Stink bug egg studies in southeastern Virginia: parasitoid survey, and susceptibility and chorion permeability to insecticides

Amanda L. Koppel

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

D. Ames Herbert, Jr., Chair
Thomas P. Kuhar, Co-Chair
Kim A. Hoelmer, Member
Douglas G. Pfeiffer, Member
Scott M. Salom, Member

March 26, 2010
Blacksburg, VA

Keywords: Pentatomidae, Scelionidae, biological control, efficacy, egg chorion

Stink bug egg studies in southeastern Virginia: parasitoid survey, and susceptibility and chorion permeability to insecticides

Amanda L. Koppel

ABSTRACT

Currently, there is little known about stink bug (Hemiptera: Pentatomidae) eggs, their natural enemies, and their susceptibility to insecticides.

A survey of stink bug egg parasitoids was conducted in row crops and vegetables in eastern Virginia. Parasitization was highest in *Euschistus servus* (Say) with 89.7 % and 49.2% of egg masses and individual eggs parasitized, respectively, followed by *Acrosternum hilare* (Say), with nearly half of all individual eggs parasitized. The most common parasitoid was *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae).

Laboratory egg-dip bioassays and field applications of acephate, λ-cyhalothrin, spinosad, and thiamethoxam, were carried out to determine efficacy against non-parasitized *E. servus* and *A. hilare* eggs, and *T. podisi* embryos developing in *E. servus* eggs. Results showed that eggs of both species were susceptible to insecticides, that there was little difference among insecticides, but there was generally greater mortality in field-treated versus dipped eggs. Developing *T. podisi* were generally more susceptible to insecticides than stink bugs.

Scanning electron microscopy was used to investigate oviposition sites as possible sites of insecticide movement into eggs. Oviposition wounds and holes made by a tungsten probe were similarly sealed by a “scab”, so it was not clear whether these wounds allow for increased insecticide movement into parasitized eggs.

Differences in chorion permeability of non-parasitized and parasitized eggs were compared by immersing them in solutions containing different ^{14}C-ammended insecticides at field application rates for 0, 30, 120 or 240 minutes. Results showed that insecticide movement into the egg increased significantly with immersion time for both acephate and λ-cyhalothrin, but there were no significant differences between non-parasitized and parasitized eggs. Neither immersion time nor egg status was significant for thiamethoxam. A model was constructed that predicts amount of insecticide entering the egg at any given time.

An 8-week survey for the brown marmorated stink bug, *Halyomorpha halys* (Stål) was conducted in Beijing and five other cities in China. Incidence of egg parasitism was recorded. Results showed that *H. halys* utilized at least four different plants throughout the summer, and insects were found in Nanjing, Kunming, and Xi'an. Parasitization of eggs was noted, and the parasitoids were identified as *Trissolcus halyomorphae* Yang (Scelionidae: Hymenoptera) by K.A. Hoelmer (USDA-ARS).

Acknowledgements

So many people have given me their valuable time, reviews, and care, and I never would have completed my education if it wasn't for them. First and foremost, my major professor, Dr. Ames Herbert, who immediately snatched me up after I sent an interest letter to the Virginia Tech Department of Entomology as an undergraduate. He has never spared any expense in my graduate career, and has always gone out of his way to make sure that the research experience I gained was in the field and in the laboratory.

My dissertation committee is an incredible group who encouraged me to be my best. Dr. Tom Kuhar always kept an eye on my research from the Eastern Shore, and asked his hard-working summer crew to help me collect data. Dr. Doug Pfeiffer encouraged me to be independent as his Integrated Pest Management teaching assistant, and supported my teaching decisions. Dr. Scott Salom kept me interested in biological control with enthusiastic lectures. His biocontrol class was one of the first courses I took in graduate school. Dr. Kim Hoelmer, USDA-ARS-BIIR, gave me the opportunity to take what I learned in the classroom and apply it in the real world—I never would have gone to China if it wasn't for him.

Many other people were indispensable through the course of my research. Dr. Sean Malone (Tidewater AREC) spent hours in the 100°+ heat index helping out with field work and never hesitated to review a manuscript or poster. Dr. Don Mullins and Sandra Gabbert (VT) dedicated their time, laboratory, and resources to my chorion research and always supported this study. The enthusiasm of Edwina Westbrook (Virginia State University), for microscopy, helped me to discover a new avenue of research and a new area of science to participate in.

Thanks also to C. Brownie (North Carolina State University), R. M. McPherson (University of Georgia), G. Chappell (VSU), J. McIntosh (Virginia Tech Institute for Critical Technology and Applied Science), J. Cranmer (Valent U.S.A. Corporation, Cary, NC), B. Feiler and J. Harper (American Vanguard Chemical Corp., Newport Beach, CA, and Seaford, DE), V. Mascarenhas (Syngenta Crop Protection, Inc., Whitakers, NC), L. Zhang, H. Chen, and H. Lu (USDA Sino-American Biological Control Laboratory, Beijing, China) for their help with my research.

I would like to thank my "momma hens" at VT and the Tidewater AREC, Carolyn Crowder, Debbie Estienne, Sarah Kenley, Kathy Shelor, and Robin Williams, for keeping an eye on me and my paperwork. My fellow grad students at VT also gave me a network of support and encouragement, and lots of company on long van rides to the ESA meetings.

If Tidewater was my home away from home, then Mike, Glynda, and Rebecca Arrington were my family away from my family. Mike and Rebecca worked tirelessly in the field with me under the worst weather conditions. At the end of the day, Glynda would welcome me to the dinner table if Rebecca and I had baking to do.

The Warriors of Inconvenience (Andrew, Matt, Scott, Lauren, Dave… and Jellybean) kept my weekends full of gaming and distractions. We did it all for science, and have no regrets. They taught me to think on my feet and to never forget the power of Oreos.

Last but not least, I'd like to thank my family for their support. My Mom and Dad might have had reservations about me becoming a bug lady, but they never showed

it if they did. My sister Rebecca just started her PhD program in psychology, and I hope that I can give her even half the support she's given me over the years.

Funding for various research projects was provided by the Virginia Agricultural Council, the Virginia Soybean Board, and the National Science Foundation East Asia and Pacific Summer Institutes program.

Table of Contents

List of Figures

Chapter Three

Chapter Four

Chapter Five

Chapter Six

List of Tables

Chapter Two

Chapter Three

Chapter Five

Chapter One

Introduction and Literature Review

An Economic Concern

Stink bugs (Hemiptera: Pentatomidae) attack many crops and commodities, with different species complexes in each region of the United States. In Virginia, for example, cotton and soybean are at risk from both brown [*Euschistus servus* (Say)] and green stink bugs [*Acrosternum hilare* (Say)]. Further south, the southern green stink bug [*Nezara viridula* (L.)] is a major problem, while in Pennsylvania, New Jersey, Delaware, Maryland, and increasing areas to the south, including Virginia since 2004, the brown marmorated stink bug [*Halyomorpha halys* (Stål)] is an invasive pest.

Stink bugs are a serious concern. Economic damage of injured soybeans in Georgia exceeded $2 million in 1993 (McPherson and McPherson 2000). The Beltwide Cotton Insect Loss Report reported a loss of over $7.6 million in cotton in 1994 (Williams 1995). In Indiana, 80% of all tomato fields are affected by stink bugs, and 8-10% crop loss may be expected without treatment (Janssen 1999). Nault and Speese (2002) has reported that stink bugs are a major pest on tomato in the spring. Soybean and cotton, in particular, are especially important to Virginia agriculture, as soybean is the second most valuable row-crop in the state and cotton is valued over $30 million (Virginia National Agriculture Statistics Service 2009).

A recent survey of the soybean-cotton ecosystem determined that the brown and green stink bugs were the most common species found in southeastern Virginia (Kamminga 2008).

Euschistus servus

Biology. *Euschistus servus* is a polyphagous insect, feeding on shrubs, trees, grasses, and various commodities; however, it prefers to eat plants which produce pods or fruits. It can survive on weedy hosts, which plays a major role in its pest status (McPherson and McPherson 2000).

The brown stink bug is bivoltine in Virginia, and begins the first generation in the spring as adults. They overwinter in crop residues and weeds along field borders, such as mullein (*Verbascum* species). Studies in northwestern Arkansas fields determined that emerging adults laid first generation egg masses beginning on April 30th. There were approximately 19-23 individual eggs per mass, and they hatched beginning on May 11th (Kendrick and Rolston 1961). Five instars of nymphs feed by puncturing food with their stylets. Their diet includes the fruiting bodies of plants such as cotton bolls or bean pods. The nymphal stage lasts approximately 33 days. The second generation of adults laid eggs between August 3rd and October 10th, and progeny emerged between August 7th and October 20th (Kendrick and Rolston 1961).

Economic Importance. On soybean, the brown stink bug was considered a minor pest because it had such a wide range of host plants, and there was sufficient overlapping of uncultivated fruiting plants such that other commodities were more attractive than soybean. Increasingly in Virginia and other states, this insect has become an important pest. *E. servus* is able to severely damage soybean, and even a single puncture on the axis of a radicle-hypocotyl on a seed can prevent its germination (McPherson and McPherson 2000). Damage done to the soybean through feeding may include reduced, terminated, or deformed seed growth, spotting on fruiting bodies, loss of plant fluids and tugor pressure, delayed plant maturation, and a reduction in yield. In addition, puncture wounds put the plant at a higher risk to contract pathogens, or

agents of decay. Moreover, *E. servus* is capable of transmitting the causative agent of yeast-spot disease to soybeans (McPherson and McPherson 2000) .

In field cages containing 6 plants and 1-4 brown stink bugs, it was found that as infestation levels increased, the number of underdeveloped beans was most pronounced in the middle third of the plant (Daugherty et al. 1964). Further, at densities of 3-4 bugs per cage, soybean maturity was severely delayed. An increase in feeding punctures leads to a decrease in bean germination, possibly due to the introduction of unidentified organisms into the plant. The highest populations of *E. servus* occur in the fall, when other species of stink bug are also most numerous, thus adding to the pest complex (Daugherty et al. 1964).

Acrosternum hilare

Biology. *Acrosternum hilare* is a polyphagous insect, which requires a series of plants with overlapping periods of seed and fruit production to achieve maximal development. The green stink bug prefers trees and bushes as hosts, although it is a known pest of commodities such as lima beans, green beans, peaches, soybean, and cotton. A combination of American elder, black locust, honey locust, elderberry, and mimosa are an excellent combination of hosts to increase their population (Underhill 1934).

There has been debate concerning whether this insect is univoltine or bivoltine in different parts of the United States (McPherson and McPherson 2000). However, it is thought that the differences in reproductive cycles may reflect differences in climate. In more favorable southern conditions, such as in Virginia, the life cycle is bivoltine. Similar to the brown stink bug, *A. hilare* overwinters as an adult, preferring leaf litter and deciduous woodlands (Underhill 1934). In early April, when the adults emerge, they immediately go to a host and deposit egg masses. It has been noted, for example, that adults in Virginia deposit eggs on peach trees, and

upon emergence, the nymphs will dimple the fruit surface, resulting in severe injury (McPherson and McPherson 2000). It takes 6-7 weeks for a full life cycle. Egg masses hatch approximately 8.4 days after being deposited on substrate, and it takes nymphs 36 days to go through five instars. Adults are the most active on days where the temperature exceeds 23.3°C. Females will lay over 90% of their eggs on leaves (Underhill 1934).

A specific life cycle has formed on soybean in the southern Atlantic states, specifically in the Carolinas. The first generation feeds and reproduces on black cherry and elderberry where they increase their population. In mid-July, these adults begin to enter soybean fields. Adult populations peak in soybean in September and early October (McPherson and McPherson 2000).

Economic Importance. *Acrosternum hilare* is one of the three major members of the soybean-stink bug complex in the United States, the others being *E. servus* and *N. viridula*. As with *E. servus*, its numbers reach their peak late in the summer, when soybean pods and seeds are most abundant. Damage is done to plants when the stylet penetrates plant tissue, and losses can be significant.

When soybean was exposed to a stink bug density of 2 or 4 bugs per 0.3 meter of plant row, there were significant reductions in yield and percent germination. Yield loss was due to decreased numbers of seeds produced and reduced seed size. In addition, at densities of 1, 2, or 4 bugs per 0.3 meter of row, there was a significant increase in percent of beans injured. In these studies, the fifth instar nymph caused the most damage (Yeargan 1977).

The most common forms of damage to soybean include decreased quality and yield of seeds, fewer seeds per pod, increased number of discolored or moldy seeds after storage, an increased percentage of small seeds, and decreased stem length of the plant. In addition, heavily damaged soybeans produce less oil than slightly damaged beans. Finally, as with *E. servus*,

excessive punctures from *A. hilare* stylets may increase the chance that a pathogen or bacterium would enter the soybean, and the bug has been shown to transmit the causative agent of yeast-spot disease (McPherson and McPherson 2000).

Monitoring for *E. servus* and *A. hilare*

Leskey and Hogmire (2005) studied how different constructions of pyramid and jar traps affected the number and kind of stink bugs caught. In the case of the former, they tested different colors of varying base materials and lures, and found that baited masonite pyramid traps in peach orchards caught the most stink bugs. The most attractive color was industrial safety yellow exterior latex gloss enamel paint, and the best bait was the *Euschistus* species aggregation pheromone, methyl (2E, 4Z)- decadienoate (Leskey and Hogmire 2005). Both brown and dusky [*Euschistus tristigmus* (Say)] stink bugs were caught most often (55% and 20%, respectively), followed by green stink bugs (16%), and miscellaneous species (9%). Pyramid traps caught more stink bugs than jar traps did. Kamminga (2008) tested collection techniques using a beat sheet and a sweep net in soybean and cotton. The beat sheet caught more brown stink bugs and the sweep net caught more green stink bugs.

Stink Bug Control

Insecticides are the most common strategy used for controlling stink bugs. In southeastern Virginia, pyrethroids are most commonly used against stink bugs for three reasons: farmers are already accustomed to using to insecticides in those classes, they are inexpensive, and they are already used for controlling other common pests like corn earworm (*Helicoverpa zea* Boddie). In addition to pyrethroids, recent efficacy tests suggest that organophosphate and some neonicotinoid insecticides are also effective (Kamminga et al. 2008).

Biological control, which relies upon natural enemies of the pest to manage the population and reduce commodity damage, could be another viable option. For example, the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) has shown potential against southern green stink bugs in soybean (Ehler 2002). Inoculative releases of 15,000 *T. basalis* adults per hectare in a trap crop of early maturing soybean reduced the stink bug density an average of 58% in the main crop of late planted soybeans (Corrêa-Ferreira and Moscardi 1996).

Pentatomid Eggs

Structure. The general structure of pentatomid eggs is well described (Esselbaugh 1946, Bundy and McPherson 2000). The egg shell is referred to as the chorion, and is composed of sclerotized proteins (Nation 2002), forming spinose, reticulate, or triangular patterns that vary among stink bug species (Bundy and McPherson 2000). At the anterior of the egg is a circular lid, the operculum, which is loosely attached to the stink bug egg and provides an opening for emergent nymphs (Esselbaugh 1946). Micropyles are tube-like hollow protrusions of the chorion which are arranged in a circle around the operculum (Esselbaugh 1946). Beament (1948) studied the properties of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) eggs, stating that it would serve as a "type specimen" for hemipterous pest-species' eggs, and determined that micropyles, which penetrate some egg shell layers, are crucial to the transport of insecticides into eggs because chorion is impermeable to ovicidal or toxic substances (Beament 1952).

Insecticide Permeability. The efficacy of insecticides on adult stink bugs and egg parasitoids has been explored in a number of studies. The organophosphate dicrotophos has been cited as having a high toxicity to various stink bug species (Tillman et al. 2004, Snodgrass et al. 2005), and it is suggested that insecticides provide more consistent control for *A. hilare* and *N. viridula*, the southern green stink bug (Willrich et al. 2003, Snodgrass et al. 2005, Kamminga

et al. 2008) than for *E. servus*. Kamminga et al. (2008) performed a number of efficacy trials on *E. servus* and *A. hilare* nymphs and adults in southeastern Virginia and found that *A. hilare* was especially susceptible to all of the pyrethroids tested.

Waddill (1978) subjected a number of parasitoids, including *Telenomus remus* (Nixon) (Hymenoptera: Scelionidae), to treatment with pyrethroids, including permetherin, resulting in high mortality. High mortality was also recorded within 6 h of exposure to methyl parathion, to *T. remus* and other hymenopteran parasitoids, including members of the families Chalcididae, Braconidae, and Ichneumonidae (Wilkinson 1975). However, another study found that almost all adult *T. basalis* survived when exposed to permetherin (Orr et. al 1989).

The uptake of specific classes of insecticides into insect eggs has also been studied. Organophosphates have been used as ovicides, but eggs have developed resistance to this group due to biochemical differences in the esterase complex of resistant-strain embryos (Smith and Salkeld 1966). Further, parathion does not act as a true ovicide on the eggs of milkweed bug, *Oncopeltus fasciatus* (Dallas), as the penetration barrier of eggs does not allow enough insecticide to flow into the eggs to kill the embryo (Zschintzsch et al. 1965). However, it has been demonstrated that symptoms of organophosphate toxicity coincide very closely with cholinesterase inhibition in *O. fasciatus* eggs (Smith and Salkeld 1966). Both cholinesterase and acetylcholine appear in these eggs at four days of development (Mehrotra 1960).

Natural Enemy Surveys

Before incorporating biological control into an IPM program for *E. servus* and *A. hilare*, natural enemy surveys were performed to assess current levels of naturally occurring parasitization. Early studies were designed to detect egg parasitoids, which "constitute the most important natural enemy [of stink bugs]" (Underhill 1934). In order to study this phenomenon,

egg masses may be collected from the field, lab-reared eggs may be placed in crops as sentinels, or both procedures can be used. Eggs that have turned dark are then brought back to the laboratory and evidence of predation is noted before they are placed in a growth chamber. There, parasitoids and healthy stink bug nymphs are reared out of the eggs, and quantitative data such as percent parasitization are recorded for both individual eggs and egg masses.

South Carolina. Jones et al. (1996) investigated the parasitism of pentatomid pests in South Carolina soybean. A total of 164 egg masses were collected from four genera of pentatomids. Egg parasitism of *N. viridula* ranged from 0-50%, and all of the parasitoids were *T. basalis*. *A. hilare* was only parasitized by *Trissolcus edessae* Fouts, and parasitism ranged from 11-25% in 1975, and from 21- 80% in 1978. Parasitism of *E. servus* ranged from 33-50%, and *T. basalis, Trissolcus euschistii* Ashmead, and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) emerged from egg masses. The final stink bug studied was *Thyanta custator accerra* McAtee, which was parasitized by *T. basalis* and had a parasitism range of 0-66.7%.

Of all the pentatomid eggs found, *N. viridula* was most heavily parasitized, followed by *A. hilare*, *E. servus*, and *T. c. accerra*. Jones et al. (1996) also investigated parasitization of adult pentatomids, which occurred far less frequently than egg parasitization. Finally, in South Carolina, it was observed that neither *T. podisi* nor *T. basalis* will attack *A. hilare* in laboratory no-choice tests.

Kentucky. Yeargan (1979) studied both parasitization and predation of stink bug eggs in Kentucky, in soybean and alfalfa. Of the laboratory-reared eggs placed in the field, no parasitism of *A. hilare* occurred. In addition, *T. podisi* was the only parasitoid recovered from *Euschistus* species and *Podisus maculiventris* (Say) eggs. In the next year of laboratory-reared egg studies, egg parasitism of *A. hilare* was much lower than it was for either *E. servus* or

Euschistus variolarius (Palisot). Evidence of chewing and sucking predators was found in all of the species' eggs. Of the eggs collected from the field, 83% of *E. servus* eggs were parasitized, and almost all of them by *T. podisi*. In contrast, *A. hilare* had 45% parasitism from *T. euschistii*.

Telenomus podisi was the most commonly encountered parasitoid in Yeargan's study, and it was the only species recovered from some of the experimentally manipulated eggs throughout the year. However, because it will parasitize eggs of five different species of pentatomids, it is a generalist, and could be a threat to beneficial pentatomids.

Louisiana. In Louisiana, Orr et al. (1986) investigated stink bug egg parasitism in soybeans. The eggs of *Euschistus* species were parasitized most heavily, with 51% of egg masses and 45% individual eggs parasitized. *A. hilare*, on the other hand, had the lowest parasitism percentages for egg mass and individual eggs, 22% and 15%, respectively. *T. podisi* was the most common parasitoid reared from *Euschistus* eggs, although other parasitoids included *T. basalis*, *T. euschistii,* and *T. edessae*. Emerging from the eggs of *A. hilare* were *T. euschistii, T. edessae, Trissolcus cristatus* Johnson*,* and *T. podisi*. In addition, *T. podisi* frequently visited the egg masses of *N. viridula*, but only parasitized egg masses already parasitized by *T. basalis*. In such instances, the ratio of individual eggs parasitized by the latter to the former was nearly 9:1. The authors were unable to explain why *T. podisi* did not parasitize these without the others' presence.

A relationship was detected between parasitism by *T. podisi* of *Euschistus* egg masses, and the seasonal trend in pentatomid egg mass densities (Orr et al. 1986). Parasitization by *T. podisi* occurred almost exclusively on *Euschistus* early in the field season, when the densities of all pentatomid egg masses was low. After those densities reached their peak levels, *T. podisi*

shifted hosts to include other available species in relation to their abundance. A similar relationship was noted between *N. viridula* egg masses and *T. basalis* (Orr et al. 1986).

Florida. Temerak and Whitcomb (1984) looked at the parasitoids of pentatomids in soybean fields in Florida. Egg masses from eight species of stink bug were recovered; three were predaceous species and the others were phytophagous. Of the parasitoids, *T. podisi* and *T. basalis* were the most abundant, and they attacked the eggs of four different stink bug species. In addition, three different scelionids were reared from the eggs of *Podisus guildinii* Westwood. One tachinid and eight sarcophagid species were reared from *N. viridula* egg masses. The hosts with the greatest percent parasitism were *P. guildinii* and *E. servus*, both of which had greater than 65% parasitism.

California. Ehler (2002) evaluated the natural enemies of *N. viridula* in California. Results showed that predation did not exceed 10%, although parasitization ranged from 0-40%. Parasitoids recovered from the egg masses included *T. basalis, Gryon obesum* Masner (Hymenoptera: Scelionidae)*, T. podisi,* and *Ooencyrtus californicus* Girault (Hymenoptera: Encyrtidae). Predation was carried out by unknown species with chewing mouth parts. On beans and tomato, *T. basalis* caused the highest rate of parasitization, sometimes as high as 74%.

For predators, the mean number of egg masses attacked ranged from 15-40% and was independent of egg mass size. In 50% of the egg masses, predators only attacked 10 or fewer eggs. In contrast, the attack rate of *T. basalis* was directly related to egg mass size, and in 90% of the egg masses, all of the eggs in a given mass were preyed upon.

Brazil. Correa-Ferreira and Moscardi (1995) investigated the seasonal occurrence and host spectrum of stink bug egg parasitoids in soybean in Brazil. The stink bug host species represented in the egg masses were: *N. viridula, P. guildinii, Euschistus heros* (F.)*, Dichelops*

melacanthus (Dallas), *Acrosternum* species, *Edessa meditabunda* (F.), and *Podisus connexivus* Berg. Twenty species from four hymenopteran families were found parasitizing eggs. Of these, Scelionidae was the most frequent and abundant family, with 12 species present. *T. basalis* and *T. podisi* were the most common scelionids. In addition, encyrtids (*Ooencyrtus*) and eurytomids (*Neorileya*) were observed on various stink bugs. In a total of 8,225 egg masses, 112,603 individual eggs, most of the eggs were *N. viridula*, and the average egg mortality was 62 % in the 1989-1990 field season, and 74% in the 1990-1991 field season. Parasitism rates were 51% for *P. guildinii*, 59% for *E. heros*, and 22% for *E. meditabunda*.

In *N. viridula* eggs, *T. basalis* was responsible for 98% of total parasitization. Other species of parasitoids, such as *Trissolcus brochymenae* (Ashmead), *T. podisi,* and *G. obesum* were found at low incidences. *E. heros* eggs were most frequently parasitized by *T. podisi*, which was responsible for 98% of total parasitization. *E. heros* was its preferred host.

Virginia—Preliminary Survey. At the Eastern Shore Agricultural Research and Extension Center in Painter, Virginia, stink bug egg masses were collected in the field from a variety of crops. Egg masses from *M. histrionica* were found on collards, *A. hilare* on soybean, and *E. servus* on soybean, corn, squash, and cucumber. These masses were placed in individual petri dishes and observed in the lab for signs of parasitism (such as darkening of the eggs). Parasitoids were collected upon emergence and percent parasitization was calculated.

Parasitoids were collected and placed in vials of alcohol, and sent to the Systematic Entomology Lab (USDA) in Beltsville, MD for identification, and all of the parasitoids keyed out to the same species, *T. podisi* [T. P. Nuhn (Scelionidae), Systematic Entomology Laboratory, Agriculture Research Service, US Department of Agriculture]. Of the egg masses, 14 were *E.*

servus, 28 were *A. hilare,* and 152 were *M. histrionica*. The parasitism on these species' egg masses was 36, 7, and 1%, respectively.

Due to its relative abundance in the field, *T. podisi* was chosen as a parasitoid to focus on in this dissertation.

Telenomus podisi

Host Range. *Telenomus podisi* has been reported in Kentucky in the eggs of *A. hilare, E. servus,* and other *Euschistus* species, in Louisiana in *A. hilare, Euschistus* species, and *N. viridula*, in South Carolina in *Euschistus* species and *N. viridula*, and in Florida in *N. viridula* (Jones et al. 1996). In addition, it was recently shown to be found in the eggs of *A. hilare, E. servus,* and *M. histrionica* in Virginia.

Host Location and Discrimination. Much research has been done to determine how *T. podisi* locates egg masses. Bruni et al. (2000) studied kairomonal activity for the attractant pheromone of *P. maculiventris* in order to determine if it attracted scelionid parasitoids, specifically *T. podisi*. Exposed *P. maculiventris* eggs were placed in field traps both with and without synthetic pheromone. The incidences of *T. podisi* from eggs of baited and nonbaited traps was not statistically significantly different, suggesting that the parasitoid does not use *P. maculiventris* pheromone as a kairomone.

Soybean and pigeon pea plants infested with *E. heros* were studied to see what effect their induced volatiles had on *T. podisi* (Moraes et al. 2005). Bioassays in a Y-tube olfactometer with female parasitoids and plants pierced by *E. heros* demonstrated that *T. podisi* was attracted by the stink bug host volatile. However, when placed in the Y-tube with plants attacked by a chewing insect, the velvetbean caterpillar, parasitoids showed no significant attraction. This suggests that host feeding makes plant volatiles attractive, as opposed to feeding by a non-host

such as the caterpillar. Further investigating this phenomenon, Moraes et al. found that *E. heros* saliva mechanically injected into soybean and pigeon pea released the same attractive volatile blend as saliva injected naturally by the stink bug. They concluded that the saliva of stink bug was what made the plant volatile attractive to *T. podisi*.

