The Effects of Insecticides on Squash Bug, its Egg Parasitoids and Pollinators in Virginia Cucurbit Production

James McKee Wilson

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Entomology

T. Kuhar, Committee Co-Chair

T. Anderson Co-Chair

J. Adams

D. Pfeiffer

A. Herbert

December 6, 2016

Blacksburg, Virginia

Keywords: Cucurbits, Squash Bug, Egg Parasitoids, Pollinator Risk

The Effects of Insecticides on Squash Bug, Its Egg Parasitoids and Pollinators in Virginia Cucurbit Production

James McKee Wilson

Abstract

My dissertation and research focused on the effects of insecticides on squash bugs, its egg parasitoid, and pollinators in the production of cucurbits in Virginia. Cucurbits, dependent on insect pollination for successful fruit set, are also susceptible to herbivorous insects. Squash bugs are capable of transmitting cucurbit yellow vine decline, and their feeding can cause significant necrotic wilt and death in many varieties. A common control practice is a tank-mixed application of broad-spectrum insecticides with the frequently applied prophylactic fungicides. Broad-spectrum insecticide applications are known to have deleterious effects on natural enemy populations, promote insecticide resistance, and have negative effects on pollinators. Squash bugs have several natural enemies, but their predominant egg parasitoid is most effective at reducing damaging populations. The scelionid wasp *Gryon pennsylvanicum* Ashmead, is a prevalent egg parasitoid in Virginia and can be negatively affected by the application of broad-spectrum insecticides. Through survey efforts I found that *G. pennsylvanicum* is widely distributed throughout Virginia and is capable of high rates of egg parasitism (>90%). This is contrary to the 20% level previously assumed for the East Coast. I explored the effects of narrow-spectrum and novel insecticides on the fate of the egg parasitoids, those developing in

the host egg and emerged adults of *G. pennsylvanicum*. Contact assays showed that λ-cyhalothrin and sulfoxaflor treatments had high adult parasitoid mortality. As new insecticides get registered for use there is often concern about their effect on pollinators, specifically the European honey bee *Apis mellifera* L. I evaluated the use of near-field assays on the sub-lethal effects of narrow-spectrum insecticides to honey bees, as a means to qualify risk. The protocol utilizes nucleus colonies of honey bees (with stores of nectar and pollen) and their feeding at a treated sucrose solution after being trained to a feeder in an enclosed arena. This choice-test style behavioral experiment shows promise in qualifying the risks associated with insecticide exposure in the field. In the case of pyrifluquinazon, colonies repeatedly choose to avoid feeding at tainted feeders even after training with no other outside sources of food present. Further researching the sub-lethal behavioral effects some insecticides have on bees in a colony can help us that better qualify risk.

General Audience Abstract

My dissertation and research focused on the effects of insecticides on squash bugs, its egg parasitoid, and pollinators in the production of cucurbits in Virginia. Plants in the cucumber family are dependent on insect pollination for successful fruit set, and are also susceptible to plant eating insects. Squash bugs are capable of transmitting cucurbit yellow vine decline, and their feeding can cause significant wilt and death in many varieties. To control for squash bug and other pests, growers commonly combine the application of broad-spectrum insecticides with the frequently applied prophylactic fungicides. Broad-spectrum insecticide applications are known to have negative effects on natural enemy populations, are capable of promoting insecticide resistance, and can have negative effects on pollinators if care in their use is not taken. Squash bugs have several natural enemies, but their predominant egg parasitoid is most effective at reducing damaging populations. The scelionid wasp *Gryon pennsylvanicum* Ashmead, is a prevalent egg parasitoid in Virginia and can be negatively affected by the application of broad-spectrum insecticides. Through survey efforts I found that *G. pennsylvanicum* is widely distributed throughout Virginia and is capable of high rates of egg parasitism (>90%). This is contrary to the 20% level previously assumed for the East Coast. I explored the effects of narrow-spectrum insecticides on the fate of the egg parasitoids, those developing in the host egg and emerged adults of *G. pennsylvanicum*. Contact assays showed that the insecticides λ-cyhalothrin and sulfoxaflor had caused high adult parasitoid mortality. As new insecticides get registered for use there is often concern about their effect on pollinators, specifically the European honey bee *Apis mellifera* L. I evaluated the use of large flight cages as a method to measure the sub-lethal effects of narrow-spectrum insecticides to honey bees, as a

means to qualify risk. The method utilizes small colonies of honey bees (with stores of nectar and pollen) and their feeding at a treated sucrose solution after being trained to a feeder in an enclosed arena. This choice-test style behavioral experiment shows promise in qualifying the risks associated with insecticide exposure in the field. In the case of pyrifluquinazon, colonies repeatedly choose to avoid feeding at tainted feeders even after training with no other outside sources of food present. Further researching the sub-lethal behavioral effects that insecticides have on bees in a colony can help us better qualify their risk.

Acknowledgements

Thank you.

Thank you to my loving and supportive wife, Molly. Thank you to my family for always encouraging me and believing in me.

I would like to thank Tom for taking me in as a student and pushing me to new limits, and for playing Devil's advocate when I needed it. It has been great to be a part of your team. I would like to thank Troy for listening to my ideas, and encouraging me to break into a new field of study. I would like to thank my graduate advisory committee for their support and feedback throughout my Ph.D. studies, their experience and input has been invaluable.

Scotty, thanks for all of the help on the high tunnels and feeders. Barry, thanks for helping me reclaim the tunnels after a couple of windy winters, they look great! Curt, the egg masses from your garden fueled a great part of my survey efforts. I look forward to sharing an office wall and a classroom or two with you in the next few years.

Nick, thanks for helping me out; in the bee yards, the class, the high tunnels, and all around. James, thanks for helping me get things started. It has been a pleasure to watch you join the team and succeed. In order of appearance, thank you to Liz, Casey, Madeline, Emily and Caroline. Your help in the field and the lab was tremendous. Thanks to the rest of the VIPR lab,

you guys have always had my back. Kathy and Sarah, thank you for looking out for me through the years.

I would like to thank the Virginia Tech Department of Entomology and the College of Agriculture and Life Sciences for giving me a home and making me feel like a part of the family. I am proud to be joining this family full-time.

Contents

Chapter 1

Literature Review

(Some of the text from this chapter has been published in: Doughty, H. B., J. M. Wilson, P. B. Schultz, and T. P. Kuhar. 2016. Squash Bug (Hemiptera: Coreidae): Biology and Management in Cucurbitaceous Crops. Journal of Integrated Pest Management (2016) 7(1): 1; 1–8.)

Cucurbits, including pumpkins, squash, melons, and cucumbers, are important vegetable crops in the U.S. planted on ~134,000 hectares and valued at nearly $800 million per year (NASS 2016). In Virginia, production of fresh market cucurbits accounts for 18% of the 11,285 ha in vegetable production (NASS 2012). Cucurbits are attacked by several key insect pests that can impact yield. These pests include striped cucumber beetle *Acalymma vittatum* (Fabr.), spotted cucumber beetle *Diabrotica undecimpunctata howardi* (Mannerheim), squash bug *Anasa tristis* (DeGeer), melon aphid *Aphis gossypii* (Glover), squash vine borer *Melitta satyriniformis* (Huebner), melonworm *Diaphania hyalinata* (L.), and seedcorn maggot *Delia platura* (Meigen). Consequently, there is a heavy reliance on insecticides in commercial cucurbit production. Often insecticides are tank mixed with fungicides that are routinely applied. In pumpkins and squash in the mid-Atlantic and southeastern U.S., one of the primary targets of the insecticides is squash bug (Doughty et al. 2016).

Being endemic to North America, the squash bug, *Anasa tristis* (De Geer) (Hemiptera: Coreidae), has a long history as a pest of cucurbits in the United States (Britton 1919, Wadley

1920, Beard 1940). The pest status of squash bug remains considerably high because of changes in cucurbit production and pest management tactics, and because of its association with cucurbit yellow vine disease (CYVD), which has occurred in several states in the United States in the past decade. This piercing–sucking insect is particularly problematic on *Cucurbita* vegetables like squash and pumpkin, but may attack other cucurbits as well (Bonjour and Fargo 1989; Bonjour et al. 1990, 1991). A discussion of the biology of the squash bug and integrated pest management options in cucurbit crops follows.

Description of Life Stages

Adult

The adult squash bug is typically a brindled grayish brown color 1.4–1.6 cm in length and 0.75 cm wide at the widest part of the abdomen (Beard 1940; Figure 1.1). Although it is a member of the family Coreidae, commonly known as leaf footed bugs, *A. tristis* does not have a noticeable widening of its hind tibiae as most coreids do. The adults are darker on their dorsum and lighter gray in color ventrally.

Egg

Eggs are deposited in groups often on the undersides of leaves (Weed and Conradi 1902, Bonjour et al. 1990; Figure 1.2). Clutch size can vary from only a few to more than 40 eggs, averaging 18 eggs per mass (Bonjour et al. 1990). As the female lays the eggs, she incorporates an adhesive to adhere each egg in the mass to the surface of the leaf. Individual eggs are oval shaped, around 1.5 mm long and 1.2 mm wide, and begin a pale off-white color that later darkens to a shiny, coppery maroon to brown color (Figure 1.3). The egg is larger on one end and does not appear to have any external openings. The pseudo-operculum, visible once eggs

have tanned, is an egg structure found throughout the Corinae subfamily (Beard 1940, Koerber 1963, Roversi et al. 2014). This structure is reported to aid in gas exchange through micropores in the chorion as well as serving as a specific point of weakness exploited during egg eclosion.

Nymph

The nymphal stage has five instars. Immediately following egg hatch, first instars (neonates) are 2–3 mm in length, and light green in color with red legs, head and thorax, which later darkens to black (Weed and Conradi 1902, Capinera, 2001; Figure 1.4). Second instars are 3mm long, a darker green color with black appendages. With each successive molt, the nymph's size increases become lighter gray in color, and the end of the abdomen widens into a tear drop shape (Figure 1.5). The fourth and fifth instars have a more distinct thorax and wing pads and are 6.5 mm and 9.5 mm long, respectively (Beard 1940).

Insect Biology

Squash bugs overwinter in the adult stage and emerge from the soil or ground litter in the spring. Cooler spring temperatures may delay emergence of adults (Nechols 1987). After emergence, overwintered adults immediately seek out cucurbit plants on which to feed and mate. Adults are cryptic and prefer to remain hidden during the day (Nechols 1987). They also can emit a distinct, unpleasant odor from their repugnatory glands, similar to stink bugs, when disturbed (Moody 1930). Gravid females can lay eggs 7–10 d after their emergence (Nechols 1987). They often prefer to deposit eggs on the undersides of leaves next to leaf veins (Figure 1.3).

The squash bug typically completes its entire life cycle in 6–8 wk, and development can be predicted based on heat-unit accumulation. Using a lower and upper temperature threshold of

58º F and 92º F, respectively, the degree-day (F) accumulation required for eggs is 193 DD, for nymphs 554 DD, and for a complete generation 747 DD (Fargo and Bonjour 1988). In the southern United States, egg development time is typically 6–15 d, and first to fifth instars last 3, 9, 8, 7, and 9 d, respectively (Weed and Conradi 1902, Beard 1940, Bonjour and Fargo 1989, Capinera 2001). Nymphs have a strong disposition toward aggregating (Palumbo et al. 1991), particularly after egg hatch on leaves (Figure 1.4) and on fruit following leaf desiccation (Figure 1.5). Both adults and nymphs prefer sheltered areas, with a partiality for the base of plants. Squash bug adults can be observed hiding in the center of plants, under large leaves, wilted leaves (Figure 1.6), and in the transplant holes of plastic mulch. Dependent upon location, the squash bug can have one to three broods (Wadley 1920). According to Nechols (1987), an overlap of over wintered adults and adults of the first summer generation can be observed.

Host Plants and Damage

Squash bug can feed on most cucurbit crops (Quaintance 1899, Beard 1935, Metcalf et al. 1962, Nechols 1985, Fargo et al. 1988, Bonjour et al. 1990, Edelson et al. 2002a). However, they strongly prefer summer squash (*Cucurbita pepo* L.) and pumpkin (*Cucurbita pepo*; *Cucurbita maxima* Duchesne) for oviposition (Bonjour et al. 1993). Moreover, incidence in the field and survival from egg to adult is significantly higher on squash and pumpkins compared to cucumbers (*Cucumis sativus* L.) or muskmelons (*Cucumis melo* L.; Bonjour and Fargo 1989, Cook and Neal 1999). In addition, watermelons (*Citrullus lanatus* Thunberg) also suffer important economic damage from squash bug in Texas and Oklahoma (Riley et al. 1998, Edelson et al. 2002b). In field surveys conducted in community gardens throughout Virginia in 2014 and 2015, we observed significantly more squash bug egg masses on zucchini squash compared with other squash varieties, gourds, or other cucurbit species (Chapter 2).

Squash bug is a piercing sucking feeder. During the feeding process, adults and nymphs pierce through the leaf with their stylets. Although it was once believed that a salivary toxin was injected during the feeding process (Surface 1902), the resulting leaf injury is chiefly explained by physiological dysfunctions of the leaf as well as disruption of the xylem (Neal 1993). The injection of these fluids interrupt the flow of water and some nutrients in the xylem and can cause its collapse along with the general vessel disruptions resulting from the feeding (Neal 1993). The subsequent wilting of the leaf tissue has been referred to as "Anasa wilt" (Robinson and Richards 1931, Knowlton 1935, Hoerner 1938). Prolonged feeding by squash bugs will lead to this condition followed by leaf necrosis, fruit rot, and plant death (Fargo et al. 1988, Neal 1993, Capinera 2001). Plants colonized by squash bug nymphs in the 2–4 leaf stage may quickly succumb to feeding (Woodson and Fargo 1991). In addition, feeding injury occurring at flowering and fruit set can significantly impact fruit production. Palumbo et al. (1993) observed reductions in yield of over 50% in untreated summer squash plots that were invaded at flowering and fruit set by squash bug. In a laboratory and greenhouse study, Woodson and Fargo (1991) reported a decrease in vegetative growth rate and ovulate flower productivity in summer squash with increasing numbers of squash bug. In watermelon, seedlings also experienced more frequent mortality with increasing squash bug density (Edelson et al. 2002a).

Squash bug feeding on fruit (Figure 1.5) creates blemishes and sunken areas most likely resulting from the phenomenon previously described as "Anasa wilt" as well as the likely introduction of pathogens during feeding. The piercing of the fruit tissue by the squash bug certainly adds to the potential for diseases by creating an opportunistic gateway for the common squash fruit pathogens described by Ramsey et al. (1938) as anthracnose, choanephora fruit rot,

gray mold rot, and rhizopus soft rot. Subsequently, a greater incidence of fruit rot in storage can be observed as a result.

In addition, squash bug can transmit the phloem-colonizing bacterium *Serratia marcescens* Bizio, which can cause significant yield losses due to CYVD (Bruton et al. 2003, Pair et al. 2004). This disease is a recent challenge for cucurbit growers, particularly since coreids or hemipterans in general are not widely known as disease vectoring insects. According to Pair et al. (2004), adult squash bugs harbor the bacterium during winter diapause and can infect plants the following spring upon emergence. CYVD can inflict heavy losses to watermelon, pumpkin, cantaloupe, and squash (Bruton et al. 2003). Occurrences of the disease have been observed most often in Oklahoma and Texas (Pair et al. 2004), but more recently in several midwestern states (Wayadande et al. 2005) and even more recently, in Pennsylvania (Gugino et al. 2014).

Management of the Squash Bug

Chemical control is generally the most commonly used strategy by conventional growers, and there are a number of insecticides that are efficacious and registered for use. However, for organic production, squash bug is especially challenging to control because of the lack of efficacious approved insecticides and because the habitat on organic farms (i.e., weedy ground cover, straw mulch, etc.) is conducive to squash bug infestations (Cranshaw et al. 2001). Snyder (2015) reviews strategies for managing squash bugs in organic farming systems, and several of these tactics are described below.

Cultural Control

Before the advent of synthetic insecticides, a number of cultural practices were recommended to reduce squash bug populations, including proper field sanitation to reduce debris and old squash plants serving as shelters for squash bug, crop rotation to eliminate host plants on the farm, and early planting to reduce infestation levels (Weed and Conradi 1902, Wadley 1920, Woodson and Fargo 1992). Each of these strategies has merit and can contribute to the integrated pest management of this pest. Because there is considerable variation among species and cultivars of squash with respect to susceptibility to damage and ability to support the development of squash bugs (Bonjour and Fargo 1989, Cook and Neal 1999, Capinera 2001), varietal selection can impact squash bug infestation levels. In Virginia, I consistently observed the highest densities of squash bug on zucchini (personal observation). Winter squash, such as green striped cushaws and Waltham butternut, are not as attractive to squash bug and the bug does not survive well on them compared to summer squash (Vogt and Nechols 1993). Other varieties of *C. moschata* Duschesne (sweet cheese squash), *C. pepo* (royal acorn squash), and *C. maxima* (pink banana squash) also demonstrated resistance or less susceptibility to damage by the squash bug (Novero et al. 1962). However, during field observations in a commercial organic farm in Virginia in 2009 and 2010, squash bug infestations and damage were quite extensive on Waltham butternut squash, *C. moschata*. Thus, there may be local adaptations and preferences among squash bug populations for certain cucurbit plants, or the availability of more preferred host plants may impact pest densities on a specific crop.

