Species survey, monitoring and management of economically important stink bug species in eastern Virginia

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

Doctor of Philosophy

In

Entomology

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October 23, 2008
Blacksburg, Virginia

Keywords:
Acrosternum hilare, *Euschistus servus*, bioassays, insecticide efficacy, organic insecticide efficacy, modeling

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ABSTRACT

Stink bugs are major pests of agricultural crops throughout Virginia and much of the United States. Knowledge of the biology, the species complex, and insecticide susceptibility can improve management. A survey was conducted in Virginia to determine the species complex in soybean and cotton and to monitor for nonnative species. Seven stink bug species were identified. *Acrosternum hilare* (Say) and *Euschistus servus* (Say) were the most common. Two sampling methods, the sweep net and the beat sheet, were assessed in soybean and cotton. There was less variability with the sweep net method compared to the beat sheet method.

Laboratory bioassays and field trials were conducted to evaluate the toxicity and efficacy of selected conventional and organic insecticides against *A. hilare* and *E. servus*. In bioassays with conventional insecticides, *A. hilare* adults and nymphs were susceptible to all pyrethroids tested. Generally, the neonicotinoids, dinotefuran and clothianidin, were more toxic to *A. hilare,* while thiamethoxam and acetamiprid were more toxic to *E. servus*. In soybean field efficacy trials, dinotefuran performed comparably to the organophosphates and pyrethroids.

Laboratory bioassays with organic insecticides resulted in moderate to high levels of mortality, and in antifeedant and repellency responses. Likewise, soybean field trials indicated that a single application can reduce stink bug numbers for up to two days after treatment; however in tomato field trials multiple weekly applications did not result in significant reductions in stink bug damage.

A weather model to predict abundance of *A. hilare* adults was developed using weekly black light trap catch data collected from 1990 to 2007 at a single location. The two weather variables that resulted in a significant model were days below freezing and mean monthly precipitation from January to April. The model was validated by

correlating five independent data sets to predicted weekly trap catch. Mean trap catch plotted over time showed three peaks. In accordance with *A. hilare* developmental rates, the peaks indicated that two generations and a partial third occur in Virginia. Cumulative trap catch estimated from the 18-yr trap catch mean showed that 10, 50, and 90% of the total seasonal catch should occur by 153, 501, and 1066 degree days, respectively.

Acknowledgements

I would like to express my appreciation to my major professor, Dr. Ames Herbert. He has guided me through my graduate studies with encouragement, support and patience. Dr. Herbert's knowledge and enjoyment of his profession will certainly influence my career for the rest of my life.

I also wish to thank my committee members, Dr. Thomas Kuhar, Dr. Douglas Pfeiffer, Dr. Donald Mullins, and Dr. Randy Huckaba, for all their support and guidance. I would especially like to express my sincere gratitude to Dr. Thomas Kuhar for all the additional assistance and encouragement. Without the benefit of his time and knowledge, my graduate research wouldn't have gone as smoothly. I am also very appreciative of Dr. Carlyle Brewster. Dr. Brewster wasn't on my committee, but spent hours assisting in the analysis of my data with a jovial attitude, especially when things went wrong.

I am indebted to Dr. Sean Malone and Mike Arrington for all the extra time spent on my research. We spent many hours bug collecting and poring over methodology and presentations. This research would also not have been possible without the field crew from Tidewater Agricultural Research Station. Rebecca Arrington and Amanda Ashton were always a positive addition to hot summer field work. This research was supported with funding provided by the Virginia Agricultural Council and the Virginia Soybean Board.

Thanks to all the faculty, staff and students at Virginia Tech. Their kindness and encouragement created a positive environment for my graduate studies. To all my friends in Blacksburg and beyond, you were my rock and my distraction. The banter and laughs always added up to a wonderful time.

Most importantly, I want to thank my mom, Joanne Kamminga, and brother, David Kamminga, for their support and encouragement though this chapter in my life. They have taught me to work hard and always maintain an optimistic outlook in all aspects in life. Thank you for always being there for me.

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Introduction

Stink bugs (Hemiptera: Pentatomidae) are significant economic pests of many agricultural crops and are frequently one of the most difficult pest groups to control in crops such as cotton, soybean, tomato and many fruit crops. Historically in Virginia, two species *Acrosternum hilare* (Say) and *Euschistus servus* (Say) have predominated in most crops. Information on the species complex in crops and the efficiency of different sampling techniques is not current in Virginia or for the Mid-Atlantic United States. This basic information is critical to developing new integrated pest management (IPM) practices. Additionally, two relatively new exotic species, *Halyomorpha halys* (Stål), the brown marmorated stink bug, and *Piezodorus guildinii* (Westwood), the redbanded stink bug, have become established in agricultural crops in nearby states. Identification of the species complex and efficient sampling techniques for stink bugs will provide valuable information that can be used in pest management in Virginia.

Insecticides in the organophosphate and pyrethroid classes are the most commonly used to control stink bugs in most crops. However, these insecticides are broad spectrum toxicants that may have environmental and human health risks and frequently destroy important natural enemies in the agro-ecosystem. Newer insecticide classes such as the neonicotinoids and others may offer control options that are less toxic and disruptive to natural enemies, and may fit better with IPM programs. Laboratory bioassays and field trials were conducted in soybean and tomato to evaluate the efficacy of selected insecticides on stink bugs.

There is an increasing demand for organic food and fiber based products. Currently, there is little known about the efficacy of Organic Materials Review Institute (OMRI) approved organic insecticides against common stink bug pest species. Some organic insecticides, along with inducing mortality, also may have sublethal effects such as hormonal disruption impacting development and reproduction (i.e., IGRs), repellency and antifeedancy. This project evaluated the efficacy of selected organic insecticides using laboratory bioassays, and repellency and feeding preference studies. These studies focused on two of the most common stink bug pest species, *A. hilare* and *E. servus*. Understanding the overall effects of organic insecticides should lead to improved stink bug management by organic growers.

In Virginia, *A. hilare* adults are caught in large numbers in black light traps that are used to monitor the activity of various agricultural pests. In this project, weather variables and degree

day models were used to develop models to predict mean weekly black light trap catch, periods of peak activity, and to estimate number of generations occurring in a season. Results of these different research projects have increased our understanding of stink bug biology and management.

Soybean and cotton research presented within this dissertation identifies stink bug species present in row crops in Virginia, and the best sampling methods for stink bugs in soybean. It also evaluates the efficacy of conventional and organic insecticides through the use of laboratory bioassays and field efficacy trials, and presents biological data relating to generational and developmental time of *A. hilare.* This information can improve the IPM strategies for managing stink bugs in soybean, cotton, and tomato.

Chapter One

Literature review

History, significance and biology of stink bugs

Importance and relevance. Stink bugs (Hemiptera: Pentatomidae) are native insects throughout the United States and have been reported as agricultural pests in Virginia since the 1880's (Curtis 1889). Stink bugs are significant economic pests of many agricultural crops and have become one of the most difficult pest groups to control in crops such as cotton, soybean, tomato (Greene et al. 2001, Gore et al. 2006) and many fruit crops (McPherson and McPherson 2000). Stink bugs usually attack developing fruiting structures such as soybean pods, cotton bolls and fruit of many vegetables. Direct damage to the fruit is caused by insertion of the stylets to suck plant fluids. Additionally, feeding sites provide entry points for pathogenic and decay organisms, which can cause great damage to the plant (Underhill 1934).

The most common stink bugs found in Virginia are the green stink bug, *Acrosternum hilare* (Say), and brown stink bug, *Euschistus servus* (Say) (Day and Kuhar 2003). Stink bugs are strong fliers and this allows them to move quickly between their wide array of host crops making them difficult to detect (McPherson and McPherson 2000). For these reasons and due to a multiple-species complex, stink bugs can be extremely difficult to manage.

Acrosternum hilare, the green stink bug, first described in 1831, is found throughout North America and is native to the United States. *A. hilare* is polyphagous and feeds on a variety of plants, but prefers woody plants (McPherson 1982). The first record of the green stink bug as a pest in Virginia was documented in 1889 (Curtiss 1889), who reported it on green beans *Phaseolus vulgaris* L. *A. hilare* is similar in appearance to *Nezara viridula* (L.), but can be differentiated by a longer ostiolar canal and the presence of a pointed abdominal spine (McPherson 1982).

Stink bugs are very sensitive to temperature. They are generally immobile around and below 10 ºC, have some activity on or below 20 ºC, and are very active above 24 ºC (Underhill 1934). *A. hilare* overwinter as adults under leaves and brush in the forest (Underhill 1934) and emerge from diapause once the temperatures exceed 18 ºC (McPherson 1982).

In the 1930's it was originally believed that only a single generation per year of *A. hilare* occurred in Virginia (Schoene and Underhill 1933, Underhill 1934). Currently, *A. hilare* is thought to have two generations a year in Virginia, although it has not been documented for this

state. McPherson and Weber (1990) recorded three distinct peaks of *A. hilare* activity in the black light traps for a season in southern Illinois. They stated that if *A. hilare* were bivoltine, the overwintered adults would reproduce on wild hosts in the spring resulting in their offspring being collected in the traps in June and July, during the first peak. Thus, the second generation collection would occur during the second peak in September and October. However, their data were inconclusive and they also reported that it was possible that the first peaks during June and July represented overwintered adults. With further research, McPherson and Tecic (1997) discovered that it is bivoltine in southern Illinois; therefore, Virginia, at a similar latitude and comparable plant growth zones, may also contain bivoltine *A. hilare*. It has also been reported as being bivoltine in South Carolina (Jones and Sullivan 1982), Arkansas (Miner 1966), and Kansas (Wilde 1969). This pest may be bivoltine in southern areas with favorable conditions, while in northern areas only univoltine (Sailer 1953).

Little research has been done to establish whether overwintered adults disperse into agricultural fields before or after copulation. Javahery (1990) reported that *A. hilare* mates after flight from overwintering sites. However, Miner (1966) wrote that first generation insects remain on wild hosts, and lab-reared *A. hilare* exhibit a preovipositional period of 43 d. This length of time appears to be abnormally long as compared to a report by Sailer (1953), who wrote that *A. hilare* had a preovipositional period of approximately 3 d, and this observation is similar in other pentatomids. *Euschistus heros* (F.) oviposits approximately 11 d after copulation (Costa et al. 1998), *Halyomorpha halys* (Stål) after 13 d (Nielsen 2008), *N. viridula* after 6 d (Alam and Ahmed 1992), and *Acrosternum marginatum* (Palisot de Beauvois) after 10 d (Hallman et al. 1992); each species is directly affected by temperature.

The ovipositional period of overwintered *A. hilare* adults begins in the middle of June and ends the first week of September; peak egg laying period is the third week in July (Underhill 1934, Javahery 1990). Time between egg mass depositions usually decreases after the first mass is laid (Underhill 1934, Nielsen 2008). The heaviest populations of first generation egg masses are found near the woods subsequent to adult emergence in the spring. This generation continues to feed on weedy hosts and migrates into nearby crops once they become succulent. The second generation remains in the cropping system throughout its life stages (Miner 1966).

Females can lay a new egg cluster every 8-10 d which is deposited vertically in clusters of 1 to 72, usually to the underside of leaves. Eggs are barrel-shaped and change from a light

green to yellow and then to light pink before hatching in about 2 wk (Miner 1966). There are five nymphal instars and adulthood is reached after approximately 36 d (Underhill 1934, Miner 1966). The first instars do not feed and remain clustered together around the egg mass. Second instars are less gregarious and begin to feed. Fourth and fifth instar feeding can result in as much economic damage to the plants as adults (Barbour et al. 1988).

Euschistus servus, the brown stink bug, is also found through-out North America and is a common pest of fruiting and field crops. There are several *Euschistus* species in North America, but *E. serv*us is of the most economic importance (McPherson and McPherson 2000). It is polyphagous and feeds on grasses and shrubs and prefers fruit and pods (McPherson and McPherson 2000). *E. servus* has two subspecies. *E. s. servus* (Say) occurs in the southern part of the United States. *E. s. euschistoides* (Vollehoven) thrives throughout Canada and the northern part of the United States. The two subspecies overlap in the area that extends from Maryland to Kansas (Sailer 1954, McPherson 1982). *E. servus* also has been found to be bivoltine, but may be univoltine in Canada and parts of the northern United States (Munyaneza and McPherson 1993, McPherson and McPherson 2000).

Euschistus servus also overwinters as an adult, but usually under crop residues and weeds, especially common mullein, *Verbascum thapsus* (L.). Following emergence in the spring in mid to late April, they are typically found on their main host plant, mullein (Munyaneza and McPherson 1993). However, Buntin and Greene (2004) have reported an abundance of brown stink bugs in winter wheat. Brown stink bugs may complete a generation in winter wheat before moving into later maturing nearby crops as they become attractive.

Female brown stink bugs deposit eggs on the host plant in clusters of approximately 21 eggs per cluster laid in multiples of seven. The eggs hatch usually around day five. The brown stink bug also has five nymphal instars, completing its development in an average of 33 d. Individual instar length varies highly with temperature (Rolston and Kendrick 1961).

Damage

Tomato. In the United States, the tomato, *Solanum lycopersicum* (L.) or *Lycopersion* spp., industry consists of two sectors: fresh market tomatoes and processing tomatoes. California is the largest producer of processing tomatoes (Davis et al. 1998), whereas, only fresh market

tomatoes are grown in Virginia. In 2007, more than 2,200 ha of fresh market tomatoes were cultivated in Virginia with a value of over 66 million dollars (USDA: NASS 2008).

Additionally, Nault and Speese (2002) conducted a survey of tomato pests in eastern Virginia from 1998-2000, and documented that stink bug crop injury was an important factor reducing marketable fruit yield of tomatoes especially in the spring crop. In response to the large pest complex, the majority of tomato growers are using IPM practices for insect control (Davis et al. 1998).

Stink bug feeding on tomatoes results in an injection of toxin into the fruit which can result in a spongy white area rendering it unmarketable for the fresh market. Stylet insertion also leads to premature ripening and smaller fruit (Lye et al. 1988). Several species of stink bugs are economic pests of the tomato (Lye et al. 1988, Lye and Story 1988, Zalom et al. 1997, McPherson and McPherson 2000). Zalom et al. (1997) reported that increasing stink bug numbers results in higher numbers of damaged fruit. However, the comparison of two common stink bugs at the same density, *Euschistus conspersus* (Uhler) and *Chlorochroa sayi* (Stahl), did not result in a significant difference in punctures. Lye et al. (1988) indicated that with increasing stink bug density there is a marked decrease in tomato size and an increase in stylet sheath numbers. Stylet sheaths are salivary deposits left by pentatomids when feeding, and have been reported by Bowling (1980) as an indicator of feeding occurrence. Lye and Story (1988) reported that *N. viridula* prefers green to red tomatoes, based on counting stylet sheaths. Fruit size, however, had no effect of feeding occurrence by stink bugs on tomatoes.

Soybean. Soybean, *Glycine max* (L.), is native to China and was introduced in the 1700's to the United States, which has since become the largest producer and exporter in the world (USDA: ERS 2005). In 2007, 200,000 ha of soybean were planted in Virginia. The majority of this crop was exported to Asia. The total value of this soybean production is estimated at 136 million dollars (USDA: NASS 2008) signifying the importance of this crop to Virginia. As a legume, soybean is commonly used in crop rotation for nitrogen fixation. In western parts of the world, most soybeans are grown for oil and animal feed; however, the soybeans grown in Asia are for human consumption including tofu and meal (Liu 1997).

The selection of the variety from the different maturity groups of soybeans largely depends on latitude because flowering is photoperiod sensitive. In the United States, there are 13 maturity groups geographically ranging from Texas to Canada resulting in early and later

maturing soybean. The soybean maturity selected for a region is based on its response to the photoperiod resulting in full season soybeans (Scott and Aldrich 1983, Liu 1997).

Every soybean production area in the world is associated with at least one economically damaging stink bug (Todd and Herzog 1980). *A. hilare*, along with *N. viridula* and *E. servus*, is one of the top three stink bug pests to attack soybeans in the Mid-Atlantic region (McPherson 1982). Stink bug feeding preference varies with the developmental progress of the crop. The insects typically move into a soybean field after flowering, remain through maturity, and migrate to more nutritious plants once the fruit is no longer succulent. Stink bug feeding can cause green stem syndrome, a plant response that results in green stems past maturity. Feeding also reduces the seed quality, slows the maturity of the plant and decreases bean germination with the introduction of pathogens, and decreases soybean yield (Underhill 1934, Emfinger et al. 2001, Medrano et al. 2007). Seeds may be rejected by importers if stink bug damage is present (Chyen et al. 1992), resulting in economic loss to the growers.

Cotton. Cotton, *Gossypium* spp. (L.), is native to subtropical regions of the world, and documents recording its use have been found dating back 2000 yr. Cotton is a perennial plant that is grown as an annual through destructive harvesting at the end of the season (USDA 1896). Currently, the United States is the third largest producer of cotton after China and India, with about 3.8 million ha in 2008 (USDA: NAAS 2008). As a crop, it is grown for production of fiber as well as cottonseed oil.

Since the early 1900's stink bugs have been considered to be pests of cotton (Morrill 1910). Stink bugs feed on young, tender cotton bolls resulting in damaged seeds, lint, and lower yield (Barbour et al. 1988, Barbour et al. 1990). However, feeding damage is difficult to discern externally. Historically, stink bugs were controlled by broad spectrum insecticide sprays used to control the cotton pest complex (Greene et al. 1997). Recently, stink bug populations in cotton have increased due to the boll weevil, *Anthonomus grandis* Boheman, eradication program and adoption of *Bacillus thuringiensis* Berliner, *B.t.,* cotton varieties, with concomitant reduction in insecticide applications.

Boll weevil eradication program. The boll weevil was an emerging pest in the late 1800's (USDA 1896), after having crossed over from Mexico in 1893. It was reported by Leiby (1928) that the damage to cotton crop by the boll weevil in 1927 reached 40% loss in some states. By the 1940's, new synthetic insecticides helped control the bollworm and boll weevil

pests which allowed for greater expansion of cotton production (Barbour et al. 1988). However, the boll weevil continued to cause great economic losses for cotton growers for the next several decades.

In 1978, the boll weevil eradication trial (BWET) began on 6,000 ha of cotton located in North Carolina and Virginia. After substantiating population numbers via pheromone trap capture, spray treatments were aerially applied at levels in accordance with the beetle population (Bachelor et al. 1995). Sterile males were also released in an attempt to decrease the next generation of weevils. After successful completion of the first year of the BWET, the program moved to Georgia, Florida, Alabama, and Arizona (Ganyard et al. 1979).

The BWET and the IPM strategies for boll weevil eradication have lead to an increase in cotton production. This program has benefited farmers by increasing the profitability of their cotton harvest, partly by decreasing the number of insecticide sprays and increasing yield through the eradication of the destructive pest. In 1978 there were only 81 ha of cotton in production in Virginia and by 2008 there were over 24,000 ha (USDA: NASS 2008).

***B.t.* gene.** In recent years, cotton varieties with the *B.t.* gene have gained acceptance with growers. It is estimated that up to 87% of the cotton acreage is now planted with *B.t.* varieties that contain Cry 1 Ac, Cry 2 Ab and/or Cry 1 F proteins (J. Faircloth, personal communication) to control many lepidopteron pests. *B.t.* crops are also available for the control of some coleopteran pests; however, they are not effective on the boll weevil and are not offered for cotton.

The successful eradication of the boll weevil and the introduction of *B.t.* cotton varieties have led to a decreased spray environment. This, in turn, has elevated stink bugs to a higher pest status in cotton production (Barbour et al. 1988, Bacheler et al. 1995, Bundy and McPherson 2000). Stink bugs are not controlled by *B.t*. crops, and with a reduced spray environment due to fewer insecticides being applied to control the boll weevil and lepidopteron pests, they are now greater in numbers (Bundy et al. 1998).

Management options

Chemical control. In the late 1800's and early 1900's, poisonous chemicals like rotenone (Underhill 1934) and kerosene emulsion (Curtiss 1889) were used for stink bug control. More recently, other highly toxic insecticides including methyl parathion and methamidophos

were used in cotton crops for stink bug management (Chyen et al. 1992, Cullen and Zalom 2007). In 1996, Congress requested the EPA to introduce the Food Quality Protection Act to reassess pesticide tolerances and safety issues. This act led to the review and reassessment of the food safety laws in the United States (US EPA 1999) and the reevaluation of all pesticides by the EPA resulting in their use being phased out of certain crops.

Organophosphates (such as acephate, methamidophos, and dicrotophos) or pyrethroids (such as λ-cyhalothrin, ζ-cypermethrin, cyfluthrin, fenpropathrin, esfenvalerate, or permethrin) are the most commonly used insecticides to control stink bugs in most crops (Kuhar et al. 2006, Herbert 2008). Organophosphates are effective on *Euschistus*, while both organophosphates and pyrethroids are frequently used to control *Acrosternum* (Willrich et al. 2003). *E. servus* has been reported as having a higher LC_{50} value than *A. hilare* for pyrethroids and organophosphates (Green et al. 2001, Snodgrass et al. 2005).

Within the insecticide classes there are also variations of response/mortality of the stink bugs. Willrich et al. (2003) determined that acephate was more toxic to the brown stink bug than dicrotophos. However, Snodgrass et al. (2005) found the opposite. Oxamyl is less effective against *E. servus* than dicrotophos; while acetamiprid, a neonicotinoid, has little activity against *E. servus* (Tillman and Mullinix 2004). Mixed results have also been reported for the efficacy of another neonicotinoid, thiamethoxam, against *E. servus* adults and nymphs (Willrich et al. 2003, Tillman and Mullinix 2004). The field efficacy trials by Cullen and Zalom (2007) indicated that a pyrethroid and neonicotinoid mix is not more effective than a pyrethroid alone against *Euschistus conspersus* (Uhler). Differences in the susceptibility of the stink bug life stages to insecticides are also common (McPherson et al. 1979, Willrich et al. 2002, Willrich et al. 2003). McPherson et al. (1979) and Willrich et al. (2002) reported that LD_{50} for late instar nymphs of *E. servus*, *A. hilare*, and *N. viridula* were higher than the adult stage.

Integrated pest management programs are being used for stink bug control in agricultural systems (Gore et al. 2006, Cullen and Zalom 2007). This includes researching neonicotinoids as alternative control methods for stink bugs in the Mid-Atlantic region. Newer insecticides may have greater efficacy on stink bugs found in Virginia's crops, and may offer an alternative to the older insecticides that are known to disrupt the natural enemy population. This would fit better with integrated pest management programs (Tillman and Mullinix 2004, Carvalho et al. 2006).

Cultural control. Trap crops are commonly used to confine a pest species to an area outside of the core cropping system. This area can then be treated with insecticides to inhibit the movement of the pest into the main crop. Due to its attractiveness to stink bugs, soybean may be a possible trap crop for cotton (Bundy et al. 1998). Green stink bugs are more numerous in early maturing soybean varieties. This increases the feasibility that early maturing soybean could also be an effective trap crop for later maturing soybean (Todd and Schumann 1988, Bundy et al. 1998). Resistant varieties of soybean have been shown to have a lower population of stink bugs than other varieties (Jones and Sullivan 1979, Gilman et al. 1982).

Biological control. Stink bugs are vulnerable to predacious and parasitic insect activity resulting in their mortality. Most of the parasitoids are the tachinids. There are also several egg parasitoids including *Trissolcus*, *Anastatus*, and *Telenomus* species. Lacewing larvae and the spined soldier bug, *Podisus maculiventris* (Say), are common predators of stink bugs (Underhill 1934, McPherson 1982, McPherson and McPherson 2000). These predators and parasitoids are good options for biological control of stink bugs. Birds have also been reported to feed on stink bugs (Underhill 1934).