Finally, Borges et al. (1999) studied both semiochemicals and physical stimuli involved in *T. podisi* host recognition. The results indicated that both *E. heros* egg adhesive and a complex of stink bug sex pheromones acted as kairomones, playing an important role in the process of parasitoid selection. The former has a lower volatility than the later, so it was hypothesized that the egg adhesive acted as a short-range kairomone. Visual stimuli are more important than semiochemicals in host finding over short distances. *T. podisi* is visually attracted by small objects which project above a surface, and as such, will move toward either eggs or small beads placed on a substrate. Color of the egg or bead was not observed to play a factor as an attractant. After seeing the egg, a female parasitoid will walk 1-2 mm to it, and then engage in antennal drumming. Next, she will mount the egg and begin ovipositor probing and extrusion, probing several locations before she drills. After oviposition, females mark the egg by rubbing their ovipositor on it (Borges et al. 1999).

T. podisi, when in a system with *T. euschistii*, has been shown to exhibit habitat partitioning and intraspecific host discrimination, although not interspecific host discrimination. Okuda and Yeargan (1988a) studied habitat partitioning between these two scelionids by raising *P. maculiventris* in the laboratory and placing its eggs on various plants in the field: alfalfa, hackberry trees, and black cherry trees. Vertical partitioning was also investigated by selecting six black cherry and six hackberry trees in a woodlot and attaching the eggs of *P. maculiventris* to leaves at two different heights. The results showed that *T. podisi* parasitized more egg masses

in alfalfa, whereas *T. euschistii* only parasitized eggs in the hackberry trees. However, there was no significant difference found in either parasitization rate between eggs placed at different heights, and thus, it was concluded there was no vertical partitioning in either species.

Okuda and Yeargan (1988b) also studied intra- and interspecific host discrimination between *T. podisi* and *T. euschisti*. Parasitoids were placed in a plastic bag with a *P. maculiventris* egg mass and observed. When all the eggs had been oviposited and marked, some of the eggs were rinsed off and allowed to dry. One rinsed and one unrinsed egg were placed in a plastic bag with a female parasitoid and observed for host preference. Further, in intraspecific testing, a female of each species was given a choice between a *P. maculiventris* egg parasitized by a member of her own species and a nonparasitized egg. In interspecific tests, females of both species were allowed to choose between a *P. maculiventris* egg parasitized by the other species and a nonparasitized egg. In the marking pheromone solubility experiment, there was no difference in the number of marked or washed eggs *T. podisi* parasitized. Further, there was a significant amount of intraspecific host discrimination; however, there was no evidence of interspecific host discrimination.

Reproductive Capability. The reproductive potential of *T. podisi* has been studied on a number of different stink bug species' eggs. Pacheco and Correa-Ferreira (1998) studied the reproductive potential and longevity of *T. podisi* on the host eggs of *E. heros, P. guildinii,* and *N. viridula.* Highest production of progeny occurred in the first 10 days of the female's life on *E. heros* and *P. guildinii*. The parasitoid sex ratio, calculated as the number of females divided by the total number of adults, in *E. heros* and *P. guildinii* hosts was 0.67 and 0.61, respectively, and the mean number of progeny per female was of 211 and 76 offspring, respectively. Finally, the longevity of *T. podisi* females on *P. guildinii, E. heros*, and *N. viridula* was 19.9, 30.9, and 40.6

days, respectively. The authors feel that their research suggests that *E. heros* and *P. guildinii* are the most adequate hosts for *T. podisi* development.

Orr and Boethel (1990) investigated aspects of the reproductive biology of two scelionids, *T. podisi* and *T. cristatus,* on the eggs of *P. maculiventris*. Neither species had a preovipositional period, and there was no significant difference in the length of time in reproductive activity. However, *T. podisi* was more prolific during that period. Its peak progeny was 3 times that of *T. cristatus*, and *T. podisi* females produced 2.4 times more progeny throughout their lifetime than *T. cristatus*. In addition, the total progeny *T. podisi* produced was 78, which was more than twice the value for *T. cristatus*. The longevity of reproductively active females was similar, as was the life span of adult females when they were denied oviposition. Finally, the sex ratios differed significantly, as production of male progeny averaged 18 and 6% for *T. podisi* and *T. cristatus*. Their intrinsic rates of increase were estimated at 0.31 and 0.23. The final conclusion was that *T. podisi* had a greater reproductive potential than *T. cristatus*.

Finally, the reproductive capability and longevity of parasitoids was studied by Yeargan (1982). *T. podisi* produced fewer offspring per female than *T. euschistii* and also had a decreased longevity. However, *T. podisi* produced more offspring in the first 24 hours of adult life, and its maximal period of oviposition was 18 days, as opposed to 49 days in *T. euschistii*.

Effect of Temperature on *T. podisi* Development. Cividanes and Figueiredo (1996) studied the development and emergence of *Trissolcus brochymenae* Ashmead and *T. podisi* at different temperatures. *T. brochymenae* development was slower than that of *T. podisi*. The emergence of both species from eggs was similar at 21, 24, and 27°C, but at the 30°C, *T. brochymenae* emerged after 12.8 days, whereas *T. podisi* emerged after 9.4 days. Also, the estimated lower development thresholds and thermal constants were 14.1°C /199.1 degree days

for the former species, and 13.2°C /150.7 degree days for the latter. With these data, the possible number of annual generations was estimated to be 22.8 for *T. podisi* and 15.6 for *T. brochymenae* in the laboratory.

This study was taken a step further, as Cividanes et al. (1998) tried to predict the emergence of *T. brochymenae* and *T. podisi* under field conditions in Jaboticabal, São Paulo, Brazil using the degree days they previously established. In the field, these two species were monitored on the eggs of *P. guildinii*. Their total emergence was observed after the accumulation of 211.2 and 229 degree days. The accuracy in predictions of more than 50% emergence was less accurate for *T. podisi*, but for both species, emergence occurred 2-3 days before the predicted date.

The effects of cold storage on the reproductive capability and longevity of two scelionids, *T. basalis* and *T. podisi*, was studied by Foerster and Nakama (2002). Females of both species survived for more than five months when stored at 15°C, but their fecundity was significantly reduced. After spending 140 days at 15°C, both species were still able to parasitize host eggs at a temperature of 25°C. Although parasitism was observed at 15°C, only 3.1% of eggs were parasitized by *T. basalis* and 0.2% of eggs by *T. podisi*. If females of *T. podisi* were maintained at 15°C for any longer than 50 days, they became unable to oviposit or produce progenies with a sex-ratio similar to any control insects kept at a lower temperature. However, the reduced longevity and reduced fecundity of these two parasitoids show that both species hibernate, and the authors believe that this arrestment can be useful as a tool for the mass production and storage of *T. basalis* and *T. podisi* in the autumn and winter.

Foerster et al. (2004) tested the emergence, longevity, and fecundity of the same two parasitoids after cold storage in the pupal stage. Adults failed to emerge when the two

parasitoids were transferred from 18 to 12°C at any of the pupal ages evaluated, demonstrating that this temperature is lethal to these parasitoids in the pupal stage. In addition, emergence of *T. basalis* occurred in all treatments at 15°C, however, no adults of *T. podisi* emerged when their pupae were stored at 15°C for more than 15 days after parasitism. Cold storage of the pupae did not affect the sex ratio of the parasitoids—the progeny sex ratio, the number of females divided by the total number of adults, ranged from 0.67 to 0.95 in *T. basalis* and from 0.71 to 0.80 in *T. podisi*. Adults that emerged after pupal storage at 15°C hibernated with a significant reduction in mobility and feeding. The greatest longevity was obtained when parasitoids were stored at 15°C one day before the predicted date of emergence. Finally, the fecundity of *T. podisi* after storage at 15°C was lower than that of females reared at 25°C, and the mean number of parasitized eggs decreased as the storage period increased.

Torres et al. (1997) investigated the thermal requirements and development of *T. podisi* and *T. brochymenae* reared on *Podisus nigrispinus* (Dallas). The development and viability of these two scelionids was studied at five temperatures ranging from 17°C to 32°C. Female development of *T. podisi* ranged from 10.8 days at 32°C, to 48.6 days at 17°C, whereas the female development of *T. brochymenae* ranged from 10.2 days at 32°C to 35.4 days at 17°C. In addition, parasitism viability ranged from 89 to 93%, although the viability of *T. podisi* decreased in both lower and higher temperatures. The lower development threshold for egg to adult female development was 9.5°C for *T. brochymenae*, and 11.1°C for *T. podisi*. Thermal requirements for that period were 214.7 degree days and 205.3 degree days for those two species, respectively. The number of generations per year were estimated to be 22.6 for *T. podisi,* and 23.9 for *T. brochymenae*. The authors concluded that *T. brochymenae* was thus better adapted to temperature variations than *T. podisi*.

Yeargan (1980) also studied the effects of temperature on the developmental rate of *T. podisi*. The relationship between temperature and percent development per day was plotted, and looking at the most linear portion of the temperature developmental-rate curve, Yeargan extrapolated a developmental threshold of 15°C was required for *T. podisi.* Using that as the base temperature, it was determined that 3 degree days would be accumulated every 24 hours at a constant temperature of 18°C. However, under a 12:12 hour fluctuating regime of 22, 14, and 0°C, 3.5 degree days would be accumulated every 24 hours. Yeargan also concluded that because the relationship between temperature and the developmental rate of *T. podisi* is non-linear, the degree days would vary with temperature, particularly at low and high temperatures.

References Cited

Beament, J. W. L. 1948. The penetration of insect egg-shells. I.—Penetration of the chorion of *Rhodnius prolixus*, Stål. Bull. Entomol. Res. 39: 359-383.

Beament, J. W. L. 1952. The role of cuticle and egg-shell membranes in the penetration of insecticides. Ann. Appl. Biol. 39: 142-143.

Borges, M., M. L. M. Costa, E. R. Sujii, M. D. G. Cavalcanti, G. F. Redígolo, I. S. Resck, and E. F. Vilela. 1999. Semiochemical and physical stimuli involved in host recognition by *Telenomus podisi* (Hymenoptera: Scelionidae) toward *Euschistus heros* (Heteroptera: Pentatomidae). Physiol. Entomol. 24: 227-233.

Bruni, R., J. Sant'Ana, J. R. Aldrich, and F. Bin. 2000. Influence of host pheromone on egg parasitism by scelionid wasps: comparison of phoretic and nonphoretic parasitoids. J. Insect Behav. 13: 165-173.

Bundy, C. S., and R. M. McPherson. 2000. Morphological examination of stink bug (Heteroptera: Pentatomidae) eggs on cotton and soybeans, with a key to genera. Ann. Entomol. Soc. Am. 93: 616-624.

Cividanes, F. J., and J. G. Figueiredo. 1996. Development and emergence of *Trissolcus brochymenae* (Ashmead) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) at different temperatures. Ann. Soc. Entomol. Brazil. 25: 207-211.

Cividanes, F. J., J. G. Figueiredo, and D. R. Carvalho. 1998. Prediction of the emergence of *Trissolcus brochymenae* (Ashmead) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) in the field. Sci. Agric. 55: 43-47.

Corrêa-Ferreira, B. S., and F. Moscardi. 1995. Seasonal occurrence and host spectrum of egg parasitoids associated with soybean stink bugs. Biol. Control 5: 196-202.

Corrêa-Ferreira, B. S., and F. Moscardi. 1996. Biological control of soybean stink bugs by inoculative releases of *Trissolcus basalis*. Entomol. Exp. Appl. 79:1–7.

Daugherty, D. M., M. H. Neustadt, C. W. Gehrke, L. E. Cavanah, L. F. Williams, and D. E. Green. 1964. An evaluation of damage to soybeans by brown and green stink bugs. J. Econ. Entomol. 57: 719-722.

Ehler, L. E. 2002. An evaluation of some natural enemies of *Nezara viridula* in northern California. BioControl 47: 309-325.

Esselbaugh, C. O. 1946. A study of the eggs of the Pentatomidae (Hemiptera). Ann. Entomol. Soc. Am. 39: 667–691.

Foerster, L. A., A. K. Doetzer, and L. C. F. D. Castro. 2004. Emergence, longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi* after cold storage in the pupal stage. Pesqui. Agropecu. Bras. 39: 841-845.

Foerster, L. A., and P. A. Nakama. 2002. Effect of cold storage on the reproductive capacity and longevity of *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). Neotrop. Entomol. 31: 115-120.

Janssen, C., S. Smith, R. Foster, R. Latin, S. Weller, and F. Whitford. 1999. Pest control in tomatoes for processing. Purdue Pesticide Program. Purdue University Cooperative Extension Service. PPP-101.

Jones, W. A., B. M. Shepard, and M. J. Sullivan. 1996. Incidence of parasitism of pentatomid (Heteroptera) pests of soybean in South Carolina with a review of studies in other states. J. Agric. Entomol. 13: 243-263.

Kamminga, K. L. 2008. Species survey, monitoring and management of economically important stink bug species in eastern Virginia. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.

Kamminga, K. L., D. A. Herbert, Jr., T. P. Kuhar, S. Malone, and A. Koppel. 2008. Efficacy of insecticides against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae) in Virginia and North Carolina. J. Entomol. Sci. 44:1-10.

Kendrick, R. L., and L. H. Rolston. 1961. Biology of the brown stink bug, *Euschistus servus* Say. J. Kansas Entomol. Soc. 34: 151-157.

Leskey, T. C., and H. W. Hogmire. 2005. Monitoring stink bugs (Hemiptera: Pentatomidae) in mid-Atlantic apple and peach orchards. J. Econ. Entomol. 98: 143-153.

McPherson, J. E., and R. M. McPherson. 2000. Stink bugs of Economic Importance in North America & Mexico. CRC Press LLC, Boca Raton, FL.

Mehrotra, K. N. 1960. Development of the cholinergic system in insect eggs. J. Insect Physiol. 5: 129-142.

Moraes, M. C. B. , R. A. Laumann, E. R. Sujii, C. Pires and M. Borges. 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. Entomol. Exp. Appl. 115: 227–237.

Nation, J.L. 2002. Insect Physiology and Biochemistry. CRC Press LLC, Boca Raton, FL.

Nault, B. A., and J. Speese. 2002. Major insect pests and economics of fresh-market tomato in eastern Virginia. Crop Prot. 21: 359-356.

Okuda, M. S., and K. V. Yeargan. 1988a. Habitat partitioning by *Telenomus podisi* and *Trissolcus euschisti* (Hymenoptera: Scelionidae) between herbaceous and woody host plants. Environ. Entomol. 17: 795-798.

Okuda, M. S., and K. V. Yeargan. 1988b. Intra- and interspecific host discrimination in *Telenomus podisi* and *Trissolcus euschisti* (Hymenoptera: Scelionidae). Ann. Entomol. Soc. Am. 81: 1017-1020.

Orr, D. B., and D. J. Boethel. 1990. Reproductive potential of *Telenomus cristatus* and *T. podisi* (Hymenoptera: Scelionidae), two egg parasitoids of pentatomids (Heteroptera). Ann. Entomol. Soc. Am. 83: 902-905.

Orr, D. B., J. S. Russin, D. J. Boethel, and W. A. Jones. 1986. Stink bug (Hemiptera: Pentatomidae) egg parasitism in Louisiana soybeans. Environ. Entomol. 15: 1250-1254.

Orr, D. B., D. J. Boethel, and M. B. Layton. 1989. Effect of insecticide applications in soybeans on *Trissolcus basalis* (Hymenoptera: Scelionidae). J. Econ. Entomol. 82: 1078-1084.

Pacheco, D. J. P., and B. S. Corrêa-Ferreira. 1998. Reproductive potential and longevity of the parasitoid *Telenomus podisi* Ashmead in eggs of different stinkbug species. An. Soc. Entomol. Brasil. 27: 585-591.

Smith, E. H., and E. H. Salkeld. 1966. The use and action of ovicides. Annu. Rev. Entomol. 11: 331-368.

Snodgrass, G. L., J. J. Adamczyk, Jr., and J. Gore. 2005. Toxicity of insecticides in a glass-vial bioassay to adult brown, green, and southern green stink bugs (Heteroptera: Pentatomidae). J. Econ. Entomol. 98: 177-181.

Temerak, S. A., and W. H. Whitcomb. 1984. Parasitoids of predaceous and phytophagous pentatomid bugs in soybean fields at two sites of Alachua County, Florida. Z. Ang. Entomol. 97: 279-282.

Tillman, P. G., and B. G. Mullinix, Jr. 2004. Comparison of susceptibility of pest *Euschistus servus* and predator *Podisus maculiventris* (Heteroptera: Pentatomidae) to selected insecticides. J. Econ. Entomol. 97: 800-806.

Torres, J. B., D. Pratissoli, and J.C. Zanuncio. 1997. Thermal requirements and development of the egg parasitoids *Telenomus podisi* Ashmead and *Trissolcus brochymenae* (Ashmead) on the predator stinkbug *Podisus nigrispinus* (Dallas). Ann. Soc. Entomol. Brasil. 26: 445-453.

Underhill, G. W. 1934. The green stinkbug. Va. Agric. Exp. Stn. Bull. 294: 1-26.

Virginia National Agriculture Statistics Service. 2009. Virginia Fact Sheets. http://www.nass.usda.gov/Statistics_by_State/Ag_Overview/AgOverview_VA.pdf

Waddill, V. H. 1978. Contact toxicity of four synthetic pyrethroids and methomyl to some adult insect parasites. Fla. Entomol. 61: 27-30.

Wilkinson, J. D., K. D. Biever, and C. M. Ignoffo. 1975. Contact toxicity of some chemical and biological pesticides to several insect parasitoids and predators. Entomophaga 20: 113-120.

Williams, M. R. 1995. Beltwide cotton insect losses 1994. Proc. 1995 Beltwide Cotton Conf. 2: 746-757.

Willrich, M. M., B. R. Leonard, and D. R. Cook. 2003. Laboratory and field evaluations of insecticide toxicity to stink bugs (Heteroptera: Pentatomidae). J. Cotton Sci. 7: 156-163.

Yeargan, K. V. 1977. Effects of green stink bug damage on yield and quality of soybeans. J. Econ. Entomol. 70: 619-622.

Yeargan, K. V. 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8: 715-719.

Yeargan, K. V. 1980. Effects of temperature on developmental rate of *Telenomus podisi* (Hymenoptera: Scelionidae). Ann. Entomol. Soc. Am. 73: 339-342.

Yeargan, K. V. 1982. Reproductive capability and longevity of the parasitic wasps *Telenomus podisi* and *Trissolcus euschisti*. Ann. Entomol. Soc. Am. 75: 181-183.

Zschintzsch, J., R. D. O'Brien, and E. H. Smith. 1965. The relation between uptake and toxicity of organophosphates for eggs of the large milkweed bug. J. Econ. Entomol. 58: 614-621.

Chapter Two

Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean and vegetable crops in Southeast Virginia

ABSTRACT Stink bugs (Hemiptera: Pentatomidae) cause significant damage to many different crops and horticultural commodities in Virginia. However, little is known about the species diversity or impact of stink bug egg parasitoids in the state. A survey was conducted in the 2005 and 2006 field seasons (May through September) in wheat [*Triticum aestivum* (L.)], soybean [*Glycine max* (L.)], and several vegetable crops by collecting natural egg masses of various stink bug species and by monitoring sentinel egg masses. A total of 570 *Euschistus servus* (Say) eggs in 26 egg masses, 11,197 *Murgantia histrionica* (Hahn) eggs in 939 egg masses, 15 *Podisus maculiventris* (Say) eggs in 2 egg masses, and 546 *Acrosternum hilare* (Say) eggs in 18 egg masses were field collected and returned to the laboratory where emerging parasitoids were identified to species. In addition, 2,512 laboratory-reared *E. servus* eggs and 230 *P. maculiventris* eggs were placed as sentinels into crop fields, collected after 7 days, and parasitoid or stink bug emergence was recorded. Four species of hymenopteran parasitoids in the family Scelionidae were recovered from stink bug eggs: *Telenomus podisi* Ashmead, *Trissolcus basalis* Wollaston, *Trissolcus edessae* Fouts, and *Trissolcus euschisti* Ashmead. In addition, one parasitoid in the family Mymaridae (Hymenoptera) was recovered. Parasitism rates were highest in *E. servus* with 89.7% and 49.2 % of egg masses and individual eggs parasitized, respectively. The predominant parasitoid species was *T. podisi*.

Key Words Pentatomidae, Scelionidae, parasitoid, *Telenomus podisi, Trissolcus basalis*

Stink bugs (Hemiptera: Pentatomidae) attack many field, vegetable, and orchard crops, with different species posing a threat in each geographical region of the United States. In Virginia, the two major pest species attacking crops are the brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Acrosternum hilare* (Say) (Kamminga et al. 2008). *Euschistus servus* is a polyphagous insect that feeds on shrubs, trees, grasses, row crops, fruiting vegetables, and fruit. It prefers to feed on plants that produce pods or fruit. It is bivoltine and can survive on weedy hosts (McPherson and McPherson 2000). *Acrosternum hilare* is also polyphagous and requires a series of plants with overlapping periods of seed and fruit production to achieve maximal development. It prefers trees and shrubs as hosts, although it is a known pest of lima beans [*Phaseolus lunatus* (L.)], snap beans [*Phaseolus vulgaris* (L.)], peaches [*Prunus persica* (L.)], soybean [*Glycine max* (L.)], tomato [*Solanum lycopersicum* (L.)], and cotton [*Gossypium hirsutum* (L.)] (Underhill 1934). The harlequin bug, *Murgantia histrionica* (Hahn), a common pest of brassica vegetables, and the spined soldier bug, *Podisus maculiventris* (Say), a common predator of lepidopteran larvae, are also present in the local stink bug complex.

In southeastern Virginia, pyrethroid insecticides are most commonly used against stink bugs, but other options for control are being explored, such as organic pesticides and biological control. With these methods, natural enemies are important in managing pest populations and reducing damage to the commodity. For example, the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) has proven effective against southern green stink bug, *Nezara viridula* (L.), in soybean (McPherson and McPherson 2000, Ehler 2002).

Before developing biological control strategies against *E. servus, A. hilare*, or *M. histrionica*, it is important to know what natural enemies are currently limiting stink bug populations in the region. Natural enemy surveys have been conducted in South Carolina (Jones

et al. 1996), Kentucky (Yeargan 1979), Louisiana (Orr et al. 1986), Florida (Temerak and Whitcomb 1984), Washington (Krupke and Brunner 2003), and California (Ehler 2002). In these studies, parasitoids such as *Telenomus podisi* Ashmead, *T. basalis*, and *Trissolcus edessae* Fouts have been collected from stink bug eggs. Information on stink bug natural enemies in the Mid-Atlantic region is very limited; however, a survey for *M. histrionica* in southwestern Virginia has been reported (Ludwig and Kok 1998). Thus, this research was conducted to determine species composition of parasitoids and rates of egg parasitism for common stink bug species in southeastern Virginia in crops where stink bug pest species are commonly encountered.

Materials and Methods

This study was conducted in 2005 and 2006 from May through September using the identical procedures each year. Any commodities known to harbor the four stink bug species in Virginia's complex, *E. servus, A. hilare, M. histrionica,* and *P. maculiventris*, were utilized. In order to detect the presence of parasitoids, naturally occurring egg masses were collected, sentinel *E. servus* egg masses were reared in the laboratory, and *P. maculiventris* eggs were obtained from Biocontrol Network (Brentwood, TN).

To produce sentinel eggs in captivity, adult field-collected stink bugs were held in the laboratory in a growth chamber [24.4°C, 85% relative humidity, 14:10 (L:D) h] in 30 cm (length) x 17 cm (width) x 8 cm (height) plastic containers containing a substrate [Viva® scrub cloths (Kleenex®, Neenah, WI)] for oviposition. They were fed snap bean [*Phaseolus vulgaris* (L.)] pods and raw peanut kernels [*Arachis hypogaea* (L.)], and were provided with cotton pads soaked in distilled water. Containers were checked daily, and newly laid egg masses were removed and stored in a 9 cm diameter Petri dish in a growth chamber [24.4°C, 85% relative humidity, 14:10 (L:D) h] until they were taken to the field the next day.

Sentinel egg masses were brought to a variety of commercial fields, of different sizes, which were previously untreated with insecticides. Egg masses, still attached to their substrate, were pinned to stems or to the midvein onto the undersides of leaves of host plants. These plants were located along the perimeter of crop fields, and were approximately 15 meters apart. After one week, masses were returned to the laboratory and kept in a 9 cm diameter Petri dish, in a growth chamber separate from the stink bugs, but with the same laboratory environmental conditions. They were observed daily and the emergence of egg parasitoids or stink bug nymphs was noted. Predation was scored once (noted for recognizable remains), at the time the eggs were collected from the field.

Field-collected egg masses were also observed for evidence of parasitism. Wheat [*Triticum aestivum* (L.)] was surveyed for stink bug eggs in late May and early June, during the heading stage. Potato [*Solanum tuberosum* (L.)] was surveyed in June, from the flowering to post-flowering stage, and vegetables were surveyed during their fruiting stages in July and August. Collards [*Brassica oleracea* (L.)] were surveyed during the vegetative stage in early August and corn [*Zea mays* (L.)] during the reproductive stage in early July. Soybean was surveyed during the pod stages, R3-R5, in late July and throughout August. Plants were visually inspected for egg masses and the handles of sweep nets were used to turn over leaves for inspection. Masses were clipped from the leaf or stem, when found, and returned to the laboratory. They were placed on moistened filter paper in a 9 cm diameter Petri dish and placed in a growth chamber [24.4°C, 85% relative humidity, 14:10 (L:D) h] where daily observations were made as described above, with sentinel eggs.

Data for 2005 and 2006 were organized using tables as presented by Yeargan (1979) and Orr et al. (1986) with percent parasitism calculated for egg masses and individual eggs. Further,

emerging parasitoids were preserved in vials filled with 80% ethyl alcohol and were sent to a taxonomist for initial identification and archival as voucher specimens in that facility [T. P. Nuhn (Scelionidae), Systematic Entomology Laboratory, Agriculture Research Service, US Department of Agriculture]. Subsequent specimens were identified to species using taxonomic keys (Johnson 1984a, 1984b, 1985a, 1985b), and more voucher specimens were sent to E. Day at the Virginia Tech Insect Identification Lab (Blacksburg, VA).

Statistical analysis. Logistic regression of binomial response variables was performed (procgenmod, SAS Institute 2002-2003) to determine if there were significant differences in parasitization rates between different host species and host crops. Contrast statements were also used to compare pairs of crops and host species. In many cases, the scale parameter was fitted to account for over-dispersion using the pscale option. Thus, in cases where proc genmod indicated a high pscale value, *P* values were used to interpret results.

Results

Of the 15,070 individual pentatomid eggs and 1,098 egg masses examined during the 2005 and 2006 field seasons, 22.3 and 66.9% were parasitized, respectively (Table 2.1). Four species of pentatomid eggs, *E. servus, A. hilare, M. histrionica,* and *P. maculiventris*, were represented, and of them, *E. servus* had the highest parasitization rates: 49.2% of individual eggs and 89.7% of egg masses. *Podisus maculiventris* had the overall lowest rates of parasitization. T-tests indicated that there was no significant difference between the parasitization rates of different host egg masses ($F = 0.29$; df = 3, 41; $P = 0.8289$); however, there was a significant difference between different species' parasitization of individual eggs ($F = 6.71$; df = 3, 46; $P = 0.0007$). Further, contrast statements suggested a strong difference between the parasitization of *E. servus* and *P. maculiventris* eggs ($F = 18.44$; df = 1, 46; $P = <0.0001$).

