom Right-*C. juncea.*

Fig. 2.3. Visual symptoms of cucurbit yellow vine disease. Plants display yellowing, wilting, and rapid decline (L). Honey-yellow discoloration of the phloem is also present (R).

Table 2.1 Timeline of events.

Trial	Squash planting date	Cover crop planting date	Squash harvest	Insect counts
1 (2014)	2 June	18 April	21 June-11 July	2 June-7 July
2 (2015)	11 Aug.	28 July	1 Sept.-26 Sept.	25 Aug.-22 Sept.
3 (2015)	5 May	9 April	27 May-3 July	18 May-3 July

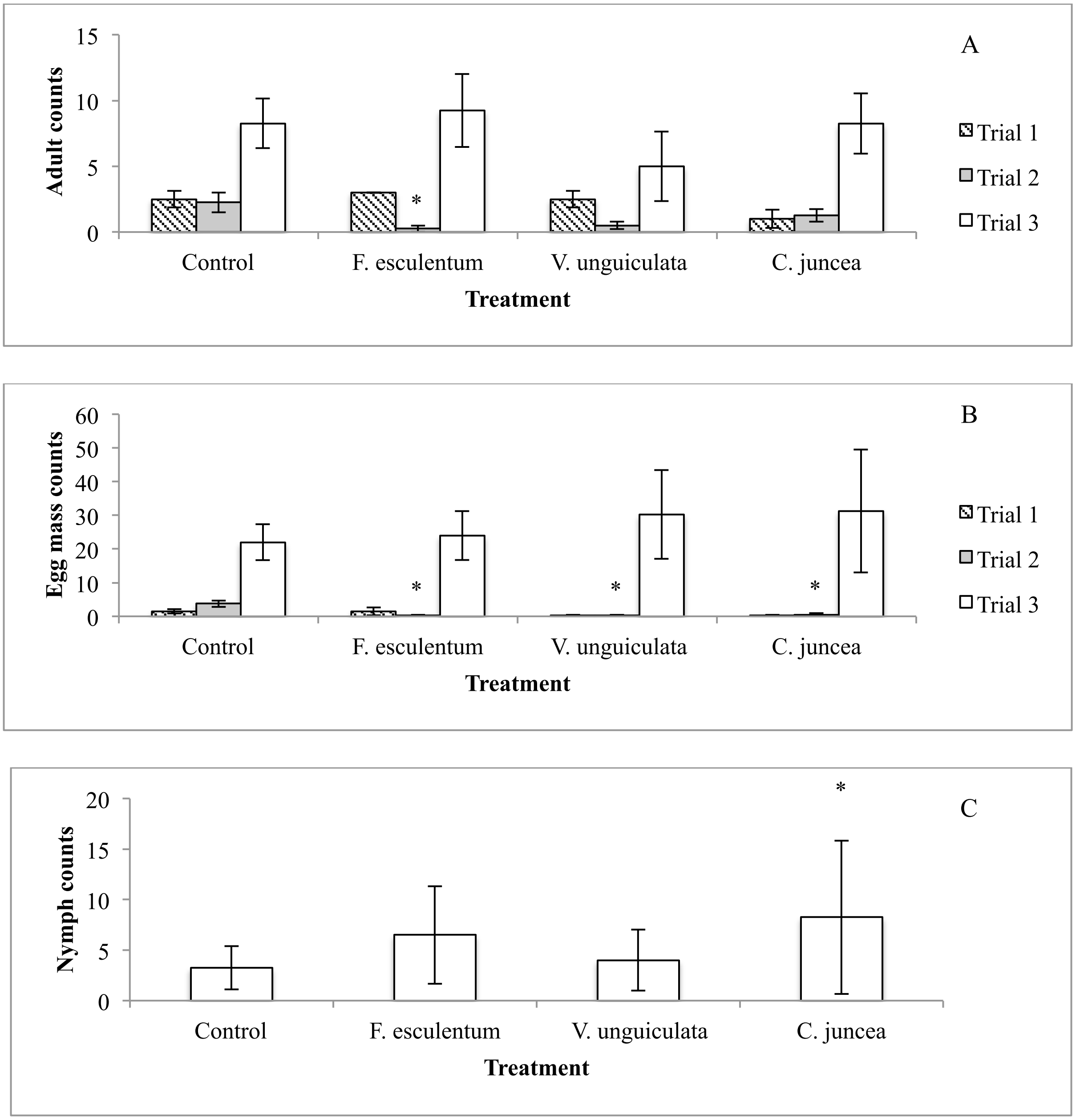

Fig. 2.4. *A. tristis* populations in treatment plots. A) Mean (+/-SE) of *A. tristis* adults for three trials. B) Mean (+/-SE) of *A. tristis* egg masses for three trials. C) Mean (+/-SE) of *A. tristis* nymphs in Trial 3. * Significant difference $P < 0.05$ compared with control

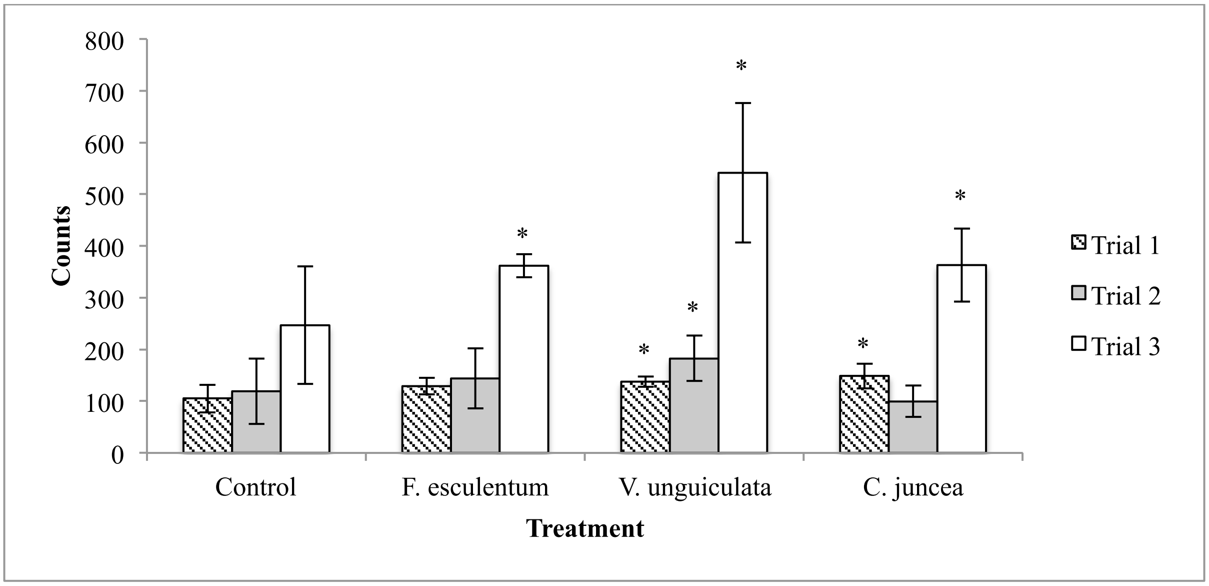

Fig. 2.5. Natural enemy populations in treatment plots. Mean (+/-SE) of all *A. tristis* predators.

* Significant difference $P < 0.05$ compared with control.