Eliminating weeds and mulch, which provide hiding places for the insect, may reduce infestation levels and damage (Cranshaw et al. 2001). As squash bugs also have been observed quite frequently hiding in the planting holes, minimizing the use of plastic mulch also may

reduce infestations. However, because there are many benefits of using both organic and plastic mulches (i.e., soil moisture retention, weed control, cleaner fruit at harvest, etc), it is often not a compatible pest management tactic to eliminate them from cucurbit production systems. Alternatively, because squash bugs have an affinity to seek shelter, the practice of placing wooden boards between the rows of crops can be used to trap bugs below where they may be crushed by stepping on the board (Weed and Conradi 1902, Smith 1910, Figure 1.7).

Polyester floating row covers can offer some respite from early infestations by the squash bug in summer squash, but populations quickly rebounded following removal of the covers (Cartwright et al. 1990). Cucurbits need to be pollinated and row covers are only practical for a short time. In previous studies, floating row covers have been shown to increase humidity and temperature in zucchini and lettuce crops (Qureshi et al. 2007, Rekika et al. 2008a), leading to some negative effects in radish crops with reduced foliage health (Rekika et al. 2008b). Other effects have shown increased vegetative growth and reduced fruit production (Gaye et al. 1991).

As the squash bug has a preference for squash or pumpkin over other cucurbits (Bonjour et al. 1993), planting these as a trap crop for cantaloupes and cucumbers can be effective (Chittenden 1899, Bonjour et al. 1990). Treating the trap crop with a systemic insecticide has been shown to increase the efficacy of this strategy (Pair 1997, Wallingford et al. 2013). Use of interplanting or companion planting with repellent plants is a strategy that needs further investigation. Essential oils of clove, spearmint, lemon- grass, rosemary, and geranium have been shown to act as a repellent to other insects including stink bugs (Zhang et al. 2014), and should perhaps be investigated for their use in squash bug control including a push-pull control strategy with an attractive trap crop.

Biological Control

Squash bug eggs, nymphs, and adults are attacked by various generalist predators such as spiders, carabids, staphilinids, geocorids, and coccinellids, all of which contribute to lowering population levels (Rondon et al. 2003, Decker and Yeargan 2008). Predation of squash bugs may be increased by employing farmscaping strategies that conserve predators (Snyder 2015). However, the most important natural enemies of squash bug are parasitoids. The orange-bodied tachinid fly, *Trichopoda pennipes* F. (Diptera: Tachinidae; Fig. 8), deposits its eggs on late instar nymphs and adults of squash bug as well as other coreids and pentatomids (Figure 1.9). The larva of *T. pennipes* will subsequently emerge and burrow into the body of its host, fastening itself onto the squash bug's tracheal trunk to ensure appropriate levels of oxygen (Beard 1940). Upon termination of its larval development, it will emerge through the posterior of its host to undergo pupation in the soil (Worthley 1925, Shahjahan 1968). Development from egg to adult averages 18 d (Shahjahan 1968). One or more eggs may be deposited by *T. pennipes* onto the body of its host (Beard 1940, Shahjahan 1968, McPherson et al. 1982), but only one larva will undergo development in the squash bug (Beard 1940). *T. pennipes* will generally undergo two generations, emerging from squash bug soon after termination of diapause, developing to deposit eggs on new hosts in July (Worthley 1925). The following generation will develop mid to late summer to parasitize second generation squash bug late summer (Worthley 1925). Beard (1940) reported that 20% of the squash bugs that were collected in Connecticut in late summer were parasitized by *T. pennipes*. In coastal Virginia in 2009, an average parasitism rate of 12% (20% in organic fields, 8% in conventional fields) was observed (Doughty et al. 2016).

Egg parasitism plays a major role in the biological control of squash bug. Recognized egg parasitoids of this pest include *Gryon pennsylvanicum* (Ashmead) (syn. Hadronotus ajax

Girault) (Hymenoptera: Scelionidae), *Ooencyrtus anasae* (Ashmead) (Hymenoptera: Encyrtidae), and *Anastatus* sp. (Hymenoptera: Eupelmidae) (Schell 1944; Nechols et al. 1989). Squash bug egg parasitism levels in Florida were reported to be about 30% (Capinera 2001), but parasitism rates can be higher in individual fields particularly later in the season (Nechols et al.1989, Olson et al. 1996). Based on a collection of over 80 egg masses from two farms in southwest Virginia in 2013, we found that 66% of squash bug eggs were parasitized predominately by *G. pennslyvanicum* (Fig. 10) (Chapter 2). As this egg parasitoid species has been shown to readily parasitize and develop on other coreids, it has been released into Europe for control of *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) (Koerber 1963, Peverieri et al. 2012).

Entomopathogenic nematodes have also been investigated as biological control agents of squash bug adults. In laboratory studies, Wu (1988) indicated the potential of *Steinernema carpocapsae* Weiser (Nematoda: Steinernematidae), as it caused death to adult squash bugs by its symbiotic bacterium. Furthermore, nymphs that emerged from egg masses laid by infected female squash bugs before their death, were also infective (Wu 1988).

Sampling and Economic Thresholds

Inspecting plants to determine squash bug density should be done prior to making the decision to apply insecticides. Sampling plans for scouting in summer squash and watermelons have been developed. The recommended economic threshold in summer squash is one squash bug adult per plant (Palumbo et al. 1991) or one egg mass per plant (Doughty et al. 2014). In watermelons, the economic threshold is one to two squash bug adults per plant (Dogramaci et al. 2006). A fixed precision sampling plan of 64 plants in summer squash (Palumbo et al. 1991) and 54 plants in watermelons (Dogramaci et al. 2006) is suggested to determine the density of one

squash bug adult per plant. Squash bug populations are indeed more heavily aggregated in summer squash than in watermelons therefore requiring a higher number of samples (Dogramaci et al. 2006). Fewer plants are required to determine egg mass density. Palumbo et al. (1991) suggested that 38 plants needed examining to determine the threshold of one egg mass per plant. In regions where CYVD is prevalent, such as Texas and Oklahoma, preventative control is recommended rather than the use of sampling and thresholds.

Chemical Control

Chemical control is the most widely used strategy to prevent crop damage from squash bug. Insecticides are often applied to target the nymphal stage, which is easier to kill than adults. Effective control of squash bug nymphs can be achieved with foliar applications of pyrethroids such as bifenthrin, cypermethrin, fenpropathrin, lambda-cyhalothrin, and others, as well as neonicotinoids such as thiamethoxam, imidacloprid, clothianidin, and acetamiprid, and organophosphates such as disulfoton and metasystox-R (Edelson et al. 2002b, 2003; Eiben et al. 2004; McLeod et al. 2003; Kuhar et al. 2005; Abney et al. 2011). However, foliar applications of pyrethroids, which are the most commonly used foliar insecticides by growers because of their low cost, have deleterious effects on natural enemy populations and can cause outbreaks of secondary pests such as melon aphids, *Aphis gossypii* Glover (Kuhar et al. 2005). All of the aforementioned foliar insecticides, with the exception of acetamiprid, are also highly toxic to pollinators (Johansen, 1977, Smith and Stratton 1986, Iwasa et al. 2004, Desneux et al. 2007, Laurino et al. 2011, Van der Sluijs et al. 2013). Thus, alternatives to their use have been explored.

One option is changing application method. Because of their ability to be taken up by the roots from the soil as systemic insecticides and transported to the foliage, neonicotinoids can be

applied to cucurbits quite effectively, efficiently, and economically via drip chemigation (Ghidiu et al. 2012). Edelson and Otieno (2003) evaluated the efficacy of imidacloprid, thiamethoxam, as well as the carbamate, carbofuran applied as a soil drench, and showed that all three insecticides were efficacious on squash bug. However, the efficacy of soil-applied neonicotinoids will decrease over time and may not provide control over the growing season, particularly when needed for squash bug later in the crop cycle (Kuhar et al. 2005). Other more IPM-friendly insecticides have been evaluated for squash bug control. Novaluron, a benzoylphenyl urea insecticide, has demonstrated efficacy against squash bug nymphs (Eiben et al. 2004). As an insect growth regulator, it offers an alternative to broad-spectrum insecticides with a better fit in an IPM program. The spinosyn, spinosad, has also been evaluated, but shown to be not very effective against squash bug (Edelson et al. 2002). Other reduced-risk insecticides that may show promise for control of this pest in the future include sulfoxaflor, flonicamid, and cyclaniliprole (Aigner et al. 2015, Wilson et al. 2015). Additional field efficacy tests are needed to confirm results from laboratory bioassays. Organic producers have fewer chemical control options. Applications of pyrethrins and azadirachtins can suppress squash bug nymphal densities. However, under heavy pest pressure, these insecticides have not provided effective or consistent control in the field.

Squash bug remains an important economic pest of cucurbit crops in the United States, particularly in areas where CYVD occurs. For conventional growers today, a number of insecticides, particularly pyrethroids and neonicotinoids, are registered that provide effective control of the pest. However, these insecticides pose risks to nontarget organisms such as natural enemies and pollinators and are not the most IPM-compatible control options. A combination of preventative and curative control measures discussed above provide a number of options to

effectively manage this pest using an IPM approach. A greater focus on scouting for the pest before applying pesticides, awareness and conservation of natural enemies in the agro-ecosystem, and use of more selective narrow-spectrum insecticides, if and when they are needed, would provide a more sustainable approach to managing this pest.

Pollinator Protection in Cucurbits

Of particular interest in recent years is the compatibility of current insecticide-based pest management strategies with pollinators. Cucurbits are pollination-dependent vegetables that rely on insect pollinators, commonly honey bees or native bees (Morse and Calderone 2000, Cane et al. 2011). The European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is the most commonly managed species of pollinator in the United States (Klein et al. 2007) and as such migratory beekeepers commonly move colonies around from crop to crop to provide pollination services (Aizen and Harder 2009).

Pollinator safety protocols require that insecticide applications be made during periods of bee inactivity, or that pesticides that are known to be acutely toxic to bees not be used during pollination (USEPA 2015). Some growers and pesticide applicators take precautions to avoid harming bees by spraying in the evening or during lower temperatures as outlined in the new EPA mandated insecticide label changes (USEPA 2013). Additional levels of safety precautions can be achieved by utilizing narrow-spectrum (or reduced risk) insecticides whenever possible. Current regulations stipulate that insecticides be screened for lethal and sub-lethal effects on honey bees specifically, prior to product registration. The US Environmental Protection Agency requires at least one tier of toxicity testing to include honey bee toxicity data specifically for registration of pesticides (USEPA 2016a). The tiered system screens data from initial laboratory assessments that are designed to make conservative assumptions that would likely overestimate

exposure risk. The initial laboratory assessments include: acute oral adult, acute contact adult, and acute larval toxicity testing, as well as 10–day adult chronic and 21-day larval toxicity studies. All Tier 1 testing is performed with the Technical Grade Active Ingredient (TGAI). Higher tiered studies may be necessary based on the estimates of risk from Tier 1 studies with TGAI. Tier 2 studies focus on colony level exposure and near-field to full field testing. The Typical End-Use Product (TEP) or TGAI may be used for this tier depending on the end use and pesticide label directions for use. In the Tier 2 studies one component can be semi-field testing in enclosed areas (tunnels). The enclosed semi-field tests are typically involved in the characterization of risk to honey bees from exposure to the TEP. While these regulations are clearly designed to provide additional protection of pollinators, they do not take into account aspects of colony behavior that affect long term survivorship like foraging.

The examination of sub-lethal insecticide effects on bee behavior may provide a more complete picture of the characterization of pesticide risk. One such study was conducted in France in an effort to examine the change in honey bee foraging behavior after exposure to sub-lethal doses of imidacloprid and fipronil (Colin et al. 2004). This study was unique in its design and implementation, where the experimenters were able to isolate extraneous factors and remove much of the observer bias and interference. Colin et al. (2004) conducted their experiments in insect-proof high tunnels and made their observances of feeding behavior with video cameras. The key to their study was quantifying foraging bee behavior in a way that could reflect changes in large numbers of bees to relate their activity levels to the abilities of the colony. The experimenters trained bees to feed from artificial feeders for a two-hour duration every day. Once adequately trained, bees were exposed to doses of insecticide that were placed in the feeders for the two-hour feeding periods during the five days of the trial. Training the bees and

limiting their access to the dose allowed for precise, known amounts of insecticide treated material to be administered and monitored. Additional research that builds on this type of near-field colony level examination of the effects of pesticides on bees are needed.

Foliar insecticides are frequently tank mixed with fungicides because they are relatively cheap and growers are making a trip over the field anyway. Current recommendations for the control of squash bug in the Mid-Atlantic region include fungicide sprays that are frequent with recommendations that call for alternation of chemistries and repeat applications (Wyenandt et al. 2016). The addition or use of narrow-spectrum or reduced-risk insecticides into this pest management system could alleviate the potential harm to natural enemy communities and pollinators that are so critical to the production of cucurbits.

Rationale

The purpose of this doctoral research project is to improve upon the sustainability of pest management practices in cucurbit production. The research focus is on *A. tristis* because the pest is a frequent target of foliar insecticide sprays on pumpkins and squash, and because all of the currently-recommended insecticides (5 pyrethroids, 3 neonicotinoids, 1 carbamate, and 2 combination products), are acutely or highly toxic to bees (USEPA 2015, 2016b, Wyenandt et al. 2016). In addition, the impact of the egg parasitoid, *G. pennsylvanicum*, on squash bug populations, as well as the risk of insecticides to it, are not known. To that end, I conducted a series of related experiments whose objectives follow:

Objective 1: To determine parasitism levels of the eggs of *Anasa tristis* throughout Virginia.

Objective 2: To assess the effects of narrow-spectrum insecticides on the eggs and nymphs of *A. tristis*, as well as its key egg parasitoid *G. pennsylvanicum*.

Objective 3: To utilize a near-field experimental protocol to characterize the risk of selected narrow-spectrum insecticides to honey bees at sub-lethal levels of exposure.

References Cited

Abney, M. R., and R. Davila. 2011. Evaluation of foliar and soil applied insecticides for control of squash bug on zucchini squash, 2010. Arthrop. Manag. Tests 36 (1): E68 (DOI: http://dx.doi.org/10.4182/amt.2011.E68)

Aigner, J., J. Wilson, L. Nottingham, J. Morehead, A. Dimeglio, and T. Kuhar. 2015. Bioassay evaluation of IKI-3106 (cyclaniliprole) for control of brown marmorated stink bug and harlequin bug, 2014. Arthrop. Manag. Tests 40 (1): L4 (DOI: http://dx.doi.org/10.1093/amt/tsv205)

Aizen, M. A., and L. D. Harder. 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Current Biology : CB 19: 915-918.

Beard, R. L. 1935. Further observations on the squash bug in Connecticut. Conn. Agric. Exp. Stn. Bull. 383.

Beard, R. L. 1940. The biology of *Anasa tristis* (DeGeer), with particular reference to the tachinid parasite, *Trichopoda pennipes* Fabr. Conn. Agric. Exp. Stn. Bull. 440: 597–679.

Bonjour, E. L., and W. S. Fargo. 1989. Host effects on the survival and development of *Anasa tristis* (Heteroptera: Coreidae). Environ. Entomol. 18: 1083–1085.

Bonjour, E. L., W. S. Fargo, and P. E. Rensner. 1990. Ovipositional preference of squash bugs (Heteroptera: Coreidae) among cucurbits in Oklahoma. J. Econ. Entomol. 83: 943–947.

Bonjour, E. L., W. S. Fargo, J. A. Webster, P. E. Richardson, and G. H. Brusewitz. 1991. Probing behavior comparisons of squash bugs (Heteroptera: Coreidae) on cucurbit hosts. Environ. Entomol. 20: 143–149.

Bonjour, E. L., W. S. Fargo, A. A. Al-Obaidi, and M. E. Payton. 1993. Host effects on reproduction and adult longevity of squash bugs (Heteroptera: Coreidae). Environ. Entomol. 22: 1344–1348.

Britton, W. E. 1919. Insects attacking squash, cucumbers, and allied plants in Connecticut. Conn. Agric. Exp. Stn. Entomol. Series No. 27. Bull. 216: 33– 51.