Sentinel eggs. Only two stink bug species, *E. servus* and *P. maculiventris,* were available in sufficient numbers to be used as sentinel species. A total of 2,512 *E. servus* eggs contained in 101 egg masses were placed in wheat, potato, squash [*Cucurbita maxima* (L.)], corn, and soybean fields (Table 2.2). Of those, 239 eggs in 16 egg masses were completely lost to unknown causes. In addition, 230 *P. maculiventris* eggs in 12 egg masses were placed in wheat, and of those, 44 eggs in three masses were lost. In both years, all parasitoids collected from these two species of sentinel eggs were identified as *T. podisi* (Scelionidae: Hymenoptera). The highest percent parasitism of *E. servus* eggs was 88.9% of surviving individual eggs (Table 2.2) and 87.5% of egg masses in potato (Table 2.3). Evidence of predation was found in *E. servus* eggs placed in wheat and soybean, and in the eggs of *P. maculiventris.*

Parasitization of *E. servus* eggs was significantly different between corn and wheat (F = 11.45; df = 1, 6; $P = 0.0148$), corn and squash (F = 7.34; df = 1, 6; $P = 0.0351$), wheat and squash (F = 43.57; df = 1, 6; $P = 0.0006$), and squash and soybean (F = 23.71; df = 1, 6; $P = 0.0028$). Since potato has a higher rate of parasitization than corn, it is assumed that the parasitization of *E. servus* eggs in potato is also significantly different from wheat and squash.

Naturally occurring eggs. Four species of stink bug eggs were found on a variety of plants, including squash, soybean, corn, potato, wheat, collards, cotton, pepper, a basswood tree (*Tilia americana* L.), cucumber, and eggplant. A total of 570 *E. servus* eggs in 26 egg masses, 15 *P. maculiventris* eggs in two egg masses, 546 *A. hilare* eggs in 18 egg masses, and 11,197 *M. histrionica* eggs in 939 egg masses were recovered (Table 2.4). The highest rate of parasitization for individual eggs was 100% of 93 *E. servus* eggs recovered in collards, and 12 *E. servus* eggs in pepper. As was the case with sentinel eggs, *T. podisi* was the only parasitoid identified from *E. servus* and *P. maculiventris* host eggs.

Three *Trissolcus* species, *T. basalis, T. edessae,* and *T. euschisti* Ashmead were identified as parasitoids of *A. hilare* eggs. Of these, the former two parasitized a combined total of 55.9% of the individual eggs and 50% of egg masses on the basswood tree. The latter species alone parasitized 68.1% of individual eggs and 50% of egg masses in squash (Tables 2.4, 2.5). In 2005 and 2006, *T. podisi* and a single specimen of Mymaridae (Hymenoptera) were recovered from *M. histrionica* eggs.

There were no significant differences in parasitization rates between the different crops in which stink bug species' host eggs were found, except between those with zero percent parasitization, or any parasitization at all.

Predation was also noted among the sentinel egg masses (Tables 2.2, 2.4). There was only a small percentage of this relative to parasitization on *E. servus* eggs, but a greater amount on *P. maculiventris*. Although no predators were captured and identified, examination of the eggs suggests one or more chewing species.

Discussion

These results suggest that there is a high percentage of stink bug egg parasitization in southeastern Virginia which, prior to this study, was unknown in the region. Virginia has a different stink bug complex than states where parasitoid surveys have been reported – most notably, *N. viridula* is absent. Despite this, egg parasitoids were the same as those reported in other studies. *Euschistus servus* was host to *T. podisi* and *T. basalis*, and *A. hilare* was host to different *Trissolcus* species, which was also the case in Louisiana (Orr et al. 1986). In addition, *P. maculiventris* is reported as a host for *T. podisi* only in Virginia and Kentucky (Yeargan 1979). The same parasitoid species were also reported from South Carolina and Virginia on *E.*

servus and *A. hilare* (Jones et al. 1996). Furthermore, in these four states, the same species of *Telenomus* and *Trissolcus* parasitoids were found.

There were some instances where more than one species of parasitoid was recovered from eggs in a given host crop. *Acrosternum hilare* egg masses recovered from basswood were parasitized by both *T. basalis* and *T. edessae*. In a 2007 parasitoid survey (A.L.K., unpublished data), *T. basalis* was a novel parasitoid found to be parasitizing *E. servus* eggs in beans, corn, and soybean, along with the previously found *T. podisi*. Also in 2007, *T. edessae* was found with *T. basalis* emerging from *M. histrionica* eggs in collards. Although more than one species emerged from the same host species in one crop, only one species emerged per individual egg mass. In southwestern Virginia, *M. histrionica* was parasitized by *Trissolcus murgantiae* Ashmead [*T. brochymenae* Ashmead] and the encyrtid *Ooencyrtus johnsoni* Howard (Ludwig and Kok 1998).

The greater percent predation of *P. maculiventris* eggs may, in part, be related to the small percent parasitization and prevalent weather. At the time the sentinel eggs were placed in the field, there was heavy subsequent rainfall. This led to a quarter of the egg masses being lost. Thus, it might have been more difficult for parasitoids to follow semiochemicals to their hosts, leaving eggs exposed to predation for longer periods.

There is a great deal of egg parasitism in southeastern Virginia, suggesting that biological control of stink bugs could be encouraged in local integrated pest management (IPM) practices. The conservation of naturally occurring parasitoids could be accomplished through changing the timing of insecticide sprays or choosing insecticides which are less harmful to beneficials, although more research is needed to determine the efficacy of locally-used pesticides on parasitized stink bug eggs. Efficacy data would provide farmers and growers with information

on which sprays could be best integrated with a biological control program to kill pest species but not beneficials in parasitized eggs. The use of such "soft" insecticides to manage nymph and adult stink bug populations, combined with stink bug egg parasitoids thriving naturally in the field, could be a good combination for an IPM program in Southeast Virginia.

References Cited

Ehler, L. E. 2002. An evaluation of some natural enemies of *Nezara viridula* in northern California. BioControl 47: 309-325.

Johnson, N. F. 1984a. Systematics of Nearctic *Telenomus*: Classification and revisions of the *podisi* and *phymatae* species groups (Hymenoptera: Scelionidae). Bull. Ohio Biol. Surv., New Series 6:3.

Johnson, N. F. 1984b. Revision of the Nearctic species of the *Trissolcus flavipes* group (Hymenoptera: Scelionidae). Proc. Entomol. Soc. Wash. 86:797-807.

Johnson, N. F. 1985a. Systematics of the new world *Trissolcus* (Hymenoptera: Scelionidae): species related to *T. basalis*. Can. Entomol. 117:431-445.

Johnson, N. F. 1985b. Revision of the new world species of the *thyantae* group of *Trissolcus* (Hymenoptera: Scelionidae). Can. Entomol. 117:107-112.

Jones, W. A., B. M. Shepard, and M. J. Sullivan. 1996. Incidence of parasitism of pentatomid (Heteroptera) pests of soybean in South Carolina with a review of studies in other states. J. Agric. Entomol. 13: 243-263.

Kamminga, K. L., D. A. Herbert, Jr., T. P. Kuhar, S. Malone, and A. Koppel. 2008. Efficacy of Insecticides Against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae) in Virginia and North Carolina. J. Entomol. Sci. (in press).

Krupke, C. H., and J. F. Brunner. 2003. Parasitoids of the consperse stink bug (Hemiptera: Pentatomidae) in North Central Washington and attractiveness of a host-produced pheromone component. J. Entomol. Sci. 38: 84-92.

Ludwig, S. W., and L. T. Kok. 1998. Phenology and parasitism of harlequin bugs, *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae), in Southwest Virginia. J. Entomol. Sci. 33: 33-39.

McPherson, J. E., and R. McPherson. 2000. Stink Bugs of Economic Importance in North America & Mexico. CRC Press LLC, Boca Raton, FL.

Orr, D. B., J. S. Russin, D. J. Boethel, and W. A. Jones. 1986. Stink bug (Hemiptera: Pentatomidae) egg parasitism in Louisiana soybeans. Environ. Entomol. 15: 1250-1254.

SAS Institute. 2002-2003. SAS version 9.0, SAS Institute Inc., Cary, NC.

Temerak, S. A., and W. H. Whitcomb. 1984. Parasitoids of predaceous and phytophagous pentatomid bugs in soybean fields at two sites of Alachua County, Florida. Z. Ang. Entomol. 97: 279-282.

Underhill, G. W. 1934. The green stinkbug. Va. Agric. Exp. Stn. Bull. 294: 1-26.

Yeargan, K. V. 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8: 715-719.

Table 2.1. Total percent parasitization of stink bug egg masses and individual eggs, 2005 and 2006, in Southeast Virginia crop fields

Host species	No. individual eggs	No. eggs failed to hatch	No. parasitized eggs	% Parasitization[a]
Euschistus servus	3,082	641	1,202	**49.2%**
Podisus maculiventris	272	64	29	**13.9%**
Acrosternum hilare	546	191	168	**47.3%**
Murgantia histrionica	11,197	3,405	1,004	**12.9%**
All pentatomids	15,097	4,301	2,403	**22.3%**
	No. egg masses	No. masses failed to hatch	No. parasitized masses	% Mass parasitization[b]
Euschistus servus	145	40	78	**74.3%**
Podisus maculiventris	14	4	2	**20.0%**
Acrosternum hilare	46	9	9	**24.3%**
Murgantia histrionica	939	402	341	**63.5%**
All pentatomids	1,144.00	455	430	**62.4%**

[a] Calculated as the number of parasitized eggs, divided by the number of hatched eggs (total - intact)

[b] Calculated as the number of parasitized egg masses, divided by the number of hatched egg masses (total - intact)

Table 2.2. Parasitization and predation of individual stink bug eggs, oviposited on filter paper in the laboratory and then placed into the field as sentinels, 2005 and 2006

					Percentage[a] of recovered eggs which were:			
Host species	Crop	Total eggs	No. lost	No. recovered	Hatched	Failed to hatch	Parasitized[b]	Predated[c]
E. servus	wheat	949	40	909	73.9	9.7	14.1	2.3
E. servus	potato	190	0	190	0.0	11.1	88.9	0.0
E. servus	squash	445	0	445	2.0	35.1	62.9	0.0
E. servus	corn	290	56	234	13.7	27.8	58.5	0.0
E. servus	soybean	638	143	495	30.3	40.4	26.9	2.4
P. maculiventris	wheat	230	44	186	53.3	34.4	1.1	11.2

[a] Percents based on the number of eggs recovered

[b] All eggs of *E. servus* and *P. maculiventris* parasitized by *Telenomus podisi.*

[c] Predated by unknown predators

Table 2.3. Parasitized and predation of stink bug egg masses oviposited on filter paper in the laboratory and then placed into the field as sentinels, 2005 and 2006

					Percentage[a] of recovered masses which were:			
Host species	Crop	Total masses	No. lost	No. recovered	Hatched	Failed to hatch	Parasitized[b]	Predated[c]
E. servus	wheat	25	2	23	21.8	30.4	39.1	8.7
E. servus	potato	8	0	8	0.0	12.5	87.5	0.0
E. servus	squash	14	0	14	7.1	28.6	64.3	0.0
E. servus	corn	16	4	12	0.0	41.7	83.3	0.0
E. servus	soybean	38	10	28	7.1	46.4	42.9	3.6
P. maculiventris	wheat	12	3	9	44.5	11.1	22.2	22.2

[a] Percents based on the number of eggs recovered

[b] All eggs of *E. servus* and *P. maculiventris* parasitized by *Telenomus podisi.*

[c] Predated by unknown predators

Table 2.4. Parasitization of individual stink bug eggs naturally found in the field, 2005 and 2006, in Southeast Virginia crop fields

			Percentage of recovered eggs which were:					
					Parasitized by:			
Host species	Crop	Total eggs	Hatched	Failed to hatch	*T. basalis*	*T. euschisti*	*T.edessae*	*T. podisi*
E. servus	squash	86	9.3	0.0	0.0	0.0	0.0	90.7
E. servus	soybean	148	46.6	17.6	0.0	0.0	0.0	35.8
E. servus	corn	20	0.0	50.0	0.0	0.0	0.0	50.0
E. servus	potato	132	0.0	47.0	0.0	0.0	0.0	53.0
E. servus	wheat	52	0.0	25.0	0.0	0.0	0.0	75.0
E. servus	collards	93	0.0	0.0	0.0	0.0	0.0	100.0
E. servus	cotton	27	96.3	3.7	0.0	0.0	0.0	0.0
E. servus	pepper	12	0.0	0.0	0.0	0.0	0.0	100.0
P. maculiventris	potato	15	35.7	0.0	0.0	0.0	0.0	64.3
A. hilare	soybean	279	43.7	46.6	9.7	0.0	0.0	0.0
A. hilare	basswood tree	145	13.1	31.0	20.7	0.0	35.2	0.0
A. hilare	squash	88	21.7	10.2	0.0	68.1	0.0	0.0
A. hilare	cotton	34	79.4	20.6	0.0	0.0	0.0	0.0
M. histrionica	cucumber	22	36.4	63.6	0.0	0.0	0.0	0.0
M. histrionica	eggplant	25	0.0	100.0	0.0	0.0	0.0	0.0
M. histrionica	collards	10,345	60.4	32.5	7.1	0.0	0.0	0.0
M. histrionica	squash	805	68.0	0.5	31.4[a]	0.0	0.0	0.0

[a] In addition, there was 1 parasitoid of the family Mymaridae (Hymenoptera), ~0.1%

Table 2.5. Parasitization of stink bug egg masses naturally found in the field, 2005 and 2006, in Southeast Virginia crop fields

			Percentage[a] of recovered egg masses which were:					
					Parasitized by:			
Host species	Crop	Total egg masses	Hatched	Failed to hatch	*T. basalis*	*T. euschisti*	*T.edessae*	*T. podisi*
E. servus	squash	4	25.0	0.0	0.0	0.0	0.0	75.0
E. servus	soybean	6	50.0	50.0	0.0	0.0	0.0	50.0
E. servus	corn	1	0.0	100.0	0.0	0.0	0.0	100.0
E. servus	potato	4	0.0	75.0	0.0	0.0	0.0	100.0
E. servus	wheat	3	0.0	33.3	0.0	0.0	0.0	100.0
E. servus	collards	6	0.0	0.0	0.0	0.0	0.0	100.0
E. servus	cotton	1	100.0	0.0	0.0	0.0	0.0	0.0
E. servus	pepper	1	0.0	0.0	0.0	0.0	0.0	100.0
P. maculiventris	potato	2	50.0	0.0	0.0	0.0	0.0	50.0
A. hilare	soybean	7	57.1	57.1	14.3	0.0	0.0	0.0
A. hilare	basswood tree	8	12.5	37.5	12.5	0.0	37.5	0.0
A. hilare	squash	2	50.0	50.0	0.0	50.0	0.0	0.0
A. hilare	cotton	1	100.0	100.0	0.0	0.0	0.0	0.0
M. histrionica	cucumber	1	100.0	100.0	0.0	0.0	0.0	0.0
M. histrionica	eggplant	1	0.0	100.0	0.0	0.0	0.0	0.0
M. histrionica	collards	875	17.8	45.7	36.5	0.0	0.0	0.0
M. histrionica	squash	62	64.5	0.0	35.5[b]	0.0	0.0	0.0

[a] Total percents may sum to more than 100 because some masses contained both parasitized and intact eggs

[b] In addition, there was 1 parasitoid of the family Mymaridae (Hymenoptera)

Chapter Three

Efficacy of selected insecticides against eggs of *Euschistus servus* (Say) and *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae) and the egg parasitoid, *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae)

ABSTRACT Brown (*Euschistus servus*) and green (*Acrosternum hilare*) stink bugs (Hemiptera: Pentatomidae) are major pests of many crops. Although various insecticides are commonly used to control nymphs and adults, little is known about how they affect eggs. Laboratory bioassays and field trials were conducted to determine insecticide efficacy against developing stink bugs and parasitoids. Common field rates of acephate, λ-cyhalothrin, spinosad, and thiamethoxam were tested on developing stink bugs in *E. servus* and *A. hilare* eggs, as well as *Telenomus podisi* (Hymenoptera: Scelionidae) parasitoids developing in *E. servus* eggs. In the bioassay, egg masses were dipped into insecticide/water solutions for 1 s and assessed for mortality after 2 wk. In the field trials, treatments were randomly assigned to field plots using a randomized complete block design. Egg masses on a cloth section were pinned to leaves in each plot and later returned to the laboratory 24 h after exposure to insecticides. Mortality was assessed after 2 wk. *Acrosternum hilare* developing in eggs which were dipped experienced greater mortality when exposed to insecticide than water ($F = 71.6$; df = 4; $P < 0.0001$); however, there were no significant differences between treatments in the field. Developing parasitoids showed high mortality when exposed to all insecticide treatments, when dipped or field-treated, and mortality was significantly greater when compared with water ($F = 11.96$; df = 4; $P < 0.0001$ in the dip test). There were no significant differences among treatments for developing *E. servus* in both the laboratory bioassays and the field trials.

Key Words Pentatomidae, Scelionidae, egg efficacy, chemical control

In Virginia, brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Acrosternum hilare* (Say), are major agricultural pests. They are polyphagous, feeding on the fruiting bodies of shrubs, trees, grasses, row crops, fruiting vegetables, and fruit, and can survive on weedy hosts (McPherson and McPherson 2000). Pyrethriod or organophosphate insecticides are typically used for control in most crops (Herbert 2008), but other control options, such as organic pesticides and biological control, are also being investigated (Kamminga et al. 2008). Pesticide sprays are timed to target stink bug nymphal and adult stages, but little research has been done to determine how these insecticides affect the stink bug eggs, or the natural enemies associated with them.

Stink bug egg parasitoids such as *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) are present throughout the stink bug field season, parasitizing up to 50% of stink bug egg masses (Koppel et al. 2009) in southeastern Virginia, and are likely to be subject to pesticide sprays, either as adults, or while developing in the stink bugs' eggs.

Waddill (1978) subjected a number of adult parasitoids, including *Telenomus remus* (Nixon) (Hymenoptera: Scelionidae), to treatment with pyrethroids, including permethrin, resulting in high mortality. A high mortality was also recorded within 6 hours of exposure to methyl parathion, and it also caused high mortality in other hymenopteran parasitoids, including members of the families Chalcididae, Braconidae, and Ichneumonidae (Wilkinson 1975). However, another study found that almost all adult *Trissolcus basalis* (Wollaston), in two repetitions, survived when exposed to permethrin (Orr et al. 1989).

The uptake of specific classes of insecticides into insect eggs has also been studied. Organophosphates have been used as ovicides, but eggs have developed resistance to this group due to biochemical differences in the esterase complex of resistant-strain embryos (Smith and Salkeld 1966). Further, parathion does not act as a true ovicide on the eggs of milkweed bug, *Oncopeltus fasciatus* (Dallas), as the penetration barrier of eggs does not allow enough insecticide to flow into the eggs to kill the embryo (Zschintzsch et al. 1965). However, it has been demonstrated that symptoms of organophosphate toxicity coincide very closely with cholinesterase inhibition in *O. fasciatus* eggs (Smith and Salkeld 1966). Both cholinesterase and acetylcholine appear in these eggs at four days of development (Mehrotra 1960).

The objective of this study was to assess insecticide efficacy on developing stink bugs and parasitoids in pentatomid eggs utilizing insecticides in four classes: neonicotinoids, organophosphates, pyrethroids, and spinosyns.

Materials and Methods

Rearing. *Euschistus servus* eggs, either non-parasitized or parasitized by *T. podisi*, and non-parasitized *A. hilare* eggs, were utilized in this study. Host eggs were obtained from a laboratory-reared colony of stink bugs as per previously described methods (Koppel et al. 2009). To obtain parasitized eggs, *T. podisi* founders were field collected. Fresh *E. servus* egg masses were pinned to the undersides of leaves or on stems of host plants, which were located on the margins of fields, on sunny days with light wind. After 48 hours, the egg masses were collected and returned to a growth chamber [24.4°C, 85% R.H., 14:10 (L:D)].

When the eggs turned black, it signaled that they had been parasitized. These parasitized eggs were placed into 47mm Millipore dishes with a few drops of honey on the lid, and returned to the growth chamber. The eggs were observed daily, and emergent adults were transferred to a

new 47mm Millipore dish, containing a newly laid (<3 days old) *E. servus* egg mass. Each dish had a 3:2 female to male ratio per egg mass, and a few drops of honey on the lid. If any of the transferred adults died, they were replaced with newly emergent adults. When the fresh egg masses darkened, *T. podisi* were transferred to a new *E. servus* egg mass. Parasitized eggs used for the efficacy study were stored in a 5°C refrigerator. All eggs utilized for laboratory bioassays and field trials were 3 days old or younger from the time they were laid or parasitized.

Laboratory Bioassay. Individual parasitized and non-parasitized egg masses were gathered for the bioassay. Acephate, λ-cyhalothrin, spinosad, and thiamethoxam were each mixed with 2500 ml of water in a mason jar, formulated for standard field rates (Table 3.1), with water as a control. Rates were determined from stink bug control recommendations on pesticide labels. Egg masses were randomly assigned treatment levels, and were dipped for 1 second. Each mass was placed into a labeled 47mm Millipore dish and returned to the growth chamber. After 2 weeks, mortality of developing insects was assessed based on the number of individual eggs within each mass that hatched or had an adult emerge.

Field Trials. Plots of Variety AG 5905 RR soybeans at the Tidewater Agricultural Research Station, planted on June 30, in the podding stage of development were selected for this study. They were divided into four, 15-foot rows per treatment, with four replicates, arranged in a randomized complete block experimental design. Each of the plots within a block was randomly assigned a treatment. An egg mass on a strip of substrate was pinned to the midvein on the underside of a soybean leaf, located near the middle of the row, no more than three in from the top of the canopy. Insecticide treatments were formulated in the same concentrations as used in the dip tests, but in 2500 ml of water. Treatments were broadcast with a spider field sprayer calibrated to deliver 154.05 L/ha at 2.04 atm through 8002VS spray nozzles spaced 45.7

cm apart on the spray boom. After 24 hours, egg masses were retrieved, placed into a labeled 47mm Millipore dish, and returned to the growth chamber. After 2 weeks, mortality of developing insects was assessed based on individual egg hatch or parasitoid emergence.

ANOVA and Fisher's least significant difference tests were performed for each data set (PROC GLM: SAS Institute 2002-2005). An arcsine-transformation was performed prior to analysis, and both transformed and non-transformed data were analyzed. Due to highly variable error variance in the *A. hilare* egg laboratory data, a Fisher's exact test was also performed on that data set using PROC FREQ (SAS Institute 2002-2005) on individual treatment comparisons.

Results

A total of 770 *E. servus* eggs, 471 parasitized *E. servus* eggs, and 1,428 *A. hilare* eggs were dipped in insecticides in the laboratory bioassay, and there were 648 *E. servus* eggs, 612 parasitized *E. servus* eggs, and 1,114 *A. hilare* eggs analyzed in the field trials. There was no evidence of partial insect emergence from eggs. All insects which hatched from eggs did so completely. Statistical analysis of transformed and untransformed data yielded similar results; untransformed numbers and statistics are reported unless otherwise noted.

There were no significant differences in mortality of developing *E. servus* among insecticide treatments in both the laboratory (Fig. 3.1) and field trials (Fig. 3.2). However, when parasitized eggs were treated with insecticides in laboratory bioassays, *T. podisi* experienced significantly greater mortality than when treated with water ($F = 11.96$; df = 4; $P < 0.0001$) (Fig. 3.3). In the field trials, there was 100% mortality to *T. podisi* in all spinosad and λ-cyhalothrin repetitions (Fig. 3.4), which was significantly greater mortality than in other treatments ($F = 19.93$; df = 4; $P < 0.0001$). When the field trial data were analyzed without these two treatments,

there was significantly greater *T. podisi* mortality in eggs treated with acephate than with thiamethoxam or water ($F = 18.24$, df = 2, $P = 0.0001$).

Developing *A. hilare* in eggs treated with any of the four insecticides in the laboratory bioassay experienced 100% mortality, which was significantly greater than when eggs were treated in water (ANOVA $F = 71.6$, df = 4, $P < 0.0001$, Fisher's exact $P < 0.0001$) (Fig. 3.5). Although there were differences in error variance between water and insecticide data, both ANOVA and Fisher's exact yielded the same statistical results. There were no significant differences among treatment groups in the field trials (Fig. 3.6).

When laboratory bioassay data from developing insects in non-parasitized and parasitized *E. servus* eggs were analyzed together, statistics indicated that insecticide treatments resulted in an overall greater mortality to developing *T. podisi* than to developing *E. servus* ($F = 84.63$, df = 1, $P < 0.0001$). Further, there was significantly less mortality of developing insects when exposed to water and λ-cyhalothrin ($F = 3.42$, df = 4, $P = 0.0013$) than the other three treatments. Due to the error variances of the *A. hilare* bioassay data, they were not included in this analysis.

It is difficult to draw definite conclusions from the combined analysis of field trial data for insects in non-parasitized *A. hilare,* and all *E. servus* eggs. Both the untransformed and arcsine-transformed data contained a significant interaction effect between egg status and treatment. Water appeared to account for this, and was removed from the analysis such that only the four insecticide treatments were analyzed. In this case, the arcsine-transformed data still indicated that there was an interaction between egg status and treatment, but the untransformed data did not. The latter analysis suggested that thiamethoxam treatments resulted in significantly lower mortality to developing insects than the other three insecticides ($F = 4.97$, df = 3, $P =$

0.0037), and that there was significantly higher mortality, overall, to developing *T. podisi* (F = 37.95, df = 2, $P < 0.0001$) than to stink bugs.

Discussion

In general, this study showed that the selected insecticides were more toxic to developing parasitoids and *A. hilare* than to developing *E. servus*. Further, *A. hilare* in bioassays experienced greater mortality when treated with insecticides than those in the field trials, which may be attributed to wind conditions when the insecticides were sprayed, decreasing foliar coverage. Developing *A. hilare* were completely submerged in insecticide solutions in the laboratory bioassay. The bioassay results suggest that developing *A. hilare* experienced greater mortality than developing *E. servus*, which is consistent with reports in the literature of *A. hilare* being more susceptible to insecticide exposure.

The efficacy of insecticides on adult stink bugs and egg parasitoids has been explored in other studies. The organophosphate dicrotophos has been cited as having a high toxicity to various stink bug species (Tillman et al. 2004, Snodgrass et al. 2005), and it is suggested that insecticides provide more consistent control for *A. hilare* and *Nezara viridula* L., the southern green stink bug (Willrich et al. 2003, Snodgrass et al. 2005, Kamminga et al. 2008) than for *E. servus*. Kamminga et al. (2008) performed a number of efficacy trials on *E. servus* and *A. hilare* nymphs and adults in southeastern Virginia and found that *A. hilare* was especially susceptible to all of the pyrethroids that were tested.

The high mortality of developing parasitoids in this study contrasts with previous research. Novozhilov et al. (1973) found that when chlorophos, an organophosphate also known as trichlorfon, penetrated stink bug eggs, most of it was absorbed by the chorion and never entered the egg interior. Further, a number of studies, including many listed by Orr (1988), and a

more recent one by Sudarsono et al. (1992), have shown that preimaginal parasitoids experienced low mortality when treated with insecticides. Developing parasitoids even experienced less mortality than developing rice stink bugs (*Oebalus pugnax* F., Hemiptera: Pentatomidae) in a study by Sudarsono et al. (1992).

