BEHAVIORAL PATTERNS OF THE BROWN MARMORATED STINK BUG AND THEIR IMPLICATIONS FOR MONITORING PROGRAMS

by

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ABSTRACT OF THE DISSERTATION

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This disseration investigates the behavior and selected monitoring methods of the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). The first chapter gives a comprehensive summary of the current and past research involving this insect and provides the basis for why the proceeding investigations were undertaken and how they added to the current body of knowledge about this important pest species. Chapter 2 explores the relationship between the number of hours past sunrise at which timed visual observations are conducted for *H. halys* and the detectable number of individuals on peach trees. The findings of this study show how the diel behavioral patterns of this insect cause large differences in the quantity observed at different times of the day ($p<0.05$; repeated measures). The variability of this metric associated with differences between observers is also investigated and shown to have a significant impact on the average number of *H. halys* seen per tree ($p<0.05$; repeated measures). Temperature was not found to be a useful indicator for estimating the number of visually observed *H. halys* ($R^2=0.0183$; regression). Chapter 3 investigates the movement of *H. halys* within and between peach trees over the course of the diel cycle. The results indicate that nymphs of this species are most active from 7 to 10 hours past sunrise. Nymphs are found more on fruit and branches during this time and a higher proportion of individuals are found on the interior of the tree ($p<0.05$; loglinear model). This chapter also discusses the percentage of newly seen individuals at each time of day and how the count of previously seen insects increases once the sun sets ($p<0.05$; loglinear model). These findings also show that low numbers of *H. halys* were found to move between trees. Chapter 4 looks at *H. halys* attraction to various light sources. This study examined the difference in attractive pull resulting from alteration of the color and luminosity of a light source. The data also shows how different nymphal instars and adult genders are affected differently by the presence of a light source. The findings of this study support previous studies that white light is an attractive stimulus for insects but that reduced attraction can be observed at very low or high light intensities. Chapter 5 decribes a study that examines *H. halys* distribution as it overwinters in urban structures. This study found that this insect was observed more in the upper floors of the buildings investigated. The findings of this study agree with the current ecological understanding of this species as *H. halys* is known to be an arboreal species in its natural habitat. The results of this work are useful for making pest management decisions that aim to reduce diapausing populations within these types of structures. Chapter 6 looks at the feasibility of constructing and designing a tower-style black-light trap that has comparable catches to the industry standard model. This project resulted in the development of blueprints for in-lab-fabrication of clear-fin black light tower traps which cost ~1/10 the price of the market standard. The new trap caught equivalent quantities of insects ($p>0.05$; repeated measures) and the method for testing comparability of the traps to one another by way of a paired-trap, multi-site, partial season capture analysis is explained and the results outlined to highlight the equivilant trap capture diversity and sample species counts. This disseration concludes with remarks in Chapter 7 that restate the major findings for each study and relate them to the field as a whole.

Acknowledgements

I would like to thank to multitude of people who helped me throughout my graduate experience. I believe it is safe to say that that I am directly responsible for several extra gray hairs sprouting around the department but I hope these are balanced by many more enjoyable memories. The patience and open mindedness of Dr. Hamilton and my other committee members cannot be overstated as they endured more than a few surprise projects, lab renovations, and incident reports. I was very fortunate to have been helped by so many students through my projects and they are each mentioned by name in the acknowledgment section of each chapter. Very little of the work I did would have been possible without their time and dedication. My family provided unwavering support (even when I was grumpy because of looming deadlines over the holidays) and for this, I am eternally grateful.

Dedication

I would like to dedicate this dissertation to my progeny. May you exist and thrive.

Publication Status

The following chapters were previously published in peer- reviewed journals:

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Chapter 5: Cambridge, J., L. Francoeur, and G. C. Hamilton. Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) Attraction to Different Light Stimuli.

Chapter 6: Cambridge, J., A. Payenski, L. Francoeur, and G. C. Hamilton. Evaluation of a Non-Commercial Ultraviolet Light Trap for Use in Pest Monitoring Programs.

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Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the brown marmorated stink bug (BMSB), is an invasive, non-native agricultural and domestic pest in North America. The brown marmorated stink bug is thought to have been first introduced into Allentown, PA in the early to mid-1990s (Hoebeke and Carter, 2003). It was initially misidentified as the native brown stink bug, *Euschistus servus* (Say), but after continual increases in the number of complaints about stink bugs entering homes, closer inspection revealed the misidentification. There are more than 40 species of stink bug species in the mid-Atlantic region which frustrated early identification and detection. *H. halys* can be easily mistaken for several native species. The most common of these are *E. servus, E. tristigmus* (Say), and *Podisus maculiventris* (Say). In Europe, characterization of the true and current distribution of *H. halys* is also being frustrated by another look-alike, *Raphigaster nebulosi* (Poda) (Garrouste *et al.*, 2014). In 2001, Karen Bernhard sent samples of the suspicious stink bug from the Lehigh County Cooperative Extension to Richard Hoebeke at Cornell University for identification (Hamilton, 2009). Hoebeke identified the stink bug as *H. halys*. Other stink bug samples from the area over the past several years were re-evaluated and more *H. halys* were identified, the oldest of which dated back to 1996 (Hoebeke and Carter, 2003). This date is not intended to be interpreted as the date of the original introduction; it is very likely that the population was established for several years in the area at low levels. The newly established population of *H. halys* began to spread from Allentown and the first report of *H. halys* in New Jersey was in 1999 in Milford, NJ (Hamilton, 2009).

Halyomorpha halys is native to China, Japan, Taiwan, and Korea (Lee et al., 2013a). It is a crop pest on a wide variety of plants but is kept in check by a complex of natural enemies, only occasionally becoming a serious pest (Lee *et al.*, 2013a). In the United States, these natural enemies are absent. In its native range, its primary hosts are suspected to be members of the genus *Paulownia* (Zang *et al.*, 1993) but it is known to feed on over a hundred different native and agricultural plants (Lee *et al.*, 2013a). *Paulownia sp.* are also currently an invasive problem in North America (Ding *et al.*, 2006) but the efect this is having on the spread of *H. halys* within the United States is unknown.

Halyomorpha halys has gained more and more attention over the past 20 years since the first detection of it in the United States and has been shown to be highly polyphagous and to cause direct damage to a wide variety of agricultural crops and ornamental species (Leskey et al. 2013d, Rice et al. 2014. It also has become an urban nuisance due to its behavior of overwintering in homes and other man-made structures (Inkley, 2012). Its suitability to such a diverse range of climatic and ecological conditions makes *H. halys* a threat to many other parts of the world where it is currently not found but where trade occurs (Holtz and Kamminga, 2010; Zhu *et al.,* 2012).

Pest Status

As of December 2014, *H. halys* was reported in 42 states in the continental United States (Leskey, 2015) and two Canadian provinces. At this time, it is considered a severe agricultural and nuisance pest in nine states: DE, MD, NC, NJ, NY, PA, TE, VA, and WV. It is considered an agricultural and nuisance pest in eight states: CT, GA, IN, KY, MI, OH, OR, and WA. Another nine states categorize it as a nuisance pest: AL, CA, IL, MA, MO, NH, SC, VT, and RI. The remaining 16 states have confirmed detections of *H. halys* but do not categorize it as established and causing problems: AR, AZ, CO, FL, HI, IA, ID, KS, ME, MN, MS, NE, NM, TX, UT, and WI. This pest have been found in Greece (Milonas and Partsinevelos, 2014), Hungary (Vétek *et al.*, 2014), Switzerland (Wermelinger, 2008), Liechtenstein (Arnold, 2009), Germany (Heckman, 2012), Italy (Pansa *et al.*, 2013; Cesari *et al.*, 2014), France (Callot and Brua, 2013), Canada (Fogain and Graff, 2011), England (Malumphy 2014), Romania (Macavei et al. 2015), Austria (Rabitsch and Friebe 2015) and Serbia (Šeat 2015). The highest densities in the United States are found in the mid-Atlantic region, corresponding to the highest levels of agricultural damage (Rice *et al.*, 2014). As new populations of *H. halys* are found in different areas around the world, reports are published by researchers in that area such as the one for the detection in Tennessee by Jones and Lambdin (2009). *H. halys* has been intercepted in other countries such as New Zealand where wild populations have not been found (Harris, 2010). This pest will likely continue to spread to new areas in the world. Those at the highest risk for this are northern Europe, northeastern North America, southern Australia, the North Island of New Zealand, Angola, and Uruguay (Zhu *et al.*, 2012). There is a great deal of work being done to understand and prevent *H. halys* from spreading and causing further damage to crops (Leskey and Hamilton, 2012).

H. halys is highly polyphagous and suspected to have over 170 host species (Nielsen and Hamilton, 2009a). Host species are quite diverse and found over many plant families. The occurrence of *H. halys* in agricultural and landscape plants changes over the course of a season (Nielsen and Hamilton, 2009a). The diversity of host plants in combination with the number of suitable habitats for *H. halys* in the mid-Atlantic landscape led to easy establishment and spread for *H. halys* (Bergmann *et al.*, 2013; Wallner, 2014). In areas where *H. halys* is newly established, the utilized hosts may not represent all of the potential hosts. In California, *H. halys* was only found over the entire season on *Ailanthus altissima* (Ingels *et al.*, 2014).

The effect *H. halys* may have on the economics of many agrosystems is to reduce grower profits. They can cause outright loss of yield but in the case of apples, peaches, sweet cherries, tart cherries, tomato, bell pepper, corn, soybean, and citrus, they can cause sufficient damage to the produce that growers are unable to sell for fresh market and must take a lower price to sell the produce for processing (Holtz and Kamminga, 2010). *H. halys* disperses and establishes in new areas easily and given the urban and agricultural impacts, and its high potential to cause negative effects in areas it invades. The United States Department of Agriculture has labeled the Pest Risk Potential of this insect to be high (Holtz and Kamminga, 2010). New Zealand has also characterized it as being a high risk for spreading and a moderate to high risk for causing economic harm (Duthie *et al.*, 2012). In Asia, *H. halys* is normally categorized as an occasional pest (Lee *et al.*, 2013a).

Host crops

Soybean**:** Even in its native range, *H. halys* is reported as one of the most important pests of soybean as reported in Japan (Kobayashi, 1981) and Korea (Kang *et al.*, 2003; Son *et al.*, 2000). It feeds primarily on the seed pods causing greening and reduced yield but is also known to cause damage to the seeds themselves. In the United

States, peak *H. halys* numbers have been recorded during the R3-R6 growth stage when seeds are particularly sensitive to feeding (Nielsen *et al.*, 2011). *H. halys* feeding during the early seed and seedpod growth stages may cause older seeds to be deformed or the entire pod to abort. Damage on a field scale can cause delayed phenological progression (also known as "greening") which can lead to harvesting and storage problems (Akin *et al.*, 2011). Feeding damage caused by *H. halys* reduces the carbohydrate and lipid content of the seeds. In laboratory experiments, the total dry weight of a soybean decreased by 42% after 24 days of feedings but increased the protein content of the seed by 13% during the same time period. *H. halys* was a more voracious feeder on soybean seeds than either *Peizodorous hybneri* (Gmelin) or the alydid, *Riptortus pedestris* (Fabricius) (Bae *et al.*, 2014). Field edges harbor more *H. halys* that other parts of a field (Akin *et al.*, 2011).

***Peaches*:** Feeding damage can be found on all portions of the peach fruit and may occur at any time during fruit formation from either adults or nymphs. *H. halys* damage on early season peaches (before mid-May) results in gummosis and deformation, while late season feeding (after mid-May) causes brown spots as well as gummosis (Lee *et al.*, 2013a). Feeding done by *H. halys* causes immediate damage as the skin and fruit flesh by the feeding site discolors (Leskey *et al.*, 2012b). When the US population of *H. halys* increased in 2010, growers in Maryland, West Virginia, and Virginia incurred extensive damage, with some growers losing 50-60% of their crop (Biddinger *et al.*, 2010). Reports have been as high at 90% loss in some mid-Atlantic orchards (Blaauw et al., 2015).

***Apple*:** *H. halys* is a pest of apple in Japan (Tada *et al.*, 2001; Funayama, 2002). Both nymphs and adults feed on the leaves, new stems, and fruit. Eggs are normally laid on the underside of leaves. Overwintering adults have been shown to immigrate to orchards carrying mature eggs (Funayama, 2002). Feeding damage on the fruit results in cat-facing, stippling, and corky tissue (Yu and Zang, 2007). Brown and Short (2010) describe the progression of damage development on fruit. Damage initially starts as discolored dots, becoming discolored dots with depressions, then discolored dots with discolored depressions. The main factor affecting what type of fruit damage was observed was the time between feeding and evaluation. Cultivar type and age of fruit were also important. This was validated by Lee *et al.* (2013a) finding cultivar differences in susceptibility; the early maturing ‘Sansa’ cultivar was more susceptible than later maturing cultivars. Funayama (2004) showed that the nutritional status of *H. halys* found in apples is inferior to that of individuals reared on peanuts and soybeans. However, the study does indicate that apple is a suitable host in the absence of better food sources. *H. halys* was also shown to damage apples in the United States (Nielsen and Hamilton, 2009b). In 2010, apple growers in the mid-Atlantic region lost 37 million dollars due to *H. halys* (United States Apple Association, 2010). Field studies looking at the prevalence of external and internal feeding damage to apples along orchard borders adjacent to forested areas, interior areas, and intermediate zones and also stratified damage within the tree by height (divided into low, middle and high) found that apples were most likely to be damaged and to have the highest rates of damage in the upper canopy of the border rows (Joseph *et al.*, 2014).

Pear**:** *H. halys* is a serious pest of pear in China. It is responsible for from 30% to 80% of fruit damage. Feeding damage caused by *H. halys* in pear is similar to peach and apple damage. The damage outcome differs depending on the age of the fruit when feeding occurred. Early season damage can cause green depressions with corked flesh but later season damage may not leave any physical symptoms on the outside of the fruit but will still cause a reduction in flesh quality (Lee *et al.*, 2013a). All life stages except eggs and first instars feed on pear fruit (Nielsen and Hamilton 2009). *H. halys* also secrete honey dew which can lead to black sooty mold on the fruit (Yingnan, 1988). In New Jersey, *H. halys* has been found to cause greater than 25% damage per tree (Nielsen and Hamilton, 2009b). Visual inspections of foliage in and around Californian pear orchards found the first egg mass on May 5th and the first nymphs in the pyramid traps on June 3rd (Ingels *et al.*, 2014). Field data suggests the *H. halys* goes through two generations in pear orchards in California.

Other tree fruit**:** *H. halys* is a pest of sweet persimmon, *Diospyros* sp. in Korea (Kang *et al*., 2003; Lee *et al.*, 2002, Kim *et al.*, 1997; Chung *et al.*, 1995; Lee, H. S. *et al.*, 2009). Field studies revealed that *H. halys* causes 100% damage to fruit when five adults were released onto a branch. The damage was most severe when feeding occurred in July and later as compared to another heteropteran pest, *Riptortus clavatus* (Thunberg), which showed the most damage by feeding in early or late season. (Lee *et al.*, 2009). *H. halys* was also found to be a pest on Yuza, *Citrus ichangensis* (Choi *et al.*, 2000) and other citrus species (Kim *et al.*, 2000) in Korea. *H. halys* can cause fruit abortion, stippling, and disfiguration on cherries (Watanabe, 1996). On Japanese bird cherry

(*Prunus grayana,* Schneider), *H. halys* nymphs were frequently found feeding and had a higher developmental ratio on ripe fruit than on immature fruit (Funayama, 2007).

Tree nuts**:** In Oregon, *H. halys* is found on hazelnuts (Wiman *et al.*, 2013) and is a significant pest (Hedstrom *et al.*, 2014). Feeding before kernel expansion caused empty shells while feeding during kernel expansion caused kernels to form irregularly (Hedstrom *et al.*, 2013). When feeding occurred after kernel formation, the kernel tissue became necrotic and corky at the feeding site (Hedstrom *et al.*, 2014). Although there are significant differences in shell thickness between cultivars, shell thickness was not affected by feeding frequency (Hedstrom *et al.*, 2014). *H. halys* was found on pecans as well. (Sutherland and Bundy, 2012)

Grapes**:** *H. halys* feeds on grapes and as the populations continue to increase and expand, they will likely represent an increasing threat to vineyards (Pfeiffer *et al.*, 2012). Damage to grapes includes aborted berries, loss of turgor, necrosis, and bunch rot (Ingels, 2014). Many of the insecticides that are effective against *H. halys* are not recommended for use in vineyards because of the risk of secondary pest outbreaks by pests such as mealy bugs which transmit diseases and cause indirect damage (Pfeiffer *et al.*, 2012). In New Jersey, damage to grapes is greater in white grapes than red (Ingels, 2014). Grapes are an early season reproductive host for *H. halys* (Basnet, 2015). They usually lay eggs on the underside of the leaves (Basnet, 2014). *H. halys* in grapes that were close to other more desirable crops such as soybean and corn moved out of the vineyard late in the season when compared to vineyards surrounded by forest (Basnet, 2014). Damage occurs as small necrotic spots at the point of stylet insertion, the spot gradually increasing in size over time (Basnet, 2014).

Vegetable crops**:** Damage on peppers, tomatoes, okra, and eggplant can be caused by both adults and nymphs. Discoloration at the site of feeding is the initial symptom but soon after, the tissue underneath will start to deteriorate and rot (Kuhar *et al.*, 2012; Lee *et al.*, 2013a). In Maryland in 2011, nearly 40% of okra was damaged by *H. halys* and other insect pests (Kuhar *et al.*, 2012). Eggplant is also a known agricultural host in Asia (Lee *et al.*, 2013a).

Field crops**:** *H. halys* feeding on corn can cause kernel disfiguration (Kuhar *et al.*, 2012). Sweet corn is also a known agricultural host in Asia (Lee *et al.*, 2013a). Many types of bean are attractive to *H. halys*. In Maryland, *H. halys* has damaged significant portions of snap, green, and lima beans (Kuhar *et al.*, 2012).

Caneberries**:** In the mid-Atlantic before to 2013, *Euschistus sp.* were the most common stink bug in caneberries, but in 2013, *H. halys* became the most common. *H. halys* causes direct feeding damage (Basnet, 2014) and surveys of caneberries in Oregon (Wiman *et al.*, 2013) have shown *H. halys* to be one of at least 16 stink bugs that feed on raspberries in the mid-Atlantic region (Basnet et al., 2014). Sampling shows that *H. halys* may be displacing the native *E. servus* as the major stink bug pest of raspberries in the mid-Atlantic region (Basnet *et al.*, 2014). Both nymphs and adults feed on this crop but the majority of individuals found were adults. No egg masses were found leading to the conclusion that *H. halys* likely does not reproduce on raspberries.

Other impacts**:** *H. halys* is also a recorded pest on many other less agriculturally import plants such as mulberry (Yoshii and Yokoi, 1984) and jujube trees (Song and Wang, 1993). In Asia, *H. halys* has been reported to feed on cucumber (*Cucumis sativus)*, saya pea (*Lathyrus odoratus*), asparagus (*Asparagus officinalis*), edamame bean

(*Glycine max*), and strawberry (*Fragaria* × *ananassa*) (Lee *et al.*, 2013a). It is also known to feed on the leaves and stems of ornamental and forest trees (Yang *et al.*, 2009). There is some debate as to whether or not *H. halys* feeds on Japanese cedar (*Cryptomeria japonica,* Thunb) cones to complete its life cycle in Japan. Funayama (2005) showed that *H. halys* cannot reproduce or readily develop on Japanese cedar alone but Kiritani (2007) reports that it is essential to the development of this species. However, Kiritani (2007) also states that there are "no substantial bug problems in Korea where significant land-use changes have not occurred" which has been debated (Williams *et al.*, 2004; Choi and Park, 2012). The feeding-damaged areas of the plant tissue fed on by *H. halys* exude liquids which are high in sugars and quickly ingested by local Hymenopterans (Martinson *et al.*, 2013). The chemicals which comprise odorous emission stink bugs are famous for, tridecane and E-2-decenal, do not transfer into the milk produced by dairy cows that are feeding on *H. halys* infested corn silage (Baldwin *et al.*, 2014).

Disease transmissions

H. halys is known to transmit a mycoplasma disease called *Paulownia witches' broom* (Doi and Asuyama, 1981; Hiruki, 1997). This disease causes reduced vigor, branch die off, poor timber quality, and ultimately death. It is transmitted by *H. halys* feeding on an infected tree and then moving to feed on another tree that was not previously infected. The most commonly infected plants are Paulownia species but other plants such as periwinkle (*Catharanthus roseus,* G.Don) can host the disease as well (Bak *et al.*, 1993).

***H. halys* as an urban pest**

Halyomorpha halys is not known to cause any direct harm to humans (bites, stings, etc.), it does not transmit human disease, and it does not cause any structural damage to homes or other buildings. Based on this, *H. halys* is categorized as an urban nuisance pest (Welty et al., 2008). However, there is some data to suggest that it may be responsible for an increased frequency of allergic responses to other insect allergens such as dust mites and cockroaches (Mertz *et al.*, 2012). *H. halys* commonly moves into urban structures to overwinter (Hamilton, 2009). Adults start to migrate into homes between early September and mid-October and then emerge again in mid-April to late May (Inkley, 2012). Exit timing of *H. halys* was positively correlated with increased daily temperature variation and increased daily high temperature (Inkley, 2012). Individuals can aggregate in clusters of over a hundred in a single area (Welty et al., 2008). This has led to an increase in homeowner-purchased products and services to treat for *H. halys* (Adams, 2010; Bozick, 2010; Holtz and Kamminga, 2010). Investigation into the dispersal pattern of *H. halys* in urban structures is discussed in a subsequent chapter here.

Biology

The brown marmorated stink bug is a member of the genus *Halyomorpha* (Mayr, 1866) and one of 4,112 species in the family Pentatomidae (Rebadliati *et al.*, 2005). Many individuals in this group are crop pests. *H. halys* is a highly polyphagous plant feeder that is known to feed on over 100 species; many of these species being of economic importance (Rice *et al.*, 2014). *H. halys* is considered a hitchhiker pest and is able to disperse over large distances in cargo containers or other man made vessels (Holtz and Kamminga, 2010). In natural landscapes, *H. halys* is considered to be an arboreal species (Bernon *et al.*, 2005).

To avoid trouble identifying *H. halys* as had happened originally in the USA, keys to distinguish *H. halys* from other look alike stink bugs have been developed (Wyniger and Kment, 2010). *H. halys* can be distinguished from other stink bugs by the presence of light bands on the antenna and legs, large size, and marmoration (alternating light and dark banding) around the outside of the abdomen (Leskey *et al.*, 2012d).

Life cycle

Adults emerge from overwintering sites in early spring and begin to sexually mature. After breaking diapause and moving out of the overwintering site to nearby host plants, the stink bugs will undergo two weeks of sexual maturation. Once this is complete, mating begins (Nielsen and Hamilton, 2009a). The mating behavior of *H. halys* was described by Kawada and Kitamura (1983). Courtship typically follows a male-induced pattern. Males will approach the female and signal her. If willing to mate, the female will lift her posterior end for the male to inspect with his antenna. If everything is satisfactory, the male turns around facing away from the female. The two insects then join posteriorly and stay coupled for approximately 10 minutes. Females are able to mate only once and lay eggs but increased fecundity and fertile egg-laying time are correlated with multiple mating (Kawada and Kitamura, 1983). Chu *et al.* (1997) reported that 53% of females undergo parthenogenesis but this has not been substantiated (Yu and Zhang, 2007). Males can be easily differentiated from females by external morphological differences. Females are generally larger than males and have a smooth posterior end where males have a "U" shaped notch (Rice *et al.*, 2014).

In the United States, the egg laying period is generally from late May to early August (Hoebeke and Carter, 2003). The minimum developmental threshold for BSMB is 14.17 °C (Nielsen and Hamilton, 2009a). Females lay egg masses generally consisting of 28 eggs. There are five nymphal instars. Eggs hatch on average 6-10 days after being laid. First instar nymphs do not normally leave the egg mass. After an average of 4.82 days on the egg mass, first instars molt and then leave to find food. The resulting, second, third, fourth, and fifth instar larva take 9.62, 7.08, 7.38, and 10.44 days to molt to the next instar, respectively (Nielsen *et al.*, 2008a). Nymphs are highly mobile and can easily move between host plants. Third and fourth instars are most mobile but all instars (except firsts) have a high dispersal capacity (Lee *et al.*, 2014b).

Symbionts

During the time that first instar nymphs sit on the egg mass, they probe with their mouthparts between the eggs and take in a gut symbiont that is found on the chorion. Without this symbiont, successful maturation to adulthood and adult fecundity, and subsequently F2 generation growth success are greatly reduced (0.3% compared to 20.8% in the controls) (Taylor *et al.*, 2014). This gut symbiont is a gammaproteobacterial mutualist held in specialized midgut gastric caeca in the V4 region (Bansal *et al.*, 2014). More than 99% of the material in these gastric caeca was identified to the genus *Pantoea* (Bansal *et al.*, 2014). This novel species of gammaproteobacteria has been given the name *Candidatus* "Pantoea carbekii" (Bansal *et al.*, 2014). Investigations into the use of these endosybionts is underway (Ioannidis *et al.*, 2014). The genome of the endosybiont has been sequenced and may be used to develop *H. halys*-specific control methods (Ioannidis *et al.*, 2014).

Saliva

H. halys saliva induces most of the physical damage response by the plant. *H. halys* has two different kinds of saliva, the sheath saliva and watery saliva. Watery saliva is used for the initial digestion of the plant tissue. It is excreted by the accessory salivary glands and is injected into the food tissue to help break down the tissues before ingestion. The sheath saliva is more of a gel. It is secreted by the stink bug and forms a protein tube around the rostrum at the feeding site. This helps form the seal for the stink bug to injest the partially digested tissues. Once secreted it rapidly hardens and remains on the plant after the insect is done feeding. The components of the two salivas are quite distinct but both contain a wide variety of proteins and enzymes. Research indicates that many of the proteins of the sheath salvia are actually derived from the plant that the stink bug is feeding on (Peiffer and Felton, 2014).

Environmental Cues

H. halys is one of many multivoltine stink bugs with generation cycles controlled exogenously (Saulich and Musolin, 2014). *H. halys* can go through different numbers of generations per year depending on the climate conditions. In China in the Hebei Province, 1-2 generations per year are found (Cuituan *et al.*, 1993) but up to six generations have been reported in the Kwangtung province (Lee *et al.*, 2013a). In the mid-Atlantic region of the US, there are 1-2 generations per year but they are not discrete and overlaps are very common (Leskey *et al.*, 2012c). In Pennsylvania, all life stages can be found simultaneously in the environment by July (Bernon, 2004).

Photoperiod and temperature can have a variety of effects on nymphal instars, including slight pigmentation differences and the induction of diapause (Niva and Takeda, 2003). They use their antennae for short-range location of other *H. halys* when aggregating for diapause (Toyama *et al.*, 2006). *H. halys* primarily use their antenna to detect food but also use visual cues once within 10 cm of the food source (Li *et al.*, 2007). Adults have a hiding behavior that causess them to stop and wait in dark locations over more illuminated ones (Toyama *et al.*, 2011).

H. halys adults are capable of long distance flights (Cuituan *et al.*, 1993). In flight mill studies, individuals were recorded traveling more than five kilometers. Adults tended to fly farther distances at the end of the summer at the time when populations are beginning to move into diapausing locations. On average, summer generation adults flew greater distances at quicker paces than did diapausing adults but flew less frequently. Overwintering adults lost a greater proportion of their body weight in a given flight than did summer adults (Wilman *et al.*, 2014).

Diapause

H. halys overwinters in the adult stage (Watanabe *et al.*, 1994). *H. halys* has a well-known behavior of moving into homes to carry out diapause (Saito *et al.*, 1964; Watanabe *et al.*, 1994). In the natural setting, *H. halys* can be found diapausing in crevasses on trees with thin or peeling bark (Oak, Locust, Paulownia, etc.) (Lee *et al.*, 2014a). Adults in the diapausing state have a preference for dark hiding spots (Toyamna *et al.*, 2011). *H. halys* use photoperiod as a diapausing cue in the fall. This is much the same as many other seed feeding heteropterans (Numata, 2004). After they break diapause, they emerge as sexually immature adults and go through a period of sexual maturation before copulating and laying the first eggs of the season (Nielsen and Hamilton, 2009a).

Every 1 C^o increase in average winter temperature will result in a 13.5% to 16.5% decrease in winter mortality (Musolin, 2007). Funayama (2013) suggests that overwintering adults may have better success when the temperatures in early spring are cooler so that they stay in diapause longer and are able to reduce the amount of time they are active without sufficient food in the environment to which they emerge.

Genetics

To confirm morphological identification of a species with many look-alikes, DNA barcoding techniques have been investigated for rapid species identification (Tembe at al., 2014). These techniques used the mitochondrial cytochrome c oxidase I sequences and are successful in distinguishing all tested members of the infraorder Pentatomorpha which includes 73 species over 5 super families (Pentatomoidea, Coreoidea, Pyrrhocoroidea, Lygaeoidea, and Aradoidea) (Tembe *et al*., 2014).

The complete mitochondrial genome of *H. halys* was sequenced and its phylogeny resolved in Lee, W. *et al.* (2009). Haplotype comparison of United States *H. halys* to populations sampled throughout the native range showed that the introduction into Allentown, PA likely occurred with individuals coming from the population of *H. halys* in Beijing, China and that the major population spread throughout the United States came from that single introduction (Xu *et al.*, 2014). Haplotype comparison revealed that the population in Canada came from the population in the United States but the introduction in Switzerland was from another area of China (Gariepy *et al.*, 2014) The population now found in Italy were likely the result of two introductions, one from the United States or Canada and the other from Switzerland (Cesari *et al.*, 2014).

Monitoring

Monitoring for *H. halys* may be done for a variety of reasons. Researchers, IPM scouts, or growers often want to quantify population levels in an area before crop damage. These measurements can also be used to characterize population growth as the season progresses, to assess if a control technique is effective, to understand the seasonal biology of the insect, or to help make the decision to apply insecticides or not. The major monitoring techniques for *H. halys* are timed visual counts, beat sheet sampling, sweep net sampling, black-light sampling, and pheromone trapping (Lee *et al.*, 2013a; Leskey *et al.*, 2012c).

Timed visual counts

Timed visual counts are a form of active, non-destructive, relative sampling. An individual observes a single plant or area for a standard duration of time and then records how many *H. halys* are seen over the course of that period. Two minutes is a common duration but observation times may be any length. During the observation, the sampler moves around the plant or area in a predetermined fashion. In tree fruit, this generally is done by circling a tree once. Observations may be broken down by life stage or aggregated for total *H. halys* found.

Timed visual counts are a useful technique to quickly generate data for a field with little preparation. The only supplies an observer requires are a stopwatch and datasheet. The weakness of this method is the difficulty in standardizing it. Results can be influenced by a myriad of biotic (observer fatigue, observer height, insect feeding history, etc.) and abiotic factors (precipitation, temperature, etc.). Investigations into this sampling method will be explored in the subsequent chapters.

