POPULATION ECOLOGY AND BIOLOGY OF THE INVASIVE STINK BUG

HALYOMORPHA HALYS (HEMIPTERA: PENTATOMIDAE) IN NEW JERSEY AND

PENNSYLVANIA

by

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ABSTRACT OF THE DISSERTATION

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an Asian species that has

become widely distributed in the Mid-Atlantic States since its confirmed introduction in

1996. Initially, there was confusion regarding the proper identification of *H. halys*

leading to a 5 year period where *H. halys* populations went unchecked, until its correct

identification by E. R. Hoebeke in 2001. One concern with invasive species is that

population densities can be higher in the introduced environment due, in part, to release

from natural enemies or lack of host plant resistance. In its native distribution, *H. halys*

has a wide host range and is considered a pest of agricultural crops. Life-history surveys

and laboratory developmental rate studies revealed that *H. halys* is univoltine in New

Jersey and Pennsylvania, requiring 538DD to complete development. Females emerging

from diapause require an additional 148DD prior to oviposition, during which time, they

utilize apple and pear trees. Surveys in ornamentals, tree fruit, and soybean demonstrate

that *H. halys* has become the predominant species of stink bug in these commodities,

outnumbering native pentatomid species. Sampling demonstrates that while beat or

sweep net sampling provides population estimates and seasonality, blacklight traps are a valuable tool to monitor adult dispersal. Monitoring for stink bugs often employs the use of traps baited with an aggregation pheromone that is attractive to all life stages. The large yellow pyramid traps baited with methyl (E,E,Z) 2,4,6-decatrientoate aggregation pheromone caught the most *H. halys* than other trap designs, especially in late-August to mid-September when imaginal ecdysis of the 1st generation adults occurs. Results show that at commercial farms, *H. halys* is present during the susceptible growing stages in apple, pear, and soybean, causing minimum of 25.9% injured fruit per tree. Laboratory insecticidal bioassays, primarily pyrethroid and neo-nicotinoids, were evaluated against *H. halys*. Pyrethroid and neo-nicotinoids caused high mortality at low concentrations. Males were significant less susceptible than females for thiomethoxam, but this was not evident for the other chemicals tested. The results demonstrate that *H. halys* has become well established in the Mid-Atlantic Region and there is a need for the development of control programs.

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INTRODUCTION

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) was introduced into
Allentown, PA around 1996 (Hoebeke and Carter 2003). It is native to South Korea,
Japan, and eastern China where it is an agricultural pest of multiple species of tree fruit,
soybean and is a homeowner nuisance because of its overwintering behaviors. Despite
being an agricultural and urban pest, little information is known (or available) about the
amount of damage inflicted by this species. In the United States, there is considerable
concern surrounding introductions of non-native species because they threaten biological
diversity, out-compete native species for resources, and can spread rapidly while
inflicting severe damage (Carruthers 2003). After introduction, the invasion process
consists of multiple steps including survival, reproduction, and then dispersal in its new
habitat. However, to be considered invasive, a non-native species must also cause
economic loss or ecological damage (Lockwood et al. 2007).

There are multiple factors that influence the ability of a species to become
invasive; specifically, a large number of founders and multiple introductions are
characteristics that can lead to a successful invasion event (Kolbe et al. 2004, Lockwood
et al. 2005, Drake and Lodge 2006). Hybridization between individuals (or species)
introduced from diverse habits can lead to superior genetic haplotypes not naturally found
in its native range (Arnold 2004). If a species is able to minimize the genetic bottleneck
that ultimately occurs during an invasion event, it will, theoretically, be a more successful
invader (Lee 2002). For example, reduced heterozygosity has resulted in reduced inter-
colony aggression in the Argentine ant, a behavior that has permitted a wider habitat
range than in its native countries (Holway et al. 1998, Tsutsui et al. 2000, Ficetola et al.

2008). Regardless of how a species enters or overcomes the hurdles surrounding

adaptation to a new environment, many insect species do this successfully each year.

Classic insect examples include the Japanese beetle, hemlock wooly adelgid, red

imported fire ant, gypsy moth, European corn borer, soybean aphid, cottony cushion scale

and now *H. halys*. One reason these species became severe pests is partially attributable

to the large lag period between invasion and detection. During this time, escape from

natural enemies, termed the "Enemy Release Hypothesis", allows a species to reproduce

unchecked before control measures are taken or native parasitoid or predatory species can

adapt to the invasive species (Williamson 1996). Only a few scelionid and tachinid

species are parasites of *H. halys* in Asia, none of which are present in the United States

(Arakawa and Namura 2002).

The life-history of *H. halys* in Asia is not fully documented, and little is known

about it since its introduction in the United States. In its native habitat, one to two

generations a year are common in temperate regions, although multiple generations are

possible in semi-tropical climates (Hoffman 1931). In temperate regions where *H. halys*

is univoltine, females will begin laying eggs around early June with their progeny

becoming adults in early to mid August (Hoffman 1931). Females lay eggs in clutches of

20-30 eggs (median 28) on the underside of leaves (Bernon 2004). Both the nymphs and

adults will feed on numerous host plants, switching to new hosts when phenologically

attractive and feeding occurs primarily on the reproductive structures of the plant.

Diapause induction is both temperature and photoperiod sensitive and is induced during

the 5th instar (Niva and Takeda 2003). Adult pentatomids go through reproductive

diapause, with under-developed oocytes, high fat content, and reduced body mass

(Borges et al. 1998). *Halyomorpha halys* adults can overwinter in large aggregations in man-made structures, a behavior that is believed to have evolved from attraction to cliff outcroppings (Kawada and Kitamura 1983). Dispersal to their overwintering locations occurs when maximum daytime temperature reaches 25°C after minimum overnight temperatures of 15°C, with peak numbers indoors after minimum temperatures fall below 10°C (Watanabe et al. 1994). Entrance into houses typically occurs through cracks in windows or the foundation. This behavior creates a considerable nuisance to homeowners due to noxious odors, tainted food supply, stained walls, and distress to inhabitants (Kobayashi and Kimura 1969). As temperatures increase in the spring, *H. halys* adults slowly move outdoors to find suitable hosts, such as apple (Funayama 2002).

Adult *H. halys* are characterized by their large size, brown or grey dorsal coloration that is mottled or marmorated in appearance with black antennae that have a band between the fourth and fifth antennal segment. The antennal characteristic is present beginning at the 2nd instar and is a key characteristic to distinguish it from native species. Adults are 12-17mm in length and 7-10mm in width, and are sexually dimorphic, with the females being larger in size (Stål 1855). Eggs are 1.6mm in length and 1.3mm in diameter, almost elliptical in shape and yellowish to pale green in color. Upon hatching, the nymphs go through five stages before imaginal ecdysis. In all life stages the body is darkly punctate and the head is broadly rounded in front. Each instar has a black head, thorax, dorsal plates. Connexiva, legs, and abdomens are off-white with red markings. First instars are tick-like in appearance, although second and third instars are tear-drop shaped. At the molt to the third instar, a white band is present on the mid-tibia and is present for the remaining life stages (Hoebeke and Carter 2003). The

scent glands are located on the dorsal surface of the abdomen and the ventral surface of the thorax.

Stål first described *Halyomorpha halys* in 1855 as *Pentatomoa halys* (Stål 1855). Since its original description, it has been reclassified several times. There are currently 37 recognized species of *Halyomorpha*, of which 16 are African, 8 are Indian, and 13 are Asian. Josifov and Kerzhner (1978) determined that only one species of *Halyomorpha* is found in Japan, Korea and eastern China and all *Halyomorpha spp.* in those localities should be referred to *H. halys*. Multiple synonyms exist for *Halyomorpha halys*: *Halyomorpha mista; Halyomorpha mysta; Halyomorpha brevis; Halyomorpha picus* (Josifov and Kerzhner 1978).

In its native range, *H. halys* is considered to be, at minimum, an occasional pest of tree fruit and soybeans (*Glycine max*) (Hoffman 1931, Kobayashi et al. 1972, Funayama 2002). The introduction of *H. halys* is concerning because of its polyphagous feeding habits and preference for these crops. In the United States, stink bugs are recognized as an increasingly important economic pest complex in many crops, due to the replacement of broad-spectrum insecticides that have historically managed populations (Todd et al. 1994, Riley et al. 1997, McPherson and McPherson 2000). The Food Quality Protection Act of 1996 restricts or eliminates the use of organophosphate and carbamate insecticides and pushes the emphasis towards management programs utilizing ecologically sound management options. Replacement or reduction of these insecticides has allowed stink bugs to become dominant or to buildup populations before dispersing to alternate hosts where they can inflict damage (McPherson and McPherson 2000, Snodgrass et al. 2005). In particular, herbivorous stink bugs are becoming a significantly greater concern in

cotton (*Gossypium spp.*) and tree fruit (Riley et al. 1997, McPherson and McPherson 2000, Leskey and Hogmire 2005). Complimenting this has been an increase in soybean [*Glycine max* (L.) Merr.] production acreage in the United States since the 1960's and increased acreage of transgenic *Bt*-cotton (Todd et al. 1994).

Stink bug populations in tree fruit are receiving more attention, primarily because of changes in insecticide practices and because feeding damage is commonly underreported (Todd et al. 1994, Riley et al. 1997, McPherson and McPherson 2000, Brown 2003, Leskey and Hogmire 2005). Damage from stink bug feeding occurs at three critical stages of fruit development, early, mid and late-season damage. In Japan and Korea, *H. halys* is an important pest of tree fruit; particularly of persimmons, apple, and pear (Kobayashi et al. 1972, Funayama 1996, Choi et al. 2000, Toyama et al. 2006). It is the dominant stink bug pest in South Korean non-astringent persimmon [*Diospyros kaki* L. (Ericales: Ebenaceae)] and Yuzu [*Citrus junos* Siebold (Sapindales: Rutgaceae)] with population peaks in mid-August (Chung et al. 1995, Choi et al. 2000). In South Korea, damage to Yuzu fruit was more severe in the lower canopy than high in the canopy and intercropping increased damage (Choi et al. 2000). Feeding damage that occurs at any of these critical growing periods can render the fruit unmarketable although immature fruit that is aborted due to feeding the occurs soon after bloom is not considered to be damage, unless fruit set is small (Rings 1957).

Halyomorpha halys is also a pest of soybeans in Asia. Most stink bug species prefer feeding on indeterminate varieties of soybean (Chang-Ki et al. 2000) and damage induced by feeding results in deformed seeds, delayed maturity, and reductions in yield and oil (Daugherty et al. 1964, McPherson 1996, Boethel et al. 2000, McPherson and

McPherson 2000). In Georgia alone, stink bug damage and control costs in 1996

exceeded $3 million from feeding by *Nezara viridula* L., *Acrosternum hilare* (Say), and

Euschistus servus (Say) (Riley et al. 1997). Before the introduction of *H. halys*,

Northeastern states such as New Jersey and Pennsylvania have not had serious problems

with stink bugs in soybeans.

Identifying monitoring methods that detect the occurrence and estimate density of

stink bugs is an essential aspect of developing an ecologically sound integrated pest

management program (IPM) (Metcalf and Luckmann 1994). Stink bugs are difficult to

sample because of their cryptic coloration, nocturnal feeding, aggregated dispersion, and

behavior of hiding or dropping off plants when disturbed, which may result in the

underestimation of population levels (Shearer and Jones 1996, McPherson and

McPherson 2000, Krupke et al. 2006). However, utilization of monitoring methods such

as pheromone traps, beat sampling, and blacklight traps can overcome some of these

behaviors (Aldrich 1988, McPherson and McPherson 2000, Leskey and Hogmire 2005,

Cullen and Zalom 2006). The use of pheromone traps has been particularly useful in

crops. Pentatomids produce aggregation pheromones for either food or mate location or

to identify overwintering habitats (Aldrich 1988). *Halyomorpha halys* males and females

are highly attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate, the aggregation pheromone of

a sympatric Asian species, *Plautia stali* Scott (Hemiptera: Pentatomidae) (Khrimian

2005, Khrimian et al. 2008). This compound has been used in Japan and South Korea for

monitoring populations (Lee et al. 2002, Adachi et al. 2007); however, the use of

pheromone trap to monitor *H. halys* has not been examined in soybean. Peak abundance

of *H. halys* in aggregation pheromone traps in early June when the adults are dispersing

to host plants from their overwintering sites and again in August and September when populations are dispersing to their overwintering sites. Pheromone traps have been used to forecast potential *H. halys* infestation rates in the early spring in apple orchards (Tada et al. 2001a). Blacklight traps in Japan have captured increasing numbers of *H. halys* in July and August (Moriya et al. 1987) but their implementation as a monitoring method has not been evaluated.