However, further studies (Koppel, unpublished data) on the chorion permeability of non-parasitized and parasitized *E. servus* egg masses showed that acephate, λ-cyhalothrin, and thiamethoxam are in fact absorbed into the interior of *E. servus* eggs. Sales (1978) found that exposure to insecticides led to high mortality of *T. basalis*. Studies from Iran on *Trissolcus grandis* Thompson (Hymenoptera: Scelionidae) have indicated that insecticide treatments have significantly decreased the emergence of this parasitoid, especially when applied to recently parasitized eggs (Garjan et al. 2005, Saber et al. 2005). Similarly, there are low rates of *Trichogramma brasiliensis* Ashmead (Hymenoptera: Trichogrammatidae) emergence in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs when they have been treated with insecticides (Varma and Singh 1987).

Since developing stink bugs in the next generation will hatch from eggs while egg parasitoids will not, it is important for growers to use insecticides only as needed. Excessive applications of insecticide could decrease the population of *T. podisi* enough to reduce naturally occurring parasitization to unnoticeable levels. Field data from Louisiana indicated that nymphal populations of stink bugs rapidly increased after applications of methyl parathion and permethrin (Orr et al. 1989), which Orr interprets as a possible reflection of stink bug predator mortality. Given the results of efficacy studies in southeastern Virginia, it is possible a similar effect would happen as a result of parasitoid mortality.

Perhaps local farmers and growers could limit their chemical applications to key times in the summer field season when stink bug populations have reached economic threshold. Leaving more time between sprays would allow natural enemies a recovery period to rebuild their populations in surrounding refugia. Efficacy studies utilizing a wider variety of insecticides are still needed to see if there are less toxic options within classes that may allow for more selective mortality of insects at the time of application.

References Cited

Garjan, A. S., K. Talebi, and A. A. Pourmirza. 2005. Effect of some pyrethroid and organphosphorous insecticides on developmental stages of *Trissolcus grandis* (Thom.) (Hymenoptera: Scelionidae). J. Sci. & Technol. Agric. & Natur. Resour., Isf. Univ. Technol., Isf., Iran. 8: 165.

Herbert, D. A. 2008. Insects: Soybeans, Pp. 61-74. *In* S.E. Hagood and D.A. Herbert [eds.], Pest Management Field Guide Field Crops. Virginia Coop. Ext. Publ. No. 456-420.

Kamminga, K. L., D. A. Herbert, Jr., T. P. Kuhar, S. Malone, and A. Koppel. 2008. Efficacy of insecticides against *Acrosternum hilare* and *Euschistus servus* (Hemiptera: Pentatomidae) in Virginia and North Carolina. J. Entomol. Sci. 44:1-10.

Koppel, A. L., D. A. Herbert, Jr., T. P. Kuhar, and K. Kamminga. 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean and vegetable crops in Southeast Virginia. Environ. Entomol. 38: 375-379.

McPherson, J. E., and R. McPherson. 2000. Stink bugs of Economic Importance in North America & Mexico. CRC Press LLC, Boca Raton, FL.

Mehrotra, K. N. 1960. Development of the cholinergic system in insect eggs. J. Insect Physiol. 5: 129-142.

Novozhilov, K. V., K. V. Kamenkova, and I. M. Smirnova. 1973. The development of the parasite *Trissolcus grandis* Thoms. (Hymenoptera, Scelionidae) under conditions where organophosphorous insecticides are used against *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Entomol. Rev. 52: 11-17.

Orr, D. B. 1988. Scelionid wasps as biological control agents: A review. Fla. Entomol. 71: 506-528.

Orr, D. B., D. J. Boethel, and M. B. Layton. 1989. Effect of insecticide applications in soybeans on *Trissolcus basalis* (Hymenoptera: Scelionidae). J. Econ. Entomol. 82: 1078-1084.

Saber, M., M. J. Hejazi, K. Kamali, and S. Moharramipour. 2005. Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). J. Econ. Entomol. 98: 35-40.

Sales, F. M. 1978. Efeito de insecticidas organossintéticos sobre o parasitóide, *Trissolcus basalis* (Wollaston). Fitossanidade 2(3): 90. (Portuguese with English summary).

SAS Institute. 2002–2005. PROC user's manual, version 9.1.3. SAS Institute Inc., Cary, NC.

Smith, E. H., and E. H. Salkeld. 1966. The use and action of ovicides. Annu. Rev. Entomol. 11: 331-368.

Snodgrass, G. L., J. J. Adamczyk, Jr., and J. Gore. 2005. Toxicity of insecticides in a glass-vial bioassay to adult brown, green, and southern green stink bugs (Heteroptera: Pentatomidae). J. Econ. Entomol. 98: 177-181.

Sudarsono, H., J. L. Bernhardt, and N. P. Tugwell. 1992. Survival of immature *Telenomus podisi* (Hymenoptera: Scelionidae) and rice stink bug (Hemiptera: Pentatomidae) embryos after field applications of methyl parathion and carbaryl. J. Econ. Entomol. 85: 375-378.

Temerak, S. A., and W. H. Whitcomb. 1984. Parasitoids of predaceous and phytophagous pentatomid bugs in soybean fields at two sites of Alachua County, Florida. Z. Ang. Entomol. 97: 279-282.

Tillman, P. G., and B. G. Mullinix, Jr. 2004. Comparison of susceptibility of pest *Euschistus servus* and predator *Podisus maculiventris* (Heteroptera: Pentatomidae) to selected insecticides. J. Econ. Entomol. 97: 800-806.

Varma, G. C., and P. P. Singh. 1987. Effect of insecticides on the emergence of *Trichogramma brasiliensis* (Hymenoptera: Trichogrammatidae) from parasitized host eggs. BioControl. 32: 443-448.

Waddill, V. H. 1978. Contact toxicity of four synthetic pyrethroids and methomyl to some adult insect parasites. Fla. Entomol. 61: 27-30.

Wilkinson, J. D., K. D. Biever, and C. M. Ignoffo. 1975. Contact toxicity of some chemical and biological pesticides to several insect parasitoids and predators. Entomophaga 20: 113-120.

Willrich, M. M., B. R. Leonard, and D. R. Cook. 2003. Laboratory and field evaluations of insecticide toxicity to stink bugs (Heteroptera: Pentatomidae). J. Cotton Sci. 7: 156-163.

Zschintzsch, J., R. D. O'Brien, and E. H. Smith. 1965. The relation between uptake and toxicity of organophosphates for eggs of the large milkweed bug. J. Econ. Entomol. 58: 614-621.

Table 3.1. Insecticides evaluated for efficacy in laboratory bioassays and field trials

Material	Product	Manufacturer	Class	Rate
acephate	Orthene 97 SP[a]	Amvac Chemical Corp.	Organophosphate	0.82 kg (AI)/ha
λ-cyhalothrin	Warrior ZT	Syngenta Crop Protection, Inc.	Pyrethroid	0.03 kg (AI)/ha
spinosad	Entrust	Dow AgroSciences LLC	Organic	0.18 kg (AI)/ha
thiamethoxam	Centric 40 WG[b]	Syngenta Crop Protection, Inc.	Neonicotinoid	0.06 kg (AI)/ha

[a] Soluble powder

[b] Water-dispersible granules

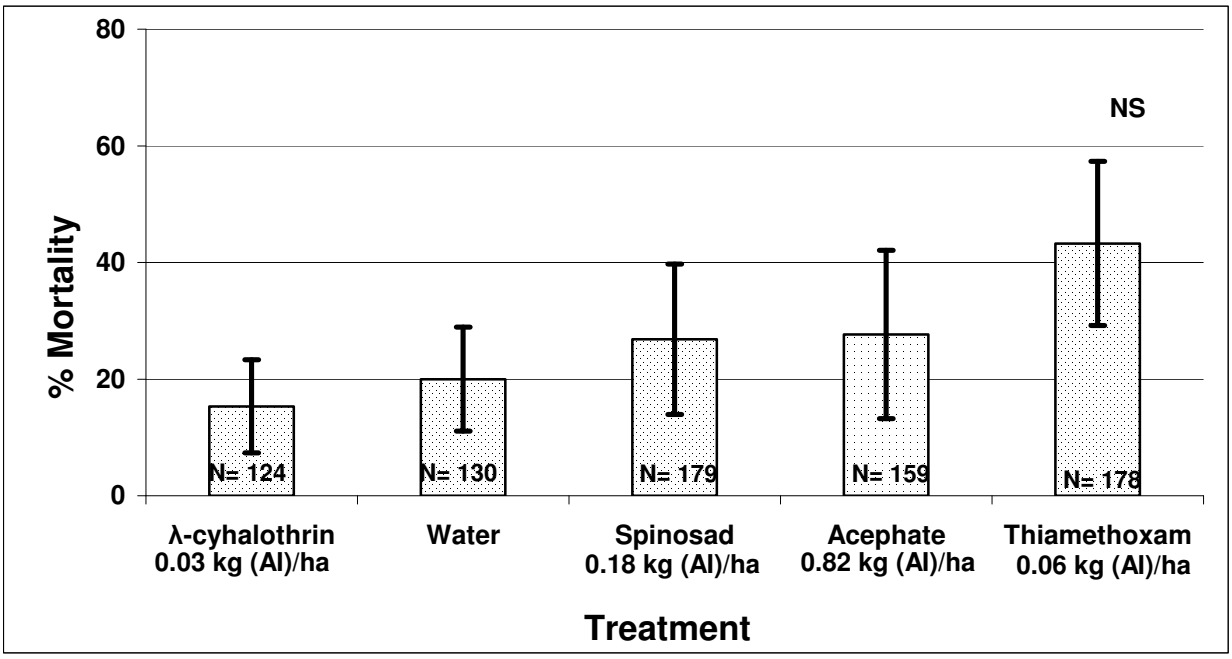

Fig. 3.1. Mortality of developing *Euschistus servus* in eggs when dipped into field-formulated insecticide solutions.

N = number of eggs
Untransformed hatch proportion data are presented, but both untransformed and arcsine-transformed data were analyzed, and produced the same analysis.
NS There were no significant differences among treatment groups.

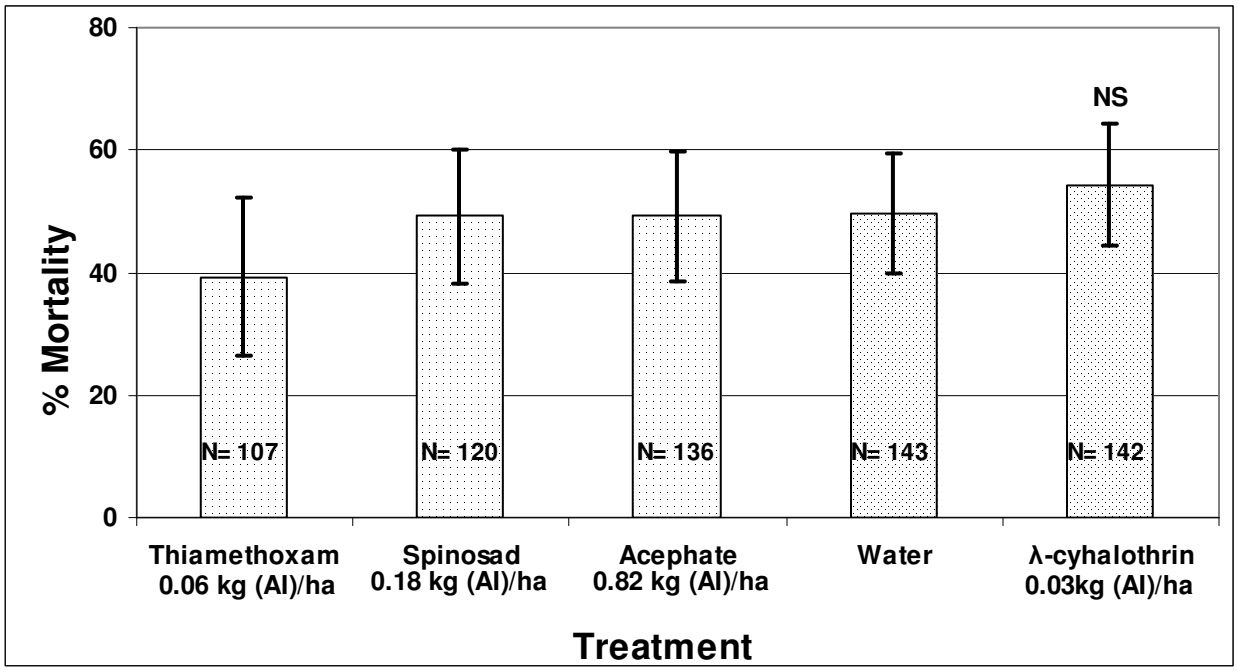

Fig. 3.2. Mortality of developing *Euschistus servus* in egg masses when placed in the canopy of soybean plants in plots, and sprayed with field-formulated insecticides at the standard rate.

N = number of eggs
Untransformed hatch proportion data were analyzed.
NS There were no significant differences among treatment groups.

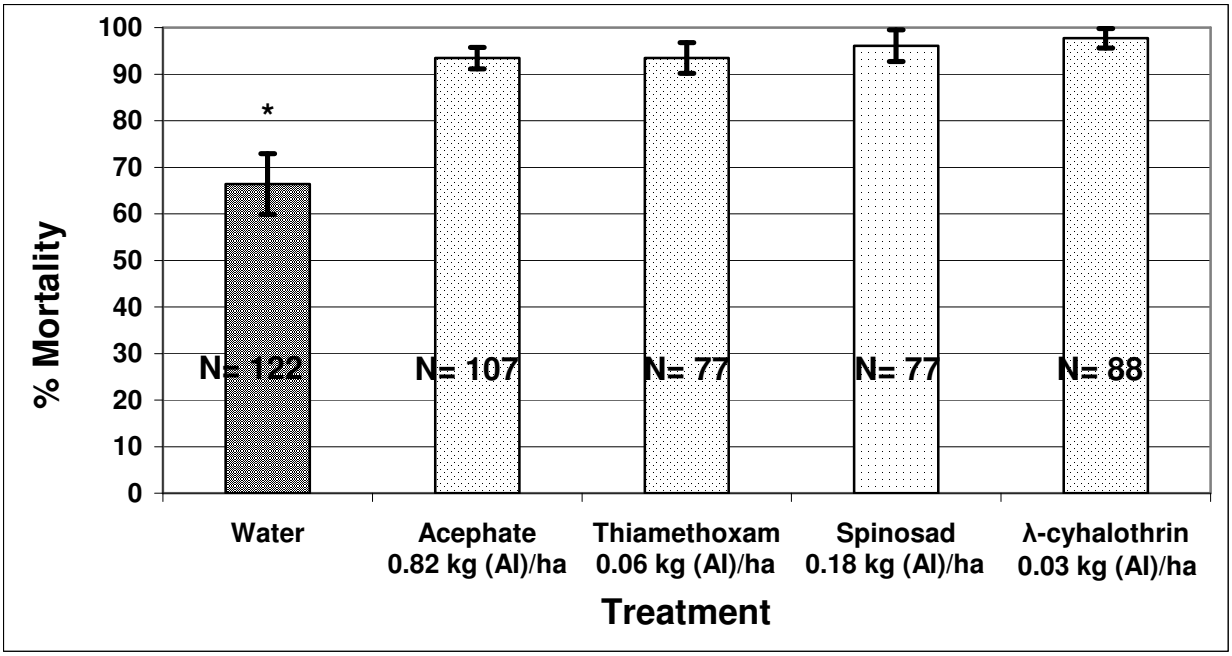

Fig. 3.3. Mortality of the parasitoid *Telenomus podisi*, developing in *Euschistus servus* egg masses, when dipped into field-formulated insecticide solutions.

N = number of eggs
Untransformed hatch proportion data were analyzed.
***** The mortality of *T. podisi* in eggs dipped in water (dark bar) was significantly lower ($F = 11.96$; df = 4; $P < 0.0001$) than the mortality of those dipped in insecticide treatments.

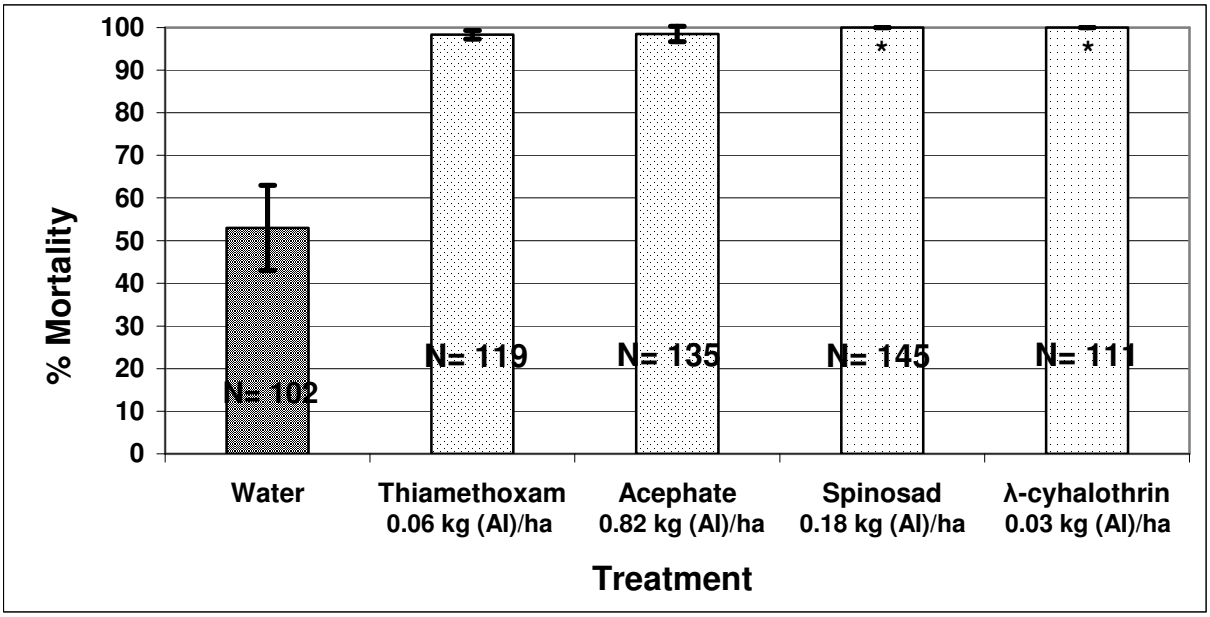

Fig. 3.4. Mortality of the parasitoid *Telenomus podisi*, developing in *Euschistus servus* egg masses, when placed into a soybean plot and sprayed with field-formulated insecticides at the standard rate.

N = number of eggs
Untransformed hatch proportion data were analyzed.
***** The mortality of *T. podisi* in eggs dipped in spinosad and λ -cyhalothrin was significantly greater ($F = 19.93$; df = 4; $P < 0.0001$) than in the other treatments.
****** When the treatments were analyzed with spinosad and λ -cyhalothrin (100% mortality) omitted, there was significantly greater mortality in *T. podisi* developing in eggs treated with acephate ($F = 18.24$, df = 2, $P = 0.0001$).

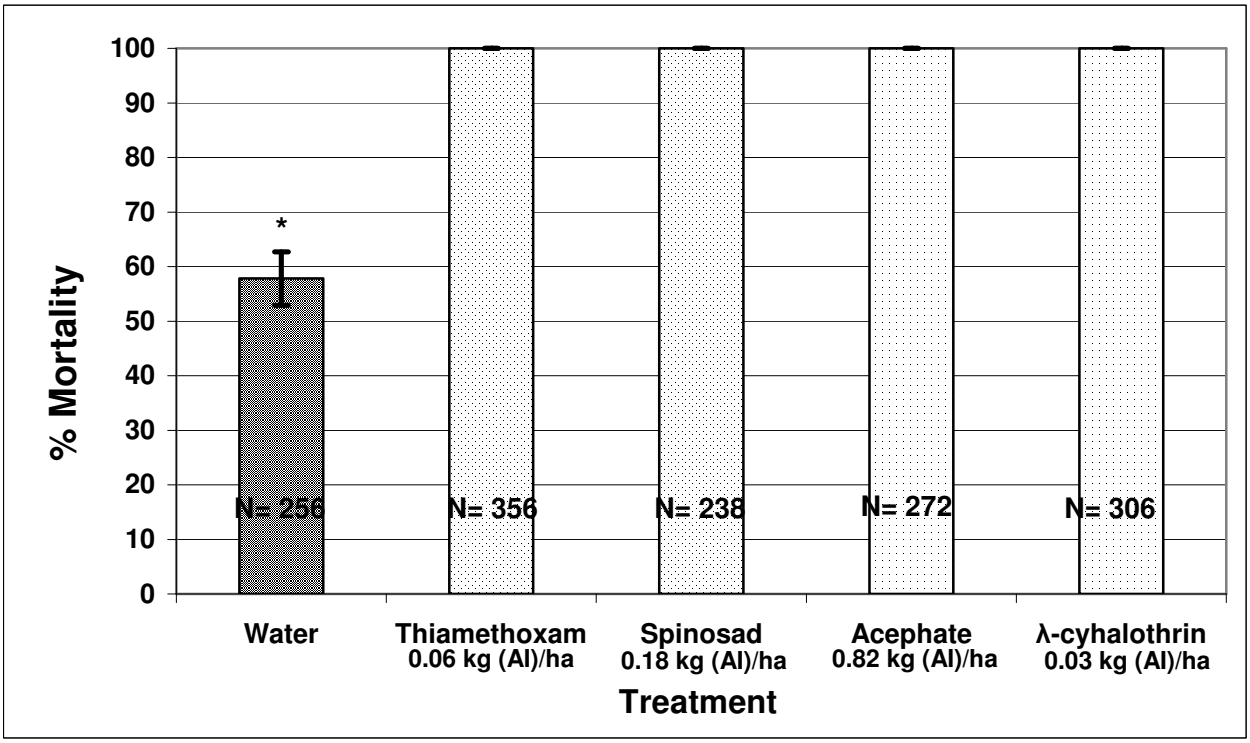

Fig. 3.5. Mortality of developing *Acrosternum hilare* in eggs when dipped into field-formulated insecticide solutions.

N = number of eggs
Hatch proportion data were normalized with an arcsine transformation.
***** The mortality of *A. hilare* in eggs dipped in water (dark bar) was significantly lower (F = 71.6; df = 4; $P < 0.0001$) than all insecticide treatments.

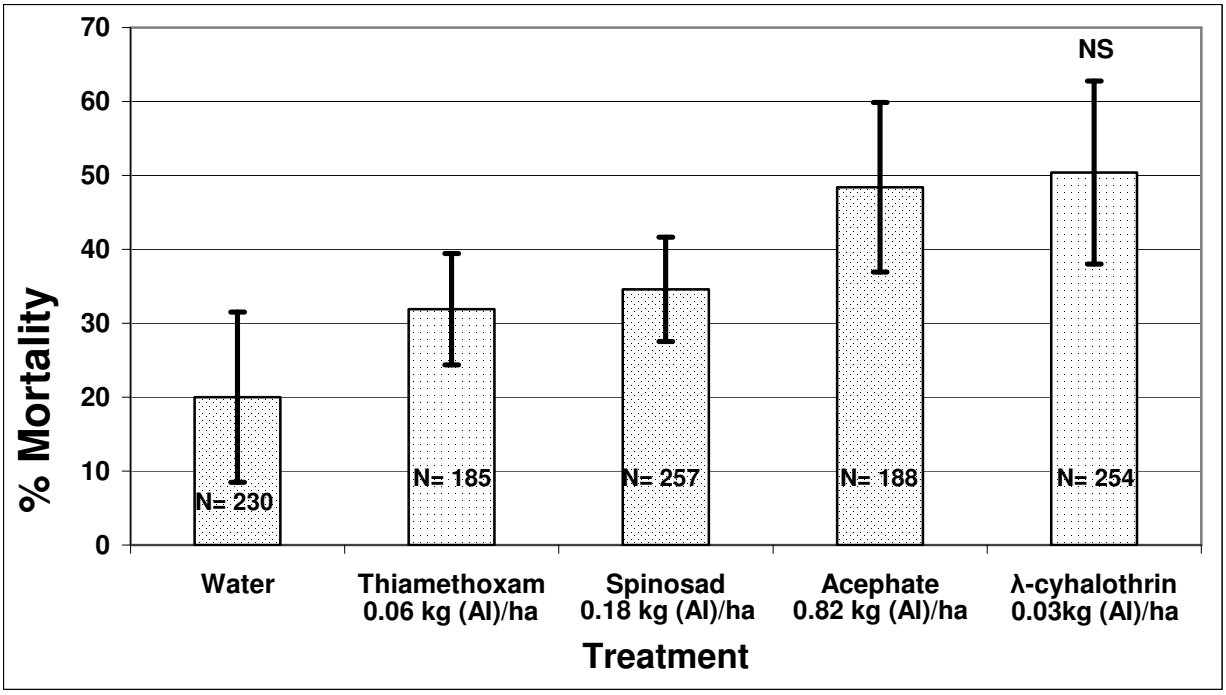

Fig. 3.6. Mortality of developing *Acrosternum hilare* in egg masses when placed in the canopy of soybean plants in plots, and sprayed with field-formulated insecticides at the standard rate.
N = number of eggs
Hatch proportion data were normalized with an arcsine transformation.
NS There were no significant differences among treatment groups.

Chapter Four

Using microscopy to assess chorion structure and parasitoid oviposition sites on stink bug (Hemiptera: Pentatomidae) eggs

ABSTRACT Previous efficacy studies found that many insecticides used by growers could be having an adverse effect on egg parasitoids (*Telenomus podisi*) developing in the eggs of the brown stink bug (*Euschistus servus*), while unhatched stink bugs experienced lower levels of mortality. Studies were initiated to define the mechanisms for this difference. One plausible explanation was that insecticides might enter parasitized eggs more readily via oviposition wounds. Studies at Virginia Tech using an FEI Quanta 600 FEG environmental scanning electron microscope examined the chorion (egg shell) of stink bug eggs parasitized by *T. podisi* and the physical damage caused by oviposition. Egg samples were coated with gold and secured to observation platforms using carbon tape. In addition, parasitized brown stink bug eggs as well as many species of non-parasitized stink bug eggs were examined using standard electron microscopy techniques at the Virginia State University Electron Microscopy Laboratory. As a "control", egg response to perforation by a tungsten probe the diameter of the parasitoid ovipositor was also studied. Microscopy images depicted the chorion surface as characterized by a matrix of ridges and apically-protruding micropylar processes in a ring around the margin of the operculum. The chorion and micropyles vary in appearance among stink bug species. Observations of oviposition sites showed a "scab" formed at sites on the egg where the ovipositor penetrated the chorion. Similar scabs also formed as a result of penetration by a tungsten probe. These scab formations appeared to be the result of fluids from inside the egg leaking out, drying, and hardening after oviposition or perforation with the probe, suggesting that

the response was not due to substances secreted by the parasitoid. The rapid scabbing over of the wound also suggests that under natural conditions, oviposition wounds would not be points of entry for insecticide movement into parasitized eggs.

Key Words Pentatomidae, egg chorion structure, oviposition sites, microscopy

In Virginia, major stink bug (Hemiptera: Pentatomidae) pests are the brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Acrosternum hilare* (Say), which are bivoltine. They are polyphagous, feeding on the fruiting bodies of shrubs, trees, grasses, row crops, fruiting vegetables, and fruit, and can survive on weedy hosts (McPherson and McPherson 2000). Insecticide applications targeting stink bugs often kill beneficial parasitoids and predators along with the target pests (Croft and Brown 1975). Stink bug egg parasitoids such as *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) are present throughout the summer, parasitizing up to 50% of stink bug egg masses (Koppel et al. 2009) in southeastern Virginia, and could be subject to insecticide sprays as adults, or while developing inside stink bug eggs.