Bruton, B. D., F. Mitchell, J. Fletcher, S. D. Pair, A. Wayadande, U. Melcher, J. Brady, B. Bextine, and T. H. Popham. 2003. *Serratia marcescens*, a phloem-colonizing, squash bug transmitted bacterium causal agent of cucurbit yellow vine disease. Plant Dis. 87: 937–944.

Cane, J. H., B. J. Sampson, and S. A. Miller. 2011. Pollination value of male bees: the specialist bee *Peponapis pruinosa* (Apidae) at summer squash (*Cucurbita pepo*). Environ. Entomol. 40: 614-620.

Capinera, J. L. 2001. Handbook of Vegetable Pests. Academic Press, New York, p. 729.

Cartwright, B., J. C. Palumbo, and W. S. Fargo. 1990. Influence of crop mulches and row covers on the population dynamics of the squash bug (Heteroptera: Coreidae) on summer squash. J. Econ. Entomol. 83: 1988–1993.

Chittenden, F. H. 1899. Some insects injurious to garden and orchard crops. USDA Div. Entomol. Bull. 19: 99.

Colin, M. E., J. M. Bonmatin, I. Moineau, C. Gaimon, S. Brun, and J. P. Vermandere. 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contam. Toxicol. 47: 387-395.

Cook, C. A., and J. J. Neal. 1999. Feeding behavior of larvae of *Anasa tristis* (Heteroptera: Coreidae) on pumpkin and cucumber. Environ. Entomol. 28: 173–177.

Cranshaw, W., M. Bartolo and F. Schweissing. 2001. Control of squash bug injury: Management manipulations at the base of pumpkin. Southwest. Entomol. 26: 147–150.

Decker, K. B., and K. V. Yeargan. 2008. Seasonal phenology and natural enemies of the squash bug (Hemiptera: Coreidae) in Kentucky. Environ. Entomol. 37: 670–678.

Desneux, N, A. Decourtye, and J. M. Delpuech 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

Domagraci, M., J. W. Shrefler, K. Giles, and J. V. Edelson. 2006. Spatial pattern and sequential sampling of squash bug (Heteroptera: Coreidae) in watermelon. J. Econ. Entomol. 97: 1999-2005.

Doughty, H. B., J. M. Wilson, and T. P. Kuhar. 2014. Squash Bug. Virginia

Cooperative Extension Publication No. ENTO-64NP.

Doughty, H. B., J. M. Wilson, P. B. Schultz, and T. P. Kuhar. 2016. Squash bug (Hemiptera: Coreidae): biology and management in cucurbitaceous crops. J. Integr. Pest Manag. 7: 1.

Edelson, J. V., and O. Otieno. 2003. Greenhouse bioassays of systemic insecticides to control squash bug, 2001. Arthropod Manag. Tests 28 (1): L11 (DOI: http://dx.doi.org/10.1093/amt/28.1.L11)

Edelson, J. V., J. Duthie, and W. Roberts. 2002a. Watermelon seedling growth and mortality as affected by *Anasa tristis* (Heteroptera: Coreidae). J. Econ. Entomol. 95: 595–597.

Edelson, J. V., J. Duthie, and W. Roberts. 2002b. Control of squash bugs on pumpkin with foliar applications, 2001. Arthrop. Manag. Tests 27 (1): E79 (DOI: http://dx.doi.org/10.1093/amt/27.1.E79)

Edelson, J. V., J. Duthie, and W. Roberts. 2003. Residual activity of foliar applied insecticides for controlling squash bug, 2002. Arthrop. Manag. Tests 28 (1): E69 (DOI: http://dx.doi.org/10.1093/amt/28.1.E69)

Eiben, J., C. Mackey, W. Roberts, and J. V. Edelson. 2004. Foliar applied insecticides for controlling squash bug, 2003. Arthrop. Manag. Tests 29 (1): E74 (DOI: http://dx.doi.org/10.1093/amt/29.1.E74)

Fargo, W. S., and E. L. Bonjour. 1988. Developmental rate of the squash bug, *Anasa tristis* (Heteroptera: Coreidae), at constant temperatures. Environ. Entomol. 17: 926–929.

Fargo, W.S., P. E. Rensner, E. L. Bonjour, and T. L. Wagner. 1988. Population dynamics in the squash bug (Hemiptera: Coreidae) squash plant (Cucurbitales: Cucurbitaceae) system in Oklahoma. J. Econ. Entomol. 81: 1073–1079.

Gaye, M. M., A. R. Maurer, and F. M. Seywerd. 1991. Honey bees placed under row covers affect muskmelon yield and quality. Sci. Hortic. 47: 59–66.

Ghidiu, G., T. P. Kuhar, J. Palumbo, and D. Schuster. 2012. Drip chemigation of insecticides as a pest management tool in vegetable production. J. Integr. Pest Manag. 3: 2012, (doi:http://dx.doi.org/10.1603/IPM10022)

Gugino, B. K. 2014. The old and the new: Yellow vine decline and update on the mildews, pp. 19–21 In Proceedings of the 2014 Mid-Atlantic Fruit and Vegetable Convention, January 28–30, 2014, Hershey, PA.

Hoerner, J. L. 1938. Controlling the squash bug. Colo. Expt. Stn. Press Bul. 93: 1–8.

Iwasa, T, N. Motoyama, J. T. Ambrose, and M. R. Roe. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot. 23: 371–378.

Johansen, C. A. 1977. Pesticides and pollinators. Annu. Rev. Entomol. 22: 177–192.

Klein, A.-M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274: 303-313.

Knowlton, G. F. 1935. The squash bug. Utah Agric. Expt. Stn. Leaflet 55. 4 p.

Koerber, T. W. 1963. *Leptoglossus occidentalis* (Hemiptera: Coreidae), a newly discovered pest of coniferous seed. Ann. Entomol. Soc. Am. 56: 229–234.

Kuhar, T. P., J. Speese, R. J. Cordero, and V. M. Barlow. 2005. Evaluation of insecticides in pumpkins, 2004. Arthrop. Manag. Tests 30 (1): E70 (DOI: http://dx.doi.org/10.1093/amt/30.1.E70)

Laurino, D, M. Porporato, A. Patetta, and A. Manino. 2011. Toxicity of neonicotinoid insecticides to honey bees laboratory tests. Bull. Insectol. 64: 107– 113.

McLeod, P., J. Diaz, S. Eaton, and L. Martin. 2003. Evaluation of insecticides for control of squash bug on summer squash, 2002. Arthrop. Manag. Tests 28: (doi:http://dx.doi.org/10.1093/amt/28.1.E72)

McPherson, R. M., J. R. Pitis, L. D. Newsom, J. B. Chapin, and D. C. Herzog. 1982. Incidence of tachinid parasitism of several stink bugs (Heteroptera: Pentatomidae) species associated with soybean. J. Econ. Entomol. 75: 783– 786.

Metcalf, C. L., W. P. Flint, and R. L. Metcalf. 1962. Destructive and useful insects: their habits and control. Fourth Edition. McGraw-Hill Book Company. New York, p. 1087.

Moody, D. L. 1930. The morphology of the repugnatory glands of *Anasa tristis* De Geer. Ann. Entomol. Soc. Am. 23: 81–104.

Morse, R. A., and N. W. Calderone. 2000. The value of honey bees as pollinators of U.S. crops in 2000, Bee Culture. A.I. Root Company, Medina, OH.

National Agricultural Statistics Service. 2012. 2012 Census of Agriculture - State Data Virginia.

National Agricultural Statistics Service. 2016. USDA Vegetables 2015 Summary, pp. 75.

Neal, J. J. 1993. Xylem transport interruption by *Anasa tristis* feeding causes *Cucurbita pepo* to wilt. Entomol. Exp. Appl. 69: 195–200.

Nechols, J. R. 1985. The squash bug: biology and management. Kans. Coop. Ext. Serv. Publ. MF 758.

Nechols, J. R. 1987. Voltinisms, seasonal reproduction, and diapauses in the squash bug (Heteroptera: Coreidae) in Kans. Environ. Entomol. 16: 269– 273.

Nechols, J. R., J. L. Tracy, and E. A. Vogt. 1989. Comparative ecological studies of indigenous egg parasitoids (Hymenoptera, Scelionidae, Encyrtidae) of the squash bug, *Anasa tristis* (Hemiptera, Coreidae). J. Kans. Entomol. Soc. 62: 177–188.

Novero, E. S., R. H. Painter, and C. V. Hall. 1962. Interrelations of the squash bug, *Anasa tristis*, and six varieties of squash (*Cucurbita* spp.). J. Econ. Entomol. 55: 912.

Pair, S. D. 1997. Evaluation of systemically treated squash trap plants and attracticidal baits for early-season control of striped and spotted cucumber beetles (Coleoptera: Chrysomelidae) and squash bug (Hemiptera: Coreidae) in cucurbit crops. J. Econ. Entomol. 90: 1307–1314.

Pair, S. D., B. D. Bruton, F. Mitchell, J. Fletcher, A. Wayadande, and U. Melcher. 2004. Overwintering squash bugs harbor and transmit the causal agent of cucurbit yellow vine disease. J. Econ. Entomol. 97: 74–78.

Peverieri, G. S., P. Furlan, S. Simoni, W. B. Strong, and P. F. Roversi. 2012. Laboratory evaluation of *Gryon pennsylvanicum* (Ashmead) (Hymenoptera, Platygastridae) as a

biological control agent of *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae). Elsevier Ltd, Oxford, UK, Biological Control 61: 104–111.

Palumbo, J. C., W. S. Fargo, and E. L. Bonjour. 1991. Colonization and seasonal abundance of squash bugs (Heteroptera, Coreidae) on summer squash with varied planting dates in Oklahoma. J. Econ. Entomol. 84: 224–229.

Palumbo, J. C., W. S. Fargo, R. C. Berberet, E. L. Bonjour, and G. W. Cuperus. 1993. Timing insecticide applications for squash bug management: impact on squash bug abundance and summer squash yields. Southwest. Entomol. 18: 101–111.

Quaintance, A. L. 1899. Some important insect enemies of cucurbits. Georgia Agric. Exp. Stn. Bull. 45: 25–50.

Ramsey, G. B., J. S. Wiant, and G.K.K. Link. 1938. Market diseases of fruits and vegetables: Crucifers and cucurbits. U.S. Dept. of Agriculture, Washington, DC.

Rekika, D., K. A. Stewart, G. Boivin, and S. Jenni. 2008a. Reduction of insect damage in radish with floating row covers. Intern. J. Veg. Sci. 14: 177–193.

Rekika, D., K. A. Stewart, G. Boivin, and S. Jenni. 2008b. Row covers reduce insect populations and damage and increase early season crisphead lettuce production. Intern. J. Veg. Sci. 15: 71–82.

Riley, D. G., J. V. Edelson, M. Miller, G. Cuperus, R. Roberts, J. Anciso, and N. Roe. 1998. Integrated pests management for cucurbit crops in the south central U.S.: Pest status, attitudes towards IPM and a plan for implementation. J. Extension 36: 4, Feature 3-3343.

Robinson, L. R., and B. L. Richards. 1931. Anasa wilt of cucurbits. Phytopathology 21: 114.

Rondon, S. I., D. J. Cantliffe, and J. F. Price. 2003. *Anasa tristis* (Heteroptera: Coreidae) development, survival and egg distribution on Beit alpha cucumber and as prey for *Coleomegilla maculata* (Coleoptera: Coccinellidae) and *Geocoris punctipes* (Heteroptera: Lygaeidae). Fla. Entomol. 86: 488–490.

Roversi, P. F., G. Sabbatini Peverieri, M. Maltese, P. Furlan, W. B. Strong and V. Caleca. 2014. Pre-release risk assessment of the egg-parasitoid *Gryon pennsylvanicum* for classical biological control of *Leptoglossus occidentalis*. J. Appl. Entomol. 138: 27–35.

Schell, S. C. 1944. The biology of *Hadronotus ajax* Girault (Hymenoptera- Scelionidae) a parasite in the eggs of squash bug (*Anasa tristis* DeGeer). Ann. Entomol. Soc. Am. 36: 625–635.

Shahjahan, M. 1968. Superparasitization of the southern green stink bug by the tachinid parasite *Trichopoda pennipes pilipes* and its effect on the host and parasite survival. J. Econ. Entomol. 61: 1088–1091.

Smith, R. I. 1910. Insect enemies of cantaloupes, cucumbers and related plants. North Carolina Agric. Exp. Stn. Bull. 205. p. 40.

Smith, T. M., and G. W. Stratton. 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Reviews: Rev. Environ. Cont. Toxicol. 93–120.

Snyder, W. E. 2015. Managing squash bugs in organic farming systems. online article downloaded November 22, 2015 from eXtension.org. (http://articles.extension.org/pages/63329/managing-squash-bugs-in-organic-farming-systems#.VlKLo41dG1s)

Surface, H. A. 1902. Insects injurious to cucurbitaceous plants. Penna. Dept. Agric. Bull. 96.

US Environmental Protection Agency. 2013. Letter to the registrants of nitrogaunidine neonicotinoid products. (https://www.epa.gov/sites/production/files/2013-11/documents/bee-july2013-letter.pdf)

US Environmental Protection Agency. 2015. EPA's proposal to mitigate exposure to bees from acutely toxic pesticide products. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0818-0002)

US Environmental Protection Agency. 2016a. Guidance on exposure and effects testing for assessing risks to bees. (https://www.epa.gov/sites/production/files/2016-07/documents/guidance-exposure-effects-testing-assessing-risks-bees.pdf)

US Environmental Protection Agency. 2016b. 2016 addendum for the proposed section 3 registration of transform wg and closer sc (sufoxaflor) for use on various crops, turf, and ornamentals. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0889-0409)

Van der Sluijs, J. P., N. Simon-Delso, D. Goulson, L. Maxim, J. M. Bonmatin, and L. P. Belzunces. 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr. Opin. Environ. Sustain. 5: 293–305.

Vogt, E. A., and J. R. Nechols. 1993. Responses of the Squash Bug (Hemiptera: Coreidae) and Its Egg Parasitoid, *Gryon pennsylvanicum* (Hymenoptera: Scelionidae) to Three Cucurbita Cultivars. Environ. Entomol. 22: 238–245.

Wadley, F. M. 1920. The squash bug. J. Econ. Entomol. 13: 416–425.

Wallingford, A. K., T. P. Kuhar, D. G. Pfeiffer, D. B. Tholl, J. H. Freeman, H. B. Doughty, and P. B. Schultz. 2013. Host plant preference of harlequin bug (Hemiptera:

Pentatomidae), and evaluation of a trap cropping strategy for its control in collard. J. Econ. Entomol. 106: 283–288.

Wayadande, A. B. Bruton, J. Fletcher, S. Pair, and F. Mitchell. 2005. Retention of cucurbit yellow vine disease bacterium *Serratia marcescens* through transstadial molt of vector *Anasa tristis* (Hemiptera: Coreidae). Ann. Entomol. Soc. Am. 98: 770–774.

Weed, C. M., and A. F. Conradi. 1902. The squash bug. New Hampshire College Agricultural Experiment Station, New Hampshire College of Agriculture and the Mechanic Arts, p.28.

Wilson, J., J. Aigner, L. Nottingham, and T. Kuhar. 2015. Bioassay evaluation of Closer SC for control of harlequin bug, brown marmorated stink bug, and kudzu bug, 2013. Arthrop. Manag. Tests 40 (1): L8 (DOI: http://dx.doi.org/10.1093/amt/tsv208)

Woodson, D. W., and S. W. Fargo. 1991. Interactions of temperature and squash bug density (Hemiptera: Coreidae) on growth of seedling squash. J. Econ. Entomol. 84: 886–890.

Woodson, D. W., and S. W. Fargo. 1992. Interactions of plant size and squash bug (Hemiptera: Coreidae) density on growth of seedling squash. J. Econ. Entomol. 85: 2365–2369.

Worthley, H. N. 1925. The biology of *Trichopoda pennipes*, Fab. (Diptera: Tachinidae). A parasite of the common squash bug. Psyche 31: 7–16.

Wu, H. J. 1988. Biocontrol of squash bug with *Neoaplectana carpocapsae* (Weiser). Bull. Inst. Zool. Acad. Sin. (Taipei) 27: 195–203.

Wyenandt, A., T. P. Kuhar, G. C. Hamilton, M. J. VanGessel, E. Sanchez, and D. Dugan. 2016. 2016 Mid-Atlantic Commercial Vegetable Production Recomendations. Virginia Cooperative Extension. Publication #456-420.

Zhang, Q., R. G. Schneidmiller, D. R. Hoover,G. Zhou, A. Margaryan, and P. Bryant. 2014. Essential oils as spatial repellents for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). J. Appl. Entomol. 138: 490–499.