There has been one successful documented case of using biological control to maintain stink bugs below the economic threshold in soybean. Corrêa-Ferreira and Moscardi (1996) released 15,000 *Trissolcus basalis* (Wollaston), resulting in control of *E. heros*, *Piezodorus guildinii* (Westwood), and *N. viridula,* reducing their populations by more than 50%. This maintained the population below the economic threshold during vulnerable growth stages.

Organic control. Botanical insecticides have been in use for over 2,000 years throughout the Middle East and Asia, and in the United States for about 150 years (Isman 2006). In the 1930's and 1940's, synthetic insecticide production and use became widespread. However, these insecticides are broad spectrum toxicants that frequently destroy important natural enemies in the agro-ecosystem (Casida 1980, Letourneau and Goldstein 2001, Tillman and Mullinix 2004). Some, like DDT, introduced deleterious effects on the environment causing harm to raptorial birds through bioaccumulation of the insecticide (Barbour et al. 1988, Isman 2006). The publication of Rachel Carson's *Silent Spring* in 1962 brought awareness and increased concerns for the environment causing the public to lobby for organic products and produce (Kristianesen et al. 2006).

With the establishment of the Organic Foods Production Act of 1990, the USDA National Organic Program (NOP) was created to regulate all aspects related to domestic organic agriculture and certification of imports. The Organic Materials Review Institute (OMRI) also became invested as a not for profit organization that determines materials appropriate for production of organic commodities following the guidelines set forth by the NOP. Standardization of natural insecticides is difficult because natural products produce natural variations. The establishment of these agencies has resulted in organic pest control compounds that are certified in accordance with governmental standards (Fetter and Caswell 2002). Organic growers now have options for pest control in cultivating certified organic produce. The three commonly used organic active ingredients are: pyrethrins, azadirachtin, and spinosad.

Pyrethrin. This natural insecticide originated in Eastern Europe, and is produced through the grinding of the dried flower, *Chrysanthemum cinerariaefolium* (Trevir). Pyrethrin has been used as an insecticide since before 1694 and became established in the United States and Japan in the late 1800's (Katsuda 1999). Cultivation and production of this botanical insecticide peaked in Japan during the 1940's, but in recent days the Japanese have been surpassed by East African countries in its production (Casida 1980, Katsuda 1999).

The flower grinding and extraction process produces an oleoresin (Isman 2006). This compound acts on the insect nervous system as a sodium channel modulator. The chemical prevents the closing of the sodium channel, the result of which is membrane depolarization, leading to convulsions and death. Pyrethrin has a rapid knockdown effect if the insects come into direct contact with the insecticide; however, in lower concentrations it can be used as a repellent (Glynne-Jones 2001). Due to rapid chemical break down by sunlight, pyrethrin has short residual activity (Casida 1980).

Spinosad. Spinosad is produced by fermentation of the soil bacterium, *Saccharopolyspora spinosa* Mertz and Yao 1990, which produces spinosyns A and D (Salgado 1998). The first application as an insecticide was in 1997, which was confirmed to be effective on many orders, primarily Lepidoptera, Diptera and Thysanoptera. However, it is moderately toxic to some beneficials such as bees, aquatic insects, as well as mollusks (Cisneros et al. 2002). Furthermore, some parasitoids were found to be highly susceptible to spinosad, especially Braconidae and Trichogrammatidae (Williams et al. 2003).

Spinosad acts on the nervous system of the insects as an acetylcholine agonist by continuously activating the cholinergic receptor. It is effective mainly through digestion and occasionally contact (Salgado 1998, Cisneros et al. 2002). This insecticide is subject to photodegradation and also has a low mammalian toxicity (Thompson et al. 2000).

Spinosad, formulated as Tracer (Dow AgroSciences LLC., Indianapolis, IN), in laboratory evaluations on predatory pentatomids has resulted in mixed results (Boyd and Boethel 1998, Viñuela et al. 1998, Budia et al. 2000, Baur et al. 2003, Mahdian et al. 2007). The differences in these findings likely result from the use of different species in the bioassays. In field trials, spinosad has not been found to be as effective against stink bug pest species in the cotton and millet systems (Bell et al. 1999, Buntin et al. 2007) as the broad spectrum counterparts.

Azadirachtin. The neem tree, *Azadirachta indica* A. Juss, native to Southeast Asia, is used in the production of two types of insecticides. Neem oil is produced by cold pressing seeds, and triterpene azadirachtin is manufactured from the neem seed residue (Schmutterer 1990, Isman 2006). The active ingredient of these products, azadirachtin, is an insect growth regulator (IGR) with anti-feeding and anti-oviposition properties (Shutter 1990). It inhibits the production of a necessary hormone, ecdysone, for chitin synthesis, causing deformation in the insects at molts (Ladd et al. 1978). Beneficial aspects of neem include low toxicity for natural enemies and mammals, and selectivity towards phytophagous insects (Weathersbee 2005, Isman 2006). Azadirachtin has been proven to be effective on chewing insects, however, its effectiveness on piercing sucking insects needs to be studied further (Riba et al. 2003).

The majority of hemipteran research with azadirachtin has been completed using *N. viridula* which feed less on food treated with azadirachtin (Seymour et al. 1995, Abudulai et al. 2003). Abudulai et al. (2003) counted stylet sheaths on azadirachtin treated cowpeas, as explained by Bowling (1979, 1980), and reported that azadirachtin-treated beans contained significantly fewer feeding punctures compared with the untreated control. Consequently, it is believed that azadirachtin acts as an antifeedant. Azadirachtin is also an ovipositional deterrent and growth regulator (Durmusoglu et al. 2003, Riba et al. 2003) for *N. viridula* in the laboratory. As an IGR, mortality is highest when applied directly to nymphs resulting in deformed adults and nymphs (Schmutterer 1990, Riba et al. 2003). When used against adults, Durmusoglu et al. (2003) suggested that it may act as an antifeedant. Barry et al. (2005) also indicated that

azadirachtin did not significantly repel blueberry maggot adults, *Rhagoletis mendax* Curran, when given a choice between a treated and untreated resting place. In field trials, Abudulai et al. (2003) determined that *N. viridula* populations were reduced in cowpea for up to 10 d after treatment with azadirachtin.

Sub-lethal effects of organic insecticides. Isman (2006) reported that it is difficult to prove field efficacy of botanical insecticides solely based on antifeedant effects. Compounds that may deter feeding by one insect may be a feeding stimulant for another. In addition, flying insects are able to leave a feeding deterrent, whereas, larvae may persist until the antifeedant stimulus diminishes. Conversely, the disruption of normal feeding stimuli can lead to an increased probing by insects by chemicals on treated food sources (Reuter et al. 1993). Azadirachtin treatments may result in a greater difficulty in finding a proper feeding site, effectively increasing the probing puncture numbers by piercing-sucking insects. Chapman (1974) researched the chemical inhibition of feeding and indicated that failure to feed could result from lack of phagostimulants and not a feeding inhibitor. Feeding inhibition occurs from the stimulation of deterrent cells or blocking the phagostimulant receptors of the insect. Toscano et al. (1997) determined that whitefly species demonstrate an aversion to pyrethrins in choice testing arenas. However, the results of azadirachtin-treated leaves varied by species and did not consistently deter insect oviposition and resting. Barry et al. (2005) tested several organic insecticides on the blueberry maggot adults and concluded that the azadirachtin treatment did not significantly repel the flies compared with the control. Furthermore, the azadirachtin, spinosad, and pyrethrin treatments did result in mortality to the flies when tested as a topical and residual exposure.

Prediction and monitoring

Using weather variables to predict insect catch. Insect movement and migration are directly affected by weather variables (Kennedy and Storer 2000). Tauber et al. (1986) reported that insects in areas of variable conditions can experience increased mortality from ending diapause prematurely. Consequently, insects experiencing excess freezing temperatures may remain in diapause until the weather fluctuations stabilize thereby decreasing their likelihood of mortality. Researchers also determined that exposure to cold temperatures may lead to acclimation resulting in a lower supercooling point and decreased mortality (Casagrande and

Haynes 1976, Elsey 1993). The supercooling point has not been determined for *A. hilare,* but is -15 ºC for *E. servus* and -11 ºC for *N. viridula* (Elsey 1993). Additionally, Hanec (1966) and Raske (1975) indicated that increased spring mortality after a cold winter may be partly attributed to a decrease in the hosts resulting in starvation. This can occur if the cold temperatures retard the emergence of vegetative growth. The insects that remain for a longer length of time in diapause after a harsh winter reduce their chance of starving.

Weather variables have been successfully used to predict insect catch in black light traps (Rodríguez-del-Bosque and Magallanes-Estala 1994, Sutherland and Baharally 2003, Flinn et al. 2004, Zou et al. 2004, Onstad et al. 2005, Edde et al. 2006, Samietz 2007, Grüebler et al. 2008). Insect development is directly related to temperature and is affected by upper and lower developmental thresholds (Tauber and Tauber 1976). These thresholds have been used to develop degree day models for forecasting critical life history events of many important pest species. Several types of degree day models have been developed including averaging (Arnold 1960), single sine, double sine (Allen 1976), single triangle (Lindsey and Newman 1956), and double triangle (Sevacherian et al. 1977). To decrease complexity and increase consistency in research, Pruess (1983) stated that either the actual day degrees or sine wave estimates should be used in degree day calculations. An evaluation of several degree day calculation methods was completed by Roltsch et al. (1999). The researchers reported that in cooler months degree day estimations resulted in the most variation between the methods; while the warmer months resulted in similar results. Their research indicated that the more complicated models, consisting of the double triangle and double sine method, did not result in a more accurate estimate of degree days than the simpler single sine and single triangle method. This conclusion resulted in the single sine and triangle method being the most frequently used models since they are less complicated, yet as accurate.

Phenology models have been established for several insect pests to predict emergence, e.g. the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, (Zou et al. 2004), emergence of some *Ephestia* species (Ahmad and Ali 1995), flight of Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), (Malinoski and Paine 1988) and European pine shoot moth, *Rhyacionia buoliana* (Denis and Schiffermuller), into pheromone traps (Regan et al. 1991). Also, phenology models have been developed to predict population abundance of the strawberry bud weevil (Bostanian 1999) and the consperse stink bug (Cullen and Zalom 2000). Developmental

thresholds have been established for several stink bug species including *N. viridula* (Egwuatu and Ant 1986), *A. hilare* (Simmons and Yeargan 1988), *E. servus* (Munyaneza and McPherson 1993), and the rice stink bug, *Oebalus pugnax* (F.) (Rashid et al. 2005). The developmental threshold for *A. hilare* was presented by Simmons and Yeargan (1988) as 15 ºC for the lower threshold, 33 ºC for the upper threshold, and 27 ºC as the optimal temperature. Identifying the upper and lower thresholds of *A. hilare* permits an appropriate degree day model to be calculated. In Virginia stink bugs are caught in large numbers in black light traps, the majority of which are *A. hilare*. Therefore, black light traps may be useful in monitoring stink bugs by improving scouting methods and control recommendations. Predictions of *A. hilare* catch in the black light have not been done. As an important pest of many crops, construction of a model for *A. hilare* catch will aid in its management and control.

Stink bug species survey in soybean and cotton. In recent years, little information has been generated regarding stink bug species in the Mid-Atlantic Region. The most current and comprehensive publication by Hoffman (1971) discusses the pentatomids identified within the state and includes minor updates to the series published in 1994 and 2002. Other published works have reported stink bug distributions in Virginia (McPherson 1980, McPherson 1982, McPherson and McPherson 2000), but there is little detailed information available within these more current publications. Moreover, the stink bug species complex in Virginia may be changing

In the southern United States soybean ecosystem, *N. viridula* is the most commonly encountered stink bug (Gore et al. 2006), while to date it has not been detected in Virginia. The introduction of invasive species into the United States, most notably *H. halys* may cause a change in the soybean pest populations in the United States. A documented pest of soybean in its host range, it was introduced from Asia into Allentown, Pennsylvania in 1996 (Hoebeke and Carter 2003) and by 2006 it was reported to be causing economic damage to fruit trees in Pennsylvania (Nielsen et al. 2008). The first specimen of the brown marmorated stink bug in Virginia was recorded in southwestern Virginia in 2004 (Eric Day, personal communication). A single specimen was captured in a black light trap in southeastern Virginia in 2006 and 2007. Furthermore, large populations were reported in northern Virginia and Roanoke in 2007.

Piezodorus guildinii is native to Brazil and is also an invasive pest of soybean (McPherson and McPherson 2000); however in the 1980's and 1990's it was not considered to

be a serious threat in the United States. In recent years its population has become more established in the southern United States as it spreads northward from South and Central America into Louisiana and Florida (Panizzi et al. 2002). Researchers have found this insect to be more economically damaging than the native southern green stink bug, raising concerns for growers (Correa-Ferrerira and Azevedo 2002). Global warming may also result in changes to the ecosystem (Kiritani 2007) resulting in shifts in species distribution.

Sampling methods for stink bugs. Insect sampling methods include absolute, relative and indirect. Absolute sampling methods measures all insects per a unit of area, i.e., on a plant, in a row or a known amount of soil. Relative sampling methods estimate insect numbers based on sampling techniques, for example the sweep net. This permits comparisons between numbers of an insect per a number of sweeps but is not on an area basis (Pedigo 1998). Indirect sampling measures insect products such as frass and damage. Pheromone and black light traps are also types of indirect sampling and are commonly used in insect surveys (Todd and Herzog 1980, McPherson and McPherson 2000).

Sampling of insects on crops is traditionally to determine pest densities in relation to economic thresholds. When pest populations reach their economic threshold, insecticides are commonly used for insect management. Sweep net and beat sheet sampling methods are recommended for monitoring stink bugs in cotton and soybean (Todd and Herzog 1980). The sweep net method requires a designated number of sweeps per sample, while the beat sheet is a sample per a length of row.

Race (1960) published the first known report on sampling stink bugs in cotton. Using a mechanical device and a 38 cm sweep net, he concluded that the mechanical device did collect more stink bugs than the sweep net, but it was not consistent enough to form a ratio between the sampling methods. Bowling (1969) found a direct correlation between stink bugs per sweep to total stink bug population in rice fields. Sane et al. (1999) reported a low correlation between absolute sampling and the cloth and vertical beat sheet methods, but high correlation between the sweep net method and absolute method in both drilled (narrow row) and conventionally (wide-row) planted soybeans. Lower variation was found for the sweep net method than the beat sheet sampling method for narrow row and conventionally planted soybean. Conversely, Todd and Herzog (1980) stated that the beat sheet method closely represents the absolute measurement of the stink bug population. Rudd and Jenson (1977) and Sane et al. (1999) also compared the

sweep net and beat sheet and determined that the sweep net was the most economical method of sampling in relation to time and precision. Rudd and Jensen (1977) also concluded that more observations are required using the beat sheet for an accurate count of the stink bug population. Consequently, the beat sheet method is the least efficient method of sampling.

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Chapter Two

A survey of stink bug species in soybean and cotton in southeastern Virginia and assessment of field sampling methods

Abstract

Soybean (9 counties, 19 fields) in 2005 (21 counties, 33 fields) in 2006 and cotton in 2005 (8 counties, 19 fields) were sampled bimonthly during the growing seasons. A 38-cm diameter sweep net (25 sweeps/sample) and a 0.9-m beat sheet (1.8 row-m/sample) were used to sample in a Z-shaped pattern across fields. Seven stink bug species were found in soybean and cotton fields during this two year study. These included *Euschistus servus* (Say), *Acrosternum hilare* (Say), *Thyanta custator accera* McAtee, *Euschistus tristigmus* (Dallas), *Oebalus pugnax* (F.), *Euschistus variolarius* (Palisot de Veauvois) and *Podisus maculiventris* (Say). Of the seven, six were considered pest species capable of doing economic damage. *Nezara viridula* (L.), and invasive species *Halyomorpha halys* (Stål) and *Piezodorus guildinii* (Westwood) were not detected in the Mid-Atlantic region soybean or cotton ecosystem during the survey. The majority of the stink bugs species found were *E. servus* and *A. hilare*. In soybean in 2005, significantly more *E. servus* than *A. hilare* were captured with both sampling methods. In cotton in 2005, there was no significant difference in the capture rate between the two species for either sampling method. In 2006, significantly more *A. hilare* were captured in soybean by both sampling methods. A linear regression of sampling methods for both single sample observation and field mean indicated high within field variability. The results indicate that the sweep net is a less variable sampling method than the beat sheet method; however, neither method could accurately predict stink bug populations in the field.

Key words: sweep net, beat sheet, sampling, stink bugs

In recent years, little information has been generated regarding stink bug species found in the Mid-Atlantic United States. The most current and comprehensive publication by Hoffman (1971) discusses the pentatomids identified within Virginia and includes minor updates to the series published in 1994 and 2002. Other published works have reported stink bug species distributions in Virginia (McPherson 1980, McPherson 1982, McPherson and McPherson 2000),

but there is little detailed information available within these more current publications on the species present in soybean or cotton. Moreover, the stink bug species complex in Virginia may be changing.

In the southern United States soybean ecosystem, *Nezara viridula* (L.) (the southern green stink bug) is the most commonly encountered stink bug (Gore et al. 2006), while to date, it has not been detected in the Mid-Atlantic region. The introduction of invasive species into the United States, most notably *Halyomorpha halys* (Stål) (the brown marmorated stink bug) may cause a change in the soybean pest populations in the United States. A documented pest of soybean in its host range, it was introduced from Asia and first documented in Allentown, Pennsylvania in 1996 (Hoebeke and Carter 2003). By 2006 it was reported to be causing economic damage to fruit trees in Pennsylvania (Nielsen et al. 2008). The first brown marmorated stink bug in Virginia was recorded in southwestern Virginia in 2004 (personal communication, Eric Day). In 2006 and 2007 single specimens were captured in black light traps in southeastern Virginia. Furthermore, large populations were reported in northern Virginia and Roanoke in 2007. *Piezodorus guildinii* (Westwood) (the redbanded stink bug) is native to Brazil and is also an invasive pest of soybean (McPherson and McPherson 2000); however in the 1980's and 1990's it was not considered to be a serious threat in the United States. In recent years it has become more established in the southern United States as it spreads northward from South and Central America into Louisiana and Florida (Panizzi et al. 2002). Researchers have found this insect to be more economically damaging than the native southern green stink bug raising concerns for growers (Correa-Ferrerira and Azevedo 2002). Global warming may also result in changes to the ecosystem (Kiritani 2007) validating the importance for baseline species documentation data.

Sweep net and beat sheet sampling methods are recommended for monitoring stink bug abundance in cotton and soybean (Todd and Herzog 1980). Todd and Herzog (1980) stated that the beat sheet method of sampling closely represents the absolute measurement of a stink bug population. Conversely, Sane et al. (1999) reported a low correlation between the absolute sampling method and both the beat sheet and the rigid beat sheet sampling methods. However, they found a high correlation between the sweep net and the absolute sampling methods in both drilled (narrow row spacing) and wide row spacing planted soybean, thereby indicating that the sweep net is the most reliable at estimating the absolute number of stink bugs. Lower variation

in numbers of stink bug caught was also determined for the sweep net than the beat sheet sampling method for both planting systems. A similar assessment was made by Rudd and Jenson (1977) who concluded that the sweep net was the most economical method of sampling in soybean for *N. viridula*.

In this study, intensive sampling of soybean and cotton in southeastern Virginia was done to provide baseline information on the stink bug species and to compare the beat sheet and sweep net sampling methods for determining stink bug numbers.

Materials and Methods

Survey and sampling methods. Nineteen soybean fields in nine counties were sampled in 2005, and 33 fields in 21 counties in 2006. Nineteen cotton fields in eight counties were sampled in 2005 (Fig. 2.1). All fields were sampled bimonthly during the growing seasons. Soybean fields were either planted in wide (76 or 91 cm) or narrow row (18 cm) spacings. Sampling began in early July once plants were large enough to sweep with a net, and continued until late August (2005) or mid September (2006). A 38-cm diameter sweep net (25 sweeps/sample) and a 0.9-m beat sheet (1.8 row-m/sample) were used to sample in a Z-shaped pattern across fields (Kogan and Herzog 1980). A cloth beat sheet was used in fields with 76 – 91 cm row centers and a rigid beat sheet was used in fields with narrower row spacing. The rigid beat sheet consists of a 0.9-m long by 0.86-m wide mesh screen attached to a wooden frame with a 10-cm wide edge. This method is commonly used in place of the cloth beat sheet in narrow-row soybeans. Five to 20 samples were taken per field (both sweep net and beat sheet) depending on the density of the population of stink bug in the fields. If high numbers of stink bugs were found during the first five samples, sampling in these fields were continued. If low numbers were found in the first five samples, sampling was discontinued. Substantially more stink bugs were found in soybean than cotton in 2005 leading us to continue the survey only in soybean in 2006. The focus was also shifted to earlier maturing soybean varieties to intercept more of the stink bug population.

The number of stink bugs captured per sample and the growth stage of the crop were recorded in each field on each sample date. Adult stink bugs were collected and identified to species using taxonomic keys in McPherson (1982), McPherson and McPherson (2000), and with assistance from Dr. David Rider of North Dakota State University.

Data analysis. Mean numbers of total stink bugs as well as numbers of *Acrosternum hilare* (Say) and *Euschistus servus* (Say) collected by each sampling method were evaluated using a paired t-test. Data were transformed using the $\sqrt{(x+0.5)}$ transformation.

A linear regression was completed using the model $y = \beta x + \alpha$, where y is the number of stink bugs caught by beat sheet; x is the number of stink bugs caught by sweep net; and β and α are regression coefficients (Rudd and Jensen 1977). Regression by single sample observation was analyzed as y representing the number caught by a single beat sheet sample (1.8 row-m) and x as the number captured for the equivalent sweep net sample (25 sweeps). The regression by field mean was analyzed as y representing the mean number of insects caught per beat sheet sample in a single field and x as the mean of the number caught per 25 sweep net sample in the same field.

Results

Species Complex. Seven stink bug species were found in soybeans and cotton during this two-year study. Of the seven, six were considered pest species capable of doing economic damage. These economically damaging stink bugs included *E. servus*, *A. hilare*, and the lesser encountered stink bugs *Thyanta custator accera* McAtee (the redshouldered stink bug), *E. tristigmus* (Dallas) (the dusky stink bug), *Oebalus pugnax* (F.) (the rice stink bug), and *E. variolarius* (Palisot de Veauvois) (the one spotted stink bug). *Podisus maculiventris* (Say) (the spined soldier bug), was also found and is a predator that regularly feeds on a variety of soft-bodied insects (Underhill 1934). The two most common stink bug species found in both years and both cropping systems were *E. servus* and *A. hilare*. *E. servus* represented 63% of the species captured in soybean in 2005, 45% in cotton in 2005, and 24% in soybean in 2006 (Table 2.1). *A. hilare* represented 30% of those captured in soybean in 2005, 45% in cotton in 2005, and 69% in soybean in 2006. *N. viridula, H. halys,* and *P. guildinii* were not found in the survey.

Stink bug numbers were more numerous in soybean in 2006 than 2005 (Table 2.2). In 2005, more stink bugs were caught in soybean than cotton. There was no significant difference between sampling methods for either crop or year.