Previous studies demonstrated that applications of acephate, λ-cyhalothrin, spinosad, and thiamethoxam result in significantly greater mortality for parasitoids developing in stink bug eggs than developing stink bugs (Koppel, unpublished data), but there is little explanation in the literature for why this occurs. A possibility is that the process of oviposition changes the physical structure of the egg chorion, leaving a large hole for insecticides to pass through.

The general structure of pentatomid eggs is well described (Esselbaugh 1946, Bundy and McPherson 2000). The egg shell is referred to as the chorion, and is composed of sclerotized proteins (Nation 2002) which may form spinose, reticulate, or triangular patterns which vary among stink bug species (Bundy and McPherson 2000). At the anterior of the egg is a circular

lid, the operculum, which is loosely attached to the stink bug egg and provides an opening for emergent nymphs (Esselbaugh 1946). Micropyles are tube-like hollow protrusions of the chorion which are arranged in a circle around the operculum (Esselbaugh 1946). Beament (1948) studied the properties of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) eggs, stating that it would serve as a "type specimen" for hemipterous pest-species' eggs, and determined that micropyles, which penetrate some egg shell layers, are crucial to the transport of insecticides into eggs because chorion is impermeable to ovicidal or toxic substances (Beament 1952).

Scanning electron microscopy was utilized to closely examine the chorion and micropylar processes of different species of pentatomid eggs, some of which were punctured with a tungsten probe. Parasitized egg chorion was also examined for evidence of physical damage caused by oviposition.

Materials and Methods

Non-parasitized and parasitized eggs. Non-parasitized eggs and host eggs were obtained from a laboratory-reared colony of stink bugs using methods described by Koppel et al. (2009). To obtain parasitized eggs of *E. servus*, *T. podisi* founders were field collected. Fresh *E. servus* egg masses were pinned to the undersides of leaves or on stems of host plants, which were located on the margins of fields, on sunny days with light wind. After 48 hours, the egg masses were collected and returned to a growth chamber [24.4°C, 85% R.H., 14:10 (L:D)] in the laboratory. When the eggs turned black, it signaled that they had been parasitized (Fig. 4.1). Parasitized *A. hilare* eggs were recovered from a soybean field located at the Virginia Tech Tidewater Agricultural Research and Extension Center (TAREC), Suffolk, VA. All eggs were stored in a 5°C refrigerator as soon as possible for optimal preservation before microscopy.

Punctured eggs. Non-parasitized eggs from the colony or the field, all of which were no more than 7 days old, were punctured with a tungsten probe to create an artificial oviposition wound. To determine an appropriate probe diameter, a female *T. podisi* adult was dissected under a Wild MP 400 Stereo Microscope. The ovipositor sheath was separated from the ovipositor, and multiple stereo microscope images were edited into one, using Syncroscopy Automontage software (Synoptics Ltd., Frederick, MD). The same software was also used to measure the diameter of the ovipositor, which was approximately 10 microns across (Fig. 4.2). A tungsten probe with a tip diameter of 10 microns was gently pressed against the egg chorion. After penetration, the probe was removed and cleaned, and the eggs were allowed to remain at room-temperature for 24 hours before being placed in a 5°C refrigerator. *E. servus*, *A. hilare*, and harlequin bug, *Murgantia histrionica* (Hahn), eggs were punctured. Although non-parasitized eggs of the spined soldier bug, *Podisus maculiventris* (Say), were observed, the number of available eggs was severely limited, and none were punctured for this study.

Microscopy. Initial microscopy was performed at the Virginia Tech Institute for Critical Technology and Applied Science (Blacksburg, VA) using a FEI Quanta 600 FEG (FEI Company, Hillsboro, OR) environmental scanning electron microscope. Eggs were coated with gold for high quality imaging and attached to small stages with carbon tape before imaging on the larger specimen stage. Further analysis was performed at Virginia State University (Petersburg, VA) using a TM-1000 Tabletop scanning electron microscope (Hitachi High Technologies America, Inc.).

Results

Non-parasitized eggs. The non-parasitized eggs of *E. servus*, *A. hilare*, *P. maculiventris*, and *M. histrionica* were observed. Observations about the eggs of the former

three species are consistent with those of Bundy and McPherson (2000). *E. servus* eggs (Fig. 4.3) were about 900 microns in diameter with a length of 50 microns per micropyle (Fig. 4.4). There was an average of 33 micropyles per egg. *A. hilare* eggs (Fig. 4.5) were comparatively large and averaged 1000 microns in diameter. There was an average of 50 micropyles (Fig. 4.5) per egg, each of which was approximately 50 microns in length (Fig. 4.6). *P. maculiventris* eggs (Fig. 4.7) were about 300 microns in diameter and had 13-15 micropyles per egg. The micropyles were the longest of the four stink bug species, about 280 microns in length (Fig. 4.8). The final stink bug species, *M. histrionica*, measured approximately 950 microns in diameter (Fig. 4.9). Each egg had between 24 and 26 micropyles, each measuring about 30-40 microns in length (Fig. 4.10). These micropyles seemed to be recessed in the egg chorion. *M. histrionica* observations were in agreement with Esselbaugh (1946).

Parasitized and punctured eggs. Parasitized *E. servus* (Fig. 4.11) and *A. hilare* (Fig. 4.12) eggs featured a scab-like protrusion at some oviposition sites. These protrusions varied in both diameter and length, but were distinct from the surface of the egg chorion. Eggs which were punctured with a tungsten probe also formed "scabs" (Figs. 4.13, 4.14, and 4.15).

Discussion

Observations suggest that when non-parasitized *E. servus* and *A. hilare* eggs are punctured by the ovipositor of a parasitoid, there is no permanent hole in the chorion. Rather, a scab forms over the oviposition wound. When punctured with a probe, there is a similar hardening of internal egg fluids on the surface of the egg. Since the scab forms as a result of oviposition and a probe wound, the scabbing process is most likely a result of egg physiology, rather than substances secreted by the parasitoid.

The scabs which formed from probe wounds were more fluid and less set in appearance than those formed via oviposition. A human hand puncturing an egg with a probe does so with less finesse than a parasitoid using its ovipositor, making it likely that the ovipositor caused a cleaner wound, despite being the same diameter as the probe. Further, although parasitized *M. histrionica* eggs were not found or successfully reared for observation, a scab formed over probe wounds. It is likely that a scab also forms after parasitization, as it did for *E. servus* and *A. hilare*.

Overall, it is suggested that while oviposition causes obvious physical differences between non-parasitized and parasitized stink bug eggs, there are no open wounds or holes which would allow for increased entry of insecticides. Further research into chorion permeability or the hardiness of developing parasitoids would provide more information about the cause for differences in mortality.

References Cited

Beament, J. W. L. 1948. The penetration of insect egg-shells. I.—Penetration of the chorion of *Rhodnius prolixus*, Stål. Bull. Entomol. Res. 39: 359-383.

Beament, J. W. L. 1952. The role of cuticle and egg-shell membranes in the penetration of insecticides. Ann. Appl. Biol. 39: 142-143.

Bundy, C. S., and R. M. McPherson. 2000. Morphological examination of stink bug (Heteroptera: Pentatomidae) eggs on cotton and soybeans, with a key to genera. Ann. Entomol. Soc. Am. 93: 616-624.

Croft, B. A., and A. W. A. Brown. 1975. Responses of arthropod natural enemies to insecticides. Annu. Rev. Entomol. 20: 285-335.

Esselbaugh, C. O. 1946. A study of the eggs of the Pentatomidae (Hemiptera). Ann. Entomol. Soc. Am. 39: 667–691.

Koppel, A. L., D. A. Herbert, Jr., T. P. Kuhar, and K. Kamminga. 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean and vegetable crops in Southeast Virginia. Environ. Entomol. 38: 375-379.

McPherson, J. E., and R. McPherson. 2000. Stink bugs of Economic Importance in North America & Mexico. CRC Press LLC, Boca Raton, FL.

Nation, J.L. 2002. Insect Physiology and Biochemistry. CRC Press LLC, Boca Raton, FL.

Fig. 4.1. Non-parasitized (light) and parasitized (dark) eggs of *Euschistus servus.*

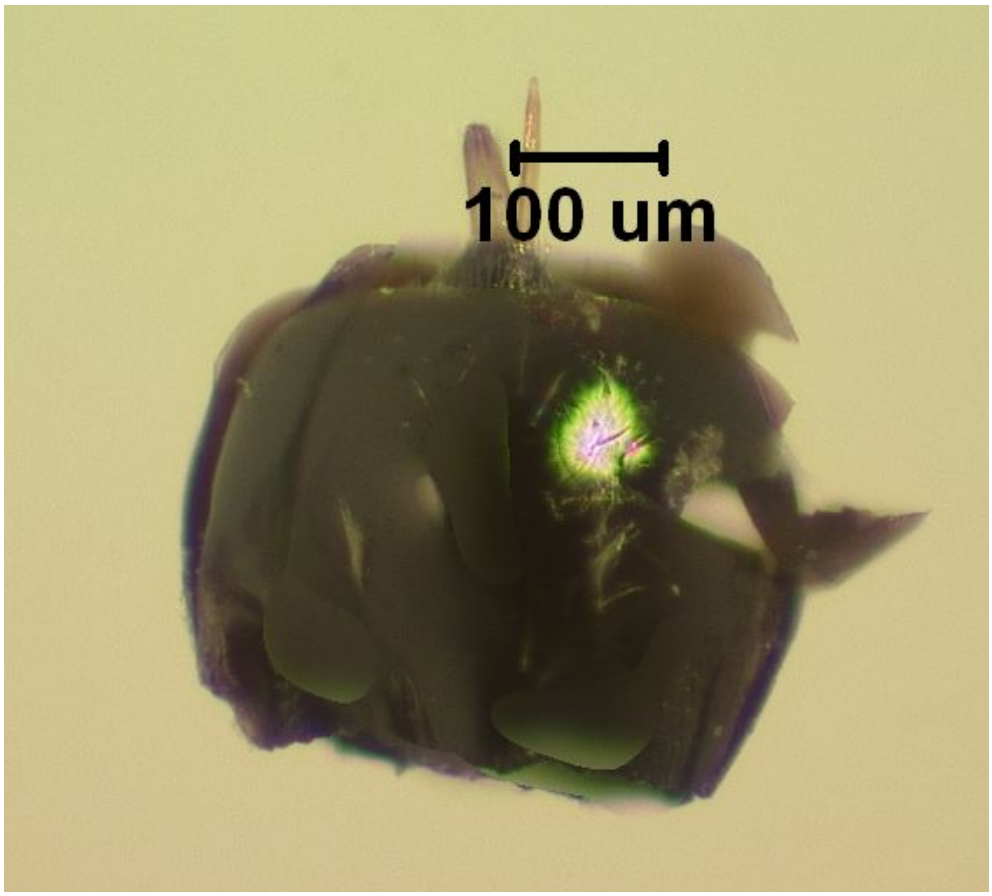

Fig. 4.2. Abdomen and ovipositor of the egg parasitoid *Telenomus podisi.*

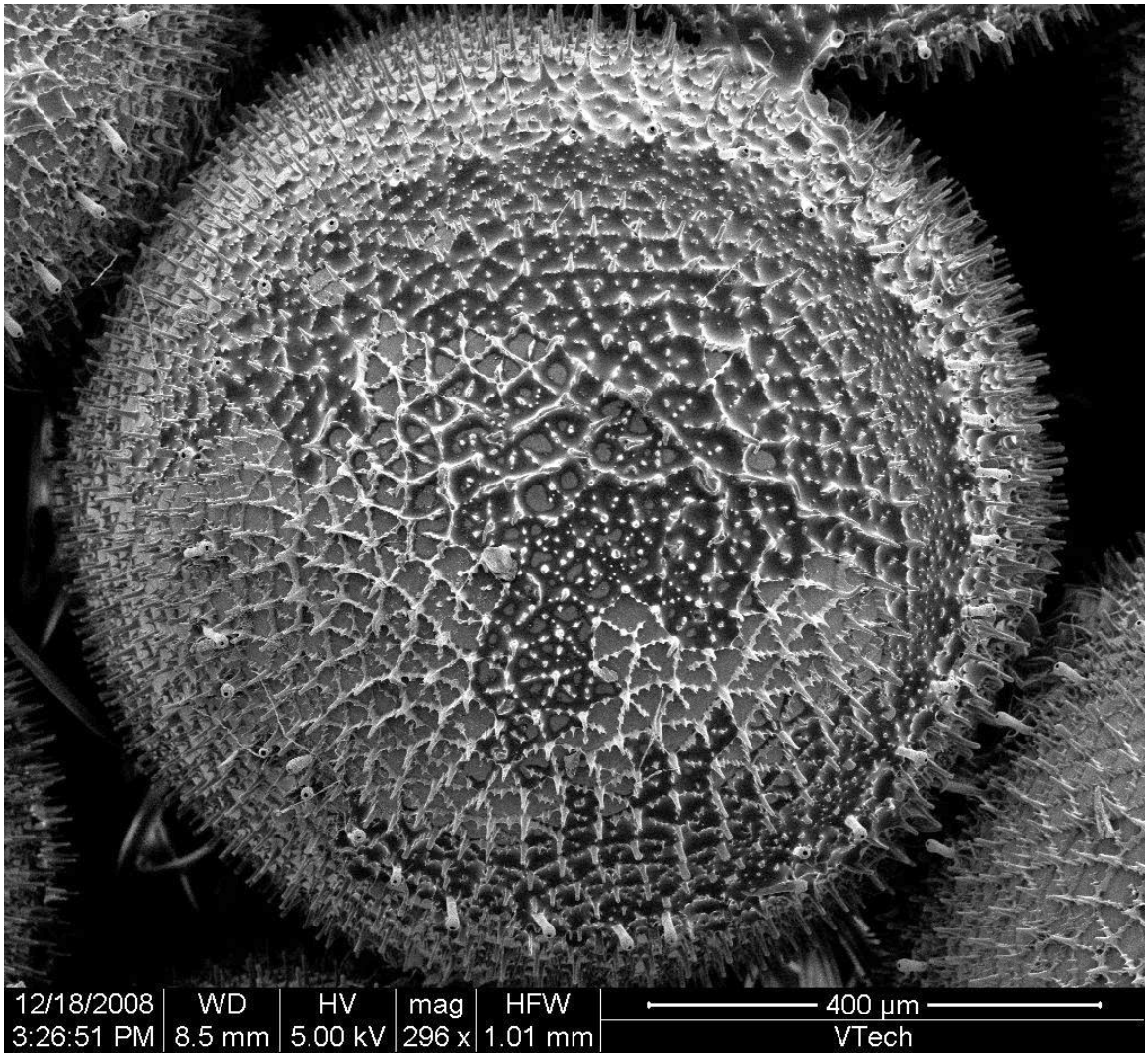

Fig. 4.3. *Euschistus servus* egg.

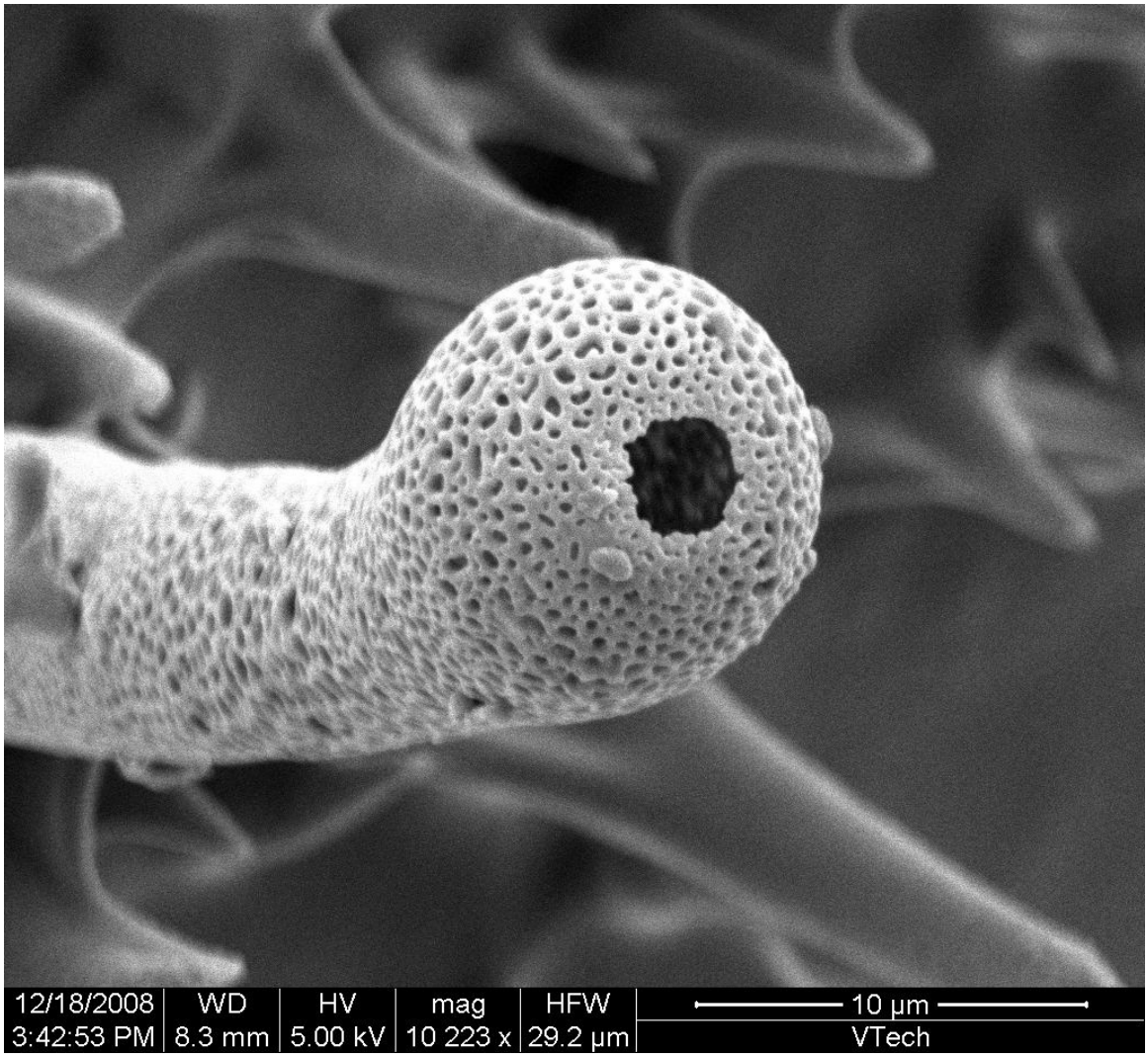

Fig. 4.4. Micropylar process of an *Euschistus servus* egg.

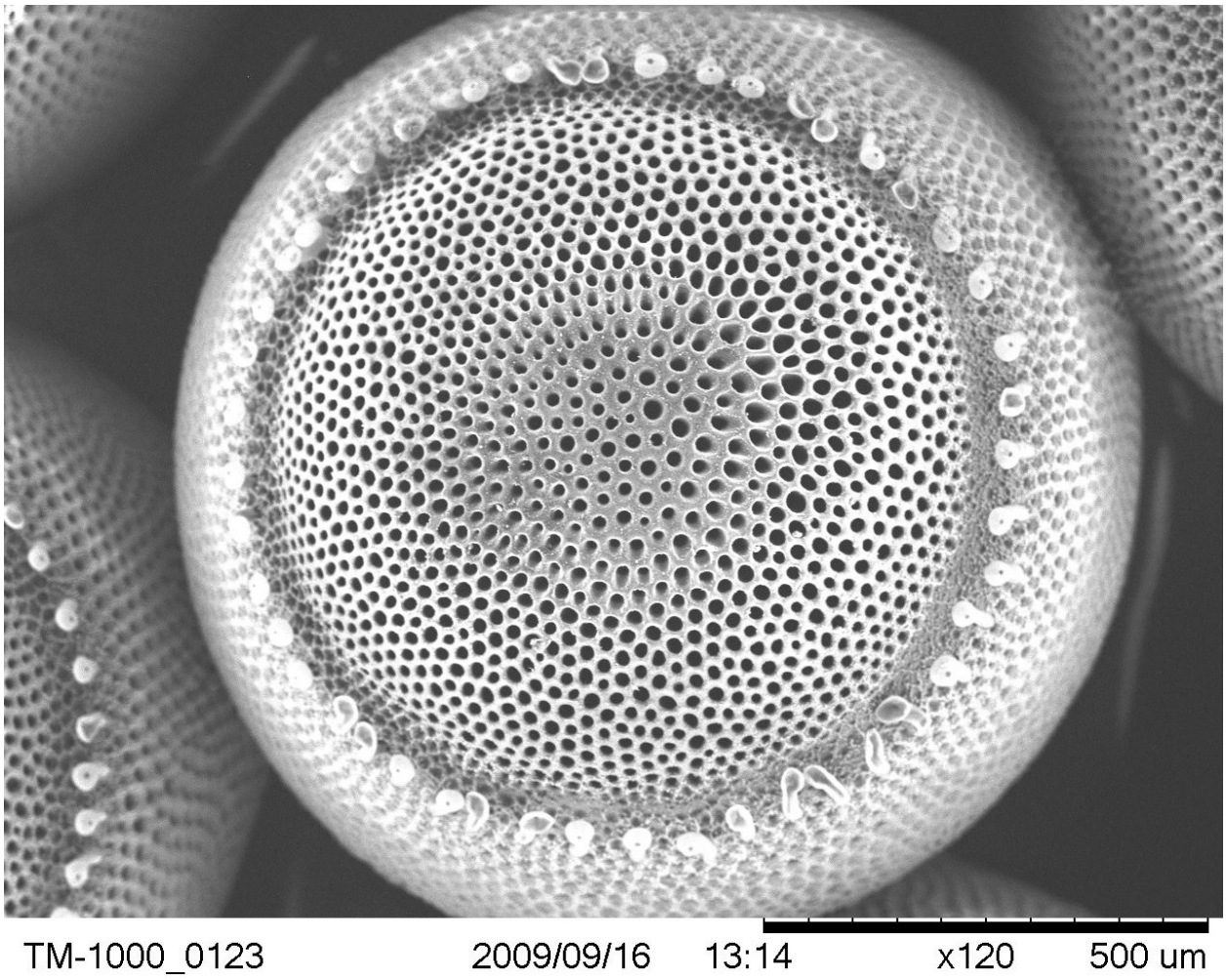

Fig. 4.5. *Acrosternum hilare* egg.

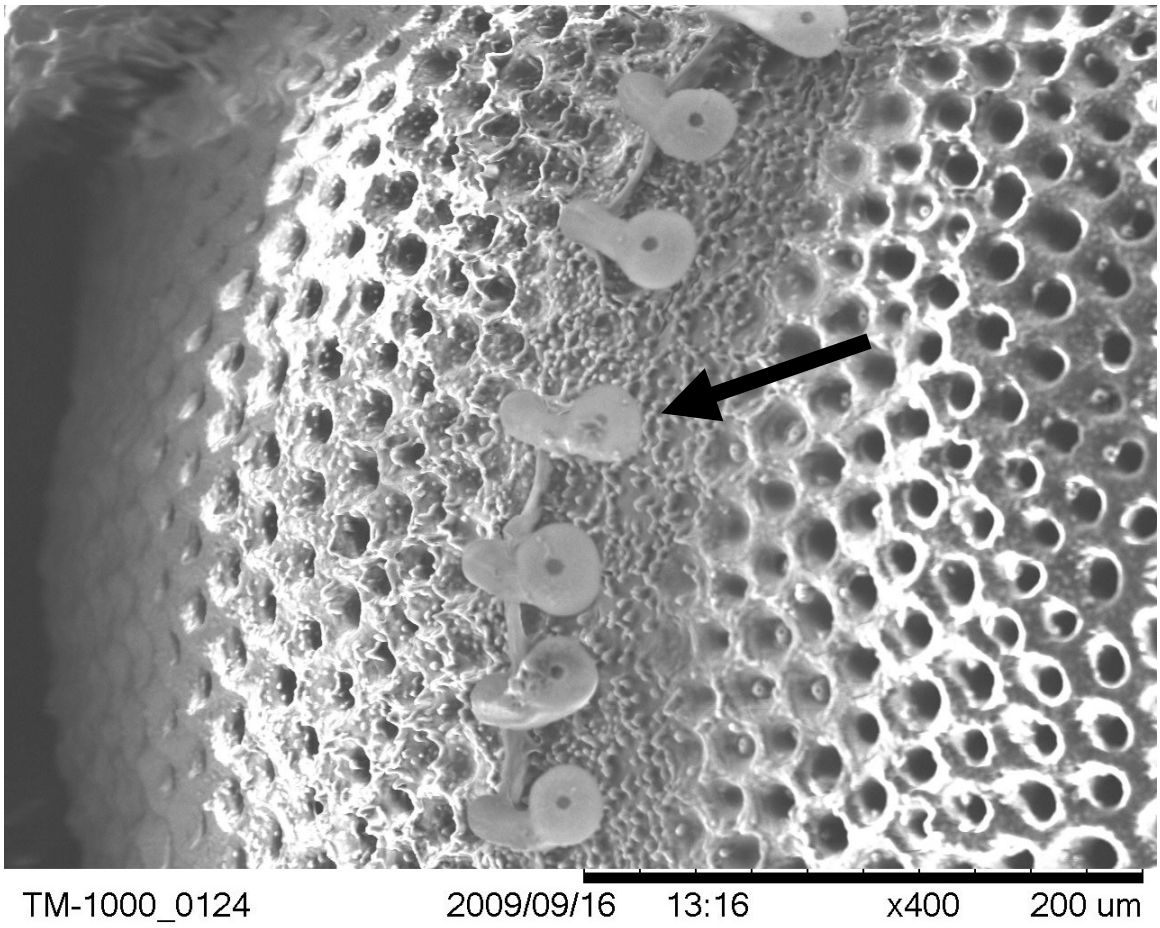

Fig. 4.6. Micropylar processes of an *Acrosternum hilare* egg.

