Integrated pest management of squash bug, *Anasa tristis*, for conventional and organic cucurbit systems in Virginia

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Integrated pest management of squash bug, *Anasa tristis*, for conventional and organic cucurbit systems in Virginia

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Academic Abstract

The squash bug, *Anasa tristis* De Geer (Hemiptera: Coreidae), is a serious pest of cucurbit crops across the US, especially within summer squash (*Cucurbita pepo* L.) systems. Using its piercing sucking mouthparts, squash bug feeds on both leaf tissue and fruits, potentially leading to leaf necrosis, seedling death, and yield loss. Currently, *A. tristis* management strategies in summer squash focus exclusively on insecticide applications. Given that continuous use of insecticides imposes negative side effects on many non-target species, the major objective of this dissertation was to identify new and understudied management strategies for minimizing *A. tristis* damage in Virginia summer squash. To accomplish this goal, we first performed experiments to test the effects of colored mulches on squash bug density. A two-year field replicated study conducted in 2019 and 2020 in southwest Virginia found that black, white, and reflective plastic mulch colors had no effect on squash bug adult, egg mass, or nymphal densities. Overall, we observed a broad plastic mulch effect, as summer squash plants grown in any plastic mulch color tended to harbor greater densities of squash bug life stages than plants grown on bare ground. Next, the potential of augmentative releases of egg parasitoid, *Hadronotus pennsylvanicus* Ashmead (Hymenoptera: Scelionidae) was assessed by deploying lab-reared parasitoids on organic farms growing summer squash in southeastern Virginia in 2020 and 2021. In both years, we found higher levels of *A. tristis* egg parasitism at *H. pennsylvanicus* release sites compared to sites where the parasitoids were not released. Further, higher egg parasitism at the release sites was observed within two weeks of the field deployment date. The third goal of this dissertation research was to characterize the relationship between squash bug life stages and marketable summer squash yield. In 2020 and 2021, summer squash fields were established in Whitethorne, Virginia and individual plants were labeled as either ‘managed’ or ‘infested’. Managed plants were subject to weekly manual removal of all squash bug egg masses and nymphs, while infested plants were left to have natural densities of squash bug life stages. We performed weekly *A. tristis* surveys on all infested plants in both years and added *H. pennsylvanicus* surveys on infested and managed plants in 2021. Also, we collected all fruit produced by each plant. Managed plants were found to produce more marketable fruit and fewer squash bug-damaged fruit than infested plants. Linear regression analyses of initial two-week accumulated squash bug life stage counts displayed adult and nymph densities to be significantly associated with marketable fruit yield per plant. However, nymphs showed a considerably stronger relationship with marketable yield than did adults. *Hadronotus pennsylvanicus* was visually sampled during each of the eight sampling weeks, but did not exhibit high egg parasitism until the latter half of the sampling interval. Lastly, we evaluated the toxicity of reduced risk insecticides on *A. tristis* nymphs and *H. pennsylvanicus* adults. To do so, we conducted laboratory bioassays exposing nymphs to insecticide treated summer squash fruit and parasitoids to treated filter paper, and recorded mortality at 24, 48, and 72 h following initial exposure. Only one reduced risk insecticide, flupyradifurone, was found to be highly toxic to squash bug nymphs and nontoxic to the parasitoids. Overall, the results of these studies will offer much needed information to improve the success and sustainability of squash bug management programs, as well as provide a fundamental basis and spark motivation for future research in squash bug biological control.

Integrated pest management of squash bug, *Anasa tristis*, for conventional and organic cucurbit systems in Virginia

Sean M. Boyle

General Audience Abstract

The squash bug is a serious insect pest of summer squash across the United States. Squash bugs inflict damage by feeding on plant leaves, stems, and fruits, leading to significant reductions in fruit yield and overall economic losses for growers. To date, farmers have few useful tools to minimize squash bug damage in their summer squash crop, and resort to over-applying insecticides. While this use of insecticides helps lower squash bug numbers, it often has negative side effects on the environment and important beneficial insect species like pollinators. To better understand the squash bug as a summer squash pest and to promote nonchemical ways to reduce squash bug damage, this dissertation research pursued answers to questions regarding: (1) the effect of plastic mulch color on squash bug numbers, (2) the use of squash bug natural enemies to control squash bugs, (3) the relationship between numbers of squash bugs and damaged summer squash fruits, and (4) whether new types of insecticides can control squash bugs and also be safe for beneficial insects. The results of this research will provide new insights on current issues with squash bug pest management. In particular, this work will provide summer squash farmers with useful knowledge that can be applied to their future efforts to responsibly control squash bugs in their fields and maximize their crop yields. With more research-supported and sustainable squash bug control tools, farmers will be able to not only adopt more environmentally-friendly food production practices, but also lower their overhead farm management costs and ultimately provide higher quality squash to consumers.

Attribution

Chapter 2: Effect of plastic mulch colors on *Anasa tristis* (Hemiptera: Coreidae) population dynamics in summer squash (*Cucurbita pepo*)

Sean M. Boyle designed and conducted the experiment, and prepared the published manuscript in its entirety. Adam A. Alford contributed to data collection, experimental design, and provided feedback on statistical analysis. Kelly C. McIntyre provided substantial edits to the manuscript preparation. Donald C. Weber, co-advisor to S.M. Boyle, provided edits and funding. Thomas P. Kuhar, co-advisor to S.M. Boyle, aided in the experimental design, statistical analysis, and provided funding (as the principal investigator). All co-authors reviewed the manuscript before publication.

Chapter 5: Effects of some selective hemipteran-targeting insecticides and one broad spectrum insecticide on squash bug and its primary parasitoid *Hadronotus pennsylvanicus*

The data included in this chapter have been published in two separate Arthropod Management Tests articles cited below.

Boyle, S. M., T. P. Kuhar, and D. C. Weber. 2022. Effects of selective hemipteran-targeting insecticides and one broad spectrum insecticide on squash bug, 2018. Arthrop. Manag. Tests. tsac073. https://doi.org/10.1093/amt/tsac073

Sean M. Boyle collected insect in the field, designed and conducted the experiment, and aided in the writing of the manuscript. Thomas P. Kuhar aided in experimental design, performed the statistical analysis, and prepared the manuscript for publication. Donald C. Weber provided edits to the manuscript and funding. All co-authors reviewed the manuscript before publication.

Boyle, S. M., T. P. Kuhar, J. Wilson, and D. C. Weber. 2022. Effects of some selective hemipteran-targeting insecticides and one broad spectrum insecticide on the parasitoid *Hadronotus pennsylvanicus*, 2018. Arthrop. Manag. Tests. tsac074. https://doi.org/10.1093/amt/tsac074

Sean M. Boyle collected insect in the field, designed and conducted the experiment, and aided in the writing of the manuscript. Thomas P. Kuhar aided in experimental design, performed the statistical analysis, and prepared the manuscript for publication. James Wilson collected insects in the field and aided in data collection. Donald C. Weber provided edits to the manuscript and funding. All co-authors reviewed the manuscript before publication.

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Introduction: *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae): A biological control agent for pestiferous leaf footed bugs (Hemiptera: Coreidae)

Overview

Hadronotus pennsylvanicus (formerly *Gryon pennsylvanicum*) (Ashmead) (Hymenoptera: Scelionidae) is a solitary egg parasitoid that attacks several species of leaf-footed bugs (Hemiptera: Coreidae), including eggs of host species in the genera *Anasa* and *Leptoglossus*, many species of which are considered serious agricultural pests of various economically important vegetable, fruit, and seed/nut crops. Over the last thirty years, there has been growing interest among biological control specialists in utilizing the parasitoid as a biological control agent to manage both native and invasive coreid pests throughout the world. In this chapter, we present a natural enemy profile for *H. pennsylvanicus* biology, life history, and relevant research concerning its potential as a classical and augmentative biological agent.

Taxonomy

The history of *H. pennsylvanicus* nomenclature is one of transience and volatility. Since it was first described as *Telenomus pennsylvanicus* by Ashmead (1893), at least eight different species names have been used to describe the wasp (Talamas et al. 2021). The most recent name change (December 2021) from *Gryon pennsylvanicum* to *H. pennsylvanicus* is most noteworthy, as nearly all the literature on the parasitoid has used the species name *G. pennsylvanicum*. Adding to the nomenclatural confusion, laboratory and field research using *G. pennsylvanicum* have been conducted with parasitoids collected from different geographic regions of North America (Masner 1983, Nechols et al. 1989, Bates and Borden 2004, Maltese et al. 2012, Cornelius et al. 2016) and even in Japan (Yasuda 1998) and Brazil (Marchiori 2002). Currently, there are ongoing efforts to implement COI barcoding to help disentangle the taxonomic ambiguity that surrounds many scelionid species worldwide (Komeda et al. 2020, Talamas et al. 2021).

Life Cycle and Morphology

Like many scelionid wasps, *H. pennsylvanicus* adults are a small, dark brown-black egg parasitoid (Fig. 1.1). Actual body size varies based on the host species egg from which the adult emerges, but generally ranges from 1 to 3 mm (Schell 1943, Masner 1983). Upon locating a suitable host egg mass, a female *H. pennsylvanicus* oviposits a single egg into each host egg by puncturing the host chorion with its ovipositor (Fig. 1.2). Depending on the host species, oviposition time per egg ranges from 5 to 20 minutes (Schell 1943, Cornelius et al. 2020). Eggs are stalked and microscopic, roughly 0.2 mm length and 0.1 mm diameter, and hatch within the host 18-20 h after oviposition (Schell 1943). The larval stage consists of three instars, beginning with a highly mobile telaform (body constricted between a large anterior part (cephalothorax) and an elongated posterior part with a tail-like caudal horn curved anteriorly) first instar that is responsible for consuming most of the host egg contents. Second and third instars assume sacciform (ovoid without features) and hymenopteriform (spindle-shaped, without thoracic legs) morphologies, respectively, with host feeding stopping by the onset of the third instar (Schell 1943).

Total development time from oviposition to adult emergence is 18-23 days (Nechols et al. 1989, Sabbatini Peverieri et al. 2012, Straser et al. 2022, Boyle, Chapter 3). Male *H. pennsylvanicus* adults emerge first and mate with their female siblings as soon as they emerge (Fig. 1.3). Females are synovigenic (i.e., oogenesis occurs after adult eclosion), but reach sexual maturity within 24 hours of emergence (Straser et al. 2022). *Hadronotus pennsylvanicus* exhibits arrhenotokous parthenogenesis: unfertilized eggs develop into male wasps. The parasitoid is relatively long-lived among parasitic Hymenoptera. Interestingly, male and female life spans are significantly affected by the presence of host eggs (Straser et al. 2022). In laboratory settings, females supplied with an optimum diet rich in carbohydrates (e.g., honey-water mixture) lived an average of 116 days in the absence of host eggs compared to 31 days when host eggs were provided. When given a continuous supply of honey and host eggs under laboratory conditions, the parasitoid's lifetime reproductive capacity is 30 to 144 offspring per female (Straser et al. 2022).

Geographic distribution of *H. pennsylvanicus* and its coreid hosts

The species name *Gryon pennsylvanicum* has been used by researchers from many different geographic locations. Its first description was based on a specimen from Louisiana (USA) that had emerged from a squash bug, *Anasa tristis* De Geer, egg mass in 1915 (Girault 1920). Subsequent studies have collected the wasp from many North American locations, including North Carolina (Schell 1943), Kansas (Nechols et al. 1989), Maryland (Cornelius et al. 2016), Virginia (Wilson and Kuhar 2017), California (Maltese et al. 2012), and British Columbia (Bates and Borden 2004, Roversi et al. 2011). Among the North American studies, there appears to be a geographic pattern in the host species from which the parasitoids emerged. Observations of *H. pennsylvanicus* hosts in the mid-Atlantic, southern, and midwestern states are comprised almost exclusively of two coreid species, *A. tristis* and *Anasa armigera* Say (Cornelius et al. 2018) (Fig. 1.4). These two hosts are pests of cucurbit crops (i.e., summer squash, winter squash, pumpkin, melon, and cucumber) throughout the continental US (Doughty et al. 2016, Cornelius et al. 2018, 2019). While both *Anasa* species can inflict damage to cucurbits, the majority of research associated with *H. pennsylvanicus* has focused on *A. tristis* in *Cucurbita pepo* L. growing systems. This is likely a function of pest abundance and severity, as *A. tristis* is more common in and damaging to *C. pepo* production (Cornelius et al. 2020) (Fig. 1.5).

Studies from the California and western Canadian localities directed their *H. pennsylvanicus* research towards *Leptoglossus* pests of tree fruit and nut orchards. The western conifer seed bug, *Leptoglossus occidentalis* Heidemann, has garnered considerable attention as a serious pest of several conifer tree species in the family Pinaceae (Strong et al. 2001, Bates and Borden 2005). In its native range of western North America, *L. occidentalis* threatens reforestation efforts for stands of ecologically significant pine species, such as lodgepole pine (*Pinus contorta* Douglas), western white pine (*P. monticola* Douglas), ponderosa pine (*Pinus ponderosa* Lawson), and Douglas-fir (*Pseudotsuga menziesii* Mirbel) (Bates et al. 2000, 2002). By feeding on cones and seeds, *L. occidentalis* causes high rates of cone abortion and 50-80% reduction in viable seeds (Strong et al. 2001). Unfortunately, the western conifer seed bug was found in Italy in 1999 and has since become a significant pest of the edible pine nut industry across many European countries (Lesieur et al. 2019). Economic losses for stone pine (*Pinus pinea* L.) seed production in Portugal, Spain, Italy, and Turkey are particularly concerning, as these four countries account for about 94% of stone pine stands worldwide (Farinha et al. 2021). Due to the growing European invasion of *L. occidentalis*, *H. pennsylvanicus* was imported to Italy under quarantine conditions in 2010 to be evaluated for its potential as a classical biological control agent (Roversi et al. 2011, 2013, Sabbatini Peverieri 2012, 2013, 2014).

Leptoglossus zonatus (Dallas), western leaffooted bug, has recently been investigated with regard to parasitism by *H. pennsylvanicus* (Straser et al. 2022). A polyphagous pest species, *L. zonatus* attacks high value nut crops in California like almonds and pistachios (Daane et al.

2005, Joyce et al. 2019), but also citrus fruit in the Gulf Coast region of the US (Xiao and Fadamiro 2010). Since broad spectrum insecticide applications dominate management plans for the pest, alternative control strategies are increasing in demand to make production systems more sustainable and environmentally friendly (Zalom et al. 2018). While little is known about the *H. pennsylvanicus* ecology in California nut orchards, wild parasitoid populations are consistently found parasitizing *L. zonatus* eggs in the field (Straser et al. 2022).

Parasitoid – Host Interactions

Parasitoids and their hosts are intimately connected by a diverse array of direct and indirect interactions (Godfray 1994). Because their hosts are only available for a short time and are inconspicuous within the environment, egg parasitoids have evolved fascinating strategies to overcome their physical and ecological limitations. Known as the *infochemical detour* strategy, egg parasitoids exploit host-related chemical cues (i.e., semiochemicals) that convey crucial information regarding the location and suitability of a potential host (Vet and Dicke 1992). While volatile compounds like herbivory-induced plant volatiles (HIPVs) and host species pheromones can indicate host general habitats (e.g., crop field or tree canopy), low-volatility chemicals deposited on plant substrates, such as frass from non-host life stages, can convey more precise information regarding a nearby host's location (Conti and Colazza 2012). Responses of scelionid parasitoids of heteropteran host semiochemicals are well-documented (Colazza et al. 2014). This research offers valuable insights on *H. pennsylvanicus* host foraging behavior.

To our knowledge, the first studies to test the ability of *H. pennsylvanicus* to exploit host-related semiochemicals was performed in the Ryukyu Islands of Japan by Yasuda and Tsurumachi (1995) and Yasuda (1998). Researchers tested the attraction of wild *H.*

pennsylvanicus to pheromone-producing male *Leptoglossus australis* (F.), a sporadic pest of vegetables and fruits, and the possible influence of male bug presence on egg parasitism on nearby cucurbit plants. By deploying cages containing male *L. australis* and cages containing female bugs in cucurbit plots, they observed *H. pennsylvanicus* were trapped only within the male-baited cages. Further, they also discovered that egg masses naturally laid on plants adjacent to male-baited traps were significantly more parasitized than eggs near unbaited control cages. Ultimately, their research suggests that *H. pennsylvanicus* exploits male coreid aggregation pheromones to locate suitable host habitat. More recent studies have examined *H. pennsylvanicus* behavioral responses to coreid adult and egg kairomones in laboratory and greenhouse settings (Hu et al. 2021). Greater percentages of female parasitoids exhibited host-foraging behaviors when exposed to volatile *A. tristis* egg extracts, compared with exposure to adult kairomone extracts in laboratory assays. Their greenhouse cage experiments baited *A. tristis* eggs with the same kairomone extracts, and then released mated female parasitoids into the cages. Similar to the laboratory results, *A. tristis* eggs baited with egg extracts displayed higher parasitism rates than eggs baited with adult kairomone volatiles.

Novel field studies conducted in the eastern US by Cornelius et al. (2022) investigated how host plant species and host habitat influence *H. pennsylvanicus* host foraging success for *A. tristis* and *A. armigera*. By deploying sentinel summer squash and cucumber plants possessing egg masses from each species into either summer squash or cucumber fields, they observed a significant effect of host plant and habitat on sentinel plant egg mass parasitism by wild *H. pennsylvanicus*. Specifically, *A. tristis* egg mass parasitism rates were highest on sentinel summer squash plants regardless of which field habitat they were located. In contrast, parasitism of *A. armigera* eggs was greatest when sentinel cucumber plants were placed in squash fields versus being placed in cucumber fields. Overall, the study presents strong evidence that *H. pennsylvanicus* may utilize different plant volatile and host-associated semiochemical combinations to locate suitable hosts in varying habitats.

Potential of *H. pennsylvanicus* as a biological control agent

Of several coreid species recorded as successfully parasitized by the parasitoid, two species, *L. occidentalis* and *A. tristis* have garnered the most attention as targets for biological control programs. Given its rapid and destructive spread as an invasive species in Europe (Farinha et al. 2021), *L. occidentalis* has been the primary target of *H. pennsylvanicus* biological control research for more than ten years (Roversi et al. 2011). Since the wasp's installation in quarantine in Italy in 2010, many of its desirable life history traits as a biological control agent have been identified. When exposed to western conifer seed bug eggs, *Hadronotus pennsylvanicus* exhibits high parasitism rates (70-80%), high fecundity (145 offspring/female), extended female adult longevity (40 days), and female-biased sex ratio (72% female) (Sabbatini Peverieri et al. 2012, Roversi et al. 2013). Additional parasitoid attributes support *H. pennsylvanicus* as a relatively robust insect for laboratory mass rearing. Parasitoid fecundity, offspring sex ratio, and development time was not significantly affected by varying *L. occidentalis* egg ages of 1-8 days old (Sabbatini Peverieri et al. 2013). *Leptoglossus occidentalis* eggs can also be stored at low (4 and - 20°C) and ultra-low (- 80 and -140 °C) temperatures for multiple months with compromising their host suitability (Sabbatini Peverieri et al. 2014). Of paramount importance for any candidate agent for classical biological control is host specificity to the invasive target pest. No-choice host range evaluations of native coreid species of Italy determined that *H. pennsylvanicus* is physiologically unable to successfully develop on any native coreid species tested (Roversi et al. 2013). To date, no field releases have been performed in any European country, most likely due to lack of additional non-target host range testing in countries outside of Italy. Yet, considering research results for *L. occidentalis* host range along with the dire threat to the highly valuable pine seed industry in Europe, the future for introduction biological control using *H. pennsylvanicus* in Europe appears to be promising, if ever approved for release.