Sampling methods. Further analysis of the data illustrated differences in the capture rates for *A. hilare* and *E. servus* between the sampling methods (Table 2.3). In soybean in 2005, significantly more *E. servus* than *A. hilare* were captured with both sampling methods. In cotton in 2005, there was no significant difference between the numbers caught of the two species for either sampling method. In soybean in 2006, there were significantly more *A. hilare* captured by both sampling methods. In cotton, the beat sheet was more effective than the sweep net in catching *E. servus* (0.03 ± 0.01 and 0.01 ± 0.01). Conversely, the sweep net was more effective in sampling *A. hilare* (0.02 ± 0.01 and 0.03 ± 0.01). In soybean in 2005, neither the sweep net nor the beat sheet was substantially more effective at catching either species. During the 2006 field season, soybean was the only crop surveyed. The most common species captured for both sampling methods in 2006 was *A. hilare.* More *A. hilare* were captured with the beat sheet or sweep net (0.50 ± 0.05 and 0.58 ± 0.05). However, fewer *E. servus* were collected with the beat sheet than the sweep net (0.21 ± 0.03 and 0.18 ± 0.02).

A linear regression of the sweep net against beat sheet methods was performed by single sample observation and field means (Table 2.4). This regression was calculated to determine if one sampling method could estimate the number of stink bugs captured by the other sampling method. There was an overall increase in R^2 when analyzing by observation to field means. Attempts at separating the data by species did not result in a higher R^2 value.

Discussion

The stink bugs found included *E. servus*, *A. hilare*, *T. c. accera*, *E. tristigmus*, *O. pugnax*, *E. variolarius*, and the predator *P. maculiventris. A. hilare* and *E. servus* were the most abundant stink bug species found in cotton and soybean in southeastern Virginia in 2005 and 2006. More stink bugs were captured in soybean than cotton. The mean number of stink bugs per sample for both species was higher for 2006 than 2005 possibly because of the focus on early maturing soybean varieties. McPherson et al. (1988) reported that the row spacing and planting date did not influence stink bug numbers, however, early maturing soybean varieties are highly attractive to stink bugs and attract in higher populations than late maturing soybean varieties (Bundy et al. 1998, McPherson et al. 2003).

The invasive *H. halys* and *P. guildinii*, as well as the nonnative stink bug to Virginia, *N. viridula,* were not found in soybeans in southeastern Virginia during 2005 and 2006. While two

specimens of *H. halys* have been captured in a black light trap in southeastern Virginia (Kamminga, unpublished data), large populations have not been reported in soybean. Additionally, *P. guildinii* has not yet been documented in Virginia, but has been documented in Louisiana, Florida (Panizzi et al. 2002) and South Carolina (Jones and Sullivan 1982). Continued monitoring of stink bug species in soybean and cotton will be important for documenting any species shifts in the Mid-Atlantic region.

The two most common methods of sampling stink bugs in soybean are the sweep net and the beat sheet (Todd and Herzog 1980). Rudd and Jensen (1977) compared these two methods and determined that the sweep net was the most economical method in relation to time and precision. Turnipseed et al. (1974) showed that the beat sheet was the most reliable method of sampling soybean; however, Sane et al. (1999) determined that the sweep net was more precise in wide row spacing planted soybeans. Rudd and Jensen (1977) concluded that more observations are required when using the beat sheet to determine an accurate count of stink bugs making it a less efficient method of sampling. My analysis of sampling methods found no significant difference between the sampling methods in soybean or cotton.

The regression of sampling methods for both observation and field mean indicated high within field variability. A low R^2 was calculated for both years of sampling. In addition, the lack of correlation by field mean indicates that the population estimated by one sampling technique may not provide a reliable estimate of the stink bug species population by the other sampling technique. This result conflicts with Rudd and Jensen (1977) who found little correlation during the analysis of observation but high correlations when analyzing by field means.

The results indicate that while there is less variability with the sweep net compared with the beat sheet, I was still unable to accurately predict the stink bug populations in the field. Future surveys should focus on what would be required to obtain a more accurate estimate of the stink bug population density.

Acknowledgements

I would like to thank the following individuals at Virginia Tech for technical assistance with the field research: H. Doughty, A. Koppel, R. Arrington, and M. Arrington. I'd also like to thank

Dr. David Rider for his assistance in stink bug species identification. This research was supported by a grant from the Virginia Agricultural Council and the Virginia Soybean Board.

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Table 2.1. Percentage of total stink bug species found for sweep net and beat sheet sampling methods in soybean and cotton in 2005, and in soybean in 2006 in southeastern VA.

Species		Soybean 2005		Cotton 2005		Soybean 2006	
		Beat sheet[1]	Sweep net[2]	Beat sheet	Sweep net	Beat sheet	Sweep net
E. servus	adults	51	53	69	25	15	17
	nymphs	14	8	0	0	13	6
A. hilare	adults	21	22	23	63	27	32
	nymphs	8	8	0	0	41	40
T. c.	adults	0	2	0	0	4	3
accera	nymphs	0	0	0	0	< 1	1
P. maculiventris		3	3	0	6	< 1	1
E. tristigmus		3	3	8	6	1	< 1
O. pugnax		0	0	0	0	0	1
E. variolarius		0	0	0	0	0	< 1
Total Bugs Found		88	95	13	16	629	683
Sample Number		374	374	320	320	833	833

Abbreviations are for the species: *Euschistus servus* (Say), *Acrosternum hilare* (Say), *Thyanta custator accera* McAtee, *Euschistus tristigmus* (Dallas), *Oebalus pugnax* (F.), *Euschistus variolarius* (Palisot de Veauvois) and *Podisus maculiventris* (Say).

[1]Beat sheet is 1.8 row-m/sample.

[2]Sweep net is 25 sweeps/sample.

Table 2.2. Comparison of mean number of stink bugs captured per sample by each sampling method in cotton and soybean in 2005, and soybean in 2006 in southeastern VA.

Crop	Year	Method	N	Mean	*P*-value
Soybean	2006	Beat sheet[1]	833	0.81 ± 0.03	0.71
		Sweep net[2]	833	0.76 ± 0.05	
Cotton	2005	Beat sheet	320	0.04 ± 0.01	0.56
		Sweep net	320	0.05 ± 0.01	
Soybean	2005	Beat sheet	374	0.24 ± 0.03	0.41
		Sweep net	374	0.25 ± 0.03	

P-value determined by a t-test.

[1] Beat sheet is 1.8 row-m/sample.

[2]Sweep net is 25 sweeps/sample.

Table 2.3. Comparison of mean number *Acrosternum hilare* and *Euschistus servus* captured per sample for both sampling methods in cotton and soybean in 2005 and soybean in 2006 in southeastern VA.

				Beat sheet[1]		Sweep net[2]	
Crop	Year	Species	N	Mean	*P*- value	Mean	*P* -value
Soybean	2006	*E. servus*	833	0.21 ± 0.02	<0.001	0.18 ± 0.02	<0.001
		A. hilare	833	0.50 ± 0.05		0.58 ±0.05	
Cotton	2005	*E. servus*	320	0.03 ± 0.01	0.078	0.01 ± 0.01	0.175
		A. hilare	320	0.01 ± 0.01		0.03 ± 0.01	
Soybean	2005	*E. servus*	374	0.15 ± 0.02	0.001	0.16 ± 0.02	0.003
		A. hilare	374	0.07 ± 0.02		0.08 ± 0.02	

P-value determined by a t-test.

[1] Beat sheet is 1.8 row-m/sample.

[2]Sweep net is 25 sweeps/sample.

Table 2.4. Regression of the sweep net against the beat sheet method by single sample observation and total field mean.

		Regression by observation				Regression by field mean			
Crop	Year	N	β	R^2	R^2 adj	N	β	R^2	R^2 adj
Soybean	2005	374	0.08	0.35	0.34	34	0.001	0.40	0.38
Cotton	2005	320	0.03	0.10	0.10	33	0.03	0.13	0.10
Soybean	2006	833	0.75	0.0003	-0.00009	45	0.34	0.29	0.28

A linear regression was completed using the model $y = \beta x + \alpha$, where y is the number of stink bugs caught by beat sheet; x is the number of stink bugs caught by sweep net; and β and α are regression coefficients (Rudd and Jensen 1977).

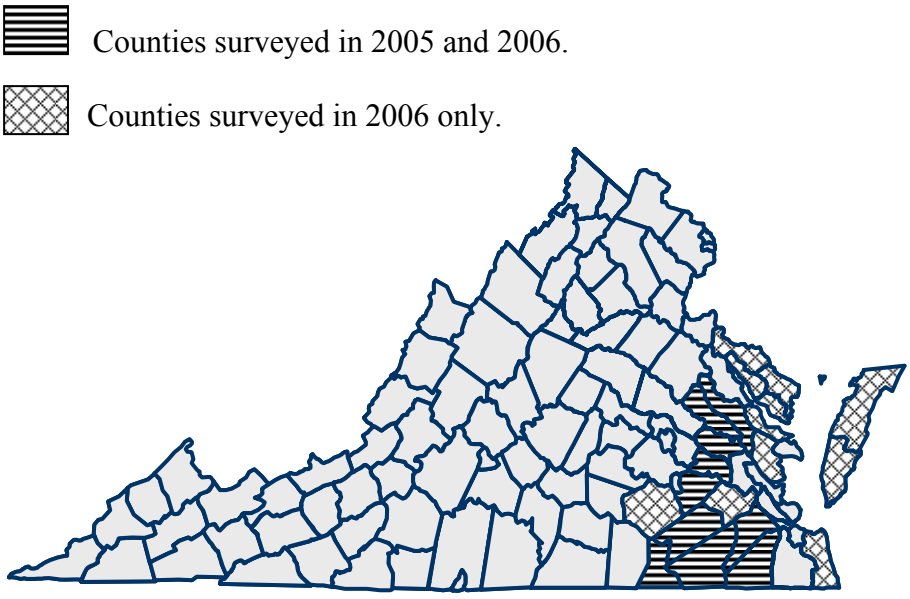

Fig. 2.1. Virginia counties surveyed for stink bug species during the 2005 and the 2006 seasons. Cotton fields (2005) were surveyed in the counties: Charles City, Greensville, Isle of Wight, King and Queen, King William, Southampton, Suffolk, and Sussex. Soybean fields (2005) were surveyed in the counties: Charles City, Chesapeake, Greensville, Isle of Wight, New Kent, Prince George, Southampton, Suffolk, and Sussex. Soybean fields (2006) were surveyed in the counties: Accomack, Charles, Chesapeake, Dinwiddie, Gloucester, Greensville, Isle of Wight, King and Queen, King William, Lancaster, Middlesex, New Kent, Northampton, Richmond, Southampton, Suffolk, Surrey, Sussex, Virginia Beach, West Moreland, and Northumberland.

Chapter Three

Efficacy of insecticides against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae) in Virginia and North Carolina[1]

Abstract

Laboratory bioassays and field trials were conducted to evaluate the efficacy of selected organophosphate, pyrethroid, and neonicotinoid insecticides, as well as a chitin inhibitor, novaluron, against two common stink bug pests in Virginia, the green stink bug, *Acrosternum hilare* (Say), and the brown stink bug, *Euschistus servus* (Say). Green bean dip bioassays revealed differences in insecticide susceptibility between the two species. *A. hilare* adults were highly susceptible to all pyrethroids tested, the organophosphates except acephate, and the neonicotinoids except acetamiprid. *A. hilare* nymphs were also susceptible to all pyrethroids tested. In general, the neonicotinoids, dinotefuran and clothianidin, were toxic to *A. hilare,* while thiamethoxam and acetamiprid were toxic to *E. servus*. In field trials in soybean, the neonicotinoids, dinotefuran, imidacloprid, and thiamethoxam were efficacious at controlling stink bugs and, in general, performed comparably to the organophosphates and pyrethroids. These results indicate that neonicotinoid insecticides offer an alternative to growers for managing stink bugs that may fit with integrated pest management programs where conservation of natural enemies is a consideration.

Key words. stink bugs, chemical control, selective insecticide, efficacy

Stink bugs (Hemiptera: Pentatomidae) are important economic pests of many agricultural crops and have become one of the most difficult pest groups to control in crops such as cotton, soybean, and tomato (McPherson and McPherson 2000, Greene et al. 2001, Gore et al. 2006) and many fruit crops (McPherson and McPherson 2000). Stink bugs usually attack developing fruiting forms, i.e., soybean seed, cotton bolls, and fruit of many vegetables. Direct damage to the fruit is caused by insertion of the stylets to suck plant fluids. Additionally,

[1] A manuscript based on this research was accepted for publication in *Journal of Entomological Science* 21 July 2008.

feeding sites provide entry points for pathogenic and decay organisms which can damage the plant (Underhill 1934, Emfinger et al. 2001)

Organophosphates (such as acephate, methamidophos, and dicrotophos) or pyrethroids (such as λ-cyhalothrin, ζ-cypermethrin, cyfluthrin, fenpropathrin, esfenvalerate, or permethrin) are the most commonly used insecticides to control stink bugs in most crops (Kuhar et al. 2006, Herbert 2008). However, these insecticides are broad spectrum toxicants that have environmental and human health risks and frequently destroy important natural enemies in the agro-ecosystem (Letourneau and Goldstein 2001, Tillman and Mullinix 2004). The efficacy of alternative insecticide classes for stink bug control have been evaluated in the southern United States (Willrich et al. 2004, Snodgrass et al. 2005), where the southern green stink bug, *Nezara viridula* (L.), is the most commonly encountered species (Gore et al. 2006), and where the brown stink bug, *Euschistus servus* (Say), is notoriously difficult to control (Emfinger et al. 2001, Snodgrass et al. 2005). In the Mid-Atlantic and northeastern United States, the green stink bug, *Acrosternum hilare* (Say) and *E. servus* comprise over 90% of the stink bug species on crops, and *N. viridula* is not found (Kamminga, unpublished data). Moreover, insecticide efficacy data on stink bugs are lacking for this region of the U.S.

Integrated pest management programs are being used for stink bug control in agricultural systems (Willrich et al. 2004, Gore et al. 2006, Cullen and Zalom 2007). Newer insecticide classes such as the neonicotinoids and others may offer control options that are less toxic and disruptive to natural enemies, and may fit better with integrated pest management programs (Tillman and Mullinix 2004, Carvalho et al. 2006). The purpose of this research was to evaluate the efficacy of commonly used insecticides as well as the newer chemistries against stink bug pest species common to the Mid-Atlantic region using laboratory bioassays and field trials.

Materials and Methods

Laboratory bioassays. Five bioassays were conducted with treatments representing a range of insecticide chemistries evaluated (Table 3.1). The first and second bioassays were conducted on *A. hilare* nymphs (4th and 5th instars) collected from soybean fields in Painter, VA (75°49'W, 37°35'N; elevation ≈ 12 m) on 12 September 2006 and from a commercial soybean field near Cheriton, VA (75°58'W, 37°17'N; elevation ≈ 5 m) on 25 September 2006. The two populations represented two separate bioassays. Insecticide concentrations were selected to

simulate recommended field rates. Bioassays were conducted using a bean-dip technique (Abudulai et al. 2003). Green beans, *Phaseolus vulgaris* L., were rinsed 3X in water, air dried, then dipped into insecticide/water solutions based on a 317.9 liter total volume ha^{-1} field application rate for 30 s. Pods were air dried for 1 h on a paper towel before being presented to the stink bugs. Nymphs were placed into Petri dishes (10 x 1.5 cm) with one pod from a respective treatment. Five nymphs in each of four replicates were assayed for each insecticide treatment and a control (water only). Petri dishes were maintained in a laboratory insectary at 27 ± 2°C, 40-70% RH, and a photoperiod of 12:12 (L:D). Numbers of live, dead, or "knocked down" stink bugs were determined after 72 h of exposure. Insects were considered "knocked down" if they appeared intoxicated (e.g., slow moving) but were able to right themselves when turned on their backs.

In 2007, a third bioassay was conducted using *E. servus* adults collected from a wheat field in Havelock, NC (76°54'W, 34°52'N; elevation ≈ 6 m) on 11 June. Five *E. servus* adults were placed in Petri dishes with a total of five replicates for each of nine insecticide treatments and a control (water only). The fourth and fifth bioassays were completed using *A. hilare* adults collected from a commercial soybean field in Camden, NC (76°20'W, 36°32'N; elevation ≈ 3 m) on 14 August and 28 September. The experimental methods, design, and analysis were the same as those used in 2006 with a total of four replicates per treatment.

2005 field efficacy trial. A field efficacy trial was conducted in soybean, *Glycine max,* (L.), at Painter, VA. This field contained greater than three stink bugs per 0.91 m (1 per 1 row foot) of row threshold for stink bugs in soybeans (Herbert 2008). The experiment was arranged in a randomized complete block design with four replicates and included an untreated control. Individual plots were four rows wide (0.76 m spacing) and 12.2 m long with an untreated border row on each side. Treatments representing a range of insecticide chemistries were evaluated (Table 3.2) including classes that are known to be effective against stink bugs (Greene et al. 2001, Willrich et al. 2003, Tillman and Mullinix 2004). Insecticide treatments were applied on 24 August using a CO_2-pressurized backpack sprayer calibrated to deliver 133.7 liters ha^{-1} at 1.22 atm using four 8002VS spray nozzles spaced 45.7 cm apart on the spray boom. Plots were evaluated at 2, 5, and 8 d after treatment (DAT) using two, 0.91 m rigid beat sheet (Kogan and Herzog 1980) samples per plot. The total number of *A. hilare* and *E. servus* adults and nymphs was recorded.

2006 field efficacy trials. In August 2006, two field efficacy trials were conducted in commercial soybean fields located in Camden, NC. Both fields also were found to have greater than three stink bugs per 0.91 m row (1 per 1 row foot), the threshold for stink bugs in soybeans (Herbert 2008). Plots were five rows wide (0.61 m spacing) and 12.2 m long with two untreated border rows on each side. The experimental design, treatment application methods, and sampling method were the same as those used in 2005. Insecticide treatments were applied on 7 August (Trial 1) and on 8 August (Trial 2). Stink bug populations were assessed at 2, 4, and 7 DAT (Trial 1) and 3, 6, and 10 DAT (Trial 2).

Statistical analysis. All data were analyzed using an analysis of variance procedure (PROC GLM, SAS Institute 2001). For all field trials, total number of stink bug adults and nymphs (all species combined) were compared among treatments using LSD procedures to separate means at the $P \leq 0.05$ level of significance. For laboratory bioassays, proportion mortality data were arc-sine square root transformed prior to analysis in order to stabilize variance.

Results

Laboratory bioassays. In 2006, the two bean-dip bioassays produced consistent results (Fig. 3.1). Treatment with the pyrethroids λ-cyhalothrin, ζ-cypermethrin, cyfluthrin, and fenpropathrin resulted in the highest mortality at 72 h post exposure. Dinotefuran performed equally well as the pyrethroids in bioassay 2, but was significantly less effective in bioassay 1. In contrast, clothianidin resulted in statistically similar mortality as the pyrethroids in bioassay 1, but was significantly lower in bioassay 2. Acetamiprid and methamidophos resulted in significantly lower mortality than all other treatments except the untreated control in both bioassays.

In 2007, the bioassay on *E. servus* adults resulted in similar results to the 2006 bioassays on *A. hilare* nymphs (Fig. 3.2). At 72 h post exposure, acephate, cyfluthrin, dicrotophos, clothianidin, thiamethoxam and acetamiprid resulted in the highest mortality and were not significantly different. Dinotefuran and imidacloprid were not as toxic as acephate but were statistically equal to thiamethoxam and acetamiprid. Also, λ-cyhalothrin did not perform as well as cyfluthrin.

The fourth and fifth bioassays on *A. hilare* adults resulted in high mortality for all treatments except acetamiprid and acephate (Fig. 3.3). Acetamiprid and acephate resulted in 85% mortality; both were numerically lower than the other insecticides tested.

2005 field efficacy trial. Two stink bug species were present in this trial, 70% *A. hilare* (86% nymphs, 14% adults) and 30% *E. servus* (56% nymphs, 44% adults). Treatments resulted in significant differences in total stink bug numbers for all days sampled after treatment. At 2 DAT, all treatments had significantly lower numbers than the untreated control (F=8.67; df=13, 39; P<0.0001) (Table 3.3). At 5 DAT, numbers of stink bugs in the imidacloprid, cyfluthrin, and acetamiprid treatments were not significantly different than in the control (F=2.77; df=13, 39; P=0.007). At 8 DAT, numbers of stink bugs in the dinotefuran treatment at 0.10 kg ai ha^{-1} was also not significantly different than the untreated control.

When averaged over the three post treatment dates, there was a significant difference in total number of stink bugs among treatments (F=9.91; df=13, 39; P<0.0001) (Table 3.3). The application of acephate, dicrotophos, dinotefuran, cyfluthrin, and oxamyl at high rates had significantly fewer stink bugs than novaluron, a chitin inhibitor, at 0.07 kg ai ha^{-1} and acetamiprid at 0.05 kg ai ha^{-1} (Table 3.3). The tank mix of novaluron and acephate did not perform significantly better than acephate alone, though it did perform significantly better than novaluron at the higher rate.

2006 field efficacy trials. For both trials, the two most common species present were 90% *A. hilare* (28% nymphs and 72% adults) and 10% *E. servus* (75% nymphs and 25% adults). In trial 1, all treatments except novaluron resulted in significantly lower stink bug numbers than the untreated control on all sample dates. Unlike results from 2005, dinotefuran and imidacloprid significantly decreased stink bug numbers at each DAT (Table 3.4). The numbered compounds GF-1796 and V-10191, and the chlorpyrifos and γ-cyhalothrin resulted in fewer stink bugs compared with the carbamate (oxamyl) at 0.28 kg ai ha^{-1}. Across all sample dates, all treatments performed significantly better than the untreated control. Dinotefuran at 0.04 kg ai ha^{-1} was found to be as efficacious as all pyrethroids and organophosphates evaluated and performed significantly better than the other neonicotinoids evaluated (Table 3.4). The least efficacious insecticides were imidacloprid and thiamethoxam at the 0.07 kg ai ha^{-1} and 0.06 kg ai ha^{-1}, respectively, followed by novaluron at 0.07 kg ai ha^{-1}.

In trial 2, all insecticide treatments had significantly lower stink bug numbers at 3 and 6 DAT than the untreated control, except novaluron at 0.07 kg ai ha^{-1} (Table 3.5). These results were similar to the field efficacy trials in 2005 and 2006 (Trial 1). Only three treatments were found to be effective at 10 DAT, the λ-cyhalothrin and thiamethoxam tank mix at 0.03 and 0.04 kg ai ha^{-1}, λ-cyhalothrin at 0.05 kg ai ha^{-1}, and the novaluron and dicrotophos tank mix at 0.03 and 0.28 kg ai ha^{-1}. Across all sample dates, all insecticides evaluated had significantly lower stink bug numbers when compared to the untreated control (Table 3.5). Dicrotophos at 0.28 kg ai ha^{-1} did not show a significant treatment effect when applied with novaluron at the 0.03, 0.04, or 0.07 kg ai ha^{-1}. However, the novaluron and dicrotophos tank mix treatments had significantly lower stink bug numbers than novaluron at 0.07 kg ai ha^{-1}.