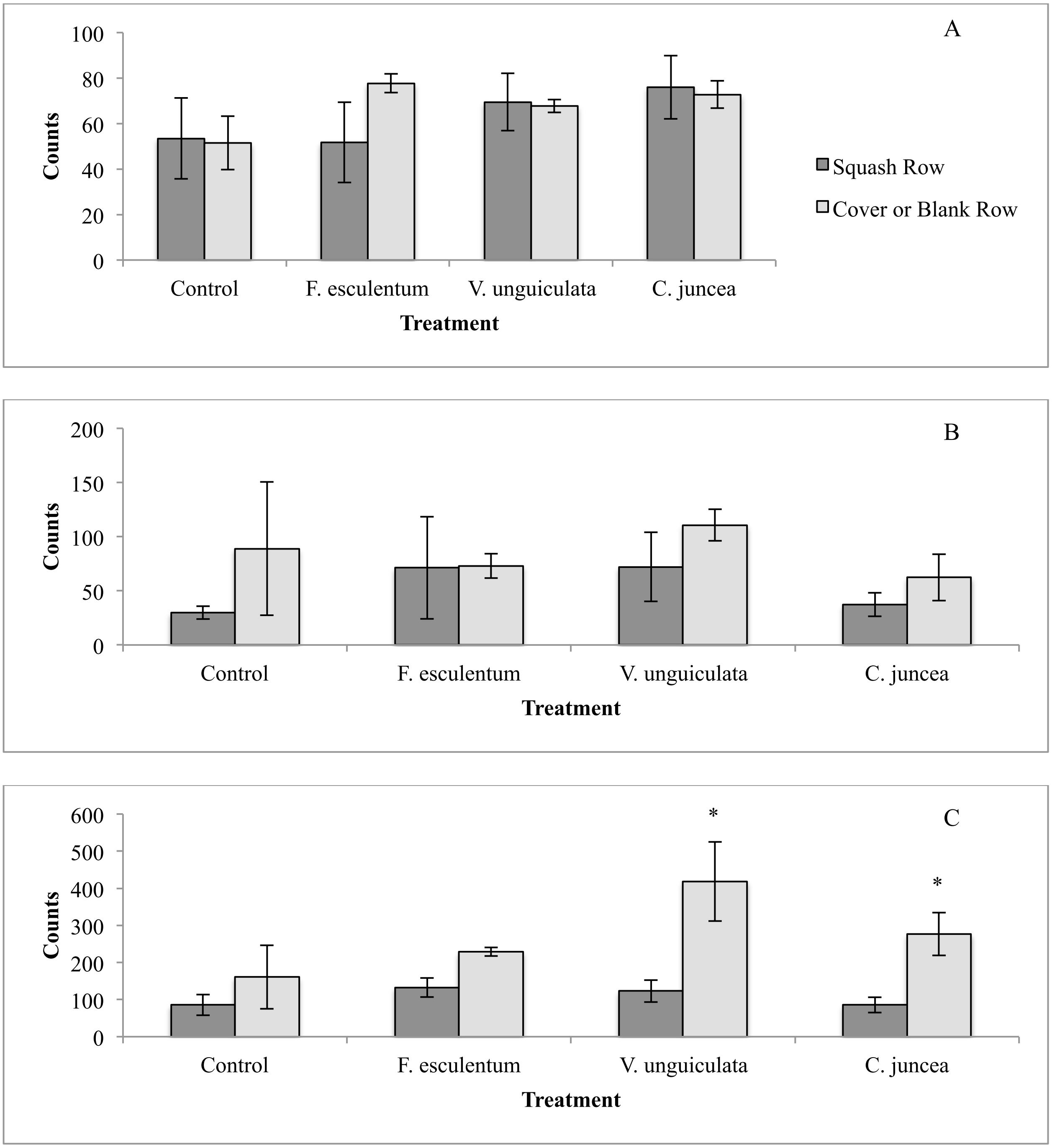

Fig. 2.6. Predator populations within squash and cover crop or control rows. Mean (+/-SE) of all *A. tristis* predators. A) Trial 1. B) Trial 2. C) Trial 3. * Significant difference $P < 0.05$.

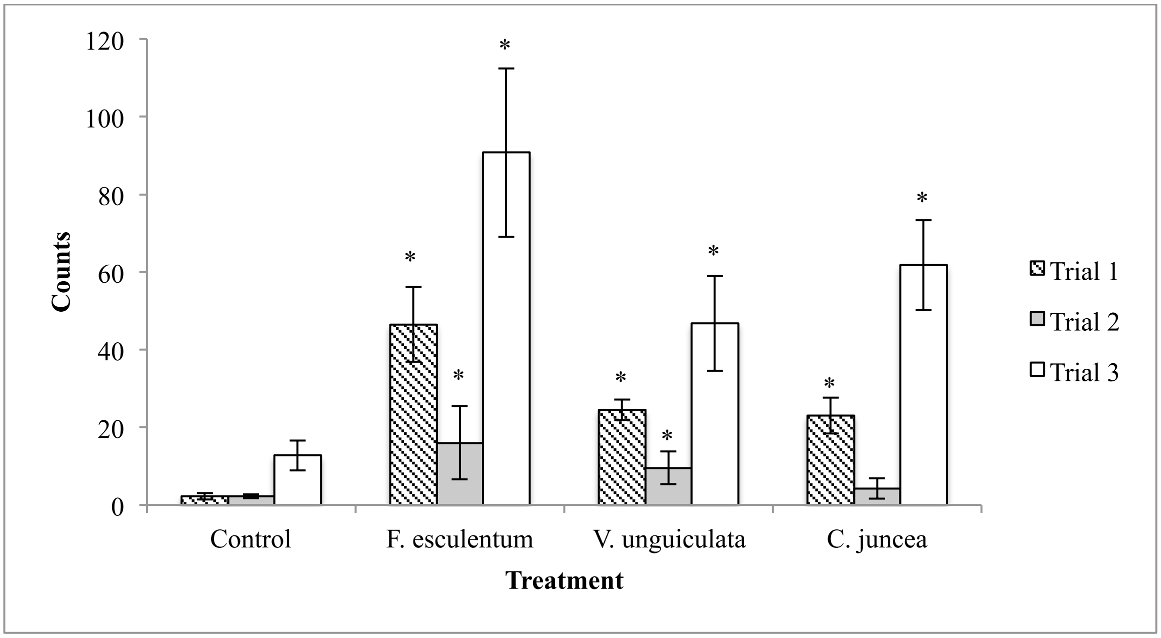

Fig. 2.7. Geocoridae populations within treatment plots. Mean (+/-SE) of Geocoridae for Trials 1, 2, 3. * Significant difference $P < 0.05$ compared with control.

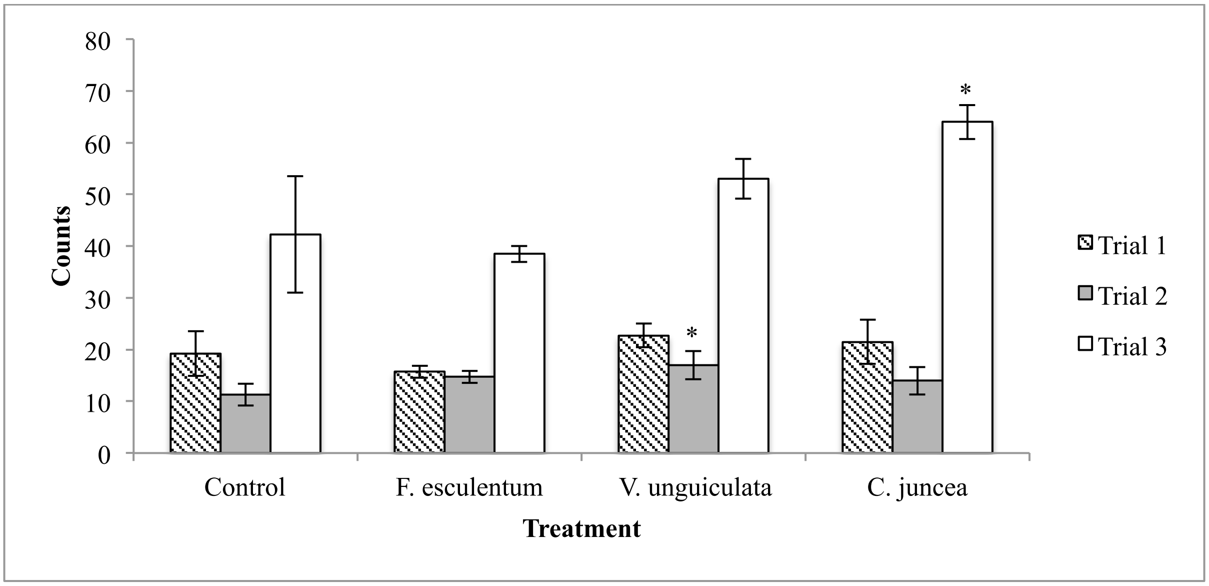

Fig. 2.8. Arenae populations within treatment plots. Mean (+/-SE) of Arenae in Trials 1, 2, 3. * Significant difference $P < 0.05$ compared with control.