The second coreid species that warrants *H. pennsylvanicus* biological control evaluations is the squash bug. Across the USA, *A. tristis* management focuses mainly on repeated applications of broad-spectrum insecticides throughout the year to reduce numbers in *C. pepo* growing systems (Doughty et al. 2016). Though effective in managing *A. tristis* and other co-occurring cucurbit pests (e.g., striped cucumber beetle, pickleworm) and inexpensive to apply, the consistent use of broad-spectrum insecticides can have detrimental effects to pollinator and natural enemy species (Kuhar et al. 2005, Desneux et al. 2007, Willis Chan 2021). Further, organic cucurbit growers have few effective approved insecticides products at their disposal, leaving them few management tactics that provide necessary *A. tristis* control. The use of *H. pennsylvanicus* as an augmentative biological control agent for squash bug may offer organic growers a much needed nonchemical management strategy, in addition to giving conventional growers alternatives to ecologically hazardous insecticide applications. Nechols et al. (1989) tested the reproductive and developmental parameters of *H. pennsylvanicus* on *A. tristis* eggs. *H. pennsylvanicus* is highly fecund when parasitizing *A. tristis:* the parasitoid produces about 80 offspring/female. It also has a high parasitism success rate (~86%) and a female-biased sex ratio. Olson et al. (1996) conducted the first augmentative releases of *H. pennsylvanicus* adults, deploying individual lab-reared adult females in small-scale pumpkin plots in Kansas. After releasing wasps at a rate of one female per 17 *A. tristis* eggs for five consecutive weeks, they were unable to observe a significant increase in *A. tristis* egg parasitism in release plots.

Other studies conducting *A. tristis* egg mass parasitism surveys in the eastern US have consistently observed high rates of parasitism, especially late in the cucurbit growing season (Decker and Yeargan 2008, Cornelius et al. 2016). Geographic differences in parasitism within Virginia were apparent in a statewide *A. tristis* egg parasitism study. Only 6.7% of egg masses collected in the southeastern region of the state were parasitized compared to > 60% in other areas of Virginia. Boyle et al. (Dissertation Chapter 3) augmentatively released lab-reared *H. pennsylvanicus* on small farms in southeastern Virginia. They deployed parasitized egg masses in summer squash fields on small (< 1 ha) organic farms early in the growing season (mid to late June), collected egg masses in two-week intervals after wasp deployment at releases sites and control (no-release) sites. The two-year study found significantly higher egg parasitism rates at farms where *H. pennsylvanicus* was released compared to the control farms. Although the study lacked large release and control site sample sizes, it showed success of a new deployment method for *H. pennsylvanicus* that could be adopted by future release studies targeting *A. tristis* and other coreid pest species. For instance, *L. zonatus* is a promising target for similar *H. pennsylvanicus* augmentative releases (Straser et al. 2022). Field deployment of the parasitoid in California nut orchards attacked by *L. zonatus* would offer valuable insights on the effectiveness of *H. pennsylvanicus* as a biological control agent in a vastly different crop system than the only previously studied cucurbit crops. Positive results from augmentative releases in California tree nut systems would likely strengthen the case for *H. pennsylvanicus* use as a classical biological agent of *L. occidentalis* in European pine nut orchards, as the two crops are more similar to each other than they are to cucurbit production systems.

Concluding Remarks

With growing global demand for organic produce and reductions in conventional insecticide use, sustainable nonchemical pest management strategies such as biological control are beginning to gain traction among current IPM programs. Ongoing unresolved issues with coreid pest management have motivated scientists to consider *H. pennsylvanicus* as a biological control candidate within many cropping systems. To date, considerable research in *H. pennsylvanicus* laboratory mass rearing techniques, developmental and reproductive trait characterization, host species preferences, and field deployment trials has formed a sturdy foundation on which large-scale biological control programs can be constructed. Continued work is needed to test *H. pennsylvanicus* release efficacy in different geographic areas, field deployment strategies in diverse cropping systems, parasitoid release rate metrics, and parasitoid-host chemical ecology, to advance our current understanding of *H. pennsylvanicus* as a classical and augmentative biological control agent.

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Figures

Figure 1.1: Adult *H. pennsylvanicus* and *Anasa tristis* egg mass

Figure 1.2: Female *H. pennsylvanicus* ovipositing in *A. tristis* egg

Figure 1.3: Recently emerged and emerging *H. pennsylvanicus* adults from *A. tristis* eggs

Figure 1.4: *Anasa tristis* (left) and *Anasa armigera* (right)

Figure 1.5: Fruit and foliar damage caused by *A. tristis* nymph herbivory

Chapter 2: Effect of plastic mulch colors on *Anasa tristis* (Hemiptera: Coreidae) population dynamics in summer squash (*Cucurbita pepo*)

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Abstract

The squash bug, *Anasa tristis* De Geer (Hemiptera: Coreidae), is a serious pest of cucurbit crops across the US, especially within summer squash (*Cucurbita pepo* L.) systems. Using their piercing sucking mouthparts, squash bugs feed on both leaf tissue and fruits, often leading to leaf necrosis, marketable fruit loss, and even plant death. To date, the relationship between squash bug presence and plasticulture has not been adequately investigated. This two-year study evaluated the effects of white, black, and reflective plastic mulch colors on the occurrence of all squash bug life stages and marketable zucchini yield in Virginia. In both years, *A. tristis* adults and egg masses were more numerous on zucchini plants grown in white and reflective plastic mulch compared to bare ground plants. Greater nymphal densities and marketable fruit yield were observed in certain plastic mulch treatments versus the bare ground treatment, yet these differences were not consistent in both years. Contrary to the repellency effects reflective mulches have on other cucurbit insect pests, our research suggests reflective and other plastic mulch colors can negatively impact squash bug management, especially in regions with high *A. tristis* pressure. Our study offers new insights for cucurbit growers to use when considering whether they should implement plasticulture in their growing systems.

Introduction

The squash bug, *Anasa tristis* De Geer (Hemiptera: Coreidae), is a formidable pest of cucurbit crops across much of the continental US, threatening an estimated $1.37 billion in cucurbit production each year (USDA-NASS 2018). *Anasa tristis* can complete its life cycle on a variety of cucurbit species, yet it prefers those in the *Cucurbita* genus, specifically *C. pepo* cultivars (e.g., summer squash, zucchini) (Bonjour et al. 1991, Bonjour et al. 1993). Using its piercing-sucking mouthparts, squash bug feeds on the xylem, phloem, and plant cell tissues of the leaves, stems, and fruit (Neal 1993). This feeding can have a variety of negative effects on the host plant, leading to localized leaf necrosis, vascular disruption, fruit rot, marketable yield loss, and even plant death (Woodson and Fargo 1991, Neal 1993, Palumbo et al. 1993). Squash bug feeding can create opportunistic entry sites for cucurbit fruit pathogens such as anthracnose, choanephora fruit rot, and grey mold rot (Doughty et al. 2016). *Anasa tristis* adults are also vectors of *Serratia marcescens* Bizio, a phloem-colonizing bacterium that causes cucurbit yellow vine disease (CYVD) in summer squash, pumpkin, watermelons, and cantaloupe (Bruton et al. 2003). Current squash bug management strategies focus primarily on the application of broad-spectrum insecticides, however, most insecticides labeled for squash bug control (e.g., organophosphates, carbamates, pyrethroids, and neonicotinoids) are harmful to natural enemy and pollinator species (Elzen 2001; Doughty et al. 2016; D'Avila et al. 2018). Additionally, repetitive use of these insecticides may result in outbreaks of secondary pests such as aphids, whiteflies, and thrips (Kuhar et al. 2005). For these reasons, alternative, non-chemical controls are needed to improve the sustainability and effectiveness of squash bug integrated pest management.

Plasticulture, or the use of plastic mulch, is commonly used in vegetable production to suppress weeds, retain soil moisture, regulate soil temperature, improve irrigation efficacy, and enhance organic matter retention (Jabran 2019). Plasticulture has also been shown to influence insect pest behavior, acting as a repellent or visual disrupter (Vincent et al. 2003, Diaz and Fereres 2007). For example, Nottingham and Kuhar (2016) observed reduced pest densities in snap beans grown on reflective plastic mulch, potentially through the augmentation of light reflectance and concomitant temperature around vegetable plants. Within cucurbit systems, reflective mulch can significantly decrease the presence of aphids, thrips, whiteflies, and cucumber beetles, and as a result, minimize the prevalence of insect-vectored plant diseases (Henshaw et al. 1991, Summers et al. 1995, Caldwell and Clark 1999, Frank and Liburd 2005). Given these benefits, investigating the impact mulch can have on incidence of squash bug seems a logical next step, however, to date only one study (Cartwright et al. 1990) has investigated the effects of different plastic mulches on squash bug presence.

In their two-year study, Cartwright et al. (1990) observed greater numbers of squash bugs but no difference in yield (i.e., mean number of fruit/plant) in reflective plastic mulch versus bare ground. Yet, these findings were inconsistent between years, planting dates, or between specific mulch colors and bare ground treatments. To clarify these varied results, the influence of various plastic mulch colors on squash bug warrants additional consideration. The findings of this single study may also not be indicative of all regions of the US, as this experiment was conducted in Stillwater, Oklahoma (Doughty et al. 2016). Here, we seek to further investigate the effects of plastic mulches on squash bug abundance and damage, using a different geographic location and increased experimental replication in Virginia summer squash systems.

Materials & Methods

Experimental Design

We conducted field experiments to test the effects of mulch color on *A. tristis* during the summers of 2019 and 2020 at Kentland and Homefield Farms in Whitethorne, VA. To do so, we constructed four treatments of plastic mulch beds (1.2 x 9.1 m /block) as a Latin square design in early June. Treatments included black, white, and reflective mulch (Berry Hill Irrigation, Buffalo Junction, VA) and bare ground. Zucchini seeds (*var.* Dunja, Harris Seeds) were sown directly in mid-June at 0.76 m spacing within each treatment bed. Seven to ten days after sowing, stand counts were taken for each germinated treatment bed. Plots with sparse germination (< 50%) were replanted with greenhouse grown seedlings (sown on the same date as in the field) so there were at least ten plants per plot. Tiffany teff grass (Hancock Seed Company, Dade City, FL) was sown between plastic mulch rows at a rate of 11 kg seed/ha, acting as a living mulch and weed suppressant throughout the experiment. A 9% concentration (total fatty acid) solution commercial blend of caprylic and capric acid (Home Plate non-selective herbicide; Certis USA, Columbia, MD) was applied to bare ground plots to manage weeds on two dates; before squash germination and immediately following germination. Subsequent weed management was accomplished through weekly manual removal of weeds.

Insect Sampling

Approximately three weeks after zucchini germination, we began sampling for squash bug at weekly intervals. Five randomly selected plants within each plot were surveyed for adults, nymphs, and egg masses. Surveys included visual checks of the entire plant and the plastic mulch directly below the plant, specifically focusing on the spaces where the plant stem meets the mulch and along the mulch teff grass margin. Additional consideration was given to these areas because previous research suggested that *A. tristis* adults and nymphs commonly reside in inconspicuous or hidden areas adjacent to the plants (Palumbo et al. 1991a, Doughty et al. 2016). In total, insect sampling was performed for six consecutive weeks, starting the first week of July and ending the second week in August.

Marketable Fruit Sampling

Once plants began to develop fruit, we harvested all undamaged, marketable zucchini from each plot three times per week from the fourth week of July through the second week of August by manually picking all market sized fruit (220-250 g). Damaged fruit was picked during each harvest date but was discarded and not included in our marketable fruit yield counts. Numerous factors, such as improper pollination, microbial pathogens, and feeding damage from other cucurbit insect species, can influence summer squash fruit quality. Since we did not specifically account for these potential predictors of fruit quality, the number of unmarketable fruit was not recorded as an additional response variable.

Statistical Analysis

Insect count and marketable yield data were separated by year. The number of marketable fruit collected per plot was summed for each sampling week and divided by the number of plants within each plot. Weekly *A. tristis* counts and marketable fruit yield/plant were normalized using a square-root transformation. To test the fixed effects of mulch treatment, sampling week, and their interaction on insect counts and yield, we used a generalized linear mixed model (GLMM) with block and site location as random factors. Although we were not interested in the effect of sampling week alone, it was included as a fixed factor in order to test its interaction with mulch treatment on *A. tristis* counts and yield. This interaction allowed us to distinguish whether the effect of mulch treatment was consistent across our *A. tristis* and yield sampling periods. Multiple comparisons between mulch treatments were conducted using a Tukey's honestly significant difference (HSD) test (α = 0.05). All statistical analyses were completed using JMP 15.0.0 software.

Results

Adults

For both years, mulch treatment and sampling week were significant predictors of *A. tristis* adult counts, while the mulch*sampling week interaction was not significant (GLMM; Table 2.1). In 2019 and 2020, a nearly five-fold difference was observed in reflective and white mulch versus bare ground plots (Tukey HSD; Figs. 2.1a, 2.2a). In 2020, significantly more adults were also found in black mulch compared to bare ground treatments.

Egg masses

Similar to the adult counts, mulch treatment and sampling week had significant effects on observed egg masses in both years (GLMM, Table 2.1). In 2020, mulch*sampling week interaction was a significant predictor of *A. tristis* egg masses, meaning the relative differences among mulch treatments was influenced by the week the egg mass counts were performed. In 2019 and 2020, roughly twice as many egg masses were found in black, white, and reflective plots than in bare ground plots (Tukey HSD; Figs. 2.1b, 2.2b).

Nymphs

In 2019, mulch treatment had no significant effect on nymph counts, and sampling week was our only significant predictor (GLMM; Table 2.1, Fig. 2.1c). In 2020, sampling week and mulch treatment significantly affected observed nymphs. Specifically, we counted over three times as many nymphs on zucchini plants in reflective and black mulch than on bare ground plants (Tukey HSD; Fig. 2.2c).

Marketable Yield

Sampling week and mulch treatment predictors had significant effects on marketable fruit/plant produced in 2019 and 2020 (GLMM; Table 2.1). The mulch*sampling week interaction was only a significant predictor of marketable fruit/plant in 2020. In 2019, plants in black and reflective mulches produced about twice as many marketable zucchini as bare ground plants (Tukey HSD; Fig. 2.1d). However, in 2020, plants in bare ground treatments produced the same marketable yield as plants in the three mulch color treatments (Fig. 2.2d).

Discussion

In our study, we observed significantly more *A. tristis* adults and egg masses on summer squash grown with white, black, or reflective mulch compared to bare ground, yet no differences in adult or egg mass numbers among the three mulch color treatments (Figs. 2.1, 2.2). The observed similarity in population dynamics for adults and egg masses was expected, as the two life stages are highly correlated throughout much of the growing season (Harmon et al. 2003). More specifically, once adults reach peak density, there is a subsequent peak in egg mass density (Fargo et al. 1988; Palumbo et al. 1991b). Our findings also support a preference by *A. tristis* adults for squash grown in plastic mulched systems, which appears to be unaffected by mulch color. Preference for plastic mulch systems may be a result of *A. tristis* adults' propensity to congregate near the crown and lower, older leaves of their host plant (Palumbo et al. 1991a). During the initial weeks of the summer squash vegetative growth phase, the plants are small and offer inadequate refuge for the colonizing adults. Holes in the plastic mulch where squash is planted provide shelter for adults to feed on the young plant's main stem and mate relatively protected from natural enemies. One of the most prominent natural enemies of *A. tristis* adults, parasitoid fly *Trichopoda pennipes* Fabr. (Diptera: Tachnidae), actively searches for the bugs in the canopy of host plants (Beard 1940). However, it is unknown whether *T. pennipes* will forage for host bugs underneath the plastic mulch. Squash bug copulation can last > 20 h for a single adult pair, leaving the bugs vulnerable for extended periods of time (Sears et al. 2020). The protective cover of plastic mulch may then allow *A. tristis* to optimize its fitness and fecundity early in the season when shelter near host plants is sparse. Future research is needed to completely discern if squash bug adults experience decreased rates of predation and parasitism when using plastic mulch as refuge.

Nymphal densities were highly variable and did not follow the pattern of the adult and egg mass numbers. Mulch treatment was only a significant predictor of nymph counts in 2020 (Table 2.1; Fig. 2.2c). Previous research suggests that while all *A. tristis* life stages exhibit an aggregated distribution, nymphs show the greatest degree of aggregation (Palumbo et al. 1991c). For this reason, nymph distribution may be patchier and require additional sampling to accurately determine nymphal abundance (Palumbo et al. 1991c). Therefore, a potentially patchy distribution of nymphs in conjunction with identical numbers of plants surveyed for all life stages may explain inconsistent results among nymphs between and within treatment groups.

Even with significant differences in *A. tristis* adult and egg mass numbers between mulched and bare ground plots, differences among mulch treatments observed in marketable fruit yield varied between years, as plants in plastic mulch produced more marketable fruit than bare ground plants only in 2019 (Table 2.1, Figs. 2.1d, 2.2d). As mentioned earlier, the use of plastic mulch in vegetable systems provides numerous horticultural advantages (Jabran 2019). In cucurbit crops, mulch increases yield and plant biomass, lowers soil moisture evaporation, and improves irrigation efficiency (Conway et al. 1989, Kirnak and Demirtas 2006, Torres-Olivar et al. 2018). The benefits of plastic mulch may have had a compensatory effect in 2019, increasing yield to even exceed reductions resultant from increased squash bug pressure. Contrarily, even with reduced *A. tristis* pressure, bare ground zucchini produced similar marketable fruit yield to mulched plants in 2020, further suggesting a compensatory/beneficial effect of plastic mulch on yield. Disentangling relationships among insect pressure, plastic mulch, and yield has the added difficulty of research lacking a well-established relationship between squash bug pressure and yield. To date, research-based threshold values for adults, egg masses, and nymphs have not been firmly established. While we did find some differences in insect pressure, mulch, and marketable yield between treatments, our study did not include numbers of fruit discarded due to *A. tristis* damage. It is possible that mulched plants with large densities of squash bugs incurred greater fruit damage than those grown in bare ground with lower bug densities. In order to correctly assess yield loss from squash bug damage, additional fruit quality predictor variables like pollination success, presence/absence of microbial pathogens, and counts of other cucurbit insect pests are required. Future experimentation attempting to connect squash bug and its influence on damaged fruit numbers should include the aforementioned predictors to accurately elucidate how *A. tristis* specifically influences overall marketable yield in summer squash.