Beat sheet sampling

Beat sheet sampling is an active, relative sampling technique that makes use of a standardized piece of capture material in conjunction with a striking bat. It is also referred to as limb jarring samples (Leskey *et al.*, 2012c). In practice, this often uses a 1x1 meter canvas square and a wiffle-ball bat. Samplers walk around the plant of interest and strike the foliage while holding the canvas square underneath the impact zone. Any *H. halys* that fall onto the sheet are counted. Sampling is standardized by the number of strikes per plant (for instance five per tree) and by areas struck (for instance foliage or fruit bearing branches and not small twigs or barren branches). After being recorded, insects can either be released back into the environment or captured.

Beat sheet sampling is a good way to gauge high populations in robust crops. Low populations may fail to be detected and delicate plants damaged with this method. Standardization can be hard as the strength of the strike or the decision about placement can impact the resulting counts. There is also ambiguity on inclusion of individuals that fly away as they fall from the plant onto the canvas sheet. The effect that the disruptive nature of this type of sampling has on the future behavior of individuals is unknown and may confound near-future population data collection.

Sweep netting

Sweep netting is an active, relative sampling technique commonly used to capture many insects and occasionally employed to survey for *H. halys* (Leskey *et al.*, 2012c). Samplers use a standard sweep net in a predetermined pattern that gives them a standardized value. The stroke used changes depending on the area being sampled (orchard trees, row crops, field crops, etc.). Once the sweep has been performed, the number of *H. halys* inside the net are counted and can be either released or kept for further study.

Black light sampling

Black light sampling is a passive, destructive sampling method that employs an ultraviolet bulb mounted on a funnel trap. Adult *H. halys* are attracted to the light source at night. When they fly towards the trap, they hit a fin blade mounted around the light and fall into a funnel cylinder. Often this cylinder also contains a toxicant that kills anything that falls into it. After a set duration of time (generally every three days to a week), the cylinder is emptied and *H. halys* are counted. Since ultraviolet light is attractive to many insects, this trap is often used to monitor multiple species at once (Shimoda and Honda, 2013).

Black light traps are an easy way to monitor the area-wide populations of *H. halys* or to examine how populations over regional areas change over time (Nielsen *et al.*, 2013; Wallner et al., 2014). Weaknesses for this method include non-selective attraction. This causes the traps to capture many different insects other than the target species. It is unknown from how far this trap attracts individuals which makes data from this method hard to use for population density estimation. This method is also only attractive to adults and as nymphs can cause damage this may lead to an underestimation of the damage potential of a *H. halys* population in an area.

Black light trapping has been used to track *H. halys* in Japan (Moriya *et al.*, 1987; Katayama *et al.*, 1993) and the USA (Nielsen *et al.*, 2013). In both countries, traps generally showed peak catches from late July to mid-August. In New Jersey, a series of black light traps has been used to track the movement and population shifts of *H. halys* across a regional level (Nielsen *et al.*, 2013). Other light types have been investigated as a means to attract this insect and white light was found to be attractive in both field and laboratory studies (Leskey et al., 2015c). Investigation into how black light traps and light attractants in general may be further used to monitor *H. halys* is investigated in the following chapters.

Pheromone trapping

Pheromone lures are generally used in combination with some other form of physical trap, else *H. halys* is attracted to the lure but is not captured. One of the most effective and commonly used trap forms is a pyramid trap. These can range in color but transluscent (Adachi *et al.*, 2007), yellow, and black traps have been shown to be more effective in field tests than other colors. Taller pyramid traps caught greater numbers of *H. halys* in soybean than did shorter pyramid traps (Nielsen *et al.*, 2011). Pyramid traps baited with methyl 2,4,6-decatrienoate attracted both adults and nymphs (Nielsen *et al.*, 2011; Leskey *et al.*, 2012c). Black pyramid traps caught more *H. halys* than did green, yellow, clear, and white and yellow traps (Leskey *et al.*, 2012c). The black pyramid traps on the ground also caught more *H. halys* than did the commercially available canopy-deployed baited traps from Japan (Leskey *et al.*, 2012c). The dark vertical structure of the pyramid trap is thought to mimic the form of a tree trunk which explains the trap's attractiveness to *H. halys* which is an arboreal species (Leskey *et al.*, 2012c).

The first pheromone used for *H. halys* was the aggregation pheromone of the brown-winged stink bug (*Plautia stali* Scott), methyl-2E,4E,6Z-decatrienoate (EEZ), which is cross attractive to *H. halys* and resulted in non-target captures (Khrimian, 2005; Leskey and Hogmire, 2007). This compound is attractive to both adults and nymphs (Khrimian *et al.*, 2008; Funayama, 2008). Sunlight can cause isomerization of the chemical but field data shows that as long as some of the EEZ isomer exists in the lure, it will attract *H. halys* (Khrimian *et al.*, 2008). This lure has also been shown to be attractant to the tachinid fly *Euclytia flava* (Townsend) which is a known parasitoid species of stink bugs in the mid-Atlantic region (Aldrich *et al.*, 2007).

Pheromone traps have been used to monitor the introduction and establishment of *H. halys* into new areas. In Beltsville, Maryland, the use of pheromone traps baited with methyl 2,4,6-decatrienoates (MDT) were used to monitor the populations of *Acrosternum hilare* (Say) and *H. halys* between 2004 and 2008. Analysis of these trap captures showed that the low, almost undetectable population in 2004 grew quickly and by 2008 outnumbered that of *A. hilare*. Work on new pheromones that are more specifically designed to attract *H. halys* (as opposed to cross attractants like methyl-2,4,6-decatrienoate) has been done. Khrimian *et al.* (2012) reported that a new compound released by *H. halys* containing (3R,6R,7R,10S)-10,11-epoxy-1-bisabolen-3-ol, (3R,6R,7R,10R)-10,11-epoxy-1-bisabolen-3-ol, (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol, (3S,6S,7R,10R)-10,11-epoxy-1-bisabolen-3-ol is attractive to *H. halys*. In 2014, a more effective aggregation pheromone was isolated by Khrimian *et al.*, 2014) from the creation of libraries of 1-Bisabolen-3-ols. Field and lab trials with this new pheromone showed it to be attractive to *H. halys* males, females, and nymphs (Khrimian *et al.*, 2014). The currently most effective pheromone lures are a combination of (3R,6R,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and MDT. Field tests showed that these two pheromones act synergistically catching 1.9-3.2 times more adults, and 1.4-2.5 times more nymphs than expected from an additive effect of the lures deployed individually. (Weber et a., 2014). Work on new pyramid trap designs is also ongoing (Schneidmiller *et al.*, 2011; Zhang *et al.*, 2014).

Pheromones are variable in effectiveness. New formulations are continuously being tested (Leskey et al., 2015b). Current formulations have greater attractiveness at certain times of the year than they do at other times. Sex pheromones are only attractive to adult males. Aggregation pheromones attract all mobile life stages but it is unknown if this attractive effect is of equal magnitude across life stages. Like black light trapping, it is unknown at what range pheromone trapping attracts individuals from the surrounding environment. This method is very effective in other species and shows promise for application with *H. halys*.

Other methods

In forested area, many trees with thin peeling bark such as oaks or locust species or any standing dead tree house serves as an overwintering home for *H. halys*. Physical and visual inspections of infested areas have revealed *H. halys* in these areas. The use of trained scent dogs has also been studied and shows promise (84% effective in lab and semi-field trials) (Lee *et al.*, 2014a).

For future studies, there is a need to be able to track individual *H. halys*. Work on how best to adhere harmonic radar trackers to *H. halys* with Krazy glue, Loctite, and FSA revealed the importance of sanding the pronotum (attachment area) to remove the wax layer. The type of glue did not matter and this proved an effective method for tracking this insect (Lee *et al.*, 2013b, 2014c). There has also been work showing the accuracy and usefulness of crowd sourced reports from online websites (Hahn *et al.*, 2016).

<u>Rearing</u>

Currently, methods for raising *H. halys* resembles other stink bug rearing protocols such as that for the southern green stink bug (Panizzi *et al.*, 2000). Individuals are kept in cages ranging in size from 0.3 to 2 square meters. These enclosures control the temperature, photoperiod, humidity, and access to food and water.

The temperature is generally held constant throughout the entire rearing process with the notable exception of colonies going through diapause. *H. halys* in diapause may be kept as low as 2-3 °C. Under non-diapausing conditions, rearing temperatures range from 22 °C to 26 °C (Funayama, 2006: Medal *et al.*, 2012; Haye *et al.*, 2014). This is well within the developmental thresholds for *H. halys* which have been found to be 17 °C to 33 °C (Nielson *et al.*, 2008a) and 20 °C to 30 °C (Haye *et al.*, 2014). *H. halys* is a facultative diapauser so colonies are not required to go through diapause to continue producing new generations (Saulich and Musolin, 2014).

Relative humidity can be maintained at 50-55% (Medal *et al.*, 2012) or 65-75% (Nielsen *et al.*, 2008a). Other stink bug rearing techniques use similar conditions (Fortes *et al.*, 2006). Humidity regulation can be achieved through either an automated system or done by hand.

A 16:8 light-dark (LD) schedule created by using fluorescent bulbs and a simple 24 hour light timer is used as a baseline for a non-diapausing colony (Medal *et al.*, 2012; Niva and Takeda, 2003; Funayama, 2006; Nielsen *et al.*, 2008a).

H. halys will readily feed on peach, apple, pear, sunflower seeds, carrots, raw peanuts, dry soybean seeds, fresh cherry tree branches, fresh tree of heaven branches, common ash branches, common ivy vines, green beans, and lima beans as a laboratory diet (Li *et al.*, 2007; Funayama, 2006; Medal *et al.*, 2012; Haye *et al.*, 2014).

Management Options

A wide variety of control measures have been tested in both the urban and agricultural setting. Control in both of these systems has primarily relied on chemical toxicants but more work is being done to investigate the utility of natural enemies, intercept traps, and deterrents.

Management in Urban Settings

Several chemicals have been shown to have toxicity or repellency to *H. halys* entering homes: dichlorvos and sumithion (Saito *et al.*, 1964), cyphenothrin and DEET (Watanabe *et al.*, 1994), phosmet, dinotefuran, acetamiprid, thiomethoxam, β-cyfluthrin, cyfluthrin, fenpropathrin, bifentrin, and λ-cyfluthrin (Nielsen *et al.*, 2008b). The use of intercept traps such "slit-traps" have been shown to decrease the number of individuals entering the building (Watanabe *et al.*, 1994). The ability of control measures to reduce the number of *H. halys* entering a building to overwinter was shown to be more efficient in new fully concrete buildings as opposed to older wooden homes (Watanabe *et al.*, 1994).

Management in Agricultural Settings

Chemical**:** The most common form of control for stinkbugs is chemical application (Akin *et al.*, 2011). Chemical treatments provide a decent amount of control but their effectiveness decreases as sprays become less frequent (Yu and Zhang, 2007). Mortality rates resulting from chemical applications decrease with decreasing exposure intervals (Lee *et al.*, 2013c). Among the most toxic to *H. halys* is the pyrethroid insecticide bifenthrin with generally no difference in toxicity between males and females despite the sexual dimorphism in weight (Nielsen *et al.*, 2008b).

The efficacy of many pesticides was evaluated in 2012 (Leskey *et al.*, 2012a). The more promising chemicals tested were dimethoate, malathion, bifenthrin, methidathion, endosulfan, methomyl, chlorpyrifos, acephate, fenpropathrin, and permethrin based on the high initial mortality rate and low recovery rate (Leskey *et al.*, 2012a). Dinotefuran (a neonicotinoid) was also investigated and was shown to be a strong potential candidate for early season control (Funayama, 2012). Deltamethrin was shown to have potential in electro auto-sprayer trials in Korea (Bae *et al.*, 2008). Often the toxicity of a chemical is assessed by looking at the proportion of individuals that are dead, moribund, and alive over time after exposure to a pesticide (Leskey *et al.*, 2012a). Occasionally another metric is used, such as how movement behaviors change 1.5-4.5 hours after exposure to the insecticide (Lee *et al.*, 2013c).

H. halys in the spring and early summer, which are mostly overwintering adults are easier to kill than *H. halys* in the middle to late portion of the summer (Leskey *et al,*. 2013). The identification of effective selective insecticides is a research priority because of the deleterious effects, such as secondary pest outbreaks, that many broad-spectrum pesticides have on the agroecosystem. The chitin biosynthesis inhibitors novaluron and diflubenzuron have already shown an ability to control nymphal *H. halys* (Kamminga, *et al.*, 2012).

The use of beating the trees with sticks to elicit anabiosis (dropping of *H. halys* to the ground) was used in field tests by Li *et al.* (2007). After causing the *H. halys* to fall to the ground, a pesticide was applied to the ground cover resulting in an 88.4% reduction in spray applied compared to previous application rates. Essential oils (lemongrass oil, ylang ylang oil, clove oil, geranium oil, rosemary oil, spearmint oil, wintergreen oil, pennyroyal oil, 1-menthone, P-menthone, eugenol, E-citral, Z-citral, pulegone, methyl benzoate, l-carvone, methyl salicylate, benzyl acetate, β-caryophyllene, geraniol, camphor, and cis-rose-oxide) have also been explored as possible tools for *H. halys* suppression (Schneidmiller and Zhang, 2012).

The detection of the Cry1Ac protein can be used to assess how much of Bt toxin an organism has been exposed to. In field-caught BSMB in Bt engineered soybean fields in China, *H. halys* nymphs feeding on leaves were found to have 1-10% of the Cry1Ac protein concentration being expressed in the leaves although they did not appear to be adversely affected (Yu *et al.*, 2014).

In orchards *H. halys* is harder to control than other pests because it does not necessarily spend its entire lifecycle in the orchard (Biddinger *et al.*, 2010). Between 2010 and 2011, apple and peach growers in the mid-Atlantic region increased the number of sprays and decreased the duration of time between sprays to treat for *H. halys* (Leskey *et al.*, 2012b). In apples and peaches, there is often higher damage in exterior rows than interior ones (Leskey *et al.*, 2012b). Work has been done in peaches to show how management practices can take advantage of the *H. halys* orchard edge aggregation behavior by only spraying in the orchard perimeter and still achieving sufficient pest suppression (Blaauw et al., 2015). Overwintering adult *H. halys* were more susceptible to pesticides than were the following generation's adults (Leskey et al., 2014).

Cultural**:** The timing of planting can be used to reduce the amount of feeding on some crops. However, effectiveness may depend on other, more attractive crops being nearby at the right time (Kuhar *et al.*, 2012). The planting of resistant cultivars is being investigated for use in soy (Bansal *et al.*, 2013). Bagging of fruit, mechanical removing of nymphs and egg masses, removal of nearby alternative hosts, and trap cropping have also been investigated as means of control in Asia (Lee *et al.*, 2013a). In Asia early maturing soybeans planted around later maturing soybean has been used as a trap crop to protect the later maturing variety (Osakabe and Honda, 2002). Sorghum, admiral pea, millet, okra, and sunflower have also been studied as potential trap crops and found that a combination of sorghum and sunflower could potentially be an effective trap crop for *H. halys* (Nielsen et al., 2016). There has been a call for further investigation into cultural practices to reduce the impact of this pest in soy beans (Akin *et al*., 2011).

Biological: Entomopathogenic fungi have been tested as potential treatment options for *H. halys*. Gouli *et al.* (2012) assayed three *Beauveria bassiana* and two *Metarhizium anispoliae* isolates. One of the *B. bassiana* types is used as the active ingredient in BotantiGard® and resulted in 85-100% mortality in 9-12 days post application. The other *B. bassiana* and *M. anispoliae* also produced mortality but *M. anispoliea* was less effective than the *B. bassiana* (Gouli *et al.*, 2012). *Metarhizium anisopliae* strain FRM515 was tested against *H. halys* as different temperatures and different concentrations. *H. halys* showed a greater resistance to this pathogen compared to *P. stali* (Scott) or *Glaucias subpunctatus* (Walker). Nearly 50% mortality was seen in *H. halys* after 10 days when a dose of $1x10^6$ conidia/ml was administered (Ihara *et al.*, 2008). There was a recently discovered iflavirus found in *H. halys* but further research into how to use this as a control has yet to be done (Sparks *et al.*, 2013).

Candidates for classical biocontrol are being investigated for release in the USA (Akin *et al.*, 2011). Several parasitoids have been investigated as possible control agents for *H. halys*. Some have been screened in the laboratory to see if they warranted further investigation. *Telenomus triptus* (Nixon) was shown to have a low potential for egg parasitism in this context (Kikuchi *et al.*, 1995). Others were recovered from field collection of eggs. In the United States, Bernon *et al.* (2005) recovered *T. podisi* (Ashmead) and *Anastatus sp*. from field collected eggs. One of the most effective parasitoids to be identified is *Trissolcus halyomorphae* (Yang) (Yang *et al.*, 2009), later reidentified as *T. japonicas* (Ashmead) (Talamas et al., 2013). Investigations into its potential as an agent of biocontrol revealed that its growth and parasitism rates are temperature dependent (Li *et al.*, 2004). *Trissolcus japonicas* has an average parasitism rate of 50% in its native range (Yang *et al.*, 2009). *Trissolcus mitsukurii* (Ashmead), *T. plautiae* (Watanabe), and *T. itoi* (Ryu) collected in Japan and reared on *H. halys* eggs at different temperatures to estimate their ability to use *H. halys* as a host (Arakawa and Namura, 2002). All three species showed successful development of both sexes from eggs when reared at or above 17.5 ^{o}C and males of each species developed faster than females. The rate of development for these species showed that they could potentially go through 11 to 15 generations per year.

Trissolcus mitsukurii was further investigated by Arakawa *et al.* (2004). This study showed that *T. mitsukurii* emerged with a larger average body size and had higher fecundity when reared on a large host such as *H. halys* as compared to smaller hosts such as *Plautia crossota stali* or *Nezara viridula* (Linnaeus). *Trissolcus flavipes* (Thomon) was found to have an egg parasitism rate of 63.3% in pear orchards (Chu *et al.*, 1997). *Trissolcus mitsukurii* was found to have an egg parasitism rate of 84.7% in pear orchards (Zang *et al.*, 1993). In order to support the natural enemies of *H. halys*, it is important to maintain insect biodiversity within an orchard (Yu and Zhang, 2007).

While *H. halys* eggs may be parasitized by native generalist parasitoids, this does not always lead to successful reproduction. In the United States, the generalist parasitoid *T. podisi* will readily lay eggs in *H. halys* egg masses but this will not lead to successful emergence of adult wasps from the eggs. This can be compared with its parasitism of another native stink bug, *P. maculiventris*, which it parasitizes at a similar rate and in which its developmental success is 98.3% (Abram *et al.*, 2014). It is also important to note though that the use of sentinel egg masses may underestimate true parasitism rates in an area (Jones, 2013).

In Maryland, the native parasites that have successfully emerged from BSMB eggs include *Anastatus reduvii* (Howard), *Anastatus pearsalli* (Ashmead), *Anastatus mirabilis* (Walsh and Riley), *Trissolcus brochymenae* (Ashmead), *Trissolcus edessae* (Fouts), *Trissolcus euschisti* (Ashmead), *Trissolcus thyantae* (Ashmead), *Trissolcus utahensis* (Ashmead), *T. podisi*, *Telenomus utahensis* (Ashmead), *Gryon obesum* (Masner), and *Ooencyrtus sp.* (Jones, 2013). *T. japonicas* was also idenfified as having established an invasive wild population in Maryland but the effect this will have on the wild *H. halys* population in the area is still unknown (Talamas *et al.*, 2015).

Augmentative biocontrol was attempted with *Anastatus sp.* in China (Zhengrong, 2009). Laboratory reared parasitoids where released around Beijingresulting in parasitism levels of *H. halys* eggs of 64.7% 52.6% for the first and second generation, respectively.

Native predators have been looked at for control of *H. halys* in Canada (Abrahm et al., 2015). Three native generalist predators, *Chrysoperla carnea* (Stephens), *Coleomegilla maculata* (De Geer), and *P. maculiventris* were tested to see if predation of stink bug eggs differed between *P. maculiventris* and *H. halys* eggs. This study focused on the difference in ability of different life stages to feed on *H. halys* eggs. Each species and life stage evaluated fed on *H. halys* eggs; the most veracious was late-instar *C. carnea* (Abram *et al.*, 2015). Other native predators, such as *Arilus cristatus* (Linnaeus), have also been reported to feed on a significant number of *H. halys* adults and nymphs (Jones, 2013).

***Integrated pest management*:** Information about how best to incorporate integrated pest management principles into control strategies for *H. halys* are a current research focus (Quarles, 2014). *H. halys* has caused many IPM programs to revert to calendar based sprays of broad spectrum insecticides (Leskey *et al.*, 2012b; Biddinger *et al.*, 2011). The negative impacts of using these broad spectrum insecticides to treat for *H. halys* include more frequent secondary outbreaks from pests like mites, aphids, and leafrollers (Biddinger *et al.*, 2011). These sprays may also affect native pollinators which are often found in orchards (Biddinger *et al.*, 2011).

Conclusions

H. halys is an invasive and highly destructive pest of a myriad of crops in the United States and across the world. Its ability to adapt to new environments makes it a serious concern for urban and agricultural pest managers in both currently invaded areas and areas where it has not yet been detected. Investigations into chemical, biological, and cultural controls are working towards determining the most ecologically safe and cost effective way to mitigate the damage caused by *H. halys*. In response to this need for easily accessible information on this destructive pest, several review papers have been complied to help distil the continuously growing amount of research (Rice *et al.*, 2014; Ingels, 2014; Lee *et al.*, 2013a; Leskey *et al.*, 2012d; Sargent *et al.*, 2011; Gill *et al.*, 2010; Holtz and Kamminga, 2010; Nielsen and Hamilton, 2009a; Hamilton, 2009; Yu and Zhang, 2007; Hoebeke and Carter, 2003; Kang *et al.*, 2003; Zhang *et al.*, 1993).

The effect *H. halys* will ultimately have on our cropping systems and native ecology are yet to be felt. More work is needed to develop an effective and accurate method for monitoring the spread and magnitude of current populations. The remainder of this thesis will cover research conducted to help standardize monitoring techniques and identify useful behavioral patterns associated with this insect.

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<u>Chpater 2</u>

Diel Sampling of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Peach Orchards

Abstract: Timed visual sampling is a common method used to monitor for the brown marmorated stink bug, *Halyomorpha halys* (Stål) in peach orchards. However the diel activity of *H. halys* and how it might bias sampling efforts is unknown. An investigation of how the number of hours past sunrise affects counts using time visual sampling was performed over a two year period at two locations in New Jersey. At both high and low population densities, observed *H. halys* exhibited significant differences in diel activity. The visually observed *H. halys* were predominantly nymphs at all sites over the course of the study. However, the ratio of adults to nymphs was found to differ between each year at each location. Observer bias was also found to affect the recorded counts. The primary findings from this study were that timed visual counts obtained at different hours past sunrise vary significantly with the highest numbers always detected at sunrise plus seven to ten hours. Timed visual observations for *H. halys* can be improved by standardizing the hours past sunrise at which sampling occurs. Sampling should include both adult and nymphal counts to accurately estimate *H. halys* abundance.

Keywords: BMSB, timed visual sampling, time of day, hours past sunrise, standardization, population monitoring

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug (BMSB), is an invasive agricultural and domestic pest in North America that is native to Asia. Since its initial identification in eastern Pennsylvania in 2001 (detected as early as 1996; Hoebeke and Carter, 2003), this pest has spread to 42 states and the District of Columbia (Leskey, 2015). This insect is known to feed on over 160 economically important plant species (Lee et al., 2013; Rice et al., 2014), which has caused many growers to increase insecticide applications in response to its arrival in their fields (Leskey et al., 2012).

Timed visual sampling is a common and effective way to sample for many insects in an agricultural setting. Examples include aphids on greenhouse tomatoes (Boll and Lapchin, 2002), thrips on cucumber and rose (Boll et al., 2007), and stink bugs in pecans (Ellis et al., 2012), macadamia nuts (Jones et al., 2001), and apples (Leskey and Hogmire, 2005). *H. halys* populations are often monitored using this method in tree crops such as peaches and apples (Blaauw et al., 2014).

Like most terrestrial organisms, insects have a circadian rhythm that influences behavioral patterns (Saunders, 2002). For example, the flight behavior and subsequent trap catches of the brown planthopper, *Nilaparvata lugens* (Stål), were found to vary over a 24 hour period in rice paddies, with peak catches recorded at sunset (MacQuillan et al., 1975). The western flower thrips, *Frankliniella occidentalis* (Pergande), was shown to increase walking, pollen consumption, and oviposition rates with increased ambient photoperiod (Whittaker and Kirk, 2004). It has been well documented that the time of day is a major factor influencing observations on species composition and abundance in visual surveys of spiders (Green, 1999). In other pentatomids such as *Plautia stali* (Scott) (Moriya and Shiga 1984) and *Biprorulus bibax* (Breddin) (James 1990), diel behaviors have been observed and documented. Given this diverse and consistent body of evidence among insects and other arthropods, it is likely that *H. halys* also exhibits such behavioral rhythms and that visual sampling outcomes are dependent on time of day.

Integrated pest management relies on the comparability of population metrics to be able to estimate the pest pressure in a field or orchard. These estimates are used to determine economic thresholds and ultimately decide when control measures are appropriate (Abrol, 2013). If an uncontrolled variable is responsible for significant changes in measured population metrics, irrespective of any actual variation in the population level, it can negate the comparability of these data. It is necessary to identify and standardize these variables before that population metric can be effectively used further. In order to evaluate the effect of time of day on *H. halys* timed visual counts, a study was conducted in two New Jersey peach orchards during 2013 and 2014. Peaches were chosen for this study because the use of timed visual counts is already an established method for monitoring *H. halys* in this system (Blaauw et al., 2014) and *H. halys* has the potential to cause serious damage on this crop (Nielsen and Hamilton, 2012b).

Methods

Study sites

This study was conducted between 1 June and 31 August in 2013 and 2014. Two peach orchards in central and southern New Jersey were used as the study sites. The first site was located at the Rutgers Fruit and Ornamental Extension Center in Cream Ridge (CR), NJ. The second site was at the Rutgers Agricultural Research and Extension Center in Bridgeton

(RAREC), NJ. Each site was sampled every other week during the study period. During 2013, RAREC was only sampled from 1 July to 31 August. At each site, the study block comprised

132 trees spread out over 6 rows.

Sampling times

Nine sampling times were set for each 24 hour period. As hours past sunrise was the variable of interest, all times were aligned by sunrise to account for the roughly one hour difference between the beginning and end of the sampling season. Sampling was conducted at 0, 2, 4, 7, 10, 12, 15, 18, and 21 hours past sunrise (HPS). The first count performed for each 24 hour sampling period was randomized to avoid bias due to observer fatigue resulting from previous samplings. Each study block was divided into n equal sized sections where n is equal to the number of observers present. Observers were randomly assigned a section at each sampling time to avoid bias due to differences in observer ability. The ambient orchard temperature (° Celsius) was recorded each time sampling was conducted.

Timed visual sampling

Sampling was done using 2.0-min visual counts during which observers walked around the tree in a circuit, recording the life stage, tree identification, and sampling time for all *H. halys* found. *H. halys* eggs and first instars were excluded from this analysis as they are non-motile (Lee et al., 2014). During these counts, the observer would make one circuit around the tree, paying equal attention to fruit, leaves, and branches. The observers did not touch or move the trees to ensure as minimal disturbance as feasible, however, they were trained to investigate the tree canopy as thoroughly and systematically as possible. As this study was conducted during both the day and night, the protocol included the use of a light source (Energizer HD4L33AE Headlamps, China) after sunset for the entire 2.0-min sampling interval.

Observer variability

To assess how different observers affected the comparability of the data, person to person variability trials were conducted. For these trials, all observers silently sampled the same set of 10 trees in sequence. Two-minute-visual surveys were conducted using the same technique as was used for the *H. halys* timed visual sampling study. These trials were replicated three times in 2013 and four times in 2014. The time of day for these trials was randomized.

Data analysis

A zero-inflated negative binomial model was fit to the timed visual sampling data. Data were then subjected to a repeated measures analysis (SAS Institute version 9.4, Cary, NC). For counts of nymphs, the first two sampling days from CR in 2013, the first sampling day from RAREC in 2014, and the first two sampling days from CR in 2014 were excluded from the statistical analysis as sampling counts were too low to estimate the effect of time of day. These exclusions fit within the current ecological understanding of *H. halys* development as first generation nymphs are not apparent until late June in New Jersey (Nielsen and Hamilton, 2009a). Backward elimination was used to reduce a fully specified model (year, site, degree day, sampling time, and all interactions) to remove the variables that did not explain an appreciable proportion of the variation. A regression of temperature and total count was also performed to assess the predictive power of that variable using (PROC GLM, SAS Institute version 9.4, Cary, NC).

Results

Two population levels (high and low) were identified from the data. High populations were defined as seeing an average of >1 *H. halys* per tree for the season in that location. Low populations were defined as seeing an average of ≤1 *H. halys* per tree for the season in that location. RAREC 2013 was found to have a high population (average of 4.50 *H. halys* per tree on each day at each time). CR 2013, RAREC 2014, and CR 2014 were all found to have low populations (0.20, 0.14, and 0.25 *H. halys* per tree on each day at each time, respectively). The averages calculated for assigning the population magnitude as high or low were calculated by combining both adults and nymphal counts.

The ratio of nymphs to adults varied between sites and years: RAREC 2013 had an average of 8.08 nymphs per adult, CR 2013 had an average of 1.34 nymphs per adult, RAREC 2014 had an average of 11.68 nymphs per adult, and CR 2014 had an average of 7.91 nymphs per adult (Figure 1).

The average number of observed *H. halys* adults and nymphs was calculated for each sampling time and associated with the number of hours past sunrise. For the low populations, the average number of nymphs found per tree was: 0.13 at 0 HPS, 0.18 at 2 HPS, 0.24 at 4 HPS, 0.26 at 7 HPS, 0.26 at 10 HPS, 0.22 at 12 HPS, 0.04 at 15 HPS, 0.02 at 18 HPS, and 0.02 at 21 HPS. These counts were significantly different between HPS ($p<0.0001$). For the low populations, the average number of adults per tree was: 0.03 at 0 HPS, 0.03 at 2 HPS, 0.04 at 4 HPS, 0.04 at 7 HPS, 0.04 at 10 HPS, 0.05 at 12 HPS, 0.06 at 15 HPS, 0.05 at 18 HPS, and 0.03 at 21 HPS (Figure 2). A repeated measures analysis showed that there were significant differences between the counts of individuals at different HPS. Counts were found to be different between HPS ($p<0.0001$).

At high population densities, the average number of nymphs found per tree was: 2.88 at 0 HPS, 4.52 at 2 HPS, 5.19 at 4 HPS, 6.82 at 7 HPS, 6.09 at 10 HPS, 5.45 at 12 HPS, 1.87 at 15 HPS, 1.54 at 18 HPS, and 1.02 at 21 HPS. Counts were found to be different between HPS ($p<0.0001$). For the high population densities, the average number of adults found per tree was: 0.47 at 0 HPS, 0.50 at 2 HPS, 0.36 at 4 HPS, 0.47 at 7 HPS, 0.47 at 10 HPS, 0.42 at 12 HPS, 0.60 at 15 HPS, 0.60 at 18 HPS, and 0.58 at 21 HPS (Figure 2). A repeated measures analysis showed that there were significant differences between the counts of individuals at different HPS. Counts were found to be different between HPS ($p<0.06$).