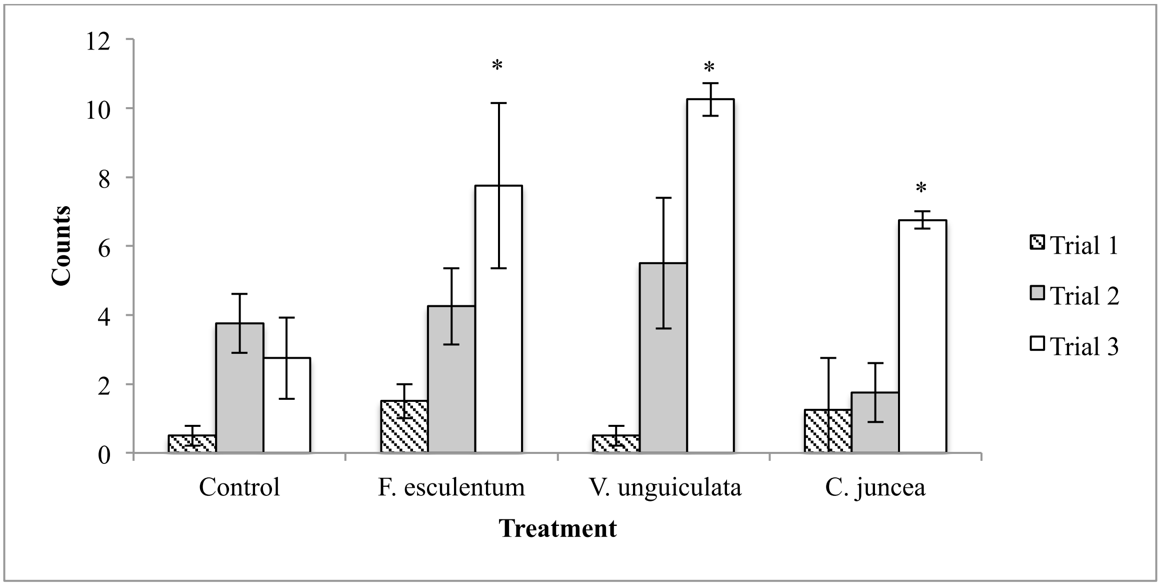

Fig. 2.9. Coccinellidae populations within treatment plots. Mean (+/-SE) of Coccinellidae for Trials 1, 2, 3. * Significant difference P < 0.05 compared with control.

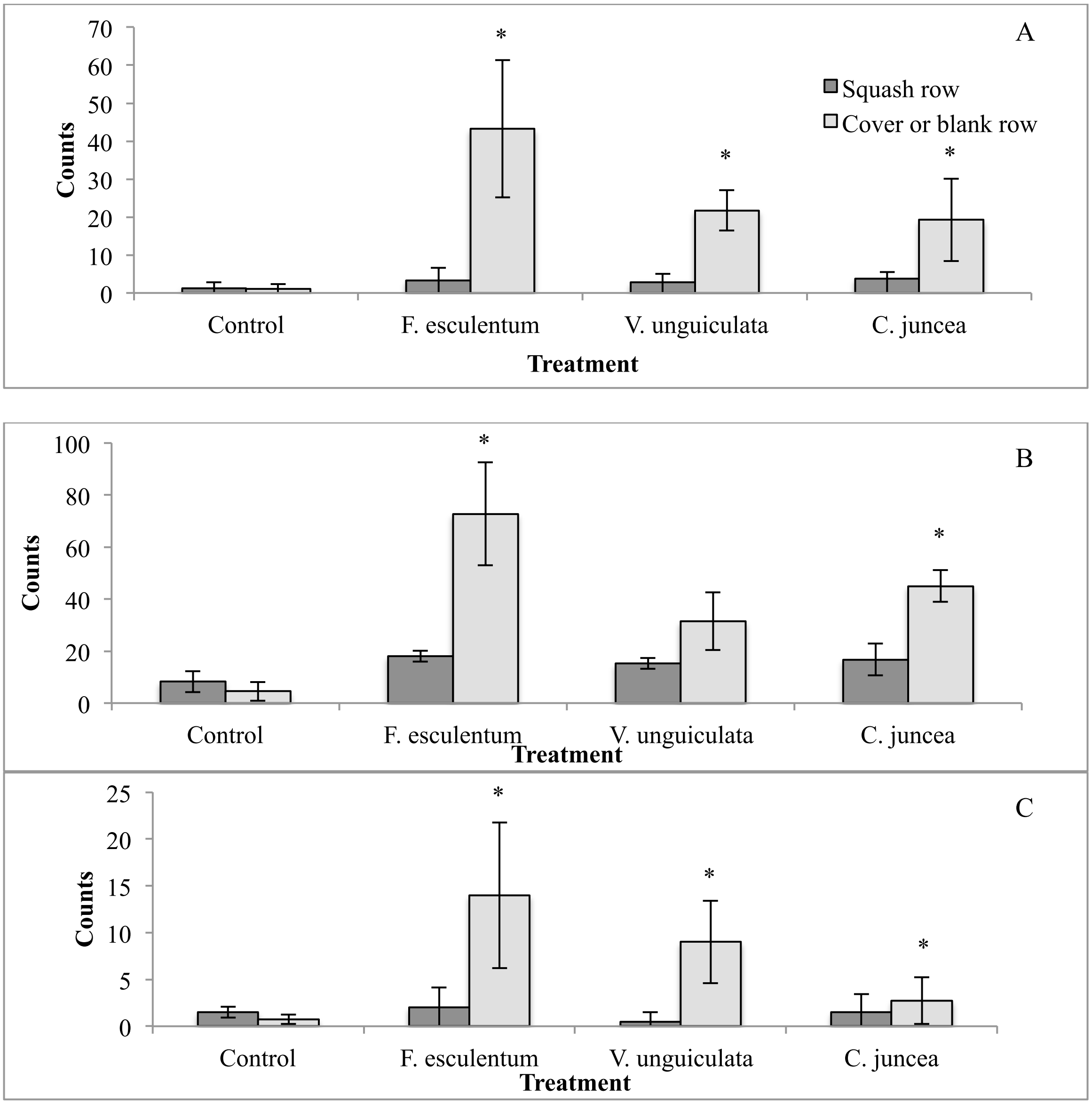

Fig. 2.10. Comparison between Geocoridae in squash and cover crop or control row. Mean (+/-SE) counts. A) Trial 1. B) Trial 2. C) Trial 3. * Significant difference $P < 0.05$ compared with control.