Chemical and biological control options can be implemented after monitoring methods determine population buildup. If *H. halys* does indeed become a pest in the eastern United States, sampling, monitoring, and control methods need to be evaluated. Chang-Ki (2000) found that feitrothion, triaazophos and carbaryl provided 83.4%, 69.5% and 87% control for stink bugs in soybeans. Similarly, Chung (1995) found that ethfenprox or phenthoate controlled *H. halys* in non-astringent persimmon orchards. With organophosphate and carbamate insecticides being pulled off the market due to the FQPA (1996) pyrethroid and neo-nicotinoid insecticides appear to be the most effective chemical insecticide against *H. halys*.

Scelionid egg parasitoids in the genus *Trissolcus* are specific to pentatomids. There are three known *H. halys* egg parasitoids, *Trissolcus mitsukurii* (Ashmead), *T. plautiae* Watanabe and *T. itoi* Ryu, in Asia (Arakawa and Namura 2002). However, these species are generalist pentatomid egg parasitoids and little is known about their ability to suppress *H. halys* populations. Studies on a different pentatomid species under conventional management programs revealed that natural enemies of *E. conspersus* had only a slight impact on the population, with a rate of less than 10% parasitism in apple (Krupke and Brunner 2003). The parasitism rate is most likely low due to suppression of

natural enemy populations from insecticidal applications and may be improved with proper insecticide management.

Halyomorpha halys is a successful invasive species. Survival of this invasive species will be aided by similarities in North American climate zones to the climate zones of its native habitat in Asia (NPAG 2001). Additionally, hitchhiking, adult flight, and a wide host range will enable this species to spread rapidly. The research presented here provides the basic biological information about development, current distribution,n and agro-ecology. Also, the seasonality and damage in agricultural crops is discussed.

CHAPTER 1

DEVELOPMENTAL RATE ESTIMATION AND LIFE TABLE ANALYSIS FOR HALYOMORPHA HALYS (HEMIPTERA: PENTATOMIDAE)

Abstract:

Egg and nymphal development were studied under constant temperatures for the newly introduced pest species, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Development was assessed at seven constant temperatures 15-35°C. Development to adult was completed at temperatures between 17-33°C with egg hatch also occurring at 15°C. The relationship between temperature and developmental rate was evaluated utilizing three developmental models. Of the models evaluated, the Briere-1 model was the best fit for the empirical data of egg and total development as well as providing accurate values for the temperature threshold. Application of the linear degree-day model estimated 537.63DD are needed for total development (egg to imaginal ecdysis). An additional 147.65DD are required for the pre-oviposition period of the female. Reproductive parameters were evaluated at 25°C and indicate a median number of 28 eggs per egg mass. Oviposition occurred at 4.32 day intervals and a female can continue to oviposit throughout its lifespan. *Halyomorpha halys* is univoltine in New Jersey and Pennsylvania but if it spreads to warmer climates in the United States could have multiple generations per year.

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an Asian stink bug species that was accidentally introduced into North America in Allentown, PA around 1996 {Hoebeke, 2003}. *Halyomorpha halys* is highly polyphagous, with over 300 reported host plants, including many agricultural crops, particularly fruits and vegetables important for fresh market sales (Hoffman 1931, Hoebeke and Carter 2003). In Japan, Korea, and China, *H. halys* is an occasional pest of soybeans and tree fruit, including apples, citrus and pear. Since its establishment in the United States, *H. halys* has been collected from multiple mid-Atlantic and New England states as well as from Oregon and California (unpublished data).

Stink bugs are increasingly serious pests in many agricultural crops, including tree fruit, because of reductions in broad-spectrum insecticide use, including organophosphates, which previously controlled stink bugs in many crops (Willrich et al. 2003, Snodgrass et al. 2005). While it is uncertain what level of economic or ecological impact *H. halys* will ultimately have in the United States, economic losses resulting from *H. halys* feeding injury have occurred in commercially grown apples, peaches and pears in New Jersey and Pennsylvania beginning in 2006. Therefore, it is important to understand the development and reproductive parameters of *H. halys* as a possible emerging pest.

In Asia, *H. halys* overwinters as an adult and produces offspring by mid-summer on host plants where the majority of the feeding damage occurs (Hoffman 1931). Many stink bug pest species like *Acrosternum* sp., *Nezara* sp. and *Euschistus* sp. exhibit similar behaviors (McPherson and McPherson 2000). The study reported here determines the

developmental biology of the New Jersey population of *H. halys* under controlled conditions. The relationship between temperature and developmental rate typically increases from a minimum threshold until the optimum temperature at which point it rapidly declines (Logan et al. 1976, Lactin et al. 1995). Comparisons between biological models have been done for Acari, Homoptera, Coleoptera, Hymenoptera, and others to aid in understanding specific developmental characteristics for insects (Lactin et al. 1995, Briere et al. 1999, Sanchez-Ramos and Castanera 2001, Roy et al. 2002, Kontodimas et al. 2004, Arbab et al. 2006). To examine the developmental rate of *H. halys* we chose models that predicted the minimum and maximum temperature thresholds (Campbell et al. 1974, Logan et al. 1976, Lactin et al. 1995, Briere et al. 1999, Roy et al. 2002). For this, we evaluated the linear degree-day model, and the non-linear Briere-1 and Lactin-2 models as descriptors of both egg and total development.

As *H. halys* expands its range in North America and increases in population density, developmental rate studies are a necessary first step to understanding its biology and ecology. Life table analysis for the New Jersey and Pennsylvania populations will help provide the necessary components for population models and the development of Integrated Pest Management (IPM) programs, should the need arise. This study presents the first laboratory data on developmental rates and life table parameters of *H. halys* in the United States under constant temperatures.

Materials and Methods

<u>Laboratory Colony</u>

Halyomorpha halys adults (N=~200) were initially collected in September 2003 at

the Rodale Organic Tree Center in Allentown, PA to establish a laboratory colony. Each

year, the colony is supplemented with individuals (N= ~200) collected from the same

location in August and September. Colony individuals are maintained on green bean

(*Phaseolus vulgaris* L.), Spanish peanut (*Arachis hypogaea* L.), corn (*Zea mays* L.) and

water under room temperature at a 16L:8D photoperiod (maintained with fluorescent

lighting) (Niva and Takeda 2003) in BugDorm2 cages (BioQuip, Rancho Dominguez,

CA). Green beans were grown without insecticides in the Rutgers University

Entomology Greenhouses and fertilized weekly [1.0 tablespoon/gallon] (Miracle Grow,

Marcusville, OH). Pest insects in the greenhouse were controlled using horticultural oil

(AllSeasons, Bonide Products, Oriskany, NY) at 1.0T/G water. When green beans were

not available, organically grown green beans were purchased from local markets. Water

was replaced every other day, food was replaced twice weekly, and nymphal cages were

replaced as needed. Developmental rate and fecundity studies using colony members

began in March 2004 and continued until March 2006. Voucher specimens are deposited

at the collections of Department of Entomology, Rutgers University (New Brunswick,

NJ).

<u>Effect of Temperature on Development of *H. halys*</u>

The effect of temperature on developmental time was examined at eight temperatures:

15, 17, 20, 25, 27, 30, 33, and 35°C, at photoperiod 16:8 (L:D) and 65 to 75% relative

humidity (RH). A long-day photoperiod was used to maintain non-diapausing conditions

(Niva and Takeda 2003) inside environmental chambers (Precision Scientific, Winchester, VA) at $\pm$ 1°C of the set temperature. Temperature and RH were recorded using a Hobo® LCD Data Logger (Onset Computers, Pocasset, MA). Individual 235ml cardboard containers (Sweetheart Jazz Co., Owing Mills, MD) with the lid replaced with organdy mesh for ventilation were used for rearing stink bugs throughout the study. A moist dental wick, piece of green bean and a Spanish peanut were placed into each container. For each temperature replicate, five egg masses oviposited within 24h were randomly chosen from the colony and placed individually into rearing containers.

Development was evaluated under constant conditions in two ways. For the first temperature experiment, twenty-four hours after egg hatch, or when first instars began moving away from the egg mass, ten 1st instars were randomly selected from each of the five egg masses and placed into the individual rearing containers. During the second experiment, cohorts were separated after the molt to the second instar and ten 2nd instars from each egg mass were randomly chosen. This was done to evaluate any benefits such as reduced mortality that could be gained during 1st instar aggregation. A total of 800 *H. halys* eggs were used with each nymph labeled according to the egg mass from which it had emerged (adapted from Rings and Brooks 1958). Individuals were checked every 24h for development or mortality. Water was replaced every other day and food was provided twice weekly. Development was documented by the presence of exuvia and nymphal characteristics as outlined in Hoebeke and Carter (2003). At the final molt to adult, all individuals were sexed.

Data Analysis and Developmental Rate Models:

For the purpose of evaluating the regression models, data from both developmental

experiments was combined. Data was subjected to the general linear model (GLM) for

equality of variance, followed by Tukey's test for mean separation $P \leq 0.05$ (SASInstitute

2002-2003). Developmental parameters, including degree-days (DD), were estimated for

egg development (time from oviposition to hatch) and for total development (oviposition

to imaginal ecdysis). We compared three developmental models for their fit to the

empirical data and definition of the biological parameters, T_o and T_m, where T_o is the

minimum temperature threshold and T_m is the maximum temperature threshold. The

linear regression model ($y = y_o + bx$; where y_o is the y-intercept and b is the slope of the

line) using mean developmental rate (1/d) estimated minimum temperature threshold as

$T_o = - y_o /b$ and estimated DD requirements ($DD = 1/b$) (Campbell et al. 1974). The

linear regression model does not calculate T_m and may not provide the most accurate

prediction of T_o since only temperatures within the linear portion of the development

curve may be used. Thus, two nonlinear models, the Lactin-2 and Briere-1 were

evaluated for their ability to predict T_o and T_m (Lactin et al. 1995, Briere et al. 1999). The

Lactin-2 model is described as:

$$r(T) = e^{\rho T} - e^{[\rho Tm - (Tm - T)/\Delta]} + \lambda$$

where $r(T)$ is the developmental rate (1/d) at temperature T; T_m is the maximum lethal

temperature; and $\rho, \Delta,$ and λ are fitted parameters. The Briere-1 model is described as:

$$r(T) = aT(T- T_o)(T_m -T)^{1/2}$$

where a is an empirical constant. Parameters were defined using the iterative function of

PROC NLIN (SASInstitute 2002-2003) according to the Marquardt method. Models

were compared by the residual sum of squares (RSS) and the calculated Akaike

Information Criterion (AIC) for goodness of fit (Briere et al. 1999, Roy et al. 2002,

Arbab et al. 2006, Zamani et al. 2007). The Briere and Lactin models differ in the number

of parameters, which can lead to better fit to the empirical data. The AIC is defined as:

$$AIC = n \ ln \ (SSE/n) + 2p$$

where n = the number of observations, ln = the natural log, SSE = the model sum of

squares term, and p = the number of parameters. This evaluation is parameter

independent permitting the Briere and Lactin models to be compared. The model with

the smallest RSS and AIC values is considered the best fit (Zamani et al. 2007).

Mean stage-specific mortality of each nymphal stage was calculated as $[d_x/l_x]$ where

d_x = the number of individuals dying stage x and l_x = the number of individual alive at the

beginning of stage x. Mean generational mortality was calculated at each temperature as

$[d_x/l_o]$ where l_o = the starting number of individuals (Wittmeyer and Coudron 2001).

Life Table Analysis

Fecundity and fertility parameters for *H. halys* were evaluated at the optimal

developmental temperature to construct a time-specific life table (Rings and Brooks

1958, Canerday 1965, Medeiros et al. 2000, Legaspi and Legaspi 2005). Incubators

(Precision Scientific, Winchester, VA) were maintained at $25^{o}C \pm 1$, photoperiod 16:8

(L:D) and 65-75% RH. Rearing conditions were similar to the developmental study,

except 710ml translucent plastic containers were used lined with a 9cm filter paper

(Fisher Scientific®, Atlanta, GA) at the bottom and organdy mesh replacing the lid.