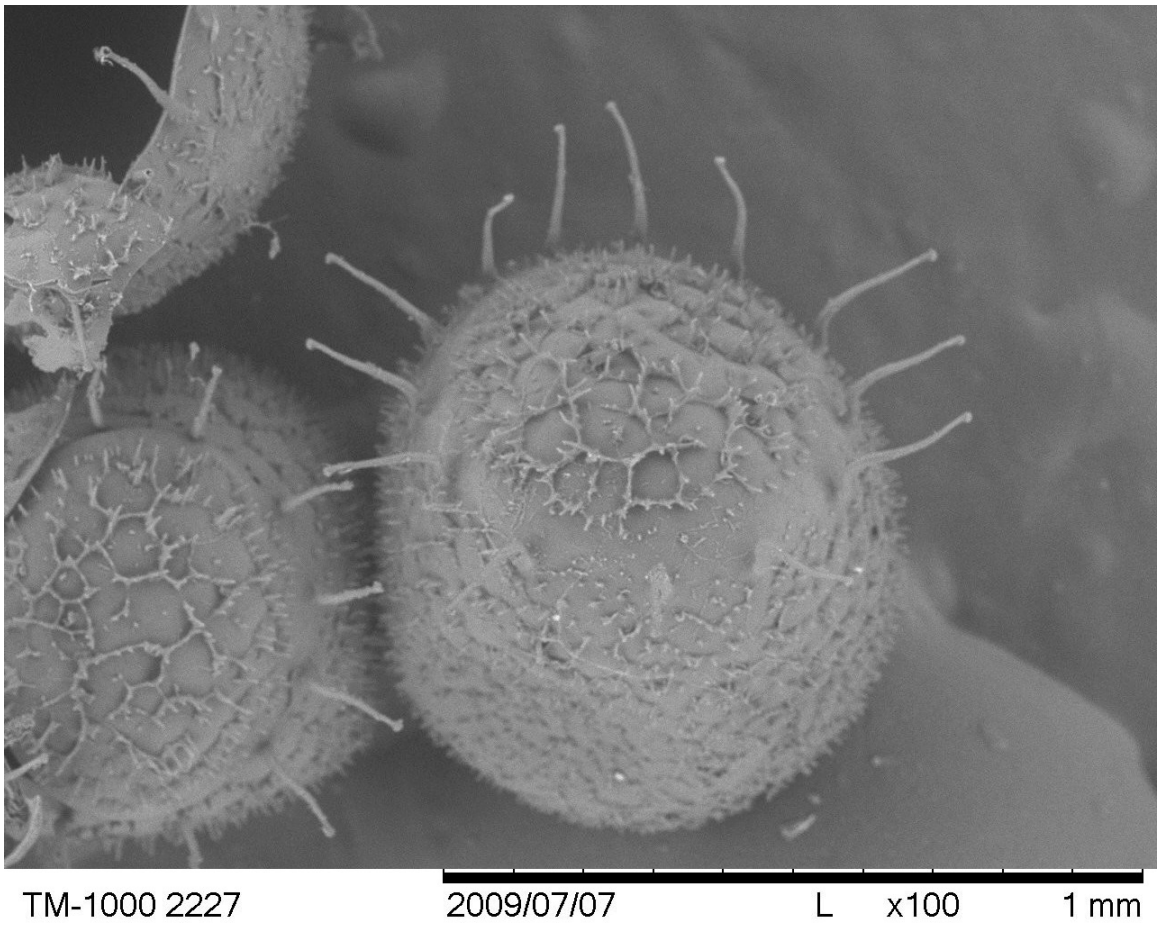

Fig. 4.7. *Podisus maculiventris* egg.

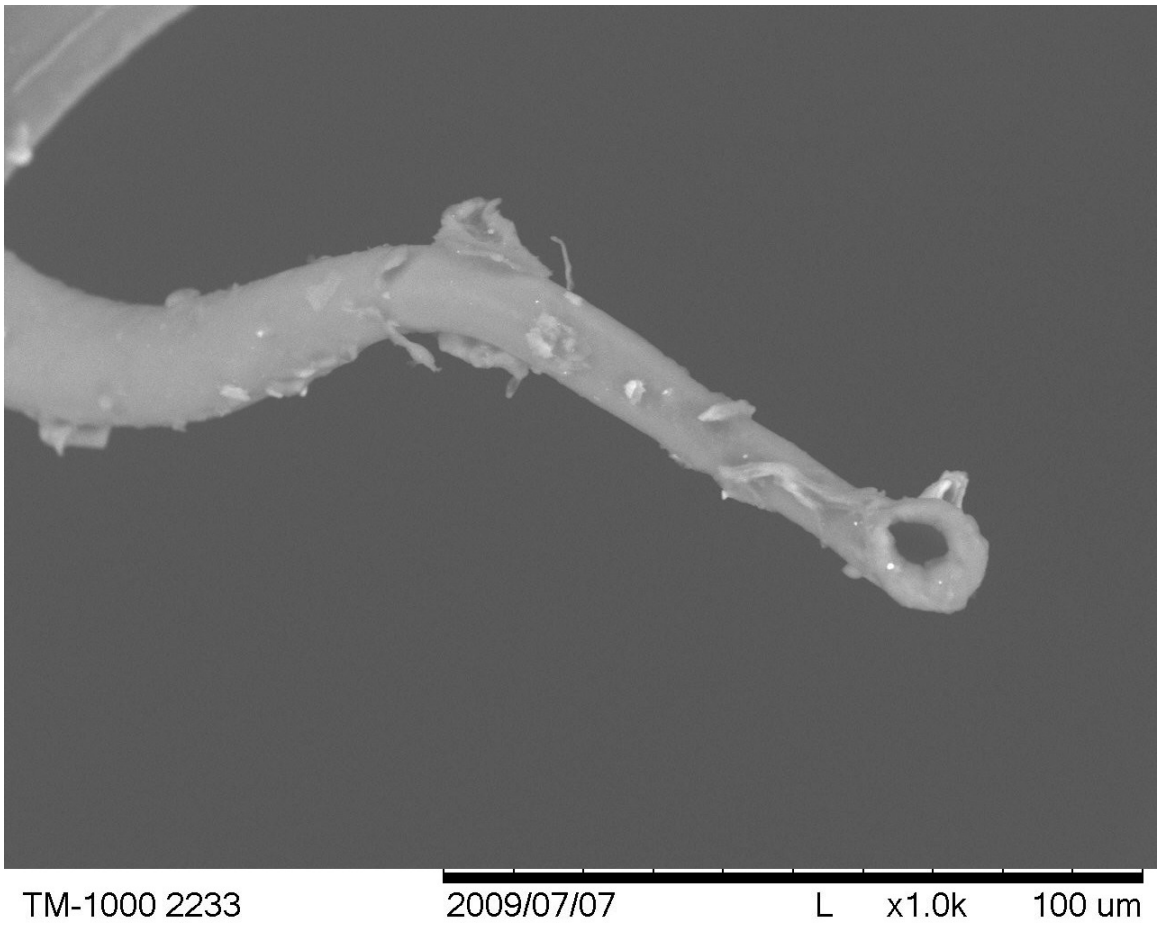

Fig. 4.8. Micropylar process of a *Podisus maculiventris* egg.

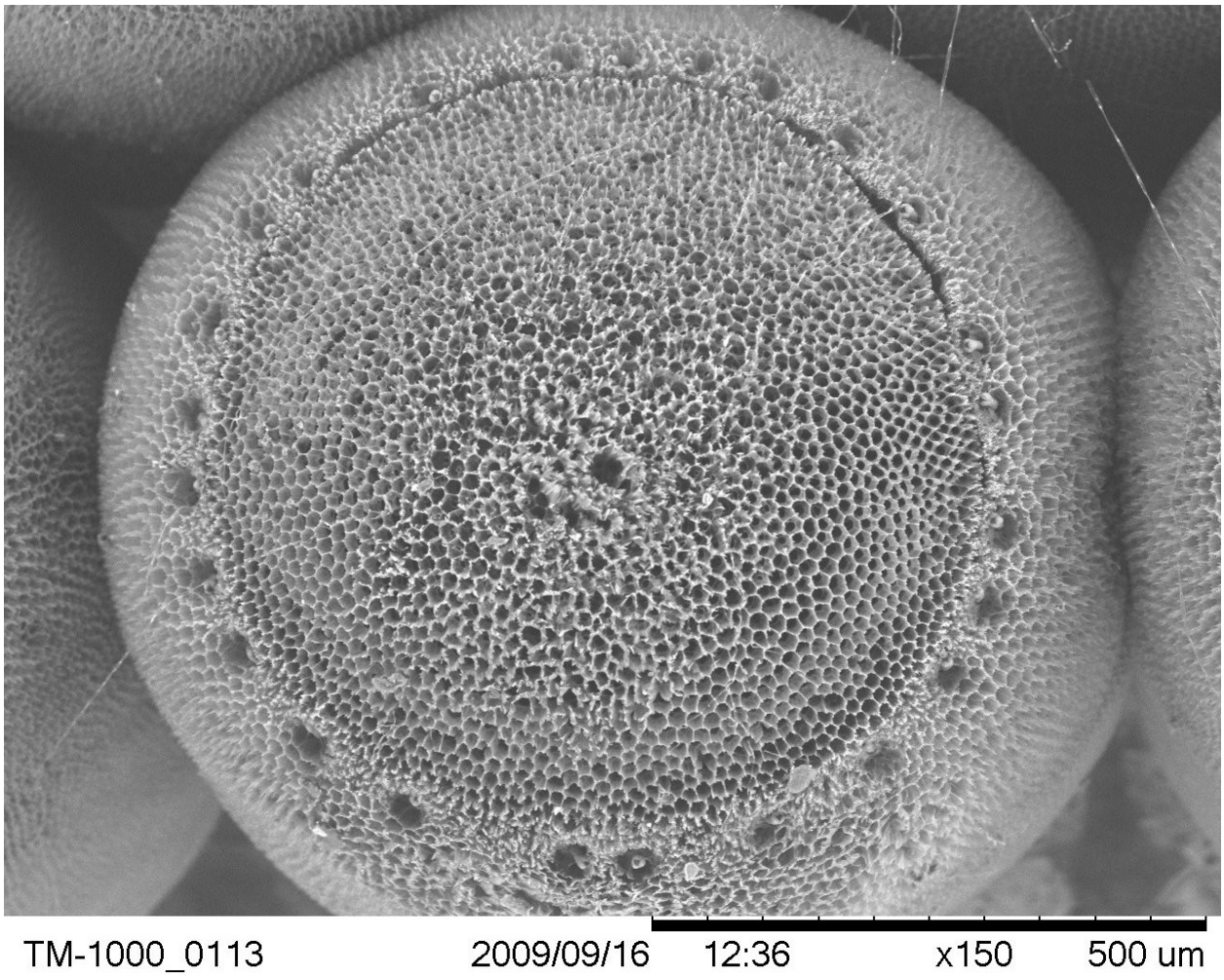

Fig. 4.9. *Murgantia histrionica* egg.

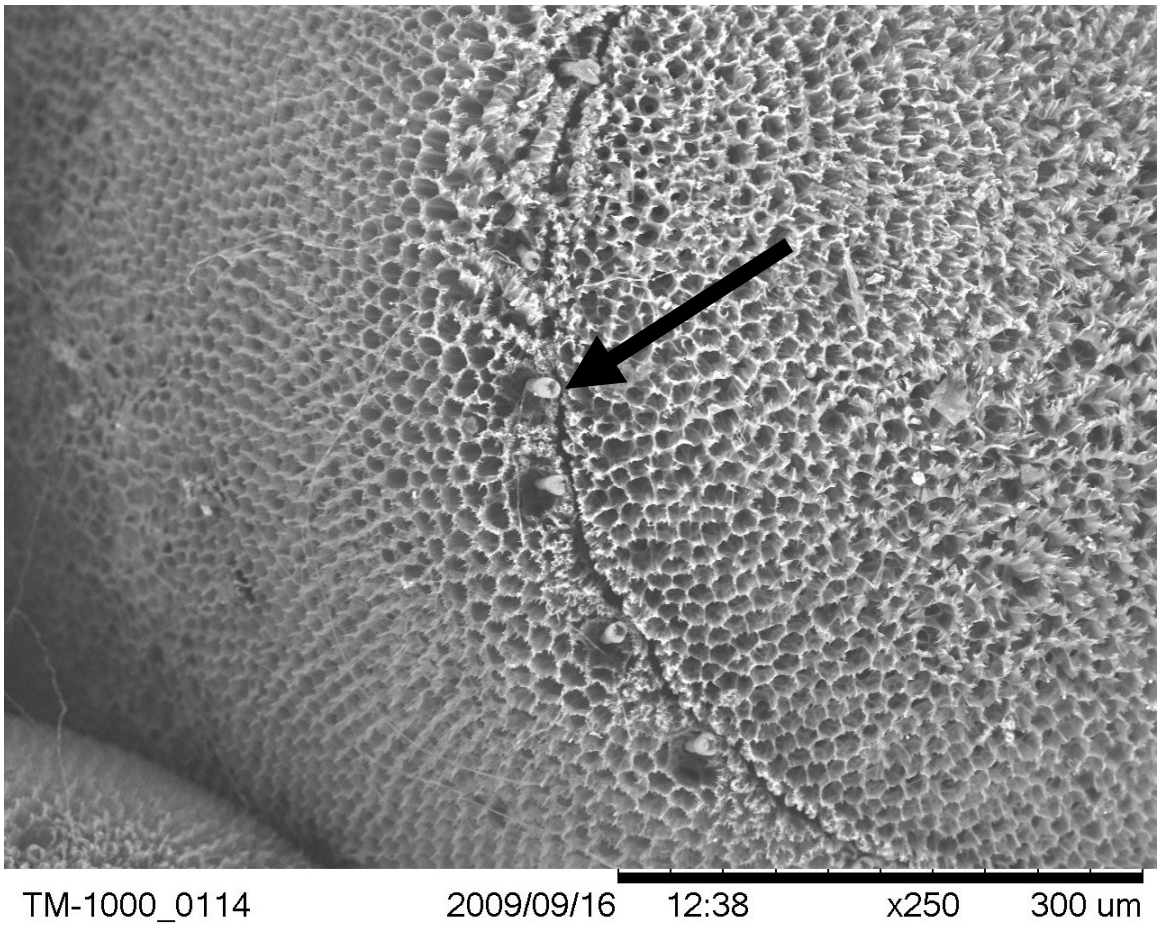

Fig. 4.10. Micropylar processes of a *Murgantia histrionica* egg.

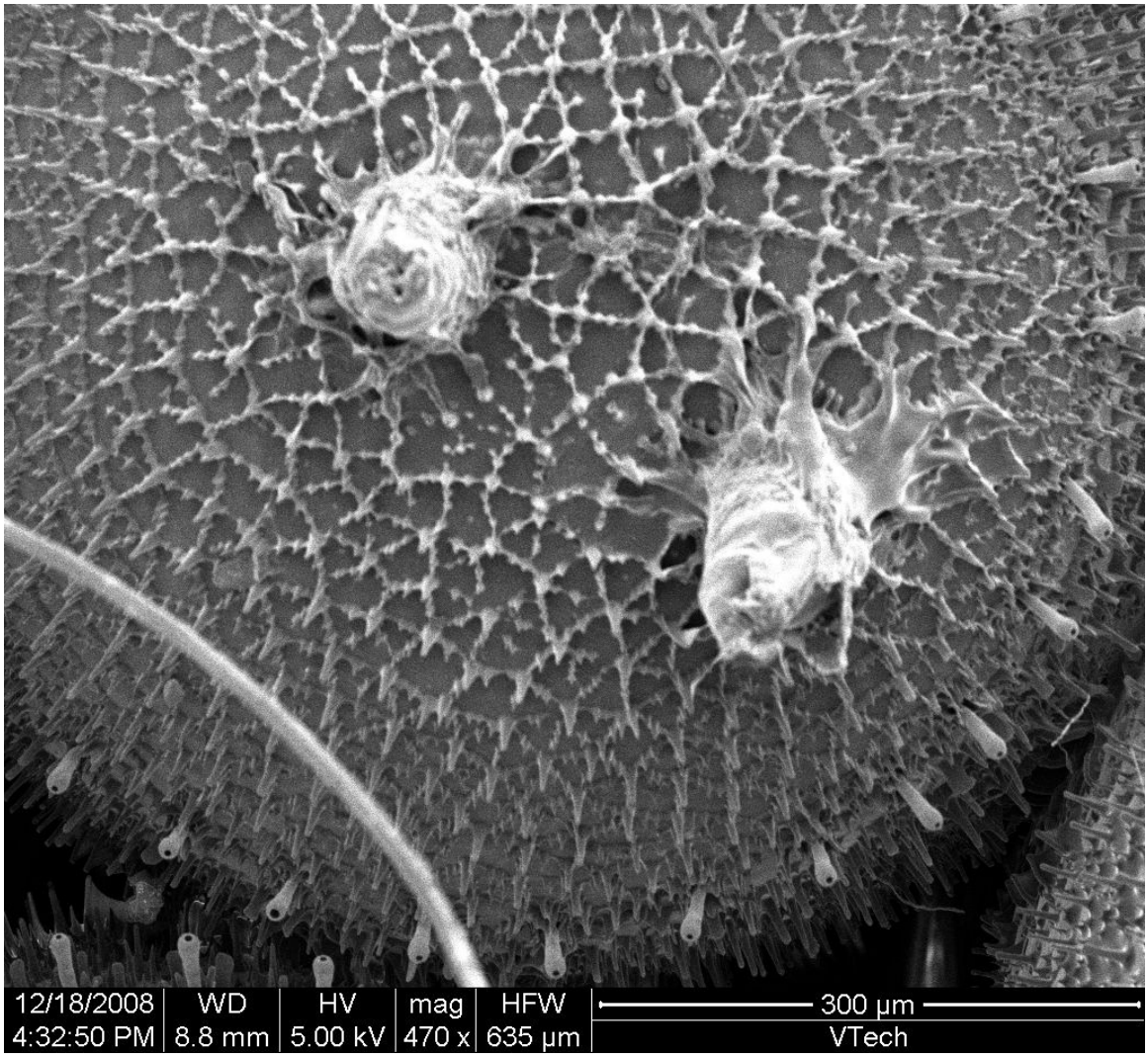

Fig. 4.11. Scabs, formed after egg parasitoid oviposition, on the chorion of an *Euschistus servus* egg.

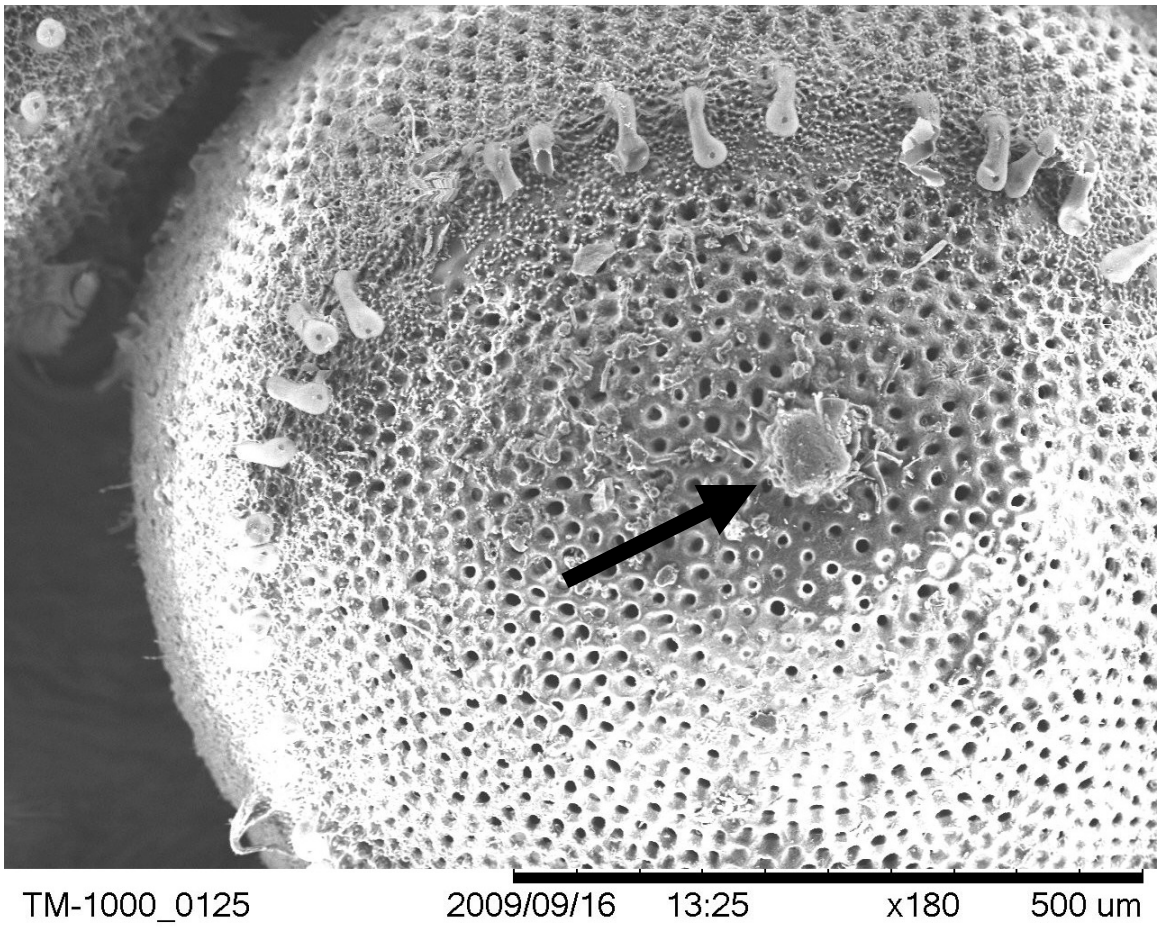

Fig. 4.12. A scab, formed after egg parasitoid oviposition, on the chorion of an *Acrosternum hilare* egg.

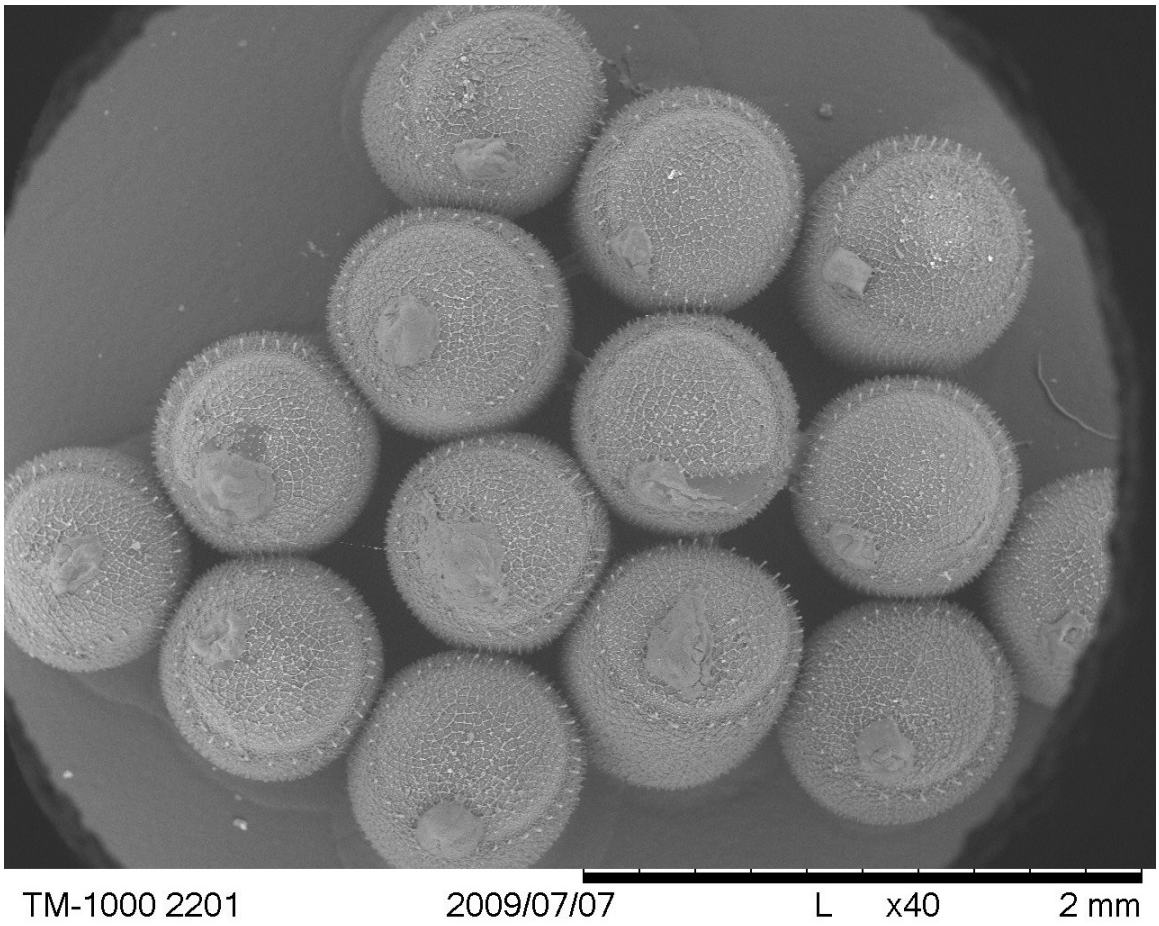

Fig. 4.13. Scabs, formed after puncturing eggs with a tungsten probe, on the chorion of *Euschistus servus* eggs.

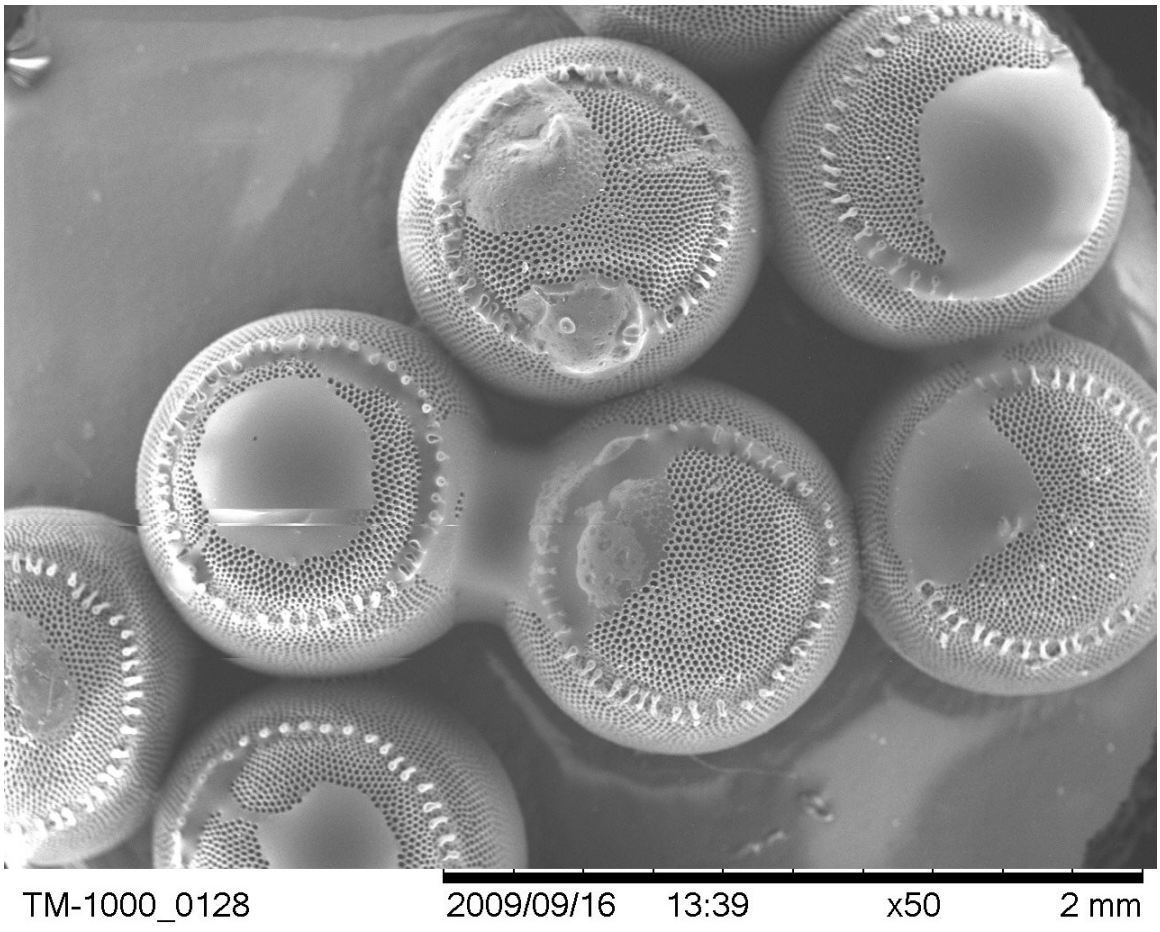

Fig. 4.14. Scabs, formed after puncturing eggs with a tungsten probe, on the chorion of *Acrosternum hilare* eggs.