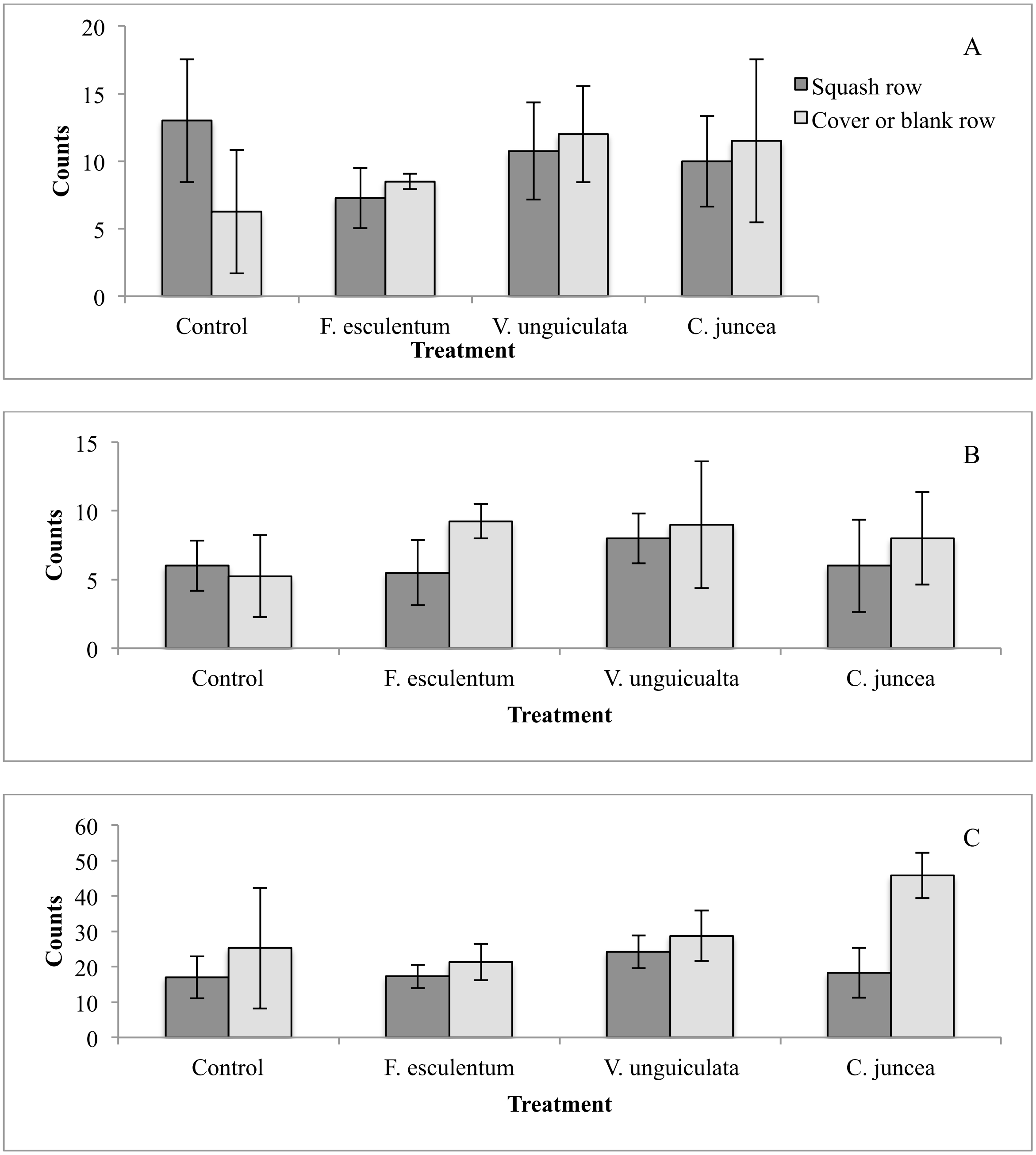

Fig. 2.11. Comparison between Arenae in squash and cover crop or control row. Mean (+/-SE) counts. A) Trial 1. B) Trial 2. C) Trial 3. * Significant difference $P < 0.05$ compared with control.

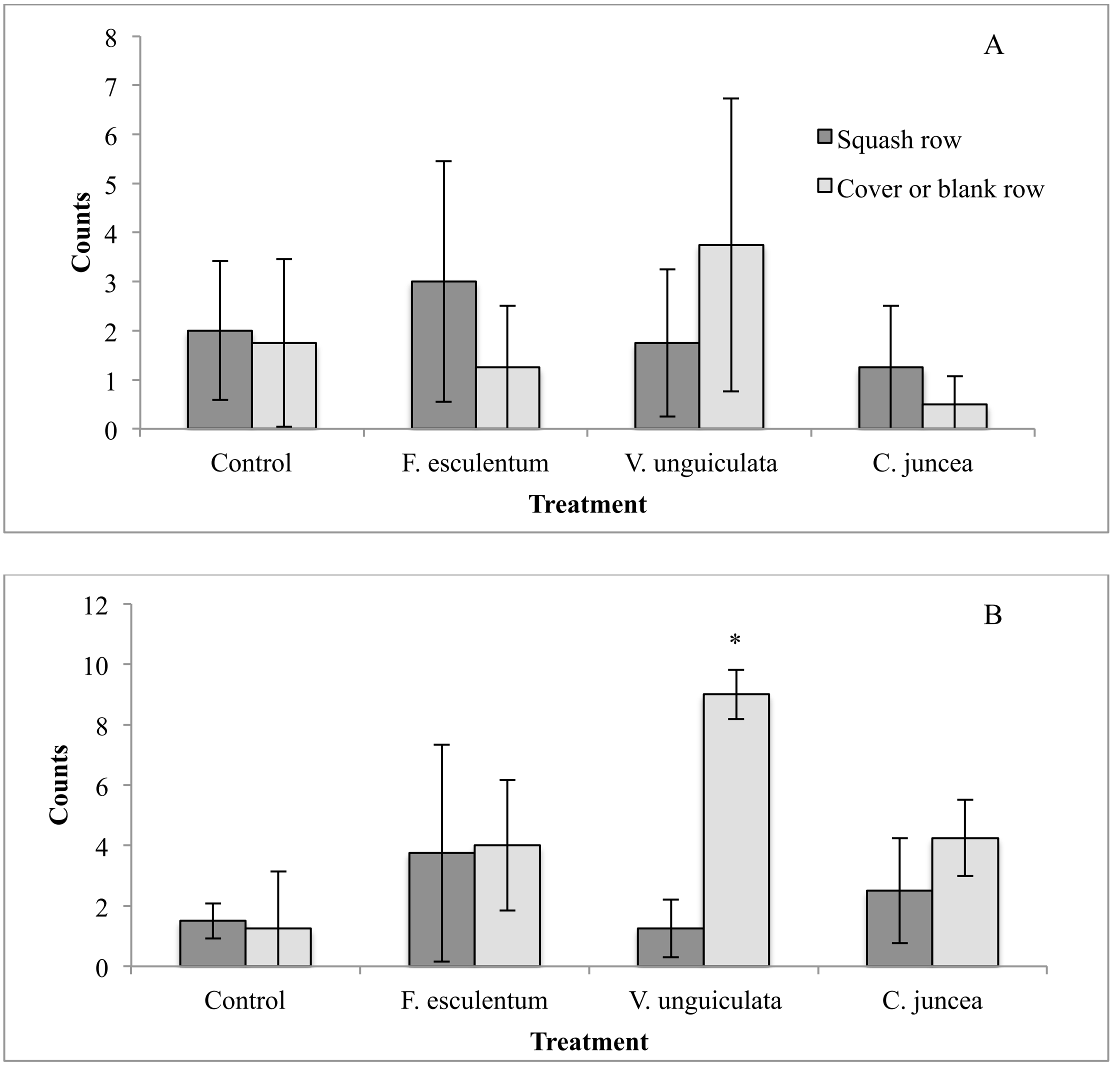

Fig. 2.12. Comparison between Coccinellidae in squash and cover crop or control row. Mean (+/-SE) counts. A) Trial 2. B) Trial 3. * Significant difference $P < 0.05$ compared with control.

Table 2.2 Effect of cover crops and control on Carabidae, Solenopsis, and Staphylinidae populations. Means +/- SE of natural enemies. Insects with * are significantly different than control at $P < 0.05$.

	Trial 1 mean +/- SE	Trial 2 mean +/- SE	Trial 3 mean +/- SE
Carabidae			
Control	6.25 +/- 1.70	0.500 +/- 0.500	5.50 +/- 0.866
F. esculentum	6.75 +/- 2.17	1.25 +/- 0.750	8.25 +/- 2.68
V. unguiculata	9.50 +/- 1.55	1.00 +/- 0.408	6.00 +/- 1.22
C. juncea	12.0 +/- 2.08*	0.500 +/- 0.289	8.75 +/- 2.06
Solenopsis			
Control	72.5 +/- 25.2	102 +/- 65.4	182 +/- 102
F. esculentum	58.8 +/- 24.7*	107 +/- 55.9	211 +/- 23.8*
V. unguiculata	67.5 +/- 12.4	148 +/- 43.7*	420 +/- 136*
C. juncea	76.8 +/- 27.0	79 +/- 28.0	217 +/- 64.0
Staphylinidae			
Control	4.25 +/- 1.97	0.75 +/- 0.478	2.00 +/- 0.707
F. esculentum	1.25 +/- 0.946*	0.75 +/- 0.25	5.75 +/- 1.31*
V. unguiculata	10.75 +/- 3.32*	2.00 +/- 0.707	5.50 +/- 1.55*
C. juncea	12.75 +/- 3.42*	0.25 +/- 0.25	3.75 +/- 0.853