Discussion

In general, results of field and laboratory trials were consistent in demonstrating the efficacy of selected pyrethroids, organophosphates, and neonicotinoids on the stink bugs, *A. hilare* and *E. servus*. Of the neonicotinoids evaluated, dinotefuran generally resulted in the highest mortality and acetamiprid the lowest. Results of the bioassays were consistent with Willrich et al. (2003) showing that the organophosphate acephate was numerically more toxic to *E. servus* adults than dicrotophos. *Acrosternum hilare* nymphs were found to be more susceptible to the pyrethroids λ-cyhalothrin and cyfluthrin than *E. servus* adults. *Acrosternum hilare* nymphs were very susceptible to all pyrethroids tested. *Acrosternum hilare* adults were highly susceptible to all pyrethroids tested, all organophosphates tested except acephate, and all neonicotinoids tested except acetamiprid. There may be differences between susceptibility of the life stages (Willrich et al. 2003) resulting in differences between the mortality of *A. hilare* nymphs and adults. In general, dinotefuran and clothianidin were toxic to *A. hilare* adults and nymphs, while thiamethoxam and acetamiprid were toxic to adult *E. servus*.

Data from field trials in soybean showed that all insecticides tested can provide control for up to 10 d. However, none of the treatments exhibited total control in the field due to possible reinvasion, natural fluctuations, or egg hatch which was not directly measured. The organophosphates and pyrethroids tended to be more effective than the neonicotinoid, acetamiprid, and the chitin inhibitor, novaluron. The field efficacy trials supported the assessment by Cullen and Zalom (2007) who showed that a pyrethroid and neonicotinoid mix is

not more effective than a pyrethroid alone against *Euschistus conspersus* (Uhler). The chitin inhibitor, novaluron, did not prove to be as effective as most other treatments, unless tank-mixed with either acephate or dicrotophos. Three neonicotinoids evaluated in 2005, dinotefuran, imidacloprid, and thiamethoxam, and dinotefuran alone in 2006, were as efficacious as the organophosphates and pyrethroids. These results indicate that neonicotinoids offer an alternative for managing stink bugs that may fit with integrated pest management programs where conservation of natural enemies is a consideration.

Acknowledgments

I would like to thank the following individuals at Virginia Tech for technical assistance with the field research: H. Doughty, A. Koppel, R. Arrington, and M. Arrington. This research was supported by a grant from the Virginia Agricultural Council and the Virginia Soybean Board.

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Table 3.1. Insecticides evaluated for stink bug efficacy in laboratory experiments conducted in Virginia from 2006-2007.

Material	Product	Manufacturer	Class
acephate	Orthene 97SP	Amvac Chemical Corp.[1]	Organophosphate
dicrotophos	Bidrin 8EC	Amvac Chemical Corp.	Organophosphate
methamidophos	Monitor 4	Bayer CropScience	Organophosphate
λ-cyhalothrin	Warrior ZT	Syngenta Crop Protection, Inc.	Pyrethroid
ζ-cypermethrin	Mustang Max	FMC Corporation	Pyrethroid
cyfluthrin	Baythroid 2	Bayer CropScience	Pyrethroid
β-cyfluthrin	Baythroid XL	Bayer CropScience	Pyrethroid
fenpropathrin	Danitol 2.4EC	Valent U.S.A. Corp	Pyrethroid
acetamiprid	Assail 30SG	United Phosphorus Inc.	Neonicotinoid
clothianidin	V-10170 50WD	Valent U.S.A. Corp	Neonicotinoid
dinotefuran	Venom 20SG	Valent U.S.A. Corp.	Neonicotinoid
imidacloprid	Trimax Pro	Bayer CropScience	Neonicotinoid
thiamethoxam	Centric 40WG	Syngenta Crop Protection, Inc.	Neonicotinoid

[1] At the time of this research, Orthene 97SP was manufactured by Valent U.S.A. Corp.

Table 3.2. Insecticides evaluated for stink bug efficacy in field experiments conducted in Virginia and North Carolina from 2006-2007.

Material	Product	Manufacturer	Class
acephate	Orthene 97SP	Amvac Chemical Corp.[1]	Organophosphate
dicrotophos	Bidrin 8EC	Amvac Chemical Corp.	Organophosphate
chlorpyrifos/ γ-cyhalothrin	Cobalt	Dow AgroSciences	Organophosphate + Pyrethroid
γ -cyhalothrin	Karate 1EC + Karate Z	Syngenta Crop Protection, Inc.	Pyrethroid
cyfluthrin	Baythroid 2	Bayer CropScience	Pyrethroid
λ- cyhalothrin + thiamethoxam	Endigo ZC	Syngenta Crop Protection, Inc.	Pyrethroid + Neonicotinoid
acetamiprid	Assail 30SG	United Phosphorus Inc.	Neonicotinoid
clothianidin	V-10170 50WD	Valent U.S.A. Corp	Neonicotinoid
dinotefuran	Venom 20SG	Valent U.S.A. Corp.	Neonicotinoid
imidacloprid	Trimax Pro	Bayer CropScience	Neonicotinoid
thiamethoxam	Centric 40WG	Syngenta Crop Protection, Inc.	Neonicotinoid
novaluron	Diamond 0.83EC	Chemtura Corp.	Chitin inhibitor
oxamyl	Vydate C-LV	Dupont	Carbamate
GF-1796	----------	Dow AgroSciences	Experimental
V-10191	----------	Valent U.S.A. Corp.	Organophosphate

At the time of this research, Orthene 97SP was manufactured by Valent U.S.A. Corp.

Table 3.3. Mean number of stink bugs (70% *Acrosternum hilare* and 30% *Euschistus servus* adults and nymphs) in soybean after insecticide applications in Painter, VA, 2005[1]

Material	kg ai ha^{-1}	Mean number per 0.91 row meter beat sheet			
		2 DAT	5 DAT	8 DAT	Avg DAT
acephate	1.09	0.25e	0.25d	1.00cd	0.50e
dicrotophos	0.28	0.25e	0.38cd	1.00cd	0.54e
cyfluthrin	0.05	0.25e	1.25b-d	0.25d	0.59e
acetamiprid	0.05	1.25cd	1.50a-c	1.50b-d	1.42bc
dinotefuran	0.10	0.25e	0.88cd	2.50ab	1.21c-e
dinotefuran	0.15	0.25e	1.13cd	0.25d	0.54e
imidacloprid	0.06	0.50de	1.5a-c	0.50cd	0.84c-e
thiamethoxam	0.06	0.63de	1.13cd	1.25b-d	1.00c-e
oxamyl	0.28	0.38de	0.63cd	0.88cd	0.63e
novaluron + acephate	0.04 + 0.54	0.75c-e	0.25d	1.00cd	0.65de
novaluron	0.07	1.63bc	0.75cd	1.75bc	1.38cd
untreated	---	3.75a	2.38ab	3.75a	3.29a
LSD		1	1.23	0.14	0.72

Different letters represent significant difference as determined by ANOVA (P<0.05) and Fishers LSD.

[1]A total of 14 treatments were evaluated, but due to confidentiality agreements only 12 are reported.

Table 3.4. (Trial 1) Mean number of stink bugs (90% *Acrosternum hilare* and 10% *Euschistus servus* adults and nymphs) in soybean after insecticide applications in Camden, NC, 2006.

		Mean number per 0.91 row meter beat sheet			
Material	kg ai ha^{-1}	2 DAT	4 DAT	7 DAT	Avg DAT
acephate	0.28	2.38b-d	0.63e	1.38f-h	1.46fg
dicrotophos	0.28	0.38f	0.75de	2.00e-h	1.04g
chlorpyrifos + γ-cyhalothrin	0.28 + 0.01	2.50b-d	3.25cd	2.00e-h	2.58d-f
bifenthrin	0.05	1.38d-f	1.75c-e	1.50f-h	1.54e-g
B-cyfluthrin	0.02	1.13d-f	2.13c-e	1.38f-h	1.54e-g
λ-cyhalothrin	0.03	0.25f	0.75de	0.63gh	0.54g
λ-cyhalothrin	0.05	1.00d-f	1.25de	0.25h	0.83g
dinotefuran	0.04	0.63ef	2.25c-e	2.25e-g	1.71e-g
imidacloprid	0.07	1.75b-f	3.88c	4.38bc	3.33cd
thiamethoxam	0.06	3.13bc	4.13c	4.13b-d	3.79c
oxamyl	0.28	1.50c-f	3.00c-e	3.63c-e	2.71c-e
novaluron	0.07	3.38b	6.88b	5.75ab	5.33b
GF-1796	0.50	1.63c-f	2.63c-e	3.13c-f	2.46d-f
V-10191	0.56	2.25b-e	2.75c-e	2.50d-f	2.50d-f
untreated	---	5.50a	9.50a	7.00a	7.33a
LSD		1.66	2.52	1.82	1.2

Different letters represent significant differences as determined by ANOVA ($P<0.05$) and Fishers LSD.

Table 3.5. (Trial 2) Mean number of stink bugs (90% *Acrosternum hilare* and 10% *Euschistus servus* adults and nymphs) in soybean after insecticide applications in Camden, NC, 2006.

		Mean number per 0.91 row meter			
Material	kg ai ha^{-1}	3 DAT	6 DAT	10 DAT	Avg DAT
dicrotophos	0.28	1.25c	0.75d	1.50ab	1.17cd
λ-cyhalothrin	0.05	0.75c	0.38d	0.63b	1.13cd
λ -cyhalothrin	0.08	0.50c	0.88d	2.00ab	0.58d
thiamethoxam	0.06	3.13b	3.38b	3.00a	3.17b
λ- cyhalothrin + thiamethoxam	0.03 + 0.04	1.00c	1.00d	1.00b	1.00cd
novaluron	0.07	7.38a	2.63bc	1.88ab	3.96b
novaluron + dicrotophos	0.03 + 0.28	0.75c	1.50cd	0.88b	1.04cd
novaluron + dicrotophos	0.04 + 0.28	1.63bc	1.00d	2.75a	1.79c
novaluron + dicrotophos	0.07 + 0.28	1.25c	0.38d	2.63a	1.42cd
untreated	---	7.25a	7.00a	2.63a	5.63a
LSD		1.53	1.6	1.61	1

Different letters represent significant difference as determined by ANOVA ($P<0.05$) and Fishers LSD.

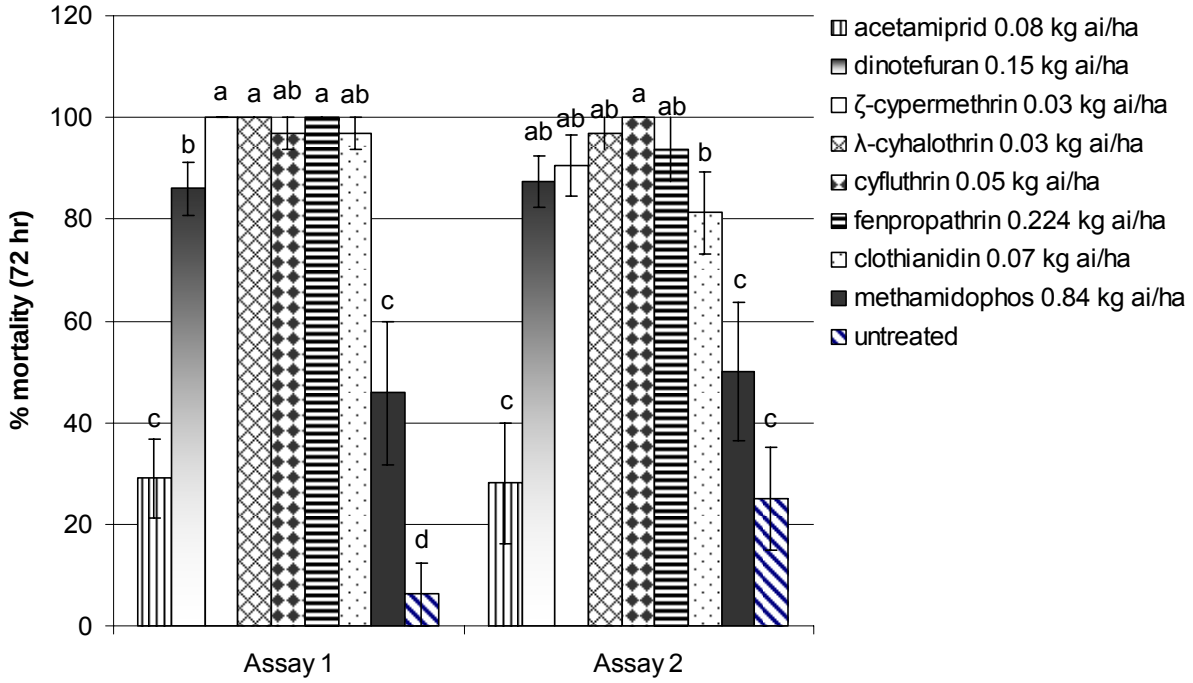

Fig. 3.1. (2006) Mean ± SE (n = 20) percent mortality of 2 bioassays (assay 1 and assay 2) of *Acrosternum hilare* nymphs collected from Cheriton, VA after 72 h exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Fishers LSD.

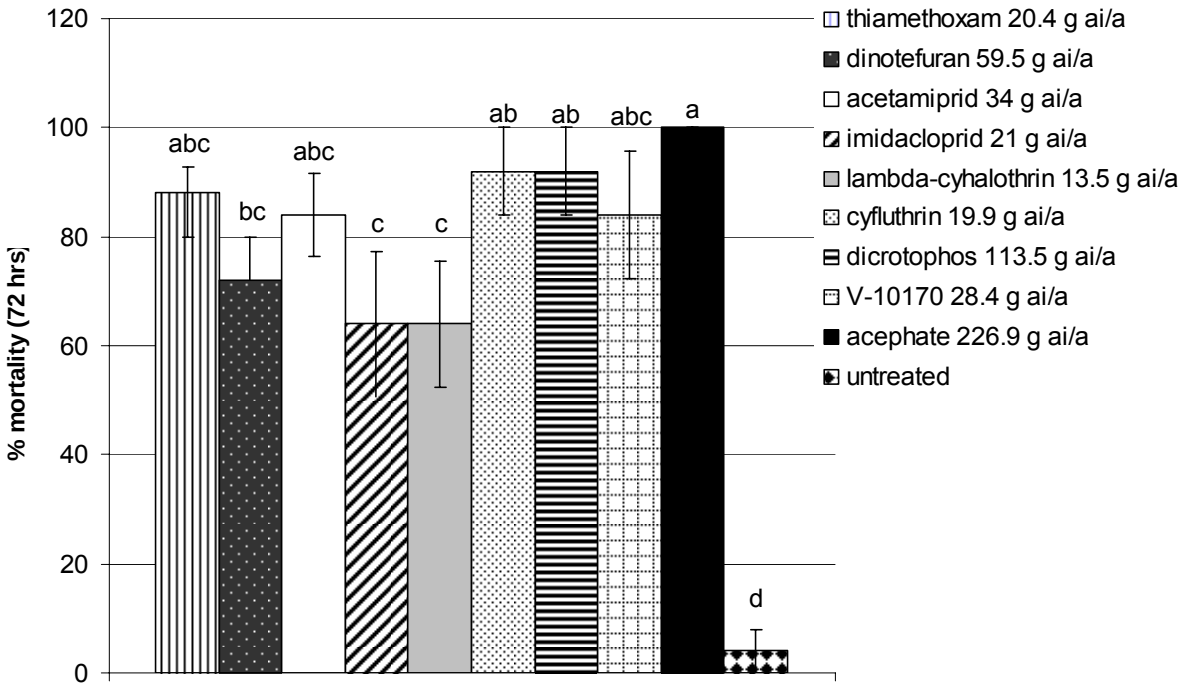

Fig. 3.2. (2007) Mean ± SE (n = 25) percent mortality of *Euschistus servus* adults collected from Havelock, NC after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Fishers LSD.

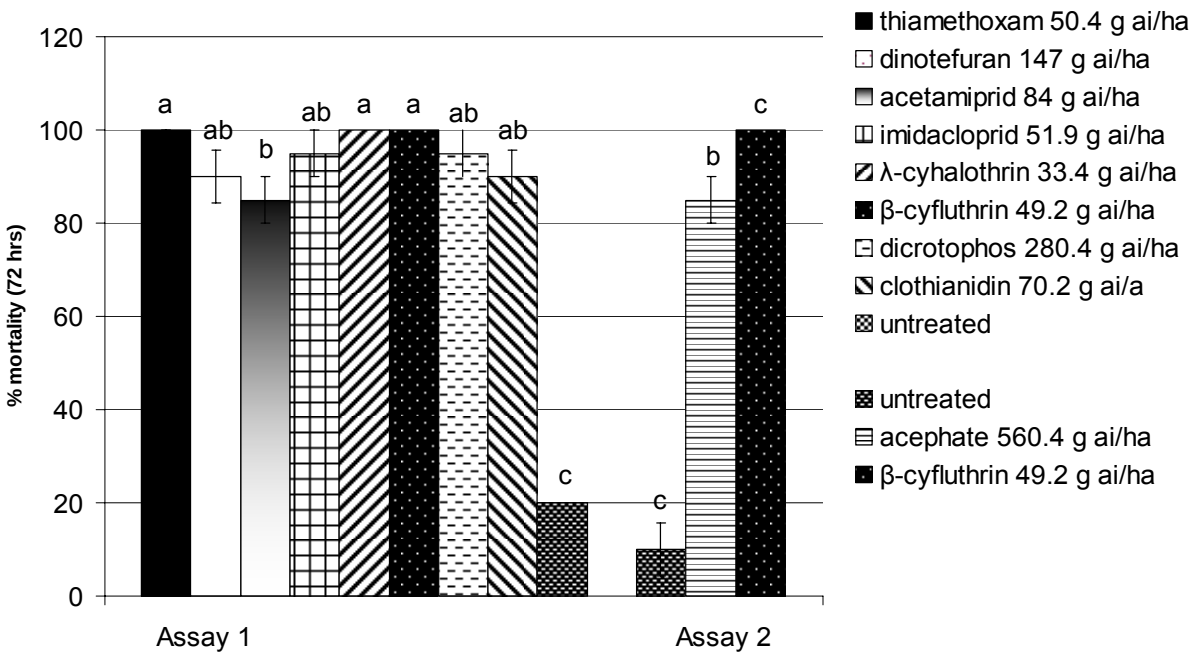

Fig. 3.3. (2007) Mean ± SE (n = 20) percent mortality of 2 bioassays (assay 1 and assay 2) of *Acrosternum hilare* adults collected from Camden, NC after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Fishers LSD.

Chapter Four

Toxicity, feeding preference and repellency of selected organic insecticides against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae).

Abstract

Experiments were conducted to evaluate the toxicity, feeding preference, repellency, and field efficacy of the organic insecticides, azadirachtin, pyrethrins, and spinosad against two stink bug species, *Acrosternum hilare* (Say) and *Euschistus servus* (Say). In laboratory toxicity bioassays using treated green bean pods, *A. hilare* was most susceptible to the conventional pyrethroid, λ-cyhalothrin as well as tank mixes of pyrethrins + spinosad. *E. servus* adults were susceptible to all treatments except azadirachtin and pyrethrins, while *E. servus* nymphs were susceptible to λ-cyhalothrin and pyrethrins. Stink bug feeding preference tests were conducted using insecticide treated tomatoes and counting the number of stylet sheaths on fruit after 24 h. Azadirachtin and pyrethrins inhibited stink bug feeding, while spinosad did not. In repellency tests conducted with filter paper half dipped into an insecticide solution, both *E. servus* and *A. hilare* were repelled by pyrethrins, exhibited no response to azadirachtin, and *E. servus* was attracted to spinosad. In field trials, each of the organic insecticides reduced the number of stink bugs in soybeans up to two days after treatment, however, none of the insecticides reduced stink bug damage to fruit in tomatoes after multiple applications.

Key words: *Acrosternum hilare*, *Euschistus servus*, spinosad, pyrethrins, azadirachtin

Stink bugs are important pests of many crops in the United States **(**McPherson and McPherson 2000). In Virginia, two species, *Acrosternum hilare* (Say) and *Euschistus servus* (Say) cause the most damage to the widest variety of crops (Nault and Speese 2002, Herbert et al. 2006). The damage to tomatoes by stink bug feeding presents as a white, spongy area on the fruit that renders it unmarketable for the fresh market and whole-peel processing (Zalom et al. 1997). In Virginia, this damage can be particularly significant for early crops of tomatoes, where up to 40% of fruit can be injured if control measures are not taken (Kuhar, unpublished data, Nault and Speese 2002). In soybeans, stink bug feeding can cause green stem syndrome, reduce the seed quality, slow the maturity of the plant and decrease bean germination with the

introduction of pathogens (Underhill 1934, Emfinger et al. 2001, Medrano et al. 2007). This feeding results in a lower quality of seed and may lead to a decrease in profit from price reduction due to seed damage and reduced yield (Chyen et al. 1992).

Management of these insects can be challenging (Chyen et al. 1992). Even in conventional agricultural crops, where most growers control the pests with multiple applications of broad-spectrum organophosphate or pyrethroid insecticides (Kuhar et al. 2006, Herbert 2008), stink bug injury can still result. For organic producers, stink bug control is an even greater challenge because there are relatively few registered insecticidal options.

Azadirachtin is a plant-derived insecticide available in several registered organic insecticide products. Azadirachtin inhibits the production of the insect hormone ecdysone, which impacts chitin synthesis and molting in many insects (Ladd et al. 1978), including the stink bug *Nezara viridula* (L.) (Abudulai et al. 2003). Research on *N. viridula* demonstrated additional effects of azadirachtin including antifeedancy (Seymour et al. 1995, Abudulai et al. 2003), ovipositional deterrence and growth regulation (Durmusoglu et al. 2003, Riba et al. 2003). In field trials, azadirachtin has also been effective at reducing the population of *N. viridula* in cowpea (Abudulai et al. 2003). Beneficial aspects of azadirachtin include a low toxicity to natural enemies and mammals, and selectivity towards phytophagous insects (Jackai et al. 1992, Abudulai et al. 2004, Weathersbee and Mckenzie 2005, Isman 2006).

Botanical pyrethrins are another organic insecticide group that cause rapid knockdown and eventual death of a wide variety of insects (Casida 1980). They have demonstrated repellency and efficacy in field trials with different insect pests such as blueberry maggot adults, *Rhagoletis mendax* Curran, and whiteflies (Toscano et al. 1997, Barry et al. 2005), but are generally not very stable in the environment and are subject to rapid photodegradation (Katsuda 1999). The efficacy of botanical pyrethrins on stink bugs has not been well studied.

Spinosad is another insecticidal option for organic growers that has demonstrated excellent efficacy against lepidopteron pests, thrips, leafminers, and certain beetle species, such as Colorado potato beetle, *Leptinotarsa decemlineata* Say. Laboratory evaluations of spinosad on predatory pentatomids have shown variable toxicity (Boyd and Boethel 1998, Viñuela et al. 1998, Budia et al. 2000, Baur et al. 2003, Mahdian et al. 2007).

The objectives of this study were to evaluate the toxicity, field efficacy, and non-lethal effects (feeding preference and repellency) of these three biologically-derived insecticides on *A.*

hilare and *E. servus*. A better understanding of the activity of organic insecticides should lead to improved stink bug management for organic growers.

Materials and Methods

Laboratory bioassays. Six bioassays using a bean-dip technique (Abudulai et al. 2003) were conducted with the organic insecticides azadirachtin (Aza-Direct at 48.4 g ai ha^{-1}; Gowan Co., Yuma, AZ), pyrethrins (Pyganic 1.4EC at 30.9 g ai ha^{-1}; McLaughlin King Co., Minneapolis, MN), and spinosad (Entrust at 168.1 g ai ha^{-1}; Dow AgroSciences, Indianapolis, IN) as well as the conventional pyrethroid, λ-cyhalothrin (Warrior ZT at 33.4 g ai ha^{-1}; Syngenta Crop Protection, Inc., Greensboro, NC). Different insecticides, alone and in combination, were assayed depending on availability of stink bugs (Table 4.1; Table 4.2). All assays included a water-only control. Insecticide concentrations were based on recommended rates per hectare for specific crops (Kuhar et al. 2006, Herbert 2008) in a total volume per hectare that simulated commonly used spray tank concentrations.