Figure 1.1: Squash bug adult (actual size, 1.5 cm long by 0.75 cm wide).

Figure 1.2: Ovipositing squash bug female and eggs.

Figure 1.3: Squash bug eggs deposited on the underside of a squash leaf.

Figure 1.4: Neonate squash bug nymphs.

Figure 1.5: Squash bug nymphs and an adult aggregating on fruit in late summer.

Figure 1.6: Squash bug nymphs aggregating on a leaf exhibiting necrosis caused by feeding injury.

Figure 1.7: Squash bug adults and nymphs found sheltering under plywood boards placed between rows in a commercial squash field in Virginia.

Figure 1.8: *Trichopoda pennipes* adult. Image courtesy of Russ Ottens, University of Georgia, Bugwood.org

Figure 1.9: Mating squash bugs revealing an egg of *Trichopoda pennipes* on the back of the bug to the right.

Figure 1.10: *Gryon pennsylvanicum*, a major egg parasitoid of squash bug and other coreids.

Chapter 2

A survey of the species composition and relative distribution of the egg parasitoids of *Anasa tristis* in Virginia

Abstract

Squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), is a major pest of squash and pumpkins in the United States. In order to better understand the importance of natural egg parasitism of this species in Virginia, I conducted a two year statewide survey. A total of 1127 squash bug egg masses were sampled from squash and pumpkins from 43 counties in Virginia from 2014 – 2015. Egg masses were brought back to the lab to record levels of squash bug nymphal emergence or adult parasitoid eclosion and identification. Over 50% of the total squash bug eggs collected statewide were parasitized. *Gryon pennsylvanicum* Ashmead was the predominant egg parasitoid accounting for over 98% of all parasitoid adults recovered. The only other species emerging from squash bug eggs was the eupelmid *Anastatus reduvii* Howard, which is a generalist parasitoid. *G. pennsylvanicum* was found in 75% of the counties surveyed with the highest levels of parasitism occurring in the Northern, Southwestern Mountain, and Western Piedmont regions of the state and the lowest levels of parasitism occurring in the Tidewater region. Based on this two year survey, *G. pennsylvanicum* was determined to be a major natural enemy of squash bug, significantly reducing the number of nymphs that emerge from deposited eggs. Conservation of this natural enemy should therefore be a priority for integrated pest management programs in cucurbits.

Introduction

The squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), is an important pest of pumpkin (*Cucurbita maxima*) and squash (*C. pepo*) causing wilt in plants with its piercing-sucking mouthparts and by potentially transmitting the bacteria that cause Cucurbit Yellow Vine Decline (Bruton et al. 2003, Doughty et al. 2016). Many commercial growers of these crops typically apply broad-spectrum insecticides such as pyrethroids to control this pest (Doughty et al. 2016). Cucurbits are pollination dependent and many of the registered insecticides recommended for use in commercial cucurbit production are known to be acutely toxic to bees (USEPA 2015, Wyenandt et al. 2016). In addition to pollinators, there are other beneficial organisms that also may be impacted by these pest management practices, for instance, natural enemies that could be keeping pests such as squash bug in check in cucurbit systems. The natural enemy complex of squash bugs has not been well studied in Virginia.

Egg parasitoids, in particular, appear to be the most important natural enemies of this pest in general (Nechols et al. 1989, Doughty et al. 2016). Out of the native egg parasitoids known for *A. tristis*, the scelionid wasp *Gryon pennsylvanicum* Ashmead, has been found to be one of the most important in the central U.S. (Nechols et al. 1989, Olson et al. 1996, Decker and Yeargan 2008), and most recently in Maryland (Cornelius et al. 2016). Among the relatively few parasitoids of squash bug, *G. pennsylvanicum* has the highest fecundity and rate of reproduction (Nechols et al. 1989). *G. pennsylvanicum* appears to be widespread in North America and was reported to occur in the mid-Atlantic U.S. as early as 1943 (Schell 1943). In Kentucky, Decker and Yeargan (2008) observed squash bug egg parasitism reaching 31% with *G. pennsylvanicum* as the predominant egg parasitoid. More recently, Cornelius et al. (2016) observed parasitism levels averaging 55.7% from wild squash bug eggs collected in Beltsville, MD. Concurrently

with that study, a sample of 81 squash bug egg masses was collected in 2013 from two counties in southwestern Virginia and revealed parasitism levels exceding 80% (JW, unpublished data). Given the high rate of parasitism recorded from the aforementioned samples in 2013, I embarked on a more extensive survey in the state of Virginia in the subsequent two years. To our knowledge a survey of squash bug egg parasitism has not been reported from Virginia. Herein, I report the results of a two year survey from 43 counties throughout Virginia to allow us to quantify the potential effects egg parasitism may have on squash bug population dynamics.

Materials and Methods

Throughout the summer months of 2014 and 2015, squash bug egg masses were collected from summer squash and zucchini (*Cucurbita pepo* L.) and pumpkin (*Cucurbita pepo*; *Cucurbita maxima* Duchesne) fields located throughout Virginia (Fig. 2.1). Virginia Cooperative Extension county agents were contacted to help find commercial squash and pumpkin fields to sample. However, it soon became apparent after visiting several commercial conventional farms, that very few squash bug eggs could be found. This was probably the result of the insecticide spray programs used in those fields. Thus, surveys subsequently shifted to squash or pumpkins located on Virginia Tech research farms, organic farms, and community gardens throughout Virginia. In 2014, a total of 722 squash bug egg masses were collected from 34 counties in Virginia (Table 2.1). In 2015, 405 squash bug egg masses were collected from 24 Virginia counties (Table 2.2).

Egg masses of *A. tristis* were removed from leaves in the field by cutting around the egg mass and placing it individually into 60 × 15 mm Petri dishes, which were kept at room temperature (~27º C) until all nymphs and adult parasitoids had emerged. Once all nymphs and

parasitoids had perished, samples were processed and parasitism and squash bug hatch rates were recorded by collection location. Data from egg masses collected in Virginia were grouped by NOAA climatic regions and analyzed with an ANOVA and a Fisher's Protected LSD test was used to compare means among NOAA climatic regions.

Results

In 2014, squash bug egg parasitism was found in 75% (30 of the 37) counties surveyed, ranging from 0% in some counties to 100% in others, with an overall statewide average of 39.4% of egg masses parasitized (Table 2.1). *Gryon pennsylvanicum* Ashmead was the predominant egg parasitoid accounting for over 98% of all parasitoid adults recovered. The only other species emerging from squash bug eggs was *Anastatus reduvii* Howard (Hymenoptera: Eupelmidae), which is a generalist parasitoid that attacks eggs from multiple insect orders (Burks 1967). In 2015, 18 of the 24 sampled counties had the egg parasitoid *G. pennsylvanicum,* with 49.4% of collected egg masses parasitized (Table 2.2).

There was a significant effect of climatic region on parasitism rates (Table 2.3). In 2014, there were significant differences among regions in egg mass parasitism ($F=3.3675$; $df=5,33$; $p<0.0166$), but not in total egg parasitism ($F=1.7080$; $df=5, 33$; $p>0.05$); however, numeric trends were the same with the highest parasitism occurring in the Northern Region(67.9% of egg masses and 46.3% of eggs) and the lowest occurring in the Tidewater (6.7% of egg masses and 0.8% of eggs) and Eastern Piedmont Regions (13.7% of egg masses and 15.1% of eggs). In 2015 there was also a significant effect of region on parasitism of egg masses ($F=4.330$; $df=5, 23$; $p<0.0092$) and eggs ($F=5.5524$; $df=5, 23$; $p<0.0029$). The highest parasitism occurred in the Southwestern Mountains (100.0% of egg masses and 96.3% of eggs) and the lowest again occurring in the Tidewater with (0.0% of egg masses).

Discussion

Sampling squash bug eggs throughout Virginia in 2014 and 2015 showed widespread occurrence of the egg parasitoid *G. pennsylvanicum,* which was recovered from 32 out of 43 counties in Virginia, causing significant egg mortality across the region, with 100% parasitism of egg masses recorded from multiple counties each year. *G. pennsylvanicum* has been previously reported from squash bug eggs in North Carolina, Kansas, and Kentucky, but at much lower parasitism levels than were found in our survey of Virginia (Schell 1943, Olson et al. 1996, Decker and Yeargan 2008). In the Southwestern Mountain region of Virginia, parasitism averaged 63.3% based on a relatively large sample of 335 egg masses collected over two years. This level is consistent with that reported recently by Cornelius et al. (2016), who observed *G. pennsylvanicum* parasitism levels averaging 55.7% from wild squash bug eggs collected in Beltsville, MD. Thus, *G. pennsylvanicum* appears to be a significant natural enemy of squash bugs in the Mid-Atlantic region of the U.S. However, there appear to be additional factors that can greatly impact the level of parasitism that occurs.

Low levels of parasitism were found in the Tidewater region of Virginia, which has the warmest climate in the state. Climate can impact survival of parasitoids as well as synchrony with the population dynamics of its host. Determining the ecological reasons for low parasitism in the Tidewater region should be a focus of additional research. The biology of *G. pennsylvanicum* has been investigated.

This scelionid wasp has a host range that appears to be limited to the leaf-footed bugs (family Coreidae), and has been recently introduced as a classical biological control agent for the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) in Italy (Peverieri et al. 2013, Roversi et al. 2014). Adult *G. pennsylvanicum* do not feed on the host

eggs (Vogt and Nechols 1993), but rather feed on the exudate from cucurbit leaf trichomes (extra-floral nectaries) that serve as sources of basic sugars and protein (Olson et al. 1996). *Gryon pennsylvanicum* has been considered for augmentative biological control of squash bug in the past (Olson et al. 1996), but was not cost efficient when compared to conventional squash bug management.

In addition to regional differences, very few squash bug eggs or parasitoids were found in conventional commercial squash and pumpkin farms in Virginia. Presumably the frequent use of broadspectrum insecticides like pyrethroids are a major limiting factor. Given the obvious biological importance of this parasitoid, conservation of this natural enemy should therefore be a priority for integrated pest management programs in cucurbits, especially in crops under reduced spray management. In Virginia, organic and or reduced spray managed squash and pumpkin fields will likely benefit from the conservation of *G. pennsylvanicum.* Using an integrated approach to squash bug management as suggested by Doughty et al. (2016) may be the most sound and sustainable way to control squash bug.

References Cited

Bruton, B. D., F. Mitchell, J. Fletcher, S. D. Pair, A. Wayadande, U. Melcher, J. Brady, B. Bextine, and T. W. Popham. 2003. *Serratia marcescens*, a phloem-colonizing, squash bug -transmitted bacterium: causal agent of cucurbit yellow vine disease. Plant Dis. 87: 937-944.

Burks, B. D. 1967. The North American species of Anastatus Motschulsky (Hymenoptera, Eupelmidae). Trans. Am. Entomol. Soc. 93: 423-432**.**

Cornelius, M. L., M. L. Buffington, E. J. Talamas, and M. W. Gates. 2016. Impact of the egg parasitoid, *Gryon pennsylvanicum* (Hymenoptera: Scelionidae), on sentinel and wild egg masses of the squash bug (Hemiptera: Coreidae) in Maryland. Environ. Entomol. 45: 367-375.

Decker, K. B., and K. V. Yeargan. 2008. Seasonal phenology and natural enemies of the squash bug (Hemiptera: Coreidae) in Kentucky. Environ. Entomol. 37: 670-678.

Doughty, H. B., J. M. Wilson, P. B. Schultz, and T. P. Kuhar. 2016. Squash bug (Hemiptera: Coreidae): biology and management in cucurbitaceous crops. J. Integr. Pest Manag. 7: 1.

Nechols, J. R., J. L. Tracy, and E. A. Vogt. 1989. Comparative ecological studies of indigenous egg parasitoids (Hymenoptera: Scelionidae; Encyrtidae) of the squash bug, *Anasa tristis* (Hemiptera: Coreidae). J. Kans. Entomol. Soc. 62: 177-188.

Olson, D. L., J. R. Nechols, and B. W. Schurle. 1996. Comparative evaluation of population effect and economic potential of biological suppression tactics versus chemical control for squash bug (Heteroptera: Coreidae) management on pumpkins. J. Econ. Entomol. 89: 631-631.

Peverieri, G. S., P. Furlan, D. Benassai, S. Caradonna, W. B. Strong, and P. F. Roversi. 2013. Host egg age of *Leptoglossus occidentalis* (Heteroptera, Coreidae) and parasitism by *Gryon pennsylvanicum* (Hymenoptera, Platygastridae). J. Econ. Entomol. 106: 633-640.

Roversi, P. F., G. Sabbatini Peverieri, M. Maltese, P. Furlan, W. B. Strong, and V. Caleca. 2014. Pre-release risk assessment of the egg-parasitoid *Gryon pennsylvanicum* for classical biological control of *Leptoglossus occidentalis*. J. Appl. Entomol. 138: 27-35.

Schell, S. C. 1943. The biology of *Hadronotus ajax* Girault (Hymenoptera - Scelionidae), a parasite in the eggs of squash-bug (*Anasa tristis* DeGeer). Ann. Entomol. Soc. Am. 36: 625-635.

US Environmental Protection Agency. 2015. EPA's proposal to mitigate exposure to bees from acutely toxic pesticide products. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0818-0002)

Vogt, E. A., and J. R. Nechols. 1993. The influence of host deprivation and host source on the reproductive biology and longevity of the squash bug egg parasitoid *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Scelionidae). Biol. Control 3: 148-154.

Wyenandt, A., T. P. Kuhar, G. C. Hamilton, M. J. VanGessel, E. Sanchez, and D. Dugan. 2016. 2016 Mid-Atlantic commercial vegetable production recommendations. Virginia Cooperative Extension. Publication #456-420

Table 2.1. Numbers of squash bug egg masses collected and percentage parasitized by *Gryon pennsylvanicum* by region and county in Virginia 2014.

NOAA Climatic Division	Location (Co.)	Number of Egg Masses Collected	Percent Parasitism	
			Egg Masses	Eggs
Tidewater	Accomack	20	20.0	2.2
	Greensville	5	0.0	0.0
	Northampton	14	0.0	0.0
Eastern Piedmont	Amelia	22	9.1	2.0
	Buckingham	25	4.0	0.7
	Caroline	4	0.0	0.0
	Cumberland	20	30.0	14.8
	Fluvanna	13	15.4	10.3
	Goochland	46	23.9	51.7
	Louisa	11	18.2	1.4
	Mecklenburg	16	18.8	50.0
	Powhatan	28	3.6	4.8
Western Piedmont	Albemarle	9	88.9	92.0
	Bedford	14	71.4	76.9
	Franklin	32	9.4	4.1
	Henry	8	0.0	0.0
	Pittsylvania	9	88.9	53.0
Northern Virginia	Clarke	2	50.0	16.7
	Fauquier	10	100.0	98.5
	Fredrick	26	96.2	64.4
	Loudoun	6	83.3	35.6
	Page	6	0.0	0.0
	Rappahannock	10	60.0	37.4
	Shenandoah	21	85.7	71.7
Central Mountain	Augusta	15	13.3	12.6
	Botetourt	3	0.0	0.0
	Roanoke	16	56.3	47.7
	Rockbridge	4	50.0	50.0
	Rockingham	22	100.0	100.0
Southwestern Mountain	Floyd	4	0.0	0.0
	Montgomery	235	68.5	37.0
	Pulaski	6	83.3	81.6
	Washington	21	28.6	13.6
	Wythe	19	63.0	40.0

Table 2.2: Squash bug egg masses collected and percentage of mass and egg parasitism by *Gryon pennsylvanicum* by region and county in Virginia 2015.

NOAA Climatic Division	Location (Co.)	Number of Egg Masses Collected	Percent Parasitism	
			Egg Masses	Eggs
Tidewater	Accomack	19	0.0	0.0
	Northampton	34	0.0	0.0
	Virginia Beach	6	0.0	0.0
Eastern Piedmont	Buckingham	16	31.3	10.6
	Cumberland	12	41.7	27.8
	Fluvanna	8	37.5	11.6
	Goochland	14	28.6	18.4
	Hanover	15	86.7	44.5
	Henrico	15	93.3	43.1
	Louisa	15	0.0	0.0
	Nottoway	15	100.0	82.8
	Powhatan	15	6.7	7.4
	Prince Edward	5	0.0	0.0
	Spottsylvania	14	71.4	33.2
Western Piedmont	Albemarle	19	63.2	30.6
	Appomattox	10	90.0	57.5
	Campbell	15	46.7	14.1
	Nelson	15	93.3	38.8
Northern Virginia	Madison	15	0.0	0.0
	Orange	17	29.4	54.5
Central Mountain	Augusta	25	100.0	99.7
	Rockingham	36	66.7	47.7
Southwestern Mountain				
	Montgomery	45	100.0	95.6
	Pulaski	5	100.0	97.3

Table 2.3. Squash bug egg mass parasitism and egg parasitism rates by region and year of *Gryon pennsylvanicum* in Virginia.