Despite many differences in methodology and experimental design, our study and Cartwright et al. (1990) identified similar patterns in squash bug presence and yield across different mulch treatments. Both studies observed more adults and egg masses on squash in plastic mulch versus bare soil plots, and inconsistent nymph presence among treatments between years. Although we demonstrated differences in yield between mulch treatments, methodological ambiguity in Cartwright et al. (1990) makes comparing yield between the two studies difficult. For example, they only recorded *total* yield in the second year of their study with no known differentiation between marketable and damaged fruit. Additionally, they offered no explanation of how or for what duration they conducted their yield collections and varied their squash planting dates and subsequent *A. tristis* sampling time frames dramatically between years. Overall, our study clarifies and expands the results described in Cartwright et al. (1990) by using uniform planting dates, increasing mulch treatment and sequential sampling replication, and including two years of marketable yield data.

When considering the implementation of plasticulture in summer squash production, growers should be mindful of the entire cucurbit pest complex, particularly which species are routinely economically destructive in their region. Despite the possible horticultural attributes provided by plastic mulch, our research conducted in southwest Virginia suggests plastic mulch can negatively impact squash bug management, especially in regions with high *A. tristis* pressure. Growers in such areas looking to minimize *A. tristis* presence may benefit from forgoing the use of plastic mulch, and instead, utilize a system that uses herbicide applications, cover crops, or other weed suppression tactics. On the other hand, growers in locations that experience significant pressure from cucumber beetles, aphids, or whiteflies can help protect their cucurbits from pest colonization with the repellency effects offered by reflective plastic mulch (Summers et al. 1995, Caldwell and Clark 1999, Vincent et al. 2003, Frank and Liburd 2005). Availability of essential farm equipment (e.g., plastic mulch layer) and added costs of specific plastic mulch colors (e.g., reflective, red, blue, etc.) will also inevitably impact whether a certain plasticulture system is implemented. In most cases, cucurbit growers will face a mixed assemblage of insect pests throughout the growing season, requiring them to conduct cost-benefit analyses of all possible cultural, biological, and chemical control strategies. Using the newly acquired knowledge put forth by this study, growers can more confidently decide if plasticulture fits within their squash bug management plans.

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Tables and Figures

Table 2.1: Estimated degrees of freedom, F statistics, and P values for the generalized linear mixed model describing the effects of mulch treatment, sampling week, and their interaction on *A. tristis* counts and marketable fruit/plant in Whitethorne, VA in 2019 and 2020.

Year	**Response variables**	**Fixed factors**	**df**	**F statistic**	***P* value**
2019	Adults	Mulch	3, 141.5	7.12	**0.0002**
		Sampling week	5, 139.5	8.23	< **0.0001**
		Mulch*Sampling week	15, 139.5	1.16	0.312
	Egg masses	Mulch	3, 141.5	10.95	< **0.0001**
		Sampling week	5, 139.5	21.03	< **0.0001**
		Mulch*Sampling week	15, 139.5	0.88	0.586
	Nymphs	Mulch	3, 141.5	1.27	0.287
		Sampling week	5, 139.5	17.06	< **0.0001**
		Mulch*Sampling week	15, 139.5	0.820	0.654
	Marketable fruit/plant	Mulch	3, 64.9	6.178	**0.0009**
		Sampling week	2, 66.2	4.893	**0.010**
		Mulch*Sampling week	6, 66.2	0.568	0.755
2020	Adults	Mulch	3, 164	8.65	< **0.0001**
		Sampling week	5, 164	2.48	**0.034**
		Mulch*Sampling week	15, 164	1.23	0.254
	Egg masses	Mulch	3, 164	12.58	< **0.0001**
		Sampling week	5, 164	19.64	< **0.0001**
		Mulch*Sampling week	15, 164	2.04	**0.015**
	Nymphs	Mulch	3, 164	5.90	**0.0008**
		Sampling week	5, 164	31.89	< **0.0001**
		Mulch*Sampling week	15, 164	1.47	0.122
	Marketable fruit/plant	Mulch	3, 80	4.66	**0.0047**
		Sampling week	2, 80	9.91	< **0.0001**
		Mulch*Sampling week	6, 80	3.00	**0.011**

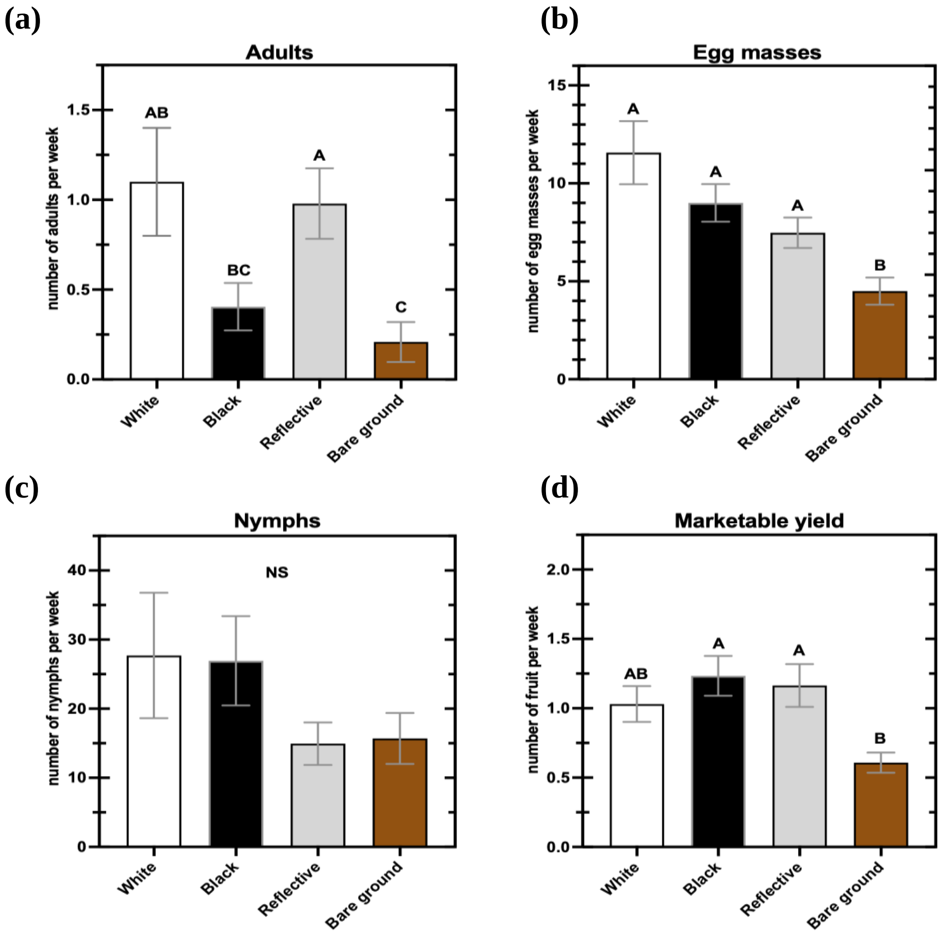

Figure 2.1: Mean counts of *A. tristis* **(a)** adults, **(b)** egg masses, and **(c)** nymphs per six-week sample period, and **(d)** mean marketable zucchini fruit produced per plant during the three-week harvest period, for different mulch treatments in Whitethorne, VA in 2019. Letters display significant differences between treatment groups (Tukey HSD, $P < 0.05$), 'NS' indicates no significant difference among mulch treatments, and bars show standard error.

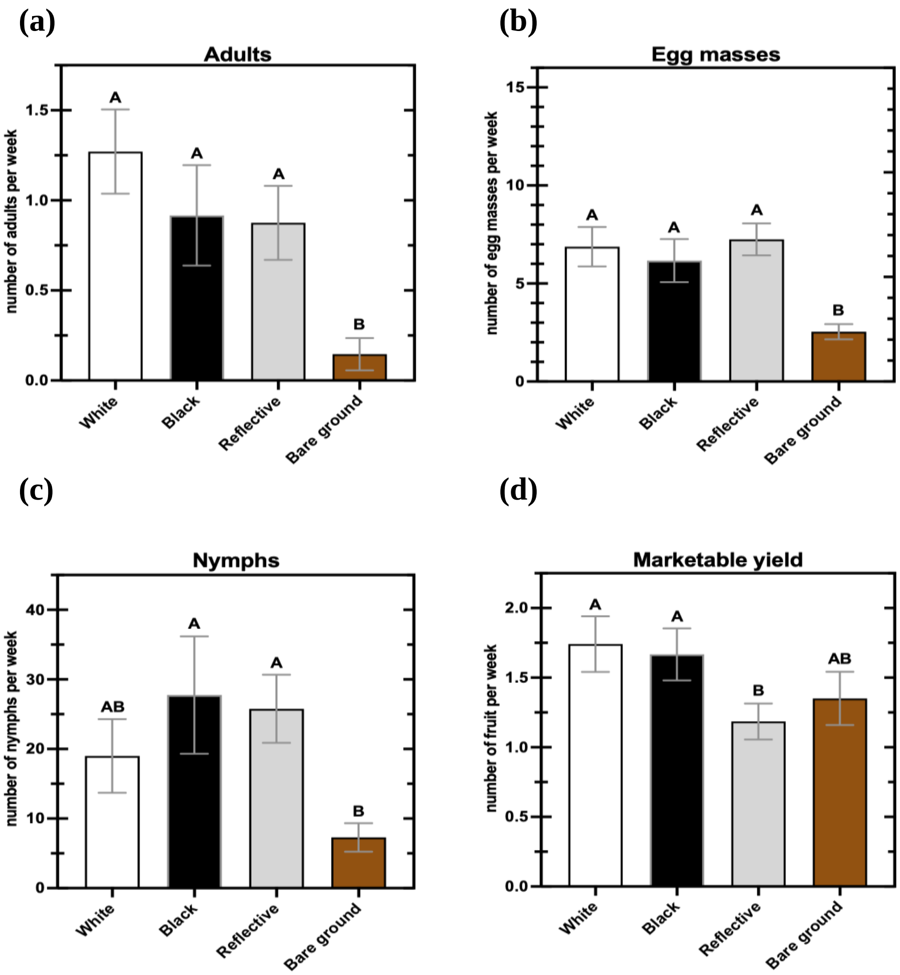

Figure 2.2: Mean counts of *A. tristis* **(a)** adults, **(b)** egg masses, and **(c)** nymphs per six-week sample period, and **(d)** mean marketable zucchini fruit produced per plant during the three-week harvest period, for different mulch treatments in Whitethorne, VA in 2020. Letters display significant differences between treatment groups (Tukey HSD, $P < 0.05$), and bars show standard error.

Chapter 3: Evaluating augmentative biological control for squash bug using releases of egg parasitoid, *Hadronotus pennsylvanicus*

Abstract

The squash bug, *Anasa tristis* (Hemiptera: Coreidae, is a serious pest of cucurbit crops across the US, especially within summer squash (*Cucurbita pepo*) systems. Conventional growers most commonly use broad spectrum insecticides to manage squash bugs, however organic growers lack these effective chemical tools and therefore rely on alternative management strategies. Biological control of squash bugs is largely understudied, specifically the potential of its natural enemy, *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae), as an augmentative biological control agent. For this reason, we performed field releases of *H. pennsylvanicus* on organic farms in southwest Virginia to test whether *A. tristis* egg parasitism would improve at parasitoid release sites. We chose organic farms growing summer squash as release sites and paired each site with a no-release site where no parasitoids were released. Parasitoids were reared in the lab and deployed as parasitized egg masses at a rate of 2-3 females wasps per plant in June 2020 and 2021. Following *H. pennsylvanicus* deployment, biweekly collections of squash bug eggs were conducted at release and non-release sites. In both years, we found greater parasitism rates of *A. tristis* eggs collected at Virginia release sites compared to no-release sites. While all eggs collected at release and no-release sites before parasitoid deployment displayed low levels of *H. pennsylvanicus* parasitism, eggs from release sites were significantly more parasitized than eggs from no-release sites within two weeks post-deployment. Our two-year study demonstrates that the releases of lab-reared *H. pennsylvanicus* can increase *A. tristis* egg

parasitism rates and subsequently decrease successful nymph hatch rates in early summer squash plantings.

Introduction

In the United States and other countries worldwide, several leaf-footed bug (Hemiptera: Coreidae) species are considered important agricultural pests within a diverse array of cropping systems (Froeschner 1988, Mitchell 2000). Particularly, coreid bugs in the genera *Anasa* and *Leptoglossus* pose significant threats to cucurbitaceous and solanaceous vegetables (Doughty et al. 2016, Braman and Westerfield 2020), as well as tree fruit crops like citrus, almond, pistachio, pecan, and pine nuts (Koerber 1963, Daane et al. 2005, Rivero and Hernández 2009, Xiao and Fadamiro 2010, Joyce et al. 2017). Current management strategies for pestiferous coreids focus mainly on repeated applications of broad-spectrum insecticides (e.g., organophosphates, carbamates, pyrethroids, and neonicotinoids). Further, broad-spectrum insecticides pose additional non-target risks on beneficial insect species, which may diminish vital pollination services for certain crops (Van der Sluijs et al. 2013, Willis-Chan and Raine 2021). Development of effective non-chemical control measures are needed to diminish the reliance on insecticide use and improve the sustainability of coreid pest management.

A major pest to cucurbits across North America, the squash bug, *Anasa tristis* (De Geer), is a prime example of a coreid species that warrants novel management considerations. *Anasa tristis* is a cucurbit specialist that prefers hosts in the genus *Cucurbita*, specifically summer squash, zucchini, and pumpkin varieties (*Cucurbita pepo* L.) (Bonjour et al. 1993). Squash bug adults and nymphs use their piercing-sucking mouthparts to feed on vascular tissues of the leaves, stems, and fruit. This feeding damage is variable, potentially leading to localized leaf necrosis, phloem and xylem disruption, fruit rot, marketable yield loss, and plant/seedling death

(Woodson and Fargo 1991, Neal 1993, Palumbo et al. 1993). Adult squash bugs are also known to transmit the pathogenic bacterium *Serratia marcescens* (Bizio), which causes cucurbit yellow vine disease in various cucurbit cultivars (Bruton et al. 2003).

Conventional growers depend largely on insecticide applications to successfully manage *A. tristis* and other cucurbit insect pests throughout the growing season (Kuhar et al. 2005). While many insecticide products are efficacious against *A. tristis*, few are approved for organic use, leaving organic growers with little to no effective means of chemical control. For this reason, organic growers employ alternative nonchemical management strategies to thwart squash bug pressure, such as covering cucurbit seedlings with floating row covers that physically exclude insect pests (Cartwright et al. 1990). Although row covers can protect young cucurbit plants from initial *A. tristis* infestations, they can only be used during the first few weeks of the season and must be removed upon flowering, as cucurbits need insect pollination to produce fruit. In addition, the use of row covers is manual labor intensive and is only feasible for smaller plantings. The effects of different plastic mulch colors on *A. tristis* abundance have also been studied as a possible alternative management tactic (Boyle et al. 2022). No effect of plastic mulch color was observed, but squash bugs were more abundant on summer squash plants grown in plastic mulch compared to plants grown on bare ground.

Despite its success in many heteropteran insect pest management programs, biological control has received little attention as an applicable control method for squash bug (Abram et al. 2020). The eggs of *Anasa* and *Leptoglossus* species are commonly parasitized by hymenopteran parasitoids (Masner 1983, Nechols et al. 1989, Decker and Yeargan 2008, Cornelius et al. 2016). The most important egg parasitoid, *Hadronotus pennsylvanicus* (previously named *Gryon pennsylvanicum*) (Ashmead) (Hymenoptera: Scelionidae), is consistently found parasitizing several coreid species in a variety of agricultural systems throughout North America (Mitchell and Mitchell 1986, Yasuda 1998, Bates and Borden 2004, Maltese et al. 2012, Cornelius et al. 2018). Field surveys of *A. tristis* egg masses in the eastern US suggest the wasp can parasitize high percentages of eggs (Cornelius et al. 2016, Wilson and Kuhar 2017). Moreover, *H. pennsylvanicus* females are long-lived and exhibit high fecundity rates with a strongly female-biased sex ratio in laboratory studies involving both *Anasa* and *Leptoglossus* host eggs (Nechols et al. 1989, Sabbatini Peverieri et al. 2012, Straser et al. 2022). Because of these desirable life history traits, *H. pennsylvanicus* is a strong candidate for potential introduction as a classical biological control agent for invasive western conifer seed bug, *Leptoglossus occidentalis* (Heidemann), in Europe (Roversi et al. 2013).

Potential also exists for *H. pennsylvanicus* as an augmentative biological control agent for *A. tristis*, yet only one previous study has attempted to test this idea. Olson et al. (1996) evaluated the impact of releasing lab-reared *H. pennsylvanicus* females on squash bug densities and yield in replicated small pumpkin plots grown in Kansas. Researchers released one female wasp per 17 *A. tristis* eggs, surveyed for five consecutive weeks, and found significantly fewer squash bugs and greater fruit weight in pumpkin plots where parasitoids were released compared to control plots. However, the study did not observe any *A. tristis* egg parasitism until late in the growing season, about five weeks following the initial release date, and did not compare egg parasitism rates between release and control plots. High rates of late season *H. pennsylvanicus* parasitism are common in field collected *A. tristis* eggs, while eggs laid on cucurbits early in the season have low rates of parasitism (Decker and Yeargan 2008, Cornelius et al. 2016, Wilson and Kuhar 2017). Squash bug infestations are generally more severe at the beginning of the growing season, as earlier plantings (i.e., May-June) experience higher levels of adult colonization, oviposition, and reduced yields compared to later plantings (i.e., July-August) (Fargo et al. 1988, Palumbo et al. 1991). With this understanding in mind, it is imperative that squash bug management strategies focus on minimizing populations early in the season.

Geographic regions with substantial cucurbit production, large annual *A. tristis* populations, and low natural *H. pennsylvanicus* parasitism rates would serve as optimal locations to examine the potential of a *H. pennsylvanicus* augmentative release program. Southeastern, or Tidewater, Virginia fits these characteristics. State-wide *A. tristis* egg mass surveys in 2014 and 2015 found only 6.7% of collected eggs in the region were parasitized compared with >60% parasitism in other regions of the state (Wilson and Kuhar, 2017). Here, we tested early season releases of *H. pennsylvanicus* in summer squash plantings for their potential to increase squash bug egg parasitism in Tidewater Virginia and other regions in the southeastern US. This study also sought to create a more field-applicable method of *H. pennsylvanicus* deployment that requires only one single release and minimal manual labor.

Methods

Insects

A laboratory colony of *A. tristis* was initially established from individuals collected from organic summer squash fields in southwestern Virginia (Whitethorne, VA, USA) between June and August 2019 and 2020. *Anasa tristis* adults and nymphs were reared in growth chambers (25±2°C, 70±10% relative humidity, 16L:8d photoperiod) and held in separate 30.5×30.5×30.5 cm mesh cages (BioQuip Products, Inc.). Bugs were given greenhouse-grown summer squash plants and organic fruit (*C. pepo*) weekly. Plants served not only as a food source, but also provided adults an optimal substrate for oviposition. Egg masses were collected daily and used to rear subsequent *A. tristis* generations and as hosts for the *H. pennsylvanicus* colony.