Whole green beans, *Phaseolus vulgaris* L., were rinsed three times in water, air dried, then dipped for 30 s into insecticide/water solutions based on a 317.9 liter total volume ha^{-1} field application rate. Pods were air dried for 1 h on a paper towel before being presented to the stink bugs. Insects were placed into Petri dishes (10 x 1.5 cm) with one pod from a respective treatment, and were maintained on lab benches at 27 ± 2 °C, 40-70% RH, and a photoperiod of 12:12 (L:D). Numbers of live, dead, or "knocked down" stink bugs were determined after 72 h of exposure. Insects were considered "knocked down" if they appeared intoxicated (e.g., slow moving), but were able to right themselves when turned on their backs.

Bioassays 1 and 2 were conducted using fourth and fifth instar *A. hilare* nymphs collected from soybean fields in Painter, VA (75°49'W, 37°35'N; elevation ≈ 12 m) on 12 September 2006 and from a commercial soybean field near Cheriton, VA (75°58'W, 37°17'N; elevation ≈ 5 m) on 25 September 2006. Five nymphs in each of four replicates were assayed for each treatment (Table 4.1). In 2007, bioassay 3 was conducted using *E. servus* adults collected from a wheat field in Havelock, NC (76°54'W, 34°52'N) on 11 June 2007. Five adults in each of five replicates were assayed for each treatment (Table 4.1). Bioassay 4 was completed using *A. hilare* adults collected from a commercial soybean field in Camden, NC (76°20'W, 36°32'N) on 16 August 2007. Five adults placed in each of four replicates were assayed for each treatment

(Table 4.1). Bioassay 5 was conducted with laboratory reared *E. servus* nymphs on 16 June 2008. Five nymphs in each of five replicates were assayed for each treatment (Table 4.1). Bioassay 6 was conducted using *A. hilare* nymphs collected from a soybean field in Camden, NC on 19 August 2008. Five nymphs in each of five replicates were assayed for each treatment (Table 4.1).

Insect rearing. Adults and nymphs were collected from commercial soybean fields and returned to the laboratory. Insects were placed into 4 liter Tupperware™ containers (11.5 x 16.5 x 8.6 cm) and fed fresh green beans and raw peanut, *Arachis hypogaea* (L.), kernels with moistened cotton as a water source. Cheese cloth strips were adhered to the sides of the box as an oviposition substrate. Egg masses were removed daily and placed into a Petri dish on a moistened piece of filter paper until egg hatch. After hatch, nymphs were removed and placed in half liter-sized ice cream containers with a water source consisting of a moistened cotton ball with green beans and raw peanut kernels as the food source. Containers were maintained in the growth chamber (27 ± 2 ºC, 14:10 (L: D), 90% RH).

Feeding preference trials. Insecticide-dipped cherry tomatoes were used to evaluate feeding preference by *E. servus* and *A. hilare* adults. Insects were collected from commercial soybean fields in Camden, NC and Painter, VA during the summer of 2007 and 2008. 'Sweet 100' variety cherry tomato seedlings were transplanted 1 June 2007 and 4 June 2008 at the Virginia Tech, Tidewater Agricultural Research and Extension Center (TAREC) (76º44W, 36º39N). Common growing practices were followed including staking and application of recommended fungicides to minimize diseases incidence. Unripened (green) tomato fruit were used in the trials with *E. servus*; ripened (red) tomato fruit were used in the trials with *A. hilare*. The difference in fruit selection resulted because feeding by *A. hilare* was inconsistent on unripened tomatoes; however, they fed consistently on ripened tomatoes. Tomatoes of approximately the same age and size in diameter (2-cm) were picked and examined under a dissecting microscope for insect feeding punctures or blemishes and if found, were discarded. Tomatoes were dipped individually into water or insecticide solution treatments for 30 s then air dried (Table 4.2). Each tomato was pierced through the center with a toothpick then adhered to the bottom of a Petri dish (6 x 1.5 cm) with a small piece of duct tape. The toothpick permitted counting stylet sheaths without handling the tomato. Three Petri dishes containing insecticide treated tomatoes and three Petri dishes containing the control tomatoes, dipped into water, were

placed lengthwise on opposite sides of shoebox-sized Tupperware™ containers (11.5 x 16.5 x 8.6 cm). Each treatment was replicated six times and side placement of treated and untreated tomatoes was alternated. For each replicate, six *E. servus* or *A. hilare* adults (three males, three females) were starved for 24 h before being placed into the container. Containers were then placed into a growth chamber (27 ± 2 ºC, 14:10 (L: D), 90% RH). Insects were removed after 24 h and the number stylet sheaths, an indicator of stink bug feeding (Bowling 1979, 1980), was determined using a dissecting microscope at 10X power.

Repellency trials. Repellency trials were performed on *A. hilare* and *E. servus* adults collected from commercial soybean fields in Camden, NC during August and September of 2007. In each trial, 40 of each species (20 male, 20 female) were evaluated for each insecticide (Table 4.2), with three trials per insecticide. One half of a piece of Fisherbrand® filter paper (P8 No. 09-790-12c) was dipped into each insecticide/water solution and the other half into water. Filter papers were air dried then placed into a Petri dish (10 x 1.5 cm) along with a single stink bug. Male and female placement and dish rotation were alternated for each trial. Adjacent dishes contained an insect of the opposite sex with treatments in reverse placement. Maintenance of the Petri dishes was the same as used in the laboratory bioassays. Insect location on either the treated (T) or untreated (U) side of the filter paper was recorded every 20 min of the first h, then every 2 h up to 10 h, with a final observation at 24 h.

Tomato field efficacy trial. In 2007, a field efficacy trial was conducted at the Virginia Tech, Eastern Shore Agricultural Research and Extension Center (ESAREC) (75°49'W, 37°35'N) Painter, VA in a field of 'Florida 47' tomatoes. Plots were one row wide (1.8 m centers, 45.7 cm between plants) and 7.6 m long with untreated border rows on each side. A randomized complete block design (RCBD) was used with four replicates. The three organic insecticides (Table 4.2) were applied alone and as tank mixes at the same rate as in the laboratory bioassays and included the neonicotinoid insecticide, acetamiprid (Assail 30SG at 83.8 g ai ha^{-1}; United Phosphorous, Inc., King Of Prussia, PA) for comparison. All treatments were applied at a total volume of 317.9 liter ha^{-1} with a 3-nozzle boom equipped with 45 cores and D4 tips using a CO_2-pressurized backpack sprayer calibrated to 2.72 atm. Treatments began at flowering, 27 June, and were repeated every 7 to 10 days until harvest on 30 July. At harvest, 50 marketable sized fruit from each plot were assessed for stink bug damage. Tomatoes were considered to be

damaged by stink bugs if white spongy areas or yellow patches surrounding a small stylet sheath feeding mark were found on the tomato surface (Nault and Speese 2002).

Soybean field efficacy trial. In September 2008, a field efficacy trial was conducted in a soybean field located at the ESAREC. Prior to treatment application, the field was found to have an average of greater than 2.4 adult or large stink bug nymphs per 10 sweeps with a 38-cm sweep net (Todd and Herzog 1980), the economic threshold for stink bugs in soybeans (Herbert 2008). The experiment was arranged in a RCBD with four replicates. Plots were three rows wide (0.91 m spacing) and 6.1 m long with two untreated border rows on each side. Insecticide treatments were applied on 9 September using a CO_2-pressurized backpack sprayer calibrated to deliver a total volume of 233.8 liters ha^{-1} at 2.72 atm using a 3-nozzle boom equipped with 45 cores and D4 tips. This application rate was based on the recommended volume per hectare rate of insect sprays in soybeans (Herbert, personal communication). The three organic insecticides were evaluated and the pyrethroid insecticide, λ-cyhalothrin (Warrior II at 35 g ai ha^{-1}; Syngenta Crop Protection, Inc., Greensboro, NC) for comparison (Table 4.2). Total number of *A. hilare* and *E. servus* adults and nymphs was assessed at 2 and 7 d after treatment (DAT) using a 10 sweep sample with a 38-cm sweep net per plot.

Statistical analyses procedures. For laboratory bioassays, proportion mortality data were arc-sine square root transformed then analyzed as a completely randomized ANOVA. The Tukey's mean separation test was used to separate the means at the $P \leq 0.05$ level of significance. Repellency trial data were pooled and analyzed as a binomial proportion that was significantly different from the probability 50% using the standard normal approximation (Ott and Longnecker 2001). For feeding preference tests, the total number of stylet sheaths per replicate was $\sqrt{(x+0.5)}$ transformed and then analyzed as a general complete block design (PROC GLM, SAS Institute 2001). All proportion data were arc-sine square root transformed prior to analysis. Tomato and soybean field efficacy trial data were analyzed as a randomized complete block ANOVA and Fisher's least significant difference (LSD) was used to separate the means at the $P \leq 0.05$ level of significance (Analytical Software 1998). For the soybean field efficacy trial, the total number of stink bug adults and nymphs (all species combined) for each treatment was compared using LSD procedures to separate means at the $P \leq 0.05$ level of significance.

Results

Laboratory bioassay with *E. servus*. Exposure of *E. servus* nymphs and adults to insecticide treatments resulted in a significant difference in mortality after 72 h ($F = 21.4$; df = 4, 24; $P < 0.001$; and $F = 10.4$; df = 7, 39; $P < 0.001$; Table 4.3). Nymphs were highly susceptible to λ-cyhalothrin at 33.4 g AI ha^{-1} resulting in 96% mortality. The bioassay with *E. servus* adults resulted in all treatments except azadirachtin and pyrethrins having significantly higher mortality than the control. The pyrethrins + spinosad tank mix resulted in 100% mortality after 72 h and was not significantly different than spinosad with 84%, azadirachtin + pyrethrins with 88%, azadirachtin + spinosad with 96%, and λ-cyhalothrin with 64% mortality.

Laboratory bioassays with *A. hilare*. Exposure of *A. hilare* to insecticides treatments resulted in significant differences in mortality in all bioassays performed (bioassay 1, $F = 38.2$; df = 4, 19; $P < 0.001$; bioassay 2, $F = 13.2$; df = 4, 18; $P < 0.001$; bioassay 3, $F = 15.2$; df = 4, 24; $P < 0.001$; and bioassay 4, $F= 15$; df = 31, 7; $P < 0.001$, Table 4.4). Bioassay 1 indicated that *A. hilare* nymphs are susceptible to λ-cyhalothrin, pyrethrins, and spinosad and percent mortality was significantly different than the control with 100, 78, and 53% respectively after 72 h. The azadirachtin treatment was not significantly different than the control with 6%. In bioassay 2, λ-cyhalothrin, resulted in significantly higher mortality than the organic treatments. The organic treatments were not significantly different than the control. Bioassay 3 showed that *A. hilare* nymphs were susceptible to the pyrethrins + spinosad tank mix and λ-cyhalothrin with 52 and 84% mortality. The azadirachtin + pyrethrins and azadirachtin + spinosad tank mixes were not significantly different than the control. In bioassay 4, there was significantly higher mortality of adults for all treatments except azadirachtin, than the control.

Feeding preference trials. For *E. servus*, all treatments, except spinosad, resulted in numerically fewer stylet sheaths on insecticide treated tomato than on the untreated controls indicating a feeding deterrent or repellency effect (Table 4.5). Differences were significant except for tomatoes treated with the azadirachtin + pyrethrin mix, and spinosad ($P = 0.11$ and $P = 0.42$, respectively).

Trials with *A. hilare* resulted in all treatments, except spinosad, exhibiting significantly fewer stylet sheaths than the untreated controls ($P \leq 0.05$) (Table 4.6). More stylet sheaths were located on the spinosad treated tomato, but the difference was not significant ($P = 0.79$).

Repellency trials. Pyrethrins significantly repelled both *A. hilare* (23 – 47% on T) and *E. servus* (20 – 39% on T) adults resulting in less time on the treated compared with the control filter paper ($P < 0.05$) (Figs. 4.1 and 4.2). Azadirachtin (*E. servus*, 48 – 58% on T; *A. hilare*, 40 – 49% on T) did not have a significant repellency effect on either species. Spinosad significantly attracted *E. servus* for seven of the ten time checks (50 – 66% on T) ($P \leq 0.05$).

Tomato field efficacy trial. In the tomato field trial, acetamiprid at 83.8 g AI ha^{-1}, resulted in significantly less stink bug damage compared with the control ($F = 1.45$; df = 7, 31; $P = 0.23$) (Table 4.7). The azadirachtin + pyrethrins tank mix and azadirachtin treatments were not significantly different than the control or acetamiprid. The other organic insecticide treatments did not result in significantly less stink bug damage than the control.

Soybean field efficacy trial. The only species present in the soybean trial was *A. hilare* (Table 4.8). There was a significant difference in number of stink bugs in plots between all the treatments and the control at 2 DAT ($F = 2.54$; df = 7, 31; $P = 0.04$). At 7 DAT only the pyrethroid, λ-cyhalothrin, and the azadirachtin + pyrethrins tank mix had significantly fewer stink bug numbers compared with the control ($F = 1.75$; df = 7, 31; $P = 0.14$).

Discussion

The majority of hemipteran research with organic insecticide compounds has been conducted on the southern green stink bug, *N. viridula* (Seymour et al. 1995, Abudulai et al. 2003, Durmusoglu et al. 2003, Riba et al. 2003). The present study documents the effects of three important organic insecticides, azadirachtin, pyrethrins, and spinosad on two other important stink bug species, *E. servus* and *A. hilare.* All organic insecticides evaluated exhibited some attribute that could contribute to improved management of *A. hilare* and *E. servus*. Generally, azadirachtin did not result in significant mortality unless tank mixed with either spinosad or pyrethrins. As an insect growth regulator, stink bug mortality from azadirachtin is highest when applied directly to nymphs resulting in deformed adults and nymphs (Schmutterer 1990, Riba et al. 2003). Durmusoglu et al. (2003) suggested that it may act as an antifeedant on adults of *N. viridula*. Abudulai et al. (2003) counted stylet sheaths on azadirachtin treated cowpeas, as explained by Bowling (1979, 1980), and reported that azadirachtin-treated beans had significantly fewer feeding punctures compared with the untreated control. Consequently, it is believed that azadirachtin acts as an antifeedant on *N. viridula* adults (Seymour et al. 1995,

Durmusoglu et al. 2003). The results with *E. servus* and *A. hilare* are consistent with these findings. The stylet sheath numbers on azadirachtin treated tomatoes were significantly lower than on untreated tomatoes illustrating its efficacy as an antifeedant. However, azadirachtin did not appear to act as a repellent for either species. In a similar study, Barry et al. (2005) found that azadirachtin did not significantly repel blueberry maggot adults when given a choice between a treated and untreated resting place.

McPherson et al. (1979) and Willrich et al. (2003) reported differences in mortality response to insecticides between species and life stages and determined the adult *E. servus* and *A. hilare* are more sensitive than their respective late-instar nymphs. *A. hilare* adults and nymphs were highly susceptible to pyrethrins in the laboratory bioassays. As a tank mix, pyrethrins were more effective combined with spinosad than azadirachtin for both species and stages. Organic pyrethrins have been listed as an effective repellent and insecticide for the blueberry maggot (Barry et al. 2005) and a repellent for whiteflies (Toscano et al. 1997). The results indicate that it is also an effective repellent for *A. hilare* and *E. servus,* resulting in significantly fewer insects resting on the treated filter paper than the untreated filter paper for the majority of the 24 h testing period. Additionally, in the feeding preference trials, pyrethrins reduced feeding on tomatoes and resulted in significantly fewer stylet sheaths than on untreated tomatoes except for a single treatment with *A. hilare* adults.

The results also indicate that spinosad is highly efficacious against *A. hilare* adults and nymphs as well as *E. servus* adults resulting in similar mortality as the standard pyrethroid λ-cyhalothrin. Applied as part of a tank mix, spinosad was effective for both stages and species of stink bugs, possibly because it is a more effective insecticide. However, during the feeding bioassays, spinosad did not inhibit feeding on tomato, but rather appeared to act as a feeding stimulant or attractant, as evidenced by a greater number of stylet sheaths on fruit than the untreated control. In this assay both species chose to feed more on spinosad-treated tomatoes, further confirming its activity as an attractant or feeding stimulant. *E. servus* was significantly attracted to spinosad during the repellency trials resulting in more time spent resting on the treated than untreated part of the filter paper.

Field trials in soybean showed that all organic treatments were efficacious for up to 2 DAT. At 7 DAT only the pyrethroid and the azadirachtin + pyrethrin tank mix was significantly different than the control. In field trials, Abudulai et al. (2003) determined that *N. viridula*

populations were reduced in cowpea for up to 10 DAT with azadirachtin. Barry et al. (2005) also reported that azadirachtin, spinosad and pyrethrins significantly reduced blueberry maggot populations up to 14 DAT. In this study, multiple field applications of the organic compounds in tomatoes did not result in less stink bug damage to fruit using standard application methods. This may be due to the need for higher application rates or more frequent spraying. Preliminary field efficacy trials with organic insecticides in 2006 indicated that precipitation within 24 h post application of the same rates as the soybean field trials may lead to the treatment being washed off the plants (Kamminga, unpublished data). This would result in no difference in stink bug numbers between the treated and the control plots.

Laboratory assays validate that organics can induce mortality, and can be a feeding deterrent, however, the field trials indicate that photodegradation or treatment wash off may result in a failure to control stink bugs in the field (Schmutterer 1990). If environmental stability or application strategies can be improved, organic insecticides may become a more important part of IPM strategies to control stink bugs. Azadirachtin and spinosad have been reported as being less toxic to natural enemies and mammals as compared with most non-organic insecticides (Schmutterer 1990, Abudulai et al. 2004, Mahdian et al. 2007) further demonstrating their potential benefit in IPM programs.

Acknowledgments

Special thanks to Helene Doughty, Sean Malone, Mike Arrington, and Amanda Koppel for help with data collection. This project was funded in part by the Virginia Agricultural Council and the Virginia Soybean Board.

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Table 4.1. Laboratory bioassay treatments on *Acrosternum hilare* and *Euschistus servus* adults and nymphs.

Treatment g ai ha^{-1}	*E. servus* nymphs	*E. servus* adults	*A. hilare* nymphs assay 1	*A. hilare* nymphs assay 2	*A. hilare* nymphs assay 3	*A. hilare* adults
λ-cyhalothrin 33.4	X	X	X	X	X	X
azadirachtin 48.4	X	X	X	X		X
pyrethrins 30.9	X	X	X	X		X
spinosad 168.1	X	X	X	X		X
pyrethrins 30.9 + spinosad 168.1		X			X	X
azadirachtin 48.4 + pyrethrins 30.9		X			X	X
azadirachtin 48.4 + spinosad 168.1		X			X	X

Table 4.2. Treatments for laboratory bioassays and field efficacy trials on stinkbugs.

Treatment g ai ha^{-1}	Laboratory bioassays	Repellency trials	Feeding preference trials	Tomato field trial	Soybean field trial
azadirachtin 48.4	X	X	X	X	X
pyrethrins 30.9	X	X	X	X	X
spinosad 168.1	X	X	X	X	X
pyrethrins 30.9 + spinosad 168.1	X			X	X
azadirachtin 48.4 + pyrethrins 30.9	X			X	X
azadirachtin 48.4 + spinosad 168.1	X			X	X
λ-cyhalothrin 33.4	X				
acetamiprid 83.8				X	
λ-cyhalothrin 35					X

Table 4.3. Mean ± SE percent mortality of *Euschistus servus* nymphs and adults after 72 h exposure to insecticide treated green beans.

Assay	Stage	Treatment g ai ha^{-1}	% mortality ± SEM (72 h)
1	nymph	λ-cyhalothrin[1] 33.4	96.0 ± 8.9a
		azadirachtin 48.4	12.0 ± 11.0b
		pyrethrins 30.9	16.0 ± 16.7b
		spinosad 168.1	12.0 ± 11.0b
		untreated	12.0 ± 11.0b
2	adult	λ-cyhalothrin 33.4	64.0 ± 11.7abc
		azadirachtin 48.4	40.0 ± 21.0bcd
		pyrethrins 30.9	24.0 ± 16.0cd
		spinosad 168.1	84.0 ±7.5ab
		pyrethrins 30.9 + spinosad 168.1	100.0 ± 0.0a
		azadirachtin 48.4 + pyrethrins 30.9	88.0 ± 4.9ab
		azadirachtin 48.4 + spinosad 168.1	96.0 ± 4.0a
		untreated	4.0 ± 4.0d

Different letters represent significant difference as determined by ANOVA (arcsine square-root transformed data) $P<0.05$ and Tukey's HSD.

[1]Pyrethroid included for comparison to organic insecticides.

Table 4.4. Mean ± SE percent mortality of *Acrosternum hilare* nymphs and adults after 72 h exposure to insecticide treated green beans.

Assay	Stage	Treatment g ai ha^{-1}	% mortality ± SEM (72 h)
1	nymph	λ-cyhalothrin[1] 33.4	100.0 ± 0.0a
		azadirachtin 48.4	6.3 ± 3.6c
		pyrethrins 30.9	78.3 ± 7.5ab
		spinosad 168.1	53.2 ± 4.3b
		untreated	6.3 ± 6.3c
2	nymph	λ-cyhalothrin 33.4	96.9 ± 3.2a
		azadirachtin 48.4	46.9 ± 13.9b
		pyrethrins 30.9	56.3 ± 6.3b
		spinosad 168.1	43.8 ± 6.3b
		untreated	16.7 ± 8.3b
3	nymph	λ-cyhalothrin 33.4	84.0 ± 4.0a
		pyrethrins 30.9 + spinosad 168.1	52.0 ± 8.0ab
		azadirachtin 48.4 + pyrethrins 30.9	20.0 ± 9.8c
		azadirachtin 48.4 + spinosad 168.1	16.0 ± 6.3bc
		untreated	4.0 ± 4.0c
4	adult	λ-cyhalothrin 33.4	100.0 ± 0.0a
		azadirachtin 48.4	15.0 ± 9.6b
		pyrethrins 30.9	75.0 ± 15.0a
		spinosad 168.1	95.0 ± 5.0a
		pyrethrins 30.9 + spinosad 168.1	100.0 ± 0.0a
		azadirachtin 48.4 + pyrethrins 30.9	90.0 ± 5.8a
		azadirachtin 48.4 + spinosad 168.1	90.0 ± 5.8a
		untreated	15.0 ± 5.0b

Different letters represent significant difference as determined by ANOVA (arcsine square-root transformed data) $P<0.05$ and Tukey's HSD.

[1]Pyrethroid included for comparison to organic insecticides.

Table 4.5. Total mean stylet sheaths for *Euschistus servus* feeding preference test on tomato after 24 h with different insecticide treatments.

Treatment g ai ha^{-1}	Total mean stylet sheaths		
	Treated	Control	*P*-value
azadirachtin 48.4	29.5 ± 6.7	83.2 ± 28.1	0.02
pyrethrins 30.9	16.7 ± 7.8	56.7 ± 14	0.02
spinosad 168.1	81.7 ± 25.6	41.8 ± 9.3	0.42
pyrethrins 30.9 + spinosad 168.1	18.5 ± 9.2	69.8 ± 26	0.01
azadirachtin 48.4 + spinosad 168.1	27 ± 8.6	115.8 ± 33.6	<0.01
azadirachtin 48.4 + pyrethrins 30.9	23.2 ± 10.6	38 ± 6.4	0.11

P-value determined by ANOVA ($\sqrt{(x+0.5)}$ transformed data).

