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DAILY BEHAVIOR AND TRAP CROPPING OF THE BROWN MARMORATED STINK BUG (*Halyomorpha halys* Stål) IN PENNSYLVANIA AGRICULTURAL SYSTEMS

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ABSTRACT

Invasive species can cause billions of dollars in economic damage every year. The brown marmorated stink bug, *Halyomorpha halys*, is a relatively new invasive pest in the United States, and in recent years has become a serious agricultural issue. Fruits, vegetables, legumes, and ornamental plants are all part of a wide host range; in the mid-Atlantic United States, *H. halys* has already caused millions of dollars in documented damage.

Most current control strategies of *H. halys* involve the use of chemical applications to protect crops, and have disrupted Integrated Pest Management (IPM) systems. In order to protect IPM management strategies in Pennsylvania, I evaluated *H. halys* activity levels in the field and the laboratory. I also evaluated feeding preferences of *H. halys* between peppers and sunflowers, and hypothetical implications of *H. halys* invasions in other countries.

I began by looking at diurnal and nocturnal behavior of *H. halys* adults and nymphs in fruit orchard arenas placed on apple, peach, and nectarine tree limbs. From this research, I found a general pattern of increased activity during the evening hours. To better explore this pattern and the driving factor behind it, I placed *H. halys* in laboratory growth chambers and monitored them during 24-hour cycles. Diel patterns emerged in keeping with the field experiment (increased activity later in the evening), which persisted in completely dark conditions, indicating that the activity patterns were linked to a circadian rhythm.

In order to determine if there is a plant preference between pepper and sunflower crops, I planted pepper fields surrounded by sunflower crops, thereby creating a trap cropping setup. The attractiveness of the sunflowers was sufficiently greater to make it a potential trap crop, although more studies need to be completed to provide recommendations for a season-long protection of vegetables.

In addition to these field and laboratory components, a hypothetical invasive species awareness program was created for Belgrade, Serbia. This was based upon surveys and a focus group questionnaire that was presented to several Serbian scientists, farmers, and agricultural consultants about the importance of invasive insect awareness in their country.

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Chapter 1

Introduction/Literature Review

A new invasive pest species

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive hemipteran pest wreaking havoc across the mid-Atlantic United States (Nielsen and Hamilton 2009a). Native to Asia, including China, Japan, Korea, and Taiwan, *H. halys* has been reported in Switzerland, Germany, France, Liechtenstein, Italy, Canada and the United States (Lee et al. 2013a; Maistrello et al. 2014). *H. halys* was most likely introduced through international commerce in bulk freight containers. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) agents had intercepted *H. halys* at ports of entry prior to the first sightings of this pest by homeowners in Allentown Pennsylvania in 1996, with positive identification in 2001 (Anonymous 2010; Hoebeke and Carter 2003).

The polyphagous *H. halys* feeds on a variety of fruit, vegetable, legume, and ornamental plant species (Maryland Cooperative Extension 2010). This insect is considered a nuisance pest for homeowners because aggregations may overwinter in homes and businesses (Hoebeke and Carter 2003). *H. halys* is a novel invasive species in North America, and better understanding of this species' biology and behavior must be established in order to develop efficient *H. halys* management practices for farmers and homeowners.

Possible economic impact of invasive species

Invasive species such as *H. halys* can threaten ecosystem stability worldwide when they hitchhike across the globe (Simberloff 2000; Pimentel et al. 2001). Unintentional introductions of invasive species are often by-products of international travel and trade, and can be economically expensive (Aukema et al. 2010; Leung et al. 2002). Of over 50,000 invasive species in the United States, approximately 4500 are arthropod species, many of which have the potential to cause direct economic harm (Pimentel et al. 2005). Overall, invasive species are responsible for up to $120 billion in economic damages and associated control costs per year in the United States (Pimentel et al. 2005). Considered "a nuisance for homeowners, and a nightmare for growers," in 2010 *H. halys* caused over $37 million in damage to mid-Atlantic apple growers alone (Sparks 2010; U.S. Apple Association 2011). Thus, *H. halys* can be a major player in destabilizing the agricultural economy, particularly since *H. halys* can have such devastating effects on a variety of crops, e.g. fruit, vegetables, field crops, ornamental plants, and native vegetation (Leskey et al. 2012a).

H. halys life history

Nomenclature

Positive identification of *H. halys* has been problematic. *H. mista*, *H. brevis*, and *H. picus* all have been misidentified as *H. halys*, creating confusion and misunderstanding in the literature. In Japan, it is still sometimes referred to as *H. mista*, while in China it may be misidentified as *H. picus* (Rider et al. 2002; Lee et al. 2013a; Yang et al. 2009). However, researchers now agree that *H. halys* is the species found in Korea, Japan, and China (Zhu et al.

2012; Lee et al. 2013a). Throughout this thesis, references to *H. halys* will retain the species name reported in the literature followed by the correct species name, e.g., *H. picus* (*H. halys*).

Distribution

The native distribution of this genus is Africa (16 species) and Asia (21 species) with eight unique species being found on the Indian subcontinent alone (Hoebeke and Carter 2003). *H. halys* is found in eastern China, Japan and Korea (Hamilton 2009; Nielsen and Hamilton 2009a). The current distribution of *H. halys* has expanded to include Europe (France, Germany, Liechtenstein, Italy and Switzerland) and North America (Canada and the United States) (Lee et al. 2013a; Maistrello et al. 2014; Wermelinger et al. 2008). North American *H. halys*, particularly those located in the United States, originated from the Beijing area of China, and are believed to have established from a single successful introduction (Xu et al. 2014).

The first recorded outbreaks of fruit-infesting bugs (e.g., hemipteran pests) in Japan were observed in 1973 and 1975. Following this outbreak, outbreaks of fruit feeding bugs, including *H. halys*, subsequently occurred in Japan at intervals of 3-6 years (Ohira 2003). *H. halys* is also a serious pest to soybeans (Toyama et al. 2006), and attacks cherry, peach, pear, and apple throughout the Japanese growing season. *H. halys* is also commonly found in wild wooded areas (Fujisawa 2003; Li et al. 2007). In Japan, *H. halys* is considered an urban pest throughout its native habitat (Watanabe et al. 1994a; Watanabe et al. 1994b; Hoebeke and Carter 2003; Lee et al. 2013).

H. halys is known to attack apple, apricot, cherry, citrus, grape, kiwi, peach, pear, persimmon, pomegranate and strawberries in China (Zhang et al. 2007). Additionally China and Korea experience damage to soybeans from *H. halys* (Toyama et al 2006). Alarmingly, *H. halys*

has been reported to be a possible plant disease vector; witches' broom disease in *Paulownia* trees is caused by a phytoplasma transmitted by *H. halys* (Jones and Lambdin 2009).

H. halys has been reported on the following crops in its introduced United States habitat: apples, grapes, pepper, tomato, eggplant, okra, corn, etc. (Leskey et al. 2012a).

Feeding preferences

One of the main areas in hemipteran research concentrates on understanding feeding mechanisms and food preferences (Rodgrigues et al. 2008). With little being known about *H. halys* biology and behavior in its new North American environment, researchers look at other, similar hemipteran, pests to gain a better understanding of *H. halys*.

Phytophagous stink bugs feed on a wide variety of plants and are important agricultural pests in a variety of crops. Rice, legumes, nuts, and fruits are among crops that face the threat of significant injury from stink bugs (Bowling 1979; Yates et al. 1991; Brown 2003). The stylet mouthpart penetrates the plants protective structures through a combination of mechanical pressure and saliva-containing enzymes that dissolve components of the cell wall and intercellular matrix (Brown 2003). When the cell wall is breached, plants can suffer decreased turgor pressure from the loss of plant fluids (Mundinger and Chapman 1932). For some species, the salivary injection can also transmit plant pathogens and delay plant maturation (McPherson and McPherson 2000).

In Japan, *H. halys* became a well-known pest post World War II because Japanese cedar, *Cryptomeria japonica*, and false cypress, *Chamaecyparis obtuse*, were the primary plants used for reforestation (Funayama 2005; Kiritani 2007). Stink bug population outbreaks began to be documented in 3-6 year intervals, and it was hypothesized that these outbreaks were dependent on cedar cone abundance (Ohira 2003; Fukuoka et al. 2002) although there is some disagreement

with this conclusion (Funayama 2005). While mature (dehiscent) *C. japonica* cones are suitable for development, the indehiscent (immature) cones are not. Dehiscent cones are not available until late fall (October), after most *H. halys* have laid their eggs and after most of the population has developed into adults. Hence, the life histories of *C. japonica* and *H. halys* do not significantly overlap, suggesting that cones are not a primary host. Additional evidence supporting this conclusion comes from Funayama (2005); salivary sheaths have been observed on the surface of cones but no attack marks were found on the cones themselves. While cyclical outbreaks of 3-6 year intervals seem to occur, the evidence supporting a relationship between *C. japonica* and *H. halys* seems to be lacking.

A variety of fruit trees, berries, and other plants have been explored as possible food sources for *H. halys*. As with Japanese cedar, fruit trees may not necessarily be the optimal host plant. While *H. halys* can complete its development feeding only on apples, *H. halys* females raised solely on apple deposited fewer eggs than females raised on a varied diet (Funayama 2002a; Zhang et al. 2007).

H. halys adults collected in coppiced woodland (grove of trees trimmed back to stumps to encourage young growth from the stools) in Akita prefecture (Japan) had a higher nutritional status (Nutritional level = live weight (mg)/ pronotum width (mm^3)) than adults raised solely on apples (Funayama 2004). However, with orchards often surrounded by other crops or woodlands, it is feasible that adult stink bugs feed on a variety of host plants (Funayama 2004).

H. halys is a polyphagous insect as indicated by the variety of plant hosts upon which it is able to feed and the higher nutritional status of individuals with a varied (non-specialist) diet. This polyphagous diet requires *H. halys* to find appropriate plants in a diverse environment. It has been hypothesized that aggregation pheromones are used by *H. halys* to identify food sources. The evidence supporting this conclusion comes from our ability to lure *H. halys* to collection traps using *Plautia stali* (brown-winged green stink bug) aggregation pheromone and the adult *H.*

halys collected in these traps have lower weights than adults collected directly from a food plant (Funayama 2008).

Feeding characteristics

The movement of hemipterans between host plants may be affected by crop variety, food availability, and crop maturity, as well as the time of year (Rodrigues et al. 2008). Feeding on fruit by various stink bugs can lead to discolored necrotic tissue, dimpling, and fruit surface discoloration-all of which can be mistaken for physiological disorders (Brown 2003). In some situations, stink bug damaged fruit can abscise (Watanabe 1996). In North America, feeding on apples and peaches can start at fruit set and continue throughout the season with damage to both young and ripening fruit (Leskey et al. 2012b). Feeding by *H. halys* has been observed on the entire surface of peach and apple fruits (Nielsen and Hamilton 2009a). In field situations, the fruit on trees located in the exterior regions of orchards seem to have greater damage than fruit in the interior part of the orchards, meaning there is a greater potential for damage along the perimeter of orchards (Leskey et al. 2012b). In certain locations in the United States, *H. halys* has become the most prevalent stink bug, making damage to orchards a definite concern for growers (Nielsen and Hamilton 2009b).

Both *H. halys* adults and nymphs are found in orchards throughout the growing season but usually fewer nymphs are found inside commercial apple orchards. This is despite the ability of *H. halys* eggs to hatch within orchards and the nymphs to actively move into orchards from surrounding areas. It is possible nymphs are more susceptible to commonly applied pesticides (Funayama 2002a).

Within apple orchards, different feeding pressure can be seen on different cultivars resulting in different levels of damage (Funayama 2002b). Damage to fruit caused by *H. halys* is

also influenced by the stage of fruit development. Apples damaged while young will show greater deformity than apples damaged at maturity (Funayama 2002b).

Overall, the feeding damage caused by different stink bug species is relatively similar. An apple damaged by stink bugs shows a depressed, discolored area on the skin. Beneath the skin, dark, corky flesh extends 0.5-1.0 cm into the fruit and looks similar to a calcium deficiency known as cork spot. Stink bug damage is expressed as uniform corking in the affect areas, while cork spot from calcium deficiency is distributed irregularly (Brown 2003). *H. mista* (*H. halys*) can damage both young and mature fruit. The young apple fruit becomes deformed while mature fruit has a white to brown spongy texture with a slight hollow. Feeding is normally concentrated on the upper periphery of fruit with salivary sheaths remaining on the fruit surface from *H. mista* (*H. halys*) sucking marks (Funayama 1996). Feeding damage to apple by *Euschistus conspersus* Uhler, another stink bug species, in Washington State was evident within one day of feeding, indicating the elapse time from initial injury to observation of that injury is short (Krupke and Brunner 2001; Brown 2003).

In cherries, brown discoloration, caving, and shriveling of the fruit can all be indicators of early feeding by *H. halys* (Watanabe 1996). Peach damage by stink bugs can be expressed as cat-facing, with depressed area on fruit surface, water-soaked lesions, or gummosis (Rings 1958; Brown 2003). During the 2010 season, some stone fruits growers in the mid-Atlantic United States lost over 90% of their crop due to fruit injuries caused by *H. halys* (Leskey et al. 2012a).

H. halys damages more than fruit; it is also a pest of vegetable, field, and ornamental crops (Li et al. 2007; Leskey et al. 2012a). Feeding by *H. halys* in cucumbers results in malformed fruit (Fukuoka et al. 2002). Damage to pepper, tomato, eggplant and okra fruits by *H. halys* can average more than 20% of crop (Leskey et al. 2012a). *H. halys* and other stink bugs have also been reported to be pests in field crops such as soybeans (Owens et al. 2013).

As an insect that inserts its stylet into plant tissue, stink bug species can potentially vector plant diseases. *H. halys* is known to vector witches' broom disease, which is a phytoplasma disease in *Paulownia* trees (Hiruki 1999). It is suspected that *H. halys* may also be a vector of disease in soybeans as other stink bug species, including *Thyanta custator* (F.), the green stink bug; *Acrosternum hilare* (Say), the brown stink bug; *Euschistus serous* (Say), the dusky stink bug; and *E. tristigmus* (Say), the one-spot stink bug, have been shown in caged studies to effectively transmit yeast-spot disease (*Nematospora coryli* Peglion) in soybeans (Daugherty 1967).

***H. halys* biology**

H. halys is sexually dimorphic, with females often larger and heavier than their male counterparts (Nielsen et al. 2008a). *H. halys* adults have a mottled brown dorsal surface with light bands on the legs and antennae, and alternating light and dark bands on the lateral abdominal margins. Nymphs are orange and black, or red and black, with a tick-like appearance and cluster around the egg mass during the first instar (Hoebeke and Carter 2003; Hamilton 2009; Medal et al. 2013). *H. halys* has five nymphal instars, with the second to fifth instars and adults being able to cause damage to crops (Medal et al. 2012).

For *H. halys*, antennae are the major organs to sense food, while vision is effective only within a 10 cm distance from their potential meal (Li et al. 2007). When antennectomized, *H. halys* did not form aggregations. This indicates olfactory or sensory cues may help guide the formation of aggregations, especially since aggregations are formed even in darkness. It has also been suggested that in addition to the olfactory cues, the mutual preference for the overwintering environment may increase aggregations (Toyama et al. 2006). *H. halys* body size, development rate, lipid accumulation and pigmentation at the nymphal stage are under photoperiodic control.

Additionally, when treated with melatonin, a hormone involved in melanization, adult male gonadal development is hampered. Although the actual mechanism of action is unknown, the use of melatonin has been suggested as a method to reduce sexual development in males (Niva and Takeda 2003).

Day length is a significant factor in *H. halys* ovarian development, and at 14:10 (L:D) ovarian development is possible, but at 13.5 (L), and shorter day lengths, ovarian development is arrested and the female may enter diapause (Watanabe 1979). To advance from egg to imaginal ecdysis, the process requires 537.63 DD, using a base temperature of 25°C. In one study, the hatch rate of *H. halys* egg masses was about 81.6%, with a single egg mass having an average of 28 eggs (Nielsen et al. 2008b). *H. haly*s ovarian development process has 9 stages (Katayama et al. 1993). *H. halys* field populations in New Jersey and Pennsylvania peak from July to August and the total number of females with mature ovaries peak in late August. Around mid-September there are no mature eggs in the ovaries as adults enter diapause (Nielsen et al. 2008b). Females entering overwintering habitats in the fall are reported to be in reproductive diapause with underdeveloped ovaries (Nielsen and Hamilton 2009b).

During a lifespan, a single *H. halys* female can produce an average of 243.78 ±27.48 eggs (Nielsen et al. 2008b). *H. halys* has repeated oviposition and has been shown to mate up to 5 times per day (Yanagi and Hagihara 1980; Kawada and Kitamura 1983a).

Natural behavior of *H. halys* influences its pest status but may also provide opportunities for its control. *H. halys* is arboreal, and will often land at, or above, mid-tree canopy levels (Lee et al. 2013c). In addition to this "arboreal" tendency, *H. mista* (*H. halys*) is known to have a "basking" behavior where it remains still on the leaves in the sunlight (Kawada and Kitamura 1983b). Dropping behavior, where *H. halys* drops to the ground from a resting position, has been observed when *H. halys* is disturbed by light or sound (Li et al. 2007). Weather and temperature can also affect the behavior of *H. halys* in a variety of ways. An increase in activity can occur

when temperatures change, and *H. halys* is most active when temperatures exceed 25°C (Li et al. 2007). However, under laboratory conditions, when temperatures exceed 33°C, *H. halys* mortality increases significantly and can reach up to 95% (Nielsen et al. 2008b). Fuller understanding of *H. halys* field behavior may provide a potential avenue of future control options.

Through the spring, summer, and fall months, *H. halys* can be easily observed in woodlands and cause damage to a variety of fruit, vegetable, field, and ornamental crops (Wermelinger et al. 2008; Nielsen et al. 2008a). In Japan, stink bug adults begin to arrive in coppices of Japanese bird cherry, *Prunus grayana*, around mid-May and populations reach their peak around mid-June. From early June to late July oviposition on a plethora of species has been observed, with females collected during this time having fully developed ovaries. Nymphs were also observed in Japanese bird cherry from mid-June to mid-September (Funayama 2007).

During the fall, this insect begins to form adult aggregations and move into overwintering shelters including human structures, e.g., houses, sheds, piles of firewood, and natural locations, e.g., under tree bark and in crevices. At this time, the insects are in reproductive diapause (Kobayashi and Kimura 1969; Niva and Takeda 2003; Toyama et al. 2006; Toyama et al. 2010). It has been demonstrated that *H. halys* responds to the aggregation pheromone of *P. stali* (Funayama 2008). It is not known if *H. halys* has an aggregation similar to *P. stali* or if they are responding to the pheromone to use *P. stali* resources.

As *H. halys* leaves crops to enter overwintering habitats, these overwintering aggregations become a serious nuisance for homeowners (Watanabe et al. 1994b; Toyama et al. 2006). *H. halys* has been known to overwinter indoors on clothing and bedding (Lee et al. 2013a, Kobayashi and Kimura 1969). While *H. halys* is not known to spread diseases to humans, if odor gland fluid enters human eyes, conjunctivitis and visual impairments may occur and skin contact may cause dermatitis. Though dose was not defined, people exposed to *H. halys* odor have been reported to experience headaches, vomiting, and loss of appetite (Saito et al. 1964). While *H.*

halys in its winter aggregations has been reported to cause various health concerns, the use of this aggregation behavior may present an opportunity to reduce its population (Toyama et al. 2006; Watanabe et al. 1994b).

Integrated pest management (IPM)

During the 2010 season, the first year with a heavy *H. halys* infestation in the Mid-Atlantic region, some growers lost over 90% of their stone fruit crop. This same year, over $37 million in damage was reported to apple crop grown in the mid-Atlantic region of United States (Leskey et al. 2012a; Leskey et al. 2012b). With such devastating economic losses caused by *H. halys* and broad-spectrum insecticides available as the only effective stink bug management tool, it has been extremely difficult to maintain IPM programs in Pennsylvania fruit orchards. The use of selective insecticides with low toxicity to beneficial orchard organisms, vigilant pest monitoring, and precise timing of necessary pesticide applications are the staple of established IPM programs. Since insecticides commonly used against *H. halys* are highly toxic to many beneficial arthropods, the preservation of currently existing balanced orchard ecosystems becomes a very daunting task. Effective monitoring tools that help detect the presence, absence, abundance and seasonal activity of *H. halys* can reduce the negative impact insecticide applications have on the overall IPM program in PA fruit orchards (Leskey et al. 2012b; Leskey et al. 2012c). With the increase in *H. halys* populations, it has become essential to retain IPM programs while properly protecting crops from stink bug feeding damage. The development of effective monitoring methods for *H. halys* and rotation of necessary pesticide chemistries can help control *H. halys* while maintaining natural predators in the field (Hull and Krawczyk 2011).