	2014		2015	
	Mean % (SE)		Mean % (SE)	
Region	Egg Masses	Eggs	Egg Masses	Eggs
Tidewater	6.7 ± 17.9bc	0.8 ± 18.1	0.0 ± 17.4d	0 ± 13.6c
Eastern Piedmont	13.7 ± 10.3c	15.1 ± 10.4	45.2 ± 9.1bc	25.4 ± 7.1c
Western Piedmont	51.7 ± 13.9ab	45.2 ± 14.0	73.3 ± 15.1ab	35.3 ± 11.8bc
Northern	67.9 ± 11.7a	46.3 ± 11.8	14.7 ± 21.3cd	27.3 ± 16.6bc
Central Mountain	43.9 ± 13.9abc	42.1 ± 14.0	83.3 ± 21.3ab	73.7 ± 16.6ab
Southwestern Mountain	48.7 ± 13.9abc	34.4 ± 14.0	100.0 ± 21.3a	96.4 ± 16.6a

*Means in columns that are not connected by the same letter are significantly different, Fisher's Protected LSD α=0.05.

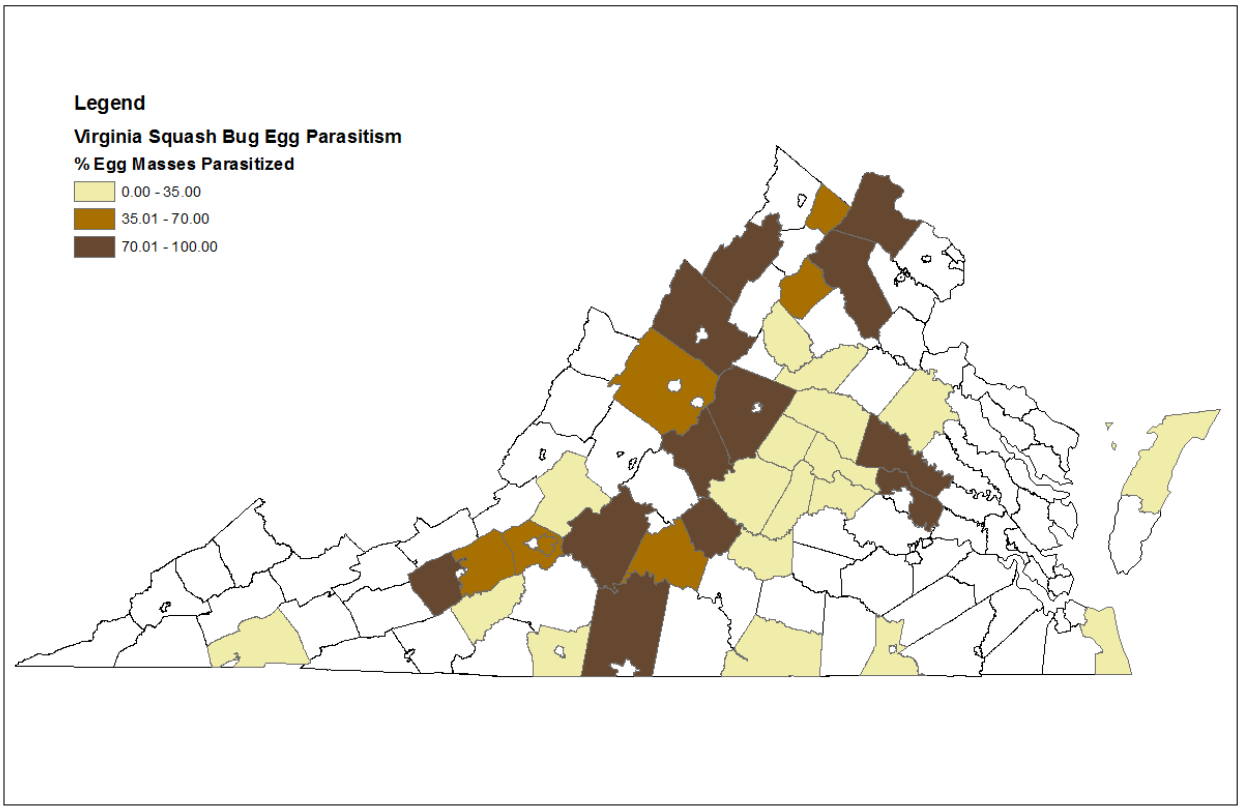

Figure 2.1: Map of Virginia and its counties showing the average mass parasitization of *Anasa tristis* egg masses by the egg parasitoid *Gryon pennsylvanicum* in representative shades of brown.

Chapter 3

Toxicity of Selected Insecticides to *Anasa tristis*, and its Primary Egg Parasitoid *Gryon pennsylvanicum*

Abstract

Squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae) is a major pest of cucurbit crops in the U.S. and currently only pyrethroids and neonicotinoids are recommended for chemical control. As there are nontarget concerns over the use of these insecticides, and because some of them also can lead to secondary pest outbreaks, there is a strong interest in finding more IPM-compatible insecticide options for squash bug control. Bioassays were conducted to assess the efficacy of several selective narrow-spectrum insecticides on squash bugs and their key biological control agent *Gryon pennsylvanicum* Ashmead (Hymenoptera: Scelionidae). In a laboratory bioassay where discs of summer squash fruit were dipped in spray tank concentrations of each insecticide and exposed to squash bug nymphs, none of the narrow-spectrum insecticides flonicamid, flupyradifurone, pyrifluquinazon, or sulfoxaflor provided more than >50% control of nymphs after 48 hrs in comparison to the pyrethroid λ-cyhalothrin, which killed virtually all nymphs. In another bioassay where field-collected squash bug eggs were dipped in insecticide treatments, there was no significant treatment effect on the number of squash bug nymphs emerging, but there was a significant effect on numbers of *G. pennsylvanicum* parasitoids emerging, with significantly fewer emerging from λ-cyhalothrin than the water control or other insecticides. Also, in filter paper dip contact bioassays, both λ-cyhalothrin and sulfoxaflor

caused significant mortality of *G. pennsylvanicum* adults after 72 hr exposure. In conclusion, the flonicamid, flupyradifurone, pyrifluquinazon, and sulfoxaflor do not appear to be effective insecticides for controlling squash bugs. However, three of the insecticides, flonicamid, flupyradifurone, and pyrifluquinazon, were shown to be less toxic to the parasitoid *G. pennsylvanicum* than λ-cyhalothrin and thus, would be more IPM-compatible options for control of other pests such as aphids on squash.

Introduction

The squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), is an endemic pest species of the Americas that feeds on plants in the family Cucurbitacea with a preference for squash and pumpkins (Beard 1940, Fargo et al. 1988, Doughty et al. 2016). With its piercing-sucking mouthparts, both nymphs and adults feed on vegetative growth, interrupting the flow of xylem, which can lead to wilt (Neal 1993). The pest status of this insect has increased in recent years because it has been shown to be the primary vector of the bacterial pathogen, *Serratia marcescens*, the causal agent of Cucurbit Yellow Vine Decline in some regions of the U.S. (Bruton et al. 2003) as well as mounting resistance of populations to host plant antibiosis that has widened its host plant range (Margolies et al. 1998). As is the case with most heteropteran pests, broad-spectrum insecticides such as pyrethroids and neonicotinoids are the primary means of controlling squash bug (Doughty et al. 2016). These insecticides, particulalry pyrethroids, are frequently tank mixed with fungicides that are routinely applied to cucurbit crops. While broad-spectrum insecticides are effective in controlling squash bug (McLeod et al. 2003, Kuhar et al. 2005, Abney and Davila 2011, Doughty et al. 2016), they can have a negative side effect by killing non-target organisms. With the slight exception of the neonicotinoid acetamiprid, all of the aforementioned foliar insecticides are also highly toxic to pollinators as well as many natural

enemy species (Johansen 1977, Smith and Stratton 1986, Desneux et al. 2007). Moreover, insecticide resistance to pyrethroid insecticides is widespread in several aphid species including melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) and green peach aphid, *Myzus persicae* (Sulzer) (Kuhar et al. 2005, Foster et al. 2007, Kuhar et al. 2011); and the use of insecticides for other insects such as squash bug frequently leads to serious outbreaks of these aphids (Slosser et al. 1989, Chapman et al. 2009, Ghidiu and Kuhar 2012, Kuhar et al. 2012). Further, aphid deposition of honeydew can increase the incidence of sooty mold that can damage large and valuable fruits like melons and pumpkins. Currently only pyrethroids and neonicotinoids are recommended for chemical control of squash bug. Although they are efficacious (Eiben et al. 2004, Abney and Davila 2011), pyrethroids are highly toxic to pollinators and natural enemies (Smith and Stratton 1986) and can result in outbreaks of secondary pests such as *A. gossypii* (Slosser et al. 1989). Neonicotinoids are also effective at controlling squash bug (McLeod et al. 2003, Eiben et al. 2004, Kuhar et al. 2005, Abney and Davila 2011, Kuhar and Doughty 2016), but are toxic to many non-target organisms and are generally not compatible with pollinator protection plans (Fairbrother et al. 2014, Pisa et al. 2014).

Hymenopteran parasitoids have been identified as natural enemies in many crop systems, but recent survey work in the Mid-Atlantic has provided a new perspective on its potential for control of *A. tristis* in cucurbit production. A recent survey of *A. tristis* egg masses in Virginia revealed that almost 40% of eggs are parasitized by *Gryon pennsylvanicum* Ashmead (Hymenoptera: Scelionidae) (Chapter 2). From an integrated pest management standpoint, it is imperative that we minimize the impact of current insecticides on the natural biological control systems, such as *G. pennsylvanicum* on squash bug and evaluate more IPM-compatible options.

Herein I evaluate several narrow-spectrum insecticides for their toxicity to *A. tristis* eggs and nymphs as well as on its key parasitoid, *G. pennsylvanicum*. Insecticides included: the sulfoximine insecticide sulfoxaflor and the novel butenolide flupyradifurone (Nauen et al. 2015), which are both neurotoxicants that act on the nicotinic acetylcholine receptor (Casida and Durkin 2013); and flonicamid (Morita et al. 2007) and pyrifluquinazon (Nesterov et al. 2015), which interferes with hemipteran feeding ability. The aforementioned insecticides are generally used to target other sucking hemipterans such as aphids, psyllids, and whiteflies (Wyenandt et al. 2016), but little is known about their efficacy against squash bug. In addition, the pyrethroid, λ-cyhalothrin was tested as a commercial standard.

Materials and Methods

Insecticides. Insecticide treatments were commercial formulations of products provided by the manufacturers. Insecticide treatments were diluted in one liter of distilled water proportional to a typical tank mix concentration based on a spray application rate of 355 liters per ha and the highest recommended field application rate listed on the label (Table 3.1).

Insects. Egg masses of *A. tristis* were collected from six locations in three counties of Virginia where parasitism of *G. pennsylvanicum* was known to occur (see Chapter 2). Collections were made when *A. tristis* eggs were prevalent and freshly laid on summer squash (*Cucurbita pepo*). These conditions were most frequently met on farms where chemical insecticides were not used. The collection period spanned the summer months in 2014 and 2015. All bioassays were set up when a sufficient number of individuals were available. An egg dip bioassay rep was conducted whenever a sufficient number of egg masses were encountered at a location (n = 60) and were used immediately. Squash bug nymphs that emerged from eggs were placed in a cage and maintained on young potted plants of either 'Lioness' yellow summer squash or 'Tigress'

zucchini grown in the greenhouse. When sufficient numbers of 2nd to 4th instars were available in this colony, they were used immediately in the nymph bioassay.

Squash bug nymph toxicity bioassay

This bioassay was conducted three times when sufficient numbers of 2nd to 4th instars (n = 120) were available from the colony from 2015 to 2016. Each of the five insecticide treatments (Table 3.1) plus a water control were poured into 500 ml beakers. Four freshly cut (8-10 cm diameter) discs of summer squash fruit were dipped in each insecticide treatment for 3 s, held vertically to allow excess solution to drip off, and then placed into 150 × 15 mm Petri dishes along with five *A. tristis* nymphs. Dishes were kept at room temperature (~ 27ºC) and mortality was assessed at 24 and 48 hours after exposure.

Squash bug egg mass dip toxicity bioassays

In 2014, five bioassays were conducted with 387 *A. tristis* egg masses. In 2015, five bioassays were conducted with over 300 egg masses. There was an average of 18 eggs per mass and 10 egg masses were dipped per treatment. Insecticides treatments were mixed and poured into 500 ml beakers as described previously. Egg masses were left on the leaf discs that they were deposited on and dipped for 1 s in insecticide treatment solutions (Koppel et al. 2011). The assays were replicated more egg masses were encountered in the field for a total of nine replications from 2014 to 2015. After treatment, eggs were returned to 60 × 15 mm Petri dishes and maintained at room temperature for 2 weeks at which time the number of *A. tristis* nymphs or adult parasitoids that emerged were recorded. Data from the nine replicates were pooled and an ANOVA was used to analyze for significant treatment effects on squash bug nymph and adult *G. pennsylvanicum* emergence.

Adult *G. pennsylvanicum* toxicity bioassays

In 2015, emerging *G. pennsylvanicum* adults were aspirated from rearing cages and were provided with a 50:50 honey: water solution ad libitum at room temperature (~ 27ºC). The adults were then utilized in a contact assay. Insecticide treatments were mixed into 500 ml beakers as described for the other bioassays and 5.5 cm P8 filter paper discs were dipped in the insecticidal solutions and allowed to dry under a ventilation hood. Once dry, these discs were then rolled up and used to line the insides 15 ml conical tubes. Adult parasitoids were gently aspirated from the colony and placed in the conical tubes evenly distributed in groups of seven or more across all six treatments. Adult mortality was assessed at 24, 48, and 72 hours after introduction to the tubes. and control mortality was corrected using Abbott's formula.

Statistics. Control mortality not exceeding 20% was corrected with Abbott's formula (Abbott 1925). Proportion mortality data of *A. tristis* nymphs and *G. pennsylvanicum* adults from direct exposure bioassays as well as numbers of *A. tristis* or *G. pennsylvanicum* adults emerging from field-collected and dipped eggs were analyzed using ANOVA in JMP 11. 0 (SAS, Cary, NC). Fisher's Protected LSD test was used to compare means among different treatments.

Results

Squash bug nymph toxicity bioassays

There was no significant effect of treatment on squash bug nymphal mortality at 24 hr; however, the λ-cyhalothrin treatment averaged approximately 70% mortality. At 48 hr, there was a significant treatment effect ($F=4.472$; $df=4, 14$; $p<0.0249$) with λ-cyhalothrin causing a

significantly higher rate of mortality (~80%) than the other insecticide treatments, none of which exceeded 30% mortality (Fig. 3.1).

Squash bug egg mass dip toxicity bioassays

There was no significant effect of treatment on the number of squash bug nymphs emerging from the dipped eggs ($F=1.7142$; $df=4, 566$; $p<0.1294$; Fig. 3.2). There was a significant effect of treatment on the number of *G. pennsylvanicum* adults emerging from dipped egg masses with λ-cyhalothrin having fewer emerging parasitoids than the other treatments ($F=4.5810$; $df=5, 566$; $p<0.001$; Fig. 3.3).

Adult *G. pennsylvanicum* toxicity bioassays

There was a significant effect of treatment on % mortality of *G. pennsylvanicum* adults exposed to treated filter paper ($F=24.5841$; $df= 4, 9$; $p<0.0017$). Both sulfoxaflor and λ-cyhalothrin had a higher % mortality when compared to the other treatments at 72 hours after treatment (Fig. 3.4).

Discussion

In this study, none of the narrow spectrum insecticides flonicamid, flupyradifurone, pyrifluquinazon, or sulfoxaflor caused significant mortality of squash bug nymphs in the lab bioassays. To our knowledge, there is not a lot of other published efficacy data on these insecticides for control of squash bug. Eiben et al. (2004) showed that flonicamid did not reduce the number of squash bugs on summer squash. However, in recent field experiments conducted in Virginia Beach, VA, both flupyradifurone and sufoxaflor resulted in significant reductions in squash bug densities compared to untreated summer squash (Kuhar and Doughty 2016). Little is

known about the efficacy of pyrifluquinazon on squash bug. Thus, the aforementioned insecticides may not be the answer for squash bug control, but they have been shown to be highly efficacious for control of homopteran pests such as whiteflies and aphids (Natwick and Lopez 2016). Thus, knowledge of their potential to at least suppress squash bug densities is important as is their impact on the key egg parasitoid of squash bug. In our bioassays, none of the narrow-spectrum insecticides had a negative effect on the number of adult *G. pennsylvanicum* emerging from dipped squash bug eggs, and only sulfoxaflor caused significant mortality in adult parasitoid exposure assays. In comparison, λ-cyhalothrin reduced the number of *G. pennsylvanicum* emerging from dipped egg masses and was quite toxic to adults.