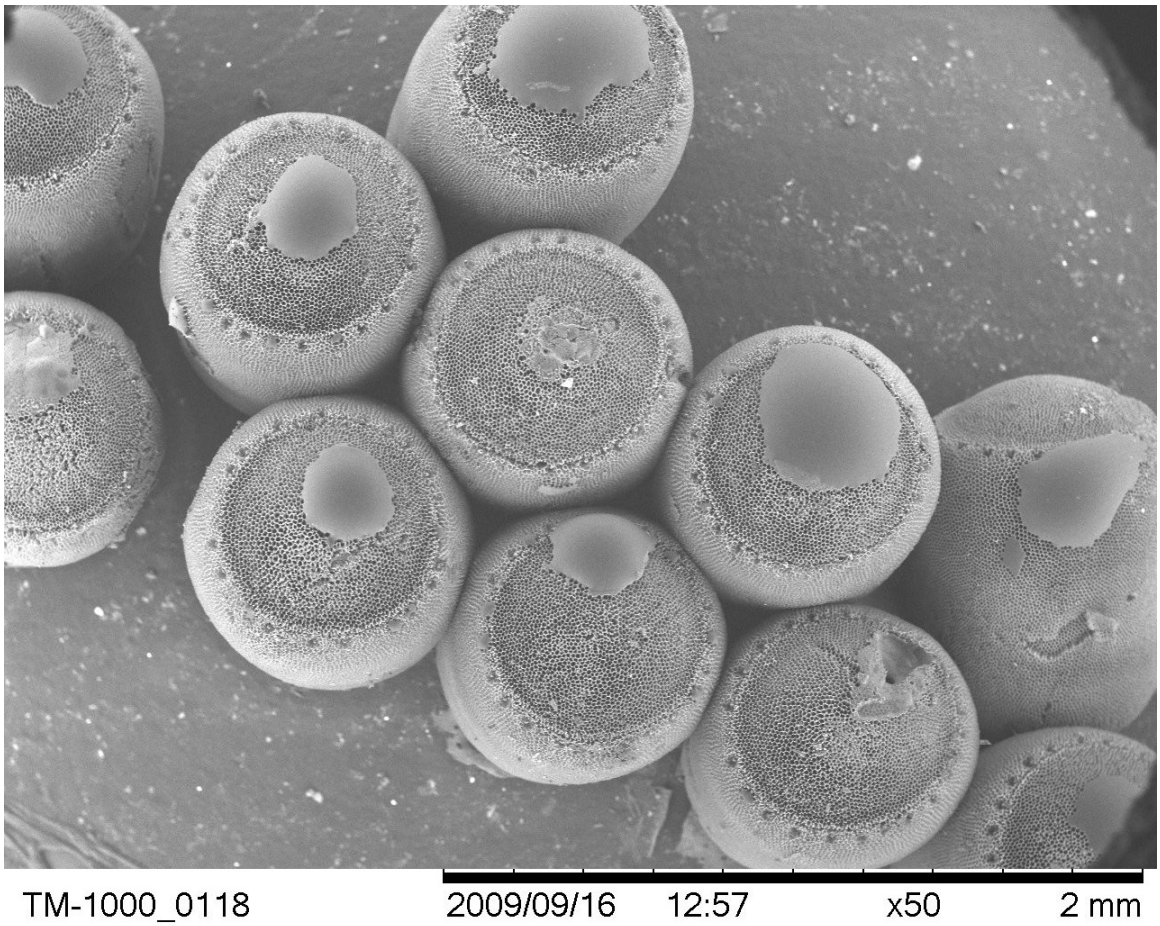

Fig. 4.15. Scabs, formed after puncturing eggs with a tungsten probe, on the chorion of *Murgantia histrionica* eggs.

Chapter Five

Assessing chorion permeability of non-parasitized and parasitized (by *Telenomus podisi,* Hymenoptera: Scelionidae) brown stink bug (*Euschistus servus,* Hemiptera: Pentatomidae) eggs to insecticides in three classes

ABSTRACT Bioassays and field investigations of the efficacy of insecticides against non-parasitized eggs and parasitoids developing in eggs of the brown stink bug indicated that there was limited mortality to non-parasitized eggs exposed to acephate, λ-cyhalothrin, thiamethoxam, or spinosad applied at field application rates. However, there was almost 100% mortality to the developing parasitoids contained in parasitized eggs. These higher rates of parasitoid mortality could be attributed to either increased sensitivity to insecticides, or increased exposure due to differences in chorion permeability. Scanning electron microscopy of parasitized eggs showed that oviposition wounds were sealed by a 'scab' but it was not clear whether these wounds might allow for increased insecticide movement. To further investigate possible differences in chorion permeability, non-parasitized (N= 420) and parasitized eggs (N = 431) were incubated in solutions containing one of three ^{14}C-insecticides at field application rates for 0, 30, 120 or 240 min. After exposure, eggs were rinsed three times with water, homogenized, and then centrifuged. All rinsates and supernatants were analyzed for radiolabel content which was used to compare differences movement rates of insecticides into eggs. Using ANOVA, we found that insecticide movement into the egg increased significantly with incubation time for both ^{14}C-acephate ($P = 0.0249$) and ^{14}C-λ-cyhalothrin ($P < 0.0001$), and a significantly greater amount of ^{14}C-λ-cyhalothrin had moved into non-parasitized eggs. Neither incubation time nor egg status was significant for ^{14}C-thiamethoxam. When incubation time, egg status, and insecticides were

analyzed together, an ANOVA model ($P = 0.0012$) was constructed that predicts amount of insecticide uptake based on radiolabel content, entering the egg at any given time.

Key Words Pentatomidae, egg chorion permeability, *Euschistus servus*, Scelionidae

Previous studies demonstrated that applications of acephate, λ-cyhalothrin, spinosad, and thiamethoxam resulted in significantly greater mortality for parasitoids developing in stink bug (Hemiptera: Pentatomidae) eggs than developing stink bugs (Koppel, unpublished data). Higher rates of parasitoid mortality could be attributed to either increased sensitivity to insecticides, or increased exposure due to differences in chorion permeability. Research using scanning electron microscopy of parasitized eggs showed that oviposition wounds were sealed by a 'scab', but it was not clear whether these wounds allow for increased insecticide movement. There could also have been differences in chorion permeability due to morphological or chemical changes caused by the parasitization process.

An important consideration in the study of insecticide efficacy on embryonic *Euschistus servus* (Say) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) is that of the structure and physiology of host eggs, which serve to protect developing embryos. Beament (1948) studied the properties of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae) eggs, stating that it would serve as a "type specimen" for hemipterous pest-species' eggs. He also proposed that micropyles, which penetrate some egg shell layers, are crucial to the transport of insecticides into eggs because chorion is impermeable to ovicidal or toxic substances, even if there are smaller molecules of methyl alcohol or acetic acid present (Beament 1952). When the micropylar regions of eggs were submerged in ovicide, it was found that many more eggs were killed than when only the rear of the egg was submerged. After exposure to stained ovicides, eggs

containing dead insects were observed to have at least one stained micropyle (Beament 1948). Further, the age of the egg determined which sub-chorial membranes contributed to ovicide resistance. Over the first six days of development, the egg became more resistant to lipophilic substances, due to wax impregnation. Formation of an epembryonic membrane layer and secondary wax caused resistance to hydrophiles to increase during development, though this resistance decreased just before eclosion (Beament 1949).

Uptake of specific classes of insecticides into the egg has also been studied. Although organophosphates have been used as ovicides, eggs have developed resistance to this pesticide group due to biochemical differences in the esterase complex of resistant-strain embryos (Smith and Salkeld 1966). Further, parathion does not act as a true ovicide on the eggs of milkweed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae), as the penetration barrier of eggs does not allow enough insecticide influx into the eggs to kill the embryos (Zschintzsch et al. 1965); however, it has been demonstrated that symptoms of organophosphate toxicity coincide very closely with cholinesterase inhibition in *O. fasciatus* eggs (Smith and Salkeld 1966). Both cholinesterase and acetylcholine appear in these eggs at four days of development (Mehrotra 1960).

The objective of this study was to assess the chorion permeability of non-parasitized and parasitized (by *T. podisi*) brown stink bug (*E. servus*) eggs to insecticides in three classes using radiolabeled insecticides.

Materials and Methods

Stock solutions. Preliminary studies with tritiated water ($^{3}H_2O$) indicated that ^{14}C-amended solutions should be formulated to have approximately 592 kilobecquerels (kBq) in an experiment. Initial radiolabeled material obtained from pesticide suppliers was diluted from

stock solutions which could be diluted with unlabeled, formulated insecticide to contain this amount of activity. We obtained radiolabel from 6,934 kBq/mL ^{14}C-acephate, 1,073 kBq/mL ^{14}C-λ-cyhalothrin, and 999 kBq/mL ^{14}C- thiamethoxam. For each experiment, a total of 5 mL of diluted ^{14}C-insecticide was prepared with the final concentration equivalent to the field application rate: ^{14}C-acephate at 0.82 kg (AI)/ha, ^{14}C-λ-cyhalothrin at 0.03 kg (AI)/ha, and ^{14}C-thiamethoxam at 0.06 kg (AI)/ha (Herbert 2010).

Experimental procedure. The general experimental procedure was similar to that reported in a study by Mullins et al. (2002). Brown stink bug egg masses, less than 14 days old and stored in a 5ºC refrigerator, selected for this study contained between eight and 10 eggs per mass, and each mass was placed into individual 1.5 mL Eppendorf centrifuge Flex-Tubes® (Eppendorf North America, Hauppauge, NY). Some of these egg masses were parasitized by the egg parasitoid *T. podisi*, no more than 14 days prior to analysis. One hundred µL of the appropriate stock ^{14}C-insecticide solution was added to each of 40 microcentrifuge tubes containing stink bug eggs. In addition, three replicate controls were made by combining 100 µL of the stock solution and 6 mL of ScintiVerse scintillation fluid (Fisher Scientific, Pittsburgh, PA) in 8 mL polyethylene scintillation counting vials.

After incubation periods of 0, 30, 120, and 240 min., the ^{14}C-insecticide incubation solution (approximately 100 µL) was withdrawn from each tube, and placed into 6 mL of scintillation fluid, in scintillation counting vials. One hundred µL of distilled water was then added back to each tube and mixed. The new rinsate (approximately 100 µL) was removed and added to 6 mL of scintillation fluid, in scintillation vials, representing the first rinsing of the incubated eggs. This rinsing procedure was repeated three more times, thus yielding a series of

five scintillation samples obtained from the original egg incubation tubes (the original stock supernatant, and 4 tubes of distilled water supernatant rinsates).

The washed eggs were then homogenized in the microcentrifuge tubes with 100 µL of distilled water and centrifuged for approximately 90 seconds at 10,000 xg (Fisher Scientific Model 59A microcentrifuge, Fisher Scientific, Pittsburgh, PA). The supernatant was combined with 6 mL of scintillation fluid in 8 mL scintillation vials. Radiolabel in the samples was determined as disintegrations per minute (DPM) of nuclear decay using a Beckman LS 6500 Scintillation Counter.

ANOVA was performed for each data set (PROC GLM: SAS Institute 2002-2005), with Fisher's least significant difference test performed on data sets where time was a significant factor. Simple effects slices for simple means were also included in the program code to analyze any interaction effects between time and egg status for further information about differences between parasitized and non-parasitized eggs. All data were log-transformed prior to analysis.

Results

The triple wash process was successful in removing insecticide from the exterior of the eggs in 110 out of 120 total samples evaluated. A successful rinse was indicated when the DPM of the egg homogenate was greater than that of the last (4^{th}) rinse (Table 5.1), and when DPM values for the last rinse were similar to background counts.

A total of 290 eggs were exposed to ^{14}C-acephate for analysis. There was a significant increase in ^{14}C-acephate movement into eggs with increasing time ($F = 3.73$; df = 3; $P = 0.0249$) (Fig. 5.1), and at 0 min., DPM values were significantly lower than at 120 and 240 min ($P < 0.05$). There was no overall difference between non-parasitized and parasitized egg absorption

of acephate. Simple effects suggested that non-parasitized eggs had a higher DPM than parasitized eggs at 0 min. and there was no interaction effect in the overall data.

There were 278 eggs exposed to ^{14}C-λ-cyhalothrin. As with ^{14}C-acephate, there was a significant increase in DPM values with increasing time ($F = 50.94$; df = 3; $P < 0.0001$) (Fig. 5.2), and according to Fisher's LSD, there was significantly less insecticide absorbed at 0 min. than at the other three times. Further, non-parasitized eggs absorbed more ^{14}C-λ-cyhalothrin than parasitized eggs did, overall. Simple effects slices indicated a significant difference in insecticide movement into non-parasitized and parasitized eggs at 120 min., and there was no interaction effect. There were no significant differences in ^{14}C-thiamethoxam uptake by eggs (N = 283), nor was there an interaction effect between time and egg status (Fig. 5.3).

Insecticides, treatment times, and egg status were compared using ANOVA. The overall analysis was significant ($F = 3.18$; df = 11; $P = 0.0012$), as were the parameters acephate ($t = -3.16$; $P < 0.0001$) and time ($t = 3.48$; $P < 0.0001$) (Fig. 5.4). From these results, an equation to predict absorption of acephate into non-parasitized and parasitized eggs was developed: $y = 0.0022 + 0.0008t$, where y is absorption of insecticides, expressed in DPM, and t is time in min. This equation may be used for any given exposure time.

Discussion

Each of the three insecticides used in the chorion assays yielded different results. While ^{14}C-acephate and ^{14}C-λ-cyhalothrin both demonstrated increased insecticide uptake with increasing time, only ^{14}C-λ-cyhalothrin was removed from the incubation solution in different quantities by non-parasitized and parasitized eggs. The ^{14}C-thiamethoxam information obtained from the incubation intervals showed no significant differences between eggs at different times or with different status. I have been unable to find any information regarding insecticide or

ovicide absorption by eggs, therefore it is difficult to suggest the cause for such differences. It could be due to differences in the structural chemistry of the insecticides and/or the formulation adjuvants which might influence movement through chorion. However, unlike insects such as *Schistocerca gregaria* Forskål (Orthoptera: Acrididae), whose eggs absorb liquid through the entire surface of chorion itself (Moloo 1970), water-soluble materials pass through the micropyles to enter hemipterous eggs (Beament 1948). The size of the opening at the apex of the micropyle was large enough to allow all ^{14}C-insecticides to enter. Alternatively, Novozhilov et al. (1973) found that when chlorophos, an organophosphate also known as trichlorfon, penetrated stink bug eggs, most of it was absorbed by the chorion and never entered the egg interior.

One similarity in our assays was an apparent decrease in insecticide absorption between 120 and 240 min. (Fig. 5.4). Again, we have not found information in the literature that might explain this. It is possible that there is a saturation curve, in which a certain point is reached where eggs cannot absorb any more liquids, and this curve could change depending on the nature of the substance entering the egg. Further studies, expanding the number of egg exposure times between 120 and 240 min., could further define the properties of such a curve, should it exist.

Previous work showed that exposure to certain insecticides caused significantly greater mortality to parasitoids developing in *E. servus* eggs than to developing *E. servus*. This work confirmed that certain insecticides move into both non-parasitized and parasitized *E. servus* eggs, so it is likely that the increased mortality of developing parasitoids is due to an increased susceptibility to insecticides. This presents a potential dilemma for growers in terms of pest management practices directed at stink bugs. Although *E. servus* parasitoids are capable of parasitizing up to 50% of egg masses in untreated fields, this number could greatly decrease if insecticide sprays coincide with when parasitoids are present in eggs. If insecticide exposure

approaches the levels simulated in our work, between 90-100% of the developing parasitoids could be killed. It is crucial to question whether the benefits of protecting developing stink bug egg parasitoids outweighs the benefits of making insecticide applications to control stink bug nymphs and adults. Further, insecticide treatments during the first generation of stink bugs, e.g., in wheat, should be carefully considered, as they will not kill second generation stink bugs developing in eggs, but will impact any developing parasitoids. These and many other questions deserve more detailed research to assess the value of conserving these important natural enemy populations. Until then, these results provide more incentive for growers to abide by existing stink bug economic thresholds and only make insecticide applications when necessary; we now know that untreated fields could be "releasing" significantly more egg parasitoid adults into the environment than treated fields.

References Cited

Beament, J. W. L. 1948. The penetration of insect egg-shells. I.—Penetration of the chorion of *Rhodnius prolixus*, Stål. Bull. Entomol. Res. 39: 359-383.

Beament, J. W. L. 1949. The penetration of insect egg-shells. II.—The properties and permeability of sub-chorial membranes during development of *Rhodnius prolixus*, Stål. Bull. Entomol. Res. 39: 467-488.

Beament, J. W. L. 1952. The role of cuticle and egg-shell membranes in the penetration of insecticides. Ann. Appl. Biol. 39: 142-143.

Herbert, D.A. 2010. Insects: Soybeans, Pp. 61-75. *In* S.E. Hagood and D.A. Herbert [eds.], Pest Management Field Guide Field Crops. Virginia Coop. Ext. Publ. No. 456-016.

Mehrotra, K. N. 1960. Development of the cholinergic system in insect eggs. J. Insect Physiol. 5: 129-142.

Moloo, S. K. 1970. Some aspects of water absorption by the developing egg of *Schistocerca gregaria*. J. Insect Physiol. 17: 1489-1495.

Mullins, D. E., K. J. Mullins, and K. R. Tignor. 2002. The structural basis for water exchange between the female cockroach (*Blattella germanica*) and her ootheca. J. Exp. Biol. 205: 2987-2996.

Novozhilov, K. V., K. V. Kamenkova, and I. M. Smirnova. 1973. The development of the parasite *Trissolcus grandis* Thoms. (Hymenoptera, Scelionidae) under conditions where organophosphorous insecticides are used against *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Entomol. Rev. 52: 11-17.

SAS Institute. 2002–2005. PROC user's manual, version 9.1.3. SAS Institute Inc., Cary, NC.

Smith, E. H., and E. H. Salkeld. 1966. The use and action of ovicides. Annu. Rev. Entomol. 11: 331-368.

Zschintzsch, J., R. D. O'Brien, and E. H. Smith. 1965. The relation between uptake and toxicity of organophosphates for eggs of the large milkweed bug. J. Econ. Entomol. 58: 614-621.

Table 5.1. ^{14}C-insecticides were successfully rinsed from the egg exterior when the DPM values decreased with increasing rinses, and when the DPM of Rinse 4 was lower than the DPM of the interior destructive sample. Two sample series from the acephate data, below.

Vial num	Sample	DPM
1	Stock	609196.63
2	Rinse 1	12732.54
3	Rinse 2	496.94
4	Rinse 3	49.87
5	Rinse 4	40.14
6	Destructive	683.73
7	Stock	769450.63
8	Rinse 1	25599.97
9	Rinse 2	1003.98
10	Rinse 3	65.10
11	Rinse 4	30.38
12	Destructive	123.65

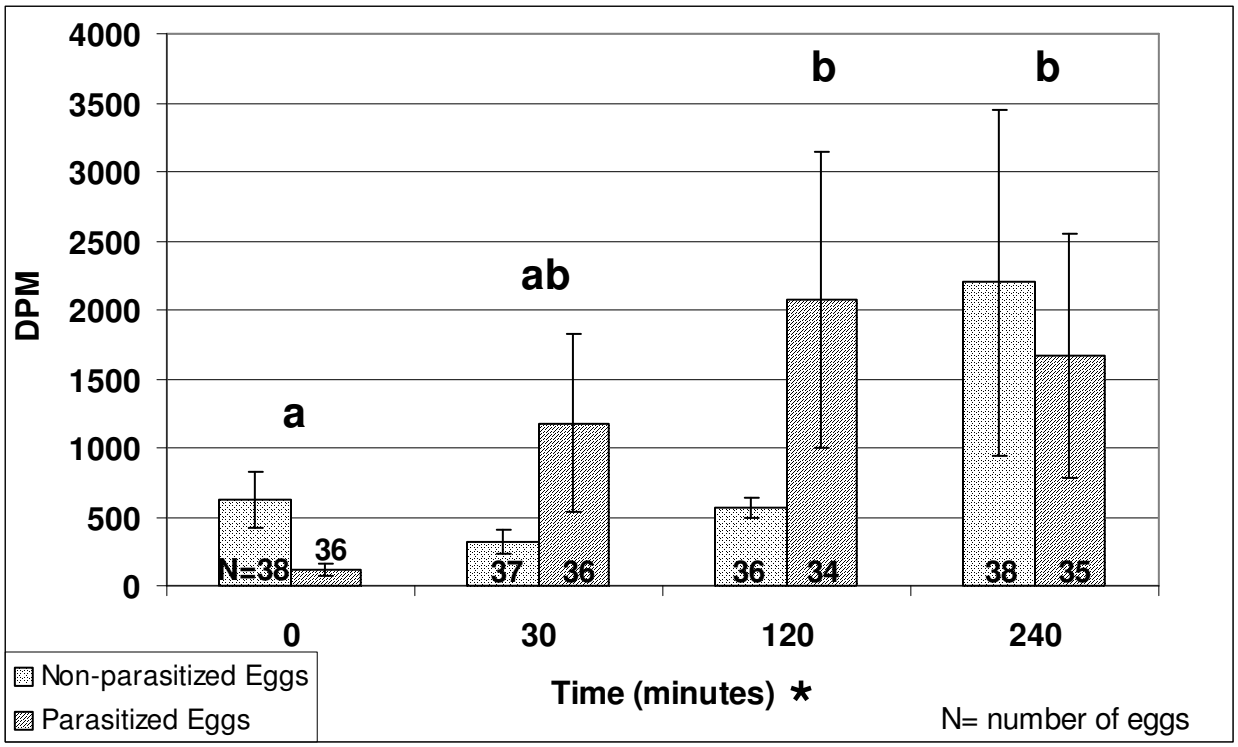

Fig. 5.1. Disintegrations per minute (DPM) of ^{14}C-acephate from the interior of non-parasitized and parasitized stink bug eggs exposed to ^{14}C-acephate for different periods of time.

***** There was significantly increasing absorption of insecticides with increasing exposure time (F = 3.73; df = 3; P = 0.0249). LSD of times in letters over bars. There were no significant differences between non-parasitized and parasitized eggs over all times, and no interaction effect.

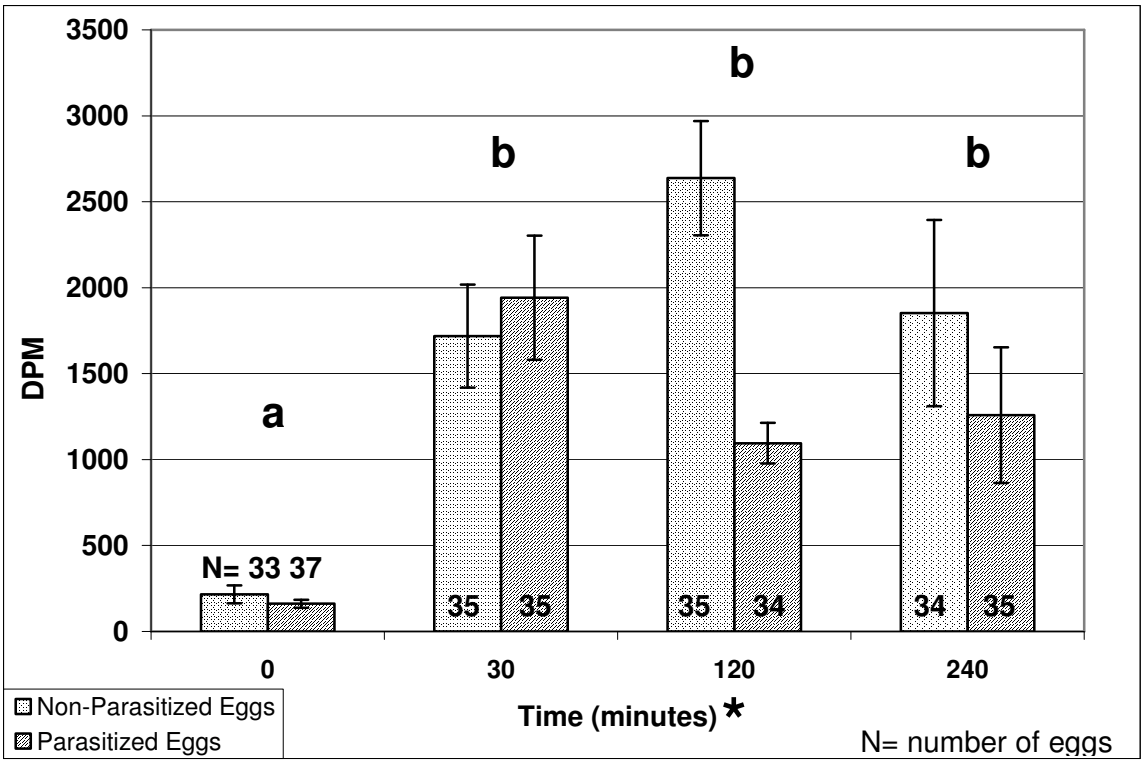

Fig. 5.2. Disintegrations per minute (DPM) of ^{14}C-λ-cyhalothrin from the interior of non-parasitized and parasitized stink bug eggs exposed to ^{14}C-λ-cyhalothrin for different periods of time.

***** There was significantly increasing absorption of insecticides with increasing exposure time (F = 50.94; df = 3; $P < 0.0001$), and overall, non-parasitized eggs absorbed more insecticide than parasitized eggs (F = 5.04; df = 1; P = 0.0343). LSD of times in letters over bars. There was no interaction effect.