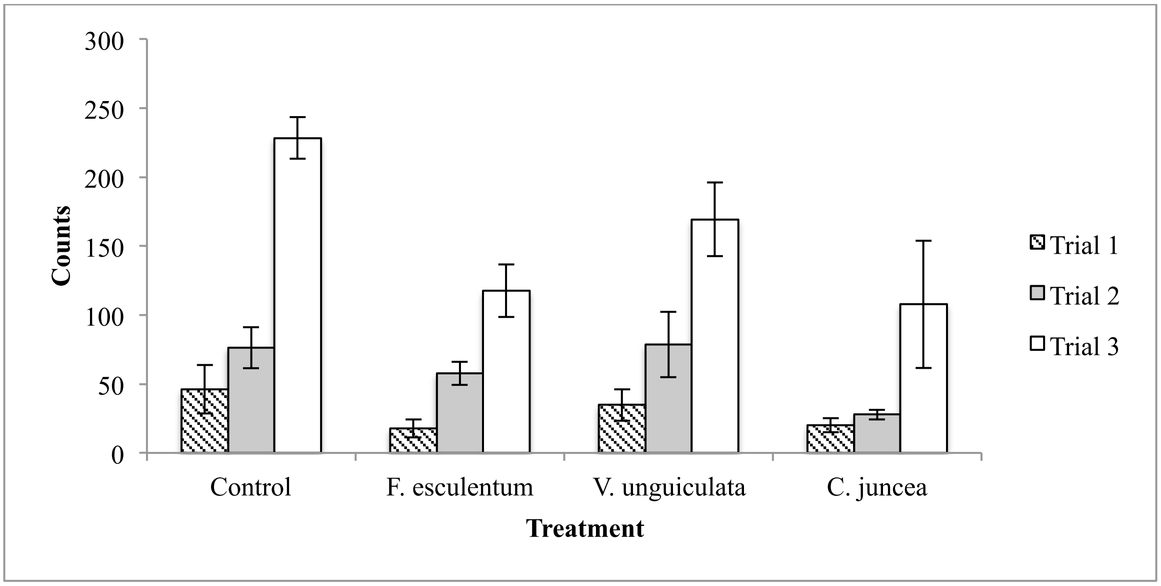

Fig. 2.13. Aphid populations in treatment plots. Mean (+/-SE) counts for Trials 1, 2, 3.

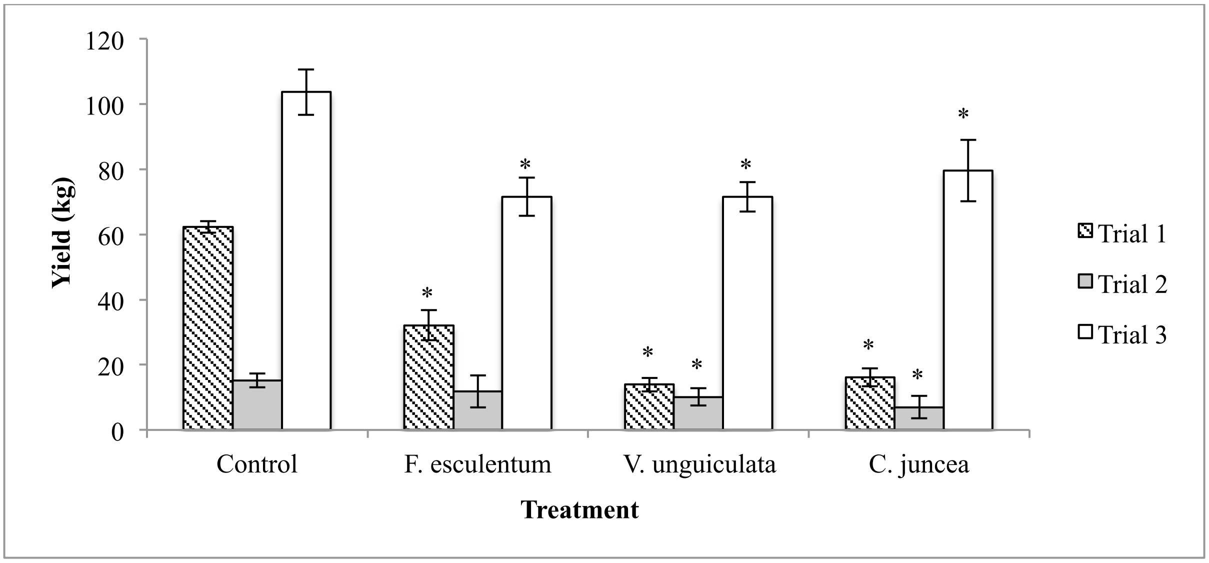

Fig. 2.14. Yield of summer squash in treatment plots. Mean (+/-SE) for Trials 1, 2, 3.

* Significant difference $P < 0.05$ compared with control.

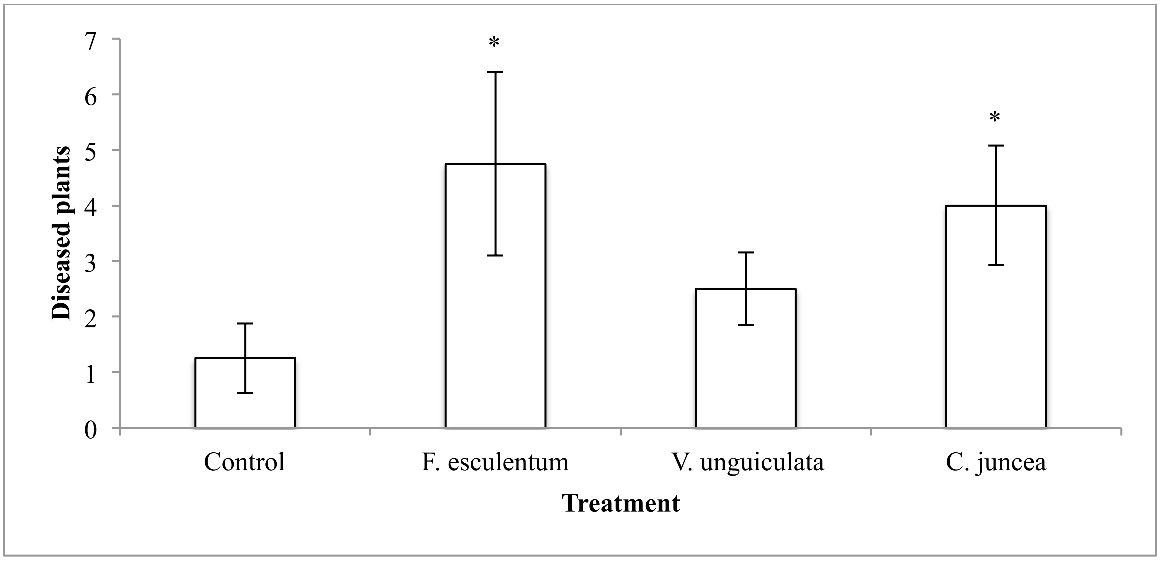

Fig. 2.15. CYVD infected summer squash plants in treatment plots. Mean (+/-SE) for Trial 3.

* Significant difference $P < 0.05$ compared with control.

CHAPTER 3

EFFICACY OF ORGANIC INSECTICIDES ON SQUASH BUG (*ANASA TRISTIS*) ADULTS AND NYMPHS[2]

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[2] L. Davies, D. Berle, P. Guillebeau, E. Little. To be submitted to *HortTechnology*

Abstract

Squash bugs (*Anasa tristis)* (DeGeer) inflict serious damage to organic cucurbit crops. They damage the crops by feeding on the plants and by transmitting the bacterium *Serratia marcescens*, which causes cucurbit yellow vine disease (CYVD). This disease varies dramatically from year to year in its effect on squash yield, depending on squash bug populations. Organic growers utilize several techniques, such as row covers, crop rotation, and trap crops, but many still struggle with yield losses due to CYVD. The purpose of this study was to evaluate organic insecticides (pyrethrin, azadirachtin, pyrethrin + azadirachtin, spinosyn A and D, K salts of fatty acids, mineral oil) to determine their efficacy. Overall, pyrethrin showed highest death rates, killing an average of 100% of young nymphs, 88% of old nymphs, and 82% of adults 24 hrs after insecticide exposure.