The influence of individual observers on observations was conducted in an orchard with high populations in 2013 and with low populations in 2014. In the high population tests, there were four observers present for all variance trials. Their averages were 11.6, 15.2, 16.1, and 14.4 *H. halys* recorded per tree. In the low population tests, six observers were present for all variance trials and their averages were 2.2, 1.7, 1.6, 0.7, 1.2, and 1.3 *H. halys* recorded per tree (Figure 3). A repeated measures analysis showed that there were significant differences between individuals in both years (high population; $p<0.0001$, low population; $p<0.0001$).

Maximum daily sampling temperatures were paired with the corresponding average number of *H. halys* per tree observed at that time to ascertain if temperature was a useful predictor of population level. The data from this study did not find the maximum daily temperature to be effective at predicting the maximum average number of *H. halys* per tree ($r^2=0.033$, $p=0.73$) (Figure 4).

Discussion

Timed visual sampling is a useful tool that researchers and IPM scouts should continue to use to monitor *H. halys*. This method allows for quick estimation of population densities in many different habitats and crops as the observers do not need to rely on having access to any tools, traps, or lures. The findings from this study indicate that *H. halys* exhibits a predictable diel behavioral cycle which results in dramatically different counts throughout the day. The number of hours past sunrise that observations are taken at must either be held constant or accounted for to allow comparability of the data between other sites or days. Future work should investigate the comparability between different HPS in more depth to identify how close sampling times must be to fall within an allowable degree of precision interval, and then determine whether or not they change over the course of the growing season.

At both high and low population levels, the nymphal instars represented the majority of total observed *H. halys* and the number of observed individuals was significantly different between most sampling times. This variation in observed population levels can lead to detecting false population increases or conversely, missing an actual population increase. Both scenarios can lead to reduced effectiveness of management programs and potentially result in unnecessary application of pesticides.

The findings from this study suggest that the diel behavior patterns of BSMB are influenced by life stage. Nymphs were found most frequently during the day with peak counts at sunrise plus 7 to 10 HPS. Night time observations of nymphs were uncommon in both high and low populations. Adult *H. halys* were found more frequently at night than during the day in the high populations. At low population levels, the only time significant differences were observed was at 15 HPS which roughly equates to sunset.

Nymphal *H. halys* showed the same pattern of increases and decreases in counts over the course of the day in both high and low population scenarios. The visually observed counts started relatively low at sunrise and steadily increased until midday (7 to

10 HPS). After midday, the detections would steadily decrease until sunset when it quickly dropped to low levels and remained low until sunrise the following day.

During the course of this study, the number of nymphal *H. halys* were much higher than that of adults. Given that observations of nymphs was so variable during the day while the adults were consistent, it initially seemed that just using the adult counts as the population metric might be a more appropriate choice. However, the ratio of adults to nymphs varied dramatically between years and locations. Since nymphs also cause unacceptable damage (Leskey et al., 2012), it is important to include their numbers in population estimates. An economic analysis of the impact of *H. halys* has not been done for many of the crops it feeds on, however in 2010 it was reported by the United State Apple Association to have cost apple growers in the mid-Atlantic region approximately 37 million dollars (United States Apple Association, 2010).

There was significant variability between observers. Observer as a variable should be treated with caution since lack of sleep, mental preoccupation, hunger, thirst, and numerous other factors have been shown to result in significant differences between attention levels (Jung et al., 2011; Furnham and Allass, 1999; Lieberman, 2007). The study design for this experiment allowed for a level of control over many of these variables as well as the training level of everyone involved but in other scenarios, this is unlikely to be the same between individuals.

The peak number of observed *H. halys* was always found at either 7 or 10 HPS, which consistently corresponded to the time of day that represented the highest daily temperature. However, only using the raw temperature data did not afford accurate estimation of the average number of *H. halys* per tree at that time. It is possible that these insects are using daily photoperiod to help accurately time their peak activity such that it corresponds to the warmest part of the day. If this is the case, we would expect that the insect would likely make the best of whatever temperature profile was to be experienced that day and normally come out at the time when the most favorable temperatures are likely to be encountered.

This study assumed that the population of *H. halys* in the area around the peach orchard did not change over the course of the 24 hour sampling period and that the samples taken each hour past sunrise were equally representative of the insect abundance in the area. The observer variance study assumed that the population of *H. halys* on each tree did not change over the course of the 20 minute sampling periods. Both of these studies assumed that conducting timed-visual sampling on a tree did not affect subsequent samplings.

We demonstrate that BSMB exhibits a diel behavioral pattern in peaches with significantly higher numbers occurring at 7 to 10 HPS for nymphs and during the nighttime for adults. Similar changes in population levels have been reported in other systems with other insects sampled with various point sampling techniques. The trend of the apparent population change will be dependent on the biology and ecology of the insect of interest. Any form of sampling that requires an individual to go out into the field and take a point measurement (timed-visual sampling, sweep net sampling, beat sheet sampling, etc.) should not assume that populations of motile insects will be consistent throughout the course of the day (Rashid et al., 2006; Wade et al., 2006).

The diel behavioral patterns of pentatomids have been well documented before and the findings of this study are consistent with other recent studies. Soergel (2014) used caged individuals on orchard trees to show that peak adult feeding occurred during the scotophase which agrees with the higher adult counts during this period recorded in this study. Shearer and Jones (1996) reported that *Nezara viridula* (L.) adult females fed more frequently during the night time. While this current study does not provide data on the number of stylet piercings by time of day, as was done with *N. viridula*, the fact that we observed more adults on the tree during the night circumstantially agrees with that finding. In 2006, Krupke et al. showed that *Euschistus conspersus* (Uhler) adults formed nightly aggregations around pheromone lures but mostly disbanded by morning. Diel aggregations have been reported for other stink bugs such as *Thyanta pallidovirens* (Stal) (Wang and Millar 1997) and *Bagrada hilaris* (Burmeister) (Huang et al., 2013). There is little available information on the diel behaviors of pentatomidae nymphs however and this study is the first record for *H. halys* in a non-caged field setting. The findings from this study will be helpful for developing accurate monitoring protocols for *H. halys* in the future and also can serve as a model system to encourage consideration for inconspicuous behavioral patterns when estimating population density for any species that have not had rigorous descriptions of its biological and ecological life history.

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Figure 1: The ratio of visually observed adult brown marmorated stink bug to nymphal brown marmorated stink bug during 2013 and 2014 RAREC and CR. Only second, third, fourth, and fifth instars are included in nymphal estimates. Ratios are based on the total yearly counts.

Figure 2: The average number (+/- SE) of *H. halys* at each hour past sunrise (A – High populations, Nymphs; B – High population, Adults; C – Low population, nymphs; D – Low population, Adults. Only second, third, fourth, and fifth instars were included in nymphal estimates. Bars with the same letter are not significantly different, $p<0.05$; Repeated Measures.

Figure 3: The average number (+/- SE) of *H. halys* for observer variability trials conducted in 2013 and 2014 (A – High population, 2014; B – Low population, 2013). *H. halys* counts represent adults and second, third, fourth, and fifth instars. Bars with the same letter are not significantly different, $p<0.05$; Repeated Measures.

Figure 4: The average number of *H. halys* per tree at the maximum daily temperature for each day in each year. The average *H. halys* represent both nymphs and adults combined. Temperature is reported in ° Celsius. $r^2=0.033$, $p=0.73$).

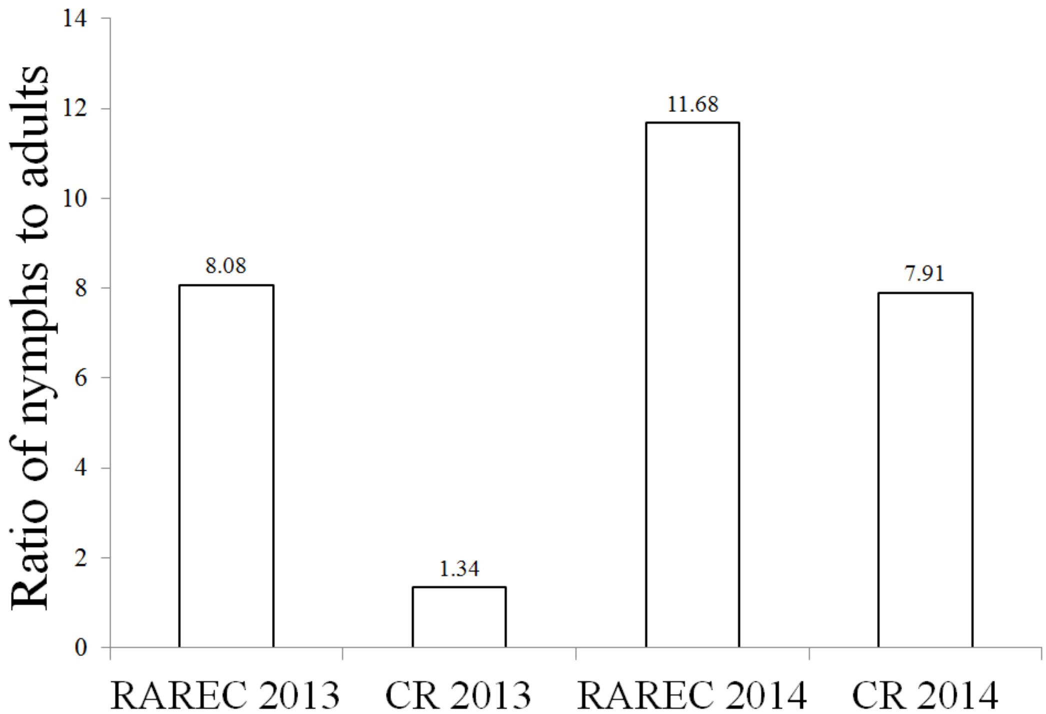

Ratio of nymphs to adults
14
12
10
8
6
4
2
0
8.08
1.34
11.68
7.91
RAREC 2013
CR 2013
RAREC 2014
CR 2014

Figure 1

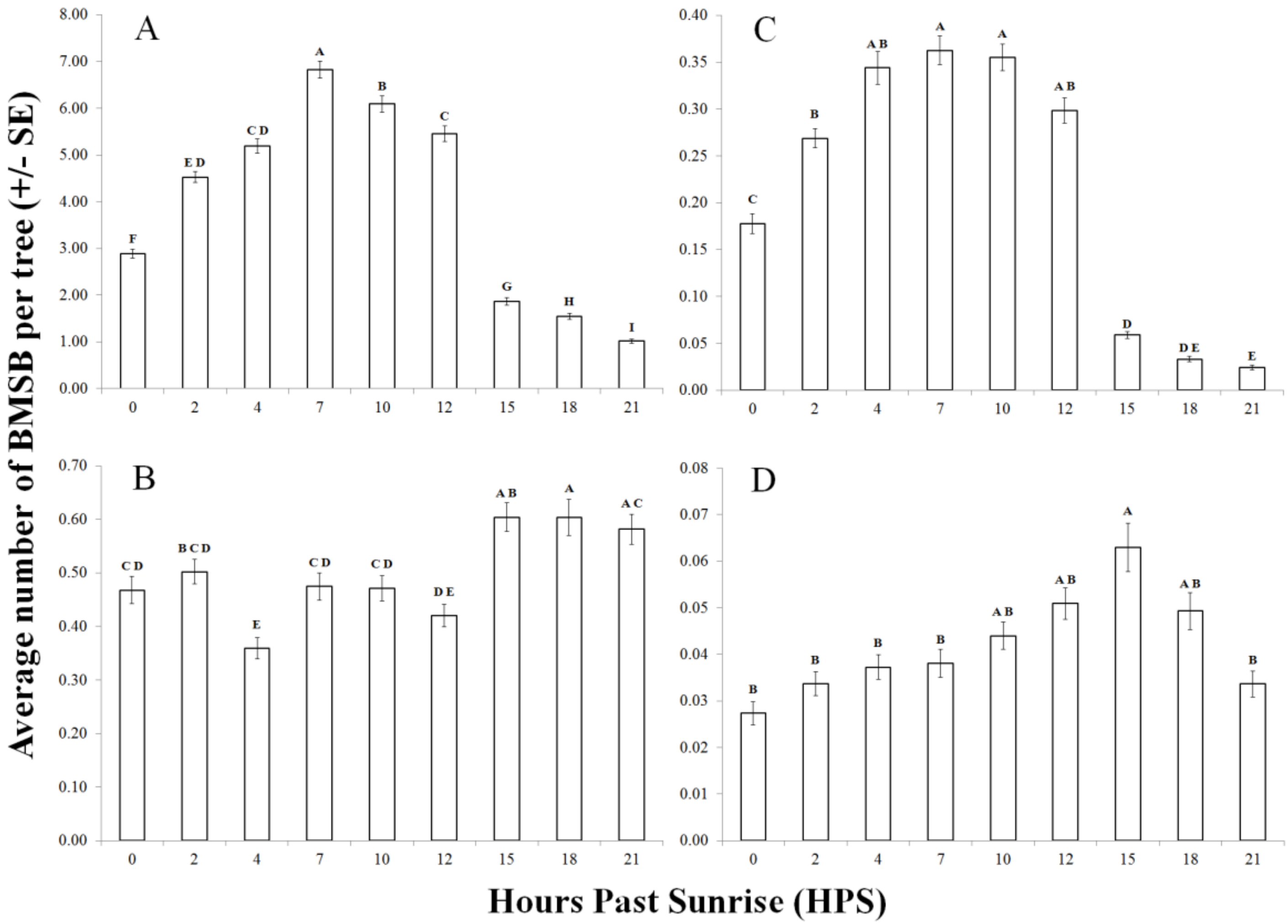

A
B
C
D
Average number of BMSB per tree (+/- SE)
Hours Past Sunrise (HPS)
0
2
4
7
10
12
15
18
21
F
E D
C D
A
B
C
G
H
I
C D
B C D
E
C D
C D
D E
A B
A
A C
C
B
A B
A
A
A B
D
D E
E
B
B
B
B
A B
A B
A
A B
B

Figure 2

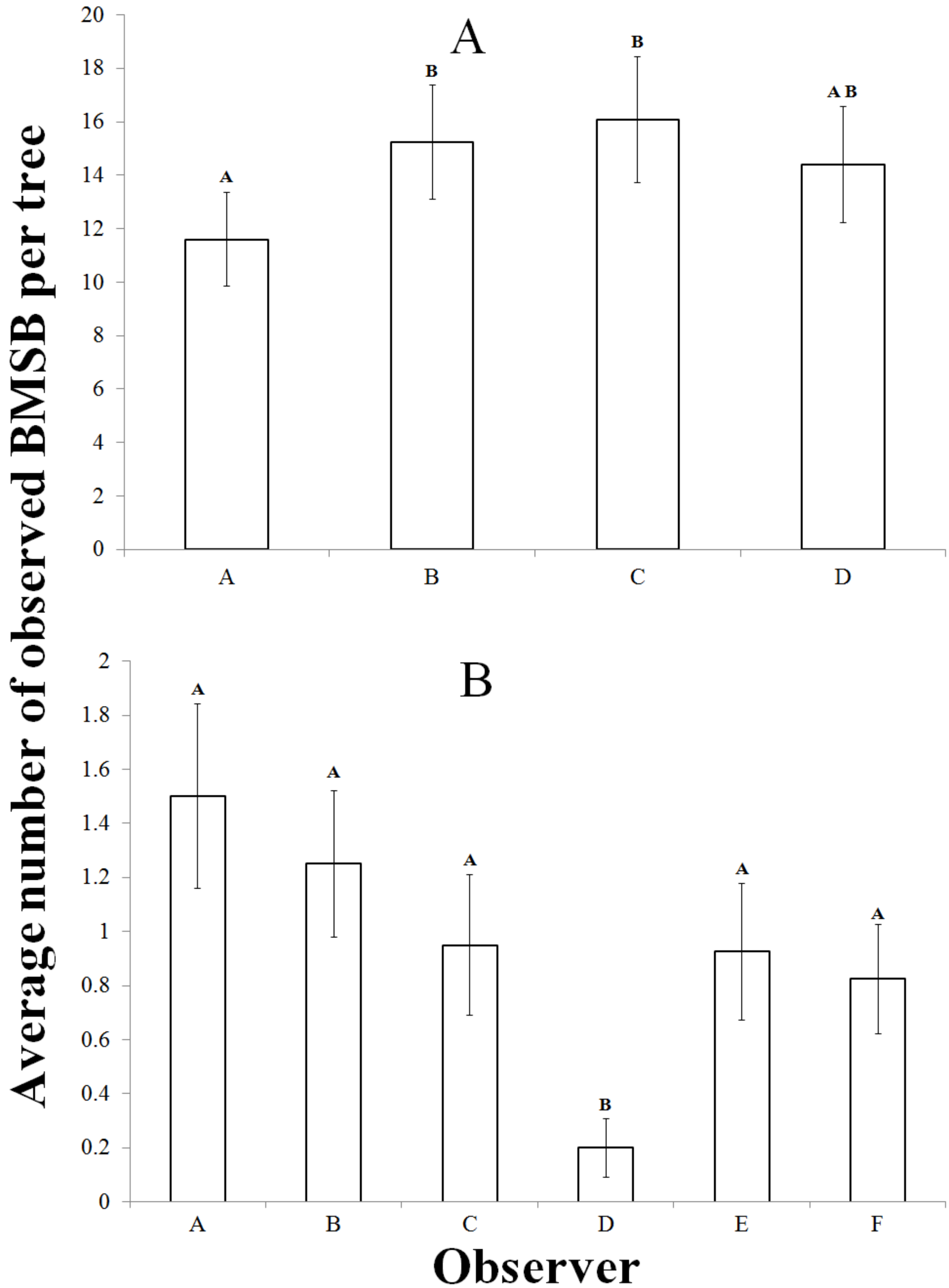
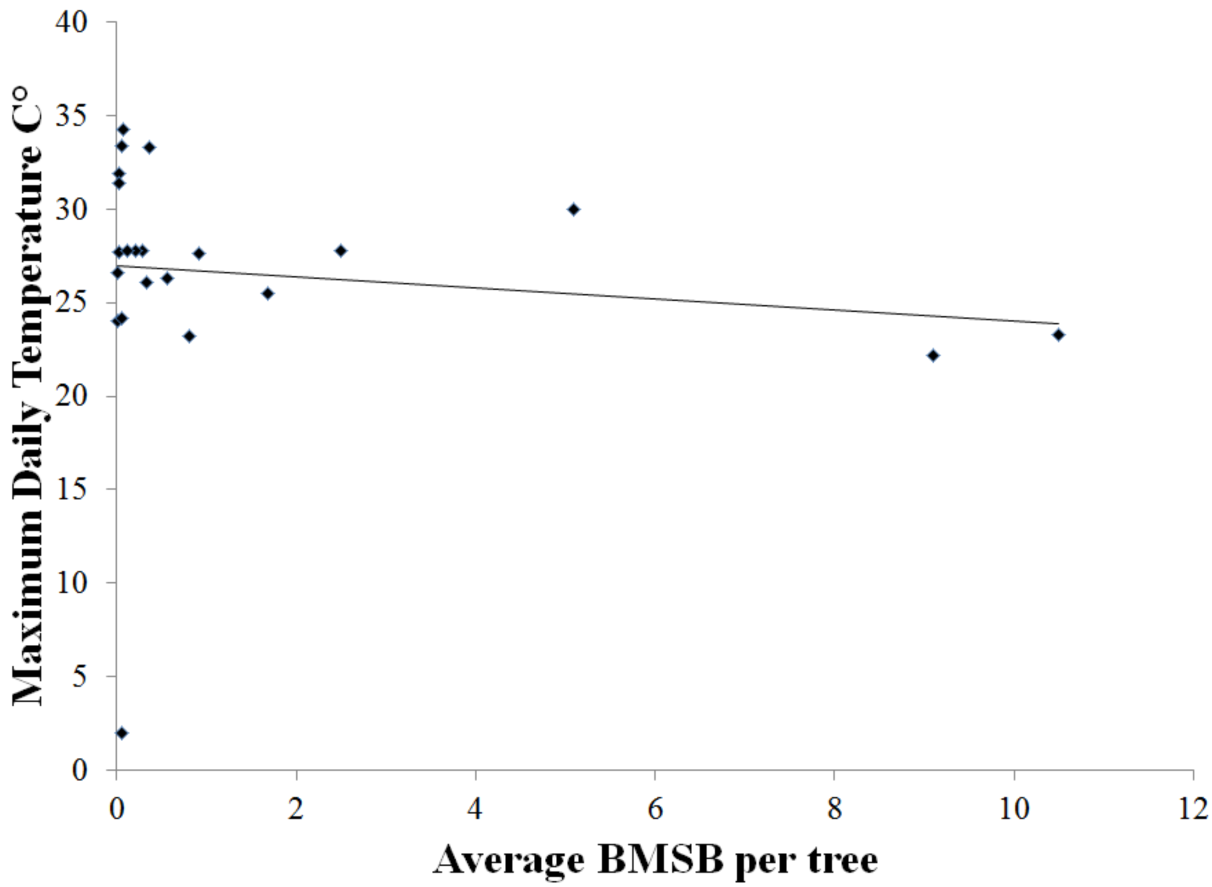

A
B
Average number of observed BMSB per tree
Observer
A
B
C
D
E
F
20
18
16
14
12
10
8
6
4
2
0
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
A B

Figure 3



Maximum Daily Temperature C°
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
Average BMSB per tree

Figure 4

Chapter 3

Within Tree Movement of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Peaches

Abstract: Timed visual sampling is a useful way to monitor for insect crop pests. Accurate prediction of where pests will be located has employed information on the specific feeding habits of each life stage, accumulated degree days towards development, and other temporal and biological indicators. This study investigated the impact that time of day has on the population distribution of *Halyomorpha halys* (Stål), the brown marmorated stink bug, within peach trees by conducting timed visual observations at nine different times over a diel period, each week in two orchards over two years. The findings suggest that during the growing season, this insect prefers to rest on leaves on the outside of the tree throughout most of the day but moves towards the fruit and interior of the tree during midday. Observers are likely seeing different individuals each time they sample, even if this is during the same day. Detection of individuals moving between trees was more common for adults than nymphs; however, neither were uncommon (31.7% and 8.2% respectively). This study shows that *H. halys* does not show the same diel behaviors in all life stages. This information should be incorporated into visual observation programs for this insect so that population monitoring can be done as effectively as possible.

Keywords: BMSB, timed visual sampling, time of day, within orchard movement, plant surface preference

Introduction

Information on a pest's biology and behavioral patterns can be used to increase the effectiveness of monitoring protocols (Quilici et al. 2007). Many insect species are known to change their preferred position on a host plant for various reasons such as thermoregulation, feeding, protection, and oviposition (Emden et al. 1969, Norton et al. 2001, Hausmann et al. 2004, Cottrell et al. 2008). Knowing locational preferences for an insect can be useful information for the description of visual inspection protocols.

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the brown marmorated stink bug (BMSB), has shown itself to be a pest in numerous agricultural systems such as tree fruit, row crops, and field crops with the number of possible hosts reaching over 100 (Rice et al. 2014). In peaches, this insect causes season-long damage resulting in a diversity of fruit injuries such as corking, catfacing, and fruit abortion. (Acebes-Doria, 2016; Nielsen and Hamilton, 2009) The temporal-spatial patterns of *H. halys* are important to characterize so that this information can be used to optimize population monitoring programs. Peaches are a particularly important system to study this in as they are susceptible to damage from this insect at all fruit growth stages (Lee at al. 2013).The distribution pattern of *H. halys* within peach trees is currently unknown. This also holds true for how the behavior of this species changes in respect to the number of hours past sunrise (HPS). The number of observed *H. halys* in peach orchards changes in a predictable way over the course of a day (Cambridge, in review). This diurnal change was previously uncharacterized other than by the simple presence or absence of individuals on peach trees. This current study provides insight into how *H.*

halys diel movement patterns can be described in terms of preferred surfaces and locations within a peach tree.

Methods

Field sites

The study was conducted in peach orchards over the growing season (June through August) in 2014 and 2015 at two Rutgers University research facilities. Each orchard included multiple varieties (Encore, Blushing Star, Fantasia, and Johnny Boy) and trees were pruned to an open V. The first site (CR) was at the Rutgers Fruit and Ornamental Extension Center in Cream Ridge, NJ and the second site (RAREC) was at the Rutgers Agricultural Research and Extension Center in Bridgeton, NJ. During 2014, 42 trees were sampled at each location. During 2015, 136 trees at CR and 76 trees at RAREC were sampled. Sampling was carried out once per week, alternating locations each week.

Sampling times

Sampling was conducted at nine times over the course of the day. Sampling occurred at 0, 2, 4, 7, 10, 12, 15, 18, and 21 hours past sunrise (HPS). Sample times were aligned by sunrise each day because the duration of light exposure was suspected to play a role in behavioral changes. At CR in 2015, 8:00am was 149 minutes after sunrise on June 1st and 96 minutes after sunrise on August 31st. This change was suspected to be enough of a difference that standard clock times throughout the summer was not deemed sensitive enough to investigate the variable of interest. The samples were taken in sequence over a 24 hour period and initial start times in HPS were randomized each week to account for any effects that could be associated with observers having been already conducting the study for a prolonged duration of time that day.

Sampling technique

Sampling was done using 6-minute visual counts per tree Six minutes is twice as long as standard visual sampling durations for stink bugs in peaches (Leskey and Hogmire, 2005). This extended time afforded the opurtunity for a greater number of *H. halys* to be observed during surveys. An ultra violet flash light (LEDwholesalers, China) was used during all sampling times to aid in the detection of marked individuals and help determine previous marking status. During sampling periods after sunset, a headlamp (Energizer HD4L33AE Headlamps, China) was used throughout the entire count.

When an individual *H. halys* was found by an observer for the first time that day, the insect was immediately marked using a fluorescent powder (Shannon Luminous Materials, Inc., Santa Ana, CA) specifically assigned to that tree. Fluorescent powder was applied to *H. halys* by using a camel hair paintbrush (Dynasty, Thailand) which was dipped in the desired powder. Three colors (pink, yellow, and blue) were used to mark the nymphs in 2014 and four colors (pink, yellow, orange, and blue) in 2015. Trees were assigned colors such that no two trees of the same color were next to each other either orthogonally or diagonally.

After marking or reporting on the previously marked color, lifestage of the insect and location (surface, height, outer or inner canopy, and direction) was recorded. Life stage was divided into small nymphs comprising the second and third instars, large nymphs comprising the fourth and fifth instars, and the adult insects. First instars were excluded from this study as they do not move from the egg mass until they molt into second instars (McPherson and McPherson, 2000). The position of each nymph was recorded in several different ways. The surface on which the insect was found on was divided into fruit, leaf, or branch tissue. Flagging tape was placed through the tree to demarcate the bottom third, the middle third, and the top third of the canopy ball. *H. halys* were determined to be on either the outer portion of the tree or the inner portion of the tree based on their proximity to the closest exterior foliage. If the stink bug was found to more than 30 centimeters from the exterior portion, it was deemed to be on the inside of the tree canopy. The interior/exterior demarcation was done using the same flagging tape. The cardinal direction, divided into north, east, south, and west, of each insect was determined by referencing preset labeled stakes placed around each tree trunk such that it was equivalent to the center of a theoretical compass.

H. halys were determined to have been previously marked if they had fluorescent powder, of any color, on their thorax or abdomen; this assessment was confirmed using an ultraviolet flashlight. *H. halys* were determined to have moved between trees if they were marked with a fluorescent powder that was of a different color than the one assigned to the tree on which they were presently being found on. Individuals that were observed in this category were not marked again. The nymphal development model described in Nielsen et al. (2008) was used to determine that individuals would have molted in between sampling times.

Statistical analysis

The population of *H. halys* in each orchard was considered low at both sites during both years as determined by detecting an average of <1 *H. halys* per tree during all samplings times. The year, sampling site, and interaction between year and sampling site did not explain a substantial portion of the variation within the effect of interest (p>0.05, df=1, glm) so data from all sites and years were pooled for the primary analysis. To assess whether or not the proportion of individuals was different within any of the categories of interest at different hours past sunrise, the data was subjected to a comparative frequency estimation using the CATMOD procedure to fit a loglinear model to test for main effects of categories and HPS as well as their interaction (SAS Institute 2014).

Results

Life stage

The majority of individuals observed were second or third instars at all times of the day. Adults were proportionally more common at night than during the day (Figure 1). The proportion of observed individuals at each life stage changed significantly over the course of the day (p<0.001, df=16; Table 1).

Location

The proportion of observed individuals on each surface type changed significantly over the course of the day (p<0.001, df=16); Table 1). The majority of individuals observed on the tree surface were on leaves at all times of the day. Towards midday, a greater proportion of *H. halys* were observed moving along the branches and feeding or resting on the fruit (Figure 2). This also corresponded to the time when the highest total counts were recorded.

Height

The proportion of observed individuals at each height changed significantly over the course of the day ($p<0.001$, df=16; Table 1). Observed individuals were commonly found in the bottom two thirds of the foliage of the tree. One individual was found on the ground crawling along the soil around the tree but was not included in subsequent analyses. Towards midday, there was an increase in the proportion of individuals found higher up in the tree (Figure 3).

Outer or Inner Canopy

The proportion of observed individuals on either the outside of the tree canopy or the inside changed significantly over the course of the day ($p<0.001$, df=8; Table 2). Observed individuals shifted from being seen primarily on the outside of the tree foliage to the inside foliage during the middle of the day (Figure 4).

Cardinal Direction

Observed individuals were consistently found on all sides of the tree (Figure 5). While there was a significant difference in the proportion of individuals found at each direction at the different sampling times, there was no apparent trend or pattern to this variable ($p<0.001$, df=24;Table 2).

Previously marked and between tree movement

H. halys that were found throughout the day were most commonly new individuals that had not been previously observed. Only 19% of individuals observed during the day had been previously marked. This changed following sunset to 52% of observed individuals being previously marked (Figure 6). Previously marked nymphs were more likely to be re-observed at night than during the day, 19% compared to 62% respectively. Adults were equally likely to be re-observed during the day and the night, both 20%. 8.2% of previously marked nymphs were found on trees that they were not initially marked on. 31.7% of previously seen adults were found on a tree that they were not initially tagged on. The total proportion of observed individuals that were previously recorded changed significantly over the course of the day ($p<0.001$, df=8; Table 2).

Discussion

The brown marmorated stink bug is a highly mobile insect that resides in different parts of the landscape at different times of the year. This seasonal movement is a response to the availability of different food sources (Bakken et al., 2015) and the need for suitable overwintering locations (Inkley, 2012). This research describes how *H. halys* behavior within peaches changes as a function of time of day (HPS).

Small instar *H. halys* were the most commonly observed at all HPS. This is in agreement with the general r-selected species model that characterizes organisms that have a high birth rate and subsequently high mortality through their development (Southwood and Henderson, 2009). The cardinal direction at which *H. halys* were found also did not appear to follow any diel trend. A tendency towards certain directions at particular HPS was considered as a potential mechanism for thermoregulation but our data did not detect this as a behavior in *H. halys* in peaches.