Our *H. pennsylvanicus* colony was started from parasitized *A. tristis* egg masses collected in organic summer squash (Whitethorne, VA) in 2019 and 2020. To rear new *H. pennsylvanicus* generations, 2-3 mated female wasps were exposed to *A. tristis* egg masses (24-48 h old) in 50 ml clear plastic snap cap vials (United States Plastic Corp.) for three days, and then removed. Parasitized egg masses were checked daily for adult emergence. Newly emerged adults were removed from plastic vials and placed in 1 L clear plastic containers (United States Plastic Corp., Lima, Ohio, USA). A new 1L colony container was used each week to keep similarly aged wasps together. Honey was provided as needed to 1L colony containers and 50 ml vials. All *H. pennsylvanicus* colony containers, vials, and parasitized egg masses were maintained in growth chambers (25±2°C, 70±10% relative humidity, 16L:8d photoperiod).

Experimental Field Sites

Small scale organic vegetable farms were chosen as experimental *H. pennsylvanicus* release sites using specific criteria. Farms needed to: (1) possess a summer squash (*C. pepo)* planting of at least 100 flowering plants, (2) refrain from applying insecticides to squash plants, and (3) experience consistent yearly pest pressure from squash bugs. Each release site was paired with at least one no-release site within the same geographic location. No-release sites were also required to meet the experimental criteria.

In 2020, *H. pennsylvanicus* were released at two organic farms in southeastern Virginia (Fritillary Farm, Suffolk, VA, USA (36.693°, -76.664°); Seafield Farm, Cape Charles, VA, USA (37.249°, -75.936°)). Two Virginia Tech Agricultural Research and Extension Centers (AREC) (Hampton Roads AREC, Virginia Beach, VA, US (36.892°, -76.177°); Eastern Shore AREC, Painter, VA, USA (37.583°, -75.821°)) were used as no-release sites. In both years, release and no-release site pairs were Fritillary Farm - Hampton Roads AREC and Seafield Farm – Eastern Shore AREC. In addition to the 2020 release sites, *H. pennsylvanicus* was released at two additional locations in 2021; another within the Virginia Eastern Shore area (Copper Cricket Farm; Machipongo, VA, USA (37.390°, -75.949°)) and one in western North Carolina (Crouching Hippo Farm; Claremont, NC, USA (35.664°, -81.162°)). Both 2021 Eastern Shore VA release sites were paired with the same no-release site (Eastern Shore AREC). Living Web Farm (Mills River, NC, USA (35.387°, -82.586°)) was used as a no-release site in 2021. We planned to release *H. pennsylvanicus* at a site near Athens, Georgia, USA in June 2021, however the parasitoids failed to emerge from deployed egg masses. Still, *A. tristis* egg mass collections were conducted at twelve separate organic farms within eastern Georgia and southwestern South Carolina to obtain natural parasitism rates for the area.

Augmentative Release Procedure

In 2020, lab reared *H. pennsylvanicus* were released at Fritillary Farm and Seafield Farm on 28 June. In 2021, parasitoids were released at Fritillary Farm, Seafield Farm, and Copper Cricket Farm on 14 June, and Crouching Hippo Farm on 1 July. Lab-reared parasitoid development time ranged from 22-26 days, so *A. tristis* egg masses were exposed to female wasps 21-22 days before each field deployment date. Successful parasitism of egg masses allocated for each release site was confirmed 10-14 days after exposure to wasps. Within this duration, all unparasitized eggs resulting in hatched nymphs would be observed in the lab and accounted for in the final number of deployed parasitized eggs per site. Parasitized egg masses were deployed in opaque plastic containers attached to a six-ft wooden stake in a central location within the summer squash planting (Fig. 3.1). A rectangular plastic cover was placed above the deployment containers to protect the parasitoids from direct sunlight. Small holes (0.31 cm diameter) were made along the bottom of the containers to allow the parasitoids to exit. A release rate of 2-3 female wasps per plant was estimated using laboratory rearing data and a squash plant count range of 100-150 plants. Among egg masses observed as parasitized in the lab, roughly 80% of eggs resulted in successful *H. pennsylvanicus* adult emergence with a 6.27:1 female to male sex ratio. Using these data, we calculated 400-450 parasitized eggs would yield our desired female parasitoid release rate, and this was used as the range of total eggs deployed for all releases. Deployed egg masses remained in the field for seven days to ensure all wasps had emerged and exited the containers.

Data Collection

In 2020 and 2021, an initial pre-release *A. tristis* egg collection was conducted during the week of the release date to determine baseline parasitism rates at each research site. Roughly two weeks following the release date, we began collecting *A. tristis* egg masses and performed biweekly collections until squash plants senesced and/or squash bug eggs were no longer available. In both 2020 and 2021, there were between three and six separate collection dates per site. On each collection date, squash bug egg masses were removed from plants and placed in petri dishes to be reared out in the laboratory at 25°C and 50-70% RH. Four weeks post-collection, *A. tristis* eggs were analyzed and all possible outcomes were counted for each egg mass. These outcomes included emerged and unemerged *H. pennsylvanicus* adults, hatched *A. tristis* nymphs, undeveloped parasitoids and nymphs, predated eggs, and non- *Hadronotus* parasitism. In 2020, specific egg mass outcomes for Seafield Farm and Eastern Shore AREC were not recorded and collected egg masses from these sites were labeled as either "parasitized" (i.e., emergence of at least one parasitoid adult) or “unparasitized” (i.e., no parasitoid emergence). We were able to record specific egg mass outcomes from all sites in 2021.

Egg mass collection data from the NC release site was omitted from statistical analysis since fewer than ten total egg masses were collected at the NC release site following *H. pennsylvanicus* deployment. Georgia-South Carolina data were also omitted from statistical tests since collections were not repeated at each site per sampling date. Specifically, eggs were collected at four sites between 25-28 May, two sites on 3 June, three sites between 23-24 June, and three sites between 8-12 July.

Statistical Analysis

Hadronotus pennsylvanicus parasitism rates (# emerged & unemerged adults)/ # eggs per mass) and *A. tristis* hatch rates (# hatched nymphs/ # eggs per mass) were calculated for all egg masses collected at each site. Collected egg mass data from our Virginia sites were grouped into release and no-release treatments. Since specific outcomes were not available for Seafield and Eastern Shore AREC sites in 2020, the ratio of parasitized: unparasitized egg masses collected were analyzed using a Fisher’s Exact Test of Independence (α=0.05) to compare egg parasitism between our release and no-release sites. In 2021, Virginia squash bug egg mass outcome data from the first three collection dates (28 June, 12 July, and 28 July) following parasitoid deployment were pooled into release and no-release groups per sampling date. Mean parasitism rates from each sample date were compared between release and no-release groups using student’s t test (α=0.05). All statistical analyses were performed with JMP Pro 16.0.0 Statistical Software for Mac (SAS Institute Inc., Cary, NC).

Results

2020 Virginia Release and No-Release Sites

Pre-release egg mass collections at both release sites and Eastern Shore AREC no-release site showed nearly zero parasitism (Table 3.1). Although the Hampton Roads AREC no-release site had 43% of collected egg masses parasitized on the release date (Table 3.1, Fig. 3.2a), the actual mean parasitism rate of all eggs collected from the site was 21% (Fig. 3.2b). Two, four, and six weeks after *H. pennsylvanicus* deployment, there were significant differences in parasitized: unparasitized egg mass ratios between release and no-release sites. A greater proportion of collected egg masses from Fritillary and Seafield release sites were parasitized compared to Hampton Roads AREC and Eastern Shore AREC no release sites ($P < 0.0001$; Table 3.1).

Two weeks following *H. pennsylvanicus* release, the parasitism rate of eggs from the Fritillary Farm release site (0.68, n=993 eggs), was double the rate of Hampton Roads AREC site (0.34, n=1019 eggs) (Fig. 3.2b). Fritillary Farm and Hampton Roads AREC sites showed equally high rates of parasitism of eggs collected at four (0.76 vs. 0.83) and six weeks (0.96 vs. 0.95) post-release. Egg parasitism was low throughout the entire sample period at the Eastern Shore AREC no-release site, as only 5 of 121 (4.1%) egg masses were parasitized by *H. pennsylvanicus* (Table 3.1, Fig. 3.2a). This rate of egg mass parasitism was markedly lower than its nearby release site, Seafield Farm, where we observed parasitism in 65 of 104 (62.5%) egg masses collected.

2021 Virginia Release and No-Release Sites

All sites displayed low parasitism rates (< 0.06 total eggs collected/site) on the *H. pennsylvanicus* deployment date (Fig. 3.3a). Two weeks following wasp deployment, rates of egg parasitism rose considerably at Fritillary (0.73), Seafield (0.55), and Copper Cricket (0.41) release sites, while remaining low at Hampton Roads AREC (0.09) and Eastern Shore AREC no-release sites (0.00). Parasitism rates remained above 0.55 at the three release sites on all subsequent collection dates (Fig. 3.3a). Squash bug egg parasitism at the two no-release sites displayed stark similarities to our 2020 data. Parasitism increased gradually at Hampton Roads AREC, reaching 0.83 and 0.92 at six and eight weeks, respectively, following the release date (Fig. 3.3b). Likewise, no egg parasitism was observed at Eastern Shore AREC until late July and stayed low through the remainder of the collection period.

We observed significantly greater parasitism rates at release sites two- ($t_{218} = 13.5$, $P < 0.0001$), four- ($t_{203} = 12.4$, $P < 0.0001$), and six-($t_{174} = 6.55$, $P < 0.0001$) weeks post *H. pennsylvanicus* deployment (Fig. 3.3b). Because the cucurbit planting at Fritillary release site suffered high plant mortality from microbial pathogens by late July, we were unable to collect *A. tristis* eggs following the 3rd post release collection date. Summer squash plants at no-release sites senesced 10 weeks after wasp deployment, so no *A. tristis* eggs were collected on the 5th collection date.

2021 North Carolina Sites

Our North Carolina release site displayed low *A. tristis* egg mass density following parasitoid deployment; only ten egg masses were found and collected from this location. Due to this low sample size, we were unable to statistically analyze the release location data. Squash bug eggs collected at the Mills River no-release site showed moderate levels of parasitism during the six-week collection period, peaking at 0.61 in the 4th week of July (Fig. 3.4a). The Mills River no-release site was the only location in the study to exhibit decreases in parasitism rates from July to August.

2021 Georgia and South Carolina Sites

Egg collections at twelve sites in eastern Georgia/southwestern South Carolina displayed moderate levels of parasitism by wild *H. pennsylvanicus* (Fig. 3.4b). Early season parasitism rates were greater than any other location in the study, as 33% and 22% of eggs collected in the 4th week of May and 1st week of June, respectively, were successfully parasitized by *H. pennsylvanicus*. Parasitism rates in GA-SC peaked on the last collection date at 53% during the second week in July.

Discussion

To our knowledge, this study is the first to augmentatively release *H. pennsylvanicus* in organic cucurbit systems at a whole farm level. In both 2020 and 2021, we found greater parasitism rates of *A. tristis* eggs collected at Virginia release sites compared to no-release sites (Table 3.1; Fig. 3.3b). While all eggs collected at release and no-release sites before parasitoid deployment displayed low levels of *H. pennsylvanicus* parasitism, eggs from VA release sites were significantly more parasitized than eggs from no-release sites within two weeks post-deployment. These findings demonstrate that releases of lab-reared *H. pennsylvanicus* can increase *A. tristis* egg parasitism rates and subsequently decrease successful nymph hatch rates in early summer squash plantings. The significant increase in squash bug egg parasitism we observed in just two weeks following field deployment contrasts that of Olson et al. (1996) who did not observe any parasitized eggs until five weeks after initial wasp deployment.

Also in both years, the Hampton Roads AREC no-release site achieved high natural parasitism rates by the end of July, similar to its paired release site (Table 3.1; Figs. 3.2, 3.3). Although high levels of parasitism at either Virginia no-release site were not expected, the gradual increase in *H. pennsylvanicus* parasitism from June to the end of July at Hampton Roads AREC aligns with previous egg survey data conducted elsewhere in Virginia (Wilson and Kuhar 2017) and throughout the US (Schell 1943, Decker and Yeargan 2008, Cornelius et al. 2016). Low early season parasitism and high late season parasitism is a common pattern shared among many naturally occurring insect host-parasitoid relationships (Heimpel and Mills 2017). From a pest management perspective, early season control of a target pest is paramount for keeping pest numbers in check and minimizing overall crop damage. Therefore, cucurbit growers cannot confidently rely on biological control of squash bug eggs by naturally occurring *H. pennsylvanicus* populations to reduce nymph densities in their crop. Squash bug adult oviposition rates and egg mass densities are greatest in the first cucurbit plantings of the season (Palumbo et al. 1991). In most temperate climatic areas of the US, initial plantings are established in May following the last frost date, and peak *A. tristis* egg mass densities in these early plantings occurs in mid to late June (Nechols 1987; Fargo et al. 1988) The high egg parasitism we observed within two weeks of the augmentative release dates suggests that the presence of large numbers of *H. pennsylvanicus* during the peak *A. tristis* oviposition period can have a significant suppressive effect on growing squash bug populations. Additional studies replicating our parasitoid releases would confirm the potential decrease in squash bug nymph densities by including weekly *A. tristis* nymph surveys following *H. pennsylvanicus* deployment.

As with any biological control program, the timing of the biocontrol agent release is critical for successful target pest suppression (Heimpel and Mills 2017, Mason et al. 2021).

When considering the release of a specialist egg parasitoid biocontrol agent, the consistent presence of suitable hosts (i.e., eggs) is vital for initial site establishment and sustained population growth. In our study, summer squash plants at all release sites were surveyed to confirm the presence of squash bug eggs and adults before deploying *H. pennsylvanicus* on 28 June 2020 and 14 June 2021 in Virginia. Releasing the parasitoid two weeks earlier in 2021 resulted in an earlier, extended time frame of high *A. tristis* egg parasitism (Fig. 3.3). Augmentative releases of *H. pennsylvanicus* may maximize their value if parasitoids are deployed when overwintered squash bug adults begin laying eggs in the first cucurbit planting of the year. Since we did not conduct squash bug adult and egg mass surveys prior to the week of parasitoid deployment, we do not know when adults colonized and began laying eggs at release and no-release sites. If sites possessed large densities of *A. tristis* egg masses at the beginning of June, high rates of egg parasitism could have been achieved sooner by deploying *H. pennsylvanicus* earlier in the season.

Successful *H. pennsylvanicus* field emergence from the deployed parasitized egg masses was achieved in five out of our six separate releases. Through the utilization of our parasitoid lab colony’s mean development time in *A. tristis* eggs, we were able to deploy *H. pennsylvanicus* at release sites near, or in certain cases, on the exact date of initial parasitoid adult eclosion. Other egg parasitoids, such as *Trichogramma* and *Trissolcus* species, have been successfully deployed within their lab-reared hosts before adult eclosion in the field (Correa-Ferreira and Moscardi 1995; Smith 1996; Wright and Diez 2011). Since no previous study has attempted to release *H. pennsylvanicus* as parasitized *A. tristis* egg masses, our results are the first to observe the method’s success and show promise for future implementation by other biological control programs targeting coreid pests. For instance, *H. pennsylvanicus* is currently being evaluated as a classical biological control agent for the invasive western conifer seed bug (*L. occidentalis*) in Europe (Roversi et al. 2013, Lesieur et al. 2019). If approved for release, our parasitoid deployment procedure can be adopted confidently with knowledge gained from our study.

Our lone incidence of unsuccessful *H. pennsylvanicus* emergence occurred with parasitized egg masses that were shipped to Athens, GA from Blacksburg, VA in 2021. Using the same shipping protocol, *H. pennsylvanicus* adults emerged successfully after being shipped to Mills River, NC. Although we shipped the egg masses in Styrofoam coolers with cold packs, we are unsure if the parasitoids were exposed to lethal high temperatures as they traveled south to Georgia during the last week in June. Further investigation is needed to determine how temperature fluctuations during the shipping process affect late-stage *H. pennsylvanicus* adult emergence. Future studies should not only identify the actual temperature ranges experienced for different packaging and shipping methods, but also examine the effects of these temperatures on *H. pennsylvanicus* during the latter portion of the parasitoid's development. This work would improve *H. pennsylvanicus* emergence success rates following the shipping process, and ultimately allow researchers to properly gauge the feasibility of shipping parasitized egg masses to geographic locations with differing climatic conditions.

Another crucial factor in developing a new biological control program is identifying the agent release rate and number of deployments necessary to attain sufficient management of the target pest. The high egg parasitism sustained at release sites for six to eight weeks post *H. pennsylvanicus* deployment supports our single deployment of an estimated 2-3 female wasps per plant significantly improved season-long squash bug egg parasitism (Table 3.1; Figs. 3.2, 3.3). This understanding is especially noteworthy for cucurbit growers in areas where wild populations of *H. pennsylvanicus* are low and *A. tristis* egg parasitism rarely occurs even late in the growing season (e.g., Eastern Shore, VA). A single parasitoid deployment early in the season is also likely to require less labor, time, and may be more cost effective overall compared to multiple deployments per location.

The cost of any insect pest management strategy is a major driver of whether a particular strategy is implemented by growers. Broad-spectrum insecticide applications are widely used to control squash bugs and other pests such as cucumber beetles and squash vine borer in conventional cucurbit systems, because they are both effective at reducing pest densities and are low cost to purchase and implement (Doughty et al. 2016). Olson et al. (1996) conducted a comparative economic analysis of squash bug control between repeated applications of the pyrethroid esfenvalerate and weekly *H. pennsylvanicus* releases in small pumpkin plots. Not surprisingly, pyrethroid applications corresponded to greater profit margins versus those estimated for *H. pennsylvanicus* releases. However, their repeated parasitoid releases improved crop profit margins by over double when compared to no management inputs. Profit margins will inevitably vary based on the targeted coreid species and specific cropping system in which *H. pennsylvanicus* is released. Parasitoid releases in high value crops damaged by coreid pests (e.g., zucchini, pine nuts, citrus) may be more financially worthwhile than in lower value crops like field pumpkin. Future studies performing *H. pennsylvanicus* releases targeting different coreid hosts in markedly differing crop systems (e.g., cucurbits vs. tree nuts) would further elucidate the versatility and cost-effectiveness of this promising biological control agent.

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Tables and Figures

Table 3.1: Ratio of parasitized: unparasitized *A. tristis* egg masses collected at each release and no-release site in 2020. Combined ratios (bold values) were compared between release and no release treatments (Fishers Exact Test of Independence). Asterisks indicate significantly larger parasitized: unparasitized egg mass ratio (*P < 0.01, *** P < 0.0001) per sample date.

Treatment	Region	Site	29 Jun	15 Jul	28 Jul	8 Aug
Release	Tidewater	Fritillary	1:20	41:11	67:7	55:1
	Eastern Shore	Seafield	0:15	17:13	38:3	10:8
			1:35	**58:24 *****	**105:10 *****	**65:9 *****
No-Release	Tidewater	Hampton Roads AREC	15:20	22:38	28:2	11:1
	Eastern Shore	E. Shore AREC	0:25	0:18	1:33	4:40
			15:35 *	**22:56**	**29:33**	**15:41**

Figure 3.1: Hadronotus pennsylvanicus field deployment containers staked within the summer squash planting at Fritillary Farm (Suffolk, VA) in 2020.