Table 4.6. Total mean stylet sheaths for *Acrosternum hilare* feeding preference tests on tomato after 24 h with different insecticide treatments.

Treatment g ai ha^{-1}	Total mean stylet sheath		
	Treated	Control	*P*-value
azadirachtin 48.4	6.7 ± 1.9	41.2 ± 6.9	<0.001
pyrethrins 30.9	8.7 ± 3.5	29.8 ± 3.5	0.01
spinosad 168.1	25.2 ± 5.3	21.7 ± 6.4	0.79
pyrethrins 30.9 + spinosad 168.1	2.3 ± 0.7	27.0 ± 6.6	<0.001
azadirachtin 48.4 + spinosad 168.1	3.3 ± 1.1	21.8 ± 2.9	0.002
azadirachtin 48.4 + pyrethrins 30.9	5.8 ± 1.9	38 ± 4.8	<0.001

P-value determined by ANOVA ($\sqrt{(x+0.5)}$ transformed data).

Table 4.7. Mean ± SE percent stink bug damaged tomatoes (n= 50) after repeated insecticide applications. ESAREC, 2007.

Treatment g ai ha^{-1}	% damage
acetamiprid[1] 83.8	3.5 ± 1.3b
azadirachtin 48.4	16.5 ± 6.3ab
pyrethrins 30.9	17.5 ± 1.0a
spinosad 168.1	20.5 ± 4.3a
pyrethrins 30.9 + spinosad 168.1	20.5 ± 7.1a
azadirachtin 48.4 + spinosad 168.1	25.0 ± 9.0a
azadirachtin 48.4 + pyrethrins 30.9	16 ± 5.5ab
untreated	19.5 ± 8.1a
LSD	2.1

Different letters represent significant difference as determined by ANOVA (arcsine square-root transformed data) $P<0.05$ and Fishers LSD.

[1]Neonicotinoid included for comparison to organic insecticides.

Table 4.8. Mean ± SE number of *Acrosternum hilare* nymphs and adults per 10 sweeps of a 38-cm sweep net in soybean after insecticide application. ESAREC, 2008.

Treatment g ai ha^{-1}	2 DAT	7 DAT
λ-cyhalothrin[1] 34.95	1.25 ± 0.6b	1.75 ± 0.6b
azadirachtin 48.4	3.25 ± 0.8b	3.0 ± 1.4ab
pyrethrins 30.9	4.5 ± 1.2b	5.25 ± 1.0a
spinosad 168.1	3.75 ± 1.0b	3.25 ± 0.9ab
pyrethrins 30.9 + spinosad 168.1	3.5 ± 1.3b	3.0 ± 1.0ab
azadirachtin 48.4 + spinosad	3.75 ± 1.1b	3.25 ± 0.8ab
azadirachtin 48.4 + pyrethrins 30.9	2.5 ± 1.0b	2.0 ± 0.7b
untreated	8.75 ± 2.8a	6.0 ± 2.0a
LSD	4	3.3

Different letters represent significant difference as determined by ANOVA ($P<0.05$) and Fishers LSD.

[1]Pyrethroid included for comparison to organic insecticides.

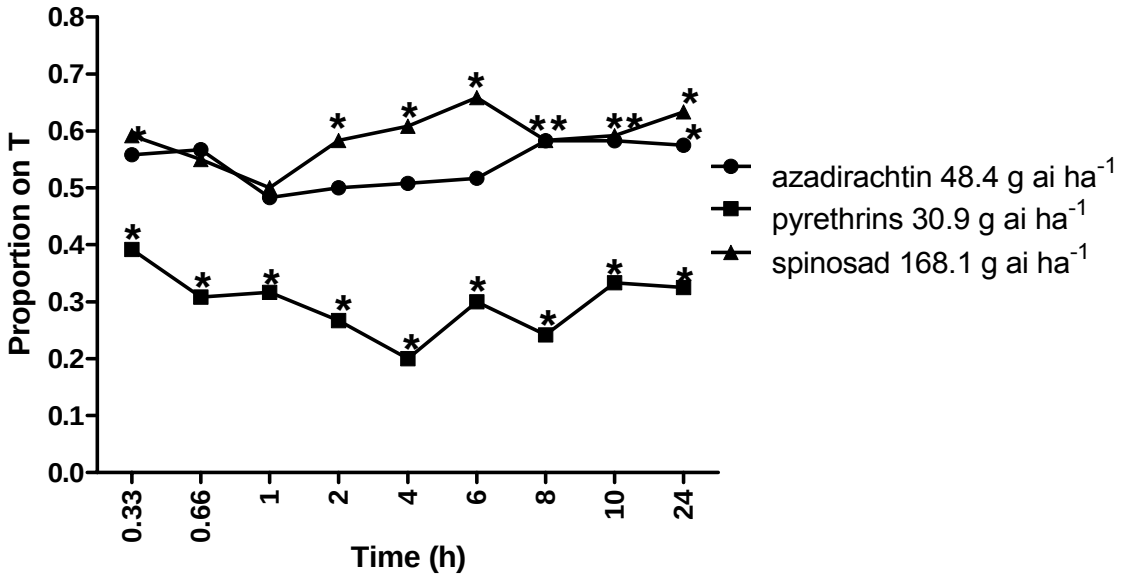

Fig 4.1. Proportion of *Euschistus servus* adults on insecticide treated paper (T) at different times after introduction. * Represents a significant difference at $P \leq 0.05$ level from the 50% probability using the standard normal approximation (Ott and Longnecker 2001).

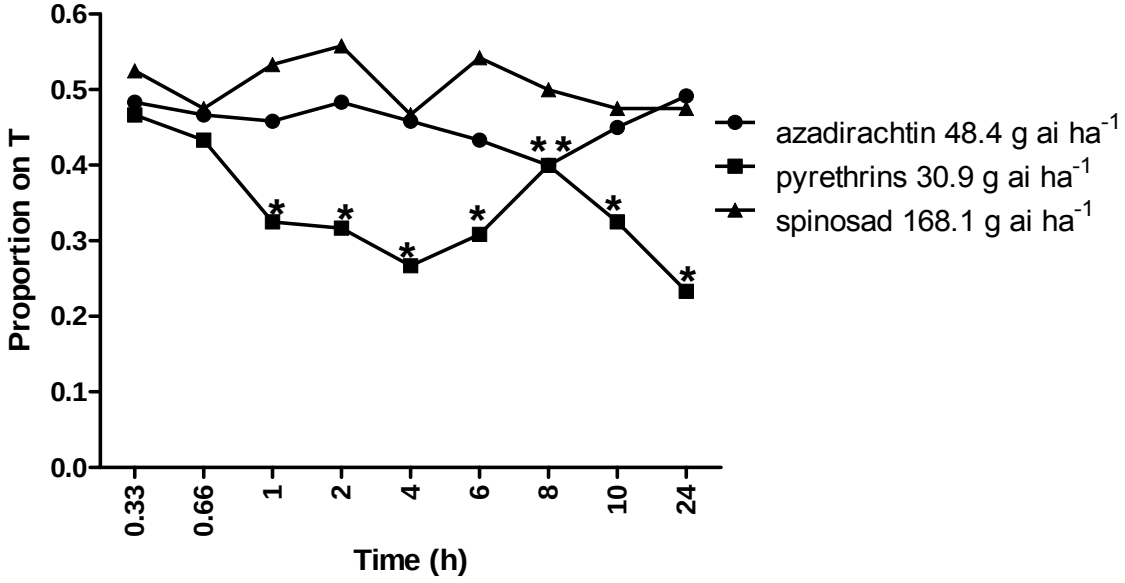

Fig 4.2. Proportion of *Acrosternum hilare* adults on insecticide treated paper (T) at different times after introduction. * Represents a significant difference at $P \leq 0.05$ level from the 50% probability using the standard normal approximation (Ott and Longnecker 2001).

Chapter Five

Predicting black light trap catch and flight activity of *Acrosternum hilare (*Hemiptera: Pentatomidae) adults

Abstract

A regression model was developed to predict the flight activity of *Acrosternum hilare* (Say) using data on the number of adults collected in a single black light trap located in Painter, VA in the 18-yr period from 1990–2007. Eighteen initial weather variables including cumulative precipitation over different time periods, mean monthly precipitation from January to April (PJA), days below freezing (DBF), and average monthly temperatures from December to July were tested in developing the regression model. Stepwise regression analysis showed that a two-variable regression model, $\log_{10} Y = 0.98 + 0.027$ (DBF) – 0.102 (PJA), was sufficient for predicting the mean weekly number of *A. hilare* adults in black light trap (Y). Validation of the model using five independent black light trap data sets (the observed) resulted in an $r = 0.98$ between observed and predicted mean weekly number of *A. hilare* adults caught in traps. Three peaks in flights of *A. hilare* adults were observed when mean trap catch was plotted over time for the 18-yr period. The peaks occurred at 319, 892, and 1331 degree days (DD) from 1 January. Based on known developmental rates, the first peak was attributed to overwintered adults, the second to first generation adults, and the third to second generation adults. This research suggests that *A. hilare* undergoes two complete and a partial third generation in Virginia. Cumulative trap catch estimated from the 18-yr mean trap catch showed that 10, 50, and 90% of the total seasonal catch should occur by 153, 501, and 1066 DD.

Key words *Acrosternum hilare*, phenology, black light trap, degree days, monitoring

The green stink bug, *Acrosternum hilare* (Say), is a major pest of fruits, vegetables, and many field crops grown throughout North America (McPherson and McPherson 2000). Adult *A. hilare* overwinter under leaves and brush in the forest (Underhill 1934) and emerge from diapause in the spring when ambient temperatures rise above 18 ºC (McPherson 1982). Individuals then feed on wild hosts until nearby agricultural crops become attractive, after which they migrate into fields. Feeding by *A. hilare* within the crop causes injury to various plant parts,

which results in crop damage and subsequent yield losses. In soybean, for example, pod feeding by *A. hilare* and other stink bugs has been shown to affect yield both directly and indirectly through the reduction in seed quality from the introduction of pathogens (Gore et al. 2006, Young et al. 2008).

The development of management programs against *A. hilare* requires that we have the ability to monitor the movement activity and population dynamics of this pest. However, as with many highly mobile and polyphagous insects, keeping track of *A. hilare* populations is not without its challenges. Monitoring stink bugs in agricultural fields by direct sampling is difficult, costly and time consuming as adults are elusive and quickly drop to the ground or fly when disturbed (Cullen and Zalom 2000). An alternative is to rely on traps, such as pheromone and light traps, to monitor the activity and abundance of the insect in and around fields and for inclusion in IPM programs against the pest. Pheromones traps have been studied and used for monitoring stink bug populations (Millar et al. 2002, Leskey and Hogmire 2005, Hogmire and Leskey 2006), but little is known about the effectiveness of black light traps as a monitoring tool for the pest (McPherson and Weber 1990).

Data on insects collected in various types of traps have been used to make predictions about the flight activity and population dynamics for several species (Rodríguez-del-Bosque and Magallanes-Estala 1994, Sutherland and Baharally 2003, Zou et al. 2004). Because insect movement and dynamics are directly affected by weather conditions (Kennedy and Storer 2000), weather variables such as temperature, rainfall, relative humidity, and wind velocity are often combined with trap catch data to make predictions about the occurrence, abundance, and seasonality of the insects (Sutherland and Baharally 2003, Edde et al. 2006). In addition, as insect development is directly affected by temperature and is sensitive at the upper and lower temperature thresholds (Tauber and Tauber 1976), pheromone or light trap catch and temperature data have also been used to construct phenology models based on degree days, for forecasting critical life history events of many important insect pests. For example, phenology models have been developed to predict the emergence of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, (Zou et al. 2004) based on light trap data, and flights of *Ephestia* spp. (Ahmad and Ali 1995), Nantucket pine tip moths, *Rhyacionia frustrana* (Comstock), (Malinoski and Paine 1988) and European pine shoot moths, *Rhyacionia buoliana* (Schiff.), (Regan et al. 1991) into pheromone traps. Phenology models also have been developed to predict population abundance

of the strawberry bud weevil (Bostanian et al. 1999) and the consperse stink bug (Cullen and Zalom 2000).

In Virginia, black light traps have been operated during the summer months for a number of years to monitor flight activity of insect pests, such as *Helicoverpa zea* (Boddie). A large number of *A. hilare* adults have also been caught in these traps. Frequently, we are asked by trap operators and other pest control personnel about the relevance of the captures of *A. hilare* adults and whether the trap catch data could be used to improve pest management activities for this insect. A unique and complete data set of black light trap catch spanning a period of 18 yr (1990–2007) provides us with an opportunity to address these questions about *A. hilare* adult flight activity and the utility of the black light trapping in management of the pest. The objective of the study, therefore, was to determine whether the historical black light trap catch data, weather variables, and degree day models could be used to predict weekly trap catch and periods of peak *A. hilare* activity.

Materials and Methods

Black light trap catch and weather data. Standard 15-watt black light traps (Gempler's, Madison, WI) were used to collect insects at three locations in Virginia. A single trap at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) was monitored from 1990–2007 (Table 5.1). Two other traps located at Tidewater Agricultural Research and Extension Center (TAREC), Suffolk, VA and Eastern Virginia Agricultural Research and Extension Center (EVAREC), Warsaw, VA were monitored for a single year in 2006 and 2007, respectively. All insects captured in the black light traps, including *A. hilare* adults, were counted weekly from April through September in each year.

Data on weather variables such as daily minimum and maximum temperatures, rainfall, and snow fall were obtained for the each of the 18 yr of black light trapping from weather station records maintained at the three locations. The weather data were corroborated with data from the VAES Weather Mesonet website (VAES Weather MESONET, 2008) for the years 2004–2007.

Regression model. A regression model was developed to predict weekly flight activity of *A. hilare* adults during the cropping season at ESAREC using weather variables and black light trap catch for 15 of the 18 yr of trapping. As described below, trap catch data for 3-yr (1990, 1999, and 2000) were randomly selected and removed from the 18-yr data set and

combined with other trap catch data to validate the regression model. The counts of *A. hilare* adults caught in the black light trap each week in a particular year were summarized as a mean weekly trap catch for that year. To satisfy the assumption of normality, the mean weekly trap catch data for the 15 yr were $\log_{10}(x)$ transformed (Bartlett 1947) and tested for normality on a Normal Quantile Plot and Shapiro-Wilk W Goodness of Fit test (JMP 7.0, 2007, SAS Institute Inc., Cary, NC).

Eighteen weather variables were initially selected from the weather station data for developing the regression model. The variables included average monthly temperature, cumulative and average precipitation, and the number of days below freezing (0 ºC) for varying time periods between December and July. Because the regression model was being developed to predict mean weekly *A. hilare* adult trap catch for the upcoming cropping season, weather variables from May onward in each year were eliminated from the analysis. Variables that included trap catches from May onward were excluded from the data set used to develop the predictive model because the cropping season at Painter, VA begins in May. The remaining 12 variables were analyzed using a mixed (forward and backward) stepwise regression (Freund and Wilson 1998) in JMP 7.0 (SAS Institute Inc., Cary, NC) to select the best set of weather variables for predicting mean weekly *A. hilare* adult catch in the black light trap.

Model validation. The model was validated using five independent data sets of mean weekly *A. hilare* adult trap catch. The validation data set consisted of trap catch data for 3-yr (1990, 1999, and 2000) that were randomly selected and removed from the 18-yr data set at the ESAREC, and two 1-yr data sets from individual traps operated at different locations in Virginia (TAREC in 2006 and EVAREC in 2007) located about 132 and 93 kilometers, respectively, from the ESAREC location (Table 5.1). Values for each of the weather variables identified in the regression analysis were selected from the validation data sets and used in the regression model to predict mean weekly *A. hilare* adult trap catch. Then a bivariate plot of the predicted and observed trap catch and used Pearson's correlation analysis to describe the strength of the relation between the two variables and to validate the utility of the regression model was developed.

Degree day calculation and modeling. Weekly degree days (DD) were calculated using the modified sine wave method described in Allen (1976). The lower and upper developmental thresholds for *A. hilare* were set at 15 ºC and 33 ºC, respectively, based on the study by Simmons

and Yeargan (1988). The average number of DD for each week of stink bug catch during the 18-yr period was calculated beginning from 1 January. DD data was used to study the relationship between accumulated DD and the time course of black light trap catches of *A. hilare* adults by two methods. First, mean weekly trap catches of *A. hilare* adults for the 18 yr were plotted against mean accumulated DD. Second, a table of accumulated DD was developed starting from 1 January of each year to each peak in *A. hilare* trap catch, and for periods between peaks.

Predicting cumulative catch. The 18 yr of black light trap catch data were averaged and cumulative weekly catch was calculated as a percentage of total trap catch (Cockfield and Mahr 1994). Accumulated DD were then calculated from 1 January and averaged over the 18-yr period. The relationship of percent cumulative catch of *A. hilare* adults to mean DD was fitted to the Weibull function

$$f(x) = 100\left(1 - e^{-(x/\alpha)^{\beta}}\right) \qquad \textbf{(1)}$$

to model *A. hilare* catch based on DD (Wagner et al. 1984). In equation (1), $f(x)$ is the percent cumulative mean trap catch at accumulated DD (x) from 1 January, α is the rate of trap catch, and β describes the shape of the curve. The fit of the data to the Weibull function was determined using nonlinear least squares regression in TableCurve 5.01(SYSTAT Software Inc., Richmond, CA).

Results

Regression model. The results of the stepwise regression analysis showed that two weather variables, number of days below freezing (*DBF*) and mean monthly precipitation from January to April (*PJA*) were the most important for predicting mean weekly *A. hilare* adult black light trap catch (*Y*). The regression model,

$$\log_{10} Y = 0.98 + 0.027(DBF) - 0.102(PJA) \qquad (2),$$

which was significant ($F = 5.90$; df = 2, 14; $P = 0.016$) with $R^2 = 0.50$ indicated that the number of stink bugs caught in the black light trap was positively related to the number of days below freezing and negatively related to average monthly precipitation from January to April. The removal of other sets of three randomly selected years from the regression analysis resulted in significant models with similar R^2-values.

Model validation. Analysis of five independent validation data sets with the regression model showed that there was strong positive relationship ($r = 0.98$) between observed and predicted mean weekly *A. hilare* adult trap catch (Fig. 5.1). The overall mean weekly catch predicted by the regression model for the 15-yr data set (the 18 trap catch years minus the three validation years) was 38.5 green stink bug adults per week (solid horizontal line in Fig. 5.2).

Degree day model. Three peaks in mean weekly black light trap catch of *A. hilare* adults for the 18 yr were observed when the data were plotted against mean DD beginning at 1 January (Fig. 5.3). The first and largest peak occurred in mid-June (319 DD), the second in the second week of August (892 DD), and the third and much smaller peak during the first week of October (1331 DD).

The mean accumulated DD from 1 January to the first, second, and third peaks were 306, 909, and 1331, respectively (Table 5.2). An average of 604 DD accumulated between the first and second peaks; 434 DD accumulated between the second and third peaks. No clear third peak was observed in years 1997, 1998, 1999, 2000, 2005 and 2006. Although accumulated DD for the peaks identified in Fig. 5.3 varied slightly from the accumulated DD to the peaks of each year in Table 5.2, the DD values from each method fell within the 95% confidence intervals (Table 5.2).

Predicting cumulative catch. The fit of the cumulative percent catch to the Weibull function was significant ($F = 3223.19$; df = 1, 31; $P < 0.0001$; Fig. 5.4) with $\alpha = 626.9$ (± 13.2 SE) and $\beta = 1.59$ (± 0.1 SE). The function explained 99% of the variability in the relationship of cumulative percent mean weekly trap catch of *A. hilare* adults and DD for the 18-yr period. The results showed that 10% cumulative catch occurred at 153 DD, 50% cumulative catch at 501 DD, and 90% cumulative catch at 1066 DD.

Discussion

Not unexpectedly, the regression model showed that mean weekly catch of *A. hilare* adults in black light traps is affected by weather conditions. Two weather variables, days below freezing and mean monthly precipitation from January to April, appear to be sufficient for explaining yearly variations in the mean weekly number of *A. hilare* adults caught in black light traps in Virginia. The regression model is also robust as it predicted the mean weekly *A. hilare* adult catch for five independent years of trap catches reasonably well.

The model showed that there is a positive relationship between days below freezing and mean weekly black light trap catch. Factors that might explain the positive relationship of trap catch and cold weather conditions include increased mortality of stink bug parasites and parasitoids, an increase in the amount of snow fall which might insulate the insects from extremely cold temperatures (Pedigo 1998), and/or an extended diapause, which may decrease mortality caused by spring freezes. Tauber et al. (1986), for example, reported that insects in areas of fluctuating spring temperature environments can experience increased mortality from ending diapause prematurely during abnormally high temperature conditions followed by late freezes. Conversely, insects experiencing excessive freezing temperatures during winter months may remain in diapause until the weather oscillations have stabilized thereby decreasing their chance of mortality. The negative relationship of mean weekly *A. hilare* catch and increased precipitation could be ascribed to higher mortality during diapause from increased precipitation. This type of relationship was reported by Slosser et al. (1975) for *Helicoverpa zea* (Boddie), who found that large amounts of rainfall can drown overwintering pupae.

The model can provide several pieces of information that could be useful for improving pest management of *A. hilare*. Based on mean trap catches determined with the regression model the expected average is about 38.5 *A. hilare* adults captured per week during the typical growing season. A prediction of higher than average catch could be used to alert growers and consultants of an impending pest problem so that they might increase their field monitoring efforts. Also, percent cumulative trap catch estimated from the 18-yr mean fitted to the Weibull function revealed that 10% of the total seasonal catch should occur by the third week in May (153 DD), 50% by the first week in July (501 DD), and 90% by the last week in August (1066 DD). This information could be useful in tracking in-season progress of this pest.

The results of this work also provide fairly clear evidence of the number of generations that *A. hilare* undergoes annually in the Mid-Atlantic region. The results showed that there are two distinct peaks and a smaller third peak of *A. hilare* adult catch during the cropping season. The three peaks of trap catch align with the known life span of *A. hilare*. In ideal conditions, *A. hilare* can live 60 d (Javahery 1990) which is in accord with the graphed peaks. McPherson and Weber (1990) also recorded similar peaks in black light traps for a season in southern Illinois. They suggest that if *A. hilare* is bivoltine, the overwintered adults may reproduce on wild hosts in the spring resulting in their offspring being collected in the traps in June and July. Thus,

collection of the second generation would occur during the second peak in September and October. With further research, McPherson and Tecic (1997) discovered that *A. hilare* is indeed bivoltine in southern Illinois; therefore, since Virginia is at a similar latitude and has comparable plant growth zones to southern Illinois, we may also expect to find bivoltine populations of *A. hilare*. The insect has also been reported as being bivoltine in South Carolina (Jones and Sullivan 1982), Arkansas (Miner 1966) and Kansas (Wilde 1969).