The most common surface on which to observe *H. halys* on a peach tree was the leaves. This behavior held true through all HPS, however, there was a significant increase in the proportion of nymphs that were found on the fruit and branches during midday. The number of nymphs on the fruit and moving around on the branches stayed high until late in the afternoon when they returned to being more commonly found on the leaves. Adults were equally likely to have been found on the fruit during the day and night, 22.6% and 23.5% of the time, respectively. The shift towards a higher proportion of nymphs on the fruit coincides with the peak number of individuals observed on most days (Cambridge, in review). This could suggest that midday is peek feeding time for *H. halys* in peaches but this study does not provide enough evidence to fully support that conclusion.

The shift towards observing a greater number and larger proportion of marked *H. halys* higher in the peach tree canopy is interpretable in several ways. The results of this study suggest that *H. halys* stay on the same peach tree more often than move between them in an orchard over the course of a 24 hour period. *H. halys* may be spending the majority of the day hidden or resting in the tops of fruit trees and then venturing to feed during the middle of the day. Joseph et al. (2014) found that *H. halys* caused more damage in the upper portions in apple trees. Our data supports this behavior in peaches as well.

An increase in the proportion of previously marked *H. halys* at night is not likely an indication of any behavioral pattern as the study design used favored this outcome.

The use of fluorescent powder makes identification of marked individuals relatively easy in the dark and the investigators believe this to be an important factor to bear in mind when interpreting the data. This bias is not thought to compromise any findings or inferences on the movement of individuals between trees. The higher percentage of adults that were found to have moved between trees is understandable given the advantage that wings confer in dispersal capability. Adults were often seen or heard flying throughout the orchard during the day and night.

The findings from this study support previous work on *H. halys* behavior in the orchard setting. Adults and nymphs were both found to spend the majority of their time resting (Soergel, 2014). Soergel (2014) also observed the peak feeding time of adults to be during the night time, which is consistent with other pentatomidae studies and not inconsistent with this study (Jones, 1996). Work with *Euschistus conspersus* (Uhler) showed that adults tend to aggregate more often at night than during the day (Krupke et al., 2006). Diel aggregations patterns have been reported in other stink bugs as well (Wang and Millar, 1997; Huang et al., 2013).

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Table 1. Proportions of *H. halys* within the stage, surface, and height categories for each sampling time (hours past sunrise) during 2014 and 2015 at CR and RAREC. All populations were <1 *H. halys* per tree at all times, characterizing these populations as low.

	Stage (% per Category)			Surface (% per Category)			Height in Canopy (% per Category)		
Hours Past Sunrise	2nd and 3rd Instar	4th and 5th Instar	Adult	Fruit	Leaf	Branch	Lower	Middle	Upper
0	73.3	14.0	12.7	15.3	81.3	3.3	37.3	44.0	18.7
2	60.4	21.5	18.1	21.5	65.3	13.2	41.0	37.5	20.8
4	69.6	16.3	14.1	15.8	78.8	5.4	42.4	25.0	32.6
7	65.9	18.9	15.1	36.8	52.4	10.8	44.3	40.5	15.1
10	69.6	11.5	18.9	36.9	41.9	21.2	34.6	35.5	29.5
12	74.1	19.8	6.1	20.8	59.4	19.8	41.3	33.1	25.3
15	52.4	23.8	23.8	16.7	81.0	2.4	19.0	52.4	28.6
18	56.8	16.0	27.2	14.8	81.5	3.7	38.3	44.4	17.3
21	70.9	12.7	16.5	10.1	81.0	8.9	63.3	22.8	13.9

Table 2. Proportions of *H. halys* within the canopy position, direction, and previously seen category for each sampling time (hours past sunrise) during 2014 and 2015 at CR and RAREC. All populations were <1 *H. halys* per tree at all times, characterizing these populations as low.

Hours Past Sunrise	Position Within Tree (% per Category)		Direction (% per Category)				When Observed (% per Category)	
	Inside	Outside	North	East	South	West	New	Previous
0	39.3	60.7	31.0	20.0	23.0	26.0	72.7	27.3
2	46.5	53.5	23.6	36.5	21.2	18.8	72.9	27.1
4	63.6	36.4	30.7	17.9	17.1	34.2	89.7	10.3
7	45.4	54.6	24.6	30.3	20.0	25.1	76.8	23.2
10	76.5	23.5	26.7	31.6	24.9	16.8	90.3	9.7
12	54.9	45.1	20.1	34.0	23.5	22.4	78.2	21.8
15	35.7	64.3	29.8	22.6	16.7	31.0	47.6	52.4
18	30.9	69.1	25.9	35.2	21.0	17.9	38.3	61.7
21	22.8	77.2	27.2	31.0	31.0	10.8	57.0	43.0

Figure 1: Percentage of observed *H. halys* by life stage and hours past sunrise on peach trees sampled in 2014 and 2015. Life stages were divided into small nymphs (2nd and 3rd instars), big nymphs (4th and 5th instars), and adults as shown in the key.

Figure 2: Percentage of observed *H. halys* by surface observed on and hours past sunrise on peach trees sampled in 2014 and 2015. Surface categories were divided up into branches, leaves, and fruit as shown in the key.

Figure 3: Percentage of observed *H. halys* by height observed and hours past sunrise on peach trees sampled in 2014 and 2015. Height categories were divided up into the top third of the foliage ball, the middle third of the foliage ball, and the bottom third of the foliage ball as shown in the key.

Figure 4: Percentage of observed *H. halys* by canopy position of tree designation observed and hours past sunrise on peach trees sampled in 2014 and 2015. The outside/inside designation is as shows in the key.

Figure 5: Percentage of observed *H. halys* by direction designation observed and hours past sunrise on peach trees sampled in 2014 and 2015. The direction designation was determined by comparing the position of the *H. halys* observed with pre-placed stakes around the outside of tree that showed each of the cardinal directions in a compass fashion with the center trunk of the tree as the origin point. Directional designations are as shown in the key.

Figure 6: Percentage of observed *H. halys* by previously observed designation and hours past sunrise on peach trees sampled in 2014 and 2015. Previous marking designations are as shown in the key.

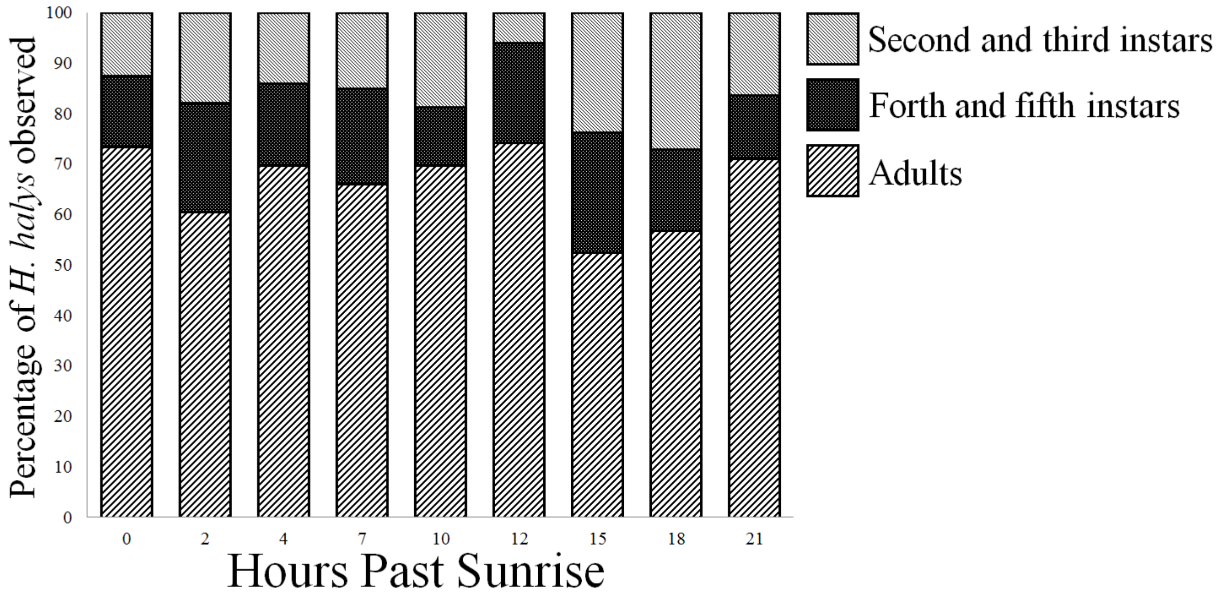
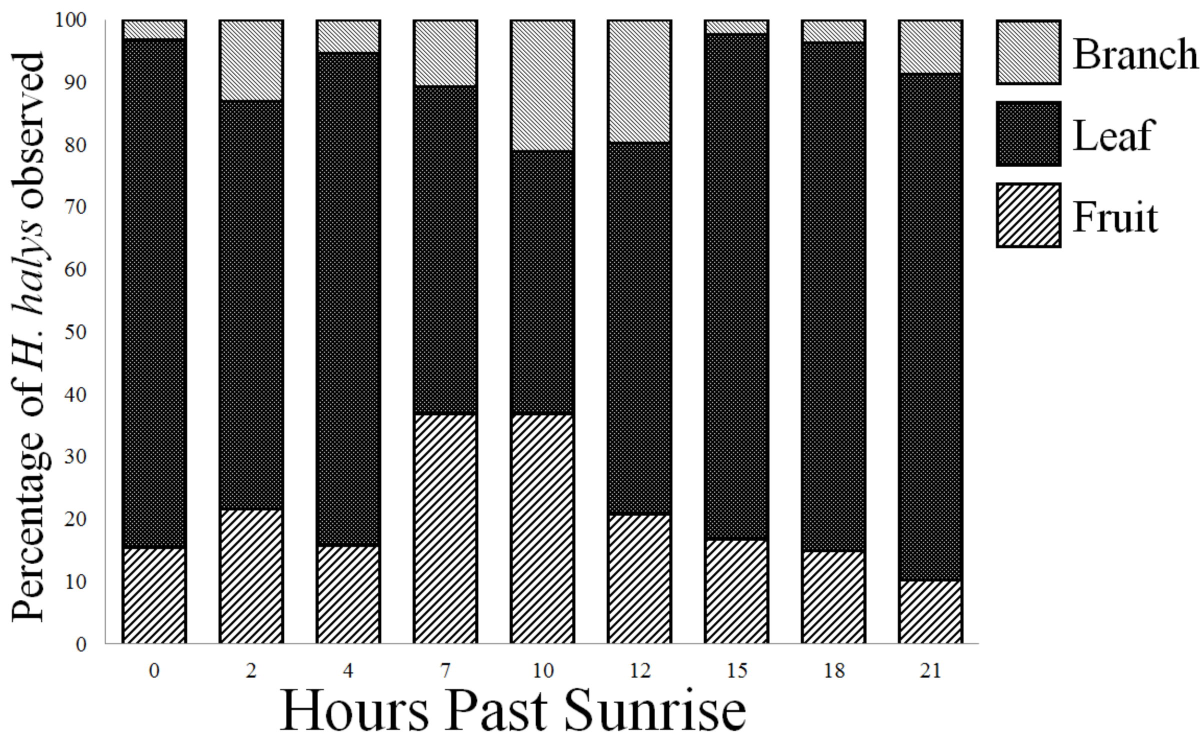


Figure 1



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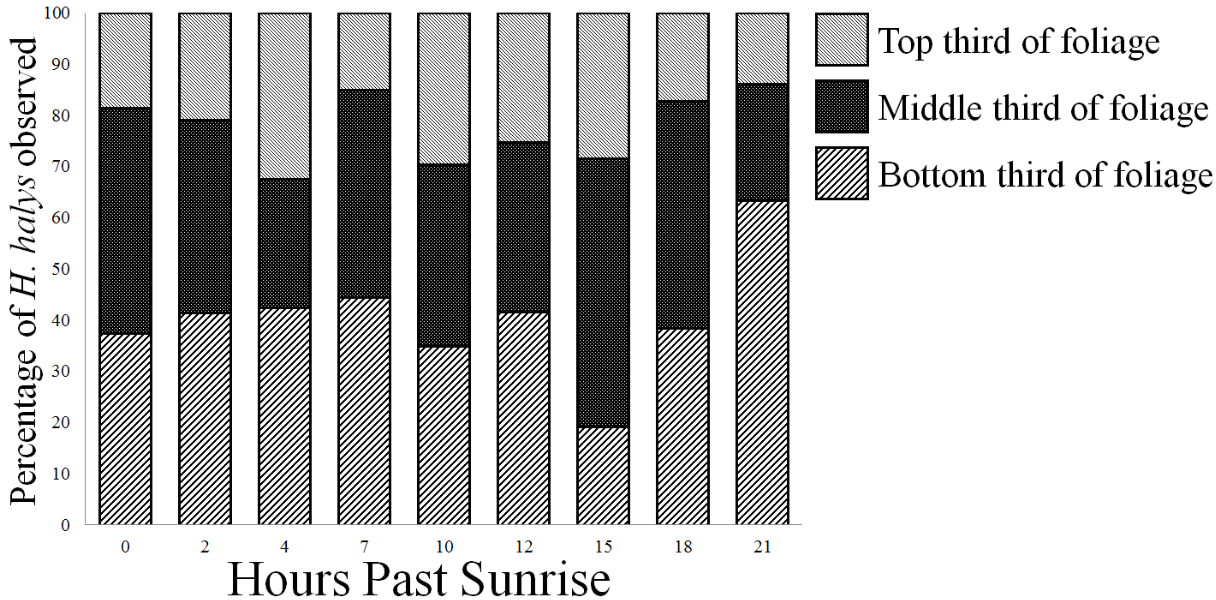

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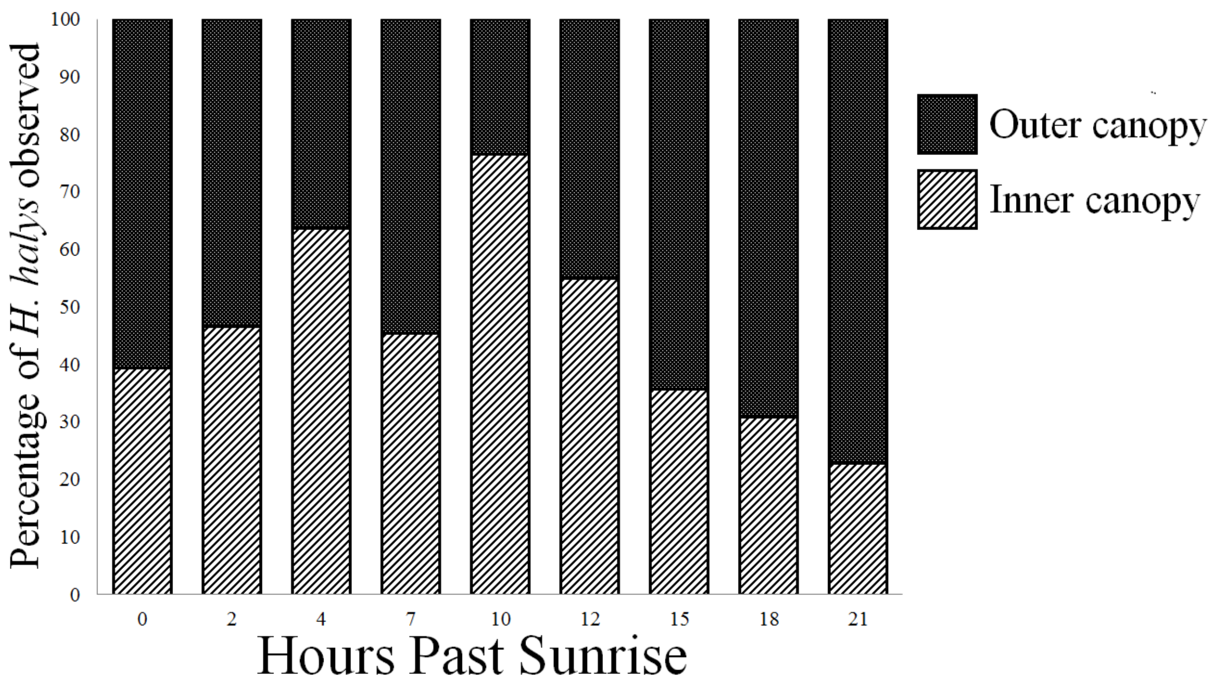

Figure 4

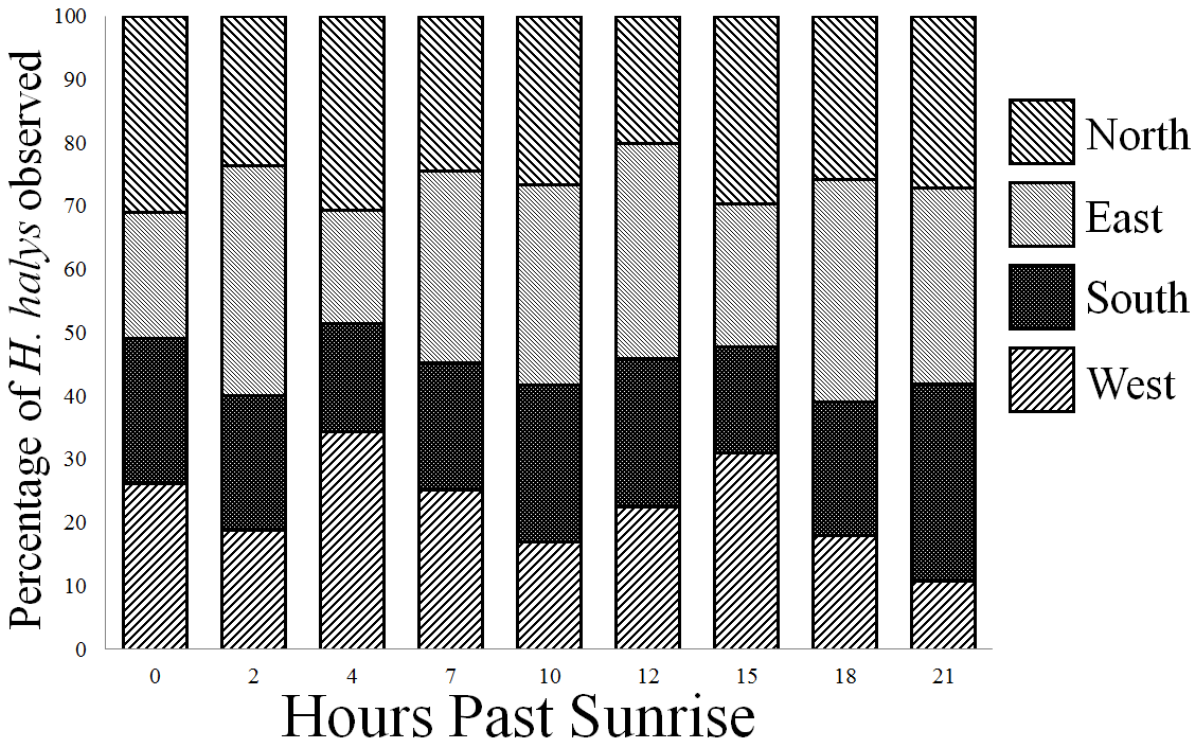

Figure 5

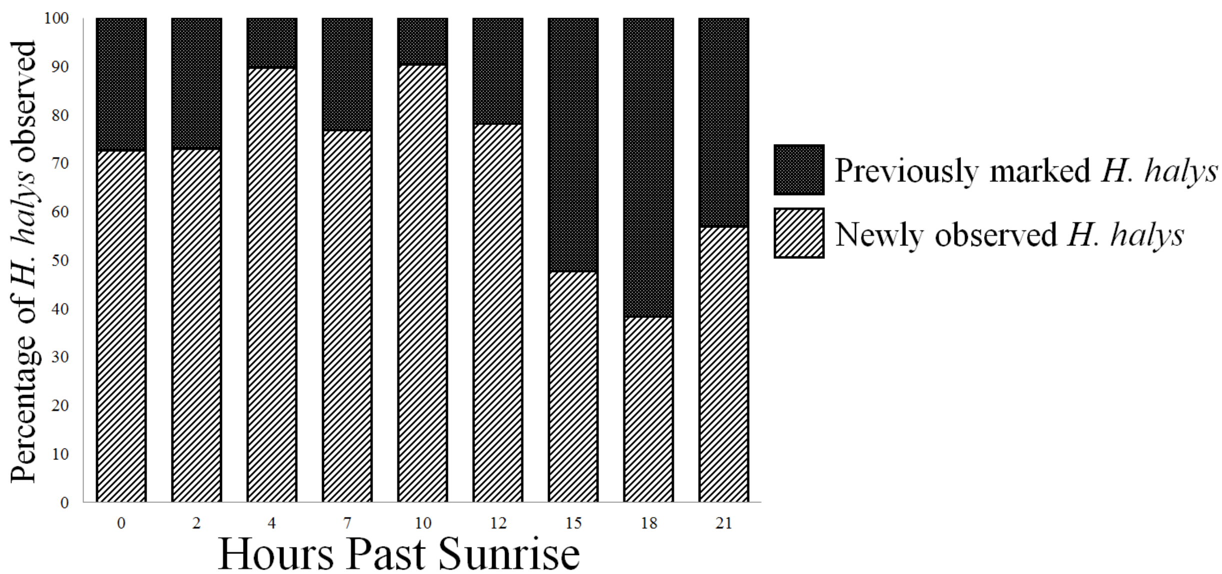

Figure 6

Chapter 4

The Distribution of Overwintering Brown Marmorated Stink Bugs (Hemiptera: Pentatomidae) in College Dormitories

Paper sections are amended to conform to the formatting requirements of the journal it was published in.

Cambridge, J., A. Payenski, and G. C. Hamilton. 2015. The Distribution of Overwintering Brown Marmorated Stink Bugs (Hemiptera: Pentatomidae) in College Dormitories. Florida Entomologist. 98: 1257-1259.

Sumary: This investigation into the pattern of overwintering brown marmorated stink bugs (BMSB) used survey data collected between Dec 2013 and Mar 2014 from residents in two 4-story dormitories on the Rutgers University Cook Campus in New Brunswick, New Jersey, USA. Results suggest that a higher proportion of *H. halys* overwinters towards the top of urban structures than towards the ground level. This finding can be used by pest control operatives for targeted applications that will reduce the total amount of pesticides needed while still suppressing the majority of urban nuisance populations.

Key Words: brown marmorated stink bug; dormitory; structure

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the brown marmorated stink bug (BMSB), is an invasive, non-native agricultural and domestic pest in North America. Since its introduction into eastern Pennsylvania in the mid 1990s (Hoebeke and Carter 2003), it has spread to or been detected in at least 42 states (Leskey 2014). Like many other pentatomids (Saulich and Musolin 2014), *H. halys* undergoes facultative diapause and overwinters as sexually immature adults (Nielsen and Hamilton 2009). Prior to diapause, this species seeks out and clusters in secluded dark areas, where it remains dormant until spring (Toyama et al. 2006, 2011). In the mid-Atlantic Region of the United States, adults begin moving into overwintering sites in Sep and Oct. They remain in these sites until they emerge in the beginning of spring between Mar and Apr (Nielsen et al. 2008). In its native range of eastern Asia, *H. halys* is known to be an arboreal species (Bernon et al. 2005) that overwinters in dead standing trees such as oaks, locusts, and paulownias (Lee et al. 2014). In addition to natural overwintering sites, *H. halys* has a well-documented behavior of moving into structures to overwinter (Kobayashi and Kimura 1969; Wantanbe et al. 1994; Hamilton 2009; Inkley 2012; Leskey et al. 2012). Entrance into these structures is thought to occur though gaps in the window and door trim, roof flashing, and other gaps around doors and ventilation holes (Welty et al. 2008).

Understanding the overwintering ecology and behavior of this insect will be critical in developing effective management techniques for suppressing it (Lee et al. 2014). To address this issue, we conducted a study in 2 student dormitories to determine how overwintering *H. halys* are distributed within this type of structure. Given the behavior of *H. halys* when seeking out overwintering locations in trees more than shrubs or ground litter (Lee et al. 2014), we hypothesized that *H. halys* utilizes the height of its overwintering locations and as such will prefer higher areas in urban structures. This is the first report on the overwintering pattern of *H. halys* inside a multi-unit residence building that details the distribution of the pest throughout the structure.

This study investigated the distribution of overwintering *H. halys* inside 2 student dormitory halls located on the Rutgers University Cook Campus in New Brunswick, New Jersey, USA. The survey portion of this study was conducted between 21 Feb and 14 Mar 2014. The survey asked the participants to identify the dorm unit in which they lived and whether or not they had observed any *H. halys* in their dorm since Sep 2013. A life-sized color picture of *H. halys* was included with each survey to help respondents with proper identification. No information about infestation magnitude or insect position within a dorm room was used in the analysis due to the non-uniformity and incompleteness of the responses. Information about observed *H. halys* in common areas, utility rooms, storage spaces, and bathrooms in the building was not collected in this study. The Perry residence hall and Voorhees residence hall contain 93 and 115 dormitory units, respectively. All rooms were of approximately equal size (~30 m3). Both buildings had nearly identical floor layouts on the second, third, and fourth floors (Fig. 1). The 1st floor of each building had fewer dorm units than the other floors because it included the common area, utility rooms, and other storage spaces. Data were combined from both dorms and analyzed using a Kruskal–Wallis test with R 3.0.1 statistical software (R Core Team 2015).

Ninety out of 113 units and 69 out of 93 units were surveyed successfully in Voorhees and Perry, respectively. From the 1st floor to the 4th floor in Voorhees, the percentage of rooms with observed *H. halys* was 20.0, 20.0, 19.2, and 34.5%, respectively. In Perry, the observed *H. halys* infestation rate was 11.1, 31.8, 40.9, and 68.8%, respectively, from the first to the fourth floor. Figure 2 shows the pooled data for both buildings on each floor. These results support the hypothesis that *H. halys* has a tendency to overwinter towards the tops of buildings ($P < 0.05$, df = 3). When tested for cardinal directionality in the buildings, results were insignificant for both individual residence halls ($P > 0.05$, df = 1).

As an arboreal species, this insect is found above ground level for much of its life cycle. A previous study looking at the distribution of overwintering *H. halys* in forests showed that individuals were much more likely to be found in dead standing trees than on the forest floor in fallen logs or leaf litter (Lee et al. 2014). This finding provides a possible behavioral explanation for the movement of *H. halys* into urban buildings through the doors, windows, and other areas higher in the structures. Our results support the hypothesis and provide evidence that *H. halys* prefers to overwinter above ground level in urban structures. Control protocols to suppress overwintering populations may use these findings to specifically target areas within an infested structure that are likely to contain the most individuals. By focusing on the upper portions of buildings, treatments may eliminate the majority of *H. halys* without having to incur the cost of treating the entire structure.

This study examined only buildings that were 4 stories tall, and the findings may not be directly translatable to taller buildings that are beyond the height of the host tree species in which *H. halys* naturally overwinters. Interpretation of these findings should also take into account the fact that the non-residential portions of the buildings were not surveyed. For the data collected, some results may also be inaccurate due to misidentification of *H. halys* or observer error, because identification was based on comparing pictures of the insect to encounters over the past several months.

Further investigation into how *H. halys* distributes itself in large, taller, and less homogeneous buildings should be done to more accurately characterize the behavior patterns governing this insect's overwintering habits. This study sampled *H. halys* locations towards the end of its overwintering period and the findings should be interpreted as such. It is possible that these insects, upon entering a structure, will continue to move around until they either find a suitable location for diapause or die. This study provides evidence on how *H. halys* are distributed in multi-floor buildings in late Feb to early Mar. Future investigations should look into where this insect can be found in these types of structures during other portions of the overwintering season.

The authors of this study do not report any conflicts of interest with the investigation. The study would not have been possible without the help of a large team of surveyors. Thank you to Mario Hernandez, Jeff Geist, Chris Alessi, David Kim, Raynee Morris, Anthony Pepi, and Kelsey Sealey. Thank you to the Rutgers University for the necessary resources and opportunity to conduct this study.

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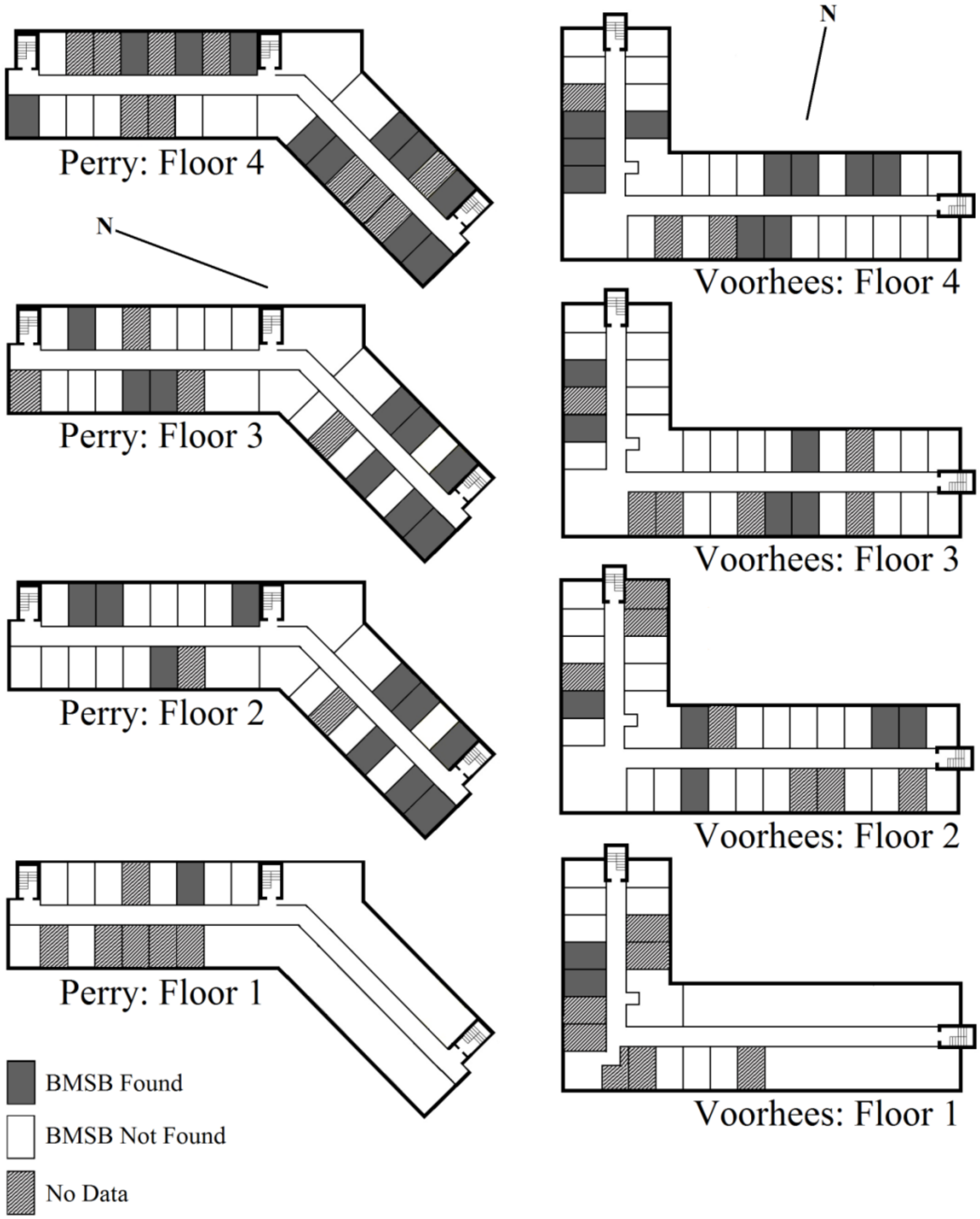
Figure 1: Floor plans for Perry (left) and Voorhees (right). Gray and white rooms represent rooms where overwintering *H. halys* was and was not observed, respectively, by the occupants. Diagonally patterned rooms represent rooms where no data were collected.