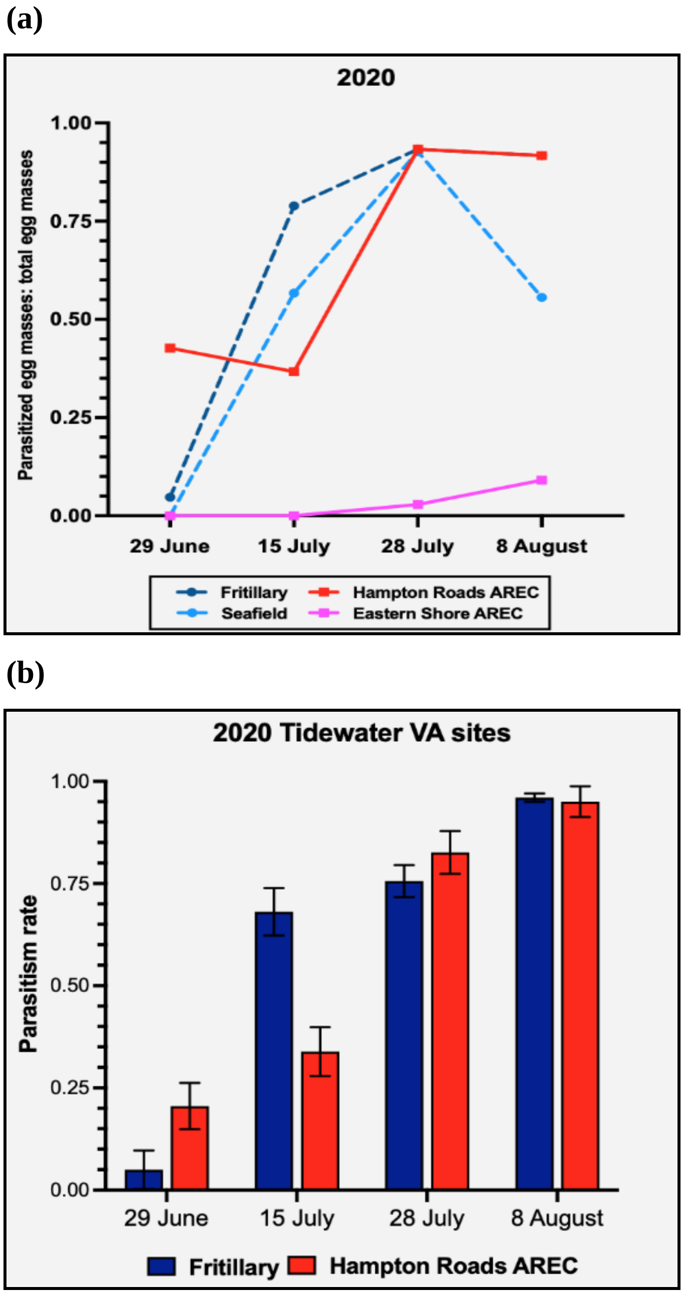

Figure 3.2: **(a)** Proportion of parasitized *A. tristis* egg masses: total egg masses collected at release (dotted lines) and no-release (solid lines) sites beginning on the parasitoid release date in 2020, and **(b)** mean *A. tristis* egg parasitism rates for eggs collected at Tidewater release (Fritillary) and no-release (Hampton Roads AREC) sites in 2020 beginning on *H. pennsylvanicus* release date. Bars display mean standard error.

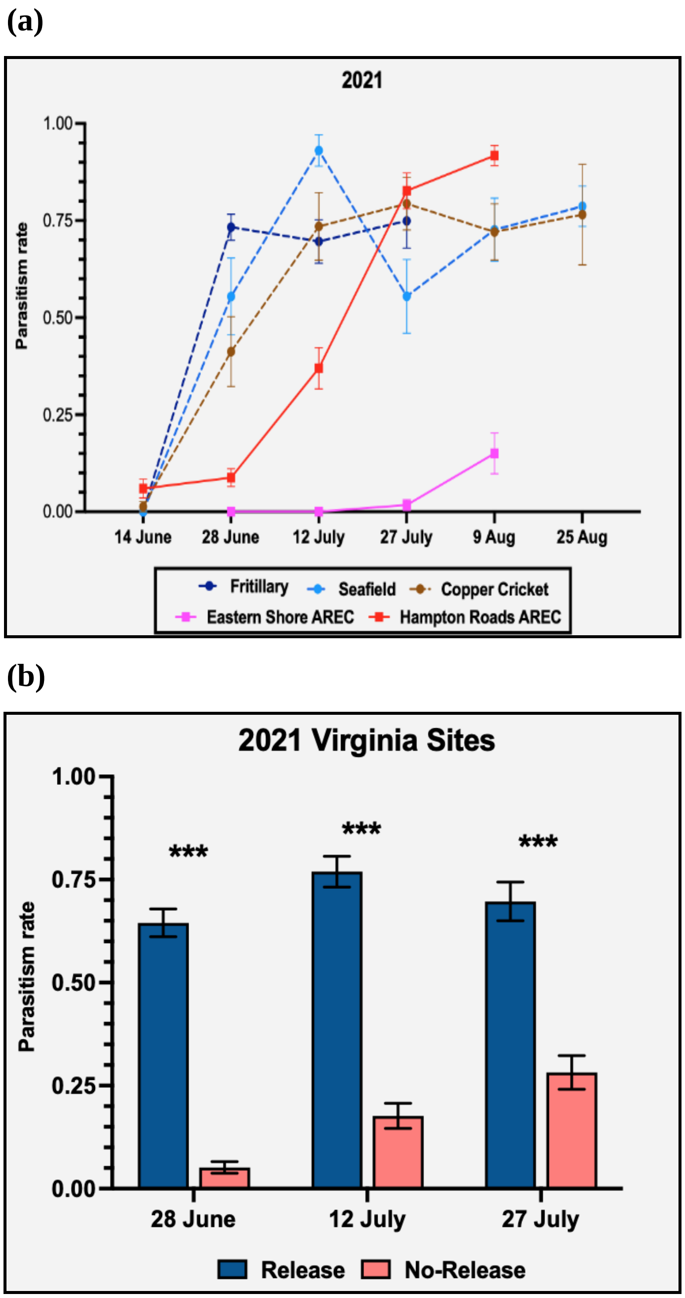

Figure 3.3: **(a)** Mean parasitism rates of *A. tristis* eggs collected biweekly at Virginia release (dotted lines) and no-release sites (solid lines) beginning on the parasitoid release date in 2021, and **(b)** combined mean parasitism rates of *A. tristis* eggs collected at Virginia release and no-release sites in 2021. Asterisks indicate significant differences between release and no-release groups per sample date (Student t test, *** P < 0.0001). Bars display mean standard error.

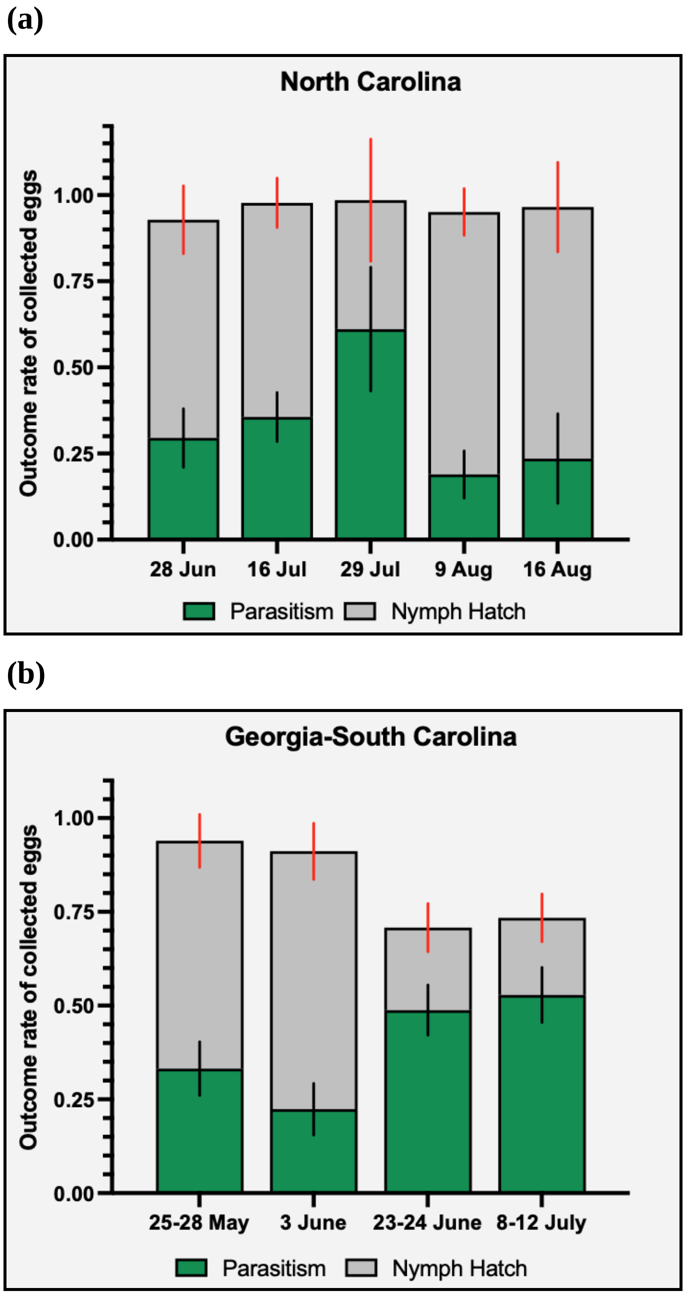

Figure 3.4: 2021 mean *H. pennsylvanicus* parasitism rate and mean *A. tristis* nymph hatch rate of eggs collected at **(a)** Living Web Farm (Mills River, NC) no-release site, and at **(b)** twelve separate sites in eastern Georgia and southwestern South Carolina. Bars display mean standard error of parasitism (black) and nymph hatch (red) rates.

Chapter 4: Relationships between squash bug life stages and marketable yield in summer squash

Abstract

The squash bug, *Anasa tristis* (Hemiptera: Coreidae), is a serious pest of cucurbit crops across the continental US, especially in summer squash (*Cucurbita pepo*) grown in Virginia. Using piercing sucking mouthparts, squash bug feeds on all above-ground portions of its host plant, often leading to leaf necrosis, marketable fruit loss, and even plant death. Although *A. tristis* nymphs contribute to much of the squash damage sustained throughout the growing season, current management actions are based on loosely established thresholds for only adult and egg mass life stages. Further, the nymphs are the most vulnerable stage to insecticides to insecticides, and therefore are an important target for effective chemical IPM strategies. For this reason, our goal was to reevaluate the relationship between squash bug life stages and marketable summer squash yield in Virginia. In 2020 and 2021, we performed weekly egg mass, nymph, and adult counts for six to eight weeks in squash fields of at least 100 plants. Plants were randomly labeled as either "managed" plants (all egg mass and nymph stages were manually removed) or 'infested' plants (no *A. tristis* egg masses or nymphs *tristis* removed). In 2021, we also performed visual presence/absence surveys for squash bug egg parasitoid *Hadronotus pennsylvanicus* Ashmead (Hymenoptera: Scelionidae) on infested plants and egg parasitism surveys on managed plants. Marketable and damaged fruit were also collected from each plant three times per week for three weeks. In both years, managed plants produced significantly more marketable zucchini and fewer squash bug-damaged fruit. Regression analyses found accumulated adult and nymph counts per plant to have significant negative correlations with the number marketable fruit produced per plant. However, nymphal counts displayed a much

stronger relationship with marketable fruit than did adult counts. *Hadronotus pennsylvanicus* was visually observed on infested plants each sampling week of the study, but high *A. tristis* egg parasitism rates were not recorded until the latter half of the sampling period. By introducing novel experimental design and sampling methodologies, our two-year study sheds new light on the relationship between *A. tristis* densities and marketable summer squash yield and offers a strong basis for which more reliable squash bug thresholds can be elucidated.

Introduction

Since its introduction by Stern et al. (1959), the economic injury level (EIL) concept has been the cornerstone of integrated pest management (IPM). An amalgam of three basic elements; economic damage, economic injury level, and economic threshold, the EIL concept connects a pest's biological and ecological traits to the pest's impact on the value of a particular commodity and the costs of enacting pest management strategies. By formal definition, the EIL is the lowest pest population density that will cause economic damage (i.e., amount of injury justifying the cost of control), while the term economic threshold describes a pest density that necessitates the initiation of control measures to prevent the pest population from reaching the EIL (Pedigo 1996). When applied to agricultural pests, determinant variables such as control costs, crop market value, amount of crop injury per pest unit, and crop response to pest injury, are used to mathematically model the EIL for a given pest and crop system. Although most IPM specialists agree that the EIL concept provides a pragmatic approach to making pest management decisions, they also realize the challenges with determining and interpreting EILs (Pedigo et al. 1986). There is concern over the quantification of determinant variables used to establish EILs, as arguments suggest they are broad oversimplifications of complex biological processes. These issues and limitations of the EIL concept, referred to as "conceptual fatigue" by Poston et al.

(1983), need to be addressed by concentrating research efforts on the specific pest-crop systems for which EIL and economic threshold ambiguity is most apparent.

One noteworthy agricultural pest and crop system that demands additional EIL and economic threshold considerations is the squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), which attacks a variety of crops in the family Cucurbitaceae grown across the continental US, but prefers *Cucurbita pepo* L. cultivars like summer squash, zucchini, and pumpkin (Bonjour et al. 1991, 1993). Both *A. tristis* adults and nymphs use their piercing-sucking mouthparts to feed on vascular tissues of the leaves, stems, and fruit, causing a wide array of damage (Doughty et al. 2016). Cucurbit host plant response to this feeding includes leaf necrosis, phloem and xylem disruption, fruit rot, marketable yield loss, and seedling death (Woodson and Fargo 1991, Neal 1993, Palumbo et al. 1993). Further, adult feeding is also known to transmit the pathogenic bacterium *Serratia marcescens* (Bizio), which causes cucurbit yellow vine disease in several cucurbit cultivars (Bruton et al. 2003).

In most growing regions, squash bug imposes season-long pest pressure on summer squash (Fargo et al. 1988). Overwintering adults emerge in spring and colonize recently established *C. pepo* (or other cucurbit) fields. Upon host colonization, female bugs begin feeding and ovipositing on young plants. It is at this point in the growing season when summer squash plants are most vulnerable to adult and early instar feeding-associated mortality, potentially leading to stand losses and delayed plant maturity (Woodson and Fargo 1991). As summer squash plants mature and set fruit (typically ~ 30 days post-germination), large infestations of *A. tristis* nymphs become more significant threats to developing fruits than are adults. If left unchecked, high nymphal densities can overwhelm fruits as nymphs aggregate and feed on areas of the plant near the crown (Palumbo et al. 1991a). Surface blemishes and scarring on the fruit caused by *A. tristis* feeding decrease the marketability of the harvested fruit and oftentimes render fruit completely unmarketable (USDA 2016).

Currently, *A. tristis* management programs are anything but *integrated*. Most growers rely exclusively on prophylactic applications of broad-spectrum insecticides to mitigate *A. tristis* in their squash (Doughty et al. 2016). Because of their efficacy in reducing numbers of squash bug and other serious co-occurring cucurbit insect pests (e.g., cucumber beetles) and their low cost, pyrethroids and neonicotinoids are commonly applied throughout the growing season (McLeod et al. 2003, Kuhar et al. 2005, Abney et al. 2011). Unfortunately, the detrimental effects these broad-spectrum insecticides have on pollinator and natural enemy species are well documented (Desneux et al. 2007, Van der Sluijis et al. 2013, Willis et al. 2021), and are particularly concerning within *C. pepo* systems, which require pollination by bees to produce fruit. In addition, arthropod natural enemies (predators and parasitoids) provide substantial biological control of pests including *A. tristis* (Olson et al. 1996, Cornelius et al. 2016), and are likely negatively impacted by indiscriminate and frequent insecticide applications with no regard for actual pest density. Specifically, the egg parasitoid *Hadronotus pennsylvanicus* (formally *Gryon pennsylvanicum*) (Ashmead) (Hymenoptera: Scelionidae) (Talamas et al. 2021) has been found to parasitize high percentages of *A. tristis* egg masses in the eastern US (Wilson and Kuhar 2017, Cornelius 2022), and this important parasitoid is susceptible to most broad-spectrum insecticides (Chapter 5).

Given the success of preventative chemical control in managing *A. tristis*, IPM principles utilizing the EIL concept have largely been ignored and remain unadopted by cucurbit growers and by IPM specialists. A small cohort of research supports a simple economic threshold for squash bug adults during the vulnerable seedling stage for watermelon (Edelson et al. 2002, 2003, Dogramaci et al. 2006), citing significant increases in seedling mortality when early season *A. tristis* adult densities reach 1-2 adults/plant. Yet, only nominal economic thresholds (i.e., experience-based/ anecdotal pest density recommendations) for squash bug adults (1 adult/plant) and egg masses (1 egg mass/plant) in *C. pepo* systems have been presented (Palumbo et al. 1991b, Doughty et al. 2014). These nominal thresholds are at best loosely based on peer-reviewed research and fail to include vital EIL determinant variables that accurately assess specific *A. tristis* sample unit – *C. pepo* yield relationships. Further, no studies have evaluated the relationships between field densities of each *A. tristis* life stage during the *C. pepo* vegetative growth stage and marketable yield, nor have studies included concurrent field surveys of the bug's primary natural enemy, *H. pennsylvanicus*. If IPM practices using the EIL concept are to be more readily employed by growers, the pest status of squash bug needs to be investigated from previously unexplored perspectives that accurately represent EIL determinants.

In this study, we seek to clarify the relationship between *A. tristis* life stage densities and marketable yield in *C. pepo* systems. By using intensive squash bug sampling techniques, explicit quantitative and qualitative yield assessments, and a *H. pennsylvanicus* field presence and parasitism survey, we take a new and more comprehensive approach to *A. tristis* as a pest in summer squash production. Ultimately, our goal is to build upon current squash bug threshold understandings so that more sustainable, IPM-friendly management programs for squash bug can be implemented.

Methods

Crop establishment and management

Experiments were conducted in the summer months of 2020 and 2021 at Virginia Tech's Homefield Farm in Whitethorne, VA, USA. Research plots consisted of five rows (1.2 m × 70 m) of plastic mulch raised beds were formed in early June. In 2020, zucchini (*Cucurbita pepo;* Johnny's Selected Seeds, Dunja F_1) seeds were sown directly on 22 June at 0.91 m spacing within each row. In 2021, zucchini seedlings were propagated in a greenhouse and transplanted in the field on 14 June once plants possessed 2 true leaves (~10 days after germination) at 1.82 m in-row spacing. We doubled our in-row spacing in 2021 to minimize movement of *A. tristis* nymphs between plants. Stand counts of zucchini transplants were performed seven days following field planting, and dead plants were replaced with new transplants of the same age. Tiffany Teff grass (Hancock Seed Company, Dade City, FL) was sown between plastic rows at a rate of 11 kg/ha to serve as a living mulch and weed suppressant throughout the experiment. Subsequent weed management was accomplished through weekly manual removal of weeds. Plots were irrigated as needed using subsurface drip tape irrigation (Berry Hill Irrigation, Buffalo Junction, VA). No insecticides or fungicides were applied during either year.