Introduction

Squash bugs can be serious insect pests for organic growers. Adults and nymphs of squash bugs feed on the xylem of cucurbits (Adam, 2006). Squash bugs can cause wilting, fruit rot, necrosis, and plant death, commonly referred to as ‘Anasa wilt’ (Doughty, 2016). Squash bugs cause damage to the plant through direct feeding and decline can also occur due to the role squash bugs play as vectors of *Serratia marcescens*, the bacterium responsible for cucurbit yellow vine disease (CYVD) (Bruton et al., 2003). This disease has been noted in several states, including Georgia (Besler, 2014). CYVD damage to squash plantings varies depending on the squash bug population, with growers seeing losses of 5-100% (Bruton et al., 2003). Plants affected by CYVD turn yellow, wilt and eventually die and the phloem of infected cucurbits turns a honey-brown color (Bruton et al., 1998).

Squash bugs harbor the bacterium while overwintering and transmit it to cucurbits when they begin feeding the following year (Pair et al., 2004). In one study, overwintering squash bug adults were found to have varying rates of infection, ranging from 10% to 50% (Besler, 2014; Bruton et al., 2003). Wayadande et al demonstrated that both adults and nymphs of *A.* tristis have the ability to transmit the bacterium multiple times (Wayadande et al., 2005). These findings suggest that controlling squash bug adults and nymphs is critical to decreasing ‘Anasa wilt’ and CYVD.

Organic growers utilize several different methods to control this insect pest, such as row covers, crop rotation, sanitation methods, trap crops, and planting cucurbits that are least preferred by squash bugs. While these methods reduce squash bug populations, many organic growers still face yield losses due to these insect pests.

Conventional growers do not have similar problems with squash bugs because there are several conventional insecticides available for squash bug control that are not permitted for certified organic production. In field studies, carbaryl has shown decreased squash bug populations when applied at the base of pumpkins and watermelons (Cranshaw et al., 2001; Dogramaci et al., 2004). In the field, Palumbo found that plots sprayed with cypermethrin had lower squash bug populations than control, and that also the timing of the insecticide spraying greatly plays a role in the success of the insecticide (Palumbo et al., 1993).

Studies using insecticides on squash bugs have focused primarily on conventional insecticides, however, there are several insecticides approved by the Organic Materials Review Institute (OMRI) that are labeled for use on squash. Some of these may provide adequate control of squash bug adults and nymphs. OMRI-approved insecticides include azadirachtin (Monterey neem oil), mineral oil (Monterey horticultural oil), spinosyn A and D (Monterey garden insect spray), pyrethrin (PyGanic), azadirachtin + pyrethrin (Azera), and K salts of fatty acids (Safer Soap). These insecticides vary in their active ingredients and effect on insects.

Azadirachtin is derived from the seeds of the neem tree, *Azadirachta indica*. Azadirachtin kills insects by inhibiting molting (Buss et al., 2002). Mineral oil works by suffocating insects when the spray is directly applied (Buss et al., 2002). Spinosyn A and D is derived from bacteria and affects the nervous system of the insect, causing paralysis and death (Bunch, 2014). Pyrethrins are derived from *Chrysanthemum cinerariaefolium.* Contact with pyrethrins causes immediate paralysis to many insects, but some are able to metabolize the pyrethrins and recover (Buss et al., 2002). Potassium salts of fatty acids and may work to disrupt the insect cuticle, although the soap may affect other aspects of the insect as well (Buss et al., 2002).

Watkins looked the effects of different conventional insecticides on eggs, young nymphs, old nymphs, and adult squash bugs and found that pyrethrin-based sprays were best at killing young nymphs and the most economically feasible for growers; other sprays did kill significant numbers of bugs, but required very high levels in order to do so (Watkins, 1946). None of the sprays were very effective at killing any other life stages of squash bugs (Watkins, 1946).

There are few studies evaluating the effects of the other organic insecticides on squash bug mortality. Some work has been done with the brown-marmorated stink bug (*Halyomorpha halys*), also a Hemipteran agricultural pest, but in the Pentatomidae family. Stink bugs exposed to spinosyn A and D, K salts of fatty acids, mineral oil, azadirachtin, and pyrethrin showed higher mortality rates than control (Bergmann et al., 2014). Pyrethrin killed almost all nymphs and adults, approximately 95%, 48 hr after exposure while the other insecticides killed at least 40% of the nymphs and adults (Bergmann et al., 2014). In another study evaluating efficacy of organic insecticides on stink bugs, pyrethrins with kaolin showed highest mortality rates, with mortality rates higher than 80%, 7 days after exposure (Lee et al., 2014). Azadirachtin, K salts, and spinosyn A and D showed similar results after 7 days, with death rates around 60% (Lee et al., 2014).

Each of these insecticides may be useful in decreasing insect pest populations, but there is little information about which is best for squash bugs and at what stage. With the exception of pyrethrin, most of the research on squash bug insecticides focuses on insecticides not approved by OMRI. The purpose of this project is to evaluate OMRI-approved insecticides for controlling squash bugs.

Materials and Methods

Insecticide Experiment (June-July 2015)

Experimental Design

Six commonly used organic insecticides advertised to kill squash bugs were selected for this study (Table 3.1). Water was used as a control treatment. Insecticides were obtained from Seven Springs Farm (Check, VA) in Feb. 2015. Highest labeled rates were applied for all treatments (Table 3.1).

Experimental Procedures

Squash bugs were collected from field plots or from farms near Athens, Georgia. The trials were completed between June and July 2015. Squash bugs were treated on the same day of collection to reduce likelihood of other causes of death. Nymphs in stage one, two, and three were classified as 'young' or 'old' if in stage four or five (Figure 3.1). Five nymphs of the same age group were put into a petri dish and sprayed once with an insecticide, which coated each of the bugs. Each spray was approximately 0.026 oz. of insecticide. Petri dishes were covered and placed in an air-conditioned room (~75° F). Death rates were recorded at 1 h and 24 h. Squash bugs were determined to be dead when not able to stand within 30 s of being placed on their back, as outlined in previous work (Watkins, 1946). Each trial consisted of the same age group sprayed with each of the 6 treatments plus the control with a total of 35 bugs in each trial replication. Six replications were completed with young nymphs, 7 for old nymphs, and 8 for adults, for a total of 735 nymphs and adults. Insecticides were mixed once a week in 32 oz. spray bottles and kept in dark, cool room to discourage chemical breakdown.

Statistical Analyses

Analysis was run using R version 3.2.2 software. Death rates were analyzed using binomial regression. If an insecticide killed all or none of the nymphs the data would be modified to show one trial had 4 deaths instead of 5 or 1 death instead of 0 in order for the model to run. As a result of this modified data, the results are slightly biased, but it did not affect the overall results or conclusions. Tukey comparisons were used to note differences among the treatments, with the single-step method used to adjust p values accordingly. Significance was noted when $P < 0.05$.

Results

Death rates after 1 hour

After 1 hour, young nymphs had the highest death rates compared to other stages of bugs tested. Among the insecticides, pyrethrin had the highest average death rate (76%) and was significantly higher than all other insecticides ($p<0.01$ for all contrasts, Fig. 3.2). Pyrethrin + azadirachtin was higher than control, with an average death rate of 43%, but was not significantly higher than any of the treatments ($p=0.0589$). Overall, K salts of fatty acids, mineral oil, spinosyn A and D, azadirachtin, and water had very similar death rates.

After 1 h, pyrethrin and pyrethrin + azadirachtin did kill older nymphs, but the death rates were not significantly higher than control (Fig. 3.2). Pyrethrin killed an average of 25% of old nymphs, while pyrethrin + azadirachtin killed an average of 8% (Fig. 3.2). Spinosyn A and D, azadirachtin, and water had no effect on older nymphs.

Only pyrethrin and pyrethrin + azadirachtin killed adults after 1 h, however there were no significant differences among these rates (Fig. 3.2).