Figure 2: Percentage of rooms where residents observed *H. halys*, shown by floor. Data are pooled from Perry and Voorhees residence halls. Bars with the same letter shown above are not significantly different as determined with a Kruskal–Wallis test ($P > 0.05$).



N
Perry: Floor 4
N
Perry: Floor 3
Perry: Floor 2
Perry: Floor 1
BMSB Found
BMSB Not Found
No Data
N
Voorhees: Floor 4
Voorhees: Floor 3
Voorhees: Floor 2
Voorhees: Floor 1

Figure 1

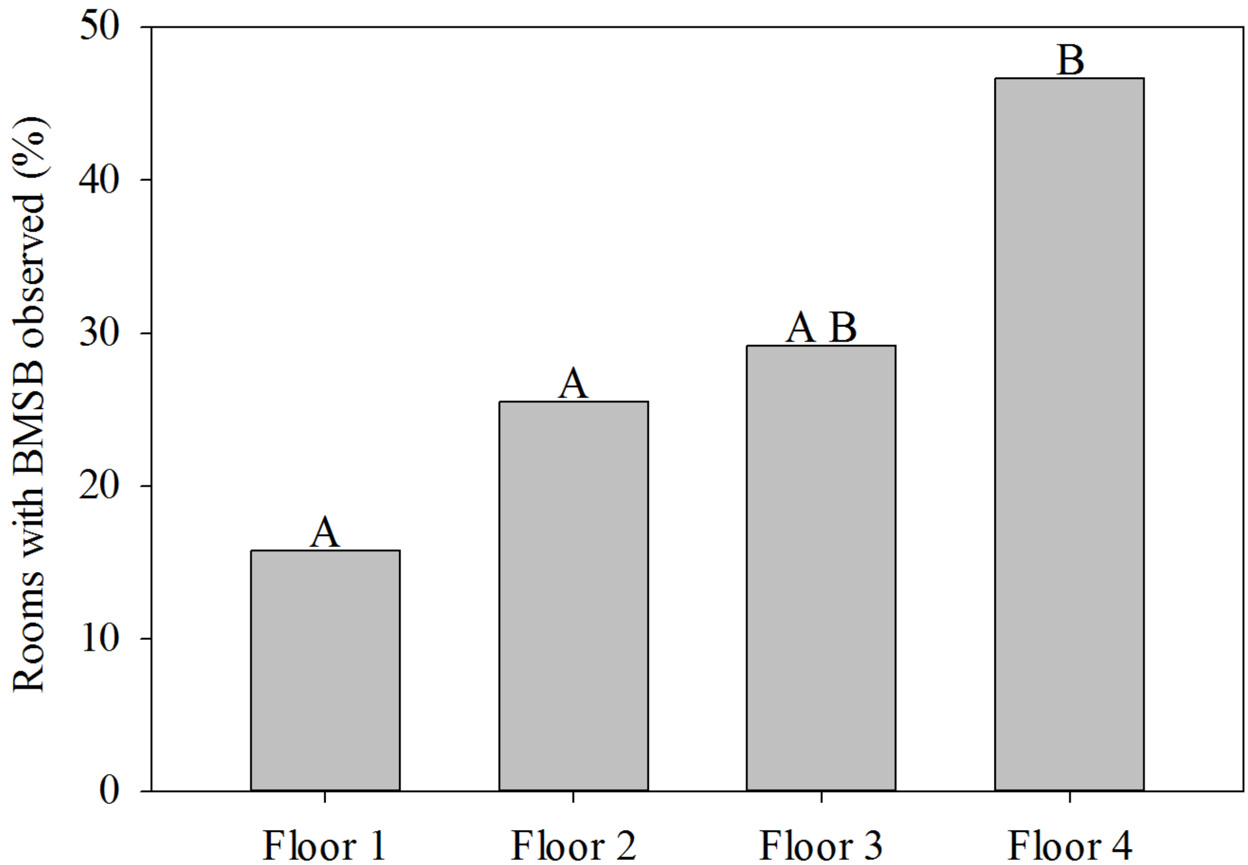

Rooms with BMSB observed (%)
50
40
30
20
10
0
A
A
A B
B
Floor 1
Floor 2
Floor 3
Floor 4

Figure 2

Chapter 5

Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) Attraction to Different Light Stimuli

Abstract: Light trapping is a common method for monitoring and capturing insects such as the invasive agricultural pest, Halyomorpha halys (Stål). Efforts to develop more effective trapping methods for H. halys have led to research investigating the response of this insect to potentially exploitable stimuli. A behavioral study was conducted to examine H. halys' response to different light stimuli. Seven intensities (0-dark, 0.1, 10, 50, 75, 100, and 155 lux) of white light were tested. The most attractive intensity was 75 lux for adult males and females. Nymphal instars 2-5, adult males, and adult females were also exposed to 75 lux white light. Adult males were significantly more attracted to the light than any other life stage. Adult *H. halys* were also exposed to green, orange, red, white, and yellow light. All colors tested were attractive to *H. halys*. White light was significantly more attractive than the other tested colors. The findings of this study suggest that the incorporation of a white light into *H. halys* traps may increase catches.

Keywords: brown marmorated stink bug, BMSB, Halyomorpha halys, light trap, intensity, wavelength, life stage

Introduction

For over one hundred years, light has been used to influence insect behavior in a variety of ways (Roth 1891; Harding et al. 1966). Insects may exhibit a positive or negative phototaxis, which can be used to either attract or repel individuals (Jander 1963; Kim et al. 2013). Traps that employ light to catch insects are most effective at capturing individuals during the night time as sunlight can negate or mask the attractive influence (Shimoda and Honda 2013). Many insects have regular circadian rhythms or other behavioral patterns which are governed by the presence or absence of light. These can be exploited to disrupt undesirable pest activities (Walcott 1969; Shimoda and Kiguchi 1995). Some insects use light cues to orient during flight or to identify suitable habitats. An understanding of these triggers has allowed growers to effectivity cloak green houses and other structures from certain nearby pests (Goodman 1965; Legarrea et al. 2010). Moreover, researchers have evaluated the consistency of these types of behavioral responses across the visual spectrum and found that different insects express peak reactions at different wavelengths (von Helversen 1972; Coombe 1981; Hardie 1989; Kinoshita and Arikawa 2000).

Investigation into the underlying biology that is responsible for these behaviors has provided insight on why variation among species occurs. While most of the insects which have been studied can be generally described as having a UV-blue-green trichromacy, there are several different pigments and configurations which insects may have within their compound eye (Briscoe and Chittka 2001; Koshitaka et al. 2008). Even within a species, males and females have been shown to have different wavelength sensitivities (Bernard and Remington 1991). Developers of light traps can use this type of information to tailor new devices to the optical peaks of the desired insect (Duehl et al. 2011).

White, UV, or yellow lights are often used for trapping mosquitoes for a variety of purposes (Li et al. 2015). In agricultural settings, ultraviolet (UV) light traps are commonly used to monitor population levels of pest species (Nielsen et al. 2013). Even when pheromones or other species specific methods for trapping an insect have been developed, it is helpful to identify the effect that light has on them so that it may be additionally incorporated into any trapping devices or protocols (Duehl et al. 2011; Leskey et al. 2015b). *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug, is a prime candidate for this type of investigation. Several semiochemicals, mainly pheromones and kairomones, have been identified and shown to be attractive to *H. halys* when used in baited traps at certain times. However, this species is such a serious pest that the need still exists for trap refining and identification of non-pheromonal synergists to attract *H. halys* and increase catches (Leskey et al. 2015a).

H. halys is a highly polyphagous, invasive agricultural pest native to Asia whose introduction into North America has been traced back to eastern Pennsylvania in or before 1996 (Hoebeke and Carter 2003). Over the past 18 years, it has spread across the continent and established populations in 42 states, the District of Columbia, and portions of Canada (Leskey 2015). This insect is responsible for damage to numerous crops including soybeans, tomatoes, peppers, apples, peaches, corn, and cane berries (Rice et al. 2014). Population monitoring and both preemptive and responsive pesticide application are currently the primary strategies employed for control of this pest (Leskey et al. 2012a, 2012b). Furthermore, UV light traps have been successfully used to monitor for *H. halys* on both field and regional scales (Nielsen et al. 2011; Nielsen et al. 2013; Wallner et al. 2014).

Recent studies have called for more comprehensive research into how different wavelengths of light affect the behavior of stink bug pest species (Shimoda and Honda 2013). Leskey et al. (2015b) investigated the potential trapping uses of different colors of light and various intensities under field conditions and in individual laboratory-based choice trails but called for future studies to look into the dynamics of these types of responses and specifically the area of arrestment around the light stimulus source. This current study further explores the potential use of light as an attractant for this insect by investigating how movement of *H. halys* differs between life stages and genders, in response to different intensities of white light, and in response to colored light across the visual spectrum in the laboratory.

Materials and Methods

H. halys adults and nymphs used in this study were taken from a lab colony maintained in the Rutgers Department of Entomology. Colony individuals were sustained on green bean (*Phaseolus vulgaris* L.), organic sunflower seeds (*Helianthus annus* L.), carrot (*Daucus carota* L.), and water at ~25 C° on a 16:8 light: dark photoperiod. Standard maintenance protocols were used in accordance with Niva and Takeda (2003) in BugDorm2 cages (BioQuip, Rancho Dominguez, CA).

The study arena consisted of a 2.3m x 1.3m x 3.0m room that was gridded into 10cm squares over all walls, ceiling, and the floor using black paint (Valspar, Minneapolis, MN). A light socket (3M Company, Flemington, NJ) was affixed to the center of four grid cells on one wall with a 1cm disk of hot glue (3M Company, Flemington, NJ). The average distance from each grid cell was then calculated and cells were grouped by their average distance from the light. The room was held at 27 C° and 30% relative humidity during all tests.

General protocol

For each trial, 10 *H. halys* were placed into an empty 1 liter polyethylene cubical holding container for 10 minutes. Individuals were then released on the floor in a standard location 155 cm away from the light source and allowed to freely move within the room for 30 minutes. At the end of each 30-minute trial the door to the room was opened and the location of each stink bug was recorded in accordance to the grid spaces. After each trial was completed, the room was aired out for 10 minutes. Individual stink bugs were not used in a trial more than once per day and were placed back into the colony at the end of each testing day. Each trial was replicated four times. Individuals who died during the trial were excluded.

Light bulbs

Color trials were conducted using flourecent light bulbs (Brightech International, Somerset, NJ) of a determined peak wavelength (560nm-green, 590nm-yellow, 750nm-red, 460nm-blue, and 640nm-orange). Dark trials used the same bulb type but turned off. Light intensity, life stage, and gender trials used an incondencent bulb. No other light sources were visible in the room during trials.

Color

One hundred and fifty eight mixed sex adult *H. halys* were tested for each color. Light bulbs in these trials had a luminosity of 30 lux as determined using the intensity protocol detailed below. The color bulbs used for this experiment were compact fluorescent lights and the luminosity was not adjustable. The white light bulb was the same type as was used in the life stages and intensity trials.

Life stage

Second, third, fourth, and fifth instars, as well as adult *H. halys* were tested using a white light set to 75 lux. Trials were conducted in a randomized order. First instars were excluded from consideration as they do not move from the egg mass under natural conditions.

Intensity

A digital lux meter (DrMeter, Union City, CA) was placed at 0.50 meters away from the white light source to determine the intensity for each trial. Trials were conducted in a randomized order. A lux reading of 0.1, 75, and 155 were used for the low, medium, and high intensity trials, respectively. An average of 40 males and 40 females were tested at each intensity using the general protocol. Males and females were tested separately to allow for comparison of the sexes.

Data analysis

The effect of the various light conditions, life stages, and gender was assessed by comparing the average distance of the individuals from that source for each trial type.

Data were analyzed using a general linear model which testsed for effects of the treatment on the average distance of *H. halys* from the light source (SAS Institute, 2014).

Results

Color

White light was the most attractive stimulus type tested. The dark trials had an ending average distance of 156.3cm which is very similar to the initial release distance of 155.0cm. All colored lights showed an attractive effect on *H. halys* ($p<0.0001$, df=5, f=23.96). Green, orange, red, yellow, and blue all had closer ending average distances than the initial release distance, 104.3cm, 112.8cm, 112.1cm, 107.8cm, and 94.4cm respectively. All colored lights elicited a significant response compared to the dark trials. White light was the most attractive with an ending average distance of 83.0cm from the light source and was significantly different than all other colors ($p<0.05$; Figure 1).

Life stage

The attractive influence of 75 lux white light was significantly different between life stages ($p<0.0001$, df=5, f=15.95). Nymphs were not attracted to the light and the responses of 2nd, 3rd, 4th, and 5th instar *H. halys* were not significantly different from one another ($p<0.05$). Nymphs averaged 113.6cm, 136.1cm, 136.6cm, and 140.1cm away from the light source at the end of the trial for 2nd, 3rd, 4th, and 5th instars, respectively. Adult males averaged 59.2cm and adult females averaged 103.5cm away from the light source. Males were significantly more attracted to the light source than all other life stages ($p<0.005$). Adult females were more attracted to the light than 4th and 5th instars (Figure 2).

Intensity and Gender

The interaction of gender and light source intensity had significant influence on the response of *H. halys* (p=0.0083, df=4, f=3.47). Intensity alone showed a significant difference between the tested lux levels ($p<0.0001$, df=4, f=9.88); however, gender did not (p=0.06, df=1, f=3.53). Males had a different response to the changes in light intensity than did females ($p<0.05$). When white light was dimmed to 20 or 0.1 lux, it did not induce a significant response from either gender of adult *H. halys* ($p<0.05$). Both males and females had the smallest average distance away from the light source at 75 lux (Figure 3).

Discussion

Laboratory studies conducted here support the previous findings that *H. halys* exhibits a positive phototactic response, orienting towards visual light sources and showing a distinct preference for white light over other colors in a laboratory setting (Leskey et al. 2015b). This investigation further explored this behavior and identified the average distance of *H. halys* around a light source to be dependent on the light type and intensity. The area of arrestment around a light source for *H. halys* is of particular interest because it can provide valuable insight into how new traps should be designed to effectively monitor this pest. The data from this study suggests that when *H. halys* are attracted to a light source, some of the individuals will cluster directly around it while others will not (Figure 4). More research should be done to investigate the individuals that do not cluster directly around the light and see if the average proportion exhibiting this behavior is consistent. If traps use light sources as an attractant, the data they produce must be interpreted in a manner that accounts for the individuals that were within the attractive range, but not induced to enter the trap. If the percentage of *H. halys* that are sufficiently attracted to a light source such that they enter a trap is not consistent, it may indicate that light is not a suitable lure for monitoring this species.

Light does not appear to be an effective way to attract nymphal *H. halys.* As light trapping for this species has traditionally failed to capture nymphs (Nielsen et al. 2011; Nielsen et al. 2013; Wallner et al. 2014; Leskey et al. 2015b), there is limited data on the behavior of nymphs to light stimulus. This is the first study to investigate the response of *H. halys* nymphs to white light. Second, third, forth, and fifth instar nymphs did not respond differently to the light source and appeared to not show any positive phototaxis throughout the trials. Adult *H. halys* males were significantly more attracted to the light source than any of the nymphal life stages and adult females were significantly more attracted to the light source than fourth and fifth instars. One explanation for these findings is that *H. halys* adults may use astronavigation for orientation during nighttime flight as has been documented in various other insects (Sotthibandhu and Baker 1979; Wehner 1984; Dacke et al. 2003). If this is the case, nymphal instars would have no use for such a behavior as they are incapable of flight.

Adults *H. halys* showed variable responses to white light at different intensities. As the light was dimmed to 20 lux and below, it appeared that the stimulus was too faint to elicit a response. Females did not show as strong an attraction to the light as did males. As the intensity increased from 20 lux to 75 lux, the average distance around the light decreased by 5.6% in females and 40.2% in males. As the intensity was increased further to 155 lux, the trend changed and the average distance increased, although there remained a significant overall attractive response in males compared to the lower intensities.

These findings suggest that there may be an optimal attractive intensity for *H. halys* adults. However, further investigation needs to be done as several issues arise from this interpretation. First, if there is an optimal intensity, a brighter stimulus might be expected to lead to a greater average group distance as the insects arrange themselves around the light at whatever distance corresponds to the location of that intensity. If the minimum threshold for light detection is greater than 20 lux in our experiments, this hypothesis could be supported; however, the investigators do not believe this to be the case and the data here does not support this trend for females at any theoretical detection threshold. Second, the large variabilty of distances at all intensities presents an issue for assigning an optimal intensity to these insects as regardless of the lux, many of them tend to disregard the stimulus while others cling to the blub. Further investigation is needed to clarify these issues. The authors also note that temperature played a role in the behavior of the insects in these conditions. All trials reported in this study were held at 27 degrees Celsius but several trials had to be excluded because the temperature either increased to over 30 C or decreased to below 23 C. During these trials, the insects did not appear to move at all. However, other research into the dispersal behaviors of this insect show that flight is indeed possible at lower temperatures (Lee and Leskey, 2014). This suggests that *H. halys* is less likely to respond to light stimulus during periods of temperature flux, regardless of the suitability of the temperatures themselves.

This study showed that *H. halys* response to light is different between life stages, light color, and light intensity. These findings can be used to develop better sampling methods for this insect but much more research to needed to fully understand all the intricacies of phototactic behaviors in this insect.

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Figure 1: Green, orange, red, yellow, and white light trials were conducted at 30 lux using mixed sex adult *H. halys*. Dark (0 lux) trials were used as the no-attractive-stimulus control. The average distance *H. halys* were found away from the light source is reported for each color of light. Error bars represent the standard error. Bars with the same letter are not significantly different, $p<0.05$; ANOVA.

Figure 2: Second, third, fourth, fifth, adult male, and adult female trials were conducted at 75 lux using white light. The average distance *H. halys* were found away from the light source is reported for each life stage. Error bars represent the standard error. Bars with the same letter are not significantly different, $p<0.05$; ANOVA.

Figure 3: Intensity trials were conducted with both male and female adult *H. halys* at 0 (dark), 0.1, 20, 50, 75, and 155 lux. The average distance *H. halys* were found away from the light source is reported for each combonation of gender and intensity. Error bars represent the standard error.

Figure 4: The distribution of *H. halys* around the light source is displayed for the adult male and female trials for both the 0 (dark) and 75 lux trials. The grid shown is a two dimensional representation of the distances for each stink bug as the actual data was not gathered on a flat plane. The light source is demarcated by the small circle in the center. The average distance for the group is shown with the black dashed circle. One standard deviation is in both directions from the mean are shown with the grey filled-in circles. Average and standard deviation values are rounded to the nearest 10cm and the angle of *H. halys* that were not found on the light wall were estimated to show general direction in this figure. The ending position of each *H. halys* is represented by a black dot.

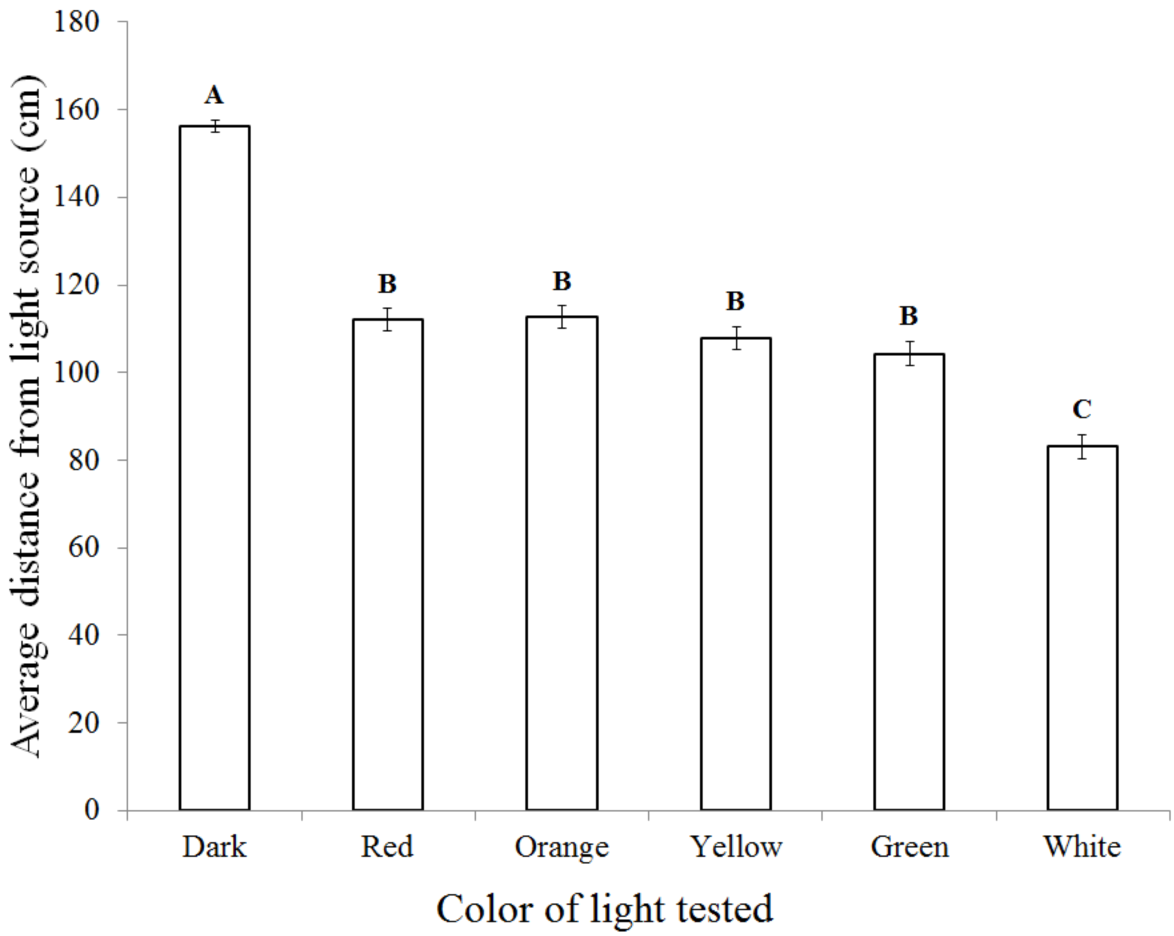

180
160
140
120
100
80
60
40
20
0
Average distance from light source (cm)
A
B
B
B
B
C
Dark
Red
Orange
Yellow
Green
White
Color of light tested

Figure 1

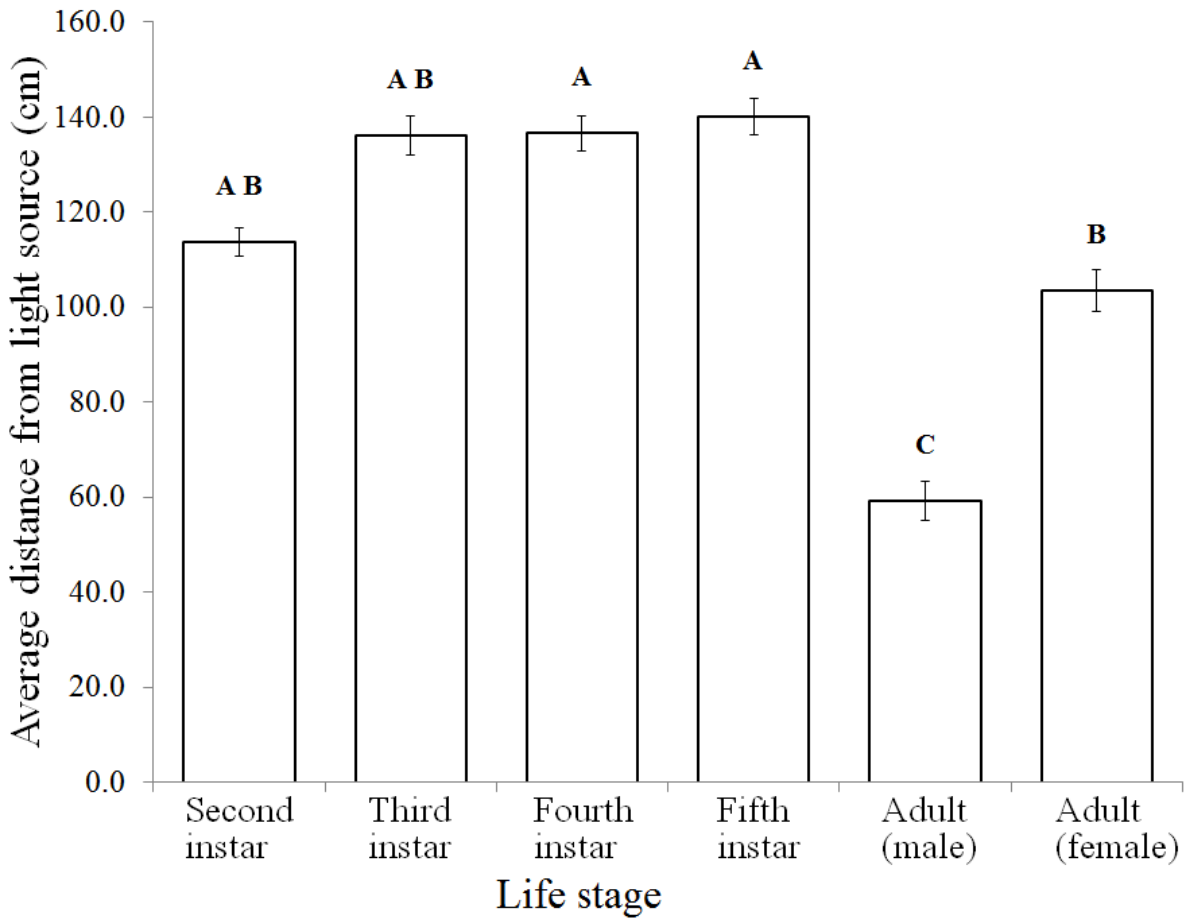
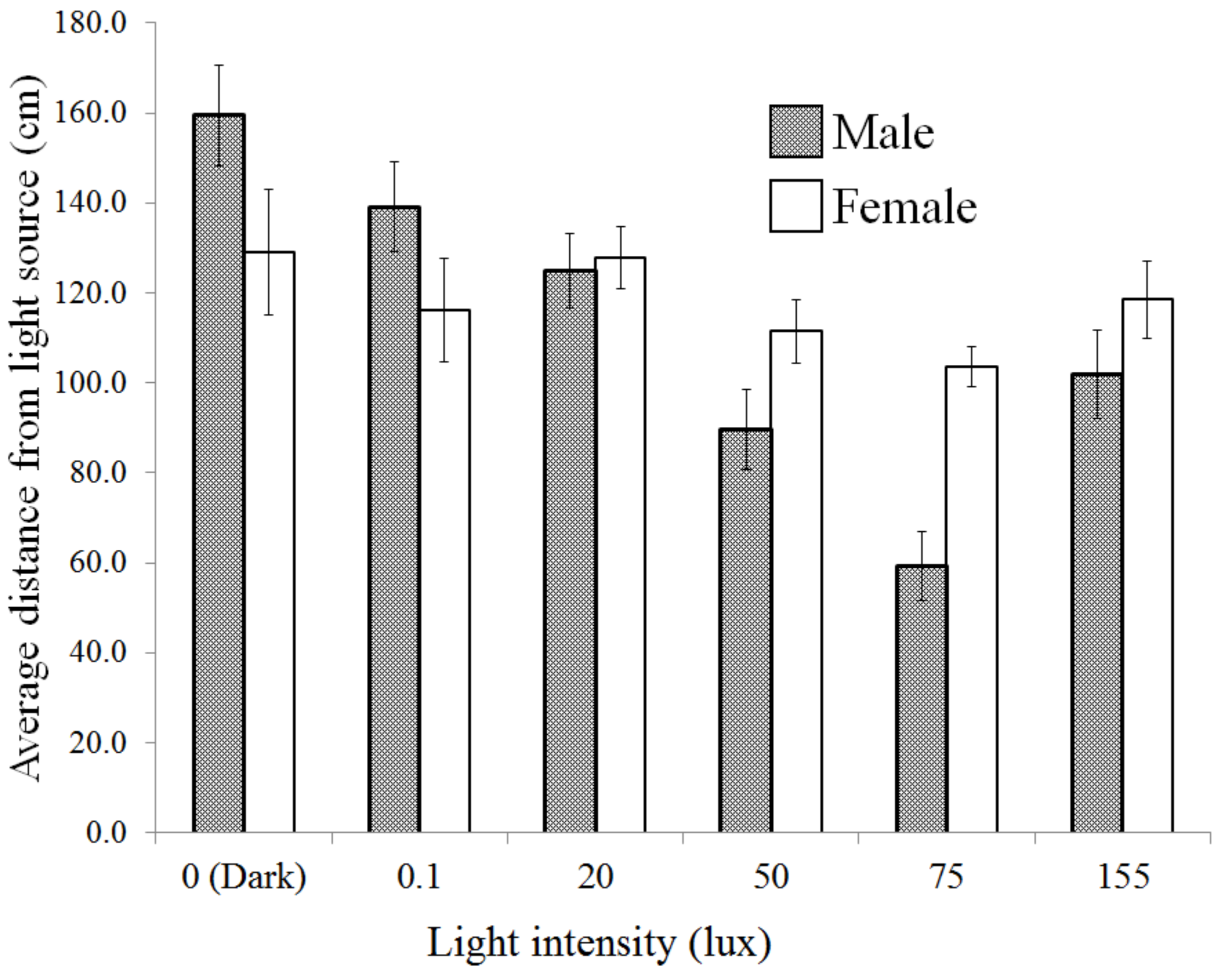

Average distance from light source (cm)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
A B
A B
A
A
C
B
Second instar
Third instar
Fourth instar
Fifth instar
Adult (male)
Adult (female)
Life stage

Figure 2


180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
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Average distance from light source (cm)
Male
Female
0 (Dark)
0.1
20
50
75
155
Light intensity (lux)

Figure 3

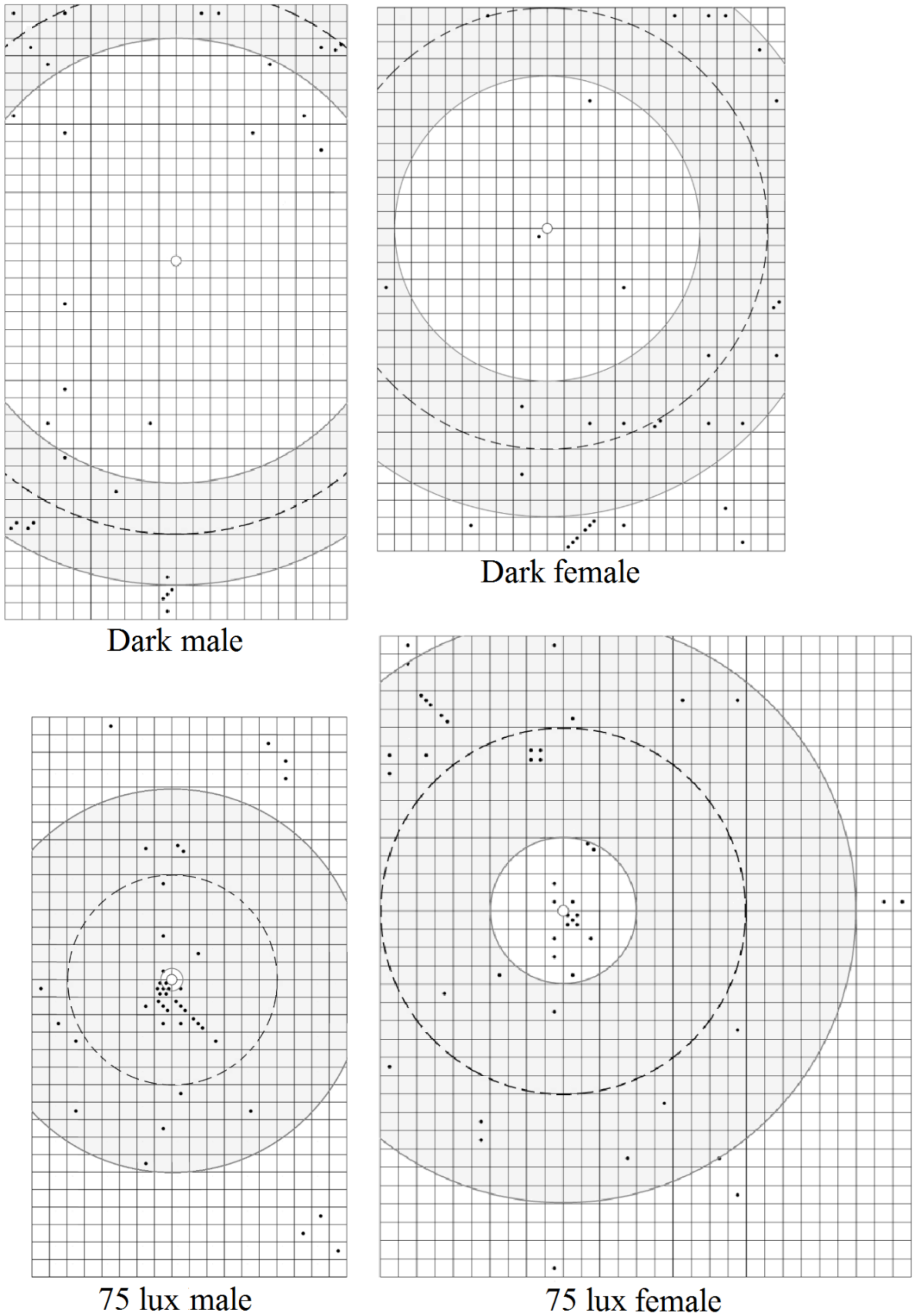

Figure 4

Chapter 6

Evaluation of a Non-Commercial Ultraviolet Light Trap for Use in Pest Monitoring Programs.