Originally it was believed that only a single generation per year of *A. hilare* occurred in Virginia (Schoene and Underhill 1933, Underhill 1934). Currently, *A. hilare* is thought to have two generations a year in Virginia, although this has not been documented. Simmons and Yeargan (1988) reported that *A. hilare* required 483 DD from egg to adult based on averages across five tested temperatures. This requirement suggests that the first peak in black light trap catch of *A. hilare* adults probably does not represent the first generation of the season as this peak occurred at only 319 DD from the first of the year. Therefore, the first peak may represent the activity of overwintered stink bugs moving from the woods into agricultural systems. The results also showed that the second peak occurred during the week of 8 August, 892 DD after 1 January and 573 DD after the first peak and may represent the first complete generation. The accumulation of 573 DD between the first and second peak is reasonable as it accounts for the degree days required for a generation (483 DD) and allows 90 DD for a preovipositional period.

The third smaller peak that was observed occurred at 439 DD after the second peak, which is well within the requirement for development of 483 DD calculated by Simmons and Yeargan (1988). This third peak could indicate the migration of second generation adults from agricultural fields back to overwintering habitats. Underhill (1934) reported that *A. hilare* began moving to wooded areas at the end of September through October. The third peak is less distinct than the first two peaks. It is likely that some overlapping of generations occur during this period resulting in a loss of a clear peak as an indicator of generation time.

In summary, the results of this work show that black light traps may be a useful tool for monitoring the flight activity and movement of *A. hilare* adults. Information using cumulative trap catch and calculated degree days could be useful in the design and implementation of field scouting programs, and for determining periods of highest risk of green stink bug activity during the cropping season. The results of this work have also suggested that *A. hilare* undergoes two complete and a partial third generation in the Mid-Atlantic region.

Acknowledgments

Special thanks the following individuals at Virginia Tech for technical assistance with black light trap operation: J. Speese and H. Doughty (ESAREC), A. Koppel, R. Arrington, and M. Arrington (TAREC), M. Beahm (EVAREC), W. Alexander (VCE ANR Agent, Southampton County), C. Estienne (VCE ANR Agent, Greensville County), D. Moore (VCE ANR Agent, Middlesex and Gloucester Counties). Also, I want to thank the volunteer cooperators, G. Chappell (Virginia State University), A. Johnson (Grower, King William County), and J. and S. Darden (Growers, Southampton County).

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Table 5.1. Location and site characteristics of the three black light traps and associated weather stations in Virginia.

Location	Site description
Eastern Shore Agricultural Research and Extension Center (ESAREC), Painter, VA. (N 37º35.32.2; W 75º49.60.7)	41 hectares of potatoes, sweet potatoes, snap beans, broccoli, cauliflower, cucumbers, peppers, squash, tomatoes, corn, cantaloupes, watermelons, small grains, corn, soybeans and cotton.
Tidewater Agricultural Research and Extension Center (TAREC), Suffolk, VA. (N 36º39.48.74; W 76º44.04.13)	136 hectares of corn, soybean, cotton, small grains, peanuts and vegetable.
Eastern Virginia Agricultural Research and Extension Center (EVAREC), Warsaw, VA. (N 37º58.27.3; W 76º45.36.6)	22 hectares of soybeans, barley, oats, corn and wheat.

Table 5.2. Degree day accumulations from 1 January for each year to distinct peaks in black light trap catch of *Acrosternum hilare* adults. ESAREC, Painter, VA.

	Accumulated Degree Days				
Year	1 Jan to 1st peak	1 Jan to 2nd peak	Between 1st and 2nd peaks	1 Jan to 3rd peak	Between 2nd and 3rd peaks
1990	392	920	528	1411	491
1991	210	1053	843	1526	473
1992	252	887	637	1173	284
1993	283	838	555	1321	483
1994	329	965	636	1358	393
1995	264	835	571	1358	523
1996	181	492	311	1139	647
1997	283	952	669	---	---
1998	495	1128	633	---	---
1999	274	1056	782	---	---

	Accumulated Degree Days				
Year	1 Jan to 1st peak	1 Jan to 2nd peak	Between 1st and 2nd peaks	1 Jan to 3rd peak	Between 2nd and 3rd peaks
2000	250	916	667	---	---
2001	321	841	519	1246	406
2002	369	953	584	1569	616
2003	381	1062	681	1285	223
2004	344	935	592	1302	366
2005	246	850	603	---	---
2006	322	850	528	---	---
2007	304	833	529	1139	306
$\bar{x}$ ± SEM					
	306 ± 18	909 ± 33	604 ± 27	1319 ± 40	434 ± 37
95% CI	268 - 344	839 - 979	547 – 661	1231 - 1407	353 - 515
Based on graphed date in Fig. 5.3	319	892	573	1331	439

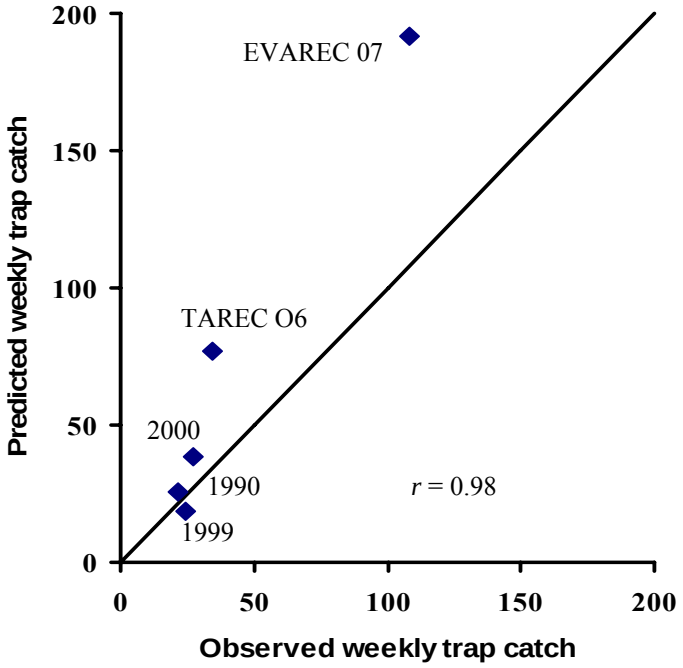

Fig. 5.1. Correlation of the observed and predicted mean weekly number of *Acrosternum hilare* adults caught by black light trapping for the five validation data sets. Years 1990, 1999, and 2000 are validated with data from Eastern Shore Agricultural Research and Extension Center (ESAREC), Painter, VA. Year 2006 (TAREC) is validate with data from Tidewater Agricultural Research and Extension Center, Suffolk, VA. Year 2007 (EVAREC) is validated with data from Eastern Virginia Agricultural Research and Extension Center, Warsaw, VA.

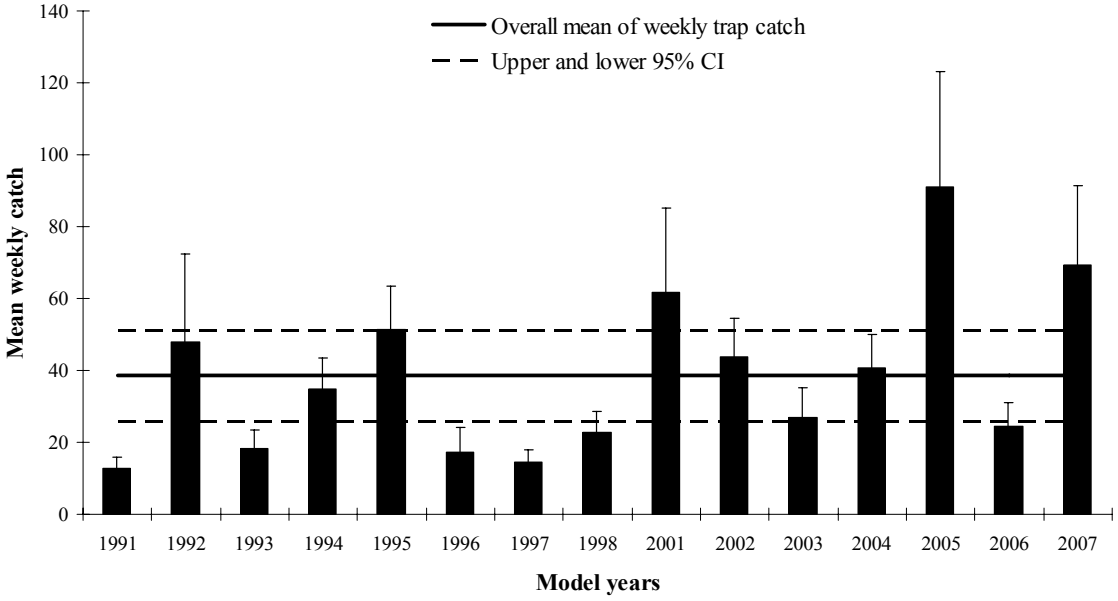

Fig. 5.2. Mean (+ SEM) *Acrosternum hilare* adults caught per week in the black light trap for each of the 15 yr that were used to develop the regression model. The horizontal line represents the overall mean weekly trap catch for the period ± the 95% CI.

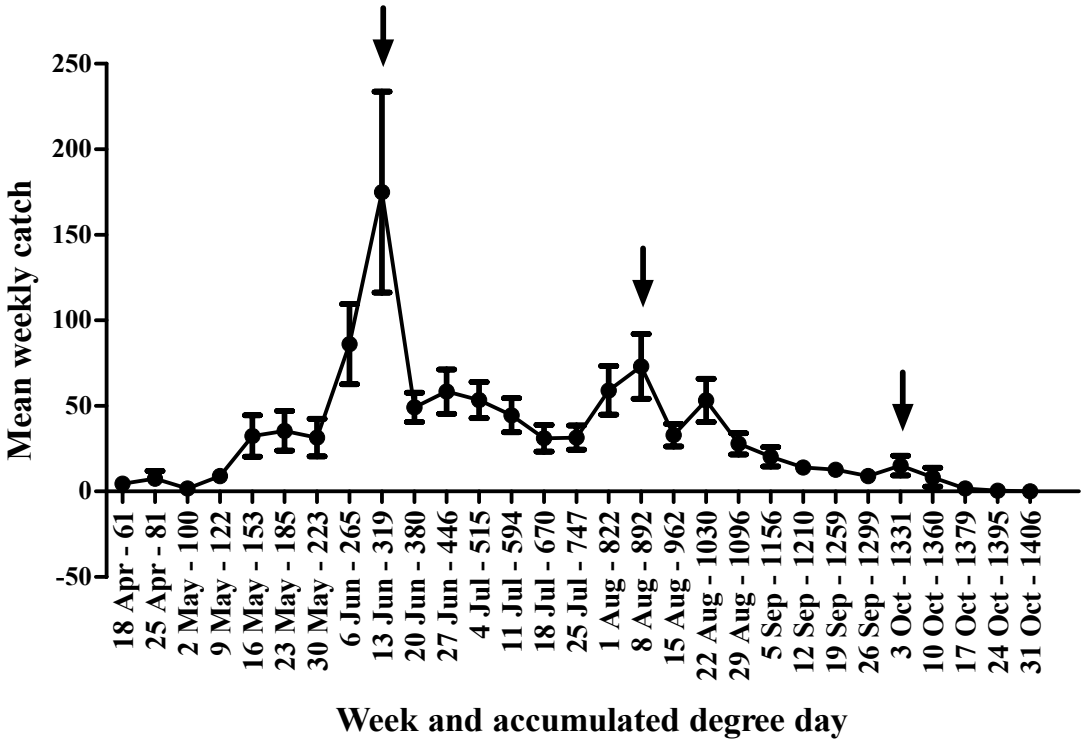

Fig. 5.3. Mean (± SEM) weekly number of *Acrosternum hilare* adults caught in a black light trap for the 18-yr study period in relation to mean accumulated degree day (DD) beginning at 1 January.

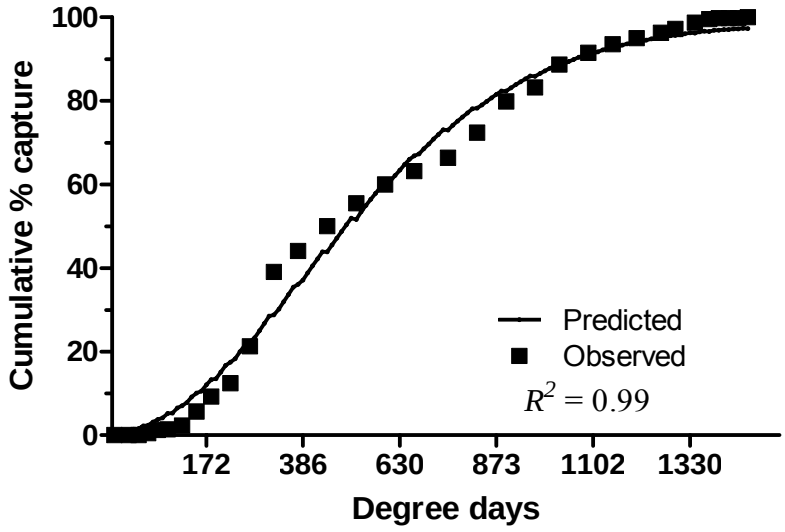

Fig. 5.4. Relationship of cumulative percent mean weekly trap catch of *Acrosternum hilare* adults to accumulated degree days fit to the Weibull function for the 18-yr mean.

Conclusion

Sampling of soybean and cotton in southeastern Virginia in 2005 and 2006 revealed seven stink bug species. These included *Euschistus servus* (Say), *Acrosternum hilare* (Say), *Thyanta custator accera* McAtee, *Euschistus tristigmus* (Dallas), *Oebalus pugnax* (F.), *Euschistus variolarius* (Palisot de Veauvois) and *Podisus maculiventris* (Say). Of the seven, six were considered pest species capable of doing economic damage. The survey determined that the two most common stink bug species were *A. hilare* and *E. servus*. Both species are native to North America and are common pests that feed on over 200 plant species (McPherson and McPherson 2000). The invasive species *Halyomorpha halys* (Stål) and *Piezodorus guildinii* (Westwood), as well as the non-native stink bug to Virginia, *Nezara viridula* (L.), were not found. Continued monitoring of stink bugs will be important for detecting the presence of invasive stink bug species in the Mid-Atlantic region. Results from the stink bug sampling method comparisons indicated that while there is less variability with the sweep net sampling method than the beat sheet sampling method, there were still difficulties with accurately predicting stink bug populations in the field (Chapter 2). Future surveys should focus on ways to improve sample precision.

Results of both field and laboratory experiments with conventional insecticides were consistent in demonstrating the efficacy of selected pyrethroids, organophosphates, and neonicotinoids on the stink bugs, *A. hilare* and *E. servus*. Of the neonicotinoids evaluated, dinotefuran generally resulted in the highest mortality and acetamiprid the lowest. Results of the bioassays were consistent with Willrich et al. (2003) showing that the organophosphate, acephate was numerically more toxic to *E. servus* adults than dicrotophos. *A. hilare* nymphs were found to be more susceptible to the pyrethroids λ-cyhalothrin and cyfluthrin than *E. servus* adults. *A. hilare* nymphs were very susceptible to all pyrethroids tested. *A. hilare* adults were highly susceptible to all pyrethroids tested, all organophosphates tested except acephate, and all neonicotinoids tested except acetamiprid (Chapter 3). In general regarding efficacy among the neonicotinoids, dinotefuran and clothianidin were toxic to *A. hilare* adults and nymphs, while thiamethoxam and acetamiprid were toxic to adult *E. servus*.

Based on efficacy trials in soybean, several pyrethroids, organophosphates and neonicotinoids can provide effective stink bug control for up to 10 days. However, none of the treatments exhibited total control in the field due to possible reinvasion, natural fluctuations, or egg hatch after applications. The organophosphates and pyrethroids tended to be more effective than the neonicotinoid, acetamiprid, and the chitin inhibitor, novaluron. The chitin inhibitor, novaluron, did not prove to be as effective as most other treatments, unless tank-mixed with either acephate or dicrotophos. Three neonicotinoids evaluated in soybeans in 2005, dinotefuran, imidacloprid, and thiamethoxam, and dinotefuran alone in 2006, were as efficacious as the organophosphates and pyrethroids (Chapter 3). These results indicate that neonicotinoids offer an alternative for managing stink bugs that may fit with integrated pest management programs where conservation of natural enemies is a consideration. Similar results were found by Fitzpatrick et al. (2001a), Fitzpatrick et al. (2001b) and Cullen and Zalom (2007).

Laboratory bioassays with selected organic insecticides, azadirachtin, pyrethrins, and spinosad showed efficacy against *A. hilare* and *E. servus*. Results suggest that azadirachtin is an antifeedant. Seymour et al. (1995) and Abudulai et al. (2003) also reported that *N. viridula* feeds less on food treated with azadirachtin. The repellency trials with pyrethrins on both species of stink bugs were consistent with Barry (2005), who also reported a repellency effect with pyrethrins on whiteflies. Spinosad was an effective insecticide, but results of the repellency trials suggest that it may be an attractant or a feeding stimulant.

Field trials in soybean found that all organic treatments were efficacious for up to 2 days after treatment (DAT), but at 7 DAT, only the conventional pyrethroid and the azadirachtin + pyrethrins tank mix was significantly different than the control. However, field trials in tomatoes did not result in effective control of stink bugs using standard application methods (Chapter 4). This may be due to the need for higher application rates or more frequent spraying.

Laboratory bioassays validated that organics can be used for inducing mortality, and as a deterrent, however, the field trials indicated that photodegradation or treatment wash off may be the cause of the failure to control stink bugs in the field (Schmutterer 1990). If environmental stability or application strategies can be improved, organic

insecticides may become a more important part of IPM strategies to control stink bugs. Azadirachtin and spinosad have been reported as less toxic to natural enemies and mammals compared with their broad-spectrum insecticide counterparts (Schmutterer 1990, Abudulai et al. 2004, Mahdian et al. 2007) further demonstrating the importance of these more selective insecticides (Chapter 4).

A model was developed using a complete 18 yr data set of weekly black light trap catch for *A. hilare*. The regression model was developed to predict weekly flight activity of *A. hilare* adults during the cropping season using weather variables and black light trap catch for 15 of the 18 yr of trapping. Two variables in the model, days below freezing and mean monthly precipitation from January to April, were significant in explaining trap catch variability. The model also accurately predicted mean weekly *A. hilare* catch for additional trap locations and supplementary seasons as verified by a strong correlation of 98% for the five validation location/years. The three peaks of mean trap catch over the 18 years, plotted over time, occurred at 319, 892, and 1331 degree days (DD) from 1 January. In accordance with known developmental rates, the first peak was attributed to overwintered adults, the second to first generation of adults, and the third to second generation adults. The results of this work have provided fairly clear evidence that *A. hilare* undergoes two complete and a partial third generation in Virginia. Percent cumulative trap catch estimated from the 18-yr mean fitted to the Weibull function revealed that 10% of the total seasonal catch should occur by the third week in May (153 DD), 50% by the first week in July (501 DD), and 90% by the last week in August (1066 DD) (Chapter 5). This information will be useful in tracking in-season pest progress. Information using cumulative trap catch and calculated degree days will also be useful in helping design and implement field scouting programs, and to help determine periods of highest risk as well as when programs can begin to relax monitoring efforts.

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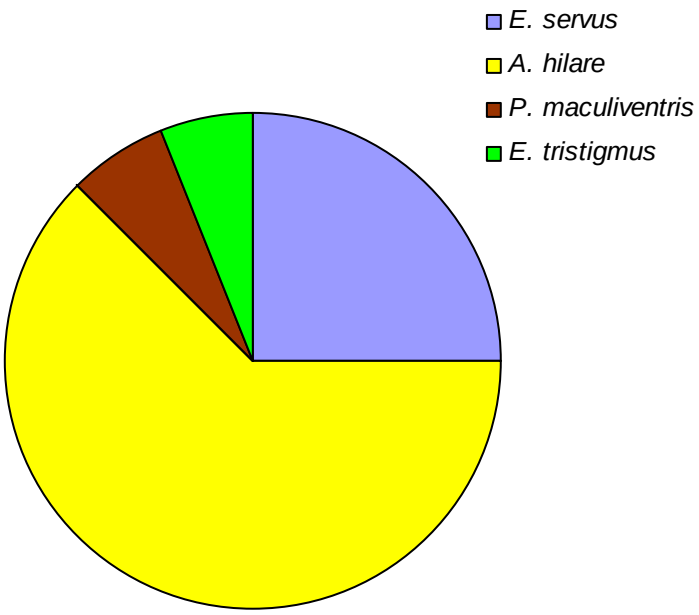
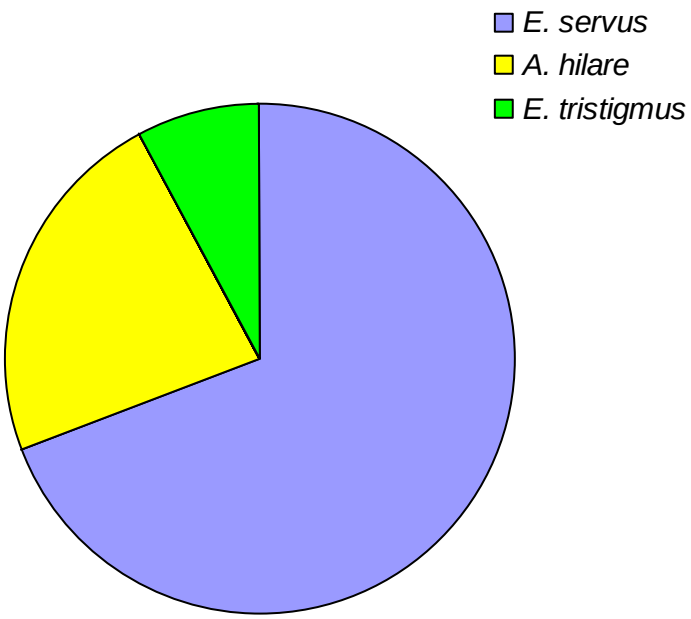
Appendix A

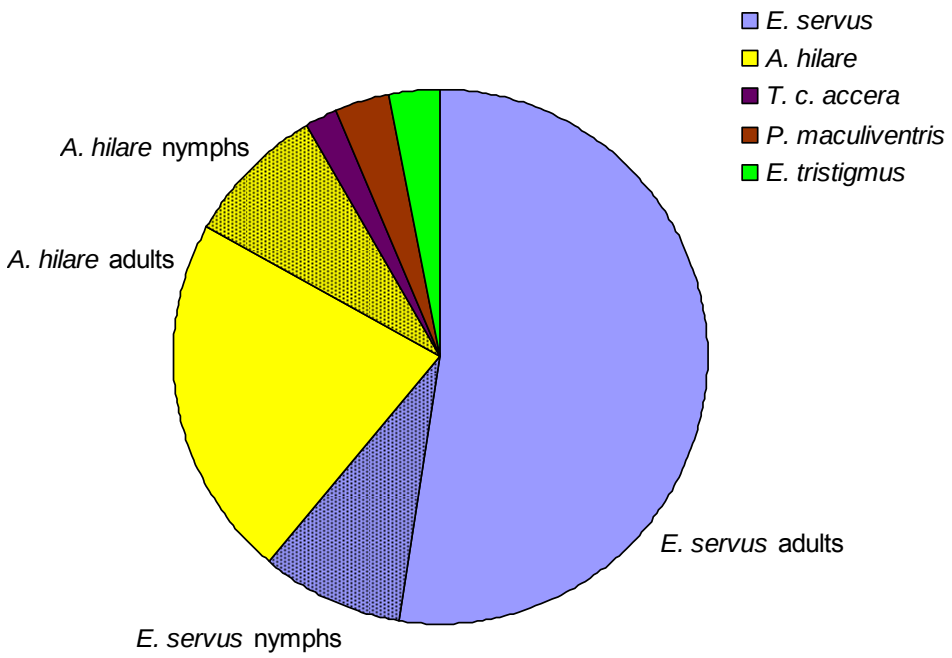
Additional figures for chapter two



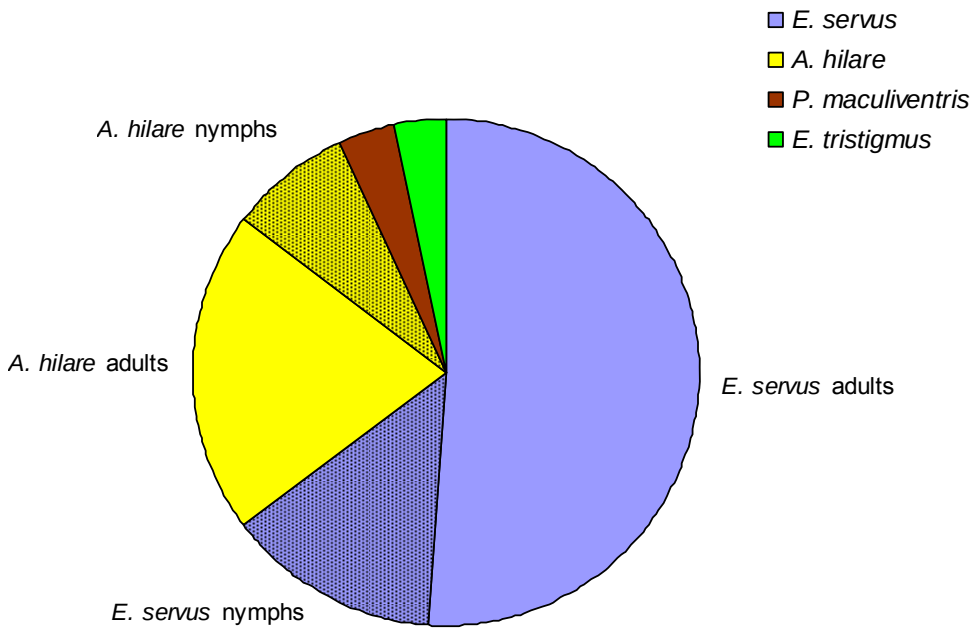
Appendix A.1. (2005) Percentage of stink bug species (n = 320, 100% adults) captured in cotton using a sweep net (25 sweeps/sample).