Newly emerging adults, within 24h since imaginal ecdysis, from the laboratory colony

were sexed and placed under the controlled conditions. Twenty-two females were paired

individually with a male of similar age and six females without access to a male were observed every 24h for oviposition and mortality (Rings and Brooks 1958). Egg masses were removed daily, labeled according to the female identifier, and placed in the incubator until hatching. The number of emerging first instars was counted and subsequently verified by counting the number of egg breakers present on the egg mass using a stereomicroscope (Leica MZ8®, Wetzlar, Germany). Males were rotated weekly between containers to reduce effects due to male fitness. When a male died, it was replaced with another male of similar age. Data was recorded until the end of the life of the female.

Mean total fecundity, egg hatch, pre-oviposition period and female longevity were recorded. Degree-day accumulations for the pre-oviposition period were calculated as:

*DD = (25^oC – lower developmental threshold) * (days spent in development)*

$$DD = (25^oC - lower\ developmental\ threshold) * (days\ spent\ in\ development)$$

(Cullen and Zalom 2006). Fecundity was defined as the number of eggs laid over the lifespan of the female and fertility was the ability to produce at least one fertile egg mass. Age-dependant fecundity was calculated by dividing the age of the females into 7-day intervals and calculating the mean number of egg masses oviposited during that week (Medeiros et al. 2000). Pre-oviposition period and female life span were compared to the unmated females.

Life table parameters were estimated for net reproductive rate ($R_o = \Sigma\ l_x\ m_x$), intrinsic rate of increase ($r_m = (ln[R_o])/T)$), finite rate of increase ($\lambda = e^{rm}$), mean generation time ($T = \Sigma\ (l_x\ m_x\ x)/\Sigma(l_x m_x)$), and doubling time ($DT = ln2/r_m$) where l_x is the proportion of individuals alive at age x, and m_x is the number of female offspring produced per female in the age interval x (Carey 1993). Eggs were not reared to adult and a 1:1 sex ratio was

assumed (Wittmeyer and Coudron 2001); (Legaspi and Legaspi 2005). Reproductive

parameters were adjusted using the number of first instars that hatched, not the number of

eggs oviposited (Wittmeyer and Coudron 2001, Legaspi and Legaspi 2005).

Results

Effect of Temperature on Development of H. halys

Egg development time was significantly impacted by temperature (F = 4933.75,

df = 6, 624; $P\leq0.0001$) and occurred from 15-33°C. Incubation was longest at 15°C (22

days) and shortest at 30°C (3 days) (Table 1). Mean developmental time was 22.00,

17.20, 11.50, 6.10, 4.87, 3.00, and 4.00 days for 15, 17, 20, 25, 27, 30 and 33°C,

respectively. High mortality (50%) occurred at 15°C. No egg development occurred at

35°C (Table 1).

Development of *H. halys* 1st instar nymphs to imaginal ecdysis successfully

occurred between 17-33°C (Table 1). Total developmental time (egg incubation to adult)

was longest at 17°C and shortest at 30°C and was also significantly impacted by

temperature (F = 424.40, df = 5, 222, $P\leq0.001$). When the two experiments are

combined, the mean developmental time from egg to imaginal ecdysis was 121.50, 81.16,

44.92, 35.81, 33.40, and 37.80 days for 17, 20, 25, 27, 30, and 33°C, respectively.

Development time from egg to adult ecdysis was associated with increasing temperature

up to 30°C. Of the temperatures where development to adult was completed, there was a

significant difference in total developmental time at each temperature ($P\leq0.05$), with

development being shortest at 30°C (33.39 ± 0.50). Development to adult did not occur

at either temperature extreme (15 and 35°C). The duration of the first nymphal stage was

typically the shortest for all temperatures; development during the fifth stadium was the longest. Development time was shorter and survival higher at 25°C (61%) than the upper threshold range (27-33°C) and should be considered the optimal temperature for *H. halys* development. Mean stage-specific mortality (d_x/l_x) of the nymphal instars was lowest during the fourth instar (Table 2). Generational mortality (d_x/l_o) was lowest at 20 and 25°C and extremely high at 17°C (97%) and 33°C (95%). High mortality throughout development at 17°C resulted in only two surviving adults, both of which were males (Table 1). At 15°C, development past the first instar did not occur (Table 2).

Mortality of the 1st instars decreased when they were allowed to aggregate at all temperatures although there was no significant difference between replicates ($P= 0.54$) (Table 3). There was no significant difference in the number of individuals surviving to the adult stage ($P=0.75$) but total development was shorter at each temperature when the 1st instars were allowed to aggregate (Table 3).

Developmental Rate Models:

A linear regression model was first applied to the egg incubation and total development by plotting development rate ($1/d$) against temperatures 15-30°C (Egg dev.: y = 0.019x - 0.271, R^2 = 0.90; Total dev.: y = 0.002x - 0.024, R^2 = 0.97). The linear regression model was not a good fit for the data at all temperatures because development rate decreased after the peak at 30°C (Fig. 1). The inverse of the slope of the linear line is used to calculate the accumulated DD requirements for insect development. Using the data for 15-30°C, the accumulated DD for egg and total development were 53.30 and 537.63 DD, respectively.

The Briere-1 nonlinear model provided a good estimation of parameters for the egg incubation period as T_o = 13.94 and T_m = 37.73. The Lactin-2 model did not converge on

the data for egg development and was not included in the analysis. Both the Lactin-2 and Briere-1 nonlinear models provided estimation of the parameters for total development of *H. halys*. Temperature thresholds for total development were calculated as $T_o = 14.17$ and 15.00 and $T_m = 35.76$ and 43.53 by the Briere and Lactin-2 models, respectively.

Life Table Analysis

At 25^oC, *H. halys* females exhibited a 13.35 ($\pm$ 0.72) day pre-oviposition period. Each egg mass contained a median number of 28 eggs (mean = 26.08 $\pm$ 0.31) and a mean interval of 4.32 ($\pm$ 0.41) days was required between oviposition events (Table 4). Twenty females laid at least one viable egg mass during the study, a fertility rate of 90.90%. Few mating events were directly observed due to *H. halys'* short copulatory duration as well as the limited time of each observation. The total mean number of eggs produced over a lifetime was 212.25 ($\pm$ 31.04) or 8.00 ($\pm$ 0.19) egg masses. Female fertility affected the number of egg masses oviposited. Females that produced more than one egg mass oviposited an average of 243.78 ($\pm$ 27.48) eggs or 9.33 ($\pm$ 1.04) egg masses over her lifespan. Mean age-specific fecundity peaked during the second and third week post-ecdysis with females producing a maximum of 1.36 ($\pm$ 0.20) egg masses per week (Fig. 2). Fertility also increased with female age as the first egg mass produced was the least fertile, 61% increasing to 80%, and remained constant for the remainder of the lifespan.

All the virgin females observed produced unfertilized egg masses but not on a specific interval. The pre-oviposition periods for mated 13.35 ($\pm$ 0.72) and virgin females 18.17 ($\pm$ 2.93) were significantly different at $P \le 0.05$. Based on the lower temperature threshold calculated by the Briere model, degree-day accumulations from imaginal ecdysis until the first oviposition event (pre-oviposition period) were calculated to be 147.65DD.

Using a mean development calculated at 25°C of 47.97 days and assuming a 1:1 sex ratio, 159.90 female eggs were produced per female. The life table parameters at 25°C, $R_o = 60.02$, $T = 56.59$ and $r_m = 0.07$ were adjusted to account for the number of 1st instars emerging.

Discussion

Developmental rate studies provide biological information that can be used in many ways, especially for understanding seasonality and population dynamics of a species. This is the first extensive laboratory study under controlled conditions for the United States population of *H. halys*. Preliminary observations of the Northeastern United States population indicated total developmental time of a cohort as 42.25 days at room temperature, data that is consistent with our study of mean development time at 25°C to be 41.32 days when 1st instar aggregation was permitted (Hoebeke and Carter 2003). Field development observed in Southern China was estimated at 27 days, and 37-45 days in Northern Japan (Hoffman 1931, Saito et al. 1964). Aggregation during the 1st instar did not have a significant effect on the survivorship to adult. There is some suggestion that the pentatomid 1st instars increase humidity regulation during aggregation or feed on the egg chorion during the aggregation and obtain symbionts which may provide physiological benefits (Hirose et al. 2006). We showed that the aggregation of 1st instars significantly reduced nymphal and total development time in *H. halys*, although the cause was not investigated.

Most previous studies on pentatomid development have been restricted to linear models. Linear regression was applied to *H. halys* development for a comparison of

temperature threshold estimations to other studies and to calculate degree-day requirements. Application of the Briere-1 and Lactin-2 models, however, provided a better fit to the temperature dependant development of *H. halys* and allowed more accurate prediction of the temperature threshold. However, the calculation of maximum threshold temperature by the Lactin-2 model of $T_m = 43.53^{\circ}C$ is unrealistic since development of *H. halys* did not occur at $35^{\circ}C$, suggesting that the Briere-1 model is the best fit for the empirical data for total development of *H. halys*. That the Briere-1 model is the most appropriate model to use for *H. halys* development is supported by AIC analysis (AIC = -10019.68). Further, the Lactin-2 model for egg development did not converge and was an unreliable model for development of *H. halys*.

The minimum temperature threshold is important not only for determining degree-day accumulations and adult emergence but also for overwintering survival and range expansion. Temperature threshold can limit the potential geographic range of *H. halys* in the United States. Its current distribution in the Northeastern United States indicates that *H. halys* is able to survive colder winter temperatures than the Southern Green Stink Bug, *N. viridula*, which is abundant in warm habitats. Similarly, in its native Japan, *H. halys* has a higher winter survival rate than *N. viridula*, presumably due to *H. halys*' lower temperature threshold and choice of overwintering habitat (Kiritani 2006). The T_o for *H. halys* egg development in Japanese provinces ranges from 9.9-$15.1^{\circ}C$, with $12.2^{\circ}C$ being the most reported. The reported T_o for total development in Japan ranges from 11.1-$12.9^{\circ}C$ (Kiritani 1997). In our study, the temperature threshold estimated was higher than reported in Japan. This suggests a possible origin from a warmer climate; the range of *H. halys* in Asia includes sub-tropical regions of China and India. Based on its distribution

in Asia and similarities with the temperature thresholds of native stink bugs, *H. halys* should be able to increase its current distribution into areas of economically important agroecosystems in the Southern United States.

Fertility and fecundity parameters for *H. halys* were estimated at 25°C (optimum developmental temperature) for use in calculating a simple life table. A high proportion (90.90%) of *H. halys* females were fertile, and of those females ovipositing more than one egg mass, an average of 243.78 (± 27.48) eggs per female were oviposited. Each egg mass contained a median number of 28 eggs, consistent with previous reports and having ovarioles in multiples of seven (Hoffman 1931, Kawada and Kitamura 1983, Hoebeke and Carter 2003, Bernon 2004). For all females, egg masses averaged a hatch rate of 81.60%. This is lower than observed hatch rates of field populations (ALN unpublished data). Artificial mating conditions could account for the lower hatch rate in the laboratory, and egg masses of overwintering females may result in a higher hatch rate (Rings and Brooks 1958). The pre-oviposition period for *H. halys* in this study averaged 13.35 (± 0.72) days with an average 212.25 (± 31.04) eggs/female. Kawada and Kimura (1983) reported a pre-oviposition period of 14 to15 days for Japanese *H. halys* and an average of 486.6 eggs per female. Rearing conditions, developmental length, geographic variation, and other variables could account for this substantial difference between the numbers of eggs produced by a female (Rings and Brooks 1958, Kawada and Kitamura 1983). Without primary egg parasitoids, *H. halys* has the potential to increase in population densities faster than native species. In the absence of species-specific natural enemies and with expanded host diversity in the United States, including host plants native to Asia plus an abundance of European and North American varieties in the same

families, it is likely that *H. halys* could become an economic concern in many cropping systems if it continues to increase in density and distribution. Life table analysis on *H. halys* will be important in future modeling efforts as well as development of biological control programs.