Current management

The brown marmorated stink bug, *H. halys,* is a pest of fruit, vegetable, ornamental and field crops. China, Korea, and Japan considered *H. halys* a significant soybean pest (Nielsen et al. 2008a; Lee et al. 2013a; Leskey et al. 2012a). While other stink bug species have a variety of controls including pheromones and natural enemies, dispersible insecticides are currently the only effective control for *H. halys* (Toyama et al. 2006; Fujisawa 2003). Chemical control, poisoned bait, and bagging of fruit have all been used to protect against *H. halys* damage in fruit (Zhang et al. 2007). However, the most effective available control involves repeated pesticide applications (Fujisawa 2003; Funayama 2003; Toyama et al. 2006). To minimize damage from *H. halys* to crops under heavy infestations observed in the United States mid-Atlantic region, the number of insecticide applications was significantly increased (Leskey et al. 2012a; Leskey et al. 2012b; Leskey et al. 2012c; Lee et al. 2013b).

Experience gained in the management of other native stink bugs can be helpful to investigate effective chemical control of *H. halys*. When trying to prevent *H. mista* (*H. halys*) from entering houses in Japan, insecticides sprayed on window frames have been reported to prevent stink bug infestation by up to 96.2% (Watanabe et al. 1994a).

In the United States, insecticides, including pyrethroids, organophosphates, and neonicotinoids, are the most effective tools available to control native stink bugs (Nielsen et al. 2008a). Pyrethroids have better residual effects than organophosphates when controlling *H. halys,* but are disruptive to established IPM programs in orchards and can negatively impact natural enemies (Funayama 2011; Funayama 2002c).

Exposure to insecticides can change the mobility of adult *H. halys,* with pyrethroids in particular causing fast neurological impairment, with uncoordinated and irregular movement in as little as ten minutes. Insecticide exposure can also affect behaviors such as reproduction, host-

finding, feeding, and dispersal/mobility activity. These altered behaviors may consequently affect insecticide efficacy (Lee et al. 2013b). The strong flight capacity of *H. halys* makes avoidance of insecticide sprays in orchards an additional common problem for farmers (Cai et al. 2008; Anonymous 2010).

Monitoring

Presently, the most effective detection and monitoring of *H. halys* can be done with light traps, black light traps, mercury lamps, sweep-net sampling, beat sampling, pheromone traps, visual observation, and radar tagging (Nielsen and Hamilton 2009a; Khrimian 2005; Yanagi and Hagihara 1980; Lee et al. 2013c). *H. halys* can be lured to light traps in peak summer if temperatures are sufficient for flight, while during spring and autumn pheromone traps can be the most effective (Saito et al. 1964; Funayama 2003; Fujisawa 2003).

H. mista (*H. halys*) outbreaks in Japan can be predicted using light traps and other trapping methods (Moriya et al. 1987). Dry traps without any liquid components have been used for *H. mista* (*H. halys*), and are generally easier to use than a wet trap for mass trapping purposes. Wet traps use water to capture many different insects species, and require frequent care, making the traps difficult to use, and making dry traps a more feasible alternative (Katase et al. 2005). Some current dry stink bug traps include kairomone traps mounted on a pyramidal base (Adachi et al. 2007; Anonymous 2010; Nielsen and Hamilton 2009a; Tillman et al. 2010). Chemical attractants such as methyl 2,4,6-decatrienoate isomers can be used to help monitor *H. halys* populations late in the season (Khrimian et al. 2008; Leskey et al. 2012b).

Funayama (2003) used apple box traps packed with straw to catch overwintering *H. halys* populations entering diapause in the fall. After overwintering, there are a variety means to capture and monitor stink bugs as *H. halys* emerge from overwintering shelters in early spring

(Hoebeke and Carter 2003). In Japan, *H. mista (H. halys)* can be captured with slit traps upon leaving their overwintering habitats (Watanabe et al. 1994a).

Biological control

While the number of documented natural enemies of *H. halys* in North America is small, there are some well-known predators and parasitoids for other pentatomid bugs. Generalist predators in the United States that prey on *H. halys* include spiders, ants, and lacewings and parasitoids such as Scelionidae (*Telenomus* sp.) and Eupelmidae (*Anastatus* sp.) (Wermelinger et al. 2008). Another parasitoid, Tachinidae are often found in conjunction with pollinator plantings, and have been known to parasitize adult stink bugs (Pease and Zalom 2010). Tachinids were also observed to be attracted to *H. halys* pheromones (Aldrich et al. 2009).

Biocontrol agents of *H. halys* found in its native habitat include parasitism by *Anastatus* sp. Both 1st and 2nd generation eggs were 64.7% and 52.6% parasitized, indicating its possible use as a biocontrol agent in the future (Hou et al. 2009). One way to check the potential of this, and other parasitoids, is to utilize sentinel egg masses to check parasitoid complexes (Tillman 2010). In addition to parasitoids, there is also promise for biological control agents such as entomopathogenic fungi, although more research is needed (Gouli et al. 2012).

Thesis objectives

The goal of this thesis is to better understand *H. halys* activity patterns and feeding preferences. Research presented in the following chapters will address diurnal and nocturnal behavior in orchards and the laboratory, feeding preferences between peppers and sunflowers, and the international implications of *H. halys* invasions in other countries.

In chapters 2 and 3, using field and laboratory observations, I characterize *H. halys* activity and the expression of feeding, moving, and resting behaviors as they relate to a 24-hour cycle. Peaches, nectarines, and two apple varieties were used to see if activity differed by crop. In addition to adult *H. halys*, I also looked at *H. halys* 2nd instar nymphal behavior patterns. Chapter 3 explores the quiescent (resting) and non-quiescent (feeding or moving) behaviors during a diel period, as well as how this relates to the expression of circadian rhythm behaviors by *H. halys.*

Chapter 4 looks at sunflowers as a potential trap crop of *H. halys* in pepper fields, particularly as it relates to crop preferences. I also look at differences in damage expression in peppers surrounded by other peppers or surrounded by sunflowers. A variation in the seasonal occurrence of *H. halys* in these two crops was also observed.

In chapter 5, I have created a hypothetical Invasive Species Awareness (ISA) program in Belgrade, Serbia based on protecting current fruit production. This program uses *H. halys* as a species of interest and a potential insect for Serbian agriculture to protect against.

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Chapter 2

Diurnal and nocturnal behavior of *H. halys* in fruit orchard arenas

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive hemipteran pest wreaking havoc across the mid-Atlantic United States (Nielsen and Hamilton 2009a). Native to Asia, including China, Japan, Korea, and Taiwan, *H. halys* has been reported in Switzerland, Germany, France, Liechtenstein, Italy, Canada and the United States and was most likely introduced through international commerce in bulk freight containers (Lee et al. 2013; Maistrello et al. 2014). The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) had intercepted *H. halys* at ports of entry prior to homeowners who reported the first sightings of this pest in Allentown Pennsylvania in 1996, with positive identification in 2001 (Anonymous 2010; Hoebeke and Carter 2003).

The polyphagous *H. halys* feeds on a variety of fruit, vegetable, legume, and ornamental plant species (Maryland Cooperative Extension 2010). The wide host range makes *H. halys* a threat to many farmers, including apple and peach growers which have experienced high levels of damage in the mid-Atlantic (Nielsen and Hamilton 2009a). Insecticide treatments are often used to manage *H. halys*. In order to minimize pesticide inputs, behavioral research in locomotion, feeding, dispersion, mating, and evasive behaviors in the field may be useful in developing future IPM control tactics (Toyama et al 2011; Lee et al. 2013; Nielsen 2008).

H. halys is also considered a nuisance for homeowners because aggregations may overwinter in homes and businesses (Hoebeke and Carter 2003). *H. halys* is a novel invasive species in North America, and a better understanding of this species' biology and behavior must

be established in order to develop more efficient *H. halys* management practices for farmers and researchers.

The objectives of this study were to determine if (1) *H. halys* has predictable diurnal/nocturnal feeding, moving, and resting behaviors, (2) these previously mentioned behaviors differ between crops, (3) these behaviors differ at different times of the day, and (4) there is a difference between adult and nymph behavior patterns. Results of this work may be used to improve future monitoring and management practices of *H. halys*.

Materials and methods

Orchard sites

Four 10 ha orchards of apples (2 cultivars), peaches, and nectarines were selected at the Penn State University Fruit Research and Extension Center (FREC) in Biglerville, Pennsylvania (39° 56' 6.2844"N, 77° 15' 19.2378"W) in 2011 and 2012 to study *H. halys* in a field setting. The FREC facility is a 72 ha research farm located in a prominent fruit production area located in south-central Pennsylvania. The four orchards used for my *H. halys* observations included apples *cv* 'Gold Rush' and 'Pink Lady', 'Red Haven' peaches, and 'SunGlo' nectarines. The trees in apple orchards at the beginning of the observations were approximately eight years old planted at row spacing of 1.8 m and within row tree spacing at 6 m (912 trees/ha). 'Red Haven' peach and 'SunGlo' nectarines were approximately 11 years old and planted at an 8.3 m and 5.1 m row and within row tree spacing, respectively (271 trees/ha). All behavioral observations, regardless of crop and variety, were conducted on limbs with fruit, foliage, and one to two year old shoots.

Behavioral sleeves

Behavioral arenas, white 30x70 cm BugDorm rearing sleeves (Taichung, Taiwan) were deployed in each block of fruit (Gold Rush apple, Pink Lady apple, Red Haven peach, and SunGlo nectarine). Each sleeve was made of breathable nylon with a transparent vinyl window in the center for easy viewing of the insects (Figure 2-1). Sleeves covered a single limb with fruit, foliage, and new plant shoots — effectively creating a miniature ecosystem for either adult or nymphal *H. halys* populations.

At all times during the summer, there were at least two sets of sleeves in each orchard. Five limbs were randomly selected each week from May through August for placement of sleeves (five sleeves observed per orchard for a total of twenty sleeves per week). During the first week, the 'preparation' mode, the limbs were contained within the behavioral arena (sleeves). The leaves, fruit and stems were inspected to determine prior feeding damage and to remove and exclude wild *H. halys* from the sleeves as a precautionary measure. At the conclusion of the first week, the sleeves shifted from 'preparation' to 'active' mode when 5-10 *H. halys* were added. While in 'active' mode, the behavior of adults and nymphs were observed 1-2 times per week for 24 hours. At the end of the second week, 'active' mode, sleeves were removed and the limb marked to prevent future selection for *H. halys* observation.

Insects

H. halys adults and second-instar nymphs originated from a colony maintained in the PSU FREC laboratory in 60x60x60 cm mesh and plastic BugDorm insect rearing tents (Taichung, Taiwan). These tents were held in growth chambers at a photoperiod of 16:8 L:D, 24-26°C and

approximately 70±5% relative humidity. Individuals for the colony were collected during the previous fall and winter, completed their overwintering stage in growth chambers and produced viable egg masses in the spring. From May 31 to June 16, 2011 ten adults (five males and five females) were placed in each 'active' sleeve for one week of observation. Different adults were used each week. The adult activity studies were concluded by June 16 due to low numbers of available *H. halys* adults. In the next, nymphal phase of the project, 'preparation' sleeves were moved to new limbs, as described above and five second-instar nymphs were placed per 'active' sleeve, as described above. The nymphal behavioral observations were conducted from July 1 to August 4, 2011.

The 2012 field observations were conducted from May through August for both nymph and adult populations. Five *H. halys* adults (three males and two females) were released into a single sleeve each week from May 12 – August 2. Nymphal observations also consisted of five second-instar nymphs released into sleeves each week from May 17- August 2.

Observation periods

During 2011, both adults and nymphs were observed between 6:00-9:30, 11:30-14:30, 15:00-17:00, 18:00-20:30, and 23:30-1:00 for five minutes per observation. During this five-minute period, I observed all individual insects and each behavior exhibited during that time frame was recorded. Weather events, such as heavy rain or thunderstorms, prevented or shortened some observations.

During the 2012 season, observations were the same as 2011 with the addition of a 2:00-4:00 observation. The only difference between 2011 and 2012 was the use of a 'snapshot' approach rather than a five-minute observation period. To eliminate possible bias in *H. halys*

natural behavior related to the observer's presence, only the first observed behavior exhibited by each individual was recorded rather than all behaviors during five-minutes of observation.

Behaviors observed

During the 24-hour observation period, I recorded the occurrence of various behaviors as well as the location on the plant (i.e. foliage, fruit, etc) where the behaviors occurred. Observed individual behaviors were grouped into three categories: 'resting', 'feeding', and 'moving'. 'Resting' behaviors were characterized by a complete lack of movement with the feeding stylet not being inserted into any plant tissue. The insertion or active probing of the stylet into any plant tissue characterized 'feeding' behaviors (Figure 2-2). 'Moving' behavior consisted of any active movement by *H. halys*, including mating, walking, or flying activity.

Analysis

All data were categorical. Independent variables were life stage (nymph versus adults), crop, and time of day and dependent variables were *H. halys* activities (resting, feeding and moving). Missing data resulted in smaller sample sizes for, primarily, the midnight observations, thus reducing the power of the statistical analysis.

After observing behaviors through the 2011 and 2012 season, the data were analyzed using Chi-Square Test of Independence analysis (SAS 9.3) to determine if:

1. the proportion of resting, feeding and moving activities differ between crops,
2. the proportion of resting, feeding and moving activities differ by time of day, and

3. the proportion of resting, feeding and moving activities differ between adults and nymphs.

Results

2011 Season

For 2011 adult *H. halys*, feeding, moving, and resting behaviors, 16%, 10%, and 74%, respectively, did not differ between the Gold Rush and Pink Lady apple cultivars (χ^2 test, DF=2; P=0.0540). Thus, the data were combined into an 'apple crop'. The crop type (apples, peaches, or nectarines) did affect the behavior that was exhibited (χ^2 test, DF=4; P=0.0014); *H. halys* was observed to be more active (moving) in apples (10%) than in nectarines (2%) and peaches (2%). [This result did not change even when apple cultivars were analyzed as separate groups.] However, irrespective of crop, overall resting behavior (83%) occurred more often than either feeding or moving behavior. More activity (feeding and moving) occurred between 18:00-20:30 (34% active, 66% resting), and 23:30-1:00 (53% active, 47% resting), and time was found to be a significant indicator of activity level (χ^2 test, DF=8; P<0.0001), with greater overall feeding, moving, and resting behaviors (Figure 2-3).

For 2011 nymphs, feeding, moving, and resting behaviors, 11%, 24%, and 65%, respectively, did not differ between the Gold Rush and Pink Lady apple cultivars (χ^2 test, DF=2; P=0.5536). Similarly, when combined into apple, peach, or nectarine crops, feeding (8%), moving (20%), and resting (72%) behaviors did not significantly differ between those crops (χ^2 test, DF= 4; P=0.3399). There was an effect of time on nymphal behavior, with resting the most common behavior (71%), and feeding (8%) less common than moving (21%) at all time periods

but midnight when nymphs were resting 100% of the time (χ^2 test, DF=8; P<0.0001) (Figure 2-4).

Overall, nymphs were marginally more active but fed less than adults in the field during the 2011 season. Adult feeding, moving, and resting behaviors, 12%, 4%, and 83%, respectively, were significantly different as compared to nymph feeding, moving, and resting behaviors, at 9%, 21%, and 70%, respectively (χ^2 test, DF=2; P=0.0016) (Table 2-1). .

2012 Season

For 2012 adults, there was no difference observed in *H. halys* feeding (12%), moving (9%), and resting (79%) behaviors between Gold Rush and Pink Lady apple cultivars (χ^2 test, DF=2; P=0.3019), so apple cultivars were combined for the remaining analysis. No effect of crop on behavior was observed, with feeding, moving, and resting behaviors across all crops being 11%, 9%, and 80%, respectively (χ^2 test, DF=4; P=0.9150). There was an effect of time on behavior, with *H. halys* adults exhibiting more activity (feeding and moving) from 18:00-20:30 (χ^2 test, DF=10; P=0.0031) (Figure 2-5).

For 2012 *H. halys* nymphs, apple cultivar did not significantly affect feeding, moving, or resting behaviors, 14%, 13%, and 73%, respectively (χ^2 test, DF=2; P=0.9541). When looking at the effect of crop on activity, apples, peaches, and nectarines were the same, 10%, 13%, and 77%, respectively (χ^2 test, DF=4; P=0.4182). However, behavior was affected by time (χ^2 test, DF=10; P<0.0001), with higher activity levels during the day that decreased at night; the highest number of resting individuals were observed from 23:30-1:00 and 2:00-4:00, both at 95% (Figure 2-6).

Overall there was no significant difference between adult and nymph behaviors throughout the 2012 season, with feeding, moving, and resting behaviors being expressed at 12%, 12%, and 76%, respectively ((χ^2 test, DF=2; P=0.4757) (Table 2-2)).

Discussion

H. halys is a particularly important economic pest because of its wide host range, and in Pennsylvania it has been observed as the predominantly collected stink bug species, replacing native species in agricultural settings (Nielsen and Hamilton 2009a). *H. halys* can inflict high levels of damage to apples and peaches, with the majority of the feeding occurring just before harvest.

Previously, anecdotal evidence has suggested an increase in *H. halys* activity towards dusk and evening (Nielsen et al. 2013). This study found dusk and evening to be the most active time for *H. halys*. Adults and nymphs experience different levels of activity throughout the day, with increased activity towards evening hours for adults, while nymphs experienced depressed activity at midnight observations in 2011.

This field study was completed in a variety of crops, including apple, peach, and nectarine, and indicated that behaviors remain consistent between all crops, particularly as resting behavior is the most prevalent behavior, regardless of crop. While we showed that behaviors are more likely to be influenced by time of day than the crop type, future studies using a wider range of fruit and adjusted times of day in tune with daylight levels and temperature would be beneficial.

It is possible to say with reasonable confidence that resting, based upon these field data and other laboratory work (Chapter 3), is the most commonly observed *H. halys* activity and that

dusk is the most likely time for feeding and other movement to occur. While the observations of resting versus moving behavior have been observed to be consistent in both field and laboratory conditions, it is possible that activities may alter at a different scale. In particular, *H. halys* activity may alter at commercial operation levels, similar to differences seen when developing trap cropping techniques (Shelton and Badenes-Perez 2006). As many commercial operations have large acreage devoted to several select crops, activity within and movement between those crops is an important consideration. In my study, *H. halys* individuals were confined to sleeves. This effectively eliminated any behavioral differences that may have occurred by *H. halys* switching between crops. In normal field conditions, *H. halys* go through a series of hosts from emergence of diapause in the spring, to preparation for diapause in the fall (Nielsen and Hamilton 2009b). Adult *H. halys* may also switch hosts depending on plant phenology, which may explain early season and pre-harvest feeding on apples and peaches. By confining *H. halys* to a single host plant, levels of feeding may be artificially higher.

Insecticides also affect locomotion and behavior of *H. halys* (Lee et al. 2013). While the arenas used in this study were not sprayed, drift from nearby sprays could have also influenced our data, and sublethal effects from drift were not evaluated. It is not expected that the impact of drift will change the observed differences in activity because the laboratory results (Chapter 3) show similar activity patterns. The impact of insecticide sprays on *H. halys* activity needs to be investigated more fully with particular attention to feeding activity.

In this study, I did not distinguish between probing and true feeding activity. Because observations were restricted and strictly visual in nature, all observations of stylet insertion into fruit were termed ‘feeding’. This feeding behavior could have been probing behavior to locate a preferable food source rather than feeding on the tissue or fruit being probed. In a study by Shearer and Jones (1996), southern green stink bug females showed diurnal feeding patterns with peak probing during the scotophase by distinguishing probing from feeding by the duration of the

stylet insertion. However, as it is unknown how much feeding or probing behavior results in damage, the clumping of all stylet insertion into a 'feeding' category acknowledges damage potential of this particular behavior.

The data for this study were collected differently in 2011 and 2012, and subsequently the data cannot be directly compared. However, the results are similar between the two years, despite the differences in collection methods. In both years *H. halys* were observed most often to be resting while activity (feeding and moving) was most often observed at dusk. The slight differences observed could be due to differences in the length of the season or by the observation method. In 2011, adult observations ran from May 31-June 16, while nymphs ran from July 1-August 4. The 2012 season, which was considerably longer, ran from May 12 – August 2 for adults, and May 17- August 2 for nymphs. The shorter data collection season for 2011 resulted in a less robust data set than for 2012. Additionally, it is possible that the 2011 data were influenced by the presence of the observer.

While *H. halys* continues to be a serious pest across the United States, chemical control is still the main means of control. By understanding *H. halys* behavior, it will be possible to develop future Integrated Pest Management (IPM) controls and lower the necessity for pesticide use.

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Figure **2-1**: A BugDorm sleeve over a limb containing foliage and fruit. These arenas were used for viewing *H. halys* adult and nymph activities over a 24-hour period.

Figure **2-2**: Feeding of *H. halys* on young apple stem, as indicated by stylet insertion.