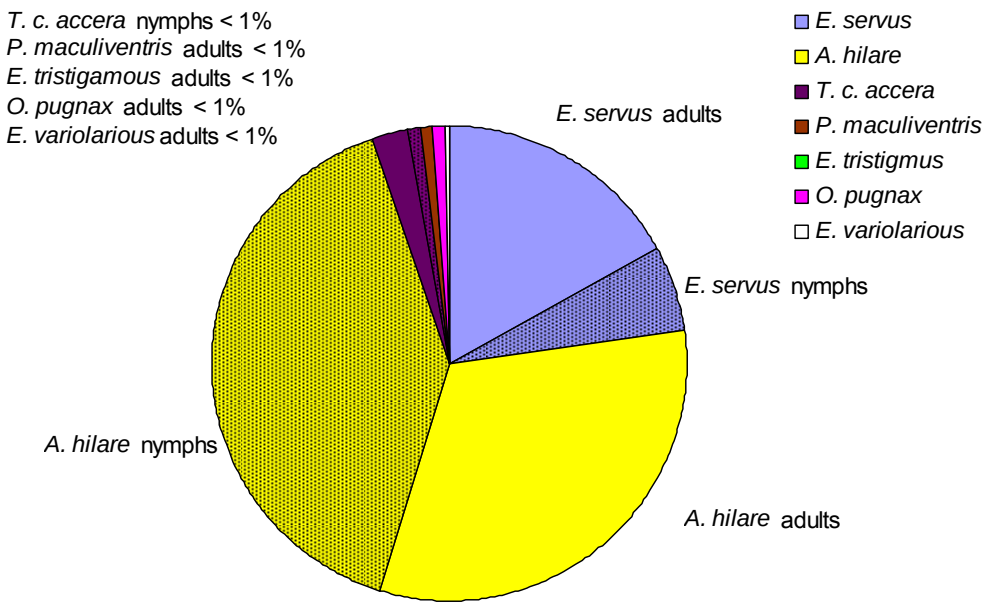
Appendix A.2. (2005) Percentage of stink bug species (n = 320, 100% adults) captured in cotton using a beat sheet (1.8 row-m/sample).



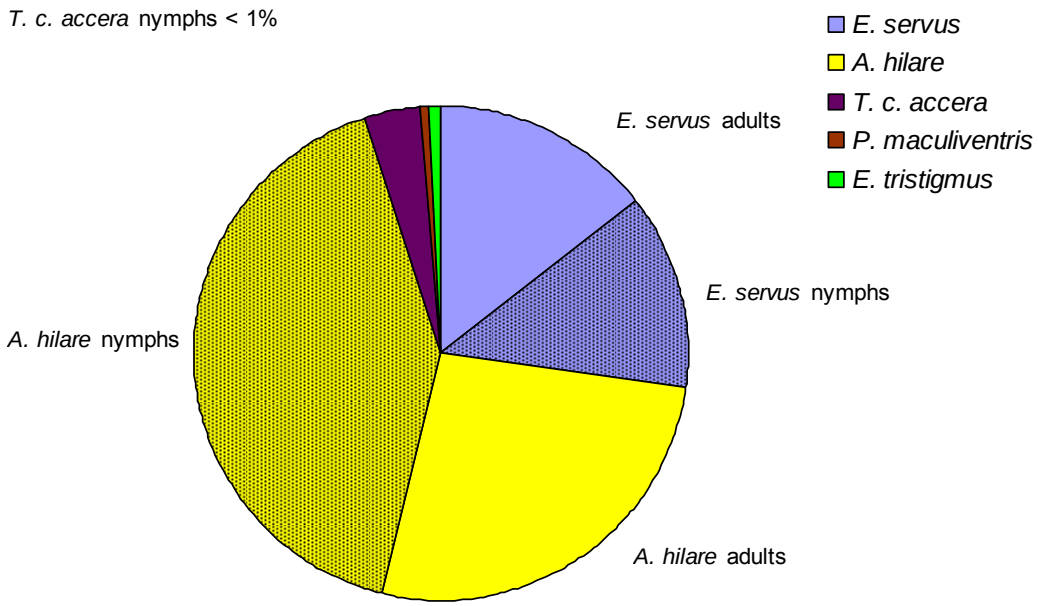
Appendix A.3. (2005) Percentage of stink bug species (n = 374) found in soybean using a sweep net (25 sweeps/sample). Solid colors represent stink bug adults and shaded colors represent stink bug nymphs.



Appendix A.4. (2005) Percentage of stink bug species (n = 374) found in soybean using a beat sheet (1.8 row-m/sample). Solid colors represent stink bug adults and shaded colors represent stink bug nymphs.



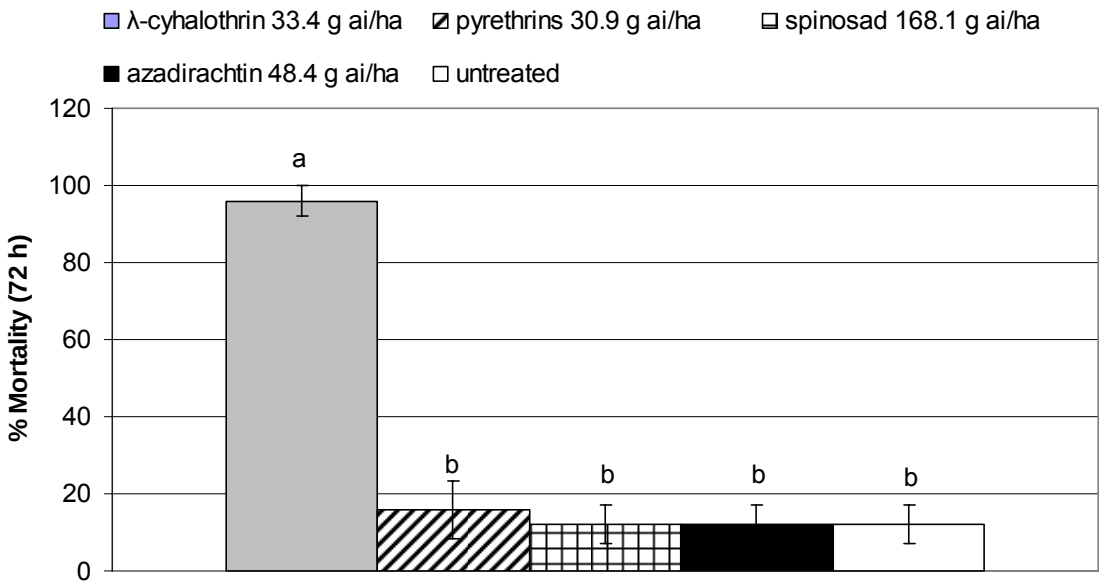
Appendix A.5. (2006) Percentage of stink bug species (n= 833) found in soybean using a sweep net (25 sweeps/sample). Solid colors represent stink bug adults and shaded colors represent stink bug nymphs.



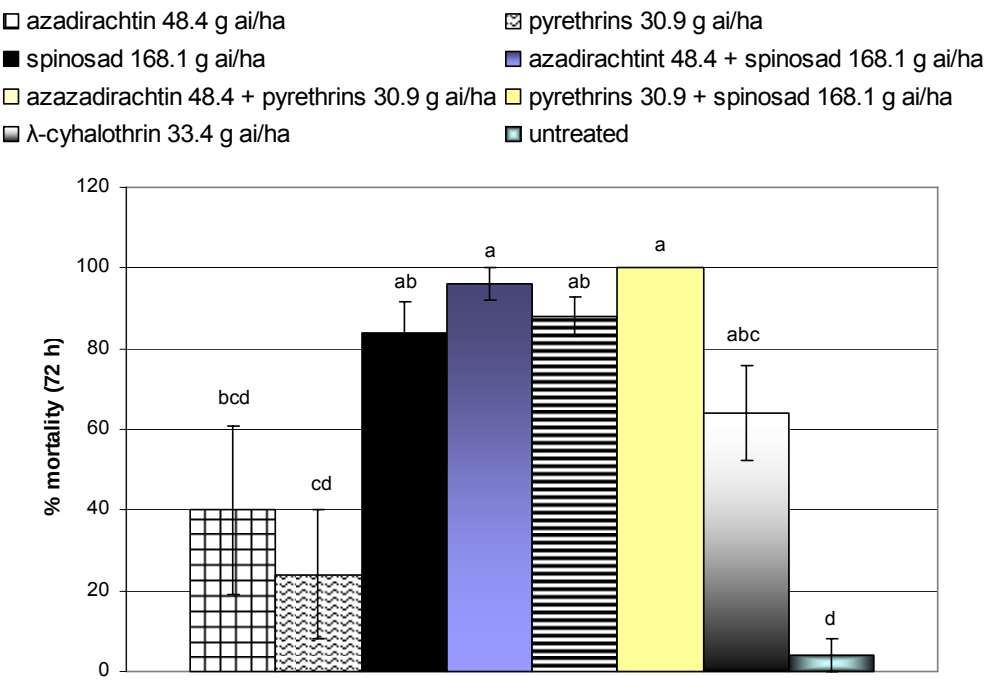
Appendix A.6. (2006) Percentage of stink bug species (n = 833) found in soybean using a beat sheet (1.8 row-m/sample). Solid colors represent stink bug adults and shaded colors represent stink bug nymphs.

Appendix B

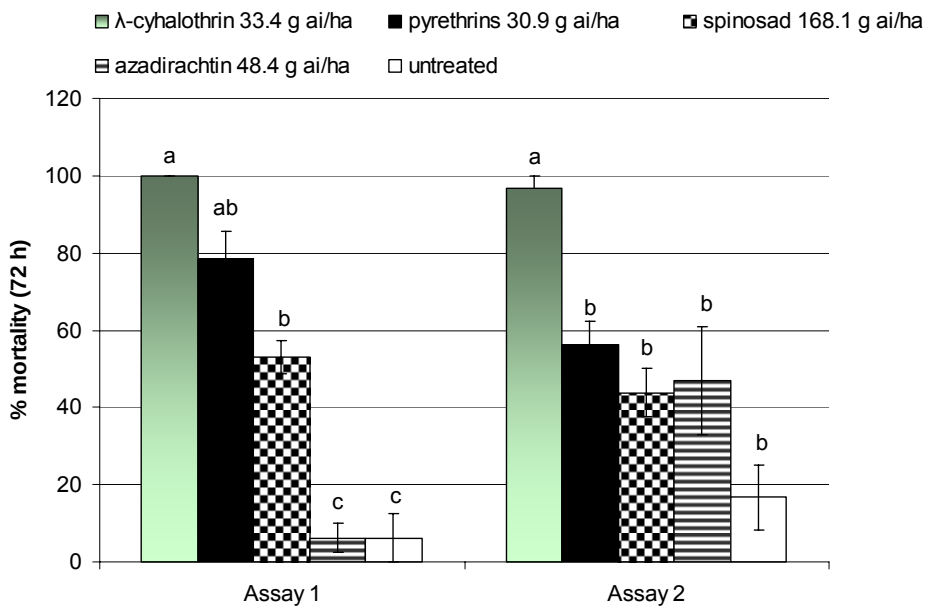
Additional figures for chapter four



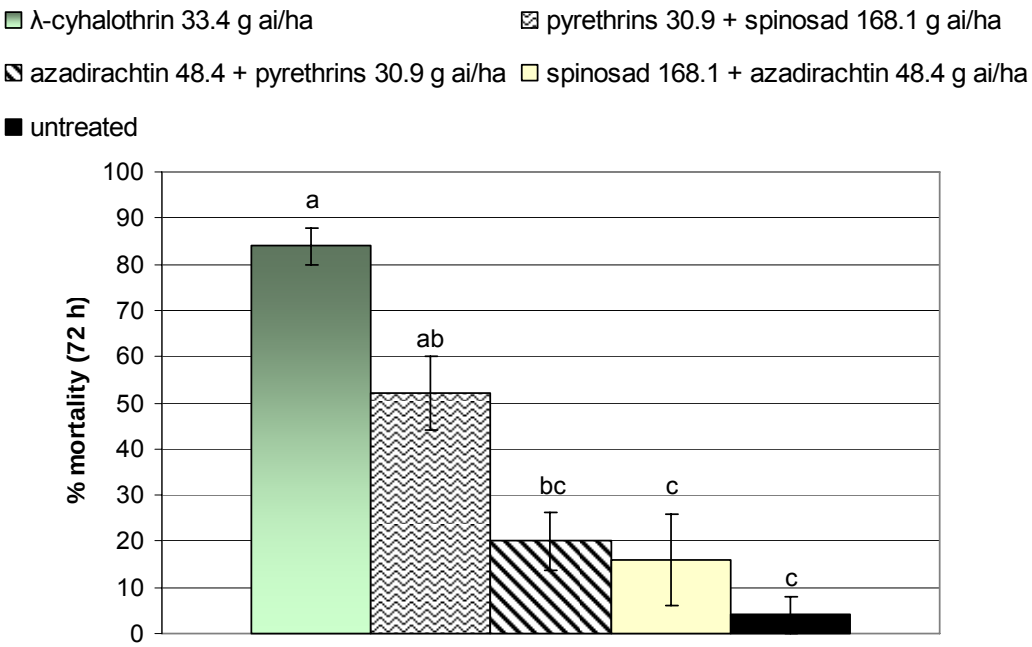
Appendix B.1. (2008) Mean ± SE (n = 25) percent mortality of laboratory reared *Euschistus servus* nymphs after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Tukey's HSD.



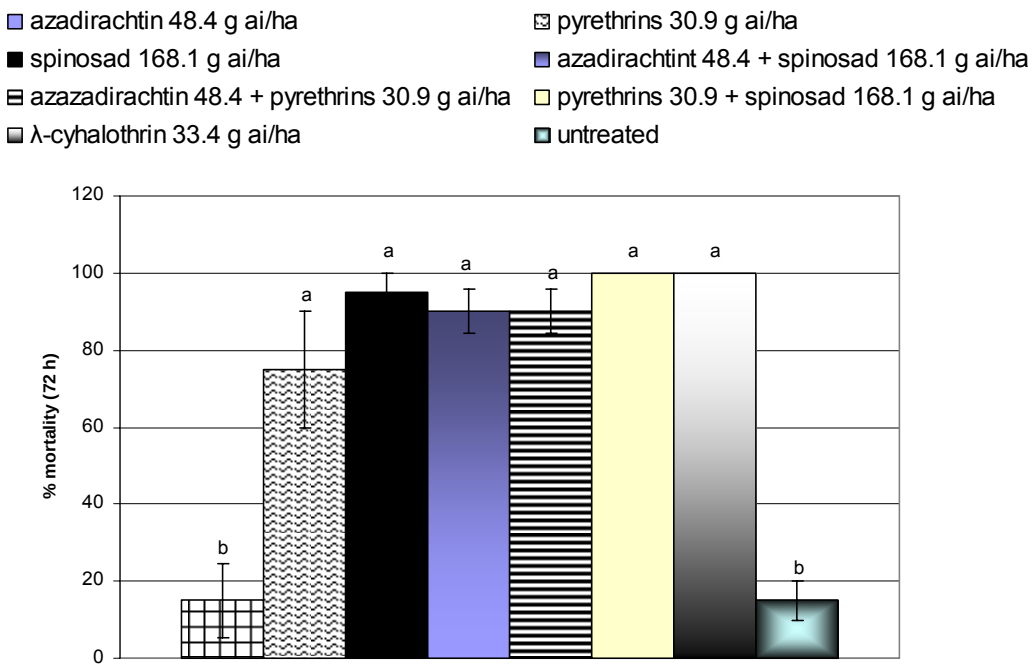
Appendix B.2. (2007) Mean ± SE (n = 25) percent mortality of *Euschistus servus* adults collected from Havelock, NC after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Tukey's HSD.



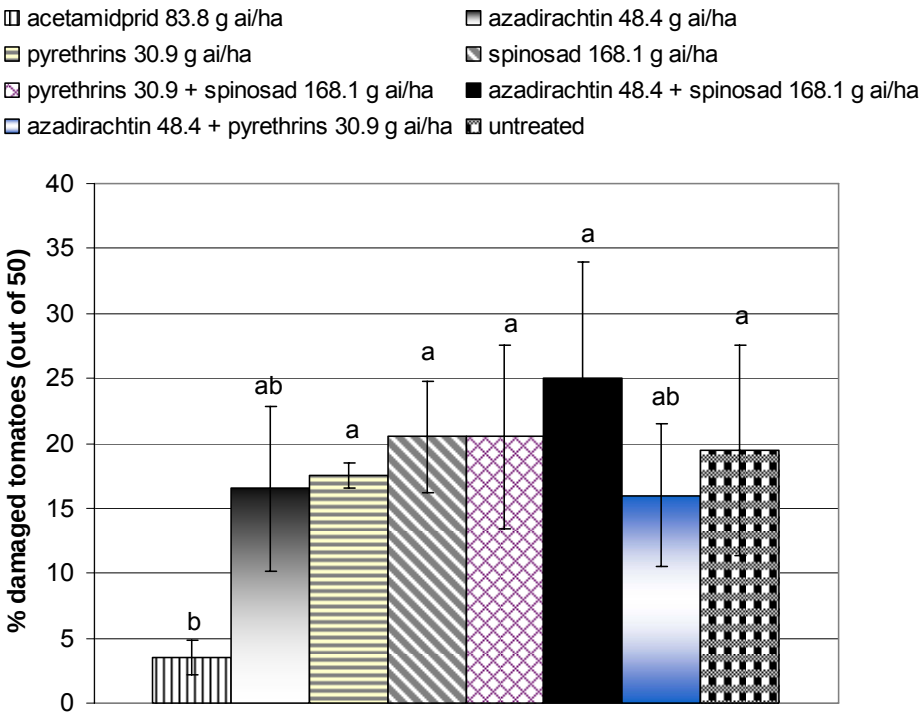
Appendix B.3. (2006) Mean ± SE (n = 20) percent mortality of *Acrosternum hilare* nymphs collected from Cheriton, VA after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Tukey's HSD.



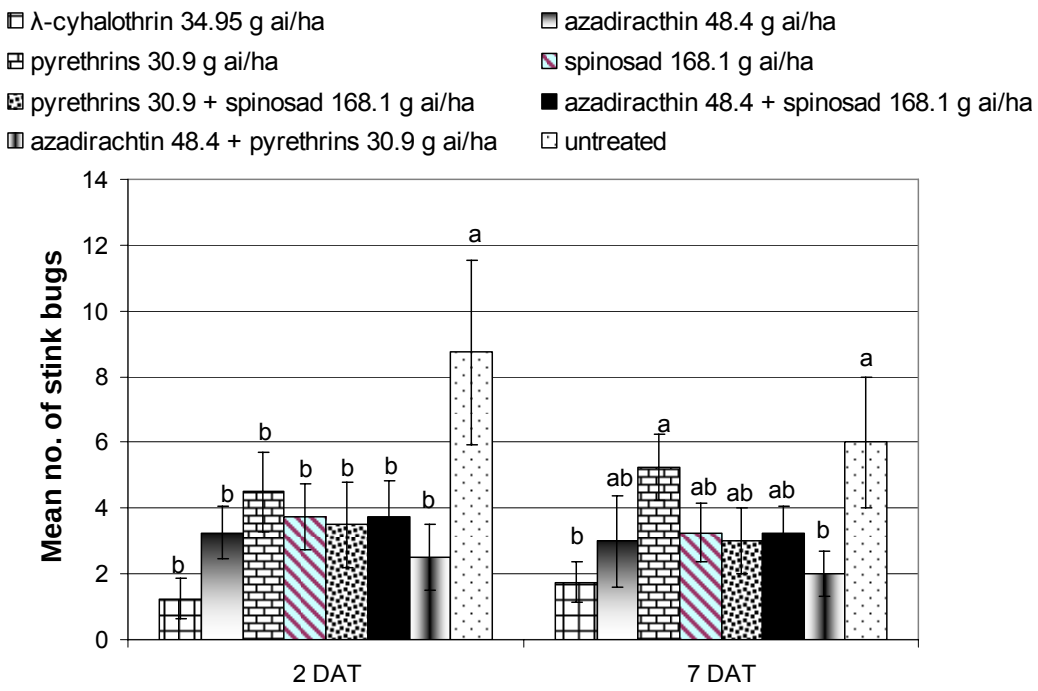
Appendix B.4. (2008) Mean ± SE (n = 25) percent mortality of *Acrosternum hilare* nymphs collected from Camden, NC after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Tukey's HSD.



Appendix B.5. (2007) Mean ± SE (n = 20) percent mortality of *Acrosternum hilare* adults collected from Camden, NC after 72 h of exposure to treated green beans. Bars with different letters are significantly different as determined by one-way ANOVA ($P < 0.05$) and Tukey's HSD.



Appendix B.6. (2007) Mean ± SE of percent stink bug damage tomatoes (n = 50) after multiple organic insecticide applications in Painter, VA. Bars with different letters are significantly different as determined by ANOVA ($P < 0.05$) and Fishers LSD.



Appendix B.7. (2008) Mean ± SE of stink bugs found in soybean with a 38-cm sweep net after a single application of insecticides at 2 and 7 days after treatment (DAT). Painter, VA. Bars with different letters are significantly different as determined by ANOVA ($P < 0.05$) and Fishers LSD.