While *H. halys* is not a highly damaging pest in Asia, we predict it will become an important addition to the phytophagous stink bug complex in the United States. The reduction in the amount of broad-spectrum insecticides previously used to control tree fruit pests and the increased planting of *Bacillus thuringiensis* (Bt)-modified cotton has increased the incidence of pest stink bugs in these crops, and may lead to increased economic losses (Willrich et al. 2003, Leskey and Hogmire 2005, Snodgrass et al. 2005). Stink bug pests are often managed as a complex in agroecosystems because of similarities in host feeding and development. The primary factor for incorporating *H. halys* into any IPM program for stink bugs will be the number of generations per year and the peak of the filial adult population, which is the most damaging stage. Nonlinear regression estimates that 537.63DD are required to complete development with an additional 147.65DD until oviposition commences. In central New Jersey and Pennsylvania, *H. halys* is univoltine with a peak in the population in late July, early August. It is likely that if *H. halys'* distribution spreads south, the number of generations per year will increase. *Halyomorpha halys* is known to be multivoltine in semi-tropical climates; such as the Canton province of southern China where up to six generations per year have been reported (Hoffman 1931). However, other environmental factors such as photoperiod length also determine the number generations per year. Maintenance of a long photoperiod and warm temperatures during nymphal development are necessary to

prevent physiological changes signaling diapause development in *H. halys* (Niva and Takeda 2003).

The developmental rate, temperature threshold, degree-day requirements and fecundity of *H. halys* are similar to native pest pentatomid species and will not limit its distribution as expands its range in the upcoming years. Based on the similarities in temperature thresholds and host plant ranges of native species, we predict that over time, *H. halys'* distribution in the U. S. will resemble that of other phytophagous species with similar host ranges, such as *N. viridula*, *A. hilare* and *Euschistus sp*. As a result, IPM programs should be modified to incorporate *H. halys* in the regions where it becomes established.

Acknowledgements:

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Table 1: Mean developmental time (days ± SE) and survivorship of *H. halys* at constant temperatures

Temp	Egg*	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Total	Percent Survival
15°C	22.00 ± 0.00a	--	--	--	--	--	--	0.00
17°C	17.20 ± 0.08b	17.01 ± 0.16a	30.36 ±1.53a	22.40 ± 4.02a	23.00 ± 1.08a	28.00 ± 0.00a	121.50 ± 0.50a	2.00
20°C	11.50 ± 0.05c	9.34 ± 0.08b	16.25 ± 0.23b	11.78 ± 0.29b	13.66 ± 0.31b	20.16 ± 0.36b	81.16 ± 0.80b	62.00
25°C	6.10 ± 0.03d	4.82 ± 0.10c	9.62 ± 0.21c	7.08 ± 0.22c	7.38 ± 0.28c	10.44 ± 0.28c	44.92 ± 0.80c	61.00
27°C	4.87 ± 0.10e	4.25 ± 0.05d	7.64 ± 0.19d	5.49 ± 0.21d	5.90 ± 0.18c	7.81 ± 0.28d	35.81 ± 0.52d	52.50
30°C	3.00 ± 0.00g	3.70 ± 0.05e	7.05 ± 0.13d	6.11 ± 0.28cd	6.11 ± 0.23c	8.47 ± 0.28cd	33.39 ± 0.50d	51.00
33°C	4.00 ± 0.00f	3.01 ± 0.01f	7.47 ± 0.23d	7.45 ± 0.53c	7.20 ± 0.40c	10.60 ± 0.81c	37.80 ± 0.86d	5.00
35°C	--	--	--	--	--	--	--	0.00

*Means within a column followed by different letters are significantly different (P <0.05)

Table 2: Stage-Specific (dx/lx)*100 and generational mortality (dx/lo)*100 of *H. halys* developmental stages

Temp	Egg	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Generational
15°C	50.00	100.00	0.00	0.00	0.00	0.00	100.00
17°C	2.00	35.71	80.95	50.00	16.67	40.00	97.00
20°C	1.00	9.09	16.67	5.00	4.00	15.28	39.00
25°C	0.00	6.00	13.83	18.52	4.55	3.17	39.00
27°C	0.00	0.00	16.25	14.93	10.53	17.65	47.50
30°C	0.00	6.00	12.77	9.76	16.22	17.74	49.00
33°C	5.00	15.79	45.00	50.00	31.82	66.67	95.00
35°C	100.00	0.00	0.00	0.00	0.00	0.00	100.00
Mean	19.75	21.57	23.18	17.90	10.47	20.06	--

Table 3: Effect of 1st instar aggregation on development time and survival to the next instar of *H. halys*

Temp	Replicate	Egg*		1st instar		2nd instar		3rd instar		4th instar		5th instar		Total Development	
		Mean	Survival**	Mean	Survival	Mean	Survival	Mean	Survival	Mean	Survival	Mean	Survival	Mean	Survival
15°C	1	22.00	n = 50	--	n = 0	--		--		--		--		--	n = 0
	2	--	n = 0	--		--		--		--		--		--	n = 0
17°C	1	16.60b	n = 50	16.71	n = 21	27.50b	n = 6	22.40	n = 5	23.00	n = 4	28.00	n = 2	121.50	n = 2
	2	17.80a	n = 50	17.16	n = 43	33.80a	n = 5	--	n = 0	--		--		--	n = 0
20°C	1	12.00a	n = 50	10.00	n = 41	16.76a	n = 38	11.36a	n = 38	13.88	n = 35	20.59	n = 32	83.68a	n = 32
	2	11.00b	n = 50	8.80	n = 50	15.73b	n = 38	12.18b	n = 38	13.44	n = 38	19370	n = 30	78.46b	n = 30
25°C	1	6.20a	n = 50	5.75a	n = 44	9.97	n = 38	7.54a	n = 37	8.11a	n = 35	11.12a	n = 33	47.97a	n = 33
	2	6.00b	n = 50	4.00b	n = 50	9.30	n = 43	6.48b	n = 29	6.46b	n = 28	9.64b	n = 28	41.32b	n = 28
27°C	1**	4.00b	n = 30	4.00b	n = 30	9.30a	n = 20	6.77a	n = 17	6.43	n = 14	8.92a	n = 13	38.92a	n = 13
	2	5.40a	n = 50	4.40a	n = 50	6.94b	n = 47	4.95b	n = 40	5.70	n = 37	7.31b	n = 29	34.42b	n = 29
30°C	1	3.00	n = 50	3.36b	n = 44	7.03	n = 37	7.57a	n = 30	6.43	n = 21	8.84	n = 19	35.05a	n = 19
	2	3.00	n = 50	4.00a	n = 50	7.07	n = 45	5.11b	n = 44	5.95	n = 41	8.25	n = 32	32.41b	n = 32
33°C	1	4.00	n = 50	3.03	n = 35	6.95b	n = 22	7.33	n = 15	7.60	n = 10	11.67	n = 3	38.33	n = 3
	2	4.00	n = 45	3.00	n = 22	8.00a	n = 7	7.71	n = 5	6.40	n = 2	9.00	n = 2	37.00	n = 2
35°C	1	--	n = 0	--		--		--		--		--		--	n = 0
	2	--	n = 0	--		--		--		--		--		--	n = 0

* Means within a column for each temperature followed by a different letter are significantly different (*P*≤0.05); ("n") signifies the number of individuals surviving indicated life stage

** Initial number for this replicate was 3

Fig 1: (A) Fit of non-linear developmental models for *H. halys* egg development (B) Fit of non-linear developmental models for total development (egg to imaginal ecdysis) of *H. halys*

A

Egg Development
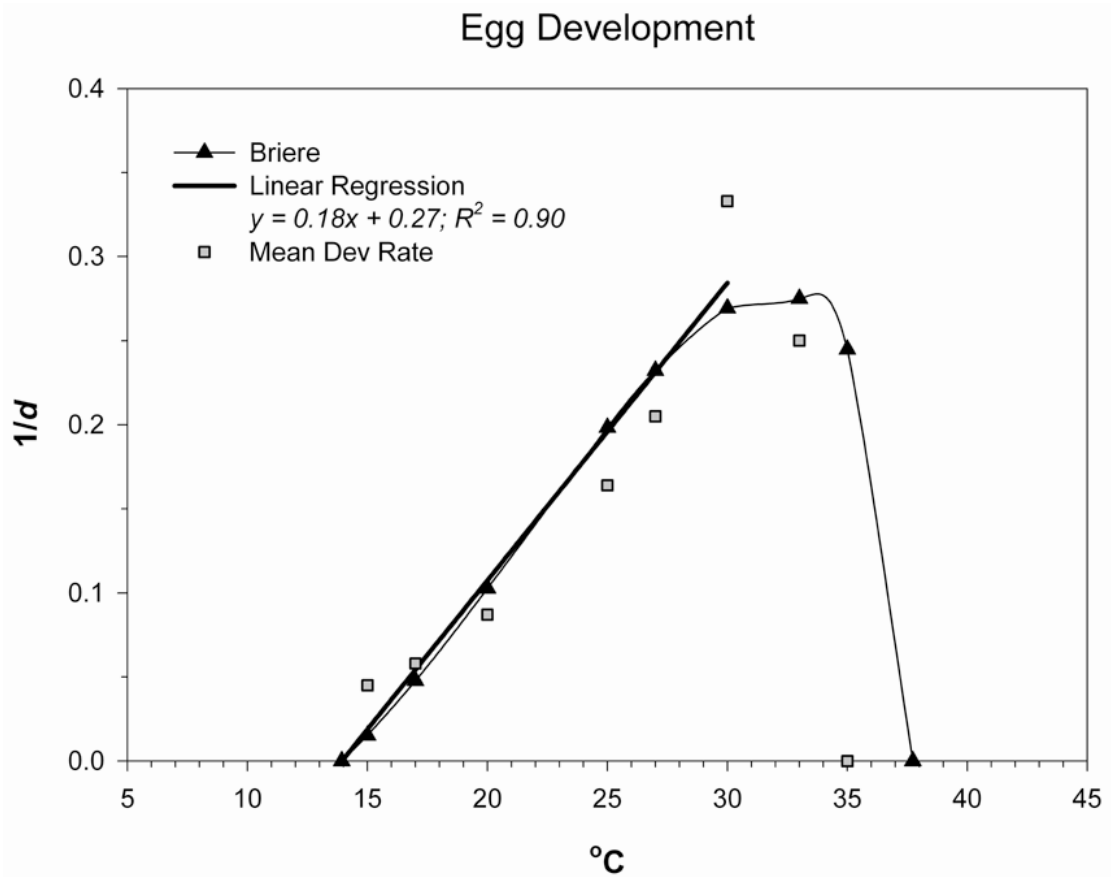
B
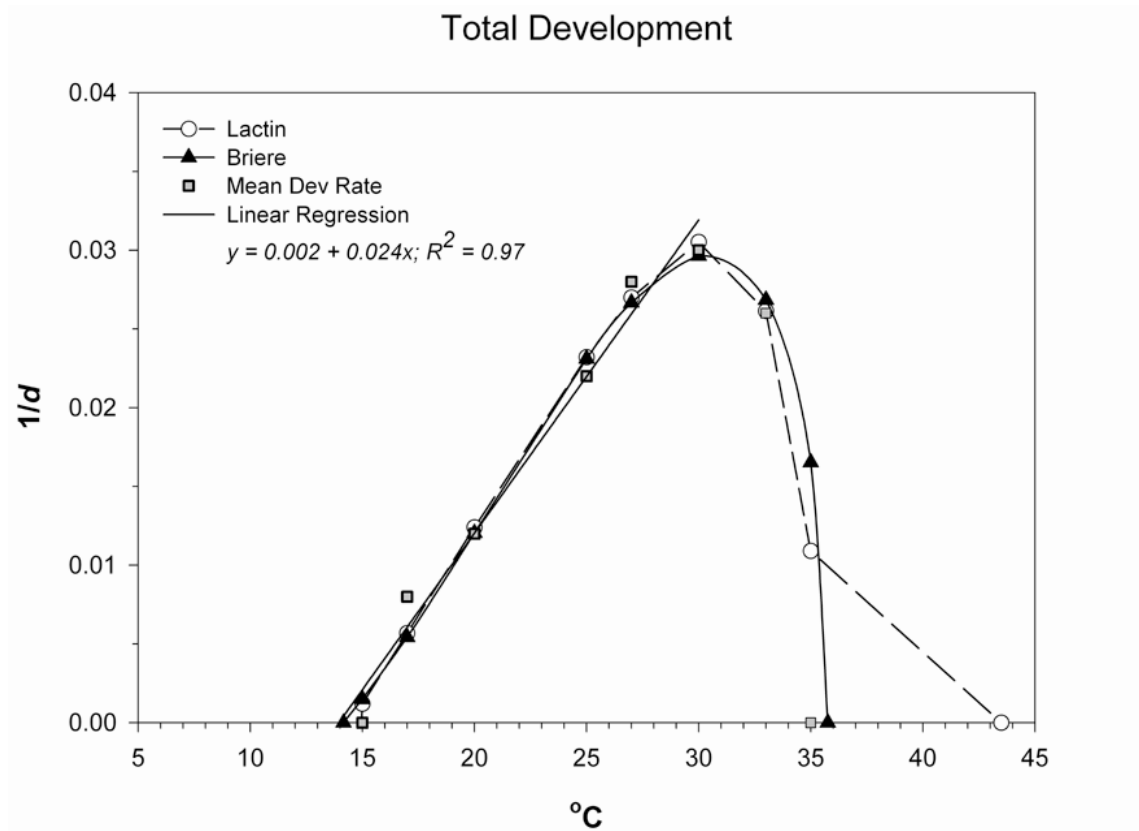
Table 4: Reproductive parameters for mated *H. halys* females