Death rates after 24 hours

After 24 h, the highest death rate was seen in young nymphs. Death rates for young nymphs were significantly higher with pyrethrin, pyrethrin + azadirachtin and spinosyn A and D than the control ($p<0.01$, $p<0.01$, $p=0.01135$, Fig. 3.3). Pyrethrin had the highest average death rate, 100%, and was significantly higher than K salts of fatty acids, mineral oil, spinosyn A and D, and azadirachtin ($p=0.002$, $p=0.0033$, $p=0.04028$, $p<0.01$). Pyrethrin + azadirachtin had the next highest average death rate, 80%, and was significantly higher than K salts of fatty acids, mineral oil, and azadirachtin ($p=0.00389$, $p=0.00833$, $p<0.01$).

The pattern of death is similar for old nymphs, with pyrethrin, pyrethrin + azadirachtin, and spinosyn A and D killing significantly more squash bugs than the control ($p<0.01$, $p=0.01202$, $p=0.04858$) (Figure 3.3). Death rates were significantly higher with pyrethrin than all other insecticides (Azera $p=0.02264$, soap, azadirachtin and mineral oil $p<0.01$, spinosyn $p=0.00177$). Pyrethrin + azadirachtin was also significantly higher than K salts of fatty acids, mineral oil, and azadirachtin ($p=0.00727$, $p=0.01225$, $p=0.01212$). Spinosyn A and D was significantly higher than azadirachtin and mineral oil ($p=0.04844$, $p=0.04818$). Azadirachtin did not kill any older nymphs after 24 h, which was actually lower than the control treatment.

After 24 h, death rates for adults showed a similar trend, but the averages were lower compared to nymph data. Pyrethrin, pyrethrin + azadirachtin, and spinosyn A and D had death rates significantly higher than control ($p<0.01$, $p=0.00189$, $p=0.02938$, Fig. 3.3). Pyrethrin killed significantly more adult squash bugs than all insecticides except pyrethrin + azadirachtin (K salts of fatty acids, mineral oil, and azadirachtin $p<0.01$, spinosyn A and D $p=0.00316$). Pyrethrin + azadirachtin killed more squash bugs than K salts of fatty acids, mineral oil, and azadirachtin (p=0.00205, p=0.00195, p=0.00199). Spinosyn A and D had higher death rates than K salts of fatty acids, mineral oil, and azadirachtin (p=0.03047, p=0.02947, p=0.02945) (Fig. 3.3).

Discussion

Results of this study suggest that pyrethrin is the most effective insecticide against squash bugs, with average death rates of 96%, 88% and 83%, for young nymphs, old nymphs, and adults after 24 h, respectively. Pyrethrin + azadirachtin was the next best insecticide, with death rates for young, old, and adults at 80%, 51% and 60%, respectively. Spinosyn A and D did little to kill bugs after 1 h but killed significantly more squash bugs than control after 24 h, and resulted in average deaths of 53%, 40%, and 35% for young, old, and adult bugs, respectively. Spinosyn takes longer to have an effect on the squash bugs. Overall, young nymphs were more susceptible to the insecticides. This is to be expected since as insects molt they are replacing their exoskeleton with a stronger one (Palumbo et al., 1993).

Azera is a combination of pyrethrin and azadirachtin. Pyretherin is the main ingredient in PyGanic, while azadirachtin is the main ingredient in Neem oil. Since Neem showed very low death rates, it is likely that azadirachtin does little to kill squash bugs and Azera is only effective because it contains pyrethrin.

Although pyrethrin appears to be the most effective at killing squash bugs, it is a broad-spectrum insecticide that can have a negative effect on non-target species. Frequency of use and timing of pyrethrin needs to be closely monitored. Honeybees can be killed from pyrethrin-based sprays (Casida et al., 1995). Pyrethrin can also kill natural enemies such as spiders and parasitic wasps and is also very toxic to fish (Cox, 2002).

Squash bugs are notorious for hiding near the base or under leaves of squash plants, making it difficult to be affected by any insecticides in the field (Palumbo et al., 1993; Palumbo et al., 1991). However, use of organic insecticides can be an important part of an organic grower's insect pest management plan if an effective material was applied at the right state of development. Based on results of this study, yield trials using pyrethrin and pyrethrin + azadirachtin should be performed to determine effectiveness on squash bugs in a field situation. It would also be useful to compare timing and targeting of spray materials to catch squash bugs in the most vulnerable stage.

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Table 3.1. Rate of insecticides. 1 oz/gallon = 7.81 L/m^3.

Dilutions used were the manufacturer's highest recommended levels.

Active ingredient	**Trade name**	**Manufacturer**	**Dilution used (oz/gal water)**
Azadirachtin (70%)	Monterey neem oil	Lawn and Garden Products, Inc.	1.0 oz
Mineral oil (80%)	Monterey horticultural oil	Lawn and Garden Products, Inc.	2.5 oz
Spinosyn A and D (0.5%)	Monterey garden insect spray	Lawn and Garden Products, Inc.	2.0 oz
Pyrethrin (1.4%)	PyGanic	McLaughlin Gormley King Company	2.0 oz
Pyretherin + Azadirachtin (1.4 + 1.2%)	Azera	McLaughlin Gormley King Company	2.0 oz
K salts of fatty acids (49.2%)	Safer Soap	Woodstream Corporation	2.5 oz

Fig. 3.1: Squash bug nymphal stages. After emerging from eggs, squash bugs have green abdomens and black or red appendages (upper left). They then molt into stage 2 and then 3 (middle left). Stage 4 (upper right) wing pads begin to form. In stage 5 (lower left) wing pads darken and they become adults after their last molt (lower right). The entire process can take 4-6 weeks and depends on weather and available food sources.

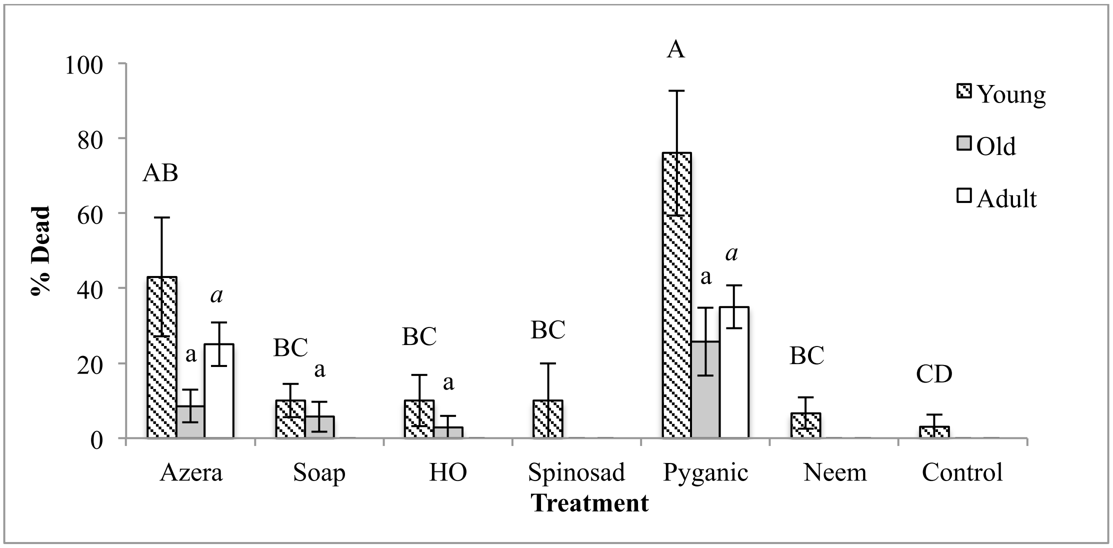

Fig. 3.2. Effects of organic insecticides on squash bug nymphal and adult survival 1 hour after insecticide exposure. Mean (+SE) of death rates for each treatment for each age group. Different letters show significant differences between treatments. Differences were compared among each age group, not between age groups.