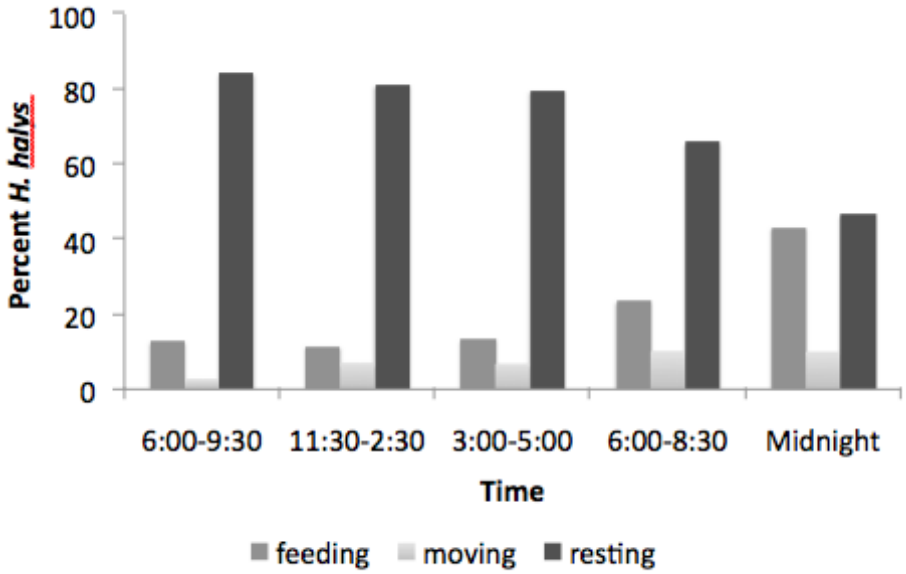

Figure **2-3**: 2011 Adult *H. halys* behaviors. Activities at each time were summed for the season, e.g., all feeding, moving and resting occurring between 6:00-8:30 pm from May 31 – June 16 were summed – 24 % of individuals were feeding 10% of individuals were moving and 66% were resting. A significant effect of time on behavior was observed (Test of Independence χ^2 test, DF=8; P<0.0001).

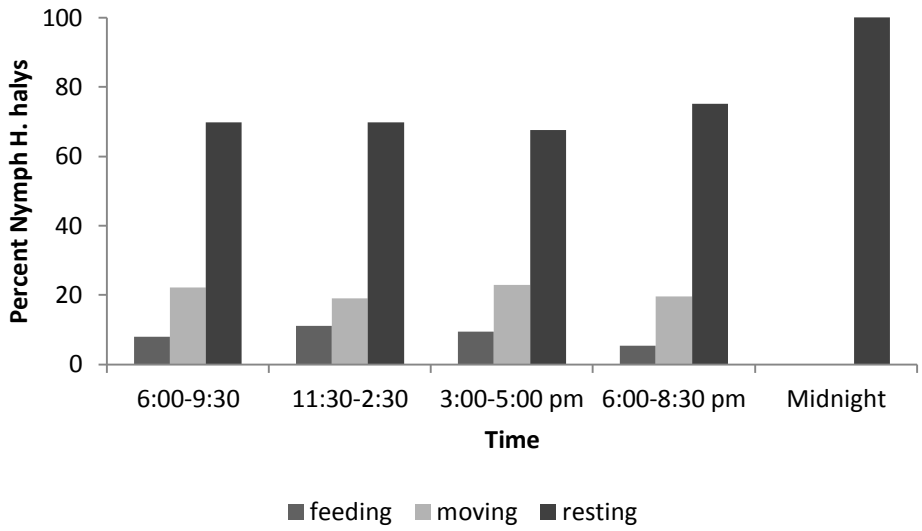

Figure **2-4**: 2011 Nymph *H. halys* behaviors. Activities at each time were summed for the season, e.g., all feeding, moving and resting occurring between 6:00-8:30 pm from July 1 – August 4 were summed – 5% of individuals were feeding 20% of individuals were moving and 75% were resting. A significant effect of time on behavior (Test of Independence χ^2 test, DF=8; P<0.0001).

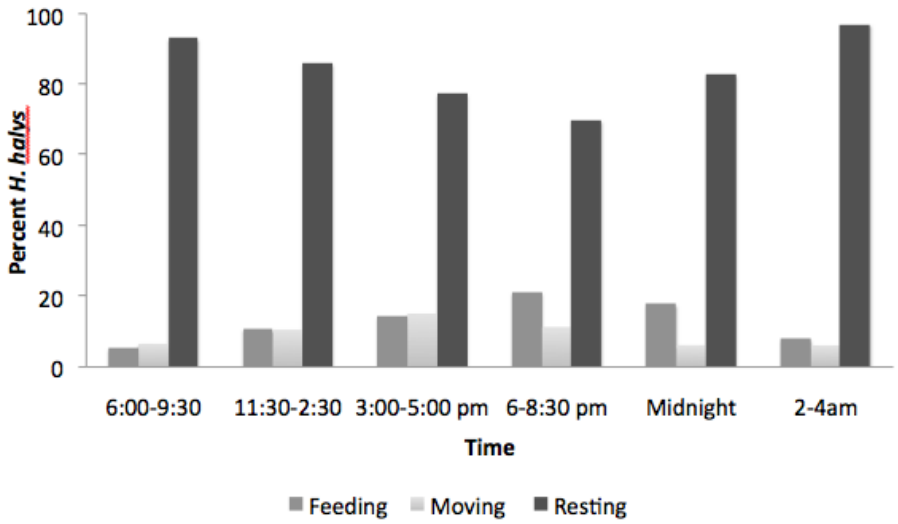

Figure **2-5**: 2012 Adult *H. halys* behaviors. Activities at each time were summed for the season, e.g., all feeding, moving and resting occurring between 6:00-8:30 pm from May 12 – August 2 were summed – 20% of individuals were feeding 12% of individuals were moving and 68% were resting. A significant effect of time on behavior (Test of Independence χ^2 test, DF=10; P=0.0031).

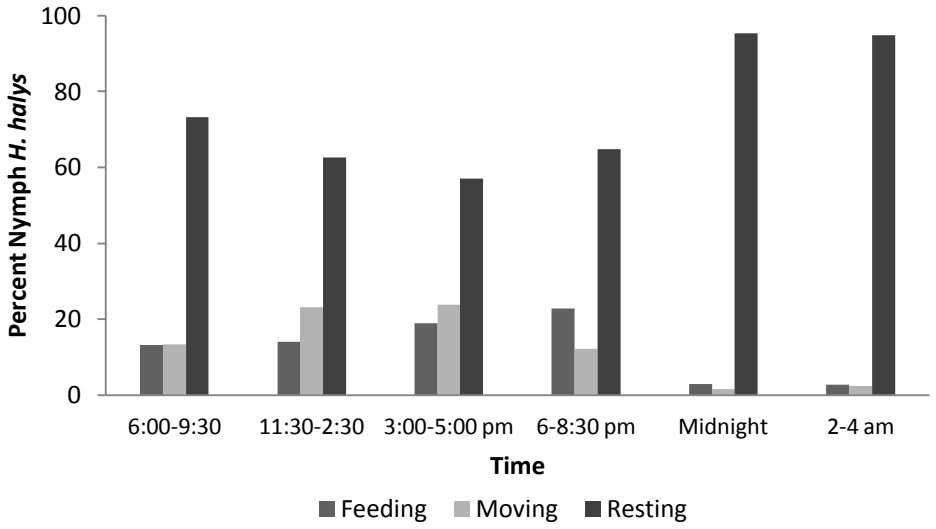

Figure **2-6**: 2012 Nymph *H. halys* behaviors. Activities at each time were summed for the season, e.g., all feeding, moving and resting occurring between 6:00-8:30 pm from May 17 – August 2 were summed – 23% of individuals were feeding 12% of individuals were moving and 65% were resting. A significant effect of time on behavior (Test of Independence χ^2 test, DF=10; P<0.0001).

Table **2-1**: 2011 Adult and nymph activity. All data were analyzed using a χ^2 Test of Independence. Nymph and adult activity in the two apple cultivars did not differ. Adult but not nymph activity in crops differed with adults being more active (10%, versus 2%) in apples versus peaches and nectarines. Activity differed by time of day in both adults and nymphs. Adults were more activity (feeding and moving) between 18:00-20:30 and 23:30-1:00 while nymphs were found to all be resting at midnight. Please note that data collection was shorter in 2011 than 2012 and no overlap between adult and nymph observation dates occurred in 2011.

2011 Adults	
	P-value
Apple cultivar	0.0540
Crop	0.0014
Time	<0.0001
2011 Nymphs	
	P-value
Apple cultivar	0.5536
Crop	0.3399
Time	<0.0001
2011 Adult vs. Nymph	
	P-value
overall	0.0016

Table **2-2**: 2012 Adult and nymph activity. All data were analyzed using a χ^2 Test of Independence. Nymph and adult activity in the two apple cultivars did not differ. Apple cultivar, and crop, did not differ in *H. halys* activity levels. Adult and nymph activity differed by time of day, with adults more active at 18:00-20:30, and nymphs with greater resting from 23:30-1:00 and 2:00-4:00. Observations ran from May-August for both adults and nymphs.

2012 Adults	
	P-value
Apple cultivar	0.3019
Crop	0.9150
Time	0.0031
2012 Nymphs	
	P-value
Apple cultivar	0.9541
Crop	0.4182
Time (with midnight)	<0.0001
2012 Adult vs. Nymph	
	P-value
Overall	0.4757

Chapter 3

Halyomorpha halys diel activity patterns and circadian rhythm

Introduction

Daily activity patterns, also known as circadian rhythms, are intrinsic patterns of mental, physical or behavior variations based on an approximately 24-hour photoperiod. Circadian rhythms have been observed in a variety of plants, birds, insects, and mammals and may be expressed as locomotor activity as well as other biological activities such as brain wave activity and hormone production (Lewis and Saunders 1987; Stephan and Zucker 1972; Beck 1968). Insects from cockroaches to house crickets and flesh flies, all exhibit circadian rhythm activities (Brady 1967; Cymborowski 1973; Lewis and Saunders 1987; Beck 1968). Since diel feeding patterns in the southern green stink bug, *Nezara viridula*, were expressed as greater feeding during the scotophase (Shearer and Jones 1996), it is reasonable that a similar pattern may be expressed by the brown marmorated stink bug, *Halyomorpha halys* (Stål).

H. halys is an invasive hemipteran pest wreaking havoc across the mid-Atlantic United States (Nielsen and Hamilton 2009). Native to Asia, including China, Japan, Korea, and Taiwan, *H. halys* has been reported in Switzerland, Germany, France, Liechtenstein, Italy, Canada and the United States, (Lee et al. 2013a; Maistrello et al. 2014) and was most likely introduced through international commerce in bulk freight containers. The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) had intercepted *H. halys* at ports of entry prior to homeowners who reported the first sightings of this pest in Allentown Pennsylvania in 1996, with positive identification in 2001 (Anonymous 2010; Khrimian et al. 2008; Hoebeke and Carter 2003).

The polyphagous *H. halys* is an economically important pest because it feeds on a variety of fruit, vegetable, legume, and ornamental plant species (Maryland Cooperative Extension 2010). In 2010 alone, *H. halys* caused over $37 million in damage to mid-Atlantic apple production (US Apple Association 2011). *H. halys* is also known to vector the phytoplasma that causes witches'-broom disease in *Paulownia* (Hiruki 1999; Leskey et al. 2012). At the end of the growing season, this insect moves to dwellings and is considered a nuisance for homeowners because aggregations may overwinter in home and business buildings (Hoebeke and Carter 2003).

H. halys is a novel invasive species in North America with a dearth of published literature, and a better understanding of this species' biology and behavior must be established in order to develop more efficient *H. halys* management practices.

The objectives of this study were to determine if (1) *H. halys* has predictable activity patterns, (2) these activity patterns are tied to external stimuli or an endogenous circadian rhythm, and (3) there is a different expression of activity between males and females. Results of this work may be used to improve monitoring and control behavior of *H. halys* in the future.

Materials and methods

Colony

H. halys individuals were collected during August and September, 2012 from agricultural settings near Lancaster, PA (39° 58' 50.2998"N, 76° 18' 36.6366"W) and placed into a laboratory colony. The laboratory colony was held in 60x60x60 cm mesh and plastic BugDorm insect rearing tent (Taichung, Taiwan). Additional overwintering *H. halys* adults collected from the Wexford, PA (40° 36' 45.1872"N, 80° 5' 31.7616"W) area supplemented the colony during the

winter months. *H. halys* were held in growth chambers at a photoperiod of 16:8 L:D, 24-26°C and approximately 70±5% relative humidity until diapause was broken and feeding resumed. Lighting was provided by 2600 lumen Phillips F32T8/TL741 Alto fluorescent light bulbs (Andover, MA) within an I36VL Percival (Perry, IA) growth chamber.

Behavior arenas

Fifteen male and fifteen female *H. halys* were placed in 450-mL, 6.5 cm-high, 11.5 cm-diameter plastic Solo® cups with lids (Solo Co. Lake Forest, IL). Each cup (arena) contained organically grown carrots, organic woodpecker birdseed mixture (Agway DeWitt, NY), and a wet paper towel (Figure 3-1). When all individuals had broken diapause, as indicated by orange frass from consuming carrots, laboratory observations of behavior began. The arenas were cleaned, and food replaced, every 2-3 days to avoid mold.

Observation periods

Observations occurred under two conditions: three days of 16:8 L:D (Experiment 1), and one day of 16:8 L:D followed by two days of complete dark (DD) (Experiment 2). Initial observations were completed with red light, and then followed by the white LED light (50-lumen, Princeton Tec headlamp, Trenton, NJ). Since no changes in behavior were observed, the white LED light was used for all observations in the diel periodicity, and circadian rhythm experiment. For both the diel periodicity and circadian rhythm experiments (Experiment 1 and Experiment 2, respectively), each individual stink bug (n=30) was observed for 15-20 seconds. After all 30 *H. halys* were observed, a second set of observations, averaging 5 seconds/individual, were completed to determine if behaviors were consistent and not altered by the presence of an

observer. In this way, it was possible to determine that behaviors were consistent throughout each observation period.

To determine if diel periodicity in behavior exists (Experiment 1) observations occurred every four hours at: 8:00, 12:00, 16:00, 20:00, 24:00, and 4:00 starting at 8:00 on Day 1 and ending at 4:00 on Day 3. Darkness on all three days began at 23:00, and ceased at 7:00.

To determine the existence of circadian rhythm (Experiment 2), *H. halys* were transitioned from a single day of 16:8 L:D (referred to as Day 4) to two days (referred to as Days 5-6) of DD to allow for observations with the external cue (light/dark) present and absent. Observations were carried out every four hours starting at 8:00 as described above.

Behaviors observed

During the 24-hour observation period, I recorded locomotor activity within the arena. Locomotor activities fell into two categories: quiescent (resting), or non-quiescent (feeding and moving) behaviors. Resting behavior was characterized by a complete lack of movement where the feeding stylet was not inserted into any seed or carrot tissue. The insertion or active probing of the stylet into any plant tissue is characterized as feeding behavior. Moving behavior consisted of any active movement by *H. halys*, including walking, or flying.

Analysis

While completing fieldwork during the 2011 and 2012 season in Biglerville, PA (39° 56' 6.2844"N, 77° 15' 19.2378"W), field observations indicated that *H. halys* seemed to be twice as likely to be resting, than to be doing any other activity. Thus it was hypothesized that *H. halys* was quiescent (resting) 50% of the time and non-quiescent (feeding or moving) 50% of the time.

A subset of data from initial observations (and not used in final analysis) was used to estimate the ratio of quiescent: non-quiescent behavior. From this subset, resting, moving, and feeding behavior was observed 55%, 8%, and 37% of the time, respectively. The proportions observed in the subset of data were consolidated to 55:45, quiescent to non-quiescent. An exact binomial tests were used to evaluate the remaining data.

Three data sets were analyzed:

1. Quiescent and non-quiescent behavior during 16:8 L:D sorted by time (e.g. 8:00, 12:00, 4:00, etc.)
2. Quiescent and non-quiescent behavior during complete dark (DD) sorted by time (e.g. 8:00, 12:00, 4:00, etc.)
3. Quiescent and non-quiescent behavior sorted by sex

Results

Diel periodicity

For the three-day diel periodicity study, at 16:8 L:D, *H. halys* was more active toward the end of the light cycle and into the first part of the dark cycle and less active toward the end of the dark cycle and into the first part of the light cycle (Experiment 1). A significant difference between the expected quiescent and non-quiescent ratio (expected 55% quiescent, 45% non-quiescent) was observed at different periods of time (Table 3-1). At 20:00, and 24:00, *H. halys* was significantly more active (non-quiescence) than expected. At 4:00 and 8:00 there was significantly more quiescence than was expected, and at 12:00 and 16:00 there was no significant deviation from the expected ratios of activity. More activity than expected was observed at 20:00

and 0:00 while less activity than expected was observed at 4:00 and 8:00 when *H. halys* experienced 16:8 L:D (Figures 3-2 and 3-3). Greater activity spanned the change from light to dark and less activity spanned the change from dark to light.

Circadian rhythm

For the two day circadian rhythm study, (DD), quiescent and non-quiescent behaviors shifted from the expected quiescent and non-quiescent ratios (expected 55% quiescent, 45% non-quiescent) and from that observed in the diel study (Table 3-2) (Experiment 2). Greater quiescence was observed at 4:00 and 12:00, rather than 4:00 and 6:00 in the diel study (Figures 3-4 and 3-5). Non-quiescent activity was greater at 20:00, but not at 0:00, as in the diel study.

Male and female activity

During the three-day diel periodicity study males were significantly more active than expected at 0:00 and 4:00 but significantly less active than expected at 20:00, while females were significantly less active than expected at 8:00 only (Table 3-3 and Table 3-4, respectively). During the two-day circadian rhythm study, activity in males and females shifted as compared to the diel periodicity study. Males were significantly less active than expected at 4:00 but significantly more active than expected at 20:00 (Table 3-5). Females were significantly more active than expected only at 20:00 (Table 3-6).

Discussion

Knowledge from laboratory-based studies of insects can be useful in determining behavior patterns and has application in future insect pest management programs (Shearer and

Jones 1996). Specifically it is important to understand the diel and circadian rhythms of an insect species to design management plans. In the Asian citrus psyllid, *Diaphorina citri*, flight behavior and host selection are regulated by circadian rhythms (Sétamou et al. 2011).

Our study shows that *H. halys* adults exhibit predictable behavior patterns under laboratory conditions. Peak activity levels for *H. halys* during a typical 16:8 L:D cycle occur at 20:00 and 0:00. This finding is similar to the southern green stink bug, *Nezara viridula*, which shows a diel periodicity based upon feeding behavior. *N. viridula* feeding was recorded via a data logger during the scotophase and photophase to determine the presence and duration of feeding on a 24-hour cycle (Shearer and Jones 1996).

For the first time, *H. halys* circadian rhythm has been demonstrated. The diel periodicity in *H. halys* activity observed during 16:8 L:D persisted when light: dark conditions changed to complete dark conditions (Fig 3-5). The observed shift in the activity pattern during the dark only cycle is to be expected if length of light: dark cycle is the external cue. Thus the periodicity in behavior is at least partly determined by an underlying circadian rhythm.

Male and female *H. halys* differed slightly in their expression of locomotor patterns. Males are more active than expected at the end of the light cycle and the beginning of the dark cycle (20:00 and 0:00) and exhibit activity levels that are lower or not different than expected from the middle of the dark cycle to nearly the end of the light cycle (4:00 to 16:00). Females exhibit less activity than expected only once and this occurs during the light cycle at 8:00.

Diurnal resting behavior, along with nocturnal activity, can make *H. halys* difficult to monitor using visual techniques. This conclusion is borne out by the observation that while *H. halys* is known to cause substantial damage to orchards, the level of damage to fruit is often difficult to correlate with visual monitoring of *H. halys* (Lee et al. 2013b). The data from this study indicate greater nocturnal activity, thus providing support for why *H. halys* respond to light traps. A better understanding of when activity is elevated may help in the deployment and use of

light trapping and monitoring (Nielsen and Hamilton 2009). As activity levels are higher in the absence of light, it appears that *H. halys* movement is not constrained by visual cues. Antennectomy of *H. halys* has also been shown to prevent the formation of aggregations, pointing to the importance of olfaction and tactile cues (Toyama et al. 2006). Regardless of the cue, a better understanding of behavioral patterns in *H. halys* may help improve future monitoring and control.

H. halys has been observed in orchards to be more active at night and less active during daylight (personal observation). Further field studies to investigate *H. halys* diurnal and nocturnal behaviors would add further evidence that *H. halys* activity level is elevated at night. Even though conditions were controlled, e.g., a constant temperature, this laboratory study provides useful data on the diel and circadian patterns of *H. halys* and points to the need for future research into behavioral patterns of *H. halys*. In particular, more research into the practical application of controlling *H. halys* through the use of these expected behavioral patterns, such as with the use of Integrated Pest Management strategies in the field, is needed.