Variable	Mated Females Mean (± SE)		Females ovipositing > 1 egg mass Mean (± SE)	
Pre-Oviposition Period	13.35	(± 0.72)	12.44	(± 0.49)
Oviposition Interval	4.32	(± 0.41)	4.83	(± 0.22)
Number of egg masses*	8.00	(± 0.19)	9.33	(± 1.04)
Number oviposited	26.08	(± 0.31)		
Number hatched**	21.30	(± 0.48)		
Total number of eggs*	212.25	(± 31.04)	243.78	(± 27.48)

* Per female
** Per egg mass

Figure 2: Average age-specific oviposition rates of *H. halys*.
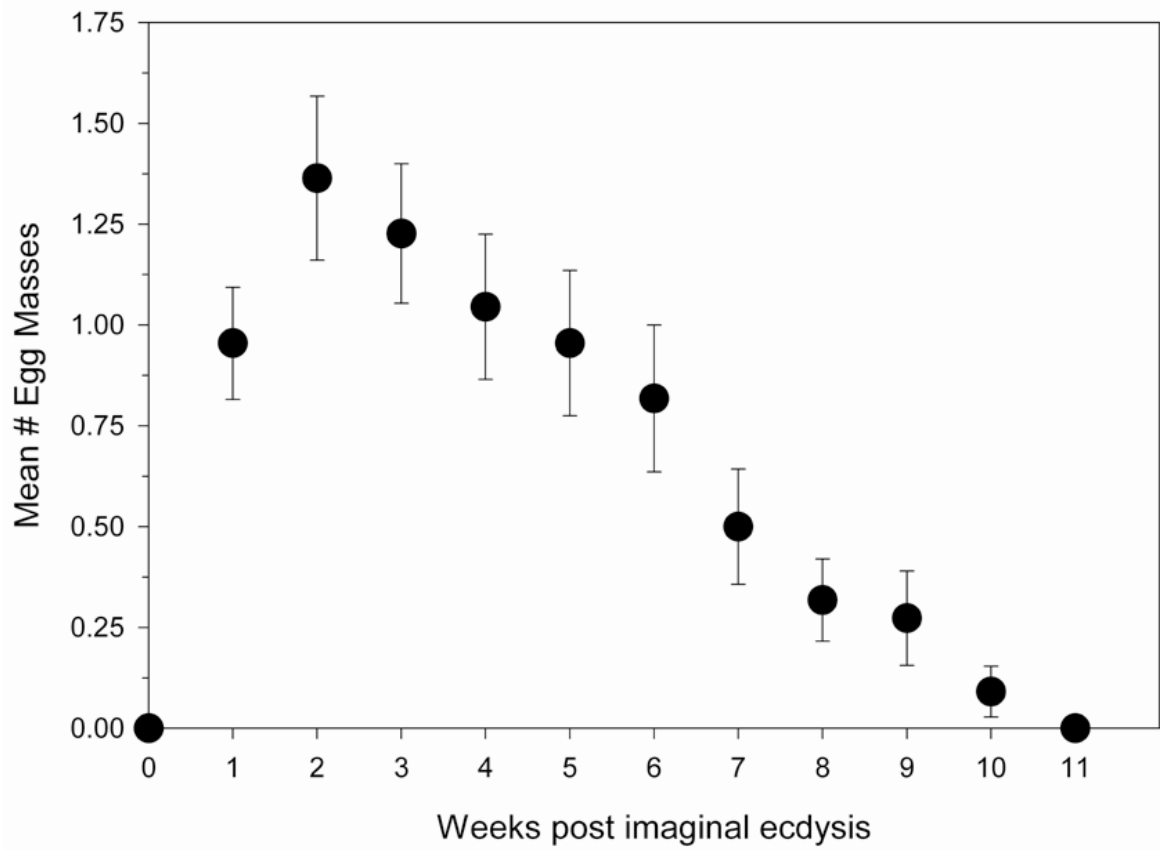
CHAPTER 2

LIFE-HISTORY AND GEOGRAPHIC DISTRIBUTION OF THE INVASIVE SPECIES *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) IN THE UNITED STATES

Abstract

Host plant utilization by nymphs and adult *Halyomorpha halys* was investigated proximal to the location of its introduction, Allentown, PA. Weekly beat samples were conducted beginning at petal fall (mid-April) in *Pyrus spp.* until the first frost (mid-October) from 2005-2007 on a variety of ornamental trees, shrubs, and agricultural crops. Egg masses were present on *Paulownia tomentosa* beginning the first week of June. In late August a large population peak was observed, which occurs just past the DD accumulation for development to imaginal eclosion. The results support laboratory studies that *H. halys* is univoltine in this region. There was an apparent difference in nymphal occurrence on host plants that was associated with the phenological stage of the plant with developing fruit. This was especially punctuated on some plant species. Distinct nymphal populations were observed on *P. tomentosa* during the 1st - 2nd instars while populations of 3rd – 5th instar nymphs were present on *Viburnum opulus* L. var. *americanum* which provides evidence for phenologically dependant host association. The species composition of *H. halys* in relation to native species showed that on the plants sampled, *H. halys* was the predominant pentatomid species present. *Halyomorpha halys* also has a unique overwintering behavior by forming large aggregations in mand-made structures. By taking advantage of the appearance in homes, a reporting website was set-up that permitted tracking the spread of *H. halys*.

INTRODUCTION

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an introduced species native to Japan, China, and Korea (Hoebeke and Carter 2003). Initial accounts of its presence in the United States were reported to Lehigh County Cooperative Extension in Allentown, PA in 1996 (K. Bernhardt, personal communication); however, the species was not properly identified until 2001 (Hoebeke and Carter 2003). Since then, *H. halys* has become established in multiple states at high densities, making it both an agricultural threat and a homeowner nuisance in the Mid-Atlantic States. At the time of its introduction in the United States, minimal information about its life-history was available in its native range. The limited literature on *H. halys* in Asia focused on its occurrence in tree fruit and soybean [*Glycine max* L. (Fabales: Fabaceae)] or on control/monitoring methods (Takashashi 1930, Hoffmann 1931, Saito et al. 1964, Kobayashi et al. 1972, Chung et al. 1995, Funayama 1996a, Watanabe 1996, Choi et al. 2000, Tada et al. 2001a, Funayama 2002).

Invasive insect species present a series of problems associated with their appearance in a new habitat. Of primary concern are the ecological damage and economic losses they will cause outside of their native host range (Lockwood et al. 2007). One approach to gauge potential pest status is to understand a species' host plant associations, seasonal dynamics, and distribution. These ecological factors are essential to implement future control methods. Alternative host plants are significant for stink bug development as they can be essential for population build-up prior to dispersing to agricultural crops (Panizzi 1997). Predicting which host plants are utilized for early

season development or as pre-oviposition hosts can be used to develop farm-scale control programs aimed at *H. halys*.

Consistent with other pentatomids, *H. halys* has five nymphal instars and oviposits on the underside of leaves (Takashashi 1930). Eggs are laid in clutches with a median number of 28 eggs at 3-4 day intervals, under laboratory conditions (Bernon 2004, Nielsen et al. 2008). Developing nymphs develop an "egg breaker" upon completion of development. After eclosion, first instars remain aggregated around the egg mass until molting to the second instar. Aggregation is believed to occur as a defensive strategy against predators, for improved humidity control, or uptake of symbionts from the eggs and is believed to shorten development time (Buchner 1965, Lockwood and Story 1986, Aldrich et al. 1991, McPherson and McPherson 2000, Hirose et al. 2006, Nielsen et al. 2008). Laboratory studies indicate that *H. halys* requires 537.63 DD to complete development (egg to imaginal eclosion) making it univoltine in Allentown, PA (Nielsen et al. 2008).

Like most phytophagous pentatomids, *H. halys* is polyphagous with a wide host range encompassing ornamental shrubs, hardwood trees, and cultivated crops such as soybean, apple, pear, cherry, and peach (Hoffmann 1931, Hoebeke and Carter 2003, Bernon 2004). Both adults and nymphs prefer to feed on plant reproductive structures (McPherson and McPherson 2000), although *H. halys* feeding site differs for some host plants, such as the Princess Tree [*Paulownia tomentosa* (Thunb.) (Scrophulariales*:* Scrophulariaceae)], where feeding occurs primarily on vegetative structures. Little taxonomic relationship exists between host plant species although there is a preference for plants in the Fabaceae and Rosaceae families (Hoebeke and Carter 2003, Bernon

2004). To investigate the relationships with host plants, we sampled ornamental host

plants, tree fruit, and soybean over a period of two to three years. Plants native to

Pennsylvania as well as non-native plants, primarily of Asian origin, were sampled. Our

efforts concentrated on plants that were believed to support *H. halys* development and

thus potential overwintering hosts were not evaluated. Flight activity was also

documented with a blacklight trap located at a commercial farm where sampling on the

cultivated crops occurred. *Halyomorpha halys* adults are known to be attracted to

blacklight traps (Tada et al. 2001a, Lee et al. 2002).

Materials and Methods

Seasonal Phenology

Weekly samples were collected from 2005-2007 at the Rodale Working Tree

Farm ("Rodale") in Allentown, PA, an organically managed arboretum located near the

believed epicenter of the population. Sampling was initiated in mid-April when pear was

in bloom and continued until mid-October or the first frost. Sampling was done with a

canvas beat sheet (71cm x 71cm, BioQuip, Rancho Dominguez, CA) at a height of 1.5 –

3.0m around the entire plant for nymphs (2nd - 5th instar) and adults. Degree days began

accumulating from the time of the first egg mass on *P. tomentosa*. Daily maximum and

minimum temperatures were downloaded from the National Weather Service for

Allentown International Airport. Visual surveys were conducted for unhatched egg

masses and 1st instars. Unhatched egg masses were primarily observed on *P. tomentosa*

as they can be difficult to observe because of their light green coloration. First instars

aggregate around an egg mass at least until the molt to the second instar. If any 1st instars

were found during beat sampling, a visual assessment was done on the host plant to look for egg masses. Preliminary sampling of wild grasses (with sweep net) did not indicate the presence of *H. halys* and were not sampled further.

In 2005, an initial survey of 12 plant species that included native and exotic plants was conducted at Rodale to document seasonality and identify potential host plants. The plants sampled were: Princess Tree [*P. tomentosa* (*Scrophulariales*: *Scrophulariaceae*)], Pear [*Pyrus sp.* (Rosales*: Rosaceae*)], Russian Olive [*Elaeagnus angustifolia* L. (Rhamnales*: Elaeagnaceae*)], Blackhaw Viburnum [*Viburnum prunifolium* L. (Dipsacales*: Caprifoliaceae*)], American cranberrybush [*Viburnum opulus* L. var. *americanum* Aiton (Dipsacales*: Caprifoliaceae*)], Asian Pear [*Pyrus pyrifolia* (Burm. f.) Nakai (Rosales*: Rosaceae*)], Rugosa rose [*Rosa rugosa* Thunb. (Rosales*: Rosaceae*)], Eastern Hemlock [*Tsuga canadensis* (L.) Carrière (Pinales: Pinaceae)], Raspberry [*Rubus spp.* (Rosales*: Rosaceae*)], Sweet Gum [*Liquidambar spp.* (Saxifragales: Altingiaceae)], Tartanian Honeysuckle [*Lonicera tatarica* L. (Dipsacales: Caprifoliaceae)], White Ash [*Fraxinus americana* L. (Lamiales: Oleacea)], and Siberian Pea Shrub [*Caragana arborescens* Lam.(Fabales*: Fabaceae*)] (USDA 2008).