Young zucchini plants were labeled as "managed" or "infested" and given an individual identification number within two weeks of field germination (2020) or seedling transplant (2021). Plants possessing at least one *A. tristis* egg masses were labeled as infested. Plants without egg masses were labeled as managed. In 2020, 50 managed and 50 infested plants were labeled, while 55 managed and 61 unmanaged plants were labeled in 2021. For the entirety of the study, managed plants were kept free of squash bug egg masses and nymphs through weekly manual removal efforts. Egg masses and nymphs on infested plants were left unabated.

Insect sampling

Infested plants were given a sample number and examined for squash bug adults, egg masses, and nymphs at weekly intervals. Surveys included visual checks of the entire plant, the plastic mulch surface directly below the plant, the plastic mulch planting hole, and along the plastic mulch/Teff grass margins adjacent to each plant. Squash bug sampling was conducted for six weeks in 2020 from 13 July to 17 August and seven weeks in 2021 from 21 June to 3 August.

In 2021, we added presence/absence observations for the egg parasitoid *Hadronotus pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae) to the infested plant surveys. Similar to our *A. tristis* life stage counts, all surfaces of infested plants were visually surveyed for parasitoid adults once a week from 21 June to 3 August. We also conducted an *A. tristis* egg mass parasitism survey of the managed plants to quantify impact of *H. pennsylvanicus* in the study. To do so, *A. tristis* egg masses removed weekly from managed plants were placed in 4 cm diam. Petri dishes and reared in a laboratory growth chamber (25±2°C) and observed under a dissecting microscope to determine specific egg fates. The number of eggs per mass, hatched nymphs, emerged parasitoids, and dead eggs (i.e., no nymph hatch or parasitoid emergence) were recorded for eggs collected from managed plants for five consecutive weeks (29 June to 28 July).

Yield collection

Once developing zucchini fruit reached adequate harvesting size (15 to 20 cm length; USDA 2016), we began recording yield for individual plants. Fruit was collected three times per week from 22 July to 21 August in 2020 and from 5 July to 6 August in 2021. The quality of each harvested fruit was evaluated and categorized as either marketable, damaged by squash bug, or damaged by cucumber beetle. Fruit evaluations were based on the USDA vegetable grade scale (USDA 2005), which describes "marketable" fruit to be free of or possess minimal signs of insect-feeding related blemishes and “unmarketable fruit” to display obvious indications of insect herbivory. Squash bug feeding damage on zucchini is discernable if fruit had discolored, grey-white marks scatter across the fruit surface (Fig. 4.1). Cucumber beetle feeding damage to fruit was confirmed by chewing marks that removed the zucchini’s soft outer skin. Fruits showing obvious signs of inadequate pollination or microbial disease were removed from plants and not included in our yield collection data.

Statistical Analysis

Yield data were separated by year. Total numbers of fruit, marketable fruit, and unmarketable fruit produced by each plant during the harvest period were used to calculate mean yield values for managed and infested plants. To determine the effect of our *A. tristis* egg mass and nymph removal efforts on yield, harvested fruit means were compared between managed and infested plants using a one-way analysis of variance (ANOVA; $\alpha = 0.05$).

Anasa tristis count data from week 1 and 2 of sampling was summed for adults and egg masses per infested plant. Since nymphs were not observed on infested plants until the second week of sampling in 2020 and 2021, nymph count data were summed for week 2 and 3. Accumulated *A. tristis* life stage densities in the initial 2-3 weeks following summer squash field establishment were used to: (1) test if early season densities of these life stages are accurate predictors of marketable fruit yield, and (2) determine the time frame in which a cucurbit grower would need to employ a pest management strategy such as insecticide application based on squash bug field density. Accumulated *A. tristis* counts were normalized using a square-root transformation.

A one-way ANOVA was performed to evaluate differences in life stage counts between years. The relationships between early season sampling of squash bug life stages and marketable fruit yield per infested plant were characterized using a simple linear regression (standard least squares, $P < 0.05$). Normalized accumulated life stage counts per plant were plotted against each plant's marketable yield. To determine a potential effect of year on the *A. tristis* count – marketable fruit relationship, indicator-variable regression was performed.

Egg parasitism and *A. tristis* nymph hatch rates of egg masses laid on managed zucchini plants in 2021 were calculated by dividing the number of emerged *H. pennsylvanicus* adults and hatched *A. tristis* nymphs by the number of eggs collected from each plant. Yield, insect count, and percentage parasitism data from managed and infested plants that did not survive through the first four weeks of our sampling period (21 June -16 July) were omitted from our analyses. All statistical analyses were completed using JMP Pro 16.0.0. software.

Results

Yield

In 2020, there was no significant effect between squash plants with squash bugs removed (managed) versus unmanaged plants in the number of total fruit per plant ($F_{1,97} = 0.417$, $P =$ 0.52.; Fig. 4.2a). However, significantly more fruit collected from managed plants were deemed marketable than fruit from unmanaged *A. tristis*-infested plants ($F_{1,97} = 5.36$, $P = 0.028$). Zucchini harvested from infested plants were also more likely to display *A. tristis* feeding injury ($F_{1,97} = 13.1$, $P = 0.0005$). In 2021, there was a significant effect of *A. tristis* management for all three yield categories (Fig. 4.2b); managed plants produced more total ($F_{1,88} = 11.5$, $P = 0.001$) and more marketable zucchini ($F_{1,88} = 20.6$, $P < 0.0001$), and had fewer fruits damaged by squash bug feeding ($F_{1,88} = 10.3$, $P = 0.002$).

Adults

Consistent patterns in squash bug adult densities were observed in both years (Fig. 4.3). Adult counts peaked within the first two weeks of sampling, followed by a gradual decline to low levels by the end of July. Mean accumulated adults per infested planted varied significantly between 2020 and 2021 ($F_{1,95}$ = 40.4, $P < 0.0001$), as plants in 2021 harbored nearly three times more adults (4.42± 2.66) than plants in 2020 (1.57± 1.64) during the first two weeks of sampling. Using an indicator-variable regression, year had no effect on the *A. tristis* adult count-marketable yield relationship (*df* = 1, *F* = 0.065, *P* = 0.799). Pooling data from both years, two-week accumulated adult numbers and total marketable yield per infested plant were shown to have a weak negative correlation ($P = 0.0136$, $R^2 = 0.062$, $y = -0.3811x + 6.764$; Fig. 4.4).

Egg masses

Like the *A. tristis* adult density data, egg mass density displayed a similar pattern in 2020 and 2021 (Fig. 4.5). The number of egg masses per infested plant peaked in mid-July, roughly one to two weeks after peak adult densities were observed. There was no difference in accumulated egg mass means during the first two weeks of sampling between 2020 (6.61± 3.22) and 2021 (8.04± 3.91) ($F_{1,95}$ = 3.37, $P = 0.0694$). Regression analysis determined sample year did not have a significant effect on the egg mass count-marketable yield interaction (*df* = 1, *F* = 1.59, *P* = 0.212). Two-week cumulative egg mass density and total marketable yield in infested plants were found to have no significant relationship ($F_{1,95}$ = 0.816, $P = 0.369$) (Fig. 4.6).

Nymphs

In 2020 and 2021, *A. tristis* nymphs were not found on zucchini plants until the second week of sampling. Nymph densities in 2020 appeared to increase gradually until reaching a peak in the fourth sampling week (3 August), while 2021 densities displayed a distinct, rapid period of growth from week 3 to 4 (5 July to 12 July) and finally reaching peak density during week 5 (20 July) (Fig. 4.7). Within the first three weeks of sampling, nymphs were over twice as numerous on infested plants in 2021 (47.3± 36.9) compared to 2020 (19.2± 20.7) ($F_{1,95}$ = 20.1, $P < 0.0001$).

There was no significant effect of sample year on the relationship between early season two-week accumulated nymph density and total marketable yield per infested plant (*df* = 1, *F* = 1.24, *P* = 0.269). Early season nymph density and marketable yield showed a significant, negative relationship ($P < 0.0001$, $R^2 = 0.147$, $y = - 0.0466x + 7.171$), as greater numbers of nymphs per plant were associated with decreases in marketable fruit yield (Fig. 4.8).

Hadronotus pennsylvanicus **presence and parasitism**

We observed the egg parasitoid *H. pennsylvanicus* on infested zucchini plants during each of the seven sampling weeks in 2021 (Fig. 4.9). Parasitoid presence was low in weeks 1 and 2, as adult parasitoids were only found on 10% (6 of 60) and 19% (11 of 58), respectively, of infested plants. *Hadronotus pennsylvanicus* was observed on the highest percentage of plants (46%, 22 of 48) during week 4 of the survey, and the season average per sampling week was 27%.

The number of *A. tristis* egg masses collected from managed plants in 2021 increased from 29 June to 5 July, when egg collections peaked at over seven egg masses per plant (Fig. 4.10). Unlike our 2021 egg mass survey data for infested plants, which peaked 12 July, managed plant egg mass density decreased from 5 July to 12 July and continued to fall until the last collection date. *Hadronotus pennsylvanicus* parasitism of squash bug eggs was low during collection week 1 (14.7 ± 29.4%) and week 2 (27.2 ± 37.6%) (Fig. 4.11). Subsequently, nymphal hatch rates during this time frame were high, as nymphs successfully hatched from 81.5 ± 30.8

and 69.6 ± 37.5 % of eggs collected in week 1 and week 2, respectively. However, egg masses collected in the latter three weeks of July displayed high egg parasitism and low nymph hatch rates. The mean percentage of eggs resulting in parasitoid emergence nearly tripled from 5 July to 12 July, and reached over 90% by 20 July.

Discussion

The results of our two-year field study offer new insights into how squash bug densities may influence marketable fruit yield in summer squash growing systems. The significant differences we observed in marketable and damaged zucchini fruit between managed and infested plant treatments in both years broadly infer the effect of *A. tristis* presence on *C. pepo* yield (Fig. 4.2). First, greater marketable yield and fewer *A. tristis*-damaged fruit collected from managed plants suggests that weekly removal of egg masses and nymphs was an effective method for representing zucchini plant response under minimal *A. tristis* pressure. However, fruit produced by managed plants still sustained low levels of squash bug damage, possibly a result of occasional feeding by adults or nymphs that had moved from infested plants to nearby managed plants. Since the *A. tristis* egg stage does not inflict damage to fruit, and adults were allowed to colonize both infested and managed plants, the continuous presence of nymphs is the most likely explanation for the decreased marketable yield produced by infested plants.

Through our efforts to sequentially monitor *A. tristis* life stages and assess yield using individual plants as our sampling units, we were able to precisely measure each plant's response to its own specific level of *A. tristis* infestation. This contrasts with all previous *A. tristis* sampling studies, as most have randomly chosen a few plants on which to count squash bug life stages within a large multi-plant sampling unit for each sampling date (Palumbo et al. 1991b, c, Dogramaci et al. 2006). Complete randomized block sampling procedures are highly effective for estimating *A. tristis* abundance, spatial distributions, and dispersal patterns across an entire cucurbit field. But unless they conduct *A. tristis* sampling within many large fields, they will lack the precision required to relate *A. tristis* densities to yield on a per-plant scale. Our sampling procedure resulted in *actual* squash bug life stage count values per plant rather than *estimated* count means per plant. If the fundamental integrated pest management EIL concept is to be implemented for squash bug in *C. pepo* systems, EIL predictor components like the pest density-host plant injury relationship need to be accurately defined using appropriate pest and yield sampling metrics.

Our regression analyses characterizing the relationship between two-week accumulated *A. tristis* life stage counts and marketable fruit produced per infested plant support the idea that nymph presence was the most influential *A. tristis* life stage on yield loss. Both accumulated adult and nymph counts per plant were found to have significant negative correlations with the number marketable fruit produced per plant (Figs. 4.4, 4.8). However, nymphal counts displayed a much stronger relationship with marketable fruit than did adult counts. Differences in the strength of these count-marketable yield relationships align with the within-field dispersal patterns of adults and nymphs. Compared to adults, squash bug nymphs have a limited capacity to move between host plants and exhibit stronger patterns of aggregation (Palumbo et al. 1991b). Considering we sampled the same plants over consecutive weeks, nymph counts may have been more accurate predictors of each plant's future marketable yield because they are less likely to leave the plant on which they were sampled. Thus, their consistent presence on the same plant is more likely to impart a consistent effect on host plant response.

To our knowledge, this is the first field study to connect *A. tristis* nymphal counts during the cucurbit vegetative growth stage (i.e., time between seedling establishment and fruit production) to marketable yield loss. This new understanding has important implications for pest management decision-making, as all previous studies have focused exclusively on adult counts during cucurbit field establishment or seedling stage (Woodson and Fargo 1991, 1992, Palumbo et al. 1991b, Edelson et al. 2003). A simple adult threshold of 1-2 adults/seedling has undoubtedly helped growers determine if a control tactic to protect seedlings is needed, yet it can be argued that the threshold is so low that it is impractical to monitor (Poston et al. 1983). Growers that experience severe yearly adult colonization pressure during field establishment are more likely to adopt preventative management plans involving repeated insecticide applications. Moreover, the adult seedling threshold has become less useful since the development and wide-spread use of systemic insecticide-coated cucurbit seed, which can provide seedlings with up to three weeks of effective control against *A. tristis* and other serious insect pests (Kuhar et al. 2020). Our data begin to bridge the knowledge gap between cucurbit pest management during the seedling stage and crop maturity. Once growers have successfully thwarted damage inflicted by initial *A. tristis* adult colonization, using our findings they can now be confident in reorienting their sampling to quantifying nymph presence.

Differences in the magnitude of *A. tristis* infestation between 2020 and 2021 were evident for all life stages (Figs. 4.3, 4.5, 4.7). At peak infestation levels, mean counts per plant for each life stage were roughly 2-3 times greater in 2021 than in 2020. In addition to *A. tristis* abundance, our infested *C. pepo* plants in 2020 produced more total fruit per plant than in 2021 (Fig. 4.2). This between-year variation in *A. tristis* density and total yield may reflect differing planting dates and planting procedures. In 2020, we established our zucchini field by directly sowing seeds on 22 June and observed complete germination during the first week of July. In 2021, we transplanted young seedlings on 8 June 2021 and confirmed field stand establishment on 15 June. Palumbo et al. (1991c) observed significantly more adults, egg masses, and nymphs on earlier *C. pepo* plantings compared to later plantings. Further, they found a significant effect of plant size (i.e., leaf area) on adult colonization, with bugs selecting more mature plants over younger plants. Despite our variation in abundance and yield between years, *A. tristis* life stages still showed similar seasonal population dynamics in 2020 and 2021 (Fargo et al. 1988). Future studies are needed to discern how planting dates within the same growing season impact plant response to *A. tristis* densities and feeding damage.

Presence/absence sampling for egg parasitoid *H. pennsylvanicus* as well as a squash bug egg parasitism survey conducted in 2021 broadened our perspective on the complexity of the *A. tristis* – *C. pepo* yield relationship. Notably, we were able to confirm the parasitoid's presence in our zucchini field during each of the seven sampling weeks (Fig. 4.9). Although little is known about *H. pennsylvanicus* population dynamics throughout the entire cucurbit growing season, the parasitoid's regular appearance, especially early in the season, was unexpected. Other studies performing squash bug natural enemy surveys in Kentucky and Kansas did not observe *H. pennsylvanicus* in field-collected *A. tristis* egg masses until July and August, respectively (Olson et al. 1996, Decker and Yeargan 2008). *Hadronotus pennsylvanicus* activity may have contributed to the nonsignificant relationship between egg mass density and marketable fruit yield (Fig. 4.6). Egg mass density and rate of parasitoid presence on infested plants peaked during the same sampling date (12 July) (Figs. 4.5, 4.9). 12 July was also the first sampling date we observed high parasitism rates (~75%) of squash bug egg masses collected from managed plants, which was nearly three times greater than the previous week's parasitism rates (Fig. 4.11). So, while we did not get egg parasitism rates from infested plants, our data suggests parasitism of eggs on managed plants was likely similar to infested plants.

This study introduces a unique outlook on squash bug management and provides reinforced scaffolding on which future integrated pest management strategies can be built. Our work presents new methodologies that bolster our understanding of how squash bug life stage densities relate to *C. pepo* marketable yield. The inclusion of *H. pennsylvanicus* surveys in our sampling efforts offers new insight on the parasitoid's role in the squash bug – summer squash yield relationship. Moving forward, squash bug integrated pest management research would greatly benefit from concurrent examinations of multiple insect and microbial pests, pollinators and natural enemies, and abiotic factors. By broadening our research approach outside of a singular pest and its host plant, we can better address the complexity of cucurbit agroecosystems and make progress towards holistic pest management strategies that meet the growing demands for sustainable food production.

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Figures

Figure 4.1: Damaged zucchini fruit displaying surface blemishes caused by *A. tristis* feeding

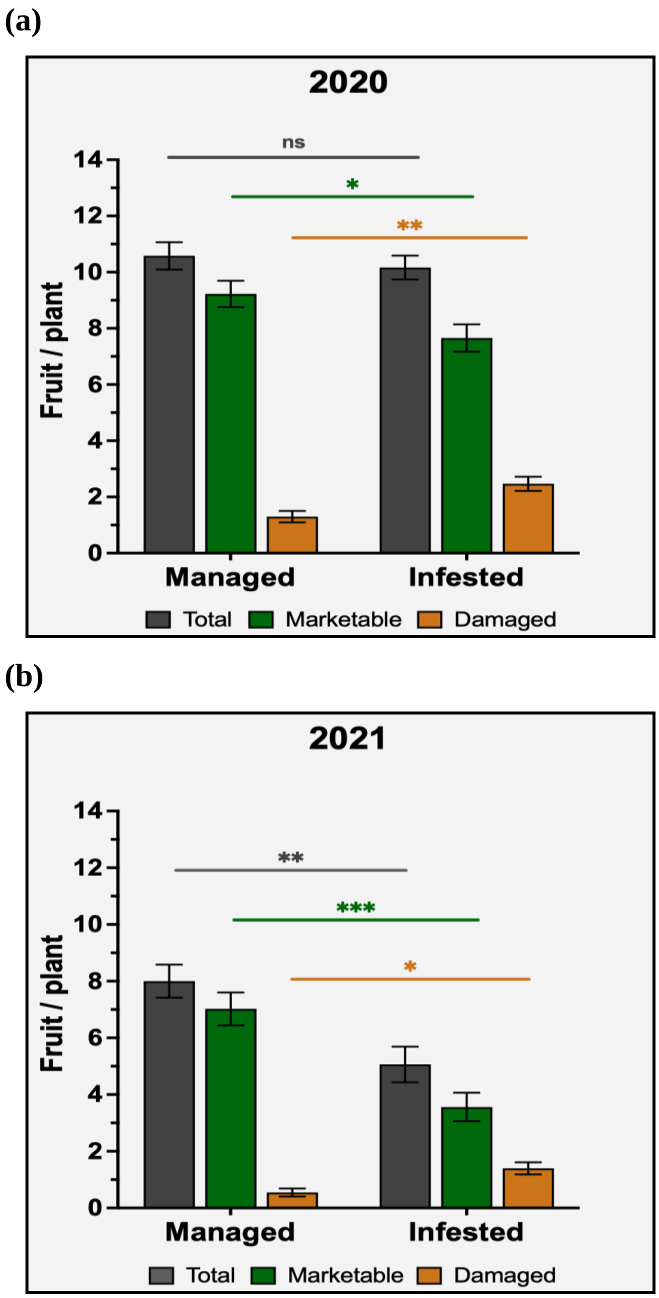

Figure 4.2: Mean total, marketable, and *A. tristis*-damaged zucchini fruit per plant produced during our yield collection period in **(a)** 2020 and **(b)** 2021. Asterisks display significant differences (ANOVA, * $P \leq 0.05$, ** $P \leq 0.001$, *** $P \leq 0.0001$) between managed and infested treatments. Bars represent mean standard error.