This research also showed little to no ovicidal activity to squash bug eggs from any of the insecticides that we tested. Ovicidal activity has been found in the neonicotinoid class of insecticides (4A) on eggs of the bollworm, *Helicoverpa zea* (Kilpatrick et al. 2005) and on the eggs of asparagus beetle *Crioceris asparagi* (Kuhar et al. 2006). We did not find the two compounds that share a similar mode-of-action (Sparks and Nauen 2015), competitive modulators of the nicotinic acetylcholine receptor, the sufoxamine (4C) or the butenolide (4D), to have significant ovicidal effects. Current pest management guidelines for squash bug recommend that insecticides be applied after nymphs can be found on plants (Wyenandt et al. 2016), and my results further support this recommendation because there is little to no ovicidal activity to squash bug, and thus, there is no reason to apply insecticides before nymphs are present. My work also provides information on the toxicity of several insecticides to the native parasitoid *G. pennsylvanicum.* Although many of the narrow spectrum insecticides will likely not replace pyrethroids for squash bug control, they may be used for control of other soft-bodied hemipteran insects and thus, knowledge of their toxicity to a key hymenopteran parasitoid is

important for developing a sound integrated pest management approach for cucurbit production systems.

References Cited

Abbott, W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abney, M. R., and R. Davila. 2011. Evaluation of Foliar and Soil Applied Insecticides for Control of Squash Bug on Zucchini Squash, 2010. Arthrop. Manag. Tests 36.

Beard, R. L. 1940. The Biology of *Anasa tristis* DeGeer with Particular Reference to the Tachinid Parasite, *Trichopoda pennipes* Fabr., Conn. Agric. Exp. Stn. Bull., 440: 593-685

Bruton, B. D., F. Mitchell, J. FLetcher, S. D. Pair, A. Wayadande, U. Melcher, J. Brady, B. Bextine, and T. W. Popham. 2003. *Serratia marcescens*, a Phloem-Colonizing, Squash Bug -Transmitted Bacterium: Causal Agent of Cucurbit Yellow Vine Disease. Plant Dis. 87: 937-944.

Casida, J. E., and K. A. Durkin. 2013. Neuroactive Insecticides: Targets, Selectivity, Resistance, and Secondary Effects. Ann. Rev. Entomol. 58: 99-117.

Chapman, A., T. Kuhar, P. Schultz, T. Leslie, S. Fleischer, G. Dively, and J. Whalen. 2009. Integrating Chemical and Biological Control of European Corn Borer in Bell Pepper. J. Econ. Entomol. 102: 287-295.

Doughty, H. B., J. M. Wilson, P. B. Schultz, and T. P. Kuhar. 2016. Squash Bug (Hemiptera: Coreidae): Biology and Management in Cucurbitaceous Crops. J. Integr. Pest Manag. 7: 1.

Eiben, J., C. Mackey, W. Roberts, and J. V. Edelson. 2004. Foliar Applied Insecticides for Controlling Squash Bug, 2003. Arthrop. Manag. Tests 29.

Fairbrother, A., J. Purdy, T. Anderson, and R. Fell. 2014. Risks of neonicotinoid insecticides to honeybees. Environ. Tox. Chem. / SETAC 33: 719-731.

Fargo, W. S., P. E. Rensner, E. L. Bonjour, and T. L. Wagner. 1988. Population Dynamics in the Squash Bug (Heteroptera: Coreidae) Squash Plant (Cucurbitales: Cucurbitaceae) System in Oklahoma. J. Econ. Entomol. 81: 1073-1079.

Foster, S. P., G. Devince, and A. L. Devonshire. 2007. Insecticide Resistanve, pp. 261-285. In H. F. van Emden and R. Harrington (eds.), Aphids as Crop Pests. CABI, U.K.

Ghidiu, G. M., and T. P. Kuhar. 2012. Chapter 16: Pepper Insects and Their Control, pp. 216-226. In V. M. Russo (ed.), Peppers: Botany, Production and Uses. CABI, U.K.

Kilpatrick, A. L., A. M. Hagerty, S. G. Turnipseed, M. J. Sullivan, and W. C. J. Bridges. 2005. Activity of Selected Neonicotinoids and Dcrotophos on Nontarget Arthropods in Cotton: Implications in Insect Management. J. Econ. Entomol. 98: 814-820.

Koppel, A. L., D. A. Herbert Jr, T. P. Kuhar, S. Malone, M. Arrington. 2011. Efficacy of Selected Insecticides Against Eggs of Euschistus servus and *Acrosternum hilare* (Hemiptera: Pentatomidae) and the Egg Parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae) J. Econ. Entomol. 104 (1): 137-142

Kuhar, T. P., H. B. Doughty, E. M. Hitchner, and A. V. Chapman. 2006. Toxicity and field efficacy of acetamiprid on asparagus beetle. Online. Plant Health Progress [doi:10.1094/PHP-2006-0818-01-RS]

Kuhar, T. P., C. R. Philips, K. Kamminga, and A. Wallingford. 2011. Pyrethroid Resistance in Green Peach Aphid in Southwestern Virginia (USA) and FIeld Efficacy of Insecticides in Peppers., pp. 8-11, Resisrance Pest Management News. http://whalonlab.msu.edu/newsletter/index.html, Online.

Kuhar, T. P., H. Doughty, K. Kamminga, A. Wallingford, C. Philips, and J. Aigner. 2012. Evaluation of Insecticides for the Control of Brown Marmorated Stink Bugs in Bell Peppers in Virginia 2011 Experiment 1. Arthrop. Manag. Tests 37 (1): E37 (DOI: http://dx.doi.org/10.4182/amt.2012.E37)

Kuhar, T. P., and H. Doughty. 2016. Evaluation of Foliar and Soil Insecticides for the Control of Foliar Insects in Summer Squash in Virginia, 2015. Arthrop. Manag. Tests 41: tsw023.

Margolies, D. C., J. R. Nechols, and E. A. Vogt. 1998. Rapid Adaptation of Squash Bug, *Anasa tristis*, Populations to a Resistant Cucurbit Cultivar. Entomol. Exp. Appl. 89: 65-70.

Morita, M., T. Ueda, T. Yoneda, T. Koyanagi, and T. Haga. 2007. Flonicamid, a Novel Insecticide with a Rapid Inhibitory Effect on Aphid Feeding. Pest Manag. Sci. 63: 969-973.

Natwick, E. T., and M. I. Lopez. 2016. Insecticide Evaluation for Aphid Control in Alfalfa, 2015. Arthrop. Manag. Tests 41: tsw025.

Nauen, R., P. Jeschke, R. Velten, M. E. Beck, U. Ebbinghaus-Kintscher, W. Thielert, K. WÖlfel, M. Haas, K. Kunz, and G. Raupach. 2015. Flupyradifurone; a Brief Profile of a New Butenolide Insecticide. Pest Manag. Sci. 71: 850-862.

Neal, J. J. 1993. Xylem Transport Interruption by *Anasa tristis* Feeding Causes *Cucurbita pepo* to Wilt. Entomol. Exp. Appl. 69: 195-200.

Nesterov, A., C. Spalthoff, R. Kandasamy, R. Katana, N. B. Rankl, M. Andres, P. Jahde, J. A. Dorsch, L. F. Stam, F. J. Braun, B. Warren, V. L. Salgado, and M. C. Gopfert. 2015. TRP Channels in Insect Stretch Receptors as Insecticide Targets. Neuron 86: 665-671.

Pisa, L. W., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, M. McField, C. A. Morrissey, D. A. Noome, J. Settele, N. Simon-Delso, J. D. Stark, J. P. Van der Sluijs, H. Van Dyck, and M. Wiemers. 2014. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Poll. Res. Int. 22: 68-102.

Slosser, E., W. E. Pinchak, and D. R. Rummel. 1989. A Review of Known and Potential Factors Affecting the Population Dynamics of the Cotton Aphid. Southwest. Entomol. 14: 302-313.

Sparks, T. C., and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic Biochem Physiol 121: 122-128.

Wyenandt, A., T. P. Kuhar, G. C. Hamilton, M. J. VanGessel, E. Sanchez, and D. Dugan. 2016. 2016 Mid-Atlantic commercial vegetable production recommendations. Virginia Coop. Ext. Publ. No. 456-420

Table 3.1: Insecticides and concentrations used in laboratory toxicity bioassays on *A. tristis* and *G. pennsylvanicum*.

Active ingredient (AI)	Product (Manufacturer)	IRAC Insecticide Group	Recommended field application rate (g ai per ha)	Concentration in mg ai per liter of water
flonicamid	Beleaf (FMC Corp.)	29	99.8	170.00
flupyradifurone	Sivanto (Bayer CropScience LP)	4D	124.9	222.17
λ-cyhalothrin	Lambda-Cy (United Phosphorus Inc.)	3A	32.3	57.00
pyrifluquinazon	Nichino America Inc.	9	46.8	84.00
sulfoxaflor	Closer (Dow Agrosciences)	4C	78.5	126.44

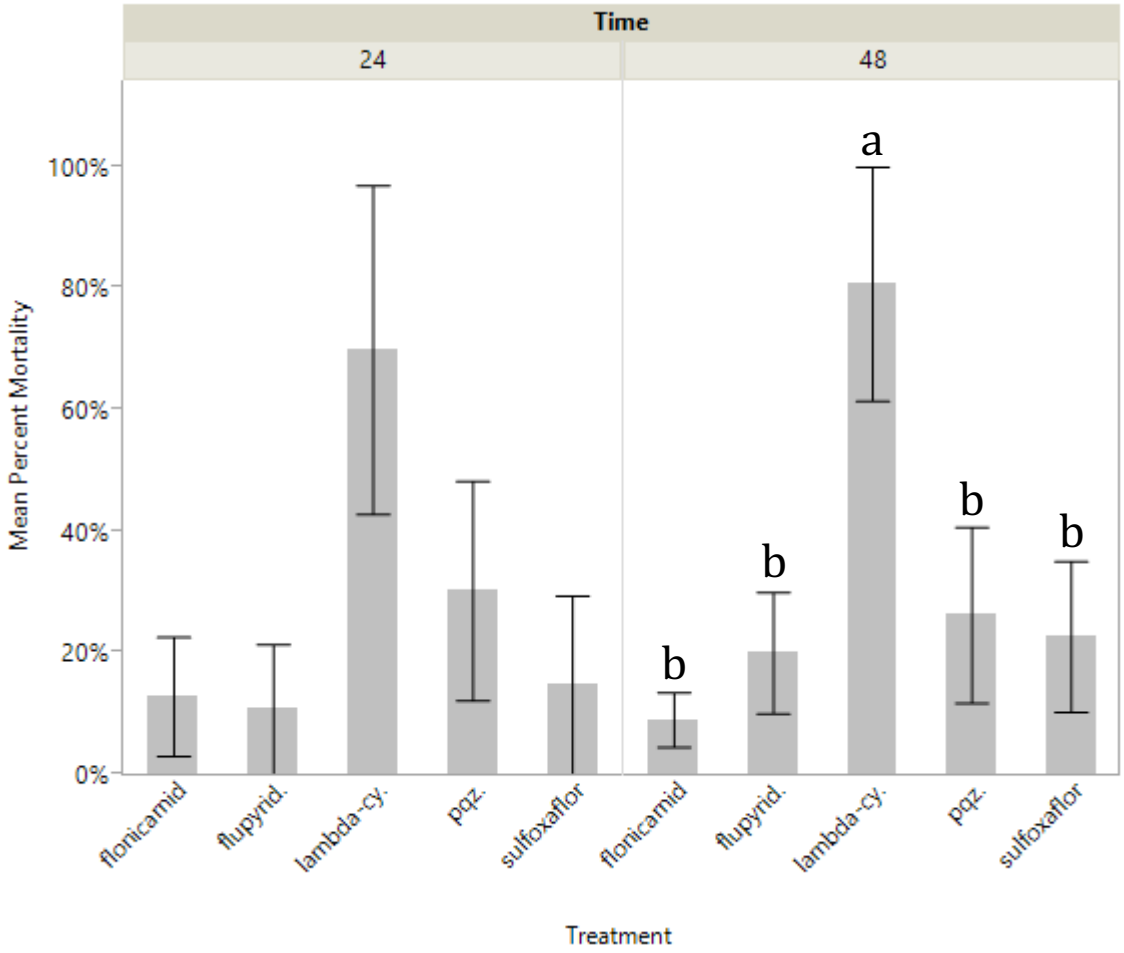

Figure 3.1: Mean ± SEM percentage mortality of squash bug nymphs from a toxicity bioassay where nymphs were held on treated (insecticide-dipped) squash discs. Columns within the same assessment time with a letter in common are not significantly different (Fisher's Protected LSD Test α = 0.05). flupyrid. = flupyridafurone; lambda-cy. = λ-cyhalothrin; pqz = pyrifluquinazon

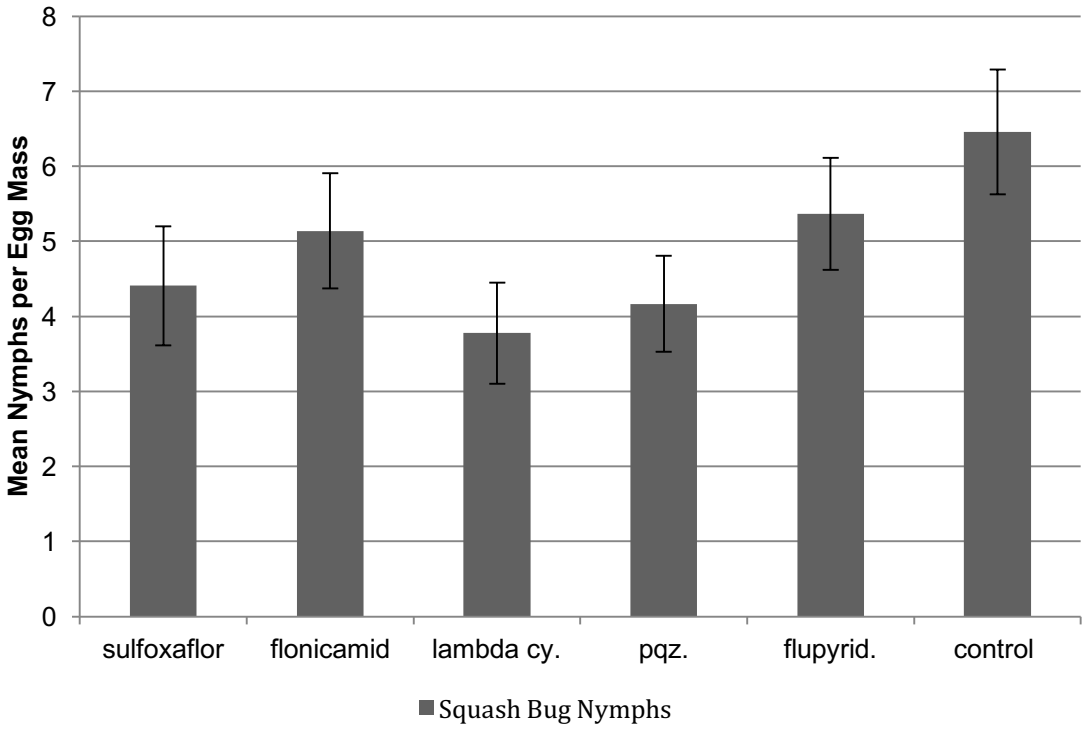

Figure 3.2: Mean ± SEM number of emerging *A. tristis* nymphs per egg mass dipped in various insecticide treatments over nine separate bioassays over the 2014 and 2015 field seasons. flupyrid. = flupyridafurone; lambda-cy. = λ-cyhalothrin pqz = pyrifluquinazon