In 2006 and 2007, host plants were selected based on the preliminary survey and host phenology. Sampling results were recorded for individual plants, except for *P. tomentosa*, where individual branches were sampled. Each year sampling occurred on *P. tomentosa*, *Pyrus spp.*, *E. angustifolia*, *V. prunifolium*, *V. opulus* var. *americanum*, *Py. pyrifolia*, *R. rugosa*, and *C. arborescens*. In 2006 and 2007, sampling also occurred at Lichtenwalner Farms in Macungie, PA, 4.7km from the Rodale site. Cultivated hosts apple [*Malus domestica* Borkh. (Rosales: Rosaceae)], *Py. pyrifolia*, *Pyrus sp.* and *G. max*

were sampled weekly from mid-April until frost. Tree fruit was sampled using methods

identical to those done at Rodale. Soybean was sampled biweekly using a 38cm sweep

net (BioQuip, Rancho Dominguez, CA) in six 10m rows, ranging 10m to 70m from the

edge of the plot. A hedge row that was located 10m from the soybean that contained

garlic mustard, Japanese stilt grass, wild raspberry, honeysuckle, multiflora rose, and

Black Walnut was sampled weekly in 2006 and 2007. At each location, all pentatomid

species collected were recorded and identified to document the relative abundance of

native species compared with *H. halys*.

Distribution Survey

The objective of this study was to document the spread of *H. halys* from the

believed epicenter to its current (as of July 2008) distribution or invasive range in the

United States. This was accomplished using two monitoring methods; a blacklight

network in New Jersey and a reporting website. The combination of these two methods

is unique by identifying the range in both the active and overwinter populations. In New

Jersey, a state-wide network consisting of approximately 83 blacklight traps has been run

since 1972 by Rutgers University Vegetable Integrated Pest Management program to

monitor the appearance and movement of corn earworm [*Helicoverpa zea* (Boddie)

(Lepidoptera: Noctuidae)] and European corn borer [*Ostrinia nubilalis* (Hübner)

(Lepidoptera: Pyralidae)]. Traps are monitored biweekly from May 1st through October

1st. Individual trap samples are sorted for *H. zea*, *O. nubilalis*, and stink bugs. In 2004,

Rutgers University and the New Jersey Agricultural Experimental Station developed a

reporting website www.njaes.rutgers.edu/stinkbug where homeowners can learn about *H.*

halys biology, compare it to native species, and report a sighting. The monitoring

website takes advantage of the unique overwintering behavior of *H. halys*. Homeowners could submit sighting reports and specimens. All specimens received were identified to species and positive locations were recorded. The distribution of *H. halys* was mapped using ArcView v3.3 (ERSI, Redlands, CA) software. Through verification of populations reported on the website, we have been able to document the current known distribution of *H. halys* in the United States (as of July 2008). We also report the distribution in Asia as reported from collection reports (K. Hoelmer personal communication) and published literature to identify the minimum geographic range of *H. halys*.

Statistical Analysis

All data were transformed using [square root(x+1)] and analyzed with a two-way GLM and Tukey's mean separation at $P \leq 0.05$. Samples were analyzed as immature, adult, and total. 'Immature' represented all nymphs documented from a given host plant; 'total' represented the sum of immatures and adults. To compare the relative abundance of *H. halys* to other phytophagous pentatomid species, data for each species was summed over each year's sampling period to account for temporal differences in abundance. Mean total abundance was calculated for each species and analyzed using a one-way GLM and Tukey's mean separation at $P \leq 0.05$. All analyses were done using SAS v. 9.1 (SAS Institute 2002-2003). Results are presented as untransformed means.

Results

<u>Seasonality</u>

Adult *H. halys* were first found on cultivated host plants in late April. Of the plants sampled, *Pyrus spp.* and *P. tomentosa* supported early season populations. A small peak, coinciding with the collection of the first egg masses was observed in mid-June each year at ~100DD (Fig. 1). First instars were found on *P. tomentosa* (2005-2007 n=1258), *F. americana* (2005-2007 n=52), *Liquidambar spp.* (2006 n=25), *V. opulus* var. *americanum* (2007 n=28), *V. prunifolium* (2007 n=6), *Pyrus sp.* (2006 n=27), and *Py. pyrifolia* (2006-2007 n=99). A large population peak of 1st generation adults was apparent from 800-1000DD which coincides with early-mid August through early September. The population decreased dramatically at the beginning of October, just prior to frost, when the adults migrated to overwintering sites. A similar pattern was observed for immatures (1st – 5th instar) (Fig. 2). A large peak occurred at 100DD (13 June 2007) when high numbers of 1st instars were observed aggregating around egg masses. The first adults were found in blacklight traps beginning 31 May 2007, indicating that the adults are active. Application of the accumulated degree days (DD) required to complete development (egg mass to imaginal ecdysis, 537.63DD) beginning on 31 May confirms that *H. halys* is univoltine in Allentown, PA. Interestingly, accumulation of DD required for one generation occurred ~200DD prior to the large peak in the adult population. DD accumulating began when the first adult was found in the blacklight or on 31 May, instead of the appearance of the first egg masses because the egg masses are difficult to observe in the field. If DD are accumulated from the time of the first egg mass, there is a difference of about two Julian days for imaginal eclosion of the 1st generation adults.

<u>Host plant definition and usage</u>

The term 'host plant' has been used in many different contexts. Here, we use the term as a plant that supports consecutive nymphal stages for multiple years. All plants sampled in 2005-2007 conform to this definition. *Paulownia tomentosa* (2006-2007) and *C. arborescens* (2006) had the highest mean number of nymphs and adults throughout the sample period (Table 1). Large increases in the population density were observed on *M. domestica* in 2006-2007.

The seasonality of *H. halys* adults and nymphs varied through time. Based on the abundance of the immature stages, it is apparent that *H. halys* utilizes different host plants at various times of the year and possibly by different life stages, which can at least, partially be explained by host phenology (Fig. 3). Stink bug nymphs can be mobile and have been observed to migrate to more suitable host plants. Adults are relatively strong fliers and thus presence on a particular host plant may not signify feeding, oviposition or mating. Early season hosts were defined as hosts supporting nymphal populations through the month of June. These included *Py. pyrifolia*, *V. opulus* var. *americanum*, and *P. tomentosa*. Mid-season hosts (July to mid-August) included *Pyrus spp.*, *V. prunifolium*, *V. opulus* var. *americanum*, *E. angustifolia*, *F. americana*, *C. arborescens*, and the uncultivated hedgerow. Late season hosts, which likely supported fifth instars preparing for diapause, included *Py. pyrifolia*, *V. pruinfolium*, *P. tomentosa*, *E. angustifolia*, *G. max*, and the uncultivated hedgerow. Utilization of these host plants are consistent with the timing of fruit or pod development. For both *G. max* and *C. arborescense* (Fabaceae), population peaks occurred at the pod-fill stage. *Paulownia*

tomentosa, which is both an early and a late season host, is colonized soon after leafing out and then again during flower bud production in late fall.

Temporally separated occurence was especially apparent between the exotic *P. tomentosa* and native *V. opulus* var. *americanum* (Fig. 4). At the Rodale property, the *P. tomentosa* flower structures produced in late fall do not bloom until the following spring and do not produce fruit pods. *Viburnum opulus* var. *americanum* produces small (8-10mm) fruit that ripen to a brilliant red shade at the end of July with a very fragrant odor. The two plants differ in plant architecture as well. *Paulownia tomentosa* is a medium size (10-20m) tree that is commonly seen in disturbed areas. It has broad heart-shaped leaves with many trichomes. *Viburnum opulus* var. *americanum* is a woody shrub with smooth maple-shaped leaves. Egg masses were not commonly observed on this host plant. Regardless of plant architecture, beat sampling should dislodge second instars and indicate a recent oviposition event. Sampling in 2006 and 2007 revealed a shift in presence between *P. tomentosa* and *V. opulus* var. *americanum*. At Rodale these plants were over 100m apart. This may indicate that *P. tomentosa* is a preferred oviposition site for females but an inferior plant for some developmental stages, although fifth instar numbers increase on *P. tomentosa* again in late August.

Relative abundance

On host plants sampled from 2006-2007, *H. halys* was significantly more abundant than native pentatomid species in both ornamentals (2006 - $F_{9, 250} = 35.28$, $P \leq 0.0001$; 2007 – $F_{8, 233} = 31.79$, $P \leq 0.0001$) and soybean (2006 – $F_{2, 75} = 11.82$, $P \leq 0.0001$; 2007 – $F_{2, 69} = 11.55$, $P \leq 0.0001$) (Table 2). Assuming *H. halys* was

introduced in 1996, there have been 14 generations in this region, in which time, it has become the predominant stink bug species in Allentown, PA at the locations we sampled.

Flight activity

Flight activity was monitored with a blacklight trap at Lichtenwalner Farms in 2006 and 2007 (Fig. 5). In 2006, *H. halys* capture in blacklight traps was much higher throughout the season than in 2007. Early season captures were low followed by a large peak at the beginning of August. Imaginal eclosion as indicated by DD accumulation (from 31 May) occurred just prior to the large peak in flight activity observed.

Distribution

Halyomorpha halys is recorded from Anhui, Fujian, Guangdon, Guangxi, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jianxi, Jilin, Liaoning, Neu Monggol, Shaanxi, Shanxi, Sichuan, Taiwan, Xizang, Yunnan, Zhejiang provinces in China (Rider et al. 2002). Collections (K. Hoelmer pers. comm.) also indicate the presence of *H. halys* in Kunming, and Xi'an provinces in China. *Halyomorpha halys* has been collected or reported from Chungcheongnam, Gyeongsangbuk, and Gyeongsangnam Provinces in South Korea (Chang-Ki et al. 2000, Choi et al. 2000, Lee et al. 2002). It is also reported in the following Japanese Prefectures: Iwate, Akita, Yamagata, Tsukuba- Ibaraki, Toyama, Chiba, and Kyoto (Watanabe et al. 1994, Tada et al. 2001b, Funayama 2002, Toyama et al. 2006, Adachi et al. 2007).

Since its introduction in 1996, *H. halys* has spread to multiple states in the United States. After populations were observed in Allentown, PA (Lehigh County) *H. halys* was found in a Milford, New Jersey blacklight trap (K. Holmstrom pers. comm.) in 1999 and in Maryland in 2004. In 2005, it was identified in Delaware and Oregon. Virginia, New

York, and Los Angeles County, CA specimens were first found in 2006, followed by

Ohio and Mississippi in 2007. Populations have been eradicated from Augusta, Maine,

and from Sacramento and San Bernardino Counties, California. It is well established in

Pennsylvania, New Jersey, Maryland, Delaware, Virginia, and Oregon (Fig. 6). The

reporting website setup by New Jersey Agricultural and Extension Service and

maintained by ALN and GCH has greatly aided in documenting the presence of *H. halys*.

As of July 2008, the website has had more than 2700 reports and Rutgers University has

received over 700 specimens, of which 96.3% were positively identified as *H. halys*.

Discussion

Since its introduction, *H. halys* has become well established in the Mid-Atlantic

States. Field sampling confirms laboratory studies that *H. halys* is univoltine in

Pennsylvania. *Euschistus servus* (Say) is the only phytophagous stink bug reported to be

bivoltine in the region (McPherson and McPherson 2000). Diapause induction in

Halyomorpha spp. is both temperature and photoperiod dependant making it unlikely to

be bivoltine in this region (Niva and Takeda 2003). However, *H. halys* is known to be

multi-voltine in its native range suggesting that the number of generations may increase

as the populations disperse south (Hoffman 1931).

Invasive species pose a multitude of problems. From the life-history data

presented here, it is evident that *H. halys* has a very wide host range which could make

control difficult. Highly polyphagous behavior could make the presence of insipient

populations difficult to detect until densities reach damaging levels. Together with

confusion related to the identification of stink bug feeding, this situation has happened in

New Jersey and Pennsylvania. The data presented here confirms that *H. halys* is present during the critical damaging periods for the crops examined and should be considered a pest. The large population build-up observed most likely results from the absence of natural enemies or lack of host plant resistance. However, over one-third of the plant species sampled were native to Asia and could have evolved host defenses against *H. halys* feeding. Escape from natural enemies is hypothesized to be an important factor that permits invasive species to become significant pests in their new habitat (Williamson 1996). In China and Japan, egg parasitoids in the *Trissolcus* genus parasitize *H. halys* (Arakawa and Namura 2002). While a different egg parasitoid species complex exists in the United States, preliminary studies suggests that parasitism by native *Trissolcus spp.* is low against *H. halys* and may account for the large populations at the site of introduction.