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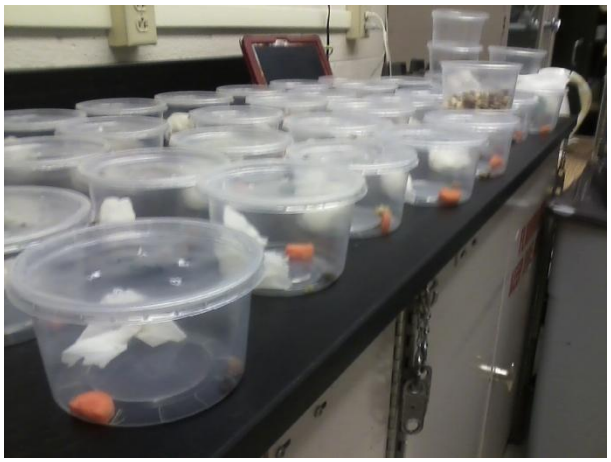

Figure **3-1**: Behavior arenas (450-mL, 6.5 cm-high, 11.5 cm-diameter plastic Solo® cups with lids) with wet paper towel and food (carrot, and woodpecker birdseed) for diel periodicity (Experiment 1) and circadian rhythm (Experiment 2) experiments.

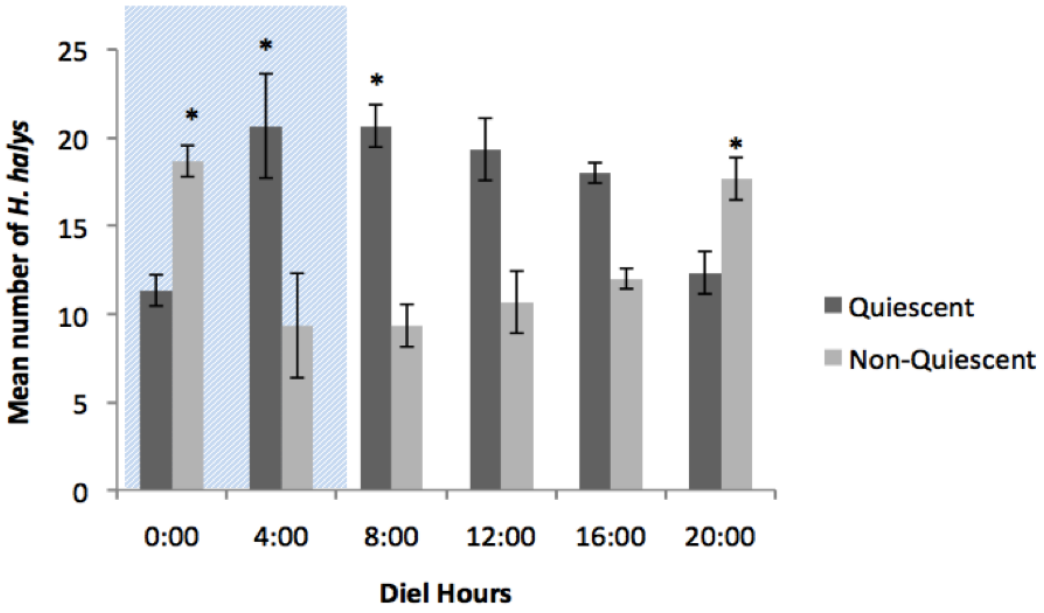

Figure **3-2**: Mean diel periodicity of quiescent vs. non-quiescent *H. halys* over three days. 16:8 L:D cycle, times of 0:00, 4:00, 8:00, and 20:00 are significantly different from the expected ratios of 55% quiescent, and 45% non-quiescent behaviors, P=0.0015, P=0.01, P=0.01, and P=0.0112, respectively. Shaded area indicates dark cycle. *significantly greater than expected.

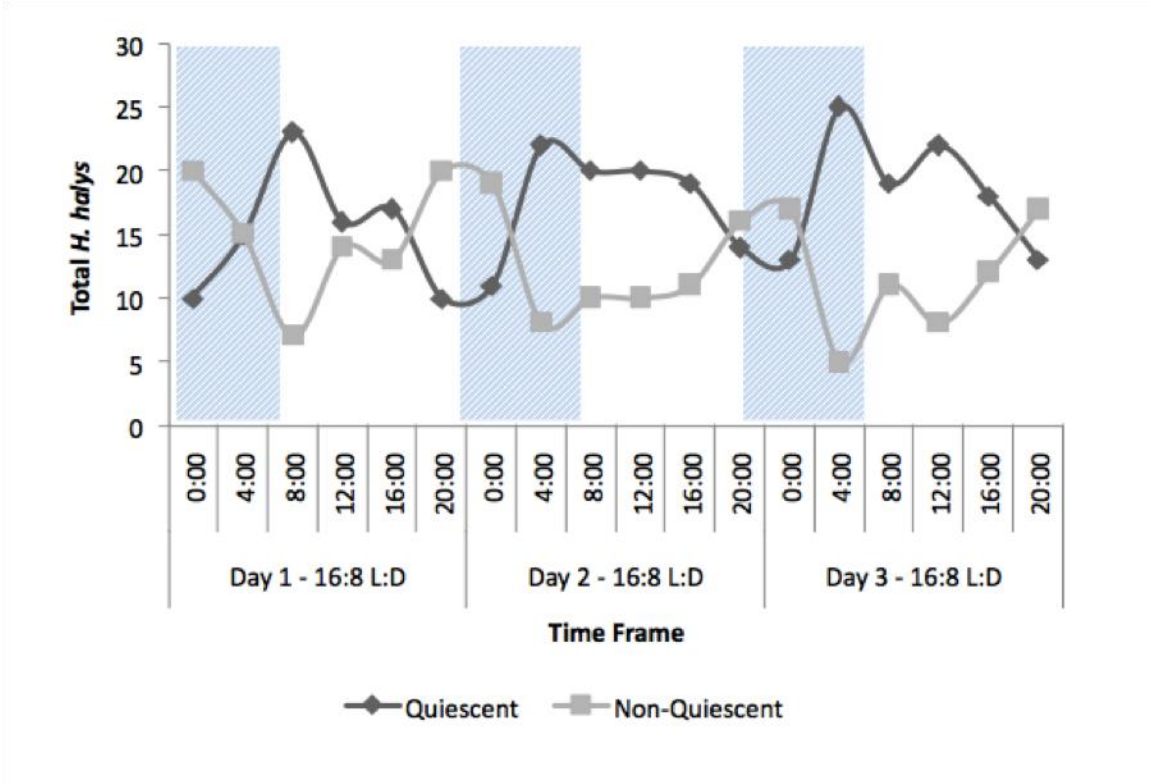

Figure **3-3**: Total number *H. halys* exhibiting each behavior from Day 1 through Day 3, all at a 16:8 L:D cycle. Lights were turned on at 7:00 and off at 23:00. Increases in activity (non-quiescent) appear at 20:00, and 0:00. Shaded area indicates dark cycle.

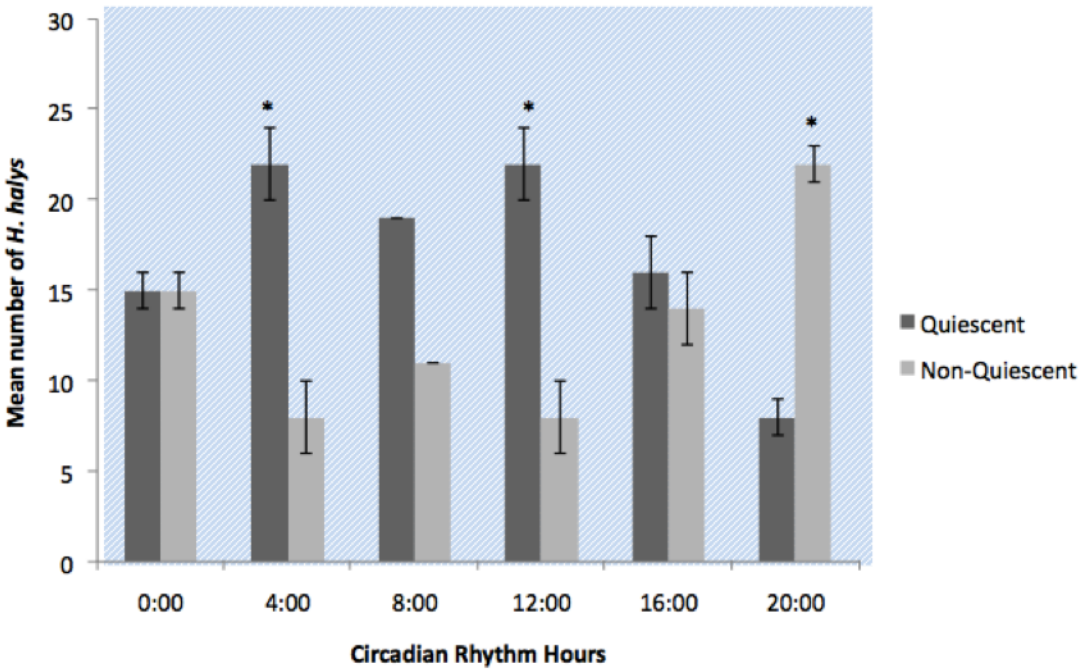

Figure **3-4**: *H. halys* circadian rhythm patterns shown from Day 5 and Day 6. The constant dark (DD) cycle resulted in significantly more quiescent behavior at 4:00 and 12:00, and significantly more non-quiescent activity at 20:00. Expected activity was 55% quiescent, and 45% non-quiescent. Shaded area indicates dark cycle. *significantly greater than expected.

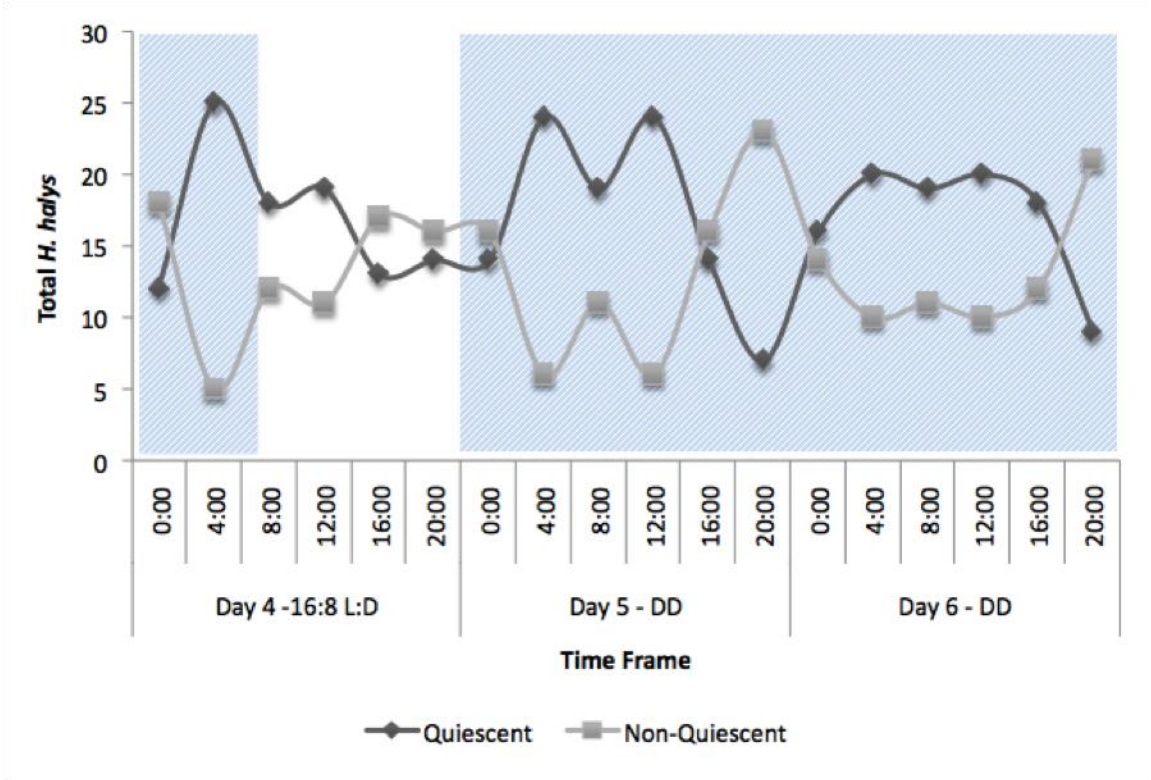

Figure **3-5**: Circadian rhythm pattern Day 4 through Day 6. At each time, the total number *H. halys* exhibiting each behavior is shown. Lights were turned on at 7:00 and turned off at 23:00 on Day 4 only while Day 5 and Day 6 were in constant dark (DD). Increased non-quiescent behavior appears around 20:00 during the constant dark. Shaded area indicates dark cycle

Table **3-1**: Results of *H. halys* diel periodicity (Experiment 1). Increased levels of activity (non-quiescent) were observed at 20:00 and 0:00 for *H halys*. Binomial analysis compared actual to expected ratios at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Percentages shown are actual data; expected activity was 55% quiescence, and 45% non-quiescence. For each time frame, n=90, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
:00	37.78	62.22*	0.0015
:00	68.89*	31.11	0.01
:00	68.89*	31.11	0.01
2:00	64.44	35.56	0.0882
6:00	60	40	0.3974
0:00	41.11	58.89*	0.0112

Table **3-2**: Results of *H. halys* circadian rhythm (Experiment 2, days five and six in complete dark). Note the increased level of activity (non-quiescent) starting at 20:00. Binomial analysis compared actual to expected ratios at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Percentages shown are actual data percentages; expected activity was 55% quiescence, and 45% non-quiescence expected. For each time frame, n=60, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
0:00	50	50	0.5152
4:00	73.33*	26.67	0.0054
8:00	63.33	36.67	0.2420
12:00	73.33*	26.67	0.0054
16:00	53.33	46.67	0.8935
20:00	26.67	73.33*	0.00002

Table **3-3**: Results of male *H. halys* diel periodicity (Experiment 1). Binomial analysis of actual to expected ratios of male *H. halys* at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Male activity (non-quiescent) levels were higher than expected at 20:00 and 0:00. Percentages shown are actual data; expected activity was 55% quiescence, and 45% non-quiescence. For each time frame, n=45, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
:00	33.33	66.67*	0 .0056
:00	73.33*	26.67	0 .0180
:00	66.67	33.33	0 .1523
2:00	68.89	31.11	0 .0819

	57.78	42.22	0
6:00			.8262
	33.33	66.67*	0
0:00			.0056

Table **3-4**: Results of female *H. halys* diel periodicity (Experiment 1). Binomial analysis of actual to expected ratios of female *H. halys* at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Female quiescent behavior (resting) was higher at 8:00. Percentages shown are actual data; expected activity was 55% quiescence, and 45% non-quiescence. For each time frame, n=45, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
0:00	42.22	57.78	0.116

			4
4:00	64.44	35.56	0.2604
8:00	71.11*	28.89	0.0402
12:00	60.00	40.00	0.6031
16:00	62.22	37.78	0.4112
20:00	48.89	51.11	0.4989

Table **3-5**: Results of *H. halys* male circadian rhythm (Experiment 2). Increased activity (non-quiescent) at 20:00 was observed. Binomial analysis compared actual to expected ratios at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Percentages shown are actual data; expected activity was 55% quiescence, and 45% non-quiescence. For each time frame, n=45, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
:00	63.33	36.67	0 .4654
:00	83.33*	16.67	0 .0033
:00	70.00	30.00	0 .1388
2:00	60.00	40.00	0 .7184
6:00	60.00	40.00	0 .7184
0:00	30.00	70.00*	0 .0100

Table **3-6**: Results of *H. halys* female circadian rhythm (Experiment 2). Increased activity (non-quiescent) was observed at 20:00. Binomial analysis compared actual to expected ratios at 0:00, 4:00, 8:00, 12:00, 16:00, and 20:00. Percentages shown are actual data; expected activity was 55% quiescence, and 45% non-quiescence. For each time frame, n=45, α=0.05, and *significantly greater than expected.

Time	Quiescent (%)	Non-Quiescent (%)	P-value
:00	36.67	63.33	0.0669
:00	63.33	36.67	0.4654
:00	56.67	43.33	1.000
2:00	45.00	55.00	0.2663
6:00	46.67	53.55	0.4618
	23.33	76.67*	0.

Chapter 4

Sunflowers as a potential trap crop of *Halyomorpha halys* in pepper fields

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive hemipteran pest wreaking havoc across the mid-Atlantic United States (Nielsen and Hamilton 2009). Native to Asia, including China, Japan, Korea, and Taiwan, *H. halys* has been reported in Switzerland, Germany, France, Liechtenstein, Italy, Canada and the United States, and was most likely introduced through international commerce in bulk freight containers. The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) intercepted *H. halys* at ports of entry prior to homeowners who first reported this pest in

Allentown Pennsylvania in 1996, with positive identification in 2001 (Lee et al. 2013; Maistrello et al. 2014; Anonymous 2010; Khrimian et al. 2008; Hoebeke and Carter 2003). Between 2004 and 2011, *H. halys* populations increased by 75% annually in New Jersey, illustrating how quickly an invasive pest can be established (Nielsen et al. 2013).

H. halys is economically important as it feeds on a variety of fruit, vegetable, legume, and ornamental plant species (Maryland Cooperative Extension 2010). In 2010 alone, *H. halys* caused over $37 million in damage to mid-Atlantic apple production (US Apple Association 2011). *H. halys* also damages a variety of vegetable crops including pepper, tomato, eggplant, okra, sweet corn, cucumbers, edamame beans, sweet corn, saya pea, asparagus and eggplant (Leskey et al. 2012; Biddinger et al. 2011; Fukuoka et al. 2002). *H. halys* can move easily between fields, and attack various vegetable crops between late July and October (Rice et al. 2014, *in press*).

H. halys feeds by inserting its stylet into plant tissue, secreting digestive enzymes, and feeding on plants fluids (Haye et al. 2014). Primary damage in vegetable crops, such as pepper and tomato, manifests as white and discolored spongy tissue beneath the skin (Rice et al. 2014, *in press*). Damage to corn results in discolored and collapsed kernels (Leskey et al. 2012). Secondary damage from infections is also a concern from *H. halys* feeding (Hiruki 1999).

With a variety of vegetables at risk from both primary and secondary feeding damage, the development of effective non-pesticidal methods of stink bug management are important to vegetable producers. Trap cropping is planting a secondary less-economically important plant stand to protect the main crop, and only works if the secondary plant stand is more attractive than the main crop. This method may hold potential for vegetable production protection against stink bugs (Hokkanen 1991). Trap cropping has been used with some success in reducing stink bug damage in soybeans (McPherson and Newsom 1984; Mizell et al. 2008). When dealing with cotton, *Nezara viridula* has been successfully trapped with sorghum (Tillman 2006). Other crops,

such as peppers, have also benefitted from trap cropping for other insect species. Boucher and Durgy (2004) saw a direct economic benefit of $153/acre with the use of a preferred pepper cultivar as a trap crop when dealing with pepper maggot, *Zonosemata electa*. In 2008, Mizell et al. recommended a mixture of trap crops for seasonal control of several stink bug species in the southern United States. To control *Euschistus servus, Acrosternum hilare,* and *N. viridula,* as well as other stink bug species, triticale, sorghum, millet, buckwheat, and sunflowers were the main trap crop species recommended (Mizell et al. 2008).

Sunflowers (*Helianthus annuus* L.) are a particularly intriguing trap crop option, as they have been used with success for Coleopteran, Lepidopteran, and Hemipteran pests (Hokkanen 1991; Shelton and Badenes-Perez 2006). *H. halys* is often kept on sunflower seeds in the laboratory, and has shown preference to sunflower seeds when compared to native Japanese cedar seeds (Aldrich et al. 2009).

The objectives of this study were to determine if (1) *H. halys* are attracted to sunflowers, (2) *H. halys* are attracted to secondary crops and non-crops surrounding the pepper and sunflower plots, (3) the population of *H. halys* varied throughout the season, (4) there is a different expression of attraction between adults and nymphs and males and females, and (5) damage in peppers differs by the type of surrounding crop. Results of this work may be used to develop innovative methods to protect crops from *H. halys* damage.

Materials and methods

Field site

During the 2012 and 2013 growing season, research plots to observe the effects of sunflower and pepper plants on the movement of *H. halys* were established at the Pennsylvania State University Southeast Agricultural Research and Extension Center in Landisville, PA (40° 7' 6.3012"N, 76° 25' 30.219"W). A single 158.5-meter long field was divided into twelve rows. In 2013, a 1.8-meter aisle was added for field maintenance. During both seasons, a field was divided into 4 blocks (Figure 4-1). Each block contained both treatments and was surrounded by a 3-meter 'alley' with no plants. Within each block a 13.7-meter area was planted with bell peppers *cv*. Revolution (Miller Plant Farm, York, PA) at approximately 46-cm spacing (treatment one). Treatment two was adjacent to treatment one and consisted of a 13.7-meter planted section where the outer two rows were planted with sunflowers, with the outer row *cv*. Giant Grey Stripe, and Mammoth Grey Stripe. The inner row of sunflowers was planted with organic sunflowers *cv*. Zohar F1 (Johnny's Select Seeds Winslow, Maine). These sunflowers were also planted in the first 1-meter of every row, surrounding a total of 8 rows planted with bell peppers (*cv*. Revolution). The layout of blocks 1 & 3 were the mirror image of blocks 2 & 4. In this way, the sunflowers were always next to another sunflower block (Figure 4-1, Figure 4-2).