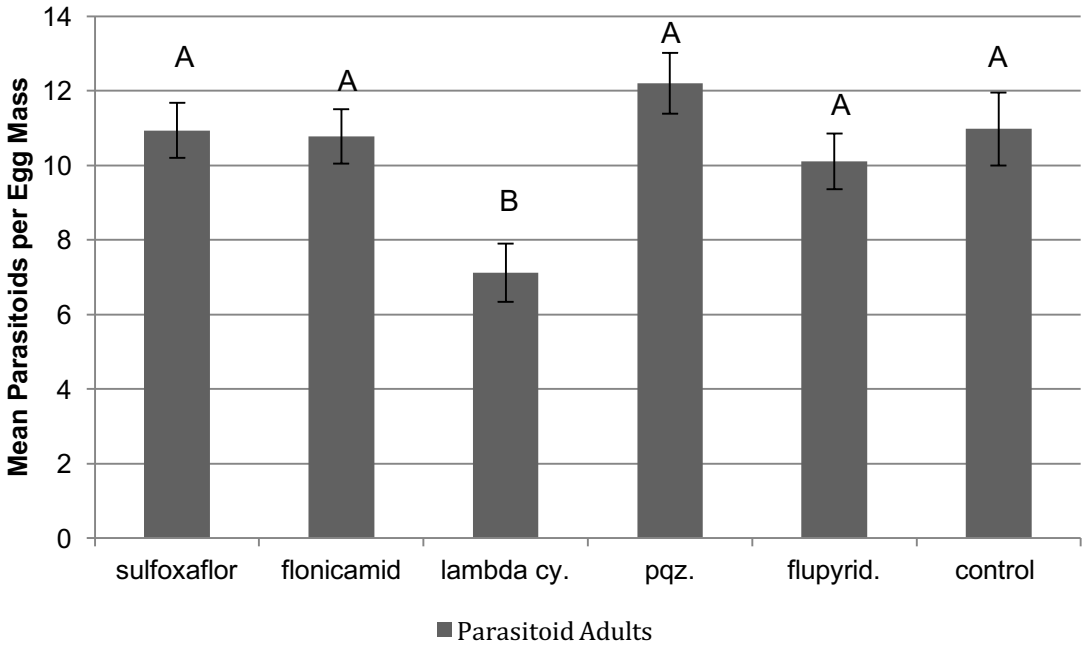

Figure 3.3: Mean ± SEM number of emerging *Gryon pennsylvanicum*s per egg mass dipped in various insecticide treatments over nine separate bioassays over the 2014 and 2015 field seasons. Columns within the same assessment time with a letter in common n are not significantly different (Fisher's Protected LSD Test α = 0.05). flupyrid. = flupyridafurone; lambda-cy. = λ-cyhalothrin pqz = pyrifluquinazon

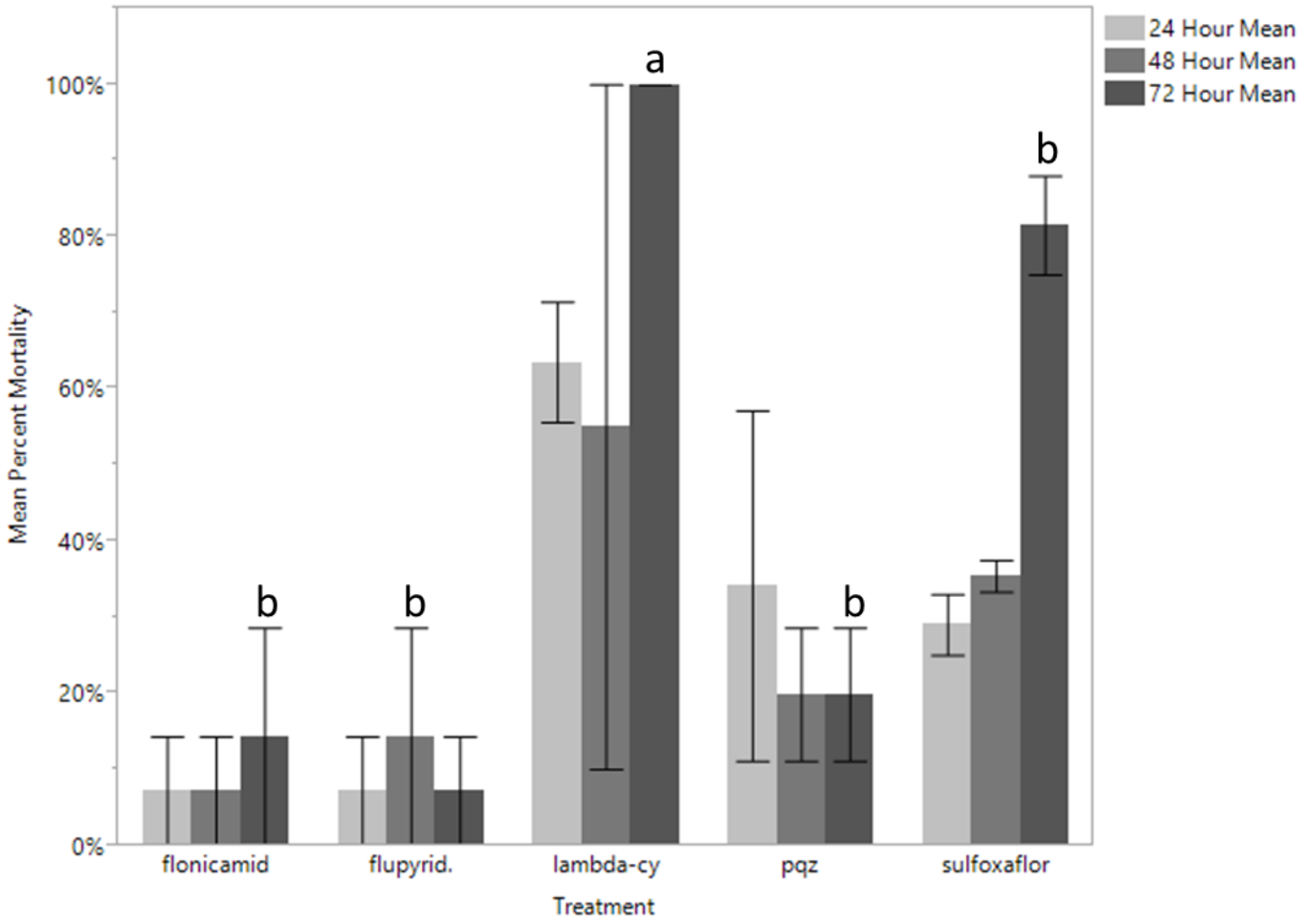

Figure 3.4: Mean ± SEM mortality of adult *Gryon pennsylvanicum* wasps after exposure to insecticide. Columns within the same assessment time with a letter in common are not significantly different (Fisher's Protected LSD Test α = 0.05). flupyrid. = flupyridafurone; lambda-cy. = λ-cyhalothrin; pqz = pyrifluquinazon

Chapter 4

Sub-lethal effects of the insecticide pyrifluquinazon on the European honey bee

Abstract

Pyrifluquinazon (PQZ) is a new IRAC Group 9 insecticide that has recently been registered for use in the U.S. for control of soft-bodied sucking insect pests. Although it has been classified as practically non-toxic to honey bees based on acute contact bioassays, additional information on sub-lethal effects of this insecticide on honey bees is lacking. Using a combination of lab assays with video movement tracking software and near-field evaluations of colonies foraging in a high-tunnel experiment, I determined that, when fed PQZ at a concentration of 84 mg ai/liter (= ppm) in sugar water a reduction in movement (lethargic behavior) by the foraging worker bees was observed. However, when provided with honey reserves in the hive, honey bees rejected the PQZ-poisoned sugar water and fed from honey reserves instead. These results indicate that if ingested at levels of 84 mg ai/liter (the high field rate), PQZ could have a negative effect on honey bee behavior; however, honey bee workers appear to be able to detect the presence of PQZ in their food and reject it.

Introduction

Pyrifluquinazon (PQZ)is a new insecticide recently categorized by the Insecticide Resistance Action Committee as a Group 9: chordotonal organ TRPV channel modulator (Nesterov et al. 2015), a limited group of pyridine azomethine derivatives (Sparks and Nauen 2015). Pyrifluquinazon has been found to be efficacious at controlling various soft-bodied sucking insects such as whiteflies (Hemiptera: Aleyrodidae) (McLeod and Rashid 2014, Palumbo 2013), and aphids (Hemiptera: Aphidae) (Kuhar et al. 2013, Haviland and Rill 2016) and will be registered in the U.S. on various vegetable and fruit crops, many of which are insect-pollinated. As part of the eco-toxicological testing requirements of the U.S. EPA for pesticide registrations, acute contact toxicity bioassays showed that PQZ is "practically non-toxic to honey bees" (*Apis mellifera* L. Hymenoptera: Apidae). In addition, an oral LD_{50} of 4.7 ug of active ingredient was submitted as part of the registration approval supplemental material for PQZ honey bee toxicity. However, beyond acute toxicity, there may still be sub-lethal risk associated with pesticide exposure to honey bees, which could significantly affect behavior and pollinator foraging ability as has been shown with other insecticdes (Desneux et al. 2007). Herein, I describe further investigations into potential sub-lethal (behavioral) effects of PQZ on honey bees at laboratory and near-field scale experimental levels.

Methods and Materials

Laboratory Experiments

Observation of sub-lethal behavioral changes in feeding bees can be difficult to quantify. Here I adapted the techniques of Teeters et al. (2012) and Ingram et al. (2015) and used a video camera and movement tracking software to assess the behavioral response of honey bees exposed to

PQZ. Bees were collected from one of the hives from the Virginia Tech Apiary near Blacksburg, VA. The hive was managed without miticides or other pesticides. Collected bees were maintained in a 9 × 9 × 7 cm container at 32°C and 70%RH for 12 h in a Percival Scientific growth chamber without food to ensure that bees would exhibit adequate feeding behavior upon the introduction of a food source.

Bees were anesthetized using carbon dioxide gas and 32 bees were selected at random from the group. Bees were placed in 16 different Petri dishes (9.5cm diam.) that were divided in half by a small segment of fiberglass window screen so that two bees occupied each dish, one on each side of the mesh. Each half of the dish contained a 0.5 × 0.5 × 1 cm sugar agarose cube that was designated as a feeding zone. Dishes in the treatment column contained two such cubes that were mixed with the field rate solution of insecticide (84 mg/liter ai of PQZ) before the cube solidified. Petri dishes that were in the control column only contained the sugar agarose cubes. A light table that was backlit using red LED light strips (620 nm wavelength) was used to provide illumination for the experimental recording without phototacticly influencing the behavior of the bees. Petri dishes were arranged in four side-by-side columns of four dishes each so that each column contained one treatment, control or PQZ treated agarose cubes.

The movements by the 32 bees were recorded simultaneously using a Basler monochrome camera suspended above the 16 Petri dishes. Movement tracks of the bees were captured using Noldus EthoVision software program, EthovisionXT (Ver. 10; Noldus Information Technology, Inc., Leesburg, VA) over a 120 min recording duration at a capture rate of 30 samples per second. Using the EthoVision software, total distance moved (cm), velocity (cm/s), and time (s) spent in the feeding zone were measured and analyzed for each pair of bees. Data were analyzed using ANOVA in JMP (JMP Pro 11; SAS Institute, Cary NC).

Near-field evaluations of the effect of pyrifluquinazon on honey bee foraging

Experimental arena

Adapting a design from Colin et al. (2004), I constructed two insect-proof high tunnels, each 22.0 m long, 7.9 m wide and 3.7 m tall during the summer of 2014 and arranged south-southeast so both tunnels received similar sunlight throughout the day at the Virginia Tech Price's Fork Research Station outside of Blacksburg, VA (37°12'40.9"N 80°29'21.8"W). Each high tunnel was a metal tube structure covered by 6 mil Clearspan™ plastic sheeting (FarmTek, Dyersville, IA), commonly used in growing season expansion high tunnel construction. The clear plastic is penetrable by the visible spectrum as well as UV light. The tunnels were used as near-field flight cages and allowed for free flight of the bees enclosed within them. Each high tunnel was divided in half by an opaque divider (the same as the ends) so that four flight arenas were created (each), the length of which allowed for colonies and feeders to be 10 meters apart within each chamber. Floors were made of gravel that was kept free of weeds.

Experimental hives

Colonies of honey bees were contained within a five frame, full depth, nucleus hive box containing a laying queen and her progeny as well as frames of capped and uncapped brood, eggs, bee bread, and honey stores. One such colony is considered a nucleus colony referred to hereafter as a "nuc". The nucs were moved into the experimental chambers at night after dusk when all foragers returned to the hive, being sure to move the bees from an offsite location. Beginning with the initial hive introduction, each chamber was equipped with a shallow source of fresh water. Each of the nucs was provisioned with a quarter of a pollen patty (, Mann Lake Ltd., Hackensack, MN) at their introduction to the experimental chamber and replaced if needed

during their pretreatment inspection. Nucs were inspected on day 0 after recording to ensure that colony provisions were adequate and egg laying was still ongoing.

Honey bee training to a feeder

The morning after the introduction of the nucs the water source was removed and replaced with a feeder containing a mix of a 50: 50 (sucrose: water) solution. The feeding apparatus was introduced to the chamber and placed directly in front of the nuc entrance. Feeders were made more attractive by mimicking easily distinguishable flower colors such as blue, which would be attractive to foraging bees. Once bees began to feed, the feeder was moved 5 meters away from the entrance, and finally 10 meters away by the afternoon of the second day if sufficient number of bees were attending and returning to the feeder. Depending on environmental conditions and colony suitability, this feeder training can take longer than two days. During the summers of 2015 and 2016, ten nucs of honey bees were trained to feed from feeders containing a 50:50 water: sucrose solution over the course of two days. The bees were then trained to locate the feeder during a fixed two-hour period for the next two days. One hour after the scheduled removal of feeders, a source of water was given and subsequently removed one hour before the next feeding event. Feeders were replaced with a water source before dusk each day. The third day of training began with only water, which was removed one hour before the target feeding time of 10:00 am EST. Honey bees are most accurate in returning to artificial feeders in the early part of a photophase, and as such, I chose the target time above (Moore et al. 1989). Feeders were then introduced at the desired time point and removed at the end of the designated exposure period. One hour after the exposure period concluded, the water source was replaced. This process mimics the ephemeral nectar reward that bees can associate with certain flowers and provided the experiment with a focused exposure period in which to collect foraging

data. The fourth day of training showed a marked increase in the response of bees to the feeder in their final location and during their specific exposure periods. This sequence was carried out simultaneously with three chambers and subsequent nucs twice during the 2015 field season and with four chambers and nucs in the 2016 field season.

Exposure and observation of trained bees

Once bees had been adequately trained to both the feeders and feeding time, video recordings using Go Pro Hero 3+ Black Edition cameras (GoPro, San Mateo, CA) were begun on day 0 to record the bees that were at the feeder and those that were actively feeding. Recordings were analyzed at five-minute intervals during the peak recruitment and feeding period (50 min) for bees that were actively feeding and bees that were present at the feeder, but not feeding. The number of actively-feeding bees relative to time was recorded as bees in "attendance". Chambers were randomly assigned treatments and their feeders were dosed with 84 mg ai/liter of PQZ in treated sucrose solutions during the exposure times in days 1 - 4. The control chambers were only given the 50:50 water: sucrose solution. Data from days 0 through 4 were analyzed as above with a Wilcoxon rank sums test (JMP Pro 11; SAS Institute, Cary NC). All five days of the experiment constituted one experimental run. The 2015 field season allowed for two such runs to be conducted with a total of three control nucs, and three PQZ treated nucs. In 2016, one run with two control nucs and two PQZ treated nucs was carried out at the same rate as in 2015.

Results

Laboratory Experiments Using Movement Tracking Software

Behavioral data from the laboratory assay consisted of the cumulative duration (s) in which the bees spent in the feeding zone, the velocity (cm/s) of bee movement in each arena, and

the overall distance (cm) moved by each bee by treatment. When comparing the 16 control bees with the 16 bees exposed to PQZ incorporated agarose cubes, there was a significant treatment effect on cumulative duration that the bees spent in the feeding zone (Figure 4.1) ($F=4.6079$; $df=1,29$; $P \leq 0.0403$), but not on total distance moved ($F=3.2890$; $df=1,29$; $P > 0.05$) or average velocity of movements ($F=0.9107$; $df=1,29$; $P >0.05$).

Near-field Evaluations of the Effect of Pyrifluquinazon on Honey Bee Foraging

In the near-field dosing experiments, the change in the attendance at the feeder was apparent on all of the first days of dosing at PQZ incorporated sugar water feeders. Pooling the five control nucleus colonies and the four PQZ dosed colonies allowed for a Wilcoxon rank sums 2-sample, normal approximation test to be performed (JMP Pro 11, SAS Institute Cary, NC). Days 0 and 1 showed no significant difference between treatments (Figures 4.2, 4.3). Days 2, 3, and 4 all had significantly different numbers of actively feeding bees in attendance at the control and PQZ incorporated sugar water feeders ($P<0.0001$ for each day). Each of the treated nucleus colonies had a marked change in the slope of the linear regression from day 0, day 1, and day 4. All four treated nucs colonies differed in their linear regressions from the pooled controls (Figure 4.4).