The ornamental host plants we selected for sampling may have biased the pentatomid species composition found. Native *Euschistus spp.* for instance, are known to prefer mullein and uncultivated hosts plants to ornamental hosts and could account for the low population levels observed (Panizzi 1997, Krupke et al. 2001). *Euschistus spp.* are known pest of tree fruits and soybeans, particularly in the Southern states. However *H. halys* was found at significantly higher levels than *E. servus* and *E. variolarius* (Palisot de Beauvois) which were the most common non- *H. halys* species encountered in soybean. *Acrosternum hilare* (Say) is believed to have a similar host range as *H. halys* (Bernon 2004), yet we found significantly higher densities of the invasive *H. halys* were present in all host plants sampled.

In a new environment, invasion success or at least initial survival may be partially enabled by having an unrestricted host range. Each host plant surveyed supported the

development of *H. halys* nymphs and were utilized by adults. Unlike other phytophagous

pentatomid species, it does not appear that grasses or uncultivated hosts play an important

role in population buildups; instead, woody shrubs and hardwood trees may act as the

desired alternate host species for *H. halys*. Of the plants surveyed, *P. tomentosa*, *C.*

arborescens, and the *Pyrus spp.* were preferred host plants in all years. Regardless of

preference, presence of *H. halys* was dependant on host phenology. The occurrence of

different life stages on specific host plants such as *P. tomentosa* and *V. opulus* var.

americanum were temporally separate in all years. The temporal differences observed

could also be the result of differential survivorship on each host plant. Future research

looking at developmental success of nymphs on wild hosts is needed.

A lag period of about 1-2 weeks between blacklight samples and collections of

first generation adults in ornamentals was observed. This lag could be because of

differential survivorship on ornamentals. Apple is believed to be an important early

season host utilized by females to feed upon while undergoing reproductive maturation.

The absence of suitable early season hosts and potential differential survivorship on the

hosts sampled could result in the lag period observed between peak imaginal eclosion in

beat samples and blacklight samples. Based on our data, blacklight traps could be used to

indicate dispersal to alternate hosts such as tree fruit or soybean, and they are an accurate

estimator of imaginal ecdysis for the 1st generation adults.

Halyomorpha halys' life history in the United States indicates that there are

suitable host plants in areas where it has become established and it is the dominant

pentatomid species in eastern Pennsylvania. It has spread to much of the Mid-Atlantic

States and isolated populations exist on the West Coast, Mississippi, and Ohio, although

the distribution may be much more wide spread than documented here. In fact, a small population was recently discovered in Zurich, Switzerland (Wermelinger et al. 2008) and is believed to be the first European population of this species. As a pest, *H. halys* may become a greater concern because it also is a nuisance pest during the winter, a behavior that may increase survivorship (Kiritani 2006). It has been well documented that a common, if not preferred, overwintering location for *H. halys* is inside man-made structures (Kobayashi and Kimura 1969, Kiritani 2006), a behavior which is believed to have evolved from attraction to north-facing cliff outcroppings. This behavior is not unique among pentatomids but is exacerbated by the large aggregations of *H. halys* that occur inside houses in the Mid-Atlantic States, creating a significant nuisance to homeowners. This nuisance behavior is initially created during the dispersal to overwintering sites in early fall and again in the spring as the adults move out of these locations. However, their presence inside is noticeable when disturbed or on warm days when they become active. The reporting website has received thousands of emails pertaining to this. Kiritani (2006) suggested *H. halys'* unique behavior increases overwintering survivorship and may positively impact its adaptation in terms of climate change.

Acknowledgements

Figure 1: Seasonality of *H. halys* adults on ornamentals from 2005-2007. Arrows (2005 ▬ · ▬ · , 2006 ▬ ▬ · , 2007 ▬▬▬) indicate the degree day accumulation for a complete generation (537DD) from May 31.
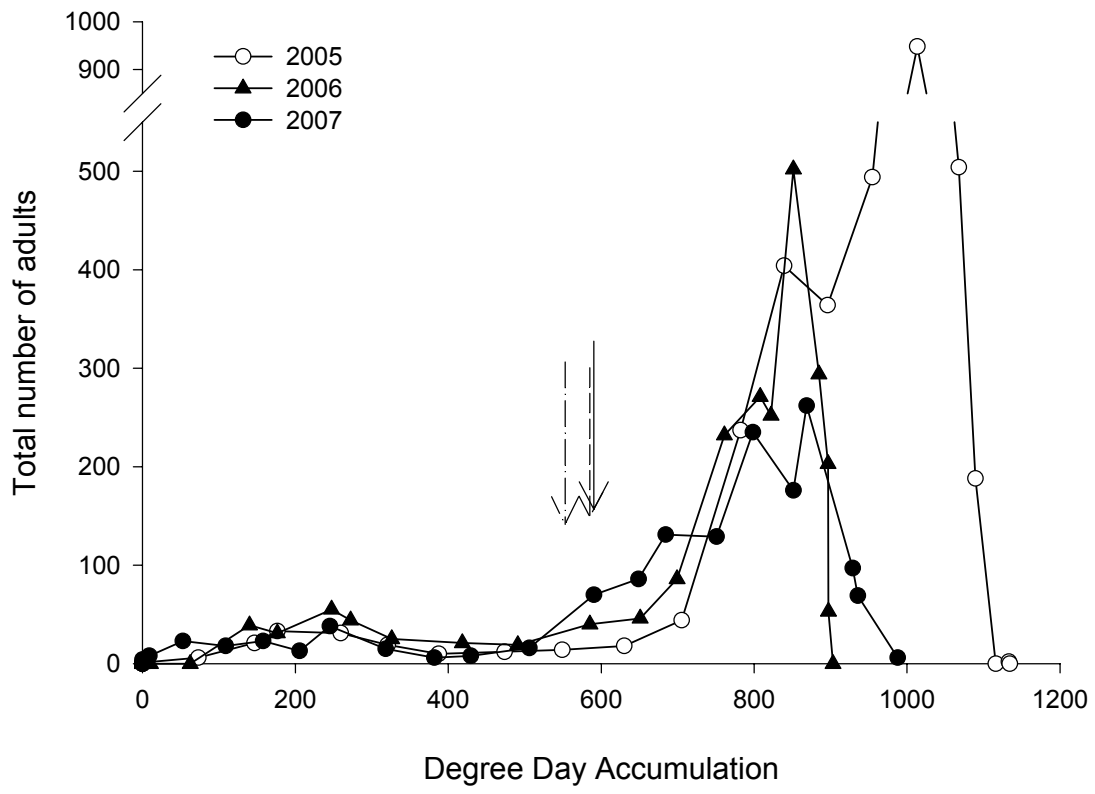
Fig 2: Seasonality of *H. halys* nymphs on ornamentals from 2005-2007. The early season peaks (in June and July) result from large numbers of 1st instar nymphs aggregating around egg masses.
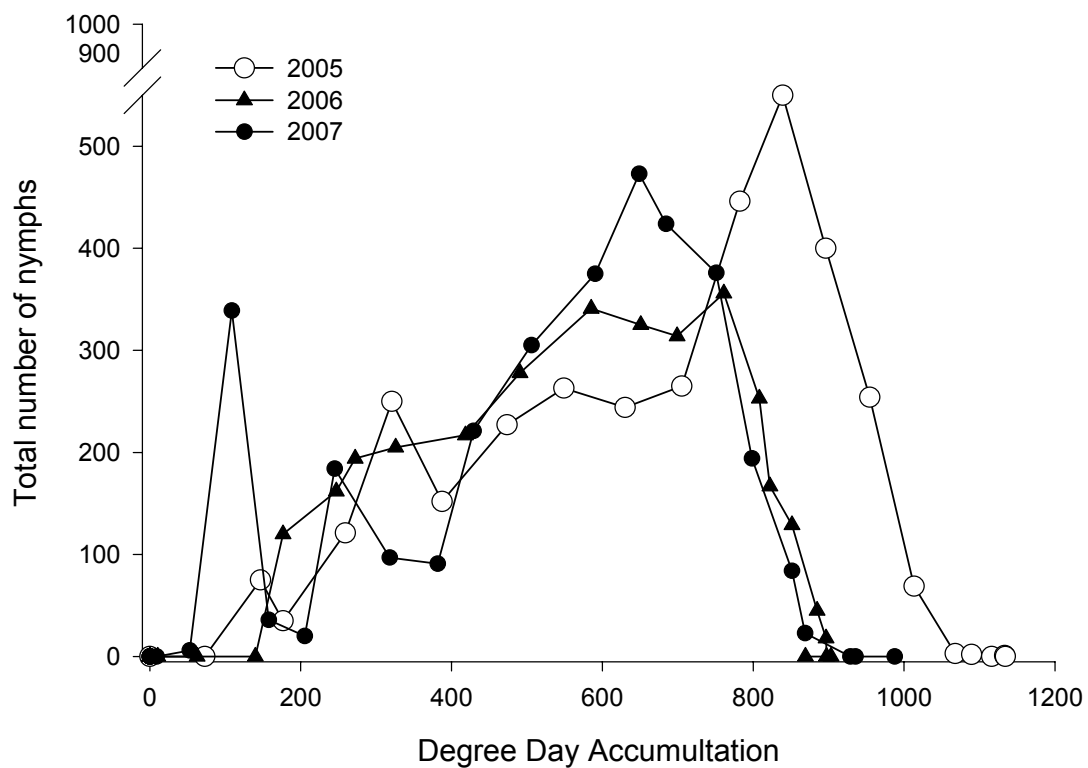
Figure 3: Presence of *H. halys* on host plants by date. Nymphal populations are indicated with a black line, adult populations with a gray line. Mean abundance per sampling date from 2006-2007 is shown.
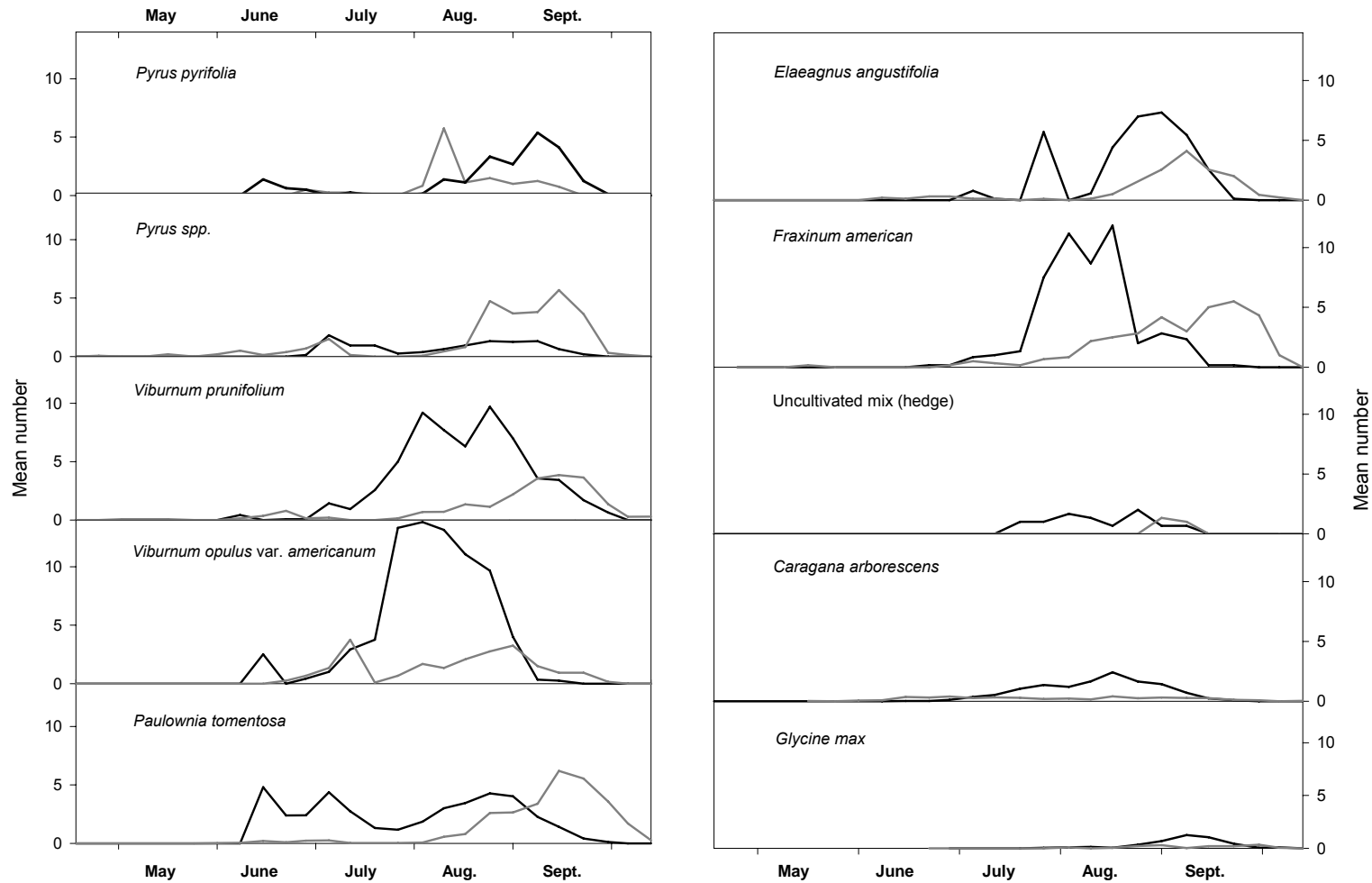
Figure 4: Association of *H. halys* nymphs with *Paulownia tomentosa* and *Viburnum opulus var. americanum* in 2006. The solid line indicates the seasonality of nymphs on each host plant. The histogram represents the amount of each life stage present on each host plant at that sample date.
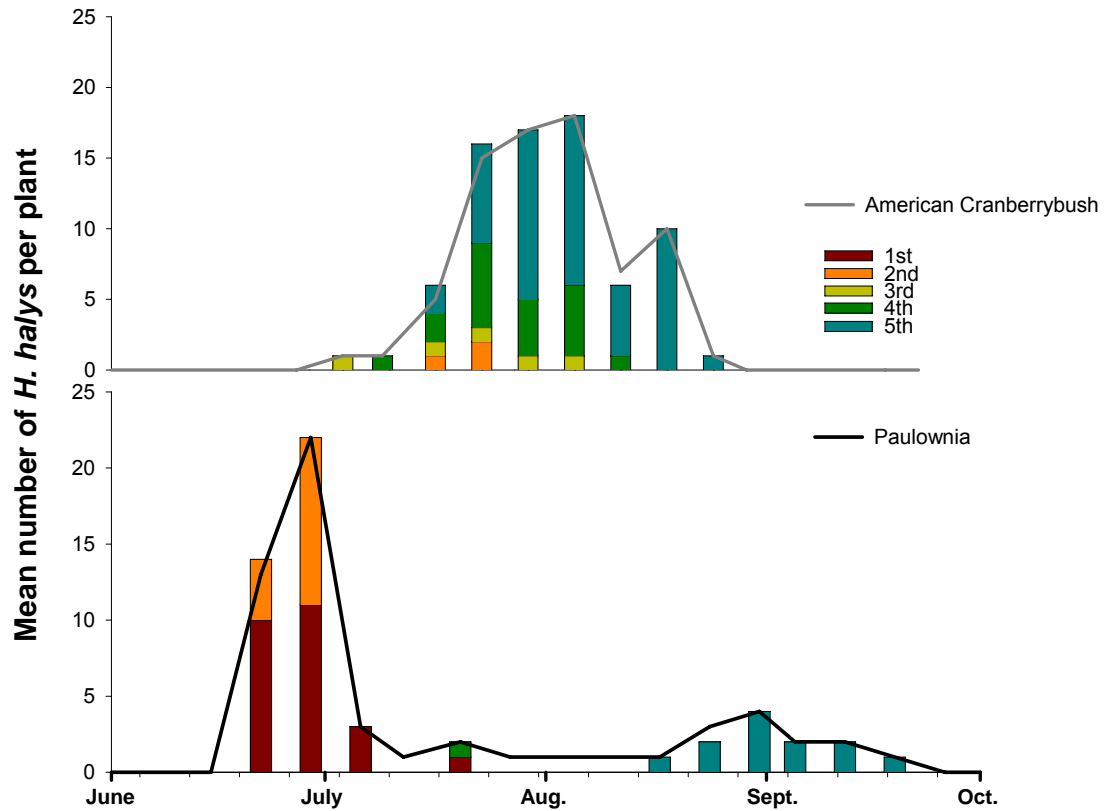
Figure 5: Seasonal flight activity of *H. halys* using a blacklight trap in Allentown, PA. Arrows (2006 — — · , 2007 ———) indicate the degree day accumulation for a complete generation (537DD) from May 31. .
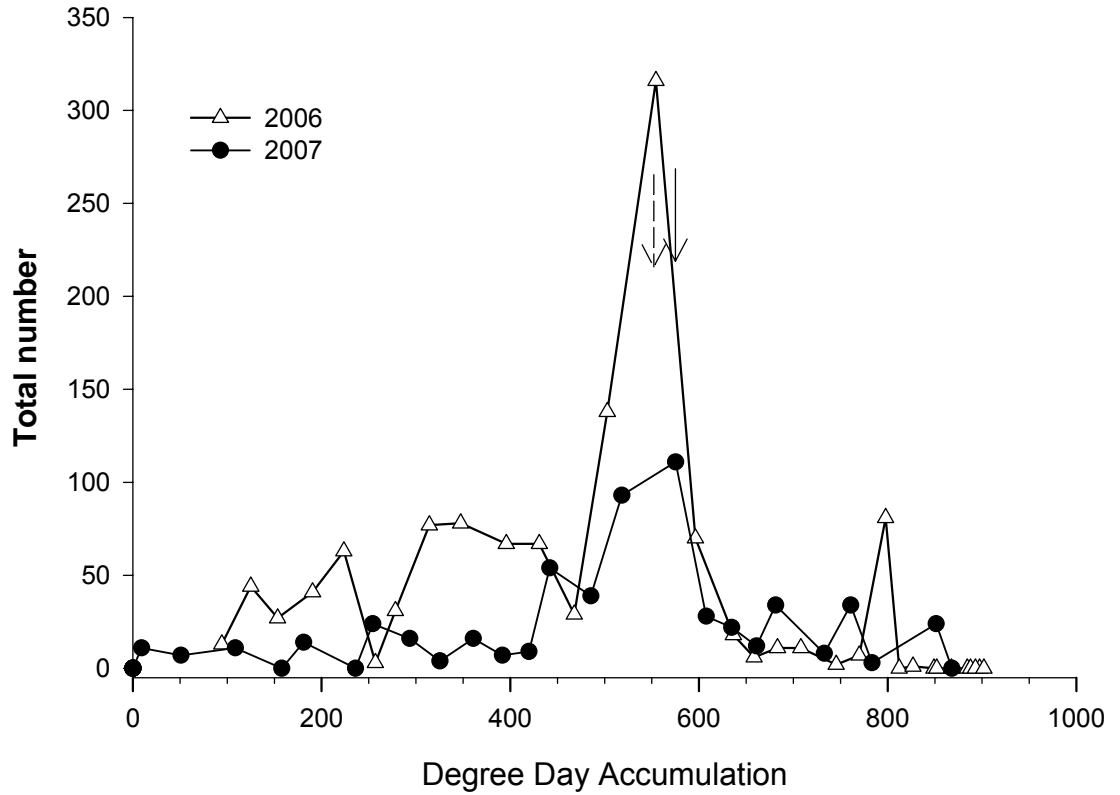
Table 1: Mean (±SE) number of _H. halys_ adults and nymphs on ornamental and cultivated hosts in 2006 and 2007.