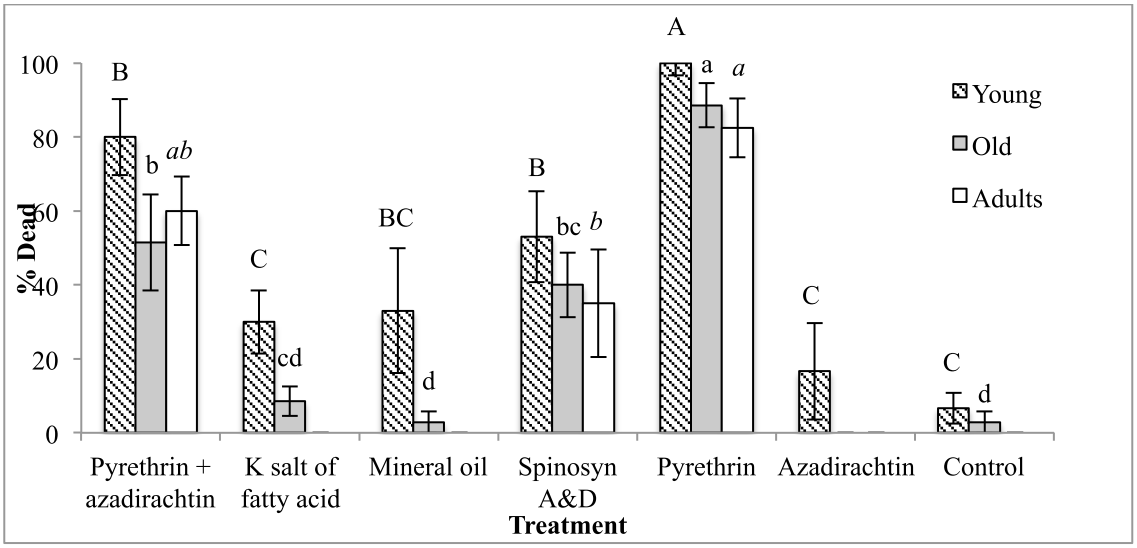

Fig. 3.3. Effects of organic insecticides on squash bug nymphal and adult survival 24 hours after insecticide exposure. Mean (+SE) of death rates for each treatment. Different letters show significant differences between treatments. Differences were compared among each age group, not between age groups.

CHAPTER 4

CONCLUSIONS

Field trials

Based solely on the results of this study, there is little benefit for planting cover crops in close proximity to summer squash. Though the squash bug population data was mixed, the general trend was that squash bugs did not proliferate within the control plots. It was expected that the control plots, with a lack of natural enemies, would have the highest populations of squash bugs.

Another study has found little evidence for utilizing diversified plantings to decrease squash bugs. Fair's research involved planting floral resources near the summer squash in an attempt to attract natural enemies. They found little conclusive evidence to recommend this practice, and they found that the floral resources attracted another insect pest, the squash vine borer, to the plots (Fair, 2015).

Squash bug populations varied from trial to trial, but were generally on the low side compared to previous years. Trial 3 had the highest counts of squash bugs, which were still somewhat low compared with observations on the same farm in years prior to this study. Data from Trial 3 is likely more consistent with what a grower would experience during a low to moderate squash bug infestation.

The lack of squash bugs for two consecutive years and three crops was clearly one of the main limitations of the study. If the experiment were repeated in the future, there should be provision for collecting and transporting squash bugs to test plots to insure there is adequate numbers present. With higher squash bug populations, the natural enemies may have made more of an impact.

Because of the elusive nature of squash bugs, even when present in high levels, a more thorough method of counting squash bugs could prove useful. Traps and visual inspections alone may not be providing the full picture of squash bug movement and habitat preferences. This was most apparent at the end of Trial 3 when plants were pulled from the test plots. After Trial 3, squash bugs were counted as plants were pulled. These numbers provided significantly higher counts in control plots compared to cover crop plots. This could be attributed to preferences to hide under leaves and at the base of the plant (Palumbo et al., 1991). Or, it could be due to greater predatory natural enemies in the cover crop treatments and not in the control.

Natural enemy populations also provided mixed results. There were generally more natural enemies within cover crop treatment plots than control, though the composition of the natural enemies fluctuated and differed between the different trials. One of the strongest trends was that big-eyed bugs were consistently attracted to the cover crop treatments, and tended to stay within the cover crop row. The big-eyed bugs may have preferred to feed within the cover crop plots and this may be one reason why squash bug populations did not decrease within the cover crop treatments. Big-eyed bugs may have been feeding on the pollen and nectar of the cover crops similar to the behavior of lacewings (Robinson et al., 2008) or they could have been eating the aphids that were prevalent in much higher numbers than the squash bugs.

Squash bug and natural enemy counts over time were not analyzed because of the fluctuations in populations due to weather and other uncontrolled variables. Whenever rainfall would occur, the pan traps would overflow and any bugs within the pan traps would be washed away. Analyzing the data over time would be useful in order to determine if squash bug and natural enemy populations were overlapping in time. If there were little overlap, this would add to the argument that this planting system provides little benefit to the squash. Future studies should take careful weather notes so to explain these patterns over time.

There are a few species of parasitic wasps that lay eggs within squash bug eggs. This data was not taken during the experiment because of difficulty in identifying parasitic wasps to species level. Parasitic wasps are specialist squash bug predators, and data collected from the experiments were concentrated on generalist predators (spiders, big-eyed bugs, lady beetles, etc). Specialist predators focus solely on squash bugs and may play a larger role in managing squash bug populations.

Yield was negatively affected by the cover crop plantings, due to competition between the cover crops and summer squash. Future studies could focus on increasing the spacing between cover crops and summer squash, or studies could focus on border crops of cover crops or cover crops planted in adjacent large plots. In general, work is needed to determine the optimal distance between the cover crops and the squash plants in order for natural enemies to move into squash while maintaining adequate yields. Yields were very high within the control plots, this could have been due to the fact that control squash had a larger amount of room to grow, compared to squash between the cover crops. The next studies could keep the same density of crops within each plot in order to get a more accurate yield (Schellhorn et al., 1997).

The long-term presence of cover crops on a farm and the stage of growth of a cover crop could also play a role in the results. Habitats for natural enemies may need several seasons or years to build up populations. If more time was used to maintain habitats, results may have been different. Besler showed that timing is also important in terms of how cucurbit yellow vine disease (CYVD) is passed on to the plants. If squash bugs do not have contact with the plants during the first three weeks of growth, the squash plants do not acquire the CYVD bacterium (Besler, 2014). Therefore, management of the bugs is crucial when plants are small, and natural enemy populations would need to be very high when the squash plants are very young in order to have any effect.

Larger plots with more distance between different treatments could have affected the results. Control plots did have fairly high counts of natural enemies, even though cover crops were generally higher. These high counts within control plots could have been due to the close proximity of the plots. Insects could have been easily flying from one plot to the next. Also, control plots had traps in the blank areas, which could have been easier for the insects to find as opposed to the traps within the cover crop rows. Cover crops surrounding pan traps within the cover crop rows should have been cut back slightly to allow greater access from insects, or the pan traps could have been raised up off the ground.

Insecticide Experiments

Overall, pyrethrin-based sprays were shown to kill the most squash bugs. In general, younger nymphs were most affected by all the sprays and higher death rates were seen after 24 hours. Pyrethrin may be useful in a squash bug pest management plan, although it should not be the only method of controlling squash bugs and should be used with caution. Squash bugs can be difficult to spray because they prefer to hide under leaves and at the base of plants. Therefore, pyrethrin, in a properly directed spray, along with row covers, crop rotation, and other strategies can help to decrease populations.

It was difficult to determine the exact nymphal stage of each bug, because of differences in growth between the individuals and sexes of squash bugs. In order to better determine the nymphal stages, nymphs could have been measured and approximate lengths for each stage determined though that would take considerable time and effort. Determining the death rates of each nymph instar separately may provide more accurate information instead of lumping them into two groups of young and old nymphs. This would have required a greater number of nymphs to work with, which is challenging because the nymphs are difficult to catch. Future studies could include a squash bug rearing component, which would ensure availability of nymphs and eliminate the need to catch them from the field. Sexing and age determination would be easier if nymphs could be pulled from a rearing chamber. Reared nymphs would presumably have more uniform availability of food and water to insure healthy specimens for the insecticide evaluation.

The experimental design of this study could have been improved by randomly assigning each bug to a treatment, this way old and new bugs would be randomly chosen for each treatment. Also, instead of including 5 bugs in each petri dish, 1 bug per dish would allow greater accuracy in results. For the experiment, the highest labeled rates of insecticide were tested and useful information may be obtained by testing different rates of each insecticide. If lower rates cause similar death rates than growers could save money by spraying less.

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