Abstract: Ultraviolet light traps have been employed as a reliable insect population monitoring tool for over 70 years. As traditional tower type black light traps are no long commercially available, a trap was designed and built using readily available materials. The trap (Clear Knight trap) was compared to the traditional tower type trap in agricultural and community garden systems to determine if trap catches were equivalent to tradition models. The results showed that the Clear Knight trap did not catch significantly different amounts of insects in 24 of the 25 families investigated when compared to the tower type trap and that no significant difference in the diversity of insects caught. These results suggest that Clear Knight traps are potentially interchangeable with the traditional tower black light traps. Full schematics and construction instructions are included.

Keywords: black light trap, population monitoring, integrated pest management

Introduction

The presence or absence of light is among the oldest biological modifiers known to man. Circadian rhythms, which is shared by nearly all living organisms (Edgar et al. 2012), were first observed in the fourth century B.C.E. by a Greek ship captain who characterized the cyclic daily movements of tamarind tree leaves (Bretzl 1903). Since then, researchers have identified a myriad of other photoperiod controlled behaviors in nearly every known taxon (Bass 2012). Insects are particularly sensitive to photo-cues as these signals are integral in numerous essential life functions such as diapause (Beck 1962; Blaney et al. 1986), flight orientation (Goodman 1965), mate finding and mating (Engelmann 1999), oviposition (Sanders and Lucuik 1975), egg hatching (Minis and Pittendrigh 1968), adult emergence (Corbet 1964), night time navigation (Dacke et al. 2003), daytime navigation (Reppert et al. 2004), feeding initiation (Hendrichs and Hendrichs 1990), host identification (Narayandas and Alyokhin 2006), and many more (Beck 2012). As early as the 1890's, knowledge about the susceptibility of insects to photo-stimulus was being used to design traps (Roth 1891) which have been repeatedly redesigned and tested in the hope of increasing performance (Boehm 1910; Baker 1912; Abresch 1918; Cherry 1928; Jacobs 1930; Gourdon 1931; Menasche 1935; Niemeyer and Elizabeth 1938; Kendrick 1945; Pohlman 1953; Emerson 1962; Takamoto 1969; Phillips 1978; Schneider 1982; Birdsong 1992; Nelson and Anderson 1994; Yates 1997; Nelson et al. 2002; Harris 2007; Child 2011; Koo et al. 2016).

Light traps are most effective at capturing insects during the night time as sunlight can negate or mask their attractiveness (Shimoda and Honda 2013). Most light traps are attractive to a wide variety of night flying insects (Blomberg et al. 1976; Henn et al. 1990). However, certain traps use specific portions of the visual spectrum to increase the specificity of attraction to particular insects, which can have the added benefit of reducing non-target catches as well (Duehl et al. 2011). Light traps may use a multitude of different bulb types and wavelengths depending on their specific use. In agricultural settings, ultraviolet (UV) light traps are often used to simultaneously monitor crop pests over the course of a growing season (Harding et al. 1966; Shimoda and Honda, 2013). The attractive power of these traps is derived from a UV light, making the use of the same bulb type an effective way to standardize these traps over time or between locations (Harding et al. 1966). Tower style black light traps (Figure 1) have been shown to be useful tools in integrated pest management monitoring programs as both solitary traps and when used in region-wide networks (NCSU 1999, 2002, 2010; Nielsen et al., 2013).

The most widely used tower type black light was most recently sold by Gempler's (Madison, WI). This trap was first sold commercially in 1963 by Ellisco and marketed as the *Ellisco General Purpose "Black Light" Trap* (Ellisco 1963). The rights to sell the trap were later obtained by Old Boy Enterprises, Inc. (K. Holmstrom, personal communication) and then finally Gempler's acquired the rights in the early 1990's (R. Smith, personal communication). In 2013, Gempler's removed this product from their catalog and it has not been offered since. However, over the course of the 50 years that it was available, many universities and pest management programs built and maintained large arrays of these traps for use in their monitoring programs (Anonymous 2010, 2011; Holmstrom et al. 2001). While decades of general use and maintenance repairs have left many of these traps in need of replacement, the data that these arrays generate is still very useful and would be difficult to reproduce without them (K. Holmstrom, personal communication).

In 2015, Rutgers University used an array of 51 tower type black light traps which were dispersed throughout New Jersey (K. Holmstrom, personal communication). This allowed for the tracking of many different pests such as European corn borer, *Ostrinia nubilalis* (Hubner), corn ear worm, *Helicoverpa zea* (Boddie), the brown marmorated stink bug, *Halyomorpha halys* (Stål), beet armyworm, *Spodoptera exigua* (Hübner), and a variety of other, primarily lepidopteran, pests on a regional scale (Anonymous 2011). Data gathered from this network is used to inform growers about pests that are about to move into their area, that resident populations are increasing, and is used to develop grower or area specific management programs. As these traps degrade each year and repairs become more time consuming and costly, the need for an alternative trap increases. This study was undertaken to assess the feasibility of building tower style black light traps that could be used in the same capacity as the Gempler's 15-watt tower type black light trap, hereafter referred to as traditional tower black light traps. During the development of these traps, efforts were made to improve any identified shortcomings of the traditional trap design and produce a standard protocol for the creation of new traps in the future.

Materials and Methods

The trap design reported in this paper is termed the "Clear Knight" (CK) trap and will be referred to as such from here on. The design and fabrication of the CK trap was conducted using facilities and equipment located in the entomology department at

Rutgers University between November of 2014 and July of 2015. Comparison of the CK trap against the traditional tower black light (TTBL) trap was done between July and August of 2015 at seven different locations throughout New Jersey.

Trap Construction

Clear Knight traps are comprised of five major sections: the top, the large cone, the body, the collection container, and the legs (Figure 1). CK traps can be broken down into these smaller component parts for easy storage and transport. These sections can also be individually fabricated on an as-needed basis for repairs and replacement parts. Detailed instructions for the construction of this trap can be found in the Appendix.

The top portion of the trap was comprised of three 20.32 x 60.96 cm clear polycarbonate (PC) (Macrolon, China) fins with a 45 cm diameter circular PC top. The electrical components and circuitry were housed in the upper center between the fins at the top. The light bulb (15 watt 18" T8 BL) (GE, Indonesia) is mounted vertically between the fins. Three Velcro® connector strips (Country Brook Design, Moulton, AL) were attached to the bottom outside of the fins for attachment to the large cone. Female PCV pipe adapters (3.175 cm diamter) were mounted on the outter center of the fins to use as leg attachments (Figure 2).

The large cone was constructed by bending a PC standard cone template shape, resulting in a cone that has a 43.18 cm diameter at the large opening, a 6.35 cm diameter at the small opening, and a height of 30.48 cm. Two rope attachment belts were strung through eyehooks around the cone to provide an anchor location for the Velcro connector strips on the top and body sections (Figure 2).

The legs were made out of telescoping PVC pipe (JM Eagle, Wilton, Iowa). A 91.44 cm long piece with a diameter of 3.175 cm was used as the outer piece and a 106.68 cm long piece with a diameter of 2.54 cm was slid inside it. Each leg had corresponding holes drilled through the diameter and a cotter pin was inserted through the holes to lock them in place in either the extended or collapsed positions. A 3.18 cm male PVC pipe adapter was mounted to the top of the leg for attachment to the female adapter on the fins. 30.48 cm spiral ground anchors (Camco, China) were mounted to the bottom of each leg for insertion into the soil to stabilize the trap (Figure 2).

The body was made from a 30.48 cm section of 20.32 cm diameter PC pipe (Petro Extrusions, Milltown, NJ). A 30.48 cm length of 10.16 cm diameter pipe (Petro Extrusions, Milltown, NJ) was formed into the rain-catch by cutting out the central 10.16 cm section and covering it with wire mesh of 0.635 cm thick (Amagabeli, China). The rain catch was then mounted through the body cylinder at a slight angle with the mesh portion in the center facing upwards. A smaller cone (20.32 cm diameter large opening, 7.62 cm diameter small opening, 10.15 cm in height) was fashioned out of PC in the same way as the large cone. The small cone was then attached to the bottom of the body cylinder. Three Velcro strips, 5.08 cm thick, were mounted to the top of the body for attachment to the large cone. A one liter bottle cap top was hollowed out and attached with silicon sealant around the small cone opening (Figure 2). A clear one liter wide mouth polyethylene collection container (Zenith Global, Howell, MI) can then be screwed on the small cone as needed. A 2.54 x 2.54 cm piece of No-Pest Strip® (Hot Shot, St. Louis, MO) was placed in the jar to kill any insects captured to prevent escape of or damage to specimens (Figure 2).

The trap was assembled by first screwing the legs onto the top. Once the trap was standing, the Velcro strips from the top and body were looped through the large cone's attachment belts and fastened in place. The collection container holding a No-Pest Strip® piece was screwed on to the body. Any trap repositioning was done at this time and then the ground anchor on each leg was sunk into the soil. The light bulb was then inserted into the top and the trap was plugged in.

Comparison of Clear Knight trap to the traditional tower black light trap

Sampling sites: CK and TTBL traps were placed out at four different locations throughout New Jersey: Davidson Mill Pond (DMP), Snyder Research and Extension Farm, Morven, and Crosswicks Farm. Davidson Mill Pond (DMP) in East Brunswick, NJ is a community garden and park area that also had small agricultural research plots set up. The Snyder Research and Extension Farm in Pittstown, NJ is a Rutgers University research farm with row, field, and orchard crops. Morven in Princeton, NJ is a community garden that was surrounded by residential homes. Crosswicks Farm in Hamilton, NJ is a commercial farm with large corn and soybean, and surrounding woods. Trap catches at each site compared from July 24, 2015 to August 20, 2015. At each site, one CK trap and one TTBL trap were set up in comparable locations between 100 and 150 meters apart. The position of CK and TTBL traps was switched weekly (Thursday) at each site to reduce the effect that microhabitat differences would have on trap comparisons. Trap catches were collected twice a week (Monday and Thursday) and all insects captured were brought back to the laboratory for analysis.

Data analysis: Weekly samples at a site were included in the analysis if both the CK and TTBL traps were functioning properly the entire time. Trap catches were compared based on the abundance of 25 insect families: apidae, arctiidae, cerambycidae, chrysomelidae, chrysopidae, cicadidae, cicindelidae, coccinellidae, coenagrionidae, corydalidae, fulgoridae, gryllidae, gryllotalpidae, mantispidae, mantidae, myrmeleontidae, oecanthinae, pentatomidae, saturniidae, siricidae, sphingidae, tabanidae, tettigoniidae, vespidae, yponomeutidae.

Average nightly trap catch for each trap and location for each family and for all families grouped together were calculated. Species diversity was calculated using Shannon-Wiener diversity index for each trap at each location. These data were analyzed using a general linear model (Proc GLM) testing for the main effects of using the effects of trap, location and the combined effect of trap by location (SAS Institute, 2014).

Results

From July 24, 2015 to August 20, 2015 the CK and TTBL traps caught a total of 1169 and 1266 insects, respectively over (Table 1). Total numbers caught across the 25 families identified ranged from 0.02 insects per night in the family apidae to 8.10 insects per night in the family coccinellidae. Other families such as carabidae and certain tricopterans had vastly higher numbers but are not reported in this study.

A comparison of the average nightly trap catches for CK trap and TTBL trap is summarized in Table 2. For all families examined no significant difference between the traps were seen ($p>0.05$) except for members of the family fulgoridae. Fulgorid’s were significantly more attracted to the TTBL traps (p=0.042, f=4.470, df=1). When average nightly trap catches were aggregated across all families no significant difference was observed between the CK and TTBL trap (p=0.736, f=0.110).

The calculation of Shannon-Wiener diversity index values resulted in similar indices at each location with the exception of the Morven site (Table 2). At three out of the four locations, higher diversity was observed for the CK trap.However, the indices were not significant different between the traps at each site (p=0.750, f=0.49, df=4).

Discussion

The CK trap was found to catch similar numbers of all insect families examined, except for members of the family fulgoridae. The Shannon-Wiener diversity indices calculated for each trap and location were also similar.

The CK trap incorporates several improvements over the TTBL traps. The most notable is the reduction in the cost. TTBL traps were sold for between $700 and $1500 per unit depending on the features requested. The CK trap costs ~$150 in parts and 10 hours to build. The price can be further reduced by buying the component materials in bulk. The electrical system used in the CK trap affords the user the following new abilities: resetting of the fuse without any disassembly or part replacement, power savings due to a photocell that automatically turns the trap off during the day, and a built in power switch. The CK trap breaks down into more easily transportable pieces that are lighter in weight require less storage space than the TTBL trap. CK trap legs have built-in attachment anchors that keep the trap standing during storm conditions or on uneven ground. In addition, the legs are mounted higher on the trap and above the center of mass creating a more stable configuration. The collection container consists of a twist-off, replaceable jar that allows for sample inspection without pouring the specimens into a transfer container. This arrangement reduces the chance that specimens will be broken or lost during transport.

The construction plans reported on here represent a third generation trap in terms of design. Three major potential improvements were incorporated. First, the use of flat sheet polycarbonate (PC) as the starting material for making the connector pieces and the formation of the cones is not recommended. Flat sheets of PC that were kept flat were found to be quite durable through the study but pieces that were bent and held under tension were likely to shatter within a month. Future construction of these traps should consider the use of either a preformed piece of PC or a different material to construct the connector pieces and cones. Second, predrilling of holes should be done using a computer-controlled machine or other automated process. If the holes are misaligned by as little as 0.5mm, fitting the pieces together is difficult. Future construction should consider drilling the screw holes in place as needed. Third, the rain catch portion of the CK trap functioned as well as the TTBL trap but neither were sufficient to exclude water during major precipitation events. More work is needed in the design of this component.

The results of this project showed that homemade black light traps, such as the CK trap, have the ability to generate comparable trap catches to commercial counterparts for a small fraction of the cost. The incorporation of several improvements does not appear to affect the function of the traps.

Light traps are a useful tool for monitoring night flying insects. Even though pheromone traps are much more target specific and as a result are causing light traps to becoming less frequently used (Henn et al. 1990), they still represent a useful way to track population changes in night flying insects. The CK trap provides a template for anyone to use who wished to cheaply incorporate this form of monitoring into their program or replace TTBL units in an existing program.

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Table 1. Total number of insects collected by family by the Clear Knight and traditional tower black light traps from July 24, 2015 to August 20. 2015 at four locations in New Jersey.

	Total Number Collected	
Family	Clear Knight	Traditional
Apidae	1	2
Arctiidae	80	56
Cerambycidae	4	7
Chrysomelidae	115	43
Chrysopidae	3	4
Cicadidae	4	3
Cicindelidae	39	9
Coccinellidae	478	535
Coenagrionidae	1	5
Corydalidae	4	0
Fulgoridae	49	208
Gryllidae	158	209
Gryllotalpidae	1	1
Mantispidae	2	2
Mantodea	2	0
Myrmeleontidae	2	0
Oecanthinae	4	0
Pentatomidae	57	38
Saturniidae	4	3
Siricidae	0	1
Sphingidae	11	15
Tabanidae	4	3
Tettigoniidae	1	2
Vespidae	106	51
Yponomeutidae	39	69
Total Number Caught	1169	1266

Table 2: Average nightly number of insects collected by family by the Clear Knight and traditional tower black light traps from July 24, 2015 to August 20. 2015 in four locations in New Jersey.

	Mean (+/-SE) Number Collected per Night			
Family	Clear Knight	Traditional	*P*-value	*F*-value
Apidae	0.02 +/- 0.01	0.03 +/- 0.01	0.560	0.350
Arctiidae	1.36 +/- 0.28	0.95 +/- 0.11	0.464	0.550
Cerambycidae	0.07 +/- 0.02	0.12 +/- 0.04	0.532	0.400
Chrysomelidae	1.95 +/- 0.65	0.73 +/- 0.27	0.327	0.990
Chrysopidae	0.05 +/- 0.01	0.07 +/- 0.03	0.777	0.080
Cicadidae	0.07 +/- 0.04	0.05 +/- 0.02	0.818	0.050
Cicindelidae	0.66 +/- 0.29	0.15 +/- 0.06	0.333	0.970
Coccinellidae	8.10 +/- 0.94	9.07 +/- 1.60	0.800	0.070
Coenagrionidae	0.02 +/- 0.01	0.08 +/- 0.05	0.439	0.620
Corydalidae	0.07 +/- 0.02	0.00 +/- 0.00	0.094	2.980
Fulgoridae	0.83 +/- 0.16	3.53 +/- 0.68	0.042	4.470
Gryllidae	2.68 +/- 0.69	3.54 +/- 0.88	0.690	0.160
Gryllotalpidae	0.02 +/- 0.01	0.02 +/- 0.01	1.000	0.000
Mantispidae	0.03 +/- 0.01	0.03 +/- 0.01	1.000	0.000
Mantodea	0.03 +/- 0.02	0.00 +/- 0.00	0.325	1.000
Myrmeleontidae	0.03 +/- 0.01	0.00 +/- 0.00	0.154	2.130
Oecanthinae	0.07 +/- 0.02	0.00 +/- 0.00	0.094	2.980
Pentatomidae	0.97 +/- 0.12	0.64 +/- 0.11	0.328	0.980
Saturniidae	0.07 +/- 0.04	0.05 +/- 0.01	0.818	0.050
Siricidae	0.00 +/- 0.00	0.02 +/- 0.01	0.325	1.000
Sphingidae	0.19 +/- 0.04	0.25 +/- 0.05	0.578	0.320
Tabanidae	0.07 +/- 0.02	0.05 +/- 0.01	0.683	0.170
Tettigoniidae	0.02 +/- 0.01	0.03 +/- 0.02	0.658	0.200
Vespidae	1.80 +/- 0.44	0.86 +/- 0.32	0.370	0.830
Yponomeutidae	0.66 +/- 0.14	1.17 +/- 0.17	0.229	1.510
Total Catch	16.77 +/- 2.18	13.93+/- 1.81	0.736	0.110

Table 3: The Shannon-Weidner diversity index for the Clear Knight and traditional tower black light traps at four locations in New Jersey. These indices are calculated based on the 25 families identified for the capture comparison shown in Table 1. There was no significant difference between the indices (p=0.750, f=0.49, df=4).

Location	Clear Knight	Traditional
Crosswicks	2.04	1.94
Davidson Mill Pond	1.27	1.88
Snyder	1.48	1.38
Morven	2.05	1.11

Figure 1: A comparison of a fully assembled Clear Knight and traditional tower black light traps. A - Clear Knight black light trap. Colors and shapes represent the five major components of the trap: the top, the large cone, the body, the collection container, and the legs. B - Traditional tower. Colors and shapes represent the five major components of the trap: the top, the large cone, the body, the collection container, and the legs.

Figure 2: Clear Knight trap component diagram. See supplementary material for full details. **A**: Top from side view. 1-Electrical housing connection piece. 2-Light bulb. 3-Electrical housing tube. 4-Photoswtich. 5-Power cord. 6-Fins. 7- PVC pipe attachment to fin. 8- Female PVC screw connector. 9-Velcro attachment washer. 10-Velcro hook strip. 11-Velcro loop strip. 12-Back of Velcro loop strip. **B**: Top fins from above view. **C**: Large cone from side view. 13-Overlap from template shape. 14-Bolt for large cone shape connections. 15-Rope attachment belt for the body. 16-Eyehook to hold attachment belts. 17-Rope attachment belt for the top. **D**: Body from side view. 18-Velcro loop strip. 19-Gasket at body to large cone interface. 20-Rain catch high side. 21-Body cylinder. 22-Connector strip between the body cylinder and the small cone. 23-Small cone. 24-Collection container screw top attachment. 25-Bolt for small cone shape connections 26-Rain catch low side. 27-Rain catch mesh. 28-Velcro attachment washer. 29-Velcro hook strip. **E**: Body from above view. 30-Rain catch low side. 31-Rain catch mesh. 32-Collection container screw top attachment. **F**: Leg collapsed for transport. 33-Lock hole for collapsed leg on inner pipe. 34-Eyehook bolt. 35-Ground anchor. 36-Cotter pin. 37-Inner leg pipe. 38-Lock hole for extended leg on inner pipe. 39-Outer leg pipe. 40-Male PVC screw connector. **G**: Leg extended for trap use. 41-Lock hole for extended leg on outer pipe.

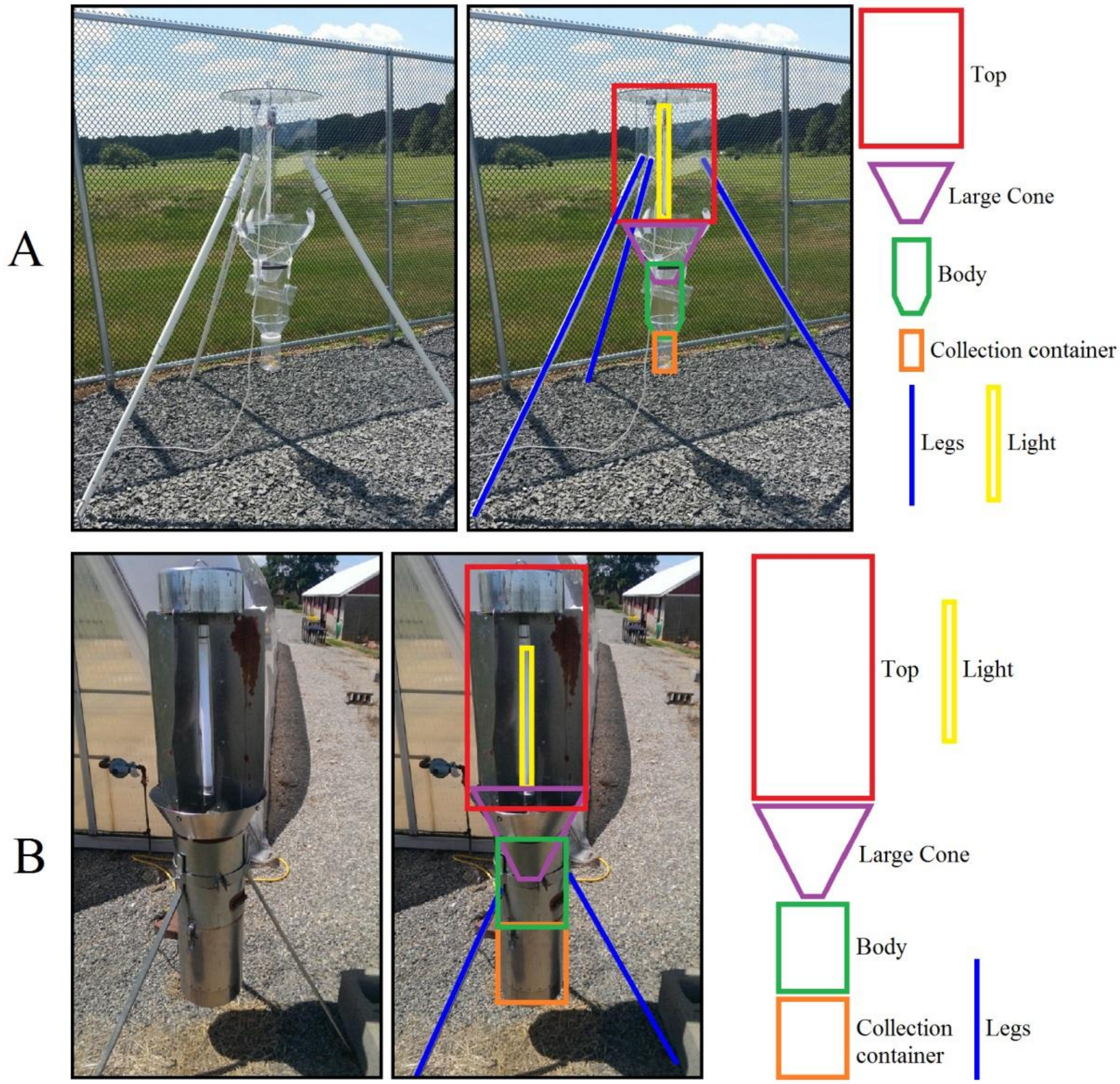
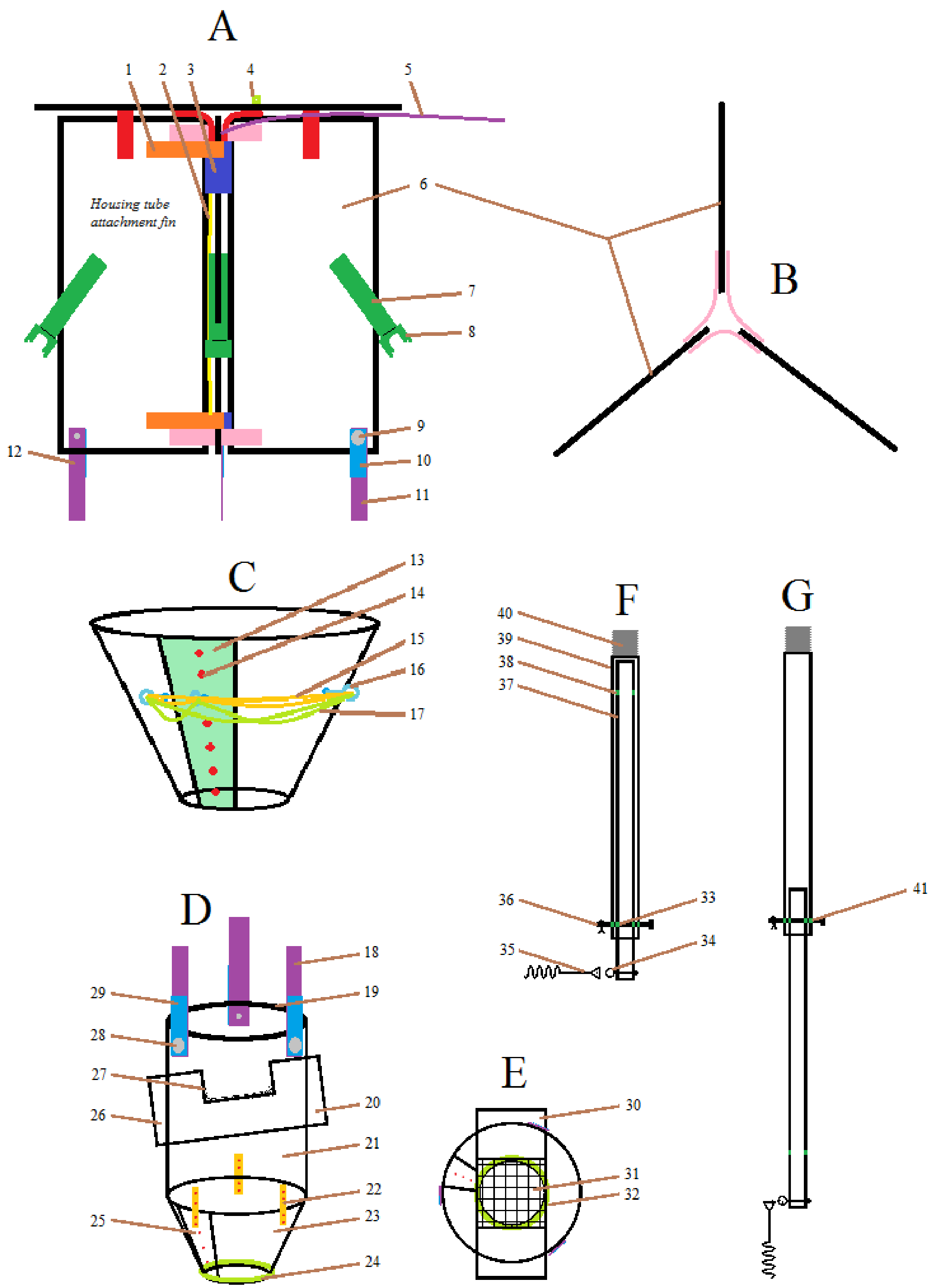

A
Top
Large Cone
Body
Collection container
Legs
Light
B
Top
Light
Large Cone
Body
Collection
container
Legs

Figure 1


A
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Housing tube attachment fin
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Figure 2

Supplementary instructions for the construction of Clear Knight traps

The top

The top portion of the trap contains the fins, the circular top, and houses the light bulb and all associated electronics. (Fig S1). The circular top functions as the first stage rain barrier and can be used as an attachment surface if the trap is to be hung. The circular top also attaches to the three fins and stabilizes them at the equally spread 120-120-120 degree orientation. The electronics are primarily housed inside a protective 10.16cm polycarbonate (PC) tube (4.45cm inner diameter, 5.08cm outer diameter) , hereafter referred to as the top electrical housing tube or just housing tube. The top fins and circle were all made out of clear 0.48cm PC sheeting (Macrolon, China). This material was chosen because of its remarkable durability and resistance to solar degradation. The transparent nature of the material was suspected to increase the attractive range of the CK trap as it did not prevent transmittance of the light like the TTBL fin material did but this hypothesis was not investigated in the study reported in this paper. 1.22x2.44 meter PC sheets of 0.48cm and 0.24cm thickness were purchased from Emco Industrial Plastics, Inc. (Cedar Grove, New Jersey).