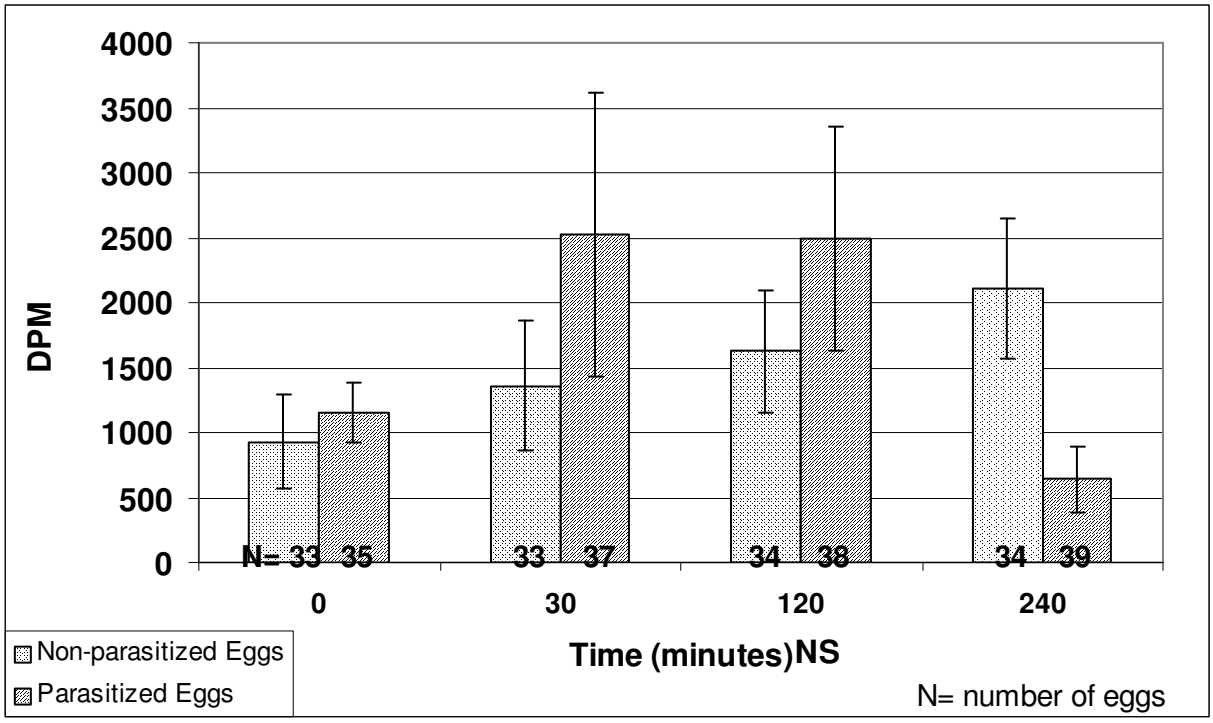

Fig. 5.3. Disintegrations per minute (DPM) of ^{14}C-thiamethoxam from the interior of non-parasitized and parasitized stink bug eggs exposed to ^{14}C-thiamethoxam for different periods of time.

NS There were no significant differences between eggs exposed to ^{14}C-thiamethoxam for different periods of time. There were no significant differences between non-parasitized and parasitized eggs. There was no interaction effect.

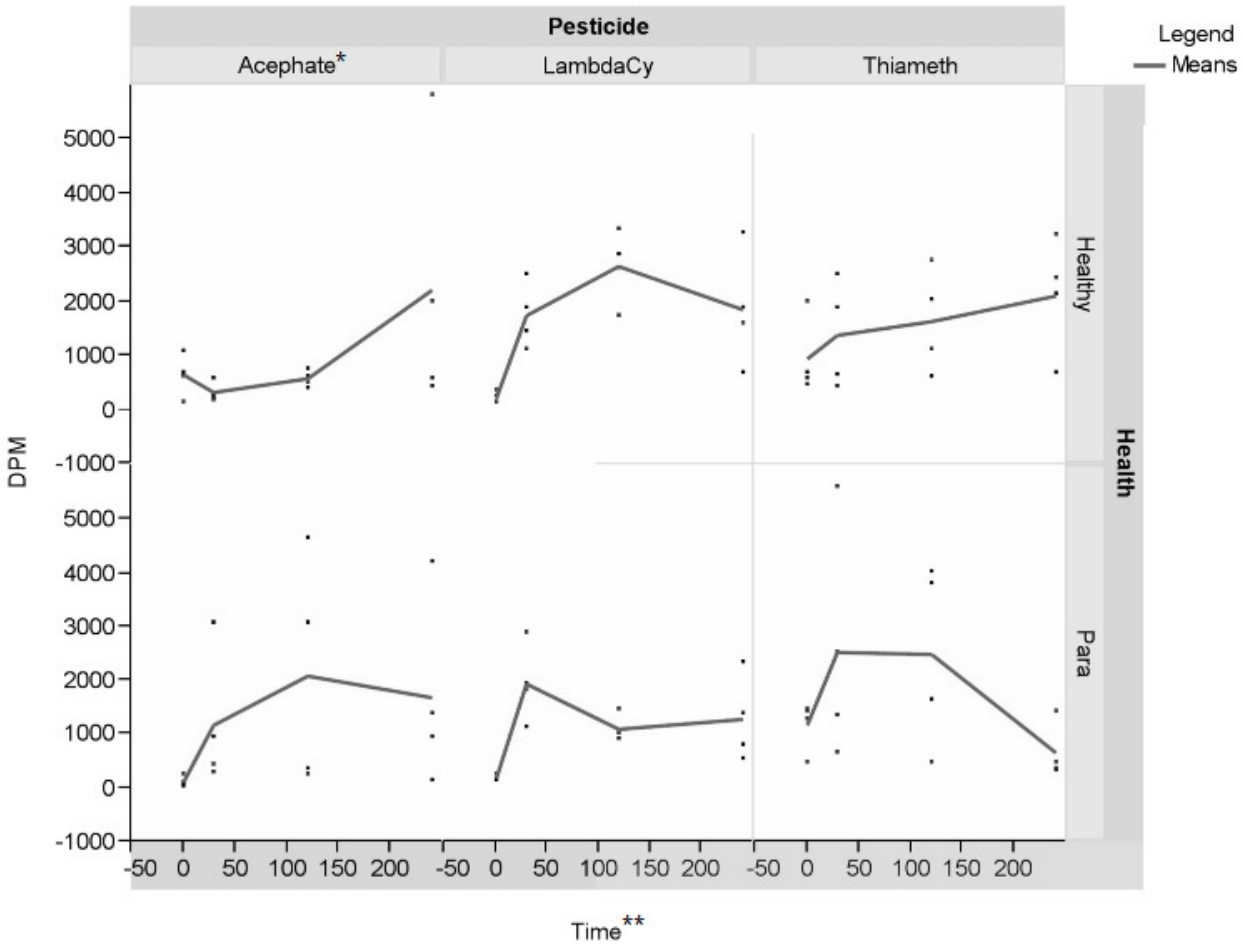

Fig. 5.4. Comparisons of disintegrations per minute (DPM) ^{14}C- amended insecticides between all treatment groups.

* ^{14}C-Acephate data are significantly different from other insecticide treatments (P = 0.0022 parameter).
** Time is significant (P = 0.0008 parameter) across all insecticide treatments.

Chapter Six

Summer temporal survey of brown marmorated stink bug [*Halyomorpha halys* (Stål), Hemiptera: Pentatomidae] host plants in Beijing, China, and parasitization

ABSTRACT The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a recently introduced insect pest from Asia that has become established in the United States. It is polyphagous and damages a number of important crops in China, Taiwan, Korea, and Japan, including many fruits, soybean [*Glycine max* (L.)], *Paulownia tomentosa* (a valuable timber tree in Asia), and other economically significant plants in its native range. Existing literature consists largely of reports where *H. halys* has been noted on crops, and there is limited information on its actual host plant range, biology, ecology, and behavior in Asia. The objective of this study was to observe *H. halys* throughout a summer field season in Beijing, China, in botanical gardens planted with trees on which the stink bug has been observed, and determine its use of different host plants in its native range. From late June to mid-August, a variety of plants, both known hosts and other nearby tree species, were surveyed with beat sheet and sweep net in Beijing and the life stage, number of *H. halys*, and host plant were recorded. Host use by different life stages of the stink bug is relevant, as it indicates development of generations through the summer field season, which may be used by researchers collecting *H. halys*. Parasitism of egg masses of *H. halys* by hymenopteran parasitoids was also recorded, and the parasitoid collected was identified as *Trissolcus halyomorphae* Yang (Scelionidae: Hymenoptera). They have been cultured in quarantine at the USDA-ARS Beneficial Insect Introductory Research Laboratory in Newark, DE.

Key Words Pentatomidae, brown marmorated stink bug, temporal host use, parasitoid

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is a recently introduced insect pest from Asia that has become established in the United States in Pennsylvania, New Jersey, Delaware, Maryland, Oregon, Tennessee, West Virginia, and Virginia. It is polyphagous and damages a number of economically important crops in China (Yuan 1984), Taiwan (Chu and Lu 1982), Korea (Chung et al. 1995), and Japan (Watanabe 1996). It feeds on many fruit species (Shiraki 1952, Chung et al. 1995), soybean (Son et al. 2000), several species of *Paulownia* (Yuan 1984), and many other hosts (Chu and Lu 1982) including economically significant plants in its native range (Chu and Lu 1982). For this reason, it has been projected that this insect will become a pest of economic significance in the United States. There are many crops at risk, including peaches, apricot, cherry, apple, citrus, berry crops, and crop commodities such as soybean. It has already been observed and documented on 73 host plants in the United States (Bernon 2004; Nielsen and Hamilton 2009), and there is preliminary evidence that it is causing economic damage in the U.S. (Nielsen et al. 2008).

Halyomorpha halys is an insect with piercing sucking mouthparts that causes damage by feeding on foliage and fruit. Although trees and ornamentals can sustain some foliar damage (Bernon 2004), fruit feeding results in necrotic areas on the outer surface (Chung et al. 1995, Choi et al. 2000) as well as 'cat-facing' (Bernon et al. 2004), which is very detrimental in markets that place a high value on the appearance of produce. In addition, *H. halys* is a vector for the phytoplasma which causes *Paulownia* witches'-broom. This pathogen causes reduced growth and vigor in trees followed by premature death. Further, the wood of infected trees is unfit for commercial use (Nakamura 1996).

From the end of September, and peaking in the third week of October, *H. halys* aggregates in large numbers on the outsides of buildings in Asia, especially Japan. It also

invades homes, schools, and other commercial establishments, producing a noxious odor. Since its introduction in 2001 (Hoebeke and Carter 2003), similar aggregation behavior has been observed in Pennsylvania and Virginia, and it has already become notable as a nuisance pest.

The first record of *H. halys* in Virginia was October 2004, when a specimen from Blacksburg was submitted by a student for her Insect Biology course. The next records were February 2005, from homes in Lynchburg and Roanoke. *H. halys* has been expanding its range since then, and was found in Fairfax County in February 2006. It is thought that the insect arrived in Virginia and expanded its range by traveling on vehicles, and is now assumed to live state-wide based on current records (E. Day, Virginia Tech Insect Identification Laboratory, personal communication 2009). Consequently, this insect will become a serious nuisance if it becomes more widely established in the United States (Hoebeke and Carter 2003, Bernon et al. 2004).

Although few control strategies have been documented in the United States, insecticidal treatments have been studied in Asia that were effective at preventing invasion of *H. halys* in reinforced concrete buildings, though not wooden ones (Watanabe et al. 1994). Also, insecticidal strategies for plant protection have been developed for use on persimmon (Chung et al. 1995), soybeans (Son et al. 2000) and fruit trees (Funayama 2002) in Asia, but, not yet in the United States although trials are underway (Nielsen et al. 2008).

Existing literature consists largely of reports where *H. halys* has been noted on crops, and there is very little information on its actual host plant range, biology, ecology, and behavior in Asia. Further, research on pest management of *H. halys* is a relatively new field of study, especially research concerning methods of control, including the use of natural enemies. The

focus of this research, in China, was twofold: to record temporal use of host plants by *H. halys*; and to begin a survey of its egg parasitoids.

Materials and Methods

This study was conducted from June through August in the summer of 2007. *H. halys* surveys were conducted in Beijing during these months, and during shorter visits to several other sites that included Nanjing (Jiangsu Province), Kunming (Yunnan Province), Xiuyan (Liaoning Province), and Xi'an (Shaanxi Province). In addition to inspecting a wide range of possible host plants in these locations, a host plant temporal study was also conducted in Beijing to document changes in host plant use during the summer months, and egg parasitoid surveys were conducted at the Beijing Botanical Institute, South Garden, where a wide range of host plants was available.

Host plant surveys. A variety of plants in parks and other public properties, including both known hosts and other plants not reported as hosts (either located in proximity to known hosts, or in plant families with known host species, or were in fruit during the survey), were surveyed on sunny days with light wind. A beat sheet was held under the selected plant, and while the branches of shrubs and shorter plants were shaken by hand to dislodge any insects, the handle of a sweep net was used to hit the branches of trees. If any *H. halys* were found, the number, life stage, and host plant were recorded. In addition, any *H. halys* which fell onto the beat sheet were collected, brought to the laboratory and placed into an insect colony.

Halyomorpha halys were separated into four different 20 cm (length) x 13 (width) x 8 cm (height) plastic containers, segregated by life stage. Each container contained a cotton wick soaked in distilled water, two snap beans, and a few sunflower seeds. Containers also contained two strips of paper towel as a substrate for stink bug egg masses, were covered with a fine mesh net, and were placed onto a lab bench next to a window. Every two days, food and water were

replaced, and the containers were checked for egg masses. If egg masses were found, they were placed into a 9 cm diameter Petri dish, and stored in a refrigerator (5°C).

Temporal survey. A variety of plant species in the Beijing Botanical Institute, South Garden, were surveyed for *H. halys* periodically over June, July, and August 2007. The same methods and criteria for selecting plant species to be surveyed were used as in the general host plant survey described above. The botanical garden was visited on a weekly schedule, whenever possible, but subject to delays due to inclement weather and transportation.

Parasitoid survey. To find *H. halys* egg parasitoids, egg masses obtained from the stink bug laboratory colony were brought into the field. They were pinned to the midvein of the undersides of leaves on a buckthorn (*Rhamnus*) bush, where parasitoids had been observed previously during surveys. After three days, the sentinel egg mass was retrieved and placed into a 9 cm Petri dish. This dish was sealed with Parafilm® and then placed into a resealable plastic bag, which was taped shut. Dishes were packaged with an ice pack and shipped by express mail to the USDA-ARS Beneficial Insects Introductory Research (BIIR) Laboratory in Newark, DE, where any emerging parasitoids would be used to begin a parasitoid colony in a quarantine facility for host range studies in the United States.

Results and Discussion

Survey outside of Beijing. In Nanjing, *H. halys* were found at only one location inspected, on *Cercis* (redbud) species in the Nanjing City Zoo. In Kunming, *H. halys* were found at only one location, on *Tecoma* species in Baohai Park. No *H. halys* were found during two days of surveys in Xiuyan, although other stink bug species were present. Xiuyan was the most northern site surveyed, and although literature reports *H. halys* from this part of China, it is possible that it may be uncommon near its northern range limit. In Xi'an, *H. halys* was found in

the Botanical Gardens on *Paulownia tomentosa* (Thunb.) and on a species of *Syringa* (lilac). This was the first documented report of *H. halys* in the Shaanxi Province. Specimens of all instars collected were shipped back to the UDSA-ARS-BIIR laboratory for future use in genetic studies.

Beijing surveys. Except for the temporal survey at the Botanical Garden, the only *H. halys* found in Beijing were collected on *Syringa* species in Zizhuyan Park, a commercial park in the northwestern part of the city. The lack of *H. halys* at other locations, such as the campuses of Beijing University and the Chinese Academy of Agricultural Science, may have been due to pesticides applied to ornamental trees in these parks.

The temporal survey (Fig. 6.1) showed that *H. halys* utilize different hosts throughout the summer. *Rhamnus* is an early and late season host, while *Catalpa* is a host later in the season. Although *Paulownia* and *Lonicera* are hosts throughout the summer field season, it was noted that as *Lonicera* lost its fruit, the number of stink bugs on the plant species decreased. The spike in population on *Paulownia* was due to an influx of late-instar nymphs, which likely came from some nearby *Lonicera*.

Parasitoid survey. Difficulties in obtaining egg mass production in the laboratory colony resulted in a low production of only three egg masses available for use as sentinels. Of the three, two masses turned dark after they were retrieved from the field, indicating parasitization. Egg parasitoids that emerged were identified by K.A. Hoelmer as *Trissolcus halyomorphae* Yang (Hymenoptera: Scelionidae), a newly described egg parasitoid of *H. halys* (Yang et al. 2009) and were placed into culture in quarantine at the USDA-ARS-BIIR Laboratory for further research.

References Cited

Bernon, G. 2004. Biology of *Halyomorpha halys*, The Brown Marmorated Stink Bug (BMSB), Final Report - USDA APHIS CPHST 2004 (one year project).

Bernon, G., E. R. Hoebeke, M. E. Carter, K. M. Bernhard, and J. F. Stimmel. 2004. Status of the Brown Marmorated Stink Bug, *Halyomorpha halys*, (Heteroptera: Pentatomidae), New Invasive Species, p. 10. *In* K. W. Gottschalk (ed.), Proceedings, U.S. Department of Agriculture Interagency Research Forum on Gypsy Moth and Other Invasive Species 2003; 2003 January 14-17; Annapolis, MD. Gen. Tech. Rep. NE-315. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station. 78 p.

Choi, D. S., K. C. Kim, and K. C. Lim. 2000. The status of spot damage and fruit piercing pests on yuzu. Korean J. Appl. Entomol. 39: 259-266.

Chu, Y. I., and C. Y. Lu. 1982. *Halyomorpha mista* Uhler (Pentatomidae). Nat. Taiwan Univ. Phytopathol. Entomol. 9: 166-173.

Chung, B. K., S. W. Kang, and J. H. Kwon. 1995. Damage, occurrence, and control of hemipterous insects in non-astringent persimmon orchards. RDA Journal of Agricultural Science, Crop Protection 37: 376-382.

Funayama, K. 2002. Residual effect of insecticides on *Halyomorpha halys* (Ståhl) (Heteroptera: Pentatomidae). 53rd Annual Report of the Society of Plant Protection of North Japan, 273-275.

Hoebeke, R. E., and M. E. Carter. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Wash. 105: 225-237.

Nakamura, H. 1996. Evaluation of primer pairs for the reliable diagnosis of paulownia witches'-broom disease using a polymerase chain reaction. Plant Dis. 80: 302-305.

Nielsen, A. L., P. Shearer, and G. C. Hamilton. 2008. Toxicity of insecticides to *Halyomorpha halys* (Hemiptera: Pentatomidae) using a glass-vial bioassay. J. Econ. Entomol. 101: 1439-1442.

Nielsen, A. L., and G. C. Hamilton. 2009. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in northeastern United States. Ann. Entomol. Soc. Am. 102: 608-616.

Shiraki, T. 1952. Catalogue of Injurious Insects in Japan. Economic and Scientific Section, Natural Resources Division. Preliminary Study No. 71. General Headquarters, Supreme Commander for the Allied Powers, Tokyo, Japan. February 1952.

Son, C., S. Park, Y. Hwang, and B. Choi. 2000. Field occurrence of stinkbug and its damage in soybean. Korean J. Crop Sci. 45: 405-410.

Watanabe, K. 1996. Characteristics of damage of *Lygocoris lucorum* (Meyer-Dur) (Heteroptera: Miridae) and *Halyomorpha halys* (Stal) (Heteroptera: Pentatomidae) on cherry. Annu. Rept. Soc. Plant Protection of North Japan 47: 143-144.

Watanabe, M., R. Arakawa, Y. Shinagawa, and T. Okazawa. 1994. Anti-invading methods against the brown marmorated stinkbug, *Halyomorpha mista*, in houses. Japanese J. Sanitary Zoology 45: 311-317.

Yang, Z., Y. Yao, L. Qiu, and Z. Li. 2009. A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha Halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Ann. Entomol. Soc. Am. 102: 39-47.

Yuan, T. L. 1984. Some studies on witches'-broom disease of *Paulownia* in China. Internat. J. Tropical Plant Diseases 2: 181-190.

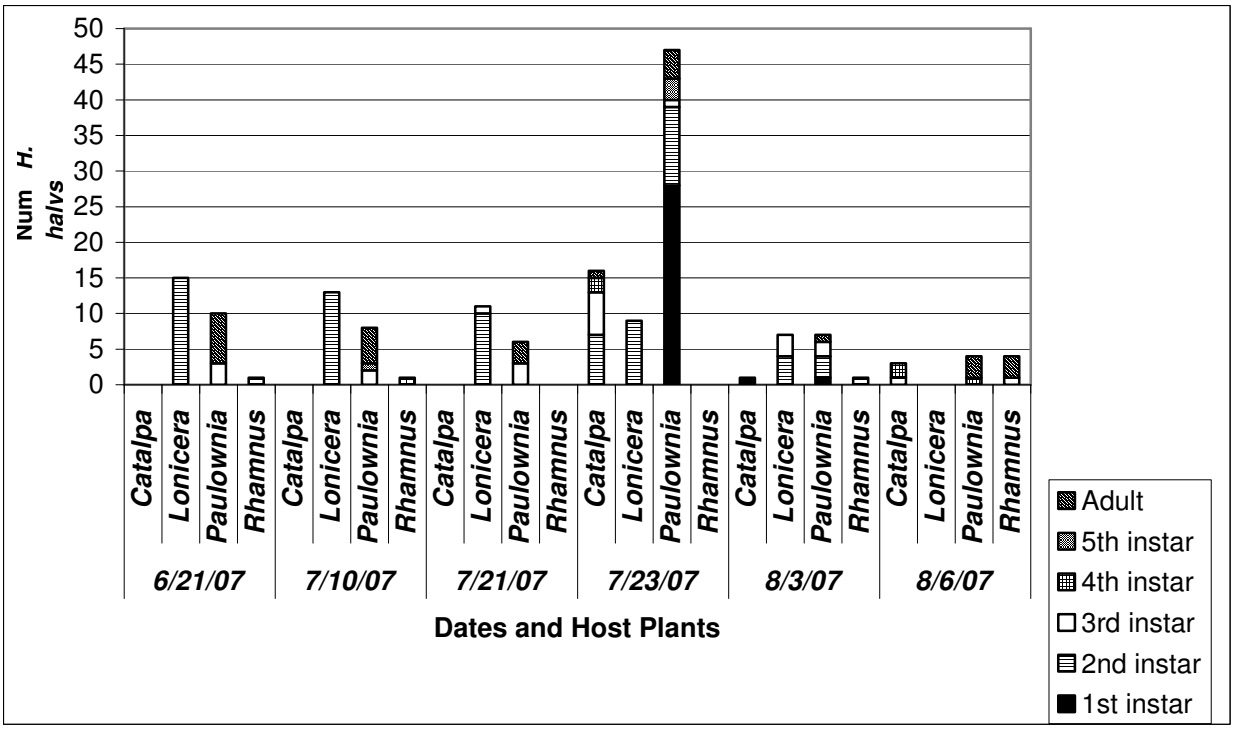

Fig. 6.1. Temporal host use by *Halyomorpha halys* nymph instars and adults in the Beijing Botanical Institute, South Garden, summer 2007.

Conclusions

Of the 15,070 individual pentatomid eggs and 1,098 egg masses examined in the natural enemy survey, 22.3 and 66.9% were parasitized, respectively. Four species of pentatomid eggs, *E. servus, A. hilare, M. histrionica,* and *P. maculiventris*, were represented, and of them, *E. servus* had the highest parasitization rates: 49.2% of individual eggs and 89.7% of egg masses. *T. podisi* parasitized *E. servus* and *P. maculiventris, T. edessae, T. basalis*. A mymarid parasitized *A. hilare* and *T. basalis* parasitized *M. histrionica*. There is a great deal of egg parasitism in southeastern Virginia, suggesting that biological control of stink bugs could be encouraged in local integrated pest management (IPM) practices.

In general, efficacy studies showed that common field rates of acephate, λ-cyhalothrin, spinosad, and thiamethoxam were more toxic to developing parasitoids and *A. hilare* than to developing *E. servus*. Further, *A. hilare* experienced more mortality in laboratory bioassays than in the field trials. The bioassay results suggest that developing *A. hilare* experienced greater mortality than developing *E. servus*, which is consistent with reports in the literature of *A. hilare* being more susceptible to insecticide exposure. However, the high mortality of developing parasitoids in this study contrasts with previous research. Since developing stink bugs in the next generation will hatch from eggs while egg parasitoids will not, it is important for farmers and growers in southeastern Virginia to use insecticides only as needed. Excessive applications of insecticide could decrease the population of *T. podisi* enough to reduce naturally occurring parasitization to unnoticeable levels.

Microscopy suggested that when non-parasitized *E. servus* and *A. hilare* eggs are punctured by the ovipositor of a parasitoid, there is no permanent hole in the chorion. Rather, a scab forms over the oviposition wound. When punctured with a probe, there is a similar

hardening of internal egg fluids on the surface of the egg. Since the scab forms as a result of oviposition and a probe wound, the scabbing process is most likely a result of egg physiology, rather than substances secreted by the parasitoid. Overall, it is suggested that while oviposition causes obvious physical differences between non-parasitized and parasitized stink bug eggs, there are no open wounds or holes which would allow for increased movement of insecticides into eggs. The scabs would not account for differences in insect mortality during insecticide efficacy testing.

When the chorion permeability of non-parasitized and parasitized *E. servus* was analyzed, each of the three insecticides utilized yielded different results. While acephate and λ-cyhalothrin both demonstrated increased insecticide movement into eggs with increasing time, only λ-cyhalothrin moved into non-parasitized and parasitized eggs at different quantities depending on egg status. No significant differences in insecticide movement was found for thiamethoxam. Due to the lack of literature concerning insecticide or ovicide absorption by eggs, it is difficult to assess the cause for such differences. A similarity between some of the assays was an apparent decrease in insecticide movement between 120 and 240 min. It is possible that there is a saturation curve, in which a certain point is reached where eggs can not absorb any more liquids, and this curve could change depending on the nature of the substance entering the egg. Further studies, expanding the times between 20 – 240 min. as an exposure period, could further define the properties of such a curve, should one exist.

Previous work showed that exposure to certain insecticides caused significantly greater mortality to parasitoids developing in *E. servus* eggs than to developing *E. servus*. This work confirmed that certain insecticides move into both non-parasitized and parasitized *E. servus* eggs, so it is likely that the increased mortality of developing parasitoids is due to an increased

susceptibility to insecticides. This presents a potential dilemma for growers in terms of pest management practices directed at stink bugs. Although *E. servus* parasitoids are capable of parasitizing up to 50% of egg masses in untreated fields, this number could greatly decrease if insecticide sprays coincide with parasitoid presence in eggs. If insecticide exposure approaches the levels simulated in our work, between 90-100% of the developing parasitoids could be killed. It is crucial weigh the benefits of protecting developing stink bug egg parasitoids relative to the benefits of insecticide applications to control stink bug nymphs and adults. Further, insecticide treatments during the first generation of stink bugs, e.g., in wheat, should be carefully considered, as they will not kill second generation stink bugs developing in eggs, but will impact any developing parasitoids. These and many other questions deserve consideration to assess the value of conserving these important natural enemy populations. Until then, my results provide additional incentive for growers to abide by existing stink bug economic thresholds and only make insecticide applications only when necessary; we now know that untreated fields could be "releasing" significantly more egg parasitoid adults into the environment than treated fields.

Information about host plant use and egg parasitoids was collected in China for an invasive species in the United States, *H. halys*. In Beijing, *H. halys* utilized different hosts throughout the summer. *Rhamnus* is an early and late season host, *Catalpa* is a host later in the season, and *Paulownia* and *Lonicera* are hosts throughout the summer field season. In Nanjing, *H. halys* was found only on *Cercis* species of trees, and in Kunming they were found only on *Tecoma* species. In Xi'an, *H. halys* was found on *Paulownia tomentosa* and *Syringa* species. This was the first documented report of *H. halys* in the Shaanxi Province. The host plant data will be useful in conducting future surveys for biological control agents of *H. halys* in Asia. Egg parasitoids were recovered from sentinel egg masses in Beijing, and identified as *Trissolcus*

halyomorphae Yang (Scelionidae: Hymenoptera), and were returned to the United States for evaluation as potential biological control agents by USDA-ARS.

Appendix A

Annotated List of Figures

Chapter Three

Chapter Four