All rows were raised beds, with black plastic and drip irrigation. Both sunflowers and peppers were planted May 29, 2012 and May 7, 2013 with a replant date of May 20, 2013 to replace plants killed by frost on May 13, 2013.

The experimental plot fields were located within different surroundings during each year of observations (Figure 4-3). In 2012, crabapple trees (east), cornfield and wood line (north),

cornfield (west), and an unplanted field (south) surrounded the experimental plot (Figure 4-4). In 2013, the field site was several hundred meters southeast of the 2012 location. Soybean, corn, maple and pine trees (east), a small dirt road (south), a hay field (west), and a barn and flower variety trial plots (north) surrounded the 2013 experimental plot (Figure 4-5).

Data collection

Each year's observations on *H. halys* presence were conducted once a week starting on June 29, 2012, and June 14, 2013. Total numbers of *H. halys* males, females, and adult and nymphal instars were recorded. Three-minute visual counts were used to monitor the *H. halys* presence on non-crop plants [crabapples, corn, and the woodland trees (2012) and crabapples, corn, soy, maple, and pine trees (2013)]. The number of *H. halys* was counted on pepper and sunflowers in two ways during each season. When *H. halys* populations were low or non-existent on the planted crops (peppers and sunflowers) at the beginning of the season, three-minute visual counts were used as follows:

1. All plants in the two exterior rows (four rows total) of peppers, and two exterior rows (four rows total) of sunflowers were scanned for three-minutes and
2. All plants in the eight rows of interior peppers of each split plot were scanned for three-minutes.

As the season progressed, and *H. halys* became more prevalent on the planted crops, data collection in the planted crop only (sunflowers and peppers) was changed to better reflect the changing numbers of *H. halys*. Starting on July 9, 2012 and July 19, 2013 every fifth plant in every other row of peppers was visually searched for *H. halys*.

While the data were collected using two methods, each reflects the best method to determine *H. halys* presence under different conditions – low versus high populations. The first observation method, the three-minute visual search, covers more plants and field area than the second method, a visual search of every fifth plant, but it provides information as to when *H. halys* first arrive. Using both methods, a determination of season-long trends was possible.

Damage to pepper fruit was evaluated at approximately 2-week intervals (3 total evaluations for 2012, and 6 evaluations for 2013), and was a minimum of 24 hours prior to harvest. Two peppers were randomly collected from each row (total of 40 peppers per block, 160 peppers per harvest), with the 8 center rows of each block and treatment being analyzed for injury (128 peppers per harvest). Injury was shown as white spongy tissue on the pepper (Figure 4-6; Figure 4-7). The number of injury spots per pepper was recorded. Injury to sunflowers was not quantified.

Analysis

Cumulative *H. halys* observations from 2012 and 2013 were analyzed with a split plot design, (PROC MIXED) and a Fisher Exact test of independence (SAS 9.3) to determine if the following factors affected *H. halys* abundance:

1. Split plot
2. Observation Year
3. Crop Type

I used the split plot design to create replicate areas for the sampling of *H. halys* in the two crops. While a split plot design was used, the area where the crops were planted is sufficiently small that no differences in soils, slopes or other factors were expected to influence the outcome.

Weekly *H. halys* observations from 2012 and 2013 were analyzed with to determine season long differences in:

1. *H. halys* abundance on pepper and sunflower
2. Adult and nymph abundance
3. Male and female abundance

To answer the first questions, these weekly *H. halys* observations from 2012 and 2013 were analyzed with serial repeated measures PROC MIXED analysis (SAS 9.3) and followed by a multiple comparison test (Tukey-Kramer) with crop, month, and year as the independent variables and the total number of BMSB as the dependent variable. The independent variables for the second question were adult/nymph, crop, month, and year with the same dependent variable. For the last question, sex, crop, month, and year were used as independent variables to determine the differences in season-long male and female abundance.

When analyzing fruit damage differences, a Fisher Exact test of independence was used to look at differences between peppers surrounded by peppers or peppers surrounded by sunflowers. Differences in damage throughout the season due to differences in BMSB in peppers surrounded by different crops were analyzed using serial repeated measures PROC MIXED analysis (SAS 9.3), followed by a multiple comparison test (Tukey-Kramer) with crop, year, and month as the independent variables.

All analyses were tested at: $\alpha \leq 0.05$.

Results

Cumulative observation of *H. halys*

Sunflowers were more attractive to *H. halys* than peppers. Significantly more *H. halys* were in sunflowers (97%) than in peppers (3%) (F=42.45; DF=1,3; P=0.0073). Additionally, there was an interaction between year and crop (F=15.21; DF=1,3; P=0.0299), with peppers experiencing significantly more *H. halys* in 2012 than 2013 (F=17.71; DF=1,3; P=0.0245 and & Fisher Exact Test; $P=5.31*10^{-272}$). In 2012, approximately 11% of the total number of *H. halys* counted on peppers and sunflowers were found in peppers, while in 2013 only 5% of the *H. halys* were found in peppers. When *H. halys* populations were lower in 2013, a greater proportion of the *H. halys* were found in the sunflowers. However, when the number of *H. halys* found on peppers when surrounded by either peppers or sunflowers was compared, no difference was observed (F=0.77; DF=1,3; P=0.4440).

Crabapples, corn, and trees within a forested area were secondary crops/non-crop plants near the experimental fields in 2012 and 2013. Observations indicate that *H. halys* were found in crabapples more frequently than on any other secondary crop/non-crop plant evaluated (Fisher Exact Test: crabapple verses other; $P=4.396*10^{-14}$). When comparing among crabapple, corn, and trees, significantly more *H. halys* were observed in crabapples, (Fisher Exact Test: crabapple versus corn versus trees; $P=5.180*10^{-106}$), approximately 91%, 3%, and 6%, respectively (Table 4-1).

Seasonal observations of *H. halys*

The crop (F=236.45; DF=1, 190; P<0.0001) and the month (F=17.31; DF=3, 190; P<0.0001) are important in determining *H. halys* abundance in the field over the season. Overall, the sunflower crop showed significantly higher numbers of *H. halys* (15.41±1.66 *H. halys* per replicate) compared to the pepper crop (1.36±1.66 *H. halys* per replicate), similar to previous analysis.

When looking at *H. halys* during each month, significantly more *H. halys* were observed in July than June (Tukey-Kramer=-5.03; DF=3, 190; P<0.001), August than July (Tukey-Kramer=-3.91; DF=3, 190; P=0.007), but not August and September (Tukey-Kramer=-1.64; DF=3, 190; P=0.3582). There appears to be a peak in *H. halys* numbers in August and September (Figure 4-11; Figure 4-12). When month and crops are controlled, the overall number of *H. halys* found did not differ by year (2012: 8.0158±1.4208; 2013: 8.7598±1.2103) (F=0.42; DF=1,190; P=0.5192).

No differences were observed in the number of adults versus nymphs (F=0.31; DF=1, 357; P=0.5809). No interaction was observed between life stage (adult or nymph) and month (F=1.8; DF=2, 357; P=0.1667) (Table 4-2). However, when looking at nymphs exclusively, more 2nd and 3rd instars were observed than the other nymphal stages (F=17.81; DF=2, 538, P<0.0001) and all nymphal stages (1st instar, 2nd - 3rd instar, and 4th - 5th instar) were observed preferentially in sunflowers (F=30.12; DF=1, 538, P<0.0001). Nymphs were most frequently observed in August (F=7.13; DF=1, 538; P=0.0009). There is also interaction between crop and month (F=5.0; DF=2, 538; P=0.0071) and nymphal stage and month (F=7.79; DF=4, 538; P<0.0001) (Table 4- 3).

A 2:1 ratio of females to males was observed for both years (F=9.23; DF=1, 357, P<0.0001) (Table 4-4). Additionally, an interaction between sex and month was observed with

more males in August than July and September and more females in September than July and August (F=3.81; DF=2, 357; P=0.0230).

Feeding damage by *H. halys*

Sunflowers did not prevent damage to peppers. Damage to the peppers fruit was compared between those inner rows of peppers surrounded by sunflowers and those inner rows surrounded by peppers (Figure 4-8; Figure 4-9; Figure 4-10). No significant difference was observed in 2012 (Fisher Exact Test, P=0.1102), 2013 (Fisher Exact Test, P=0.7905), or when the two years were combined (Fisher Exact Test, P=0.0922).

Discussion

H. halys is a particularly economically important pest because of its wide host range. Many vegetable crops, including peppers (personal observation) and beans and peas in the southern United States (Mizell et al. 2008) are threatened by *H. halys*. Understandings of *H. halys* host preferences are useful in creating future farm-based management tools.

In both years of this experiment significantly more *H. halys* were observed in sunflowers than peppers. While it may be possible to use sunflowers as a trap crop the proximity of the trap crop (sunflowers) to the cash crop (peppers) in our experimental plots leaves a number of questions to be answered (Figure 4-13). Factors such as distance of sunflowers from cash crop, size of trap crop relative to cash crop, and other variables need to be determined prior to implementation of this technique for *H. halys* control. Additionally, my study did not have a

comprehensive combination of season-long trap crop potentials. While I was able to show that sunflowers are attractive to *H. halys*, this study does not show that sunflowers alone will work as an effective trap crop. Mizell et al. (2008) recommended a variety of different flower/crop species (triticale, sorghum, millet, buckwheat, and sunflower) throughout the season to control native stink bug populations in the southern coastal plain. Despite the lack of variety trap crops tested in my experiment, sunflowers do hold potential as an attractive component in a trapping crop blend.

H. halys were mostly found in the outer row of Giant/Mammoth Grey Stripe sunflowers (Figure 4-14). Height of plants may be a factor in host choice of sunflowers (Krawczyk, unpublished data). The giant/mammoth grey stripe sunflower variety height is 2.4-3.6 m, while Zohar F1 is approximately 1.2 m tall. Surrounding the outside of the pepper plants with these tall sunflower varieties may have acted as a barrier or trap crop to protect the peppers. However, more research would need to be done to determine how critical the height of the trap crop is to *H. halys*.

Our data suggest crabapples as a possible trap plant. In data from both 2012 and 2013 91% of *H. halys* in the surrounding non-crop plants were observed in crabapples. While the 3-minute visual observations used to assess *H. halys* may not have been as robust later in the season when *H. halys* populations were higher, this bias is the same across all non-crops plants that were assessed. Converting to individual plant observations, as done within the research plot, was not practical for several reasons including the height of crabapples and the other trees and the density of the foliage. Even though it is likely the stink bug counts later in the season were under-counts of the actual population, evidence does suggest that *H. halys* are in surrounding crops and non-crop plants, especially crabapples, and not exclusively drawn to sunflowers (Table 4-1).

The stage of *H. halys* development, i.e. adult or nymph, did not differ by crop, nor were there more adults or nymphs observed. However, as expected, nymphal stages (1st instar, 2nd-3rd

instar, and 4th-5th instar) did show variation throughout the season with more 2nd & 3rd instars found in August and September and more 4th and 5th instars found in September. Ideally, egg mass information and the level of egg viability would be recorded in future field research in order to monitor female fecundity throughout the season. Unfortunately, very few egg masses were observed during this study, and the data were not comprehensive enough to address these questions. Interestingly, more females than males were observed. It would be useful to determine if the sex ratio of females to males remains constant and use this information to produce a predictive model of the future size of the *H. halys* population from early season counts of *H. halys*. It would be interesting to know if the sex ratio remains constant irrespective of plant host or over time.

Even though a clear seasonal trend of increasing *H. halys* population was observed in 2012, during 2013 only a slight peak in *H. halys* numbers towards the later part of the season was observed. Overall the mean number of *H. halys* was much lower in 2013 (Figure 4-8; Figure 4-9). Interestingly, differences in the population of *H. halys* by year were eliminated if the effect of crop and month (but not adult/nymph, nymphal stage or sex) are used in a repeated measures analysis. This may be due to the population of *H. halys* being more evenly distributed throughout the 2013 season while large peak abundance was observed in August and September in 2012.

This study showed that there was no significant difference in damage to the pepper fruit or the number of *H. halys* found within the inner rows of peppers whether the inner pepper plants were surrounded by other peppers or by sunflowers. Damage data was only compared within the internal 8 rows of peppers, while damage to the external 2 rows on each side was not assessed. Because *H. halys* and other stink bugs have been shown to cause damage in edge regions of other crops, such as soybean (Tillman et al. 2009; Leskey et al. 2012), an edge effect may exist. Thus it may be possible to use this edge effect to reduce damage to the interior crop.

While feeding damage did not differ significantly between the peppers surrounded by sunflowers or by peppers, a possible trend to significance was observed. In 2012 when the *H. halys* population was high, less damage than expected was observed in pepper fruit surrounded by sunflowers than in pepper fruit surrounded by peppers. Additionally, when the data from both years were combined the results were nearly significant (P=0.0922). Thus it may be possible that larger sunflower plants, greater distance of sunflower plots from cash crop or other factors could lead to reduced crop damage, especially in years with high population pressure.

While feeding damage was measured in this study, all feeding damage cannot be directly attributed to *H. halys* as several native stink bugs (i.e. the green stink bug [*Acrosternum hilare* (Say)], the dusky stink bug [*Euschistus tristigmus* (Say)], the brown stink bug [*E. servus* (Say)], the red-shouldered stink bug [*Thyanta custator accerra* (McAtee)], etc.) and tarnished plant bugs [*Lygus lineolaris* (Palisot de Beauvois)] were located in the pepper field throughout the season. It is likely, however, that *H. halys* causes the majority of damage as the numbers of native stink bugs were low (personal observation).

In both 2012 and 2013, *H. halys* were observed more frequently on sunflowers planted surrounding the pepper field. Future research should evaluate the impact of distance of sunflowers from the crop and determining optimal placement of a variety of trap crop mixtures. If the mixture is attractive enough, there is potential to prevent *H. halys* from invading cash crops, benefit pollinators, and minimize pollinator disruption through pesticide usage.

It is important to recognize that sunflowers are attractive to a variety of native pollinators, and so care must be taken if chemical control is used within the sunflower trap crop to reduce *H. halys* populations. Visual observations during the 2013 field season included an informal midnight observation of sunflowers to determine if *H. halys* remain in the sunflower crop overnight. These anecdotal observations suggest *H. halys* remain in fields at night, rather than leaving for relatively nearby wood lines. While the nighttime presence of *H. halys* may present

an opportunity for the application of chemical controls, it is important to note that native pollinators, e.g., bumble bees, are observed on flowers early in the morning or late at dusk (Kapustjanskij et al. 2007) so direct and residual effects on pollinators need to be taken into consideration.

Sunflowers planted as a trap crop may hold potential for reducing pesticide applications and increasing the use of integrated pest management. Additionally sunflowers can be a specialty crop on small farms as cut flowers. Another benefit of sunflowers as a trap crop on small farms participating in agro tourism is the ability to raise awareness of sustainable management practices on these small farms. Many consumers are aware of *H. halys* as a pest both within their homes, and local agricultural communities. Control of *H. halys* through trap cropping that minimizes pesticide load has potential for community support and economic gain. However, to achieve these goals, more research into *H. halys* trap cropping must be done.

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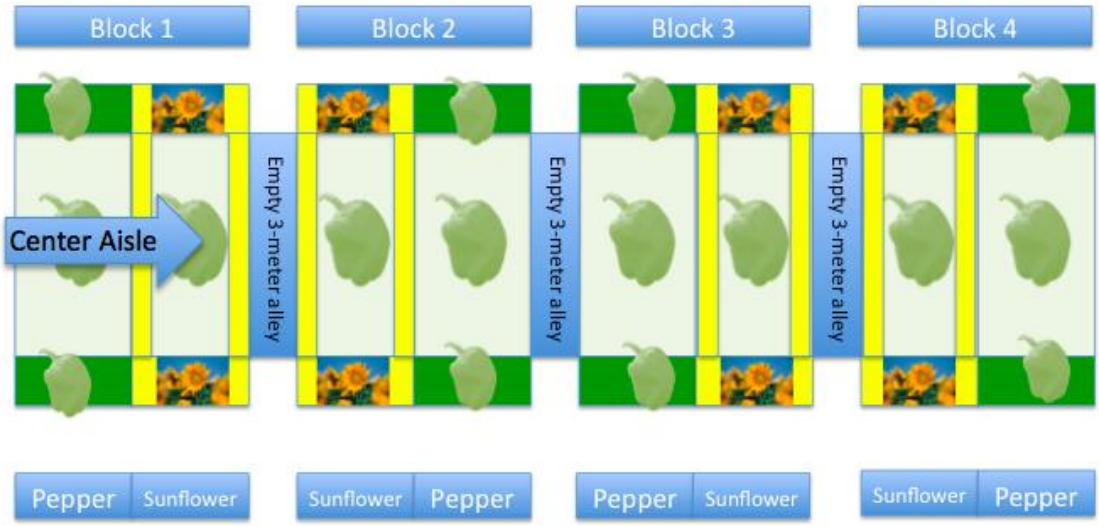

Figure **4-1**: The 2012 plot layout of pepper and sunflower blocks. Either peppers, or sunflowers surrounded inner peppers. 2013 included a center aisle, as indicated by the center aisle arrow.

Figure **4-2**: Outer two rows of sunflowers surround inner peppers. A small alley lies between this block and the next sunflower block.

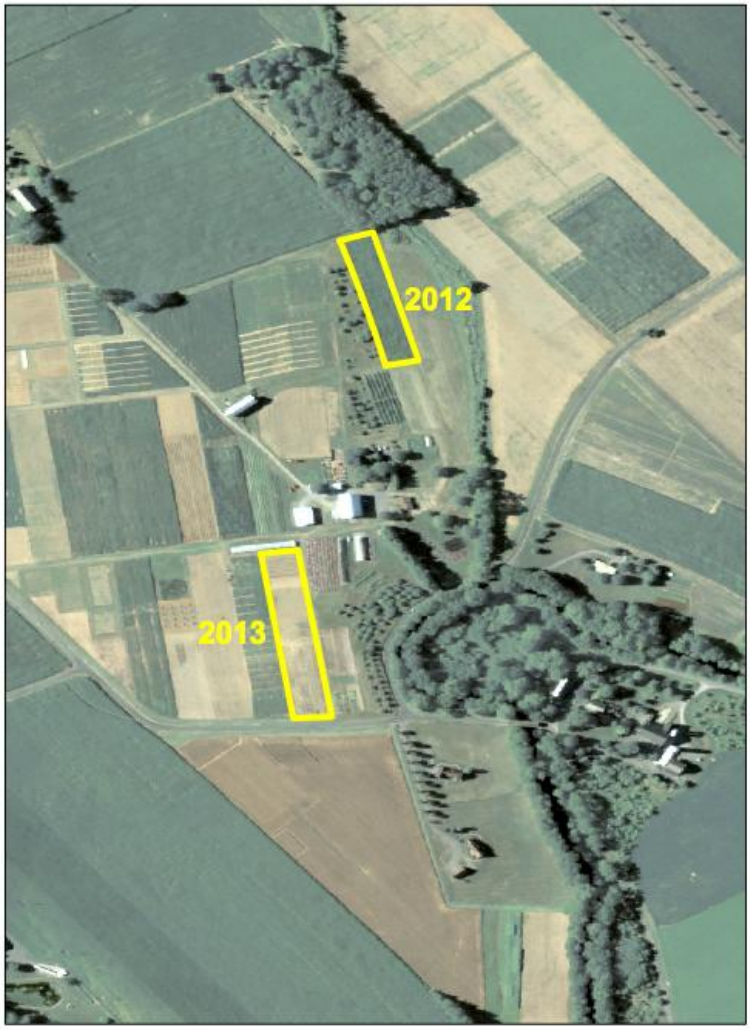

Figure **4-3**: Location of field plots at the SEAREC research station in 2012 and 2013. In 2012, the field plot was close to a wood line, while in 2013 the field plot was close to pine and maple trees.

Figure **4-4**: Pepper field and surrounding environment, 2012 field season. In 2012, the field plot was next to corn, and crabapples, while adjacent to a wood line.

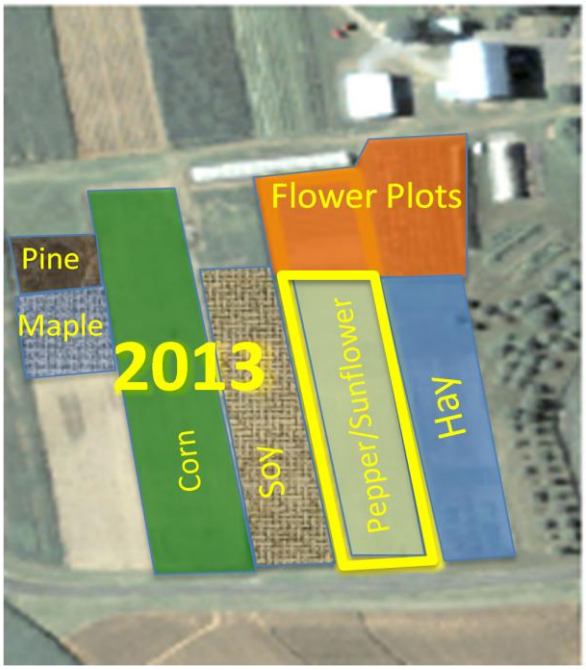

Figure **4-5**: Pepper field and surrounding environment, 2013. In 2013, the field plot was next to soy, corn, hay, and flower fields, and near to pine and maple trees.