The fins and top circle of the CK trap can be made from a 63.5x111.76cm rectangle of 0.48cm PC sheet. The fin connector pieces, the electric housing tube mounting pieces, and the connector pieces for the reminder of the trap parts can be made out of a 53.34x15.24cm rectangle of 0.24cm PC sheet. The three fins were each cut to be 20.32x60.96cm using a table saw (RYOBI, China) with a 21.50cm diameter, 80T blade (Tenryu, Hebron, KY). Throughout this and all other measurements and cuts, it was important to take into account the thickness of the saw blade as that resulted in appreciable material loss and would lead to improperly sized pieces if disregarded. For the creation of the CK traps, all saw blades were 0.3175cm thick. Two 5.08cm diameter circles were cut out of the remaining piece of 0.24cm PC sheet using a 45.72cm band saw (Craftsman, China) with a 45.72cm metal/wood blade and then placed to the side for later use. The top circle was cut to be a 24.77cm radius circle using a router (Craftsman, China) with a 0.635cm wood bit. Sixteen 2.54x15.24cm pieces of 0.24cm PC sheeting were cut to use as the fin connection and electric mounting pieces with a table saw. All holes were drilled immediately after all the template shapes of a particular type were cut out so that the production of multiple traps could be done in a standardized way as efficiently as possible. All holes were drilled with a 14.4 volt drill (RYOBI, China) using a standard 31 piece drill set (ROYBI, China). Unless otherwise stated, holes are assumed to be 0.47cm in diameter and go entirely through the piece of material being drilled into; an exception to this is when drilling into a tube, the hole is only assumed to go through one of the sides of the tube. Each of the 2.54x15.24cm fin connector pieces and small cone connector pieces had holes drilled at 1.27, 3.81, 11.43, and 13.97cm along the center line. The electric mounting pieces had a similar hole arrangement but lacked the hole at 11.43cm. To drill the holes in the fins, one of the short sides was designated to be the top and then one of the corners assigned the inner position. Each top-inner corner had one hole drilled 2.54cm in and 2.54cm down, and then another drilled 5.08cm in and 2.54cm down. The bottom inner corner of each fin had corresponding holes drilled 2.54 cm in and 2.54cm up and then 5.08cm in and 2.54cm up. Each of the fins then had holes drilled in the top of the center at 2.54cm and 5.08cm down. The bottom, outer corner of each fin also had a hole drilled 7.62cm up and 2.54cm in, termed the Velcro hole. Two holes are created in the middle of the outer portion of each fin to give the legs a good attachment point. The first leg hole was set 27.94cm up from the bottom and 2.54cm in from the outer edge and the second was set 38.1cm up from the bottom and 5.08cm from the outer edge. One of the three fins was chosen to be the housing tube attachment fin and then had four more holes drilled into it; two were 7.62cm below the top edge 5.08cm and 7.62cm out from the center and then the other two were 7.62cm up from the bottom edge of the fin 5.08cm and 7.62cm away from the center. The circular top had a 0.635cm hole drilled into the center to set the router pivot in. After the circle was cut, lines were drawn along the top to indicate where the fins would line up. These lines were set to be equally positioned apart by having their departure from one another at the circle center equal to 120 degrees. A perpendicular line was then drawn on each of these lines 20.32 cm from the center of the circle. Marks were made on the perpendicular lines so that drill holes could be made 2.54cm and 5.08cm from the fin location line in both directions. The final hole made in the top was the 1.59cm photo switch hole. This hole was drilled 5.08cm off of the center, directly opposite one of the marked fin lines. In positioning the photo switch hole, the opposite fin was then assigned to be the housing tube attachment fin. Two more set of holes were need on the housing attachment fin. The top electrical housing attachment holes were drilled 7.62cm down from the top of the fin and 7.29cm and 8.56cm in from the center. The bottom electrical housing holes were drilled 3.18cm up from the bottom of the fin and 7.29cm and 8.56cm in from the center. (Fig S2).

Once all the PC sheet parts were cut and drilled, assembly of the top began. The three fin blades were set on the circular piece and arranged at their proper angle by using the lines marked on the top. The inner corner of each fin was set 1.54cm away from the circle center. Six of the 2.54x15.24cm connector pieces were used to attach the fins together. (Fig S3). Unless otherwise stated, all connections between pieces were screwed together using 1.27cm, gauge 8, rounded Philips head machine screws (Everbilt, Singapore) with one washer and one nut. Once the top and fins were completed, the electronic housing tube was created.

A piece of housing tubing was cut into a 10.16cm section for the top piece and a 1.27cm section for the bottom piece. The top piece was then prepared for circuitry installation and mounting. Two rows of three 0.47cm holes were drilled in the top of the cylinder, henceforth called the wire holes. The first row was 1.27cm down from the upper lip of the cylinder and all holes were spaced at least .64cm away from any others. A 1.27cm diameter hole was drilled 1.27cm from the top lip of the tube and 1/3 of a turn to the left of the six newly drilled holes. A 0.32cm thick, 1.27cm high light access notch was cut in the bottom lip, directly beneath the 1.27cm diameter hole using a MultiPro Dremel (Dremel, Racine, WI) with a 2.54cm abrasive bit. The last hole was one which went straight-through both sides of the tube 5.08cm down, perpendicular to the access notch. These holes will be referred to as the mounting holes. The 1.27cm housing tube had a light access notch of the same dimensions carved into the top lip and then a set of mounting holes drilled through the tube .64cm off the bottom perpendicular to the mounting holes. (Fig S4).

The creation of the CK trap circuitry began with the disassembly of the GE Fluorescent Fixture 18” (GE, Indonesia). The plastic casing snapped apart to reveal the circuit board, on/off switch, and tube sockets. This circuit board is used to convert the alternating current delivered from the normal 110 volt municipal electricity into direct current which can then be used to power the fluorescent light bulb. The entire circuit was easily removed by gently prying the components loose one at a time. At this stage it was useful to place labeling tape on all wires and wire attachments to make sure that nothing was placed back into the wrong location when reassembling the circuit later. The light socket that was attached closest to the circuit board was not altered or disturbed; this was the top tube socket. The wires leading to the socket that was on the end of a 45.72cm pair of wires was cut 7.62cm away from the circuit board; this was the bottom tube socket. Using 16 gauge wire (Southwire, Blauvelt, NY), 40.64cm of wire was spliced onto each of the newly cut 7.62cm wires leading to the bottom tube socket. These wires were threaded through a 47cm section of 0.79cm outer diameter, 0.48cm inner diameter clear vinyl tubing (Watts, North Andover, MA) to protect them before reincorporating the bottom socket back into the circuit. The black and white wire power cord attachment points on the circuit board were cut 5.08cm from the board. At this point, a 6.1 meter Yard Master 992222 outdoor rated extension cord (Yard Master, Detroit, Michigan) was prepared for incorporation into the circuit by cutting the female end off and separating the first 15.24cm of the three smaller encased wires inside. This type of cord was chosen because it is rated to withstand environmental stressors such as rain, sunlight, and high wind, all of which were expected at the agricultural sites these traps were later to be tested in. The extension cord was plugged into a 30439005 type ground fault circuit interrupter (GFCI) pop fuse (Tower, Shenzhen, China) and then that fuse was plugged into whatever socket was to be used in each trap comparison location. These fuses provide a level of protection for the delicate electronics which could short out if water were to get into the housing tube or if any electrical surges were experienced by the circuit the trap was plugged into. A P18100 photoelectric switch (Premier Lighting Manufactures, China) and CE KN3(C)-101 toggle on/off switch (CE, China) were then unpackaged and set out to be added to the circuit. The photo switch increased the efficiency of the trap by turning the light off during the day when the trap is not attractive to insects and the toggle on/off switch was used as an override control to turn the light off if desired. Both of the switches came with rubber gaskets and nuts that screw down the sensor or toggle portion of the device to create a seal if they are mounted in the outer casing of something. This was indeed how they were to be used in this project so the gaskets and nuts were set aside for future use. For all wires being spliced into the circuit, the last 2.54cm of wire was stripped of the outer casing such that the interior conductive portion was exposed.

The three wires from the power cord were fed through the lower of the six wire holes in the housing tube. The three wires for the photo switch were then passed through the upper three remaining wire holes. The toggle switch was put into the cylinder and pushed through the 1.27cm diameter hole. The rubber gasket was then fitted over the exposed toggle portion and tightened against the tube with the factory-provided nut to create a watertight seal. The final circuit was then ready to be assembled. (Fig S5).

The outgoing hot wire (black) from the power cord was spliced together with the incoming hot wire (red) on the photo switch and then the outgoing hot wire (black) from the photo switch was then connected to the incoming hot wire (either wire works) on the toggle switch. The outgoing hot wire (the other wire) on the toggle switch was then connected to the incoming hot wire (black) on the circuit board. The neutral wire (white) from the power cord was then spliced together with two other wires, the neutral wire (white) on the circuit board and the remaining neutral wire (white) on the photo switch. The ground wire (green) from the power cord was not used and was capped off. Once all the wiring was completed, The circuit board and top tube socket were carefully fit into the housing tube such that the top socket was facing outwards, lined up with the access notch, and flush with the lip of the tube. Before the positions of the inner electronics were totally set, a 6.35cm mounting screw was placed through one of the 2.54x15.24cm electric mounting pieces. The screw was then carefully threaded through the mounting holes and a second electric mounting piece was put on the screw on the other side. The screw was then fitted with a washer and nut and left for later use. One of the 5.08cm diameter circles cut from the 0.24cm PC sheet was then affixed to the top of the housing tube by applying silicone sealant all around the edges and clamping the top in place until it was dry. This sealant was then used to secure the top tube socket by filling in all remaining gaps between the side of the tube and socket. The bottom of the bottom housing tube was then sealed with the other 5.08cm diameter PC circle and allowed to dry. The bottom socket was placed into the housing tube and the two remaining electric mounting pieces were fitted on to the bottom tube housing in the same fashion as was done for the top. The black light bulb (GE - 15 watt 18" T8 BL) (GE, Indonesia) was then placed into the sockets to check that the circuit worked before continuing. The relative location and orientation of most of the electrical components previously mentioned was flexible and the shifting of a few millimeters in one directions or the other did not impact the functionality of the trap. However, the light tube sockets needed to be positioned very carefully to ensure a solid connection with the bulb contact pins.

The orientation of the light access notch and the distance the sockets were mounted apart from one another had to be within a 1mm tolerance to function. Once the circuit was confirmed to work, the bulb was left in the fixture and the bottom tube socket glued into place using the silicone. During the drying process, any excess length of wire between the top and bottom sockets was pushed into the bottom housing tube so that the bulb fit as snugly as possible.

After completely drying, the electrical housing was installed by lining up the holes on the electric mounting pieces to the holes on the housing tube attachment fin and screwing the components together. (Fig S6). Once the housing tube was fully mounted, the photo switch was installed into the circular top by pushing it through the photo switch hole and screwing down its rubber gasket and nut. The trap was fitted with eye hooks to allow for hanging capabilities but these were never used in practice and are not necessary for functionality.

After the electronics were fully installed, the Velcro ties (Country Brook Design, Moulton, AL) and leg mounts were installed so that the top could attach to the other portion of the CK trap. The Velcro ties were attached to the bottom outer hole on each fin. Each tie consisted of a 5.08x15.24cm piece of hook Velcro on top of a 5.08x30.48cm piece of loop Velcro strip, each facing in the same direction. The strips were aligned at one end and then a hole drilled through both pieces on that end in the center 2.54cm from the tip. A 2.54cm diameter washer was placed on a screw and then the screw pushed through the new hole in the strips. The Velcro ties were then attached to tops using the Velcro holes at the bottom outside of each fin. Ties were attached to the fins such that the hook/loop sides were always facing clockwise if looking down on the trap from above.

All PVC pipe and adapters were JM Eagle brand and manufactured in Wilton, Iowa. Legs were attached to the fins using 3.175cm PCV female screw adapters. The PVC adapter was glued to a 20.32cm section of 3.175cm standard PVC pipe with regular PVC cement (Oatey, Cleveland, Ohio). The 20.32cm section was cut 15.24cm lengthwise from the open pipe end at a width of 0.48cm. Holes were drilled in each piece of pipe perpendicular to the lengthwise cut at 2.54cm and 9.72cm away from the cut end to correspond with the outer central holes on the fins. The 15.24cm slit in the pipe was slide on to the fin to line up with the holes in the pipe and the leg attachment holes. Two 5.08cm screws were used to connect the leg attachments to the fins. (Fig S7).

The large cone

The large cone is made from a single piece of 0.24cm PC sheeting. As the process for creating this part of the trap is almost identical to the fabrication of the small cone used in the body and rain catch portion, the process of making both will be detailed here. In order to create the necessary shape for either the large or small cone, a router was used to make two semi-circular cuts using the same pivot point. The radius of each semi-circle was calculated by using freely available internet software that easily determines the measurements of a template shape needed to create a cone of predetermined dimensions (Russell 2013). Exact measurements are detailed in Figure S8. Once each of the paired semi-circles was made, the final strait cuts were sketched out on the PC sheet using a ruler and marker so that they could be finished with a table saw. The calculated arc length for each of the cones was not used as the final cut line as the resulting cone would not have any overlapping lip to use for attaching the sides together. However, the calculated arc length was labeled on the shape and used as a guild to tell when the shape was properly bent. Cutting an overlap of roughly 10 degrees in excess of the calculated arc was standard during the cone forming process. Once a flat cone template was cut, the shape was bent by hand until the edges lined up with the calculated arc length. While one person held the template in this position, another person would drill through and then screw down the overlap lip so that cone held itself together. Screws were placed every 5.08cm from the top to the bottom of the overlap, resulting in 7 per large cone and 3 per small cone. Once the screws were in place, the template bender could let go and the cone was finished. At this point, the small cones were incorporated into the body portion of the trap which is detailed below in the next section. The large cones underwent further modification.

Large cones were fitted with 3 eye hooks to hold attachment belts which were used as anchors for the Velcro strips on the top and body trap portions. The eye hooks were placed around the cone 12.7cm down from the large opening. This corresponded to the third attachment screw and so positioning was based on that point. The initial screw was replaced with a 2.54cm, gauge 8 eyehook with a 1.27cm opening at the end (Stanley, China). The other two eye hooks were of the same type and were spaced out equally around the cone at the same level such that they formed an equilateral triangle if connected. Two separate loops of 0.635cm dimeter rope (Crown Screw and Bolt, Indianapolis, IN) were threaded through the eye hooks and tied closed. The smaller attachment belt measured 92.71cm in circumference and was used for an attachment belt to the body and rain catch. The longer attachment belt had a circumference of 106.68cm and was used as an attachment belt for securing the top and fins to the lower portions of the trap. (Fig S9).

The body

The primary functions of the trap body was to house the rain catch and funnel insects into the collection container. The body was comprised of three major parts; the body cylinder, the rain catch, and the small cone. The body cylinder is a 30.48cm long, 20.32cm outside diameter PC tube with 0.3175cm thick walls. 4.88 meters of this tubing was purchased from Petro Extrusions in Milltown, New Jersey and then pieces were subsequently cut down into 30.48cm lengths with a table saw. A 10.16cm door hole bit (Milwaukee, Brookfield, WI) was used in a 30.48cm drill press (Craftsman, China) to cut two 10.16cm holes on opposite sides of the body cylinder. The lower hole was centered 13.97cm from the bottom of the tube and the high hole was drilled on opposite side but centered 19.05cm from the bottom lip. These holes were used to fit the rain catch into the body.

The rain catch consisted of a 30.48cm long PC tube with an outside diameter of 10.16cm and a wall thickness of 0.3175cm. This tubing was purchased from the same vendor and at the same time as the body cylinder tubing. 20.32cm sections were cut using the table saw as well. The center 10.16cm of the rain catch tube was cut in such a way as to remove half of the pipe in a 10.16cm square. This opening was then covered with 0.635cm grid size, steel hardware cloth mesh (Amagabeli, China) and this secured in place with a 50watt electric hot glue (Dewalt, China). In order to fully cover the gap, the mesh was bent around the sides of the tube and then any excess material was trimmed away after the glue hardened. The mesh functioned to allow rain to pass through the holes and then flow out the low end while keeping the majority of insects from escaping. When all the trap components were put together, the rain catch mesh sat 5.08cm below the bottom opening of the large cone. This close distance was chosen to increase the amount of precipitation caught by the rain catch and reduce the amount of water accumulating in the collection container.

Three Velcro ties of the same type as previously described were attached to the top of the body cylinder in the same equally spaced arrangement as was used on the large cone. The Velcro strips were screwed 7.62cm down from the upper lip of the body cylinder. In addition to the Velcro, a 63.5cm long, 2.54cm thick line of foam weather stripping (Thermwell Products, Mahwah, NJ) was fixed around the inside of the upper rim of the cylinder to ensure a solid seal with the large cone. This weather stripping was attached to the cylinder using the sticky coating that came standard on the back of this product.

The small cone was attached to the body cylinder with three 2.54x15.24cm connector pieces, all previously cut during the top section construction. The connectors were lined directly below the body cylinder Velcro strips and holes were drilled 3.81cm and 6.35cm up on the cylinder in each alignment slots. Corresponding holes were then drilled on the small cone 3.81cm and 6.35cm down from the upper rim. All three connector strips were then attached to the body cylinder and the cone placed in the middle. If any of the holes did not line up for attachment of the small cone, the cone was turned 30 degrees and new holes were drilled using the preexisting holes in the connector pieces as guides. Once the small cone was attached, any remaining holes or gaps were filled up with silicone sealant and allowed to set for 24 hours.

The last part to be added to the body portion was the attachment lid for the collection container. For this part, a 10.8cm diameter polyethylene screw-on lid corresponding to the collection container was used. The lid was hollowed out using a Dremel until only a 0.76cm lip of the interior rim and the cap threading remained and then 100 small 0.16cm holes were drilled around this cap rim. The top was then fit on to the 10.16cm hole at the bottom of the small cone and affixed with silicon sealant such that the gel was pushed through each of the 100 small holes but the threads were not compromised or clogged by the gel. After the cap was secured, the body of the CK trap was complete. (Fig S10).

Collection container

A one liter, wide mouth polyethylene jar (Zenith Global, Howell, MI) was used as the collection container for the CK trap. A 2.54x2.54cm piece was cut out of a no-pest strip (Hot Shot, St. Louis, MO) and was placed into the jar to kill any insects that came into the trap. The screw-on lid for the jar was built into the bottom of the body and rain catch portion of the trap so when a sample was ready to be taken, the jar was unscrewed and the no-pest strip piece removed. The collected jar was then sealed with a spare lid and a new jar had the pest strip placed into it. This new container was then screwed on the body and the trap was reset.

Legs

The CK trap legs are made out of two telescoping pieces of PVC pipe. (Fig S11). The inner pipe measures 106.68cm in length and is 2.54cm in diameter. The outer pipe is 91.44cm long and 3.175cm in diameter. Each pipe section was cut from larger 2.44 meter pieces using a table saw. A 3.175cm male PVC screw connection adapter was attached to the top of the outer pipe using standard PVC cement and allowed to set for 24 hours. The interlocking telescope was created by inserting the smaller pipe into the larger one and lining up the ends on one side. That side was assigned to be the top. The pipes were kept in that position and then a 1.11cm hole was drilled all the way through both sides of both pipes on the end not lined up, 10.16cm up from the end of the outer pipe. These holes were used as the collapsed-leg locking hole. The inner pipe was then pulled out of the larger pipe until the total length of the combined pipes was 172.72cm including the male adapter. Another hole was drilled all the way through the same spot on the larger pipe such that only the inner pipe was actually pierced. This new set of holes was used as the extended-leg locking hole. A 1.11x8.89cm clevis pin (Crown Screw and Bolt, Indianapolis, IN) was placed through the pipes in the collapsed leg locking hole for the remainder of the leg construction. A hole was then drilled all the way through the inner pipe 2.54cm from the bottom so that a 3.81cm, gauge 8 eyehook screw (Stanley, China) could be bolted on. A 5.72cm Spring Link carabineer (Keeper, Oceanside, CA) was then clipped on the eyehook loop and used to attach the leg to an anchor when the trap was deployed in the field. Each leg was outfitted with a 30.48cm spiral ground anchor (Camco, China) that could be clipped to the carabineer and used to anchor the trap into the ground when needed.

Putting it all together

Once all of the component pieces were constructed, the full trap could be assembled. Each of the legs was screwed into a mounting socket on the fins and set to the extended-leg position. Once the trap was standing, the top was attached to the large cone by placing the bottom of the fins on the large opening of the cone and then running the bottom of each of the 30.48cm loop Velcro strips between the side of the cone and the 106.68cm attachment belt. The Velcro loop strips were then looped back up and tightly attached to their corresponding hook strip. The body section were attached to bottom of the large cone in the same manner as the top and fins but using the 92.71cm attachment belt. A collection container was set with the no-pest strip chunk and then screwed onto the bottom. A light bulb was placed into the light fixture and the plugged in and tested to ensure it was in working order. At this point, the trap was ready to be used.

Figure S1: Clear Knight trap selected parts. 1-the circular top. 2-attachment hook. 3-GFCI pop fuse. 4-photoelectric switch. 5-toggle on/off switch. 6-electrical housing tube. 7-GE - 15 watt 18" T8 black light bulb. 8-power cord. 9-fin. 10-large cone. 11-leg attachment piece. 12-low tube socket housing. 13-attchement belt and Velcro strip for the body to large cone connection.

Figure S2: Pieces template for top section assembly. Figure is not drawn to scale. A-63.5x111.76cm rectangle of .48cm PC sheeting with the template to cut the fins and top pieces; each of the three rectangles are 20.32x60.96cm and the circle has a diameter of 45cm. B- 15.24x63.5cm rectangle of 0.24cm PC sheet with template to cut out the connector pieces and circuitry tube housing caps. All red dotes represent the location of holes drilled through the shape. The electrical housing tube attachment fin was opposite the photo switch hole (shown as P in the figure) as indicated by the green arrow. Fin lines were drawn on to the top circle (shown as dashed lines in the figure) at 120 degree departures from one another. Each of these lines then had a perpendicular line drawn 20.32cm away from the circle center to determine the proper placement of the fin connector holes. The blue dots on the top represent optional eye hook mounting locations that can be used if the trap was going to be hung.

Figure S3: Top assembly. Figure is not drawn to scale. Pink strips represent the 2.54x15.24cm connector pieces holding the fins together and red strips represent the 2.54x15.24cm pieces holding the fins to the circular top. Connector strips must be forcible bent to get into the right position. Holes for all connections are drilled prior to this point and alignment should be done using those holes. A-top view before the circular piece is put in place. B-side view looking directly on one of the fins after the top piece has been attached. The housing tube attachment fin is identifiable by the extra set of holes it has on the inner top and bottom.

Figure S4: Inside circuitry of the CK trap and diagram of the electrical housing tube. 1-power cord attachment to circuit board; black is live and white is neutral. 2-circuit board. 3-top tube socket. 4-wires to bottom tube socket after they have been initially cut but before they have been extended and enclosed in clear rubber tubing. 5-photo switch. 6-electrial housing tube. 7-connection wire from the circuit board to the bottom tube socket. 8-toggle on/off switch. 9-1.27cm hole for the toggle on/off switch. 10- A 0.32cm thick, 1.27cm high access notch in the housing tube for the tube socket insertion. 11-holes for the photo switch wires (top three) and the power cord wires (bottom three) set 1.27cm down from the cylinder lip and no closer than .64cm to each other. 12-power cord. 13-mounting holes drilled strait through the diameter of the tube positioned 5.08cm down from the top lip and perpendicular to the access notch.

Figure S5: CK trap circuit schematic. 1-outgoing hot wire from the power cord connects to incoming hot wire on the photo switch. 2-outgoing hot wire from photo switch connects to the incoming hot wire on the toggle on/off switch. 3-outgoing hot wire from the toggle on/off switch connects to the incoming hot wire on the circuit board. 4-outgoing neutral wires from the power cord, photo switch, and circuit board all connect together. 5-ground wire capped on its own. 6-outgoing tube socket wire from circuit board connects to incoming wire extension on bottom tube socket. 7-incoming tube socket wire from circuit board connects to outgoing wire extension on the bottom tube socket. 8-power cord. 9-photo switch. 10-toggle on/off switch. 11-circuit board. 12-top tube socket. 13-bottom tube socket.

Figure S6: Top with electrics installed. 1-upper electric mounting piece. 2-wires from the circuit board to the bottom tube socket encased in rubber tubing. 3-top housing tube. 4-photo switch. 5-power cord. 6-lower electric mounting piece. 7-bottom housing tube.

Figure S7: Completed top (installed electrics, leg attachments and Velcro ties). 1-20.32cm long, 3.175cm diameter PVC pipe with 0.48cm wide cut running down the center of the pipe 15.24cm. 2-female 3.175cm diameter PCV pipe screw adapter. 3-15.24cm slit in 3.175cm diameter PVC pipe. 4-two inch washer. 5-5.08x15.24cm hook Velcro strip, hook side facing out. 6-5.08x30.48cm loop Velcro strip, loop side facing out. 7-Velcro tie facing the opposite direction.

Figure S8: Cones are made from flat PC sheets by bending the shape shown in part A. Determining the three measurements shown in part B allows for the creation of a template shape (part A) which can be used to precisely create a cone of given dimensions. For the large cone in the CK trap, R1= 6.15cm, R2= 41.76cm, the Arc angle is 186.16°, L1= 6.35cm, L2= 43.18cm, and L3= 30.48cm. The small CK cone used in the body and rain catch portion of the trap R1=7.19cm, R2=19.18cm, the Arc angle is 190.8°, L1= 7.62cm, L2= 20.32 cm, and L3= 10.16 inches. In order to have a lip to screw the sides of the cones together on, extra pieces of the arc were cut out and tucked under the opposite side when folding the shape. C-To create the double circle outline, a router was used to cut semi-circles and then the resulting arcs were cut with a table saw to form the final shape.

Figure S9: The large cone with both attachments belts installed. 1-overlap region of the template shape. 2-screw holding the side together. 3-short attachment belt with a circumference of 92.71cm used for attachment to the body portion of the trap. 4-2.54cm, gauge 8 eye hook with a 1.27cm opening at the end used to hold the belts in place. 5-long attachment belt measuring 106.68cm and used for securing to the top of the trap.

Figure S10: Body and rain catch fully assembled. A-side view of the body cylinder, rain catch, and small cone. B-top-down view of the body cylinder and rain catch. 1-5.08x15.24cm hook Velcro strip, hook side facing out. 2-5.08cm washer. 3-rain catch 0.635cm hole-mesh filter. 4-rain catch low end. 5-screws hold the small cone in shape on the overlap. 6-5.08x30.48cm loop Velcro strip, loop side facing out. 7-63.5cm long, 2.54cm thick strip of foam weather stripping. 8-rain catch high end. 9-body cylinder. 10-2.54x15.24cm small cone connector pieces. 11-small cone. 12-attchment lid to the collection container.

Figure S11: CK trap legs. A-legs collapsed for transport. B-legs extended for trap use. 1-male 3.175cm PVC screw connection adapter. 2-outer 3.175cm diameter PVC pipe measuring 91.44cm long. 3-lock hole for extension of leg on inner pipe. 4-inner 2.54cm diameter PCV pipe measuring 106.68cm in length. 5-1.11x8.89cm clevis pin and locking loop. 6-30.48cm spiral ground anchor. 7-lock hole for collapsed leg on inner pipe. 8-3.81cm, gauge 8 eyehook bolt attached to inner leg with 5.72cm Spring Link carabineer attachment to garden screw top. 9-lock hole for outer pipe.