Discussion

Pyrifluquinazon is a new selective insecticide that will soon be registered for use on various food crops in the U.S. Although it has been classified as practically non-toxic to honey bees (USEPA 2012), further examination of sub-lethal insecticide dose effects on bee behavior could provide a more complete picture of the pesticide risk equation to pollinators. The

relationship between sub-lethal exposure and pollinator functional behavior has not been clearly defined (Cresswell 2011). Monitoring sub-lethal effects of pesticides through behavior and interpreting the value of behavioral changes in honey bee foraging is challenging, some authors have chosen to create arenas that eliminate present observers in an effort to eliminate bias (Teeters et al. 2012, Ingram et al. 2015). I adopted those methods as an initial screening of the sub-lethal effects that PQZ may have on honey bees. In no-choice bioassays, we showed that bees fed on PQZ incorporated agarose cubes but spent significantly less time in the feeding zones compared to agarose cubes alone, suggesting a potential antifeedant response to the insecticide. Consequently, I employed a larger scaled assay conducted in high tunnels with video cameras to examine how colonies with foraging bees and honey reserves within their hive reacted to PQZ exposure. Near-field bee foraging experiments using insect-proof high tunnels with video cameras have been previously used by Colin et al. (2004) in France to examine the change in honey bee foraging behavior after exposure to sub-lethal doses of imidacloprid and fipronil. This study was unique in its design and implementation, where the experimenters were able to isolate many extraneous factors and remove much of the observer bias and interference by using video cameras. The key to their study was quantifying foraging bee behavior in a way that could reflect changes in large numbers of bees to relate their activity levels to the abilities of the colony. I utilized a similar approach in an effort to characterize the risk to honey bee colonies from sub-lethal doses of PQZ. Because my experimental nucleus colonies were well provisioned with stores of honey, nectar and bee bread, the foraging bees from the colony could choose to feed from that source or to actively avoid it. I found that after 24 hr of exposure to PQZ in their food that trained foraging bees began avoiding it, and by day 4 virtually no bees fed on PQZ poisoned food. This suggests learned behavior and avoidance. By further researching

the sub-lethal behavioral effects that some insecticides have on bees, particularly in a colony, we can better qualify the risk. In this study, the avoidant behavior that honey bees exhibited to PQZ in their food could potentially limit the risk of this insecticide in the field.

References Cited

Colin, M. E., J. M. Bonmatin, I. Moineau, C. Gaimon, S. Brun, and J. P. Vermandere. 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contam. Toxicol. 47: 387-395.

Cresswell, J. E. 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotox. 20: 149-157.

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81-106.

Haviland, D. R., and S. M. Rill. 2016. Citrus thrips control in southern highbush blueberries in california, 2014: Table 1. Arthrop. Manag. Tests 40 (1): C11 (DOI: http://dx.doi.org/10.1093/amt/tsv021)

Ingram, E. M., J. Augustin, M. D. Ellis, and B. D. Siegfried. 2015. Evaluating sub-lethal effects of orchard-applied pyrethroids using video-tracking software to quantify honey bee behaviors. Chemosphere 135: 272-277.

Kuhar, T. P., H. Doughty, K. Kamminga, C. Philips, A. Wimer, and J. Aigner. 2013. Evaluation of insecticides for the control of green peach aphids in bell peppers in Virginia - 2012. Arthrop. Manag. Tests 38 (1): E38 (DOI: http://dx.doi.org/10.4182/amt.2013.E38)

McLeod, P., and T. Rashid. 2014. Toxicity of pyrifluquinazon against greenhouse whitefly on tomato produced in greenhouses. Adv. in Entomol. 2: 115-119.

Moore, D., D. Siegfried, R. Wilson, and M. A. Rankin. 1989. The influence of time of day on the foraging behavior of the honeybee, *Apis mellifera*. J. Biol. Rhythms 4: 305-325.

Nesterov, A., C. Spalthoff, R. Kandasamy, R. Katana, N. B. Rankl, M. Andres, P. Jahde, J. A. Dorsch, L. F. Stam, F. J. Braun, B. Warren, V. L. Salgado, and M. C. Gopfert. 2015. TRP channels in insect stretch receptors as insecticide targets. Neuron 86: 665-671.

Palumbo, J. C. 2013. Evaluation of pyrifluquinazon for control of sweetpotato whitefly in fall cantaloupes, 2012. Arthrop. Manag. Tests 38. (1): E18 (DOI: http://dx.doi.org/10.4182/amt.2013.E18)

Sparks, T. C., and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121: 122-128.

Teeters, B. S., R. M. Johnson, M. D. Ellis, and B. D. Siegfried. 2012. Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.). Environ. Tox. Chem. / SETAC 31: 1349-1354.

US Environmental Protection Agency. 2012. Ecological risk summary for the section 3 new chemical registration of pyrifluquinazon for indoor greenhouse use. (https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0971-0004)

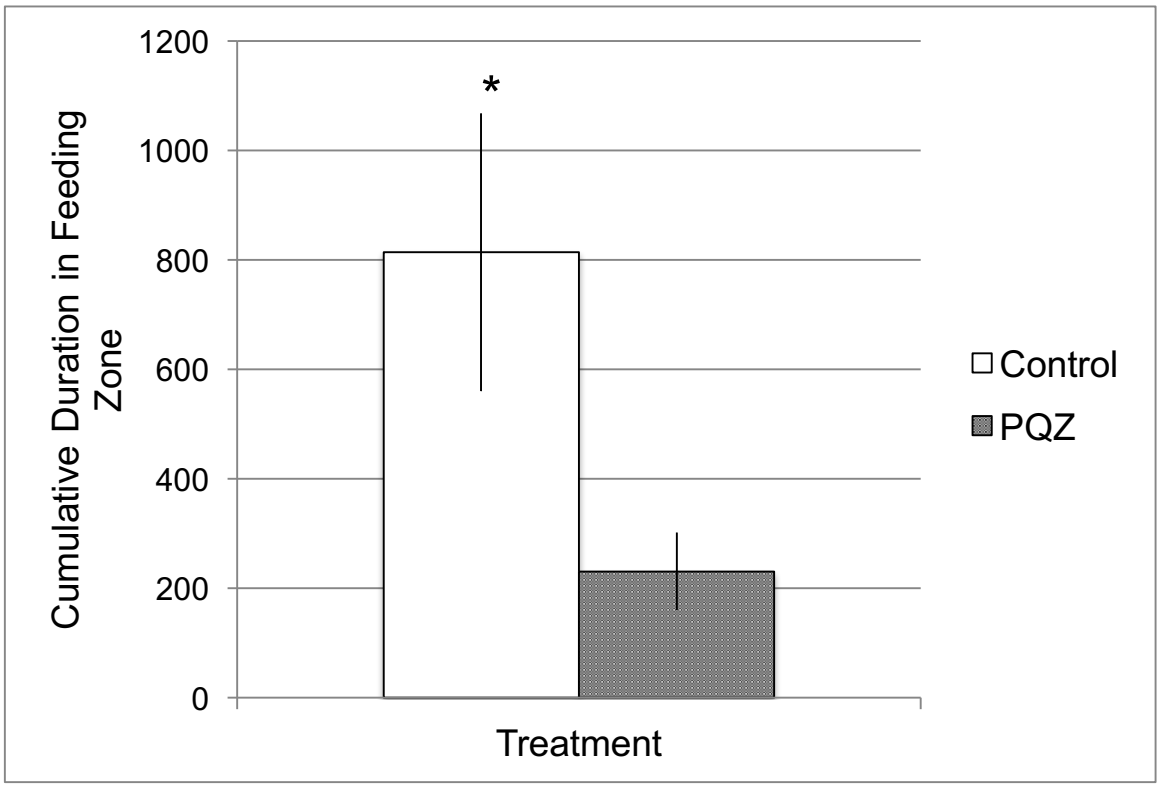

Figure 4.1: Cumulative duration of bees in feeding zones by treatment (control agarose cubes or PQZ incorporated agarose cubes) in seconds (s) from the laboratory evaluation of pyrifluquinazon. Asterisk denotes statistical significance (Fisher's Protected LSD Test α = 0.05).

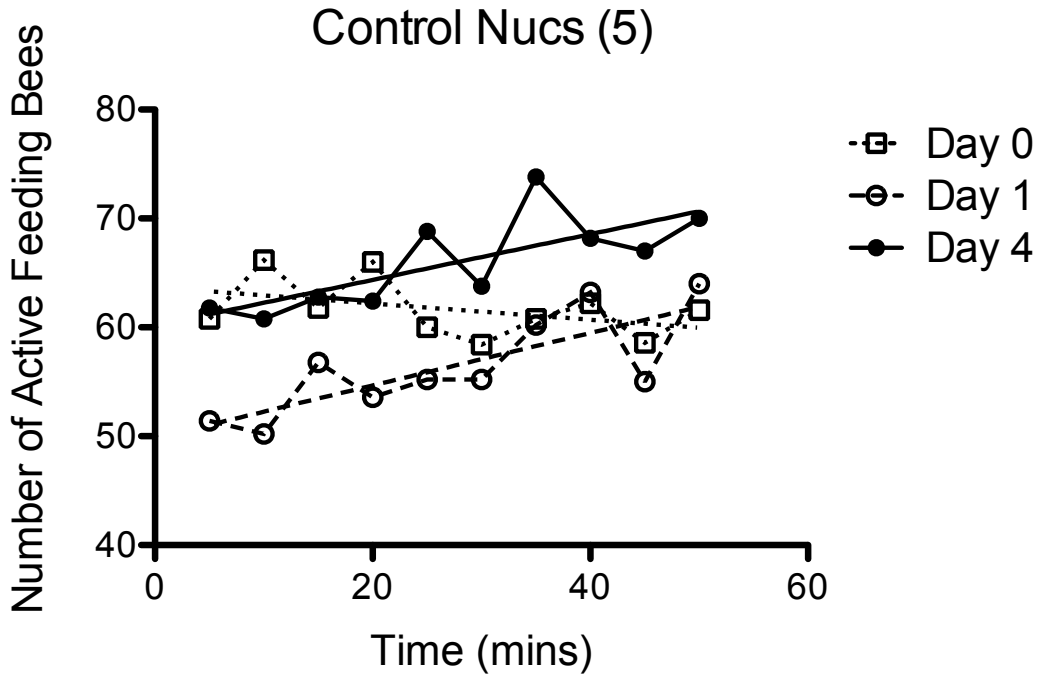

Figure 4.2: Numbers of actively feeding bees by day. Results are given for 5 pooled control nuclei. Data shown are from days 0, 1, and 4.

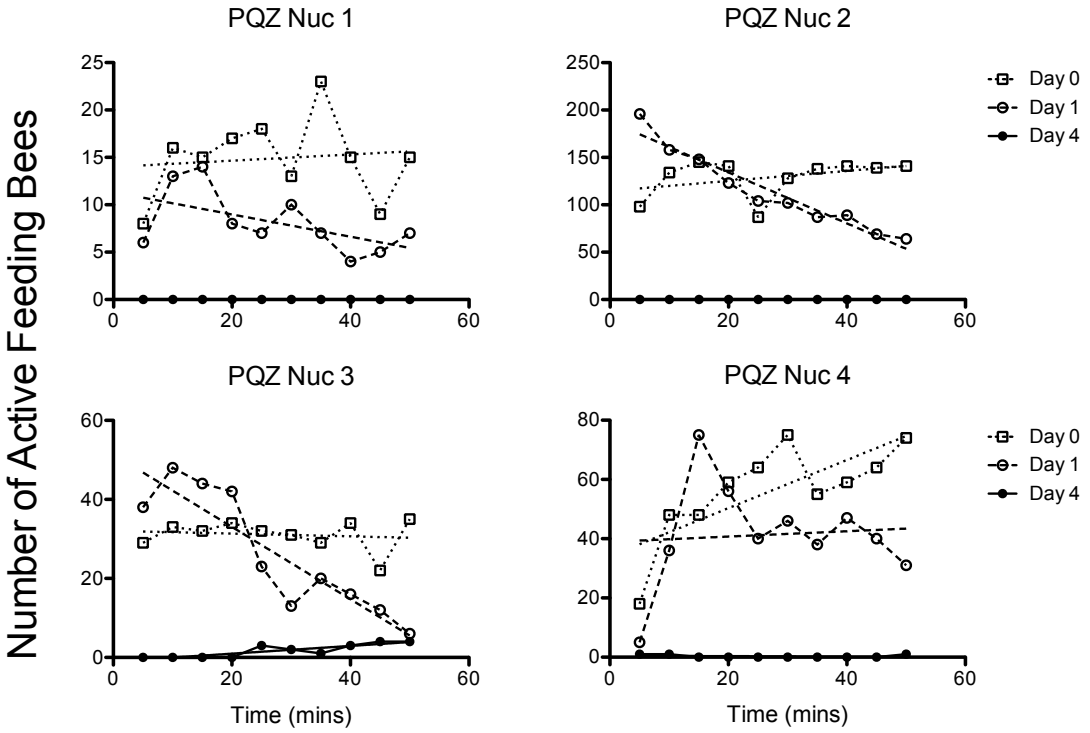

Figure 4.3: Numbers of actively feeding bees by day. Results are given for four PQZ nuclei. Data shown are from days 0, 1, and 4.

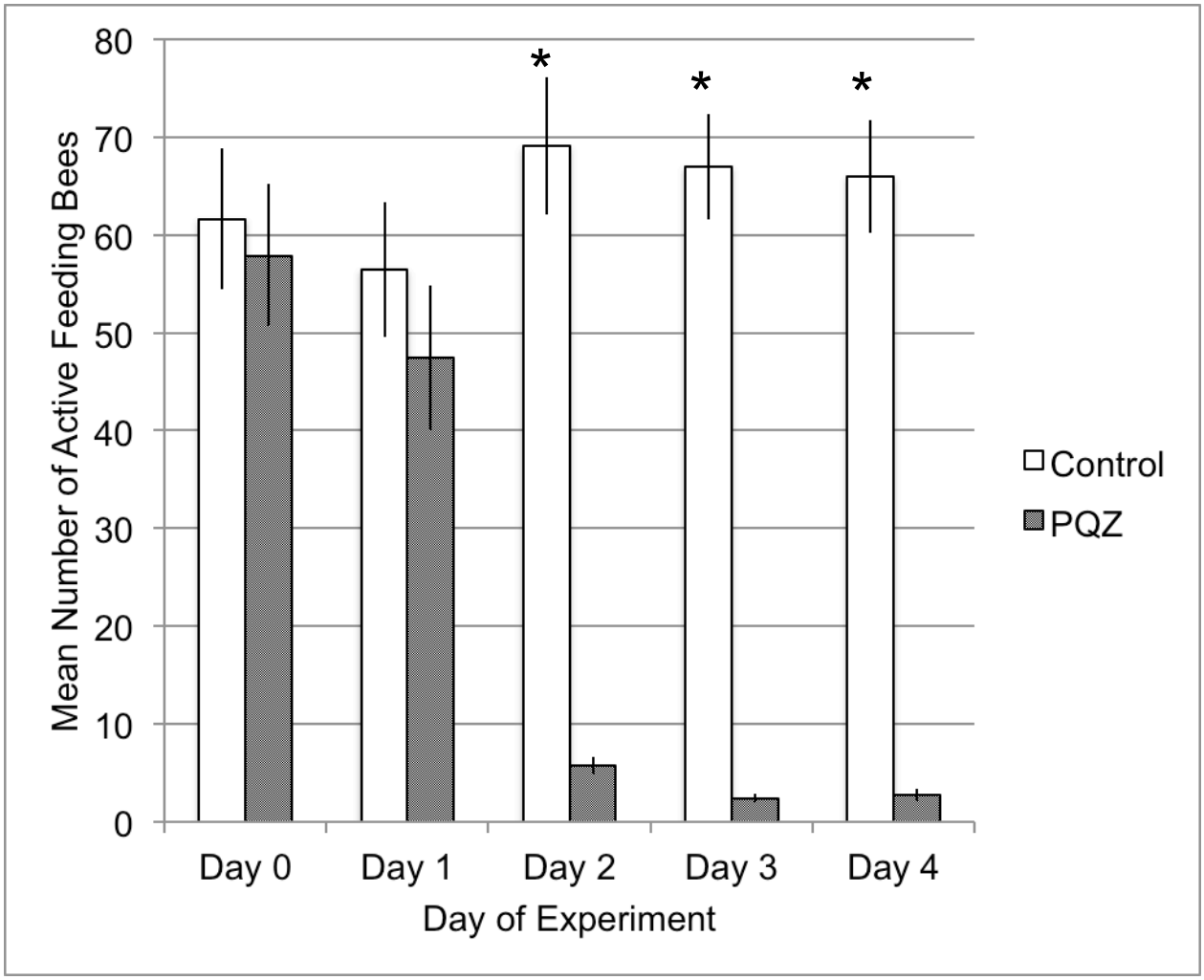

Figure 4.4: Mean number of bees actively feeding at sugar water provisioned feeders (Control), and PQZ incorporated sugar water feeders (PQZ) at five minute recording intervals for each day of the experiment. Asterisk denotes statistical significance (Wilcoxon Ranked Sums Test).