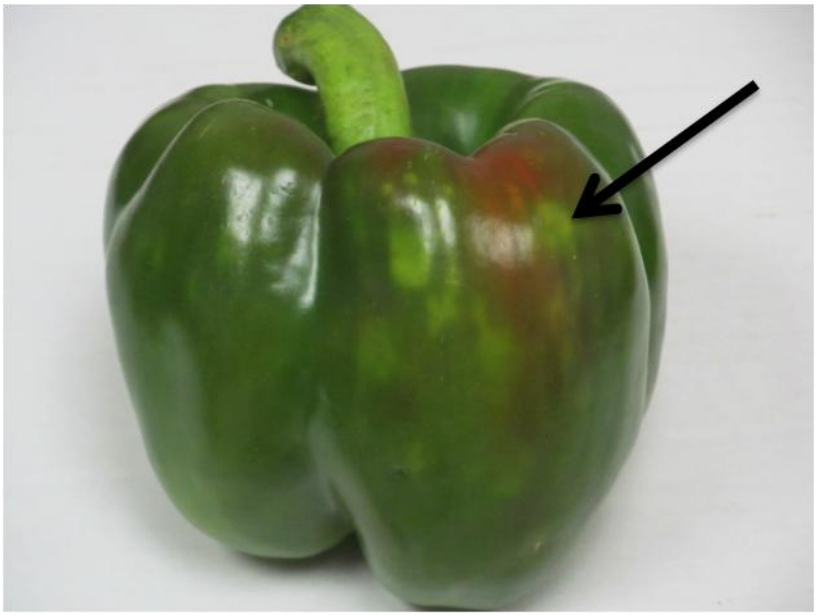

Figure **4-6**: External pepper damage from stink bug feeding. The external, pale discoloration is indicative of damaged tissue.

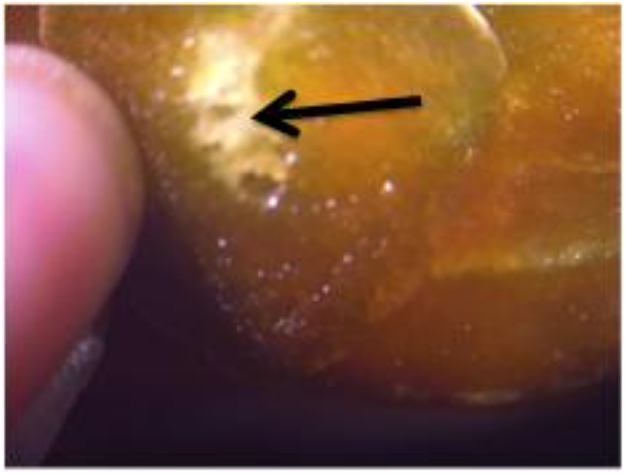

Figure **4-7**: Internal injury to pepper as a result of stink bug feeding. The flesh where feeding occurred is spongy and pale.

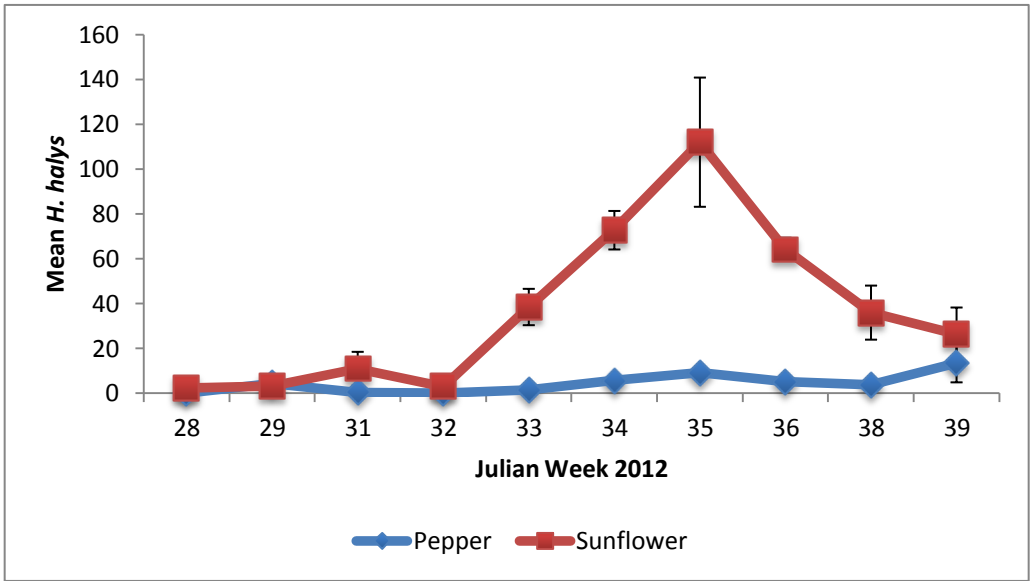

Figure **4-8**: Mean number of *H. halys* found in pepper and sunflower crops in 2012 season. Peak number of *H. halys* occurs around week 35, the first week of September, with sunflowers being a favored crop.

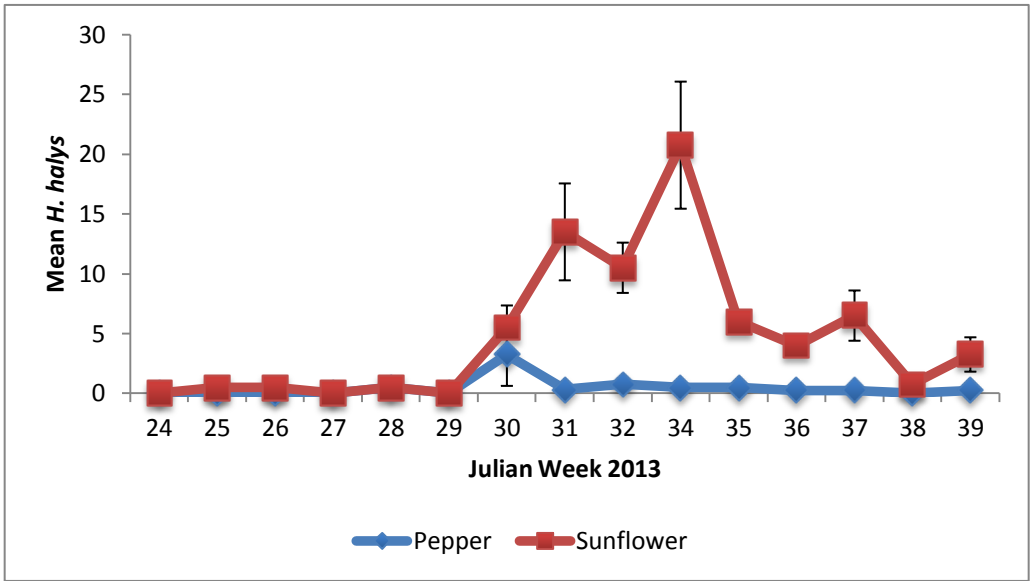

Figure **4-9**: Mean number of *H. halys* found in pepper and sunflower crops per Julian week in 2013 season. Number of *H. halys* peak around week 34, the end of August. Overall numbers were lower than the 2012 season. *H. halys* were more commonly found in sunflowers.

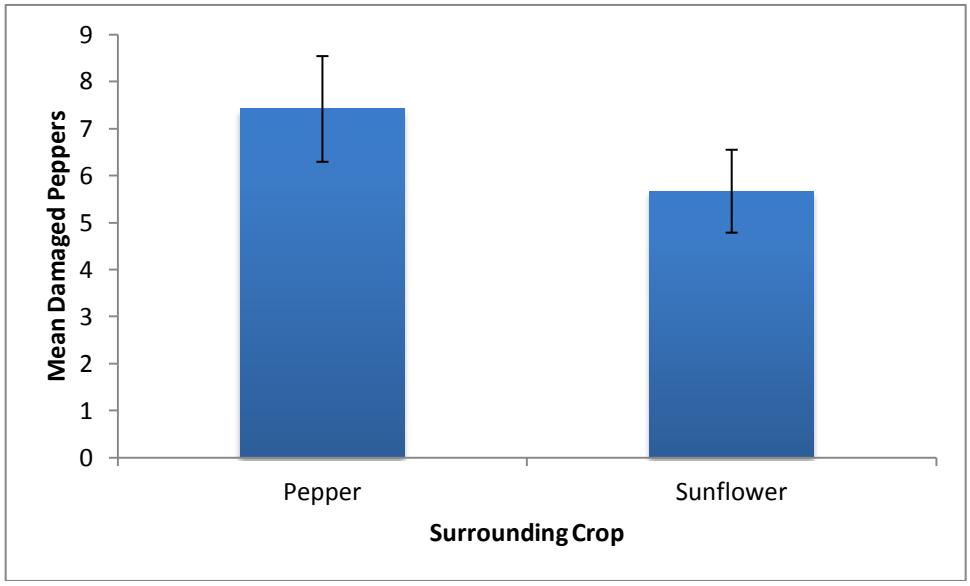

Figure **4-10**: Inner rows of mean pepper damage during 2012 field season. There was no significant difference between damage levels to inner peppers, regardless of surrounding pepper or sunflower rows (Fisher Exact Test, P=0.1102). Damage data was collected at approximately two-week intervals, with three evaluations in 2012. Each evaluation examined 128 peppers for damage.

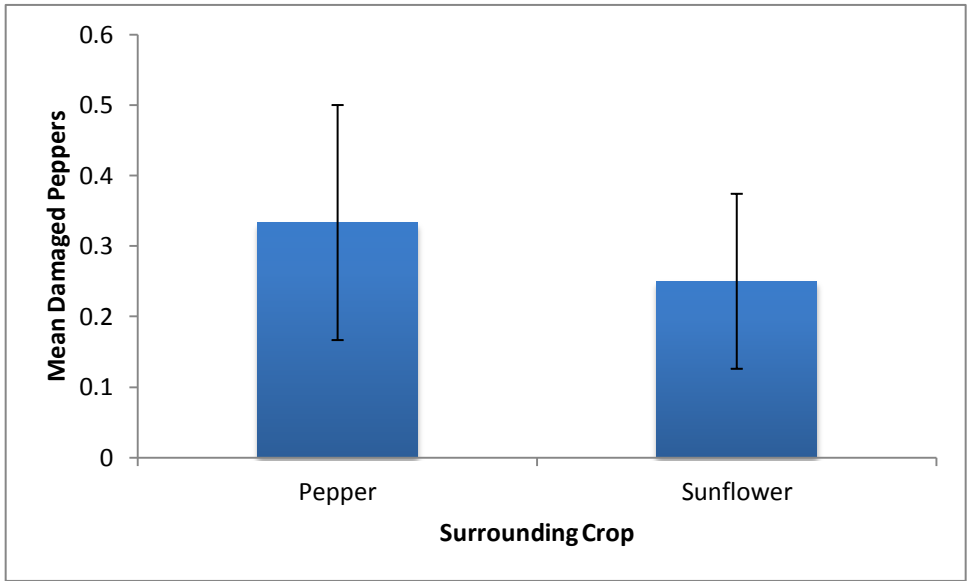

Figure **4-11**: Inner rows of mean pepper damage during 2013 field season. There was no significant difference between damage levels to inner peppers, regardless of surrounding pepper or sunflower rows. (Fisher Exact Test, P=0.7905). Damage data was collected at approximately two-week intervals, with six evaluations in 2013. Each evaluation examined 128 peppers for damage.

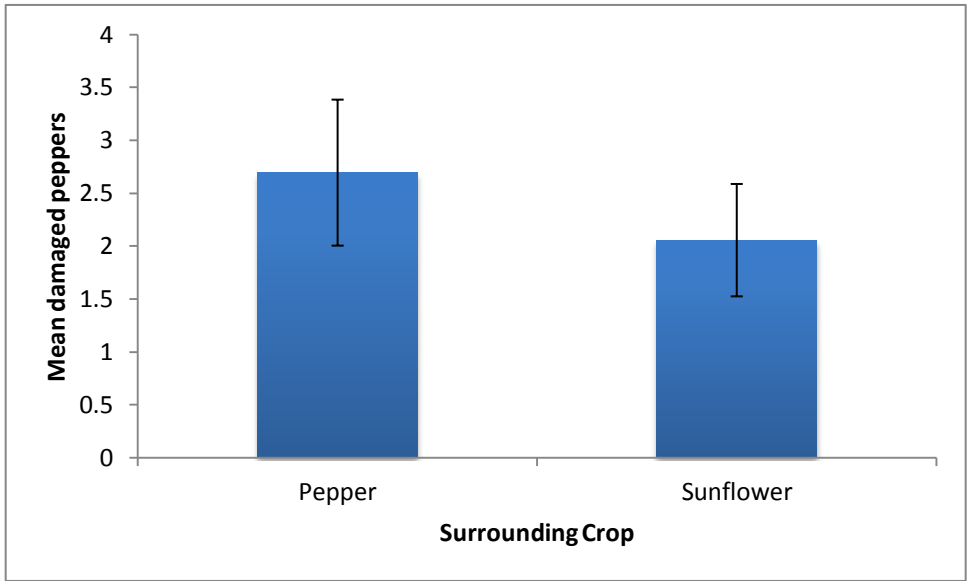

Figure **4-12**: Inner rows of mean pepper damage across both seasons. Either peppers, or sunflowers surrounded the inner pepper crop. There was no significant difference between damage done to the inner peppers, regardless of the surrounding crop. (Fisher Exact Test, P=0.0922)

Figure **4-13**: Pepper block next to sunflower block in 2013 field. While sunflowers hold potential as a trap crop, some questions still need to be answered, such as proximity to the cash crop, and the size of the trap crop relative to the size of the cash crop.

Figure **4-14**: *H. halys* was often found on sunflowers. *H. halys* nymph feeding on sunflower stem, as indicated by stylet insertion.

Table **4-1**: Presence of *H. halys* in secondary crops of crabapple, corn, and forest. *H. halys* were found most frequently in crabapples (Fisher Exact Test; $P=5.180*10^{-106}$).

Crop	Frequency	Percent
Crabapple	293	91.28
Corn	8	2.49
Forest	20	6.23

Table **4-2**: Analysis of life stage (adult vs. nymph) indicating the significance of crop (F=61.81; DF=1; 357 P<0.0001), and the interaction of crop*month (F=20.88; DF=2, 357; P<0.0001).

	F-value	Degrees of Freedom	P-value
Crop (Pepper Sunflower)	61.81	1, 357	<0.0001
Life Stage (Adult Nymph)	0.31	1, 357	0.5809
Year	3.41	1, 357	0.0656
Crop*month	20.88	2, 357	<0.0001
Life stage*month	1.8	2, 357	0.1667

Table **4-3**: Analysis of nymphal stage (n1, n2-n3, n4-n5) shows the significance of the stage (F=17.81; DF=2, 538, P<0.0001), and the month (F=7.13; DF=1, 538; P=0.0009). Also shown is the interaction between crop and month (F=6.0; DF=2, 538; P=0.0071) and nymphal stage and month (F=7.79; DF=4, 538; P<0.0001).

	F-value	Degrees of Freedom	P-value
Nymph Stage (n1, n2-3, n4-5)	17.81	2, 538	<0.0001
Month	7.13	1, 538	0.0009
Crop*month	6.0	2, 538	0.0071
Life stage*month	7.79	4, 538	<0.0001

Table **4-4**: Analysis of *H. halys* showing the significance of the crop (F=50.77; DF=1, 357; P<0.0001), year (F=16.98; DF=1, 357, P<0.0001), month (F=28.50; DF=2, 357; P<0.0001), and sex (F=9.23; DF=1, 357, P=0.0025). Interactions between crop and month (F=33.84; DF=2, 357, P<0.0001), and sex and month (F=3.81; DF=2, 357; P=0.0230) were also shown.

	F-value	Degrees of Freedom	P-value
Crop	50.77	1, 357	<0.0001
Year	16.98	1, 357	<0.0001
Month	28.50	2, 357	<0.0001
Sex	9.23	1, 357	<0.0001
Crop*month	33.84	2, 357	0.0025
Sex*month	3.81	2, 357	0.0230

Chapter 5

Invasive species awareness program in Belgrade, Serbia

Introduction

International travel and trade may be important to global markets, but people and goods are not the only ones to travel. Invasive species are a common, and constant, threat to natural and agricultural ecosystems around the world (Mills 2006). However, not all non-native species are actually considered invasive. Instead, the word invasive is commonly used to discuss a creature seen as disrupting human endeavors (Riley 2008). Not only do they threaten natural populations, capable of displacing an area's native creatures, they can also cause heavy economic damage (Lodge and Shrader-Frechette 2003). International trade and travel have increased the number of species being globally transferred. While only 1% of the species being transported may actually establish in a new environment (Mooney & Cleland 2001), invasive species are becoming a greater threat to biodiversity and food security.

One example of a devastating invasive species in the United States is the brown marmorated stink bug, *Halyomorpha halys* (Stål). First confirmed in 1996, in Allentown Pennsylvania, this pest caused over $37 million in damage to United States' Mid-Atlantic apple production in 2010 alone (US Apple Association). This polyphagous hemipteran has a host range that includes over 300 plant species, ranging from legumes to vegetables, fruits, and even ornamental plants (Nielsen 2009; Maryland Cooperative Extension 2010; Sparks 2010). This invasive species has created havoc for the United States and was also identified in Switzerland in 2007, and Italy in 2014 (Wermelinger et al. 2008; Maistrello et al. 2014).

With *H. halys* possessing the capability to cause extreme economic impacts, it is important that a program to control accidental introductions of this pest be in place. Conceptual models of species invasions in ecosystems compare economic impacts of the costs of prevention vs. remediation, and at times a greater investment in prevention is much more beneficial (Leung et al. 2002).

The purpose of this chapter is to propose a theoretical framework to help establish an invasive species awareness (ISA) program in Serbia. This chapter looks at governmental, educational, and economical systems as three key components of invasive species control (Figure 5-1). In particular, this chapter will focus on the potential importance of invasive species (particularly as it relates to *H. halys*) Serbian agriculture, and the development of a theoretical *H. halys* ISA program.

The importance of Invasive Species Awareness (ISA)

Invasive species such as *H. halys* can threaten ecosystem stability worldwide when they hitchhike across the globe (Simberloff 2000; Pimentel et al. 2001). Unintentional introductions of invasive species are often by-products of international travel and trade, and can potentially be economically expensive (Aukema et al. 2010; Leung et al. 2002). As of 2005, there were an estimated 50,000 various invasive species in the United States with over 95% of these species accidentally introduced, many with the potential to cause direct economic harm (Pimentel et al. 2005). The invasive emerald ash borer, *Agrilus planipennis*, is expected to cost nearly $10 billion over the next decade for landscape tree treatment and removal alone (Kovacs et al. 2010) while pests such as the Formosan termite, *Coptotermes formosanus,* already cost $1 billion per year in structural damage in the southern United States (Pimentel et al. 2005). Overall, invasive

species are responsible for up to $120 billion in economic damages and control costs per year in the United States (Pimentel et al. 2005).

Prior to successful colonization of new environment by any species, there are several abiotic and biotic factors such as climate, food, and habitat availability known to affect and possibly limit distribution ranges of invasive insects in new habitats (Musolin 2007). Typical insect colonization within a new area follows four distinct stages:

1. Introduction and survival
2. Establishment
3. Range expansion and adaptation to the new environment
4. Divergence

All of these four steps have to be fulfilled before any species, including *H. halys*, can successfully establish in a new environment (Musolin 2007). In the case of *H. halys*, this species was most likely introduced into the United States in shipping containers (Hoebeke and Carter 2003). By now, *H. halys* has already proven adept at surviving in large parts of North America, and has the potential to expand to new global areas (Zhu et al. 2012).

One important aspect of invasive pest control is to identify optimal and cost-effective approaches to manage the costs of controlling an invasive species while effectively guiding search efforts (Buhle et al. 2005; Sharov and Liebhold 1998). However, effective search strategies of a new pest can be difficult to develop and establish. Questions such as where to search, and at what intensity searches should be conducted, can be very difficult to answer (Baxter and Possingham 2011). There is a difference between preventing and controlling an invasion with main trade-offs existing between the cost of surveillance and the cost of management (Leung et al. 2002). Surveillance costs and damage caused by a new pest frequently increase with delayed detection, therefore greater monitoring efforts may be warranted in areas that do not already have established populations (McCarthy et al. 2012). Spatial prioritization of

search efforts are important as the value of the detection and surveillance method directs how much surveillance needs to be done and where to direct it (Hauser and McCarthy 2009).