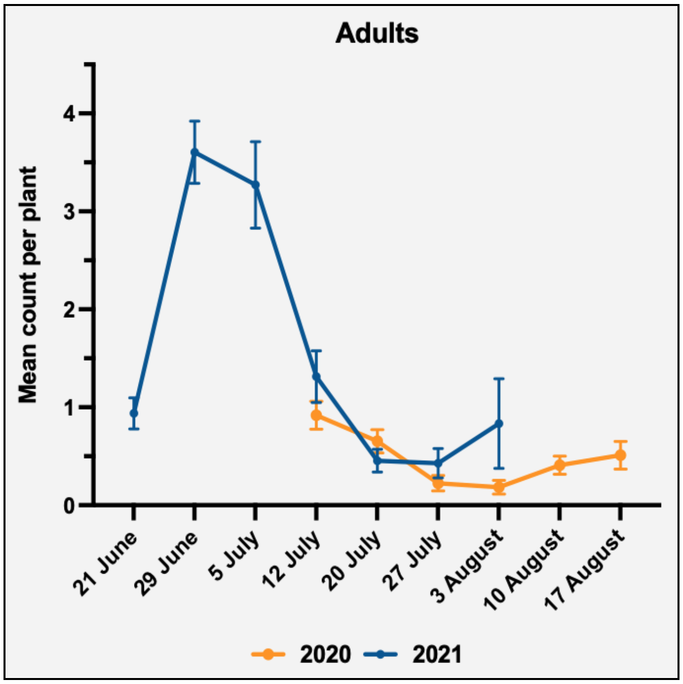

Figure 4.3: Mean number of *A. tristis* adults surveyed per infested plant on each collection date in 2020 and 2021 at Homefield Farm (Whitethorne, VA). Bars represent mean standard error.

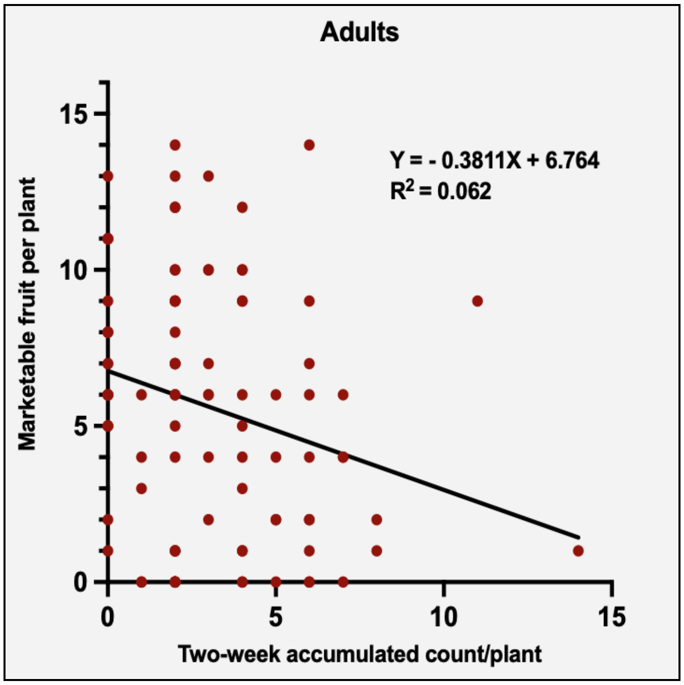

Figure 4.4: Linear regression of accumulated *A. tristis* adult counts from the first two weeks of sampling and total number of marketable fruit produced per infested plant in both years. Least-squares regression fitted to the data (P = 0.0136; R^2 = 0.062).

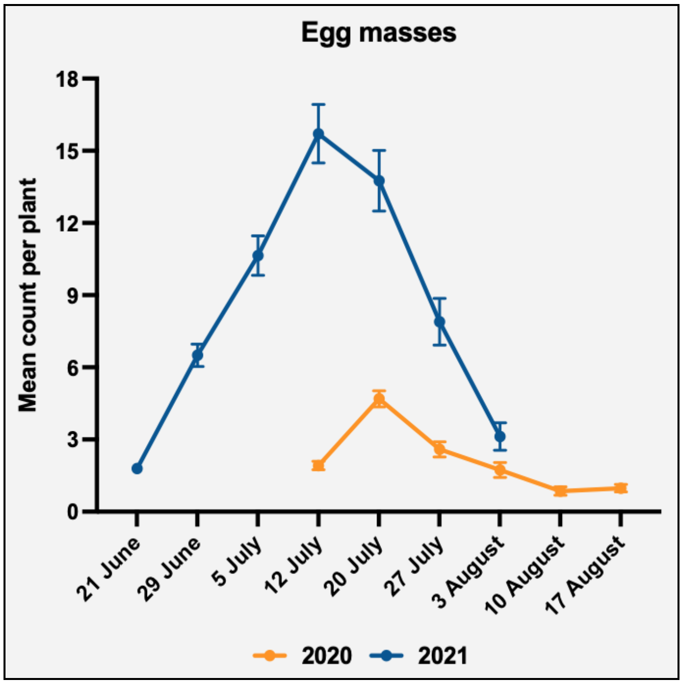

Figure 4.5: Mean number of *A. tristis* egg masses surveyed per infested plant on each collection date in 2020 and 2021 at Homefield Farm (Whitethorne, VA). Bars represent mean standard error.

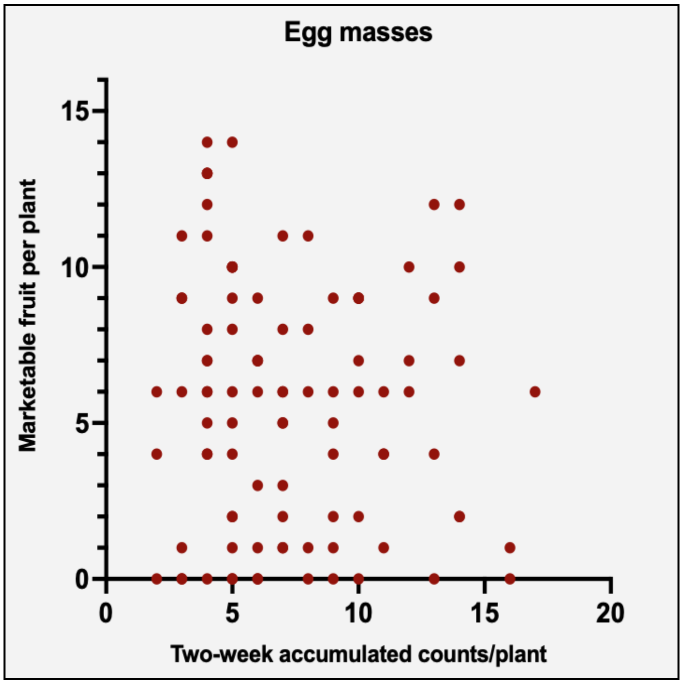

Figure 4.6: Linear regression of accumulated *A. tristis* egg mass counts from the first two weeks of sampling and total number of marketable fruit produced per infested plant in both years. Least-squares regression found no correlation between count and yield data (*P* = 0.369).

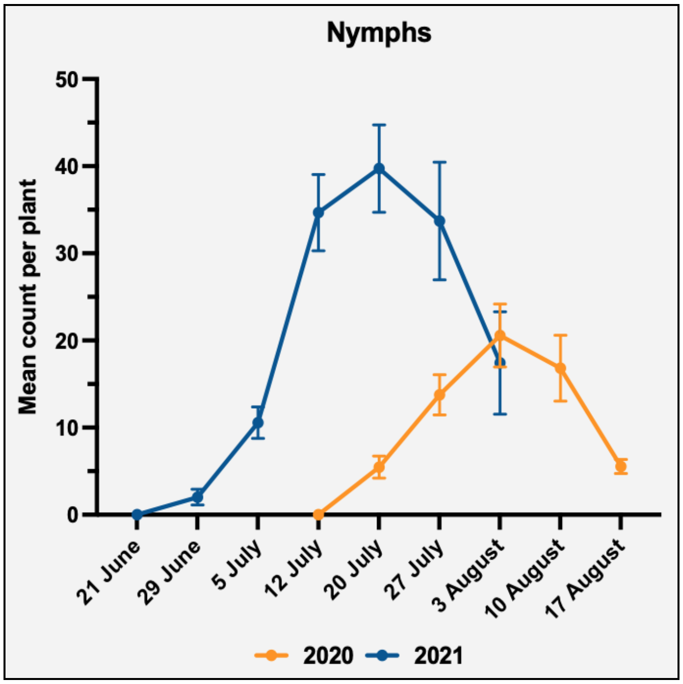

Figure 4.7: Mean number of *A. tristis* nymphs surveyed per infested plant on each collection date in 2020 and 2021 at Homefield Farm (Whitethorne, VA). Bars represent mean standard error.

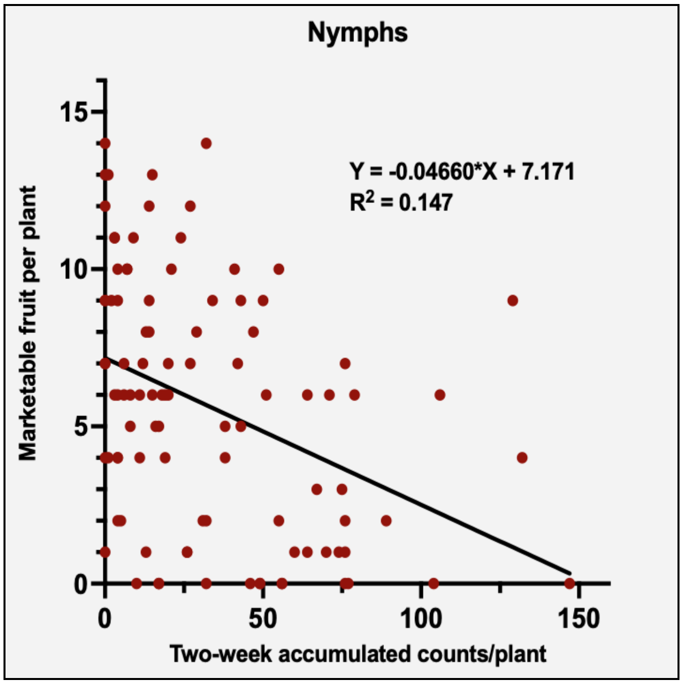

Figure 4.8: Linear regression of accumulated *A. tristis* nymph counts from week two and three of sampling and total number of marketable fruit produced per plant in both years. Least-squares regression fitted to the data (P = 0.0001; R^2 = 0.147).

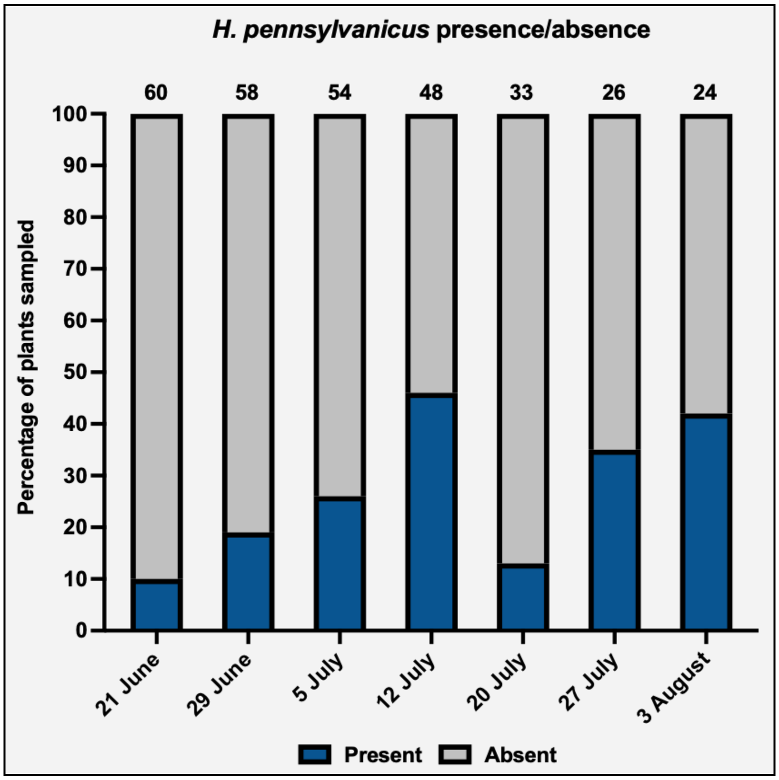

Figure 4.9: Percentage of infested zucchini plants on which *H. pennsylvanicus* was observed present (blue) or absent (grey) at Homefield Farm (Whitethorne, VA) each sampling week in 2021. Numbers above each bar represent the total number of infested plants sampled per week.

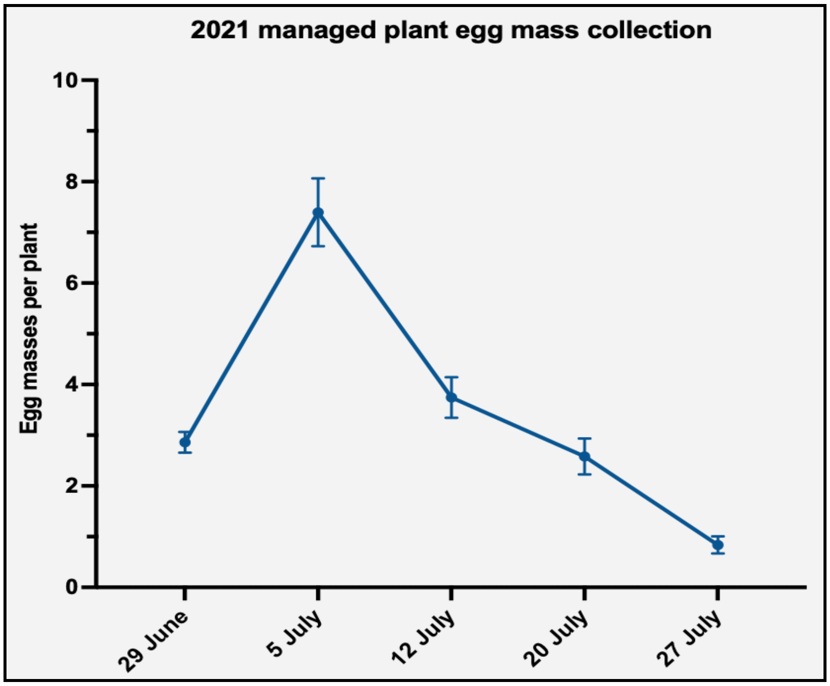

Figure 4.10: Mean number of *A. tristis* egg masses collected from managed plants per sample date in 2021 at Homefield Farm (Whitethorne, VA). Bars represent mean standard error.

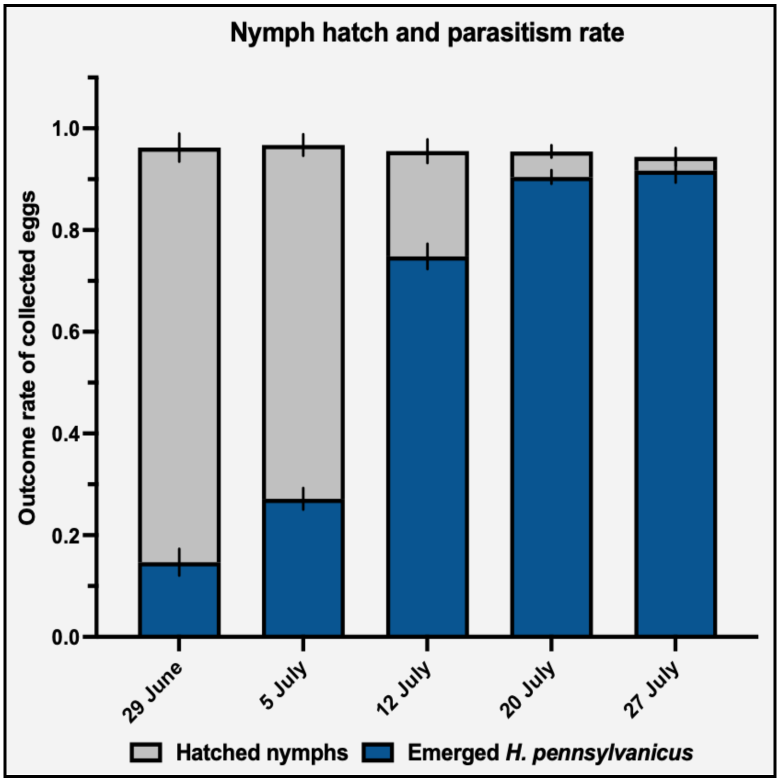

Figure 4.11: Mean rates of egg parasitism (blue) by *H. pennsylvanicus* and *A. tristis* nymph hatch (grey) of collected egg masses from managed plants per sample date in 2021 at Homefield Farm (Whitethorne, VA). Bars represent mean standard error.

Chapter 5: Effects of some selective hemipteran-targeting insecticides and one broad spectrum insecticide on squash bug and its primary parasitoid *Hadronotus pennsylvanicus*

(The results included in this chapter have been published in two separate Arthropod Management Tests articles cited below)

Boyle, S. M., T. P. Kuhar, and D. C. Weber. 2022. Effects of selective hemipteran-targeting insecticides and one broad spectrum insecticide on squash bug, 2018. Arthrop. Manag. Tests. tsac073. https://doi.org/10.1093/amt/tsac073

Boyle, S. M., T. P. Kuhar, J. Wilson, and D. C. Weber. 2022. Effects of some selective hemipteran-targeting insecticides and one broad spectrum insecticide on the parasitoid *Hadronotus pennsylvanicus*, 2018. Arthrop. Manag. Tests. tsac074. https://doi.org/10.1093/amt/tsac074

Abstract

The squash bug, *Anasa tristis*, is a serious pest of cucurbit crops throughout the United States. Current *A. tristis* pest management plans rely almost entirely on repeated applications of broad-spectrum insecticides. Despite their effectiveness in controlling *A. tristis* and other cucurbit pests, broad-spectrum insecticides are notoriously harmful to beneficial insects like pollinators and natural enemies. One natural enemy of particular concern is squash bug egg parasitoid *Hadronotus pennsylvanicus*, which provides significant control of squash bug eggs during the latter half of the growing season. Several reduced risk insecticides claim high efficacy against piercing-sucking pests along with minimal non-target effects on beneficial insect species. Since the toxicity of these reduced risk insecticides has yet to be evaluated on *A. tristis* nymphs or *H. pennsylvanicus*, we conducted laboratory bioassays to better understand how they may affect both insects compared to broad-spectrum compounds. We exposed lab-reared *A. tristis* nymphs and *H. pennsylvanicus* adults to insecticide-treated summer squash and filter paper, respectively. Mortality for each insecticide treatment was recorded at 24, 48, and 72 h after initial exposure for each species. Percent mortalities of *A. tristis* nymphs exposed to reduced risk insecticides sulfoxaflor and flupyradifurone were statistically the same as nymphs exposed to broad-spectrum pyrethroid lambda-cyhalothrin and neonicotinoid acetamiprid treatments. Adult parasitoids exposed to sulfoxaflor-treated filter paper exhibited the same percent mortality as parasitoids exposed to filter paper treated with lambda-cyhalothrin. However, *H. pennsylvanicus* percent mortality of flupyradifurone exposure was found to be statistically equal to the water control treatment. Overall, our study identified flupyradifurone as the only reduced risk insecticide to be highly toxic to *A. tristis* nymphs and nontoxic to *H. pennsylvanicus* adults.