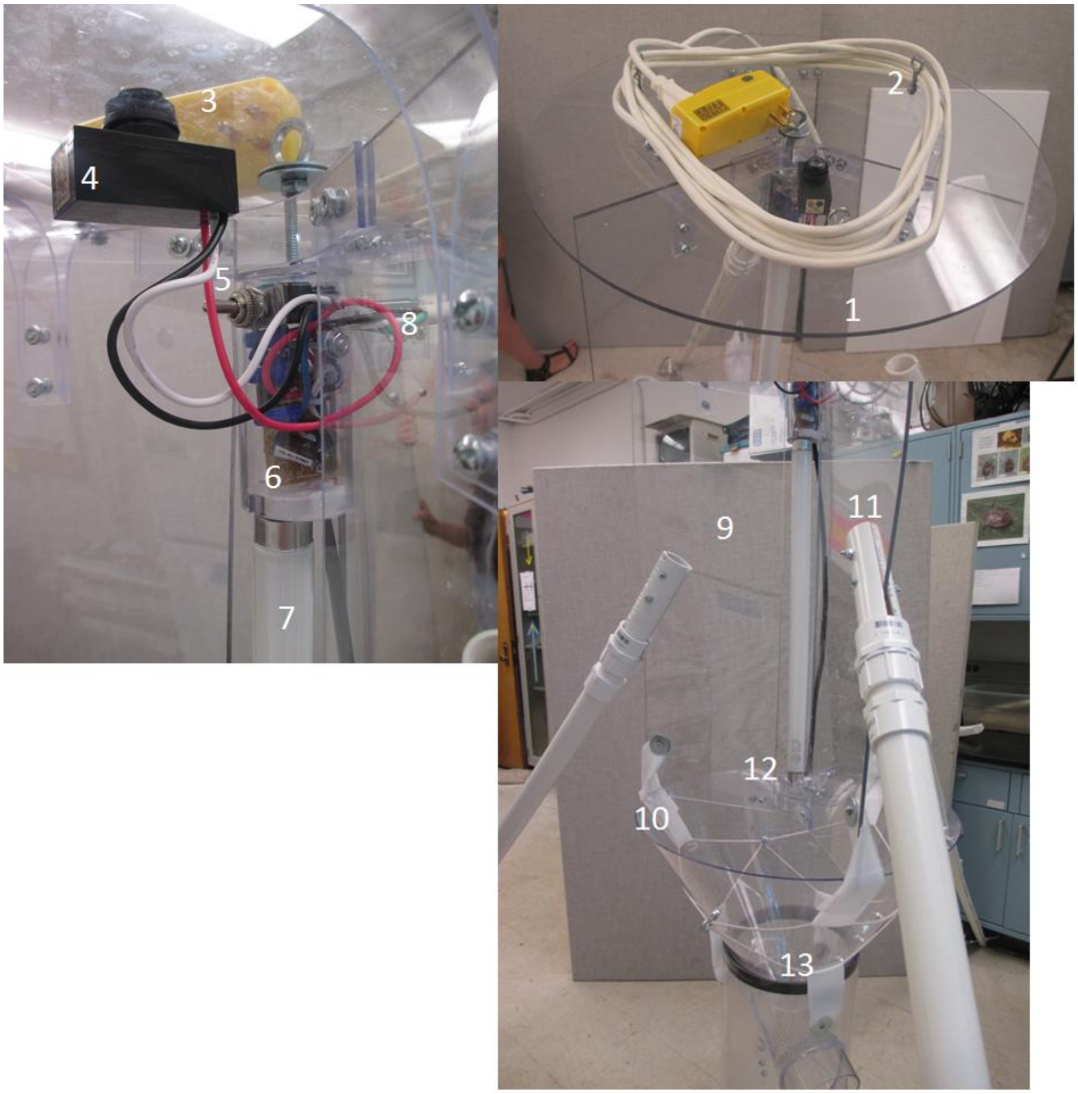
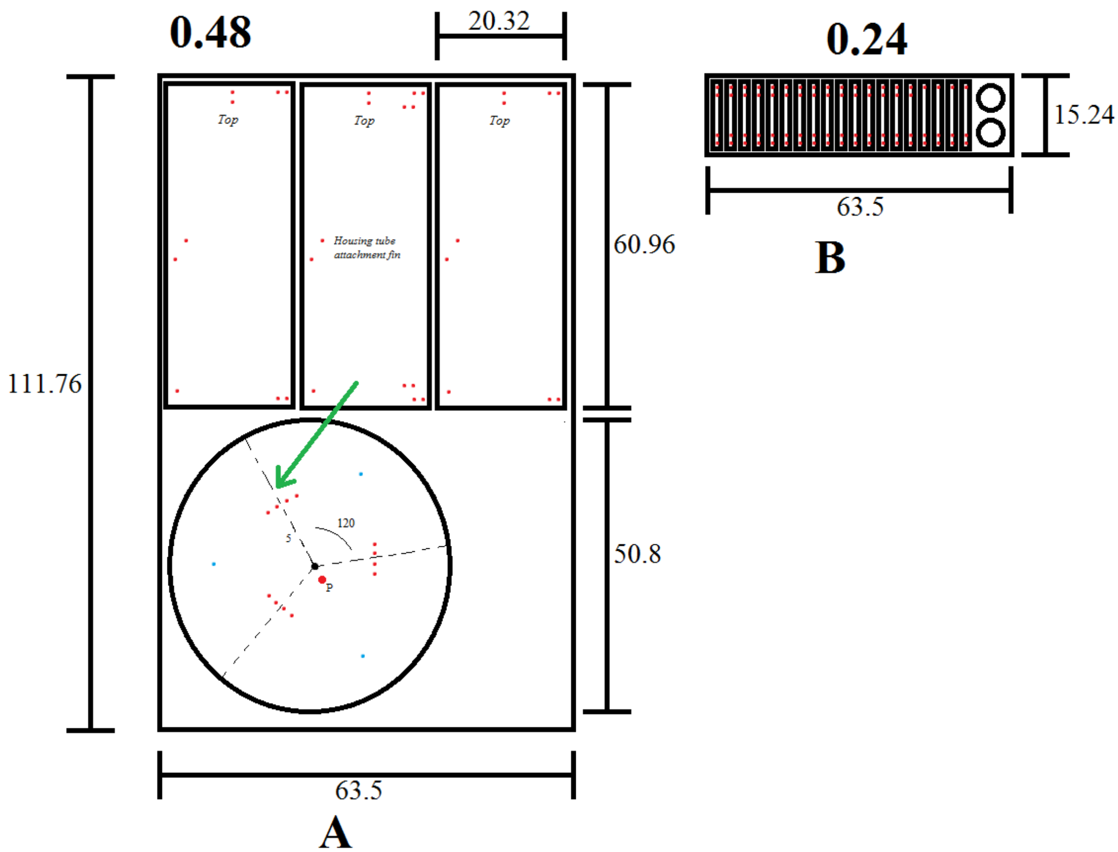
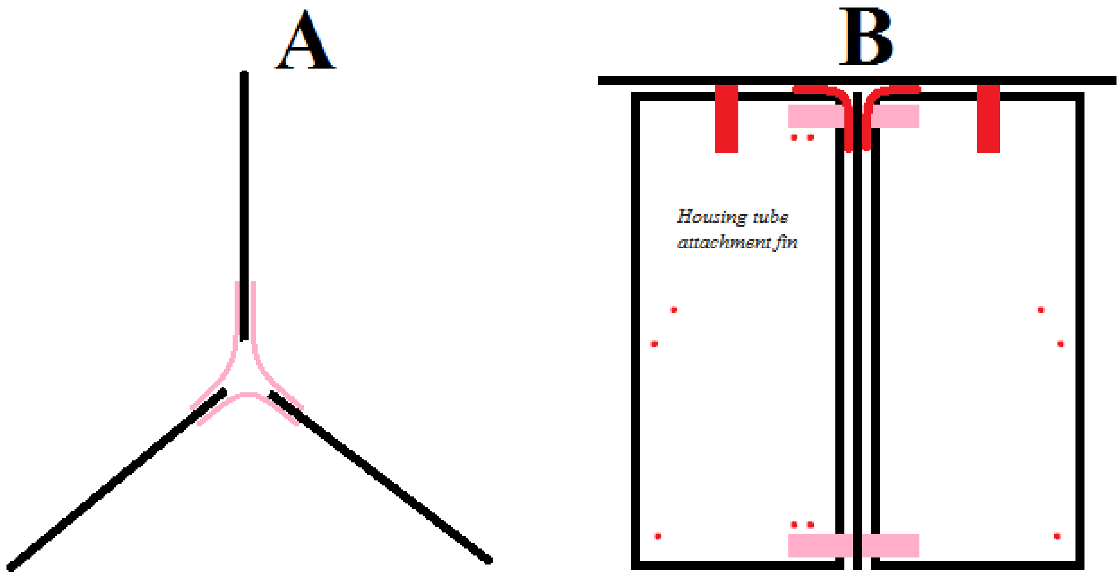


Figure S1



0.48
20.32
Top
Top
Top
Housing tube attachment fin
111.76
60.96
120
5
P
50.8
63.5
A
0.24
15.24
63.5
B

Figure S2



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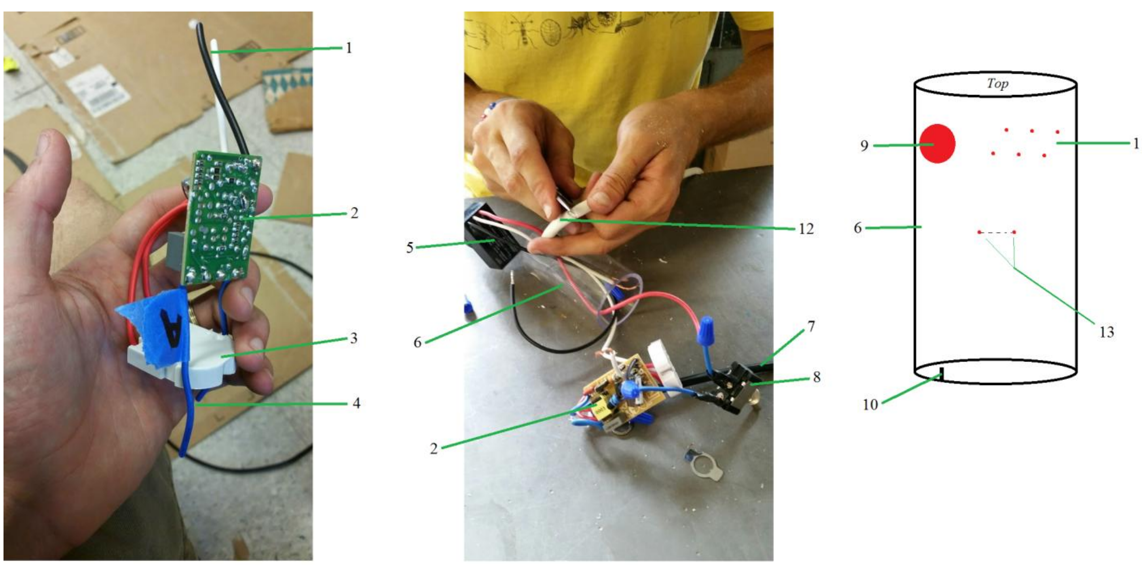

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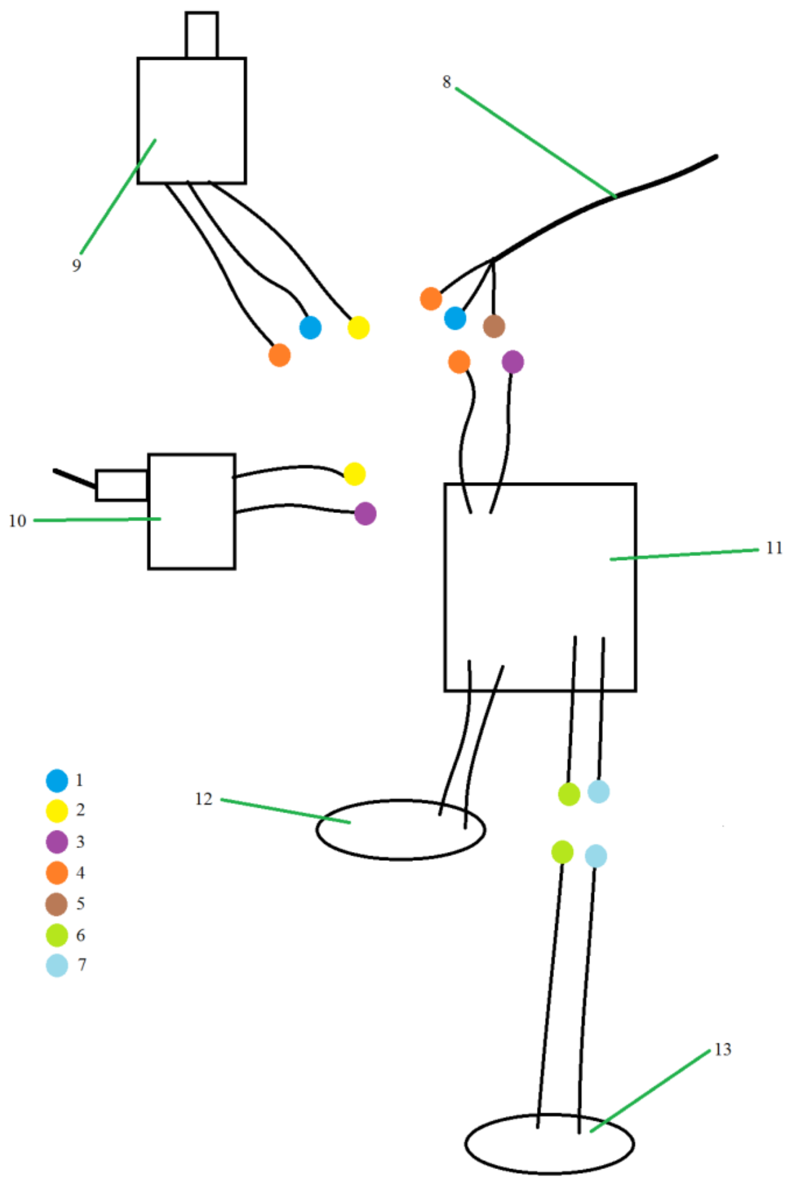

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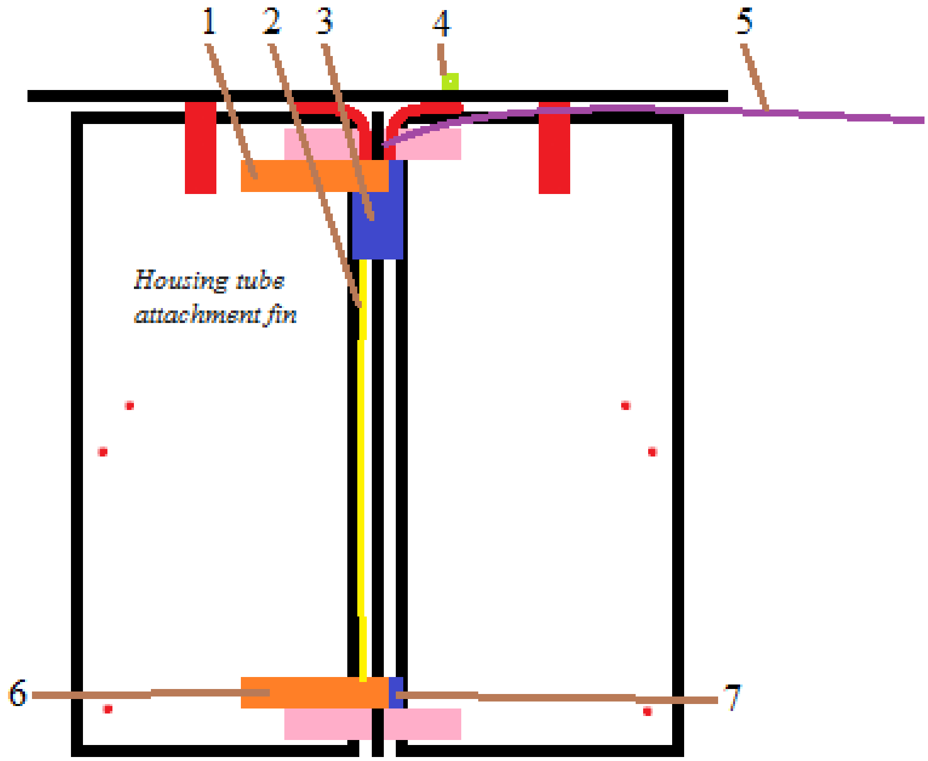

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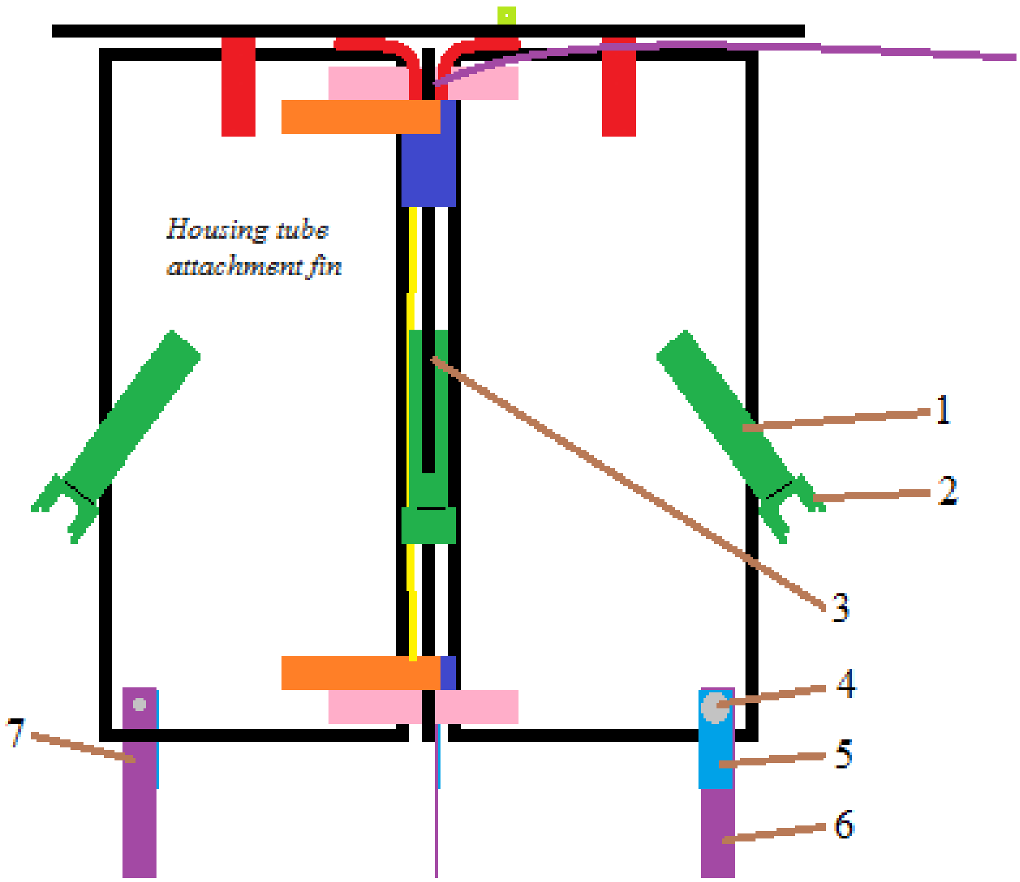

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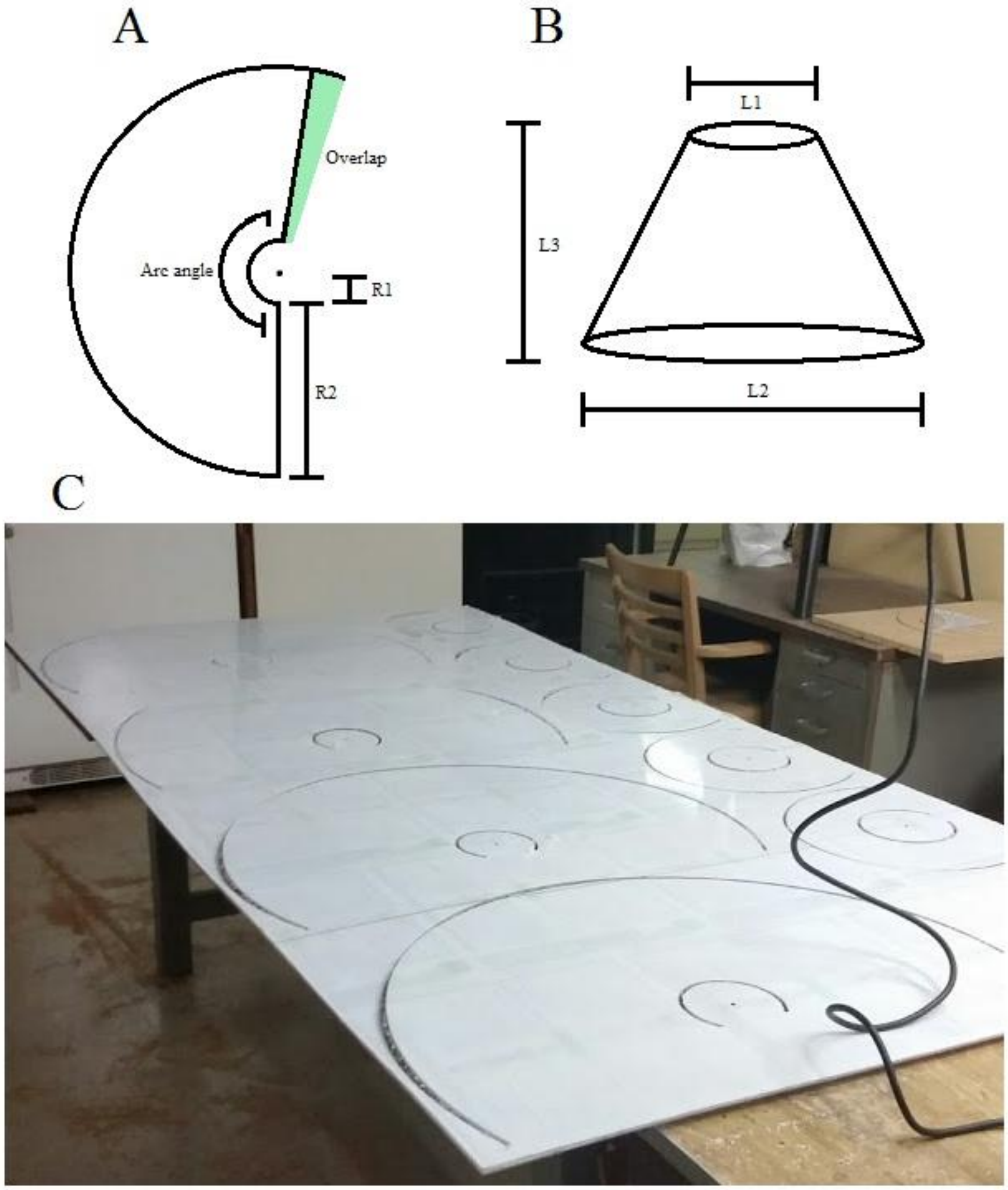

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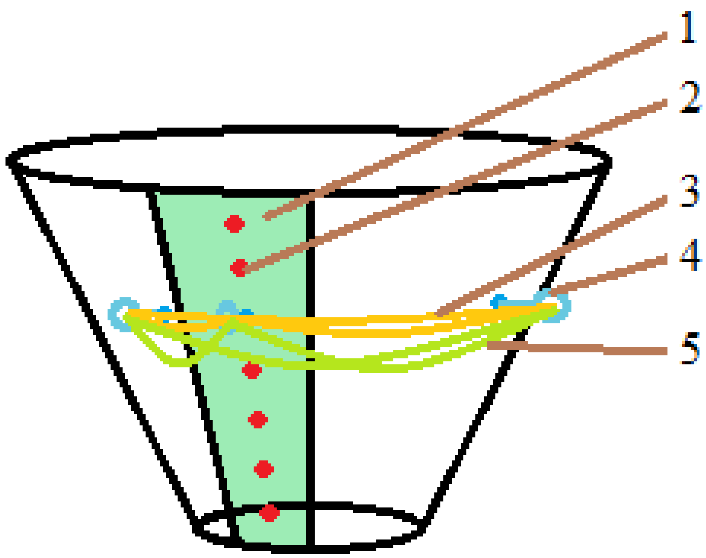

Figure S9

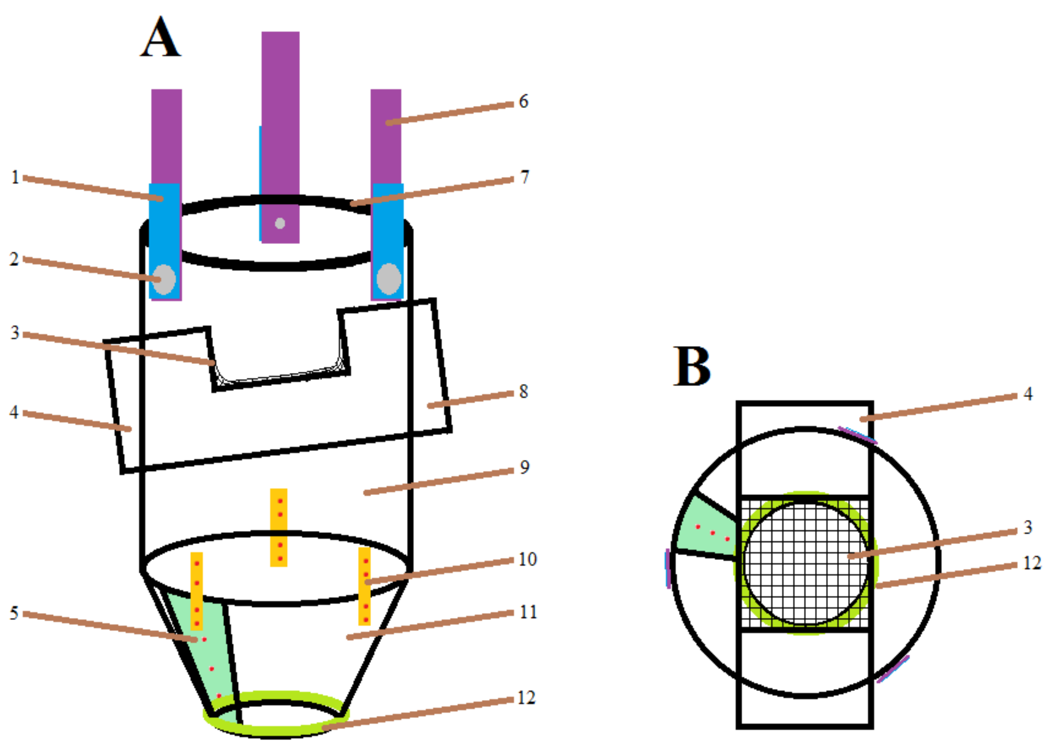

Figure S10

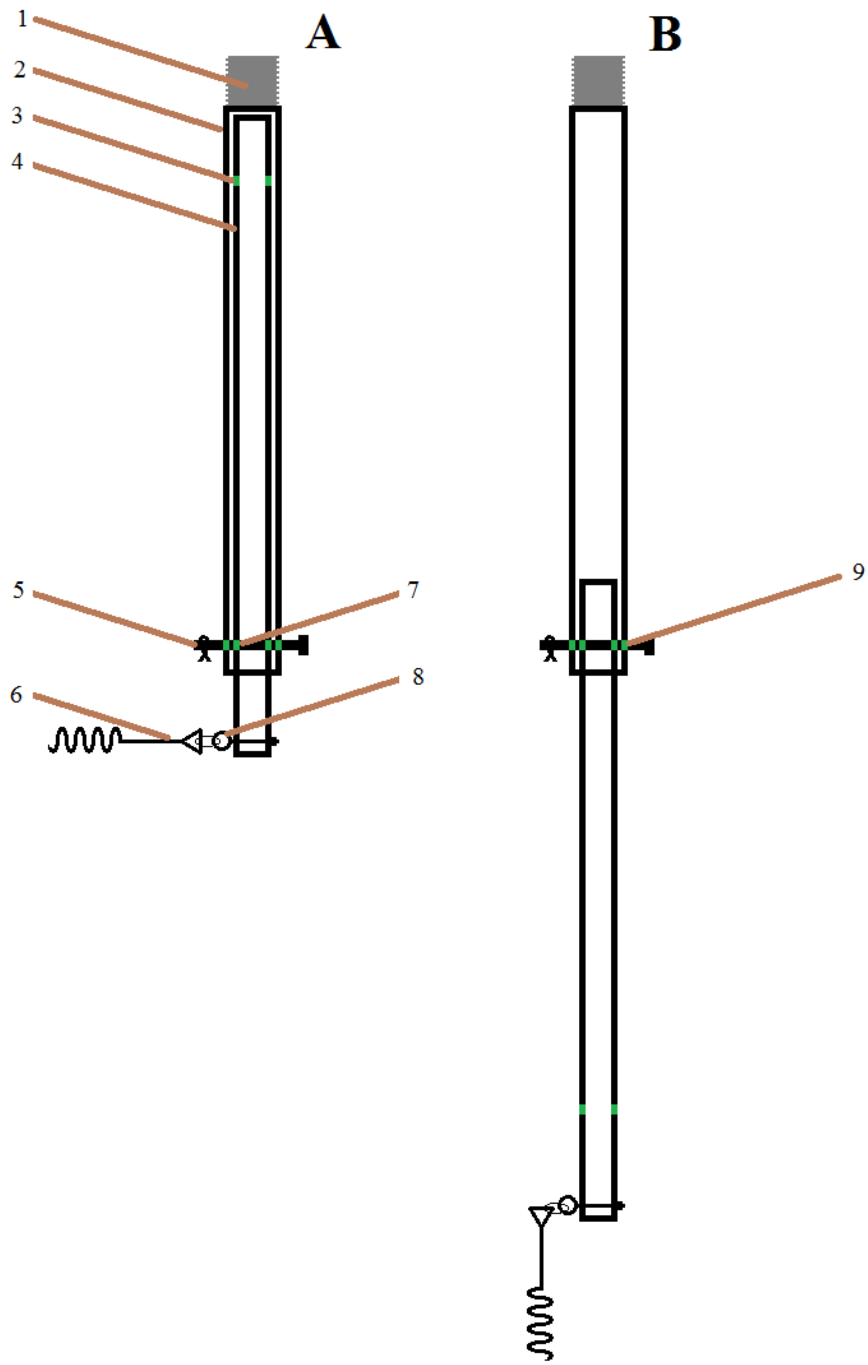

Figure S11

Chapter 7

Conclusions

The work described in this thesis explores the behavior of the important agricultural and urban pest species, *Halyomorpha halys* Stål, commonly known as the brown marmorated stink bug (BMSB) and how different sampling methods can be used to monitor the population size in an area. Research was and continues to be needed in this area to help develop better management practices for *H. halys* as its populations continue to expand across North America and Europe. The wide range of host crops that this insect can feed on has caused growers in many different systems to implement new control strategies, many of which are not yet optimized. Accurate population monitoring is the backbone of informed integrated pest management decisions and this thesis furthers our ability to detect changes in this pest's abundance.

The diel behavior of *H. halys* in peach orchards was found to significantly affect the number of individuals seen at a given time. Nymphs showed a distinct increase in observed individuals from sunrise until midday. The peak of observed counts was found at sunrise plus 7 or 10 hours. After midday, numbers decreased until sunset when they would decrease dramatically. Adult counts were fairly consistent throughout the day and night. This trend was consistent in both low and high populations. The data made a strong case for needing to standardize the time of day at which sampling occurs. This work helps better inform procedures for timed visual observations of *H. halys* in orchard systems and will lead to more accurate population estimates.

The distribution of *H. halys* within peach trees at each of the hours past sunrise used in the diel behavior study shifted in a predictable fashion over the course of a 24 hour period. Regardless of life stage, the most common surface to find these insects on was leaves. Towards the middle of the day, individuals become more frequently observed on the fruit and moving along the branches. The individuals were found to occasionally move between the trees or be re-observed on the same tree in an orchard. Most often when a *H. halys* was found, it was the first time that the individual had been identified. This information can be used to define new timed visual observation protocols and help direct observers to the best locations in the tree to detect this insect.

The study of the overwintering distribution of *H. halys* in college dormitories found that diapausing *H. halys* have a preference for locations above the ground level floors in the urban structures that they enter. The findings from this study can be used to recommend targeted sampling to detect and reduce pesticide application to manage this species in multistory man made structures.

A laboratory study to infer differential attraction of *H. halys* to light stimuli of different colors (red, orange, yellow, green, blue, and white) found all colors to be attractive to *H. halys* adults with white light showing the highest level of attraction. Males showed a greater propensity to cluster closely around the light source than females or nymphs. These findings can be used to inform future trap designs that may incorporate light as an attractant and how to interpret the data generated by these traps.

The creation of the Clear Knight black light trap was described. The trap can be used to monitor the population of numerous insects, including *H. halys*, in an agricultural setting. The Clear Knight trap was compared to the traditional tower type black light trap. This study showed that the Clear Knight trap catches similar amounts of nearly all insect families analyzed and that the diversity of insects caught by each trap was not significantly different. A detailed instruction guide for the creation of the Clear Knight trap is provided and can be used to cheaply and quickly create more of these traps for future programs.

The work in this thesis is useful for understanding the behavior of *H. halys* and how monitoring techniques are impacted by them. This work furthers the current understanding in some areas and is pioneering in others. More work is needed to fully understand *H. halys* and how best to monitor for it but several important questions have now been tentatively answered herein.