One of the biggest challenges in documenting the presence of a new pest is the low probability of detecting invasive species when they are rare in an area. However, the likelihood of detecting a population increases with a larger invasive species population (McCarthy et al. 2012). Thus, surveillance resources and search must be properly allocated to detect the invasive species and minimize the cost of potential control/management programs. Program budgets need to account for the duration of the control program, allocations for surveillance efforts and the cost of the control measures. Control requires the optimum allocation of resources between the eradication and the containment of an invasive species, both expensive to enact (Cacho 2011).

Serbian agriculture

Serbia, an eastern European country, formerly part of Yugoslavia, is one example of a country that could benefit from an 'ounce of prevention' when related to invasive pests. Serbia is an emerging democracy in Europe, close to Romania and Montenegro near the Adriatic Sea. The country is landlocked, but has a variety of rivers and tributaries crisscrossing the nation, the most important of which are the Danube and Sava Rivers, which meet in Belgrade, the capital (Stavrianos, 1958).

In Serbia, agriculture constituted over 5,093,000 ha of the land use (European Environment Agency 2011). Around 310,000 hectares of agricultural land are committed to fruit production. Major fruit crops include plums, blackberries, strawberries, peaches, cherries, raspberries, apples, pears, grapes, and apricots (SIEPA report 2012). The majority of growers have small farms; 75% of the farms are less than 5 ha (USAID 2008). With all of this fruit production, apples were identified as the second most valuable tree fruit and the fifth most

valuable overall crop of Serbia in 2006 (USAID 2008). Fruit production can bring increased income to individuals, as well as improved health (Veljkovic).

As Serbia begins it's journey into joining the European Union, farmers will have to comply with greater restrictions on pesticide and herbicide, and the quality of their fruit exports will need to be improved. An introduction of invasive species like *H. halys*, could be very destructive for Serbian agriculture.

As of 2011, 17.3% of the Serbian labor force was linked with agriculture (Serbian Government 2012), while industry constituted 19.5% and services 58.6% of the work force (FAO 2010). Unfortunately, 23.7% of Serbians were unemployed (FAO 2011), and this unemployment rate is expected to rise. Any additional disturbance in such a delicate labor situation, such as a disturbance caused by the introduction of an invasive pest, could potentially place the country's agricultural workforce at risk. Learning how to rapidly recognize new invasive species such as *H. halys* could help in at least preventing unnecessary crop loses and related challenges.

A model predicting the suitability of habitat for *H. halys*, suggests that the Serbian area provides a highly suitable range for *H. halys* and places Serbia in an area of high risk (Zhu et al. 2012). However, it is important to note that this does not necessarily mean invasion is imminent.

Evaluation of interest in a Serbian ISA program

When trying to determine the need, and feasibility, of an invasive species awareness program for Serbia, I went through two stages of evaluation. The initial evaluation stage involved a visit to Serbia for preliminary information gathering and assessment, while the second stage involved the development of a survey and questionnaire to help develop the ISA program.

Assessment trip

Prior to developing the theoretical ISA program, a preliminary information gathering was required. In June 2012, a group of researchers and I visited Belgrade, Serbia and the surrounding countryside. During this visit, farmers, researchers, and extension personnel from both the United States and Serbia discussed the current status of Serbian fruit production and several potential invasive species of interest, among them *H. halys*. Small fruit producing farms (around 1-2 ha) were visited along with large production orchards up to 240 ha in size. During these visits, an interest in improving fruit production practices was common-along with an interest in protecting crops from invasive species. Following this assessment visit to Serbia, I decided to develop a survey and focus group questionnaire to establish a better understanding of the challenges facing Serbian agriculture.

Surveys and focus group questionnaires

In July 2013, I asked a small group of Serbian farmers, educators, and agricultural consultants visiting Pennsylvania if they would participate in a short survey and focus group questionnaire (IRB approval number 43069; See Appendix B, and Appendix C). Ten individuals participated in the survey and their assistance was invaluable when trying to gain insight into the issues faced by growers. I asked the participants about the current status of Serbian agriculture and their current level of invasive species awareness. Representatives of the U.S. Embassy in Belgrade, the University of Belgrade, state and private extension personnel, and farmers participated in the survey. All of them had worked in the Serbian fruit industry for a minimum of one year, with one individual having served the industry for over twenty years. Most participants

were involved in the fruit industry for 11-20 years and were familiar with various aspects of tree fruit production in Serbia.

I asked participants if they were currently involved in any invasive species monitoring or reporting programs. An overwhelming number of them were not involved, or uncertain, about their participation in a standing program related to invasive species. It was interesting to note that farmers would report invasive species to their extension advisors, and then the extension/research personnel were aware of requirement of reporting these observations to the Ministry of Agriculture, the Institute of Plant and Environmental Protection or the Institute for Phytomedicine.

I also asked participants what are some of the current agricultural issues facing Serbian farmers. In response, I learned that horticultural issues were often seen as the most important issues, with plant nutrition, rootstocks, pruning, and irrigation listed as 'very important'. Fruit harvesting and marketing were both seen as 'very important' issues, with post harvest handling, storage, and transport to quality markets being an important aspect of fruit production. The absence of active Serbian fruit grower association(s) was also perceived as an obstacle, with a commonly held belief that greater unity among the growers was important for the fruit community.

Pest awareness was most important for insect pests, as compared to lower interest in weed and mammalian pests. Invasive insect species other than *H. halys* that could be addressed by this program, included *Drosophila suzukii*, *Tuta absoluta*, *Tettigonia viridisma*, and *Phthorimae operculella*.

In Serbia, farmers often met with their advisors on a weekly basis, and advisors were often on a daily or weekly schedule with their farmers, but invasive species monitoring topics were discussed rarely. Extension agents often monitored for invasive species, but most of the research personnel and farmers searched for invasive species only periodically or never.

However, most respondents felt well-informed about invasive species, and were interested in learning about/participating in new invasive species awareness program as an addition to the current invasive species awareness systems functioning in Serbia, similar to the Penn State University PestWatch program.

Throughout conversations with Serbian participants, the overarching need seemed to be the development of an effective way to get information to growers. Pamphlets, lectures, Internet, local television, and local radio stations are used to disseminate information to growers, with larger-scale agricultural issues reaching larger television and radio stations in order to reach a larger audience. However, with funding an issue in many places, it is difficult to get information to all of the farmers who need assistance. It was suggested by participants, that greater participation with local governments could help Serbian farmers to access information.

Theory behind the ISA program

By creating a program to enable farmers and extension educators to recognize new invasive species, it will be possible to protect the agricultural production as well as building leaders of the agricultural community. As such, I propose to create a model program that will build agricultural community leaders through invasive species awareness. This model program can also be utilized in other systems if proper adjustments for the culture and area are taken into account

The program itself will focus on a practical application of pre-emptive action and increasing awareness of invasive insect pests to farmers and extension educators. The proposed program is based on three important components: Maslow's Hierarchy of Needs, the Wilkinson Community Field, and the Penn State Cooperative Extension Program Development Model.

Maslow's hierarchy of needs

Maslow's hierarchy of needs (Figure 5-2) makes it is possible to tailor invasive pest awareness programs to their most important components. This program deals with a combination of physiological and safety needs. Food security and quality in the fruit industry are the most important needs to be addressed when developing an ISA program.

Wilkinson social fields

In 1999, Wilkinson classified the community field to include components like recreation, housing, transportation, social services, health, education, economy, faith and religion, government. All of these factors influence the community field (Figure 5-3) and the effectiveness of the program. While this program is designed for the agricultural community, a variety of factors (as previously listed) influence decision making in this field.

Penn State cooperative extension program development model

By using the Penn State Cooperative Extension Program Development Model (R.B. Radhakrishna 2012, personal communication), I hope to balance program activities and help lay the framework for invasive species identification activities and agriculture protection (Figure 5-4). The first step in this model involves situational analysis and problem identification. As previously mentioned, our project is a pre-emptive program to stop invasive species such as *H. halys* from establishing in Serbia.

Theoretical ISA program

Location

The initial implementation of this model will be a pilot program in Belgrade, Serbia. Not only is the city easy to reach for program implementers but the capital is located centrally within Serbia. By focusing on this area, participants from wealthier northern regions and poorer southern regions should be able to reach the capital for program attendance. With 1.6 million people in and around Belgrade, there are a wealth of small growers and residents with small 1-2 ha apple orchards that can be used for a field component of the program. For the information acquisition sessions, Belgrade will have available technology, facilities, and resources for holding a successful program.

The audience

This program is designed for two tiers of audience: extension educators and government officials as the primary audience and growers as the secondary audience. It is important that the primary audience support the program in order to effectively disseminate invasive species information to the secondary audience.

The secondary audience consists of growers between the ages 18-70, growers with less than 5 ha of apple orchards, and large producers. With only a limited number of primary audience members able to travel and search for invasive species, this secondary audience is where the actual program is necessary to increase invasive species knowledge and identification.

Objectives

1. After attending the first week of the program, 60% of Serbian extension educators and farmers will increase their knowledge of invasive species recognition and reporting by 20% as measured by a pre- and post- test. The real purpose of this objective is to get an increase in knowledge (cognitive objective).
2. After the second week, 80% of extension educators and farmers will be able to identify invasive species, and 80% will be able to properly report them to the correct authorities. This will be evaluated based on the individual's ability to demonstrate proper identification of the pest and proper monitoring tools for each pest (psychomotor objective).
3. Following the third week of the program, 60% of extension educators and farmers will have an increased likelihood of recognizing and 40% of extension educators will have an increased likelihood of reporting invasive species. This will be measured by a 25-question survey consisting of a 15-question post-test, and a 10-question attitude and aspiration survey (affective objective).

Curriculum guide

The main portion of this program will be carried out during three weeks in June. There will be one session per week. The first week (Table 5-1) will be focused on cognitive aspects. Primary and secondary audiences will be introduced to some of the most threatening fruit pests that are likely to affect them. They will be given information and fact sheets on recognition and identification of these pests.

In the second week, (second day of the on-ground program), participants will attend a field workshop (Table 5-2). Participants will become familiar with insect monitoring tools and setups of the pest monitoring equipment. They will also be shown proper maintenance of traps that will be set up at strategic locations throughout the country. Small hand-held magnifying lenses will be distributed to participants to improve identification of important insect characters. During this field day, participants will also meet, and become aware of the proper government and extension authorities to contact should identification of an invasive species pest occur. The evaluation for this day will be measured by participant's ability to identify pests and match the pest to the proper monitoring tool. Participants must also demonstrate ability to set-up, check, and to report trap findings through a mock finding of the pest.

The third and final week on the ground in Serbia will involve a discussion among extension educators, government officials, and farmers (Table 5-3). Program leaders will facilitate this and cover the knowledge obtained, concerns, and future directions they wish to take with the program. An evaluation will be completed in a 25-question survey (post-test of 15 questions; attitude and aspiration 10 questions).

Both primary audiences and secondary audiences will seek to improve knowledge about invasive pests and monitoring. Primary audiences will also be seeking knowledge of how to disseminate knowledge to secondary audiences. Meanwhile, farmers will be increasing their knowledge of the proper communication/ reporting routes to follow when an invasive species is spotted. Please see 'Time Frame' for longer-term curriculum (Table 5-4).

Community members involved

Local community members, and government officials, will need to be included in this program in order to ensure its success (Figure 5-5). The main member of the primary audience

will be the personnel of the Ministry of Agriculture, Trade, Forestry, and Water Management. It is essential that a memorandum of understanding (MOU) be developed with this group in order to have their support for the program, make them available to participants, and to encourage a future nation-wide training system.

The University of Belgrade, the Fruit Research Institute of Čačak, Serbia, and the Plant Protection Society of Serbia will be essential from a research and continued investment aspect. These groups will help with a continuous search for invasive species, technology development, and verification of monitoring techniques within Serbia.

Consulting groups and agencies such as Nedeljkovic Consulting, co-operatives such as Prima, and other NGOs and farm associations will be essential for disseminating information and keeping farmers involved in the program. The extension group will have to work on planning programs for when invasive species are properly identified. This program will also help with disseminating information, as discussed in the 'Advertising the Program' section of this document.

On a smaller scale, it will be very important to make this program accessible to individual farmers- particularly with regards to the target audience previously identified. On a large-scale, particularly important for funding and future progress, there are multiple line agencies that will be important through the duration of the project period for both technical and financial support. Some of these agencies include the USAID, IFAD, UNDP, World Bank, the Millennium Challenge, USDA Aphis, the National Bank of Serbia, CNFA, IPARD, and United States Universities including, but not necessarily limited to, the Pennsylvania State University.

Time frame

This program is based on a 2-year time frame. The first five months will require proper preparation, and if this program is able to start at the beginning of the year, the timeframe can be found in Tables 5-5, 5-6, and 5-7.

If interest is high enough, it will be encouraged that identified Serbian leaders have a 2-hour workshop every six months to help reinforce monitoring and identification of invasive pests for both previous participants, and newly interested parties.

Advertising the program

To attract the target audiences, advertising will need to be carried out through farmer field schools (included in this program), mass media, publications, workshops and seminars (to be held every six months at the discretion of Serbian extension educators), and farmer responses spreading the word of the program and the importance of monitoring.

Prior to implementing the program, it is imperative that program coordinators meet with people responsible for local implementation. The program will need to be available in English, Serbo-Croatian, and Romani languages to reach educators, farmers, and migrant gypsy workers as needed. The program will be advertised at small local gatherings, small farms within the Belgrade area, within a Belgrade business building where this program will take place, and at the Belgrade Horticultural Fair.

Evaluation process and forms

There are several ways the program will be evaluated: both during, and at the completion of, the program. During the program:

1. For Objective 1, there will be a pre and post-test consisting of 15 questions.
2. For Objective 2 there will be a demonstration of skills learned, and a matching checklist of monitoring techniques and the pests they may relate to.
3. For Objective 3 there is a 25-question survey consisting of a 15-question post test, and a 10 question attitude and aspiration survey.

At each follow-up teleconference there will be a pre- and post- test assessing the level of the knowledge adoption.

At the completion of the program, the results of these exams will be quantified, and retention of information calculated, along with a final 10 question aspiration survey to see if there has been a long-standing change in attitude about the dangers of invasive species and participants willingness and ability to report invasive species.

Tracking forms

In order to quickly check in on the progress of the program, there is a 2-year checklist that has been included to keep track of the completed activities. If there is questioning about the objectives, it is important to identify how the objective and goal can be clarified or improved as needed (Tables 5-5, 5-6, and 5-7).

Tips

Some suggestions with this program need to be taken into consideration by both program providers and program participants.

1. It is very important to realize that admission to the European Union makes this program even more essential. With the change in regulations that will include the loss of certain pesticides

and herbicides, as well as a reduction in the amount of chemicals that can be used, it is important that invasive species be recognized early. Without quick recognition and targeted action, the country will most likely find themselves with a loss of product quality and economic difficulties.

2. This program is designed to get people involved and working on training themselves. The point of this program is to establish an initiative, but at the completion of the program all external presence should be eliminated and Serbians take charge of educational activities in their country and food security themselves.
3. While there are many invasive species that could be a problem, it is important to keep it simple enough for farmers to understand, and economical that the extra time required for monitoring will not make it unattractive. This is also why only apple production is being targeted for this program.
4. Serbia is a very proud country with a rich culture and history. It is important to remember that they have great pride in their country and their produce; they know they can have a good product, this program is just to help them keep a good product in the future.
5. This program can be adapted to a variety of areas/cultures/situations, but it is important to tailor this for specific audiences you wish to reach.
6. Long-term benefits - how will this benefit people at the completion of the program?

Discussion

Serbia is a nation that will benefit with the protection of its fruit industry, and the assurance of food quality and security. Invasive pest species can become very devastating for any country, but could be particularly devastating for Serbia's agriculture as the country enters the European Union. While it may be difficult to convince people of its merits, a pre-emptive action

program designed to increase knowledge of invasive pests will be the most cost-effective educational program in the long run.

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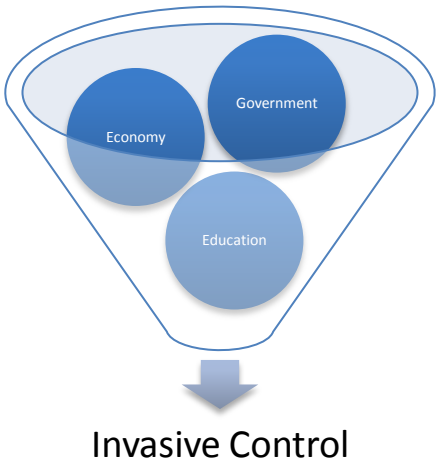

Figure **5-1**: The three factors that appear to be most closely related to invasive species control. Government, Economy, and Education. All three of these factors are of particular importance with the proposed ISA program in Serbia.

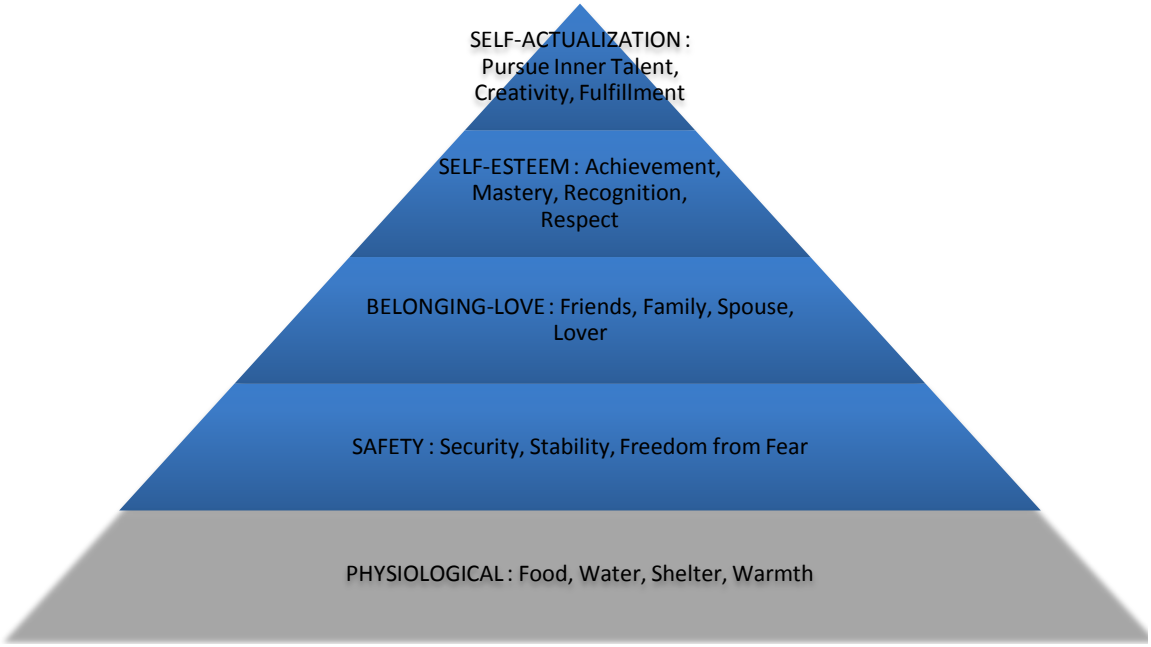

Figure **5-2**: Maslow's Hierarchy of Needs. Note Safety and Physiological needs. When developing a program, it is important to realize that there are a variety of inputs that will affect decision-making, and physiological and safety needs are the most important.

Figure **5-3**: Wilkinson (1999) Community Fields. Note that a variety of factors influence and make-up the community field. (Wilkinson 1999). A variety of inputs, including economy, education, government, and social services, all influence decision that community members will make.