Introduction

Squash bug, *Anasa tristis* DeGeer (Hemiptera: Coreidae), is a major pest of cucurbit crops in the United States (Fargo et al. 1988, Doughty et al. 2016). Both nymphs and adults feed on vegetative growth by imbibing plant fluids with their piercing-sucking mouthparts, which can lead to plant wilt and leaf necrosis (Neal 1993, Palumbo et al. 1993). Additionally, squash bug feeding on fruit can impact the quality and marketability of the crop. The most common management strategy for squash bug is routine broad spectrum insecticide applications of pyrethroids or neonicotinoids (Doughty et al. 2016). While these insecticides are effective in controlling squash bug (Eiben et al. 2004, Abney and Davila 2011, Doughty et al. 2016), they can have negative side effects on non-target organisms including pollinators and arthropod natural enemies (Smith and Stratton 1986, Slosser et al. 1989, Kilpatrick et al. 2005, Desneux et al. 2007). For this reason, there is growing motivation for reduced risk alternatives to pyrethroids and neonicotinoids to control squash bug.

Here we evaluate several reduced risk insecticides and one broad spectrum insecticide for their toxicity to *A. tristis* nymphs as well as *Hadronotus pennsylvanicus* (formerly *Gryon* *pennsylvanicum*) (Ashmead) (Hymenoptera: Scelionidae), a key egg parasitoid of squash bug found throughout the pest's North American distribution (Talamas et al. 2021). To date, no research has identified mortality risks of insecticide exposure to *H. pennsylvanicus.* However, other scelionid egg parasitoid species exhibit both lethal and non-lethal effects when exposed to broad-spectrum insecticides (Waddill 1978, Mani and Krisnamoorthy 1986, Bayram et al. 2010) By conducting simple laboratory exposure bioassays, we sought to determine a baseline understanding of how insecticides claimed to be reduced risk to non-target organisms affect *A. tristis* nymphs and *H. pennsylvanicus*.

Reduced risk insecticide treatments were chosen based on a few criteria. We included the neonicotinoid acetamiprid (IRAC 2016, Group 4A) since it has a lower toxicity to pollinators compared to other neonicotinoids (Decourtye and Devillers 2010). Insecticides targeting arthropod pests with sucking mouthparts were also included. More specifically, we chose insecticides that have yet to be tested on squash bug but have been extensively evaluated on and considered highly efficacious for small Hemipteran pests like aphids, whiteflies, thrips, and mites. Two of these insecticides were sulfoxaflor (IRAC 2016, Group 4C) and flupyradifurone (IRAC 2016, Group 4D), which act on the nicotinic acetylcholine receptor (nAChR) in a different manner than other group 4 insecticides (Casida and Durkin 2013, Sparks et al. 2013). The compounds pyrifluquinazon (IRAC 2016, Group 9) and afidopyropen (IRAC 2016, Group 9D), which act on the chordotonal organ and inhibit sucking arthropod feeding behavior, were also used. The final reduced risk insecticide used that targets Hemipteran pests was flonicamid (IRAC 2016, Group 29), a compound in its own IRAC group that inhibits feeding and reproductive behavior (Morita et al. 2007, Nesterov et al. 2015). While its effects on sucking pests are similar to insecticides in Group 9, flonicamid has a unique mode of action that targets potassium channels in malpighian tubules and salivary glands (Swale et al. 2017, Ren et al. 2018). Additionally, we included a commonly used broad spectrum insecticide in cucurbit pest management, cyclaniliprole (IRAC 2016, Group 28), which is known to be effective against coleopteran and lepidopteran pests while its effects on squash bug are largely unknown. The pyrethroid, λ-cyhalothrin, was tested as a broad-spectrum commercial standard for which many negative non-target effects have been identified (Smith and Stratton 1986).

Materials and Methods

Squash bug nymph toxicity bioassays.

During the 2018 field season, *A. tristis* egg masses from field plots of summer squash (*Cucurbita pepo* L.) at the Virginia Tech Kentland Research Farm (Whitethorne, VA, USA) were placed in a mesh cage (30.5×30.5×30.5 cm, BioQuip Products, Inc.) at room temperature. Once nymphs emerged, they were placed on greenhouse-grown ‘Tigress’ zucchini (Harris Seeds, Rochester, NY, USA) plants. When sufficient numbers of 2nd - 4th instars were available, nymphs were used immediately in the bioassay. All insecticides were obtained as formulated commercial products and were mixed at label rates using an estimated spray output of 30 gal per acre (Table 5.1). Fresh cut discs (8 -10 cm diameter, 0.6-0.7 cm width) of summer squash fruit were dipped in insecticide solution for 5 seconds then placed into 150 × 15 mm petri dishes along with 10 nymphs per dish. Nymphal mortality was recorded at 24, 48, and 72 h after treatment. Four repetitions were conducted for each insecticide treatment. Proportion mortality data were arcsine square-root transformed to normalize variances then analyzed using a one-way ANOVA followed by Fisher’s protected LSD to separate treatment means.

Adult *H. pennsylvanicus* toxicity bioassays

Parasitized squash bug egg masses were collected from summer squash (*C. pepo*) at the Virginia Tech Kentland Research Farm (Whitethorne, VA, USA) in August 2018 and held in mesh cages until *H. pennsylvanicus* adults emerged. Treatments followed those outlined in Table 5.1 and P8 filter paper discs (5.5 cm diameter, Sigma Aldrich Inc., St. Louis, MO, USA) were dipped in the insecticidal solutions and allowed to dry under a ventilation hood. Once dry, filter paper discs were then used to line 15 ml plastic conical tubes (Fisher Scientific, Podunk, IL, USA). At least five adult parasitoids were gently aspirated from the colony and placed in 15 ml conical tubes. Mortality of the wasps was recorded at 24, 48, and 72 h after exposure. Ten repetitions were conducted for each insecticide treatment. Proportion mortality data were arcsine square-root transformed to normalize variances and analyzed using a one-way ANOVA followed by Fisher's protected LSD to separate treatment means.

Results

Squash bug nymph toxicity bioassays

There was a highly significant treatment effect on mortality of *H. pennsylvanicus* adults exposed to treated filter paper for 24 h ($F_{9,30} = 13.218$, $P < 0.0001$), 48 h ($F_{9,30} = 9.461$, $P < 0.0001$), and 72 h ($F_{9,30} = 14.956$, $P < 0.0001$). At each time interval, acetamiprid and Lambda-cyhalothrin had the highest mortality followed by sulfoxaflor then flupyradifurone (Table 5.2). The aforementioned four treatments were the only insecticides to result in effective (>80%) mortality of squash bug nymphs after 72 h.

Adult *H. pennsylvanicus* toxicity bioassays

There was a highly significant treatment effect on mortality of *H. pennsylvanicus* adults exposed to treated filter paper for 24 h ($F_{9,30} = 14.529$, $P < 0.0001$), 48 h ($F_{9,30} = 6.026$, $P < 0.0002$), and 72 h ($F_{9,30} = 14.956$, $P < 0.0001$). Lambda-cyhalothrin caused the highest mortality followed by sulfoxaflor, and these were the only two treatments whose mortality was significantly higher than the water control after 72 h (Table 5.3).

Discussion

Pyrethroids or neonicotinoids have been the insecticides of choice for chemical control of squash bug for the past three decades (Doughty et al. 2016, Wyenandt et al. 2016). Although these insecticides are efficacious at controlling squash bug nymphs (Eiben et al. 2004, Kuhar et al. 2005, Abney and Davila 2011, Kuhar and Doughty 2016), most are also toxic to non-target arthropods and are generally not compatible with IPM or pollinator protection plans (Smith and Stratton 1986, Fairbrother et al. 2014, Pisa et al. 2014). In this study, acetamiprid, lambda-cyhalothrin, sulfoxaflor, and flupyradifurone all provided effective control of squash bug nymphs in lab bioassays (Table 5.2). These insecticides have also resulted in significant reductions in squash bug densities in field experiments (Kuhar and Doughty 2016). Acetamiprid, sulfoxaflor, and flupyradifurone are considered less toxic to pollinators than pyrethroids, while also providing control of aphids, whiteflies, and other small piercing-sucking pests. Repeated applications of pyrethroids, on the other hand, often lead to outbreaks of aphids by suppressing natural enemy abundance and promoting insecticide resistance (Slosser et al. 1989, Chapman et al. 2009).

With regards to insecticide impact on the parasitoid *H. pennsylvanicus*, lambda-cyhalothrin and sulfoxaflor caused the highest mortality of adult wasps compared to flupyradifurone, pyrifluquinazon, and flonicamid. High rates of *H. pennsylvanicus* mortality in the lambda-cyhalothrin treatment was not surprising, as lethal and non-lethal effects have been observed with other scelionid egg parasitoid species (Salerno et al. 2002, Bayram et al. 2010). It was surprising to observe high mortality rates for adult parasitoids exposed to sulfloxaflor since it is absorbed systemically by plant tissues and acts on sucking pests that imbibe the compound when feeding on treated plant fluids (Sparks et al. 2013). The *H. pennsylvanicus* adults were exposed to all treatments via contact by walking on treated filter papers. There is recent evidence that supports this result, as detrimental effects of contact exposure to sulfoxaflor were found with three distinct *Trichogramma* parasitoid species (Jiang et al. 2019). Future research should add field-related complexity to laboratory assays, such as different insecticide application methods for whole *C. pepo* plants (e.g., foliar, chemigation, seed treatments), to elucidate more realistic insecticide exposure effects on *H. pennsylvanicus* adults.

Based on our bioassays, flupyradifurone was the only reduced risk insecticide found to be both highly toxic to *A. tristis* nymphs and nontoxic to *H. pennsylvanicus* adults. Our laboratory bioassays align with results from field trials displaying significant reductions in *A. tristis* abundance on summer squash plants sprayed with flupyradifurone (Kuhar and Doughty 2018). Although no previous research has studied the effects of flupyradifurone exposure on scelionid parasitoids, Tabebordbar et al. 2020 showed that parasitoid *Trichogramma evanescens* has reduced lethal and sub-lethal effects from contact exposure to field rates of the flupyradifurone compared to pyrethroid deltamethrin exposure. Additional studies are needed to identify if there are sub-lethal behavioral and life history effects of insecticide exposure on *H. pennsylvanicus*. In particular, research focused on determining potential effects on *H. pennsylvanicus* ability to successfully search for and locate *A. tristis* egg masses, as well as on parasitoid fecundity and longevity, would provide valuable information concerning the overall risk broad and narrow spectrum insecticides pose to *H. pennsylvanicus*.

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Tables

Table 5.1: List of insecticides and concentrations used in laboratory toxicity bioassays on *A. tristis* and *H. pennsylvanicus.*

Active ingredient (AI)	**Commercial Product**	**IRAC Insecticide Group**	**Recommended field application rate (kg AI per ha)**	**Insect(s) tested**
λ-cyhalothrin	Lambda-cy	3A	0.0323	both
acetamiprid	Assail 30SG	4A	0.084	*A. tristis* only
sulfoxaflor	Transform	4C	0.0785	both
flupyradifurone	Sivanto HL	4D	0.1249	both
pyrifluquinazon	PQZ	9	0.0468	both
afidopyropen	Sefina	9D	0.050	*A. tristis* only
cyclaniliprole	Harvanta 50SL	28	0.040	*A. tristis* only
flonicamid	Beleaf	29	0.0998	both

Table 5.2: Mean ± SEM proportion mortality of squash bug nymphs from three separate toxicity bioassays where nymphs were confined to treated (insecticide-dipped) squash discs.

		mortality (%)		
Treatment	**Rate kg AI per ha**	**24 h**	**48 h**	**72 h**
Lambda-cyhalothrin	0.0323	67.5a	80.0a	97.5a
Cyclaniliprole	0.040	22.5bc	25.0cde	67.5bc
Sulfoxaflor	0.0785	22.5bc	60.0ab	95.0a
Flupyridifurone	0.1249	27.5b	50.0bc	87.5ab
Pyrifluquinazon	0.0468	12.5bcd	17.5de	32.5de
Flonicamid	0.0998	10.0bcd	35.0bcd	57.5cd
Afidopyropen	0.050	12.5bcd	12.5de	20.0ef
Acetamiprid	0.084	72.5a	82.5a	100.0a
Water	-	0.0d	0.0e	5.0f

Means within columns followed by a letter in common are not significantly different ($P < 0.05$).

Table 5.3: Mean ± SEM mortality of adult *Hadronotus pennsylvanicus* wasps after contact exposure to insecticide-treated filter paper.

		mortality (%)		
Treatment	**Rate kg ai per ha**	**24 h**	**48 h**	**72 h**
Lambda-cyhalothrin	0.0323	75.1a	84.6a	88.2a
Sulfoxaflor	0.0785	23.0b	48.0b	72.0ab
Flupyradifurone	0.1249	2.0c	15.7c	37.7c
Pyrifluquinazon	0.0468	4.0bc	26.0bc	48.0bc
Flonicamid	0.0998	10.0bc	28.0bc	50.0bc
Water	-	10.0bc	18.0bc	35.0c

Means within columns followed by a letter in common are not significantly different ($P < 0.05$)

Conclusion

This dissertation investigated previously understudied and unknown aspects of squash bug, *Anasa tristis*, pest management. In particular, this work focused on: (1) testing the effects of colored plastic mulches on squash bug abundance and summer squash yield, (2) the potential of augmentative squash bug biological control using releases of egg parasitoid *Hadronotus pennsylvanicus*, (3) characterizing the relationship between *A. tristis* life stages and summer squash marketable yield loss, and (4) identifying if newer, hemipteran-specific insecticides are both effective against squash bug and innocuous for *H. pennsylvanicus*.

In a two-year study, squash bug abundance and marketable fruit yield for summer squash plants grown in black, white, and reflective plastic mulch plots were compared with plants grown in bare ground plots. In both years, *A. tristis* adults and egg masses were more numerous on summer squash plants grown in white and reflective plastic mulch compared to bare ground plants. Greater nymphal densities and marketable fruit yield were observed in certain plastic mulch treatments versus the bare ground treatment, yet these differences were not consistent in both years. Contrary to the repellency effects reflective mulches have on other cucurbit insect pests, our research suggests reflective and other plastic mulch colors can negatively impact squash bug management, especially in regions with high *A. tristis* pressure. Using these findings, summer squash growers can be more confident in determining if plasticulture fits into their squash bug management plans.

Augmentative releases of *H. pennsylvanicus* on small organic farms in southeastern Virginia proved successful in both 2020 and 2021. Deployed as parasitized *A. tristis* egg masses, the parasitoids were able to emerge from their lab-reared host egg masses and disperse within the targeted summer squash planting. Squash bug egg masses collected at release sites two and four weeks after deployment exhibited higher rates of *H. pennsylvanicus* parasitism than egg masses collected from sites where parasitoids were not released. Significant differences between release and no-release site *A. tristis* egg parasitism rates were even more stark in areas where wild *H. pennsylvanicus* populations are known to be low throughout the entire growing season. This study was the first of its kind. It was the first to show successful deployment using lab-parasitized egg masses, as well as the first to show significant efficacy with high parasitism rates at release sites within two weeks of the wasp deployment date. Ultimately, this work can be used as a steppingstone not only for future squash bug biological control programs, but also for the biological control of other coreid pest species in different crop systems and geographic regions in North America and Europe.

Through the use of intensive squash bug sampling techniques, explicit quantitative and qualitative yield assessments, and the inclusion of *H. pennsylvanicus* surveys, we conducted a comprehensive approach to characterizing the relationship between *A. tristis* and marketable summer squash yield. The results from our two-year field trials indicate broad overarching effects of squash bug presence on summer squash plant yield response, as plants that were subject to weekly *A. tristis* egg mass and nymph removals produced significantly more marketable fruit than plants that were free to establish natural *A. tristis* infestations. Accumulated adult and nymph counts per infested squash plant within the first few weeks of sampling were found to have significant, negative linear relationships with the number of marketable fruit produced per plant. Furthermore, the accumulated nymph counts displayed a stronger relationship with marketable fruit yield than did adult counts. The presence of *H. pennsylvanicus* on infested plants was observed during each week of sampling in 2021, and parasitism rates of eggs from managed plants revealed a gradual increase in *A. tristis* egg parasitism from the beginning to the end of the study. Overall, this work indicates *A. tristis* nymph numbers early in the season are more accurate predictors of total marketable yield production in summer squash plantings. This information can be utilized to establish more reliable threshold values that growers can use to improve the efficacy of their management tactics for *A. tristis*.

Laboratory bioassays evaluating several hemipteran-specific insecticides and one broad-spectrum insecticide for their toxicity to both *A. tristis* nymphs and *H. pennsylvanicus* found overlap in one product that was toxic to *A. tristis* nymphs and exhibited low toxicity to *H. pennsylvanicus*. Squash bug nymphs feeding on fruit treated with reduced risk insecticide flupyridifurone (Sivanto HL) showed similar 72-h mortality rates to broad spectrum insecticides like lambda-cyhalothrin (Lamda-cy) and acetamiprid (Assail 30SG). *Hadronotus pennsylvanicus* mortality rates when exposed to Sivanto HL were the same as the control (water) treatment and significantly lower than Lambda-cy treatments. Sulfoxaflor (Transform), an insecticide previously considered a reduced risk product for non-target insects, had statistically similar mortality rates to lamba-cy for both *A. tristis* nymphs and *H. pennsylvanicus* adults. These simple laboratory bioassays can be viewed as initial investigations for further field-applicable research which tests insecticide products for both squash bug nymph efficacy and reduced toxicity for *H. pennsylvanicus* and other beneficial insects in cucurbit crops systems.

Altogether, results from each of the preceding dissertation chapters provide valuable information on various facets of squash bug integrated pest management. With this research, growers and IPM specialists alike can bolster their understanding of squash bug as a summer squash pest and implement our findings to improve and modernize their current *A. tristis* management strategies.