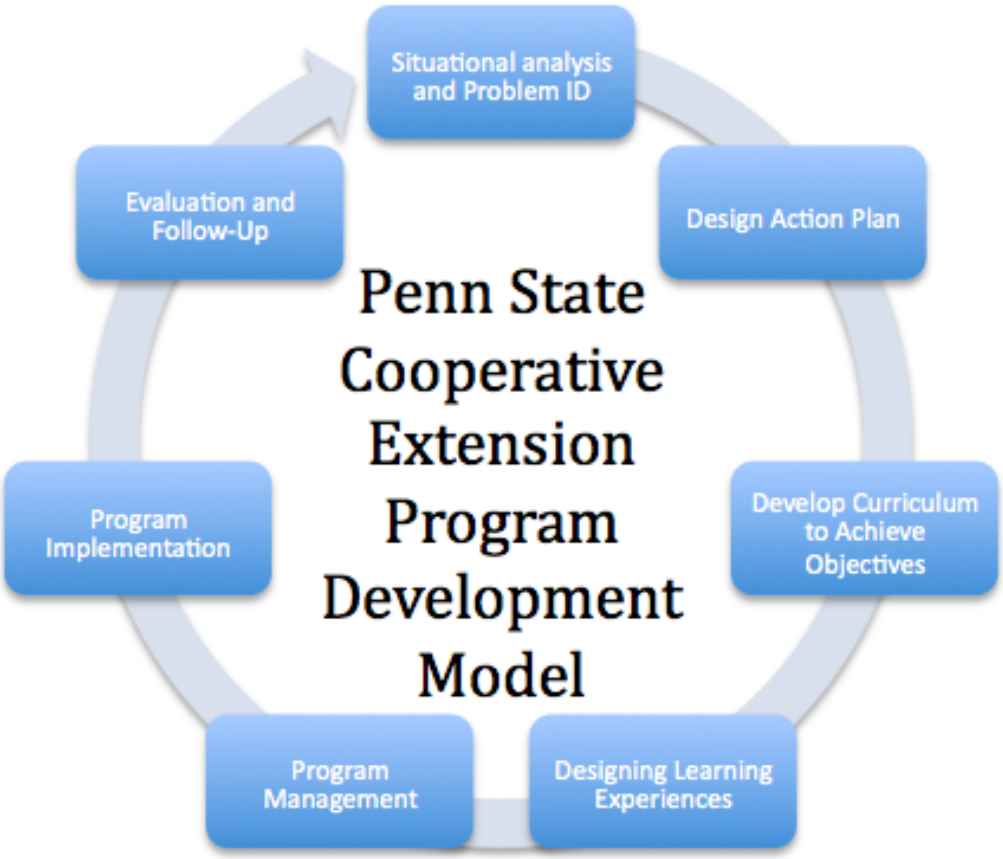

Figure **5-4**: The Penn State Cooperative Extension Program Development Model. This model is the basic framework for the Serbian program, and can be applied to other situations as needed. Identifying issues of concern, and working through to evaluation and follow-up, are important for a successful program.

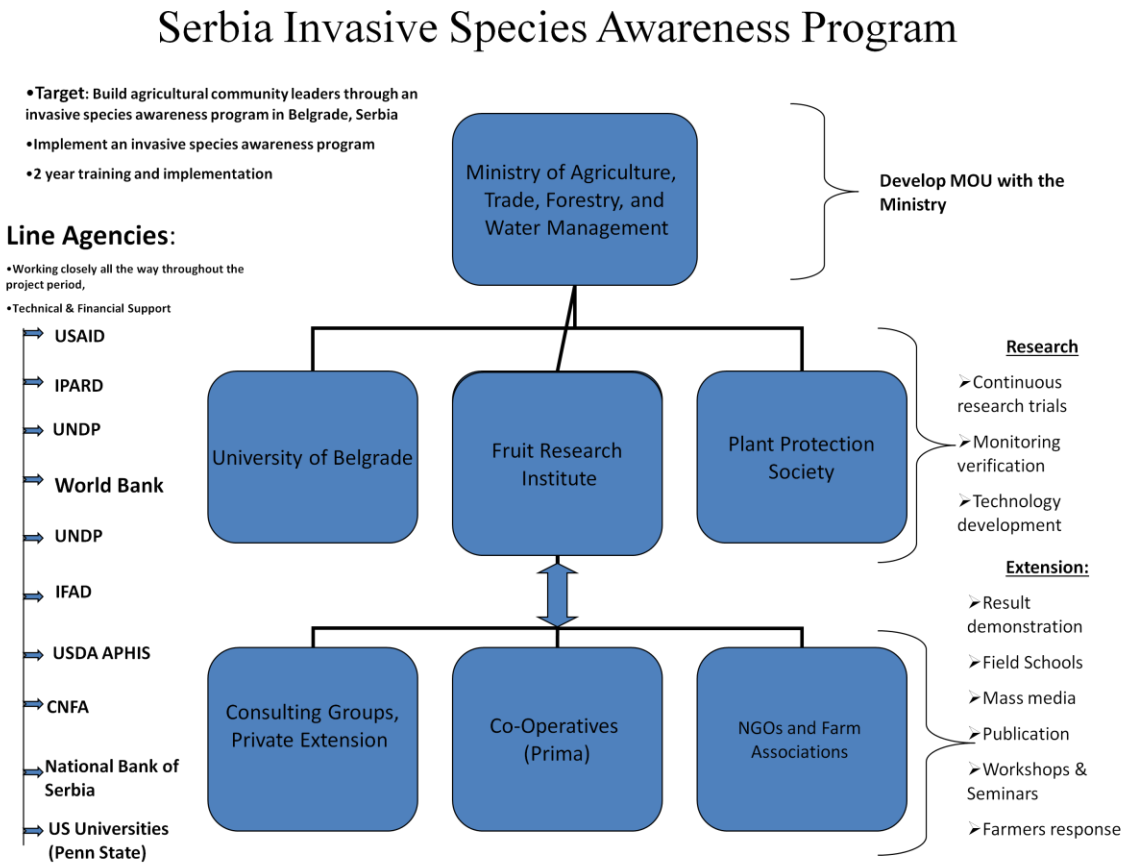

Figure **5-5**: Key community members in creating an invasive species awareness program. Reaching members of the Ministry of Agriculture, Trade, Forestry, and Water Management is a key point to establishing communication with the Serbian government. Research and extension follow, and are key players in creating networks throughout the country.

Table **5-1**: Week one of ISA program in Serbia. This week is focused on cognitive aspects, and the education of Serbian stakeholders and farmers.

Time (Day 1)	Activity
8:00-9:00 am	Registration period. Previously registered and up to 20 newly interested parties may sign in. Coffee will be provided and participants may mingle.
9:15-9:40	Welcoming Session, overview of what will be covered through the day, distribution of pre-test.
9:45-10:15	Topics will include overall threat of invasive species to Serbia.
10:30-11:00	First invasive pest biology, behavior, damage, and possible impacts
11:05-11:35	Second invasive pest biology, behavior, damage, and possible impacts
11:40-12:45	Lunch
13:00-13:30	Third invasive pest biology, behavior, damage, and possible impacts
13:35-14:05	Fourth invasive pest biology, behavior, damage, and possible impacts
14:15-14:45	Sponsor and stakeholder information and awareness
14:50-15:35	Distribution of post-test; distribution of informative factsheets; Concluding statements of the day
18:00	Dinner with stakeholders for program manager

Table **5-2**: Week two of ISA program in Serbia. Hands-on learning and education through a field workshop. Depending on the species of concern, models and dead samples may be the most effective ways to introduce new information, although any established populations of an invasive species in the country would provide a better education tool.

Time (Day 2)	Activity
8:00-9:00	Meet at business building and have registration. Coffee will be provided and mingling encouraged
9:15-10:15	Shuttle participants to a small farm that was previously identified
10:30-12:00	Pest identification with models and dead samples. Damage identification.
12:15-13:15	Lunch
13:30-15:30	Monitoring Techniques for each pest set up in different stations around the orchard
15:45-17:00	Participants demonstrate ability to identify pests. Complete matching worksheet to show they can match proper monitoring tools with the proper pest
17:15-18:15	Transportation back to business building in Belgrade center

Table **5-3**: Week three of program, discussion among extension educators, government officials, and farmers. This is the time to voice questions, concerns, and what participants would like to plan for the future.

Time (Day 3)	Activity
8:00-9:00	Registration. Coffee provided, mingling encouraged.
9:15-11:15	Discussion
11:30-12:30	Lunch
12:45-14:15	Discussion
14:30-15:15	Survey completion
15:30-16:00	Sponsor and stakeholder concerns

Table **5-4**: Time frame of ISA program in Serbia. This is a long-term curriculum based on start of the year calendar, and includes the set-up, the on-ground training, and follow-up plans.

Month	Activity
January	Discussion of program and funding requirements will be carried out with stakeholders
March	The program curriculum and funding acquisition will be coming to a close, with final adjustments being completed
April	Advertising of the program, identification of participants and the identification of farm and meeting locations will begin.
June	Actual program will be completed over a course of three weeks.
September	A group of participants and interested individuals will advertise the program and share what was learned at the International Horticultural Fair in Novi Sad (which is north of Belgrade).
December	First 6-month follow-up teleconference will be held to discuss any questions or concerns participants have, disseminate new information, and to check knowledge retention. First evaluation will be submitted to stakeholders
June	Second teleconference (1 year) to discuss concerns, check knowledge retention, and disseminate any new information.
December	Third (18 month) teleconference to discuss concerns, check knowledge retention, and disseminate any new information.
June	Final (2 year) teleconference to discuss concerns, check knowledge retention, and disseminate any new information. Final survey distributed. Final evaluation submitted to stakeholders.

Table **5-5**: Two-year program with flexible dates, this table includes program set-up, and is a useful framework for adjusting by species of interest.

ACTIVITY	PARTICIPANTS	DATE
CONTACT STAKEHOLDERS	AS PREVIOUSLY LISTED; INCLUDING PROGRAM DESIGNER	
ON-GROUND CONTACT	WITH STAKEHOLDERS AND POSSIBLE PARTICIPANTS; INCLUDING PROGRAM DESIGNER	
SOLIDIFY FUNDING ACQUISITIONS	STAKEHOLDERS; PROGRAM DESIGNER	
IDENTIFY PARTICIPANTS OF PRIMARY AUDIENCE	PROGRAM DESIGNER; STAKEHOLDERS	
IDENTIFY PARTICIPANTS OF SECONDARY AUDIENCE	PROGRAM DESIGNER; STAKEHOLDERS; PRIMARY AUDIENCE	
DEVELOP PROGRAM CURRICULUM AND PESTS TO INCLUDE	PROGRAM DESIGNER; INPUT FROM PRIMARY AND SECONDARY AUDIENCES	
BEGIN ADVERTISEMENT OF PROGRAM	PROGRAM DESIGNER; PRIMARY AUDIENCE SUPPORT	
RESERVE A BELGRADE BUSINESS BUILDING FOR THE MEETINGS 1 AND 3	PROGRAM DESIGNER; INPUT FROM PRIMARY AND SECONDARY AUDIENCES	
RESERVE A SMALL APPLE PRODUCERS FARM FOR MEETING 2 (FIELD DAY)	PROGRAM DESIGNER; INPUT FROM PRIMARY AUDIENCE	
RESERVE TRANSLATORS	PROGRAM DESIGNER; PRIMARY AUDIENCE SUGGESTIONS	
RESERVE TICKETS AND MATERIALS FOR GOING ABROAD	PROGRAM DESIGNER	

Table **5-6**: Two-year program with flexible dates, this table includes program duration. On-ground program training and field days are included.

Activities	Participants	Materials	Date
Day 1 Information session	Primary and secondary audience; program designer; translators	Media availability; computers; informative powerpoint/media; lunch; proper translator facilities; pre- and post-test papers and writing utensils; notepads	
Day 2 Field session	Primary and secondary audience; Program designer; translators	Transportation to sight; visual representations of pests and their damage; hand-held magnifying glasses; trapping materials; lunch; writing utensils; notepads	
Day 3 Discussion session	Primary and secondary audience; Program designer; translators	Media availability; computers; evaluation forms; notepads; writing utensils; lunch; proper translator facilities	
Advertising at completion of program	Primary and secondary audience; program designer; stakeholders	Advertisement avenues; funding; volunteers for fairs	
Follow-up at month 6	Primary and secondary audience; Program designer; translators	Media availability (skype or teleconference); computers; discussion	
Follow-up at month 12	Primary and secondary audience; Program designer; translators	Media availability (skype or teleconference); computers; discussion	
Follow-up at month 18	Primary and secondary audience; Program designer; translators	Media availability (skype or teleconference); computers; discussion	
Final session follow-up month 24	Primary and secondary audience; Program designer; translators; Stakeholders	Media availability (skype or teleconference); computers; discussion; final evaluation survey	

Table **5-7**: Two-year program with flexible dates, this table includes program completion and evaluation of ISA program.

Activity	Participants	Date
Final Discussion Session	Primary and secondary audience; program designer; translators; stakeholders	
Final evaluation	Primary and secondary audience	
Final stakeholder report	Program designer; stakeholders	
Thank you	Primary and secondary audience; program designer; translators; stakeholders	

Chapter 6

Conclusions and future directions

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is a serious invasive pest in the United States United States (Nielsen and Hamilton 2009a). Native to Asia, including China, Japan, Korea, and Taiwan, *H. halys* has been reported in Switzerland, Germany, France, Liechtenstein, Italy, Canada and the United States and was most likely introduced through international commerce in bulk freight containers (Lee et al. 2013a; Maistrello et al. 2014; Anonymous 2010). The wide host range makes *H. halys* a threat to many farmers (Nielsen and Hamilton 2009a). As chemical control is commonly used for management of *H. halys*, in order to minimize pesticide loads, behavioral research may be useful in development of future IPM control tactics (Toyama et al 2011; Lee et al. 2013; Nielsen 2008).

The diurnal and nocturnal behavior of *H. halys* in orchards and the laboratory may provide future opportunities for the development of more effective pest management. In Pennsylvania orchards, *H. halys* has predictable activity patterns, which are consistent regardless of the crop type. There is a pattern of greater activity towards the evening, and both adult and nymph activity patterns are related to the time of day. In the laboratory, *H. halys* was shown to have predictable behavior patterns that were related to an endogenous circadian rhythm. Males exhibited lower activity for the duration of the night than females. Results from these combined field and laboratory observations may be used to improve future monitoring and practical management strategies of *H. halys*.

Future research into the diurnal and nocturnal behavioral patterns in both the field and laboratory should take a closer look at activity patterns being exhibited. My study did not differentiate between probing and feeding thus future work should determine whether *H. halys is* probing to determine host suitability versus actual feeding behavior. The relative size of arenas

used in this work were small thus the use of larger arenas to evaluate flight dispersal behaviors of adults and behavior in relation to external stimuli (such as an agricultural sprayer, fruit pickers, etc.) should be explored. The behaviors I observed resulted from limited number of stimuli (e.g., light versus dark, plant-host odors); thus behaviors may be expressed differently when additional factors are introduced. In addition, altered behavior in relation to pesticide applications should focus on diurnal and nocturnal expressions of behavior.

When looking at the feeding preferences between peppers and sunflowers, I saw that *H. halys* were preferentially attracted to sunflowers. However, this attraction did not prevent damage in internal rows of peppers. There were no real differences observed in the number of adults versus nymphs, although nymph stage was important to where it would be found. In crop and non-crop plants surrounding the pepper and sunflower plots, I observed crabapples to be very attractive to *H. halys*.

While it may be possible to use sunflowers as a trap crop, factors such as distance of sunflowers from cash crop, size of trap crop relative to cash crop, and other variables need to be determined prior implementation of this technique for *H. halys* management. Future research should also look at sunflowers in the context of other potential trap crops to help with season-long control. If a season-long trap crop mixture is attractive enough, there is potential to prevent *H. halys* from invading cash crops, to benefit and enhance pollinators, and to minimize pollinator disruption through pesticide usage. Additionally, I observed more females than males in the sunflower trap crop. The potential importance of differential attractiveness of plant hosts to males and females should be explored. Future research should also determine the sex ratio of females to males to produce a reliable predictive model of the *H. halys* population based on early season *H. halys* observations.

In chapter 5 of this thesis, I looked at potential international implications of *H. halys* invasions in other countries. Invasive pest species can become very devastating for any country,

including my target country Serbia. I designed an invasive species awareness program that involves local farmers, government agencies, universities and research institutes, and consulting groups. I suggested the program start in Belgrade, Serbia although the program lends itself to the entire country. While it may be difficult to convince people of its merits, a pre-emptive action program designed to increase knowledge of invasive pests will be the most cost-effective educational program in the long run.

In conclusion, this research provides information on *H. halys* diurnal and nocturnal behavioral patterns and sunflower trap cropping potential in Pennsylvania. An invasive species awareness program for Serbia can help with preventing agricultural losses through increased communication. Further research is needed to provide additional, more detailed information related to *H. halys* behavior, trap cropping potential of sunflowers and other plants, as well as the effectiveness of pre-emptive invasive pest educational programs.

Appendix A

Glossary of terms

Circadian rhythm- being, having, characterized by, or occurring in approximately 24-hour periods or cycles

Diapause- a period of hormonally controlled quiescence, especially in immature insects, characterized by cessation of growth and reduction of metabolic activity, often occurring seasonally or when environmental conditions are unfavorable.

Diel- involving a 24-hour period that usually includes a day and the adjoining night

Diurnal – daytime, or light period, of a 24 hour cycle

Locomotor- an act or the power of moving from place to place

Quiescent- being at rest; quiet; still; inactive or motionless

Scotophase- The dark segment of a light-dark cycle

Phenology- periodic biological phenomena that are correlated with climatic conditions

Photoperiod- A light-dark cycle

Photophase- The illuminated segment of a light-dark cycle

Polyphagous- feeding on or utilizing many kinds of food

Appendix B

Serbian survey

Pennsylvania State University

College of Agricultural Sciences

SERBIA INVASIVE INSECT AWARENESS PROGRAM

Initial Survey

Authors:
Deonna Soergel
Ed Rajotte

Dear Respondent,

Objective: To evaluate the feasibility of an invasive species awareness program for Serbia.

Definition: Invasive Species – a species not native to the area that may disrupt the local ecosystem. Examples include Fireblight, Aphids, Gypsy Moth, etc.

Awareness- to have knowledge of a particular issue or problem, in this case it is related to knowledge of invasive species.

Directions: Please take a few minutes to fill out the survey by filling in or circling the correct answer**.**

Please Note:

There are four sections to this survey. For section II,

The scale of measurement is:

Daily	Weekly	Monthly	Yearly	Periodically	Never
1	**2**	**3**	**4**	**5**	**6**

For section III,

The scale of measurement is:

Strongly agree	Agree	Neutral	Disagree	Strongly Disagree
1	**2**	**3**	**4**	**5**

Part I

1. For Serbian fruit producers, please indicate the importance of each of the following issues:

Horticultural Issues

Plant Nutrition

Not important Somewhat Important Important Very important Uncertain

Rootstocks

Not important Somewhat Important Important Very important Uncertain

Pruning

Not important Somewhat Important Important Very important Uncertain

Please list any other horticultural issues:_____

_____

Pests

Weed pests

Not important Somewhat Important Important Very important Uncertain

Insect pests

Not important Somewhat Important Important Very important Uncertain

Mammalian pests

Not important Somewhat Important Important Very important Uncertain

Fruit harvesting

Not important Somewhat Important Important Very important Uncertain

Marketing

Not important Somewhat Important Important Very important Uncertain

Please list any other issues you find important in relation to the fruit industry:

_____

_____

2. Please list the 3 invasive insect species you are most concerned with.

a._____

b._____

c._____

Part II

Questions 3 and 4 are specific to individuals. If you are a farmer, please answer questions 3 and 4; if you advise farmers, please answer questions 5 and 6. Please answer all other questions normally.

3. If you are a *farmer*, how often do you interact directly with farming advisors?

Daily Weekly Monthly Yearly Periodically Never

4. How often do you speak with farming advisors about monitoring for invasive species?

Daily Weekly Monthly Yearly Periodically Never

5. If you are a *farming advisor*, how often do you interact directly with farmers?

Daily Weekly Monthly Yearly Periodically Never

6. How often do you speak with farmers about monitoring for invasive species?

Daily Weekly Monthly Yearly Periodically Never

Please answer questions 7 and 8 normally:

7. How often do you monitor for invasive species?

Daily Weekly Monthly Yearly Periodically Never

8. Have you ever reported an invasive species?

Yes No Uncertain

Part III

For Questions 9-11 a series of statements have been provided. Please state if you agree or disagree with them.

9. I am well informed about invasive species

Strongly agree Agree Neutral Disagree Strongly Disagree

10. I am interested in learning about invasive species and their control

Strongly agree Agree Neutral Disagree Strongly Disagree

11. I am likely to participate in an invasive species awareness program

Strongly agree Agree Neutral Disagree Strongly Disagree

12. An invasive species program is important for the Serbian fruit industry

Strongly agree Agree Neutral Disagree Strongly Disagree

13. The current invasive species awareness system in Serbia is very effective

Strongly agree Agree Neutral Disagree Strongly Disagree

Part IV

14. What is your job title? _____

15. What institution or agency do you work for?

_____

16. How many years have you worked with the fruit industry?

< 1 year 1-3 years 4-10 years 11-20 years >20 years

17. Do you currently participate in any invasive species monitoring or reporting program?

Yes No Uncertain

18. Who would you report invasive species to? _____

Thank you for your time!

Appendix C

Serbian focus group questionnaire

Pennsylvania State University

College of Agricultural Sciences

SERBIA INVASIVE INSECT AWARENESS PROGRAM

Focus Group Questions

Authors:
Deonna Soergel
Ed Rajotte

1. What are the greatest issues that Serbian Growers face?

2. What are the greatest issues that you face while trying to get information to growers?

3. How do you commonly access information? (internet, research, other means, barriers faced)

4. How do your farmers commonly access information?

5. How have you spread past awareness about agricultural issues to farmers? How was it received?

6. What is the status of invasive species awareness within your country? Insects?

7. Do you try to raise awareness for invasive species?

8. What are some common issues you face when trying to spread invasive species awareness?

9. Would you be interested in an invasive species awareness program?

10. How would you like to see this progress?

Thank you for your time!