Plant Family	Common Name[a]	Plant Species	2006 Nymphs[b]	2006 Adult[b]	2007 Nymphs[b]	2007 Adult[b]
Rosaceae	Asian Pear[a]	_Pyrus pyrifolia_	3.9±1.8cde	6.4±2.1cde	2.8±1.0cdef	2.9±0.8def
Rosaceae	Asian Pear[a]	_P. pyrifolia_ (cultivated)	3.7±1.4bc	10.3± 3.2bc	6.0±2.5c	4.1±1.3c
Rosaceae	Apple	_Malus domestica_ (cultivated)	0.3±0.2defg	0.0±0.0def	0.8±0.5b	4.37±1.2b
Rosaceae	Pear	_Pyrus spp._	5.3±1.5cd	10.7±3.8cd	1.5±0.5cdef	6.4±2.1cde
Rosaceae	Pear	_Pyrus spp._ (cultivated)	3.6±1.4b	12.4±5.0b	0.8±0.7cd	2.6±0.8cd
Rosaceae	Rugosa Rose[a]	_Rosa rugosa_	1.4±0.4fg	0.2±0.1f	0.5±0.1h	0.2±0.1i
Oleacea	White Ash	_Fraxinus americana_	4.4±1.9efg	4.9±1.7ef	7.2±2.6fgh	2.8±0.8hi
---		Uncultivated hedge	0.2±0.1g	0.3±0.2f	0.9±0.4gh	1.1±0.8hi
Caprifoliaceae	Highbush Cranberry	_Viburnum opulus_ var. _americanum_	16.5±6.2cd	4.9±1.2efg	19.3±5.9cde	3.7±1.1efg
Caprifoliaceae	Blackhaw Viburnum	_Viburnum prunifolium_	13.2±3.5bcd	7.5±2.4cde	19.0±5.8cd	3.9±1.2ef
Scrophulariaceae	Princess Tree[a]	_Paulownia tomentosa_	43.3±10.6a	42.0±14.9a	55.7±15.9a	28.0±9.0a
Elaeagnaceae	Russian Olive	_Elaeagnus angustifolia_	5.4±2.4def	2.27±0.7def	6.8±3.1efgh	3.1±1.3gh

Fabaceae	Soybean[a]	*Glycine max* (cultivated)	0.9±0.4defg	0.72±0.2def	2.1±0.7defg	0.4±0.20fgh
Fabaceae	Siberian Peashrub	*Caragana arborescens*	20.4±6.2a	6.9±1.2a	6.4±1.6b	2.0±0.4b

[a] Indicates Asian origin (USDA 2008)
[b] Transformed means in the same column with the same letter are not significantly different (Tukey's, $P \leq 0.05$).

Table 2: Mean (±SE) number of pentatomid species found on ornamentals and in soybean.

Species	2006		2007	
	Ornamental[a]	*G. max*[a, d]	Ornamental[a]	*G. max*[a, d]
Euschistus servus	1.00±0.36b	0.08±0.05b	0.31±0.14b	0.73±0.25b
E. tristigmus	2.73±0.56b	-	1.15±0.30b	
E. variolarious	0.23±0.10b	0.54±0.20b	0.00±0.00b	0.90±0.26b
Euschistus spp.[b]	4.92±0.10b	-	4.69±0.94b	
Banasa spp.	0.04±0.04b	-	0.39±0.21b	
Thyanta spp.	0.35±0.21b	-	0.04±0.04b	
Acrosternum hilare	14.15±3.08b	-	11.69±3.69b	
Halyomorpha halys	206.23±39.51a	2.04±0.50a	300.38±66.59a	6.21±1.77a
Other[c]	0.15±0.12b	-	1.58±0.47b	

[a] Transformed means in the same column with the same letter are not significantly different (Tukey's, $P \leq 0.05$).

[b] *Euschistus* nymphs were not identified to species and were recorded as "Euschistus".

[c] Other phytophagous pentatomid species include *Murgantia historonica* and *Bronchymena spp.* which were collected very infrequently in the ornamentals.

[d] In soybean, only *E. servus*, *E. variolarious*, and *H. halys* were found consistently. Populations of *A. hilare* and *Thyanta sp.* were collected infrequently and at very low numbers.

Figure 6: Current known distribution of *H. halys* in the United States by county based on Rutgers University website reports (as of March 2008). Counties highlighted in yellow are established populations of *H. halys*. Sacramento and San Bernadino Counties, CA and Augusta, Maine.underwent eradication procedures by state officials and are indicated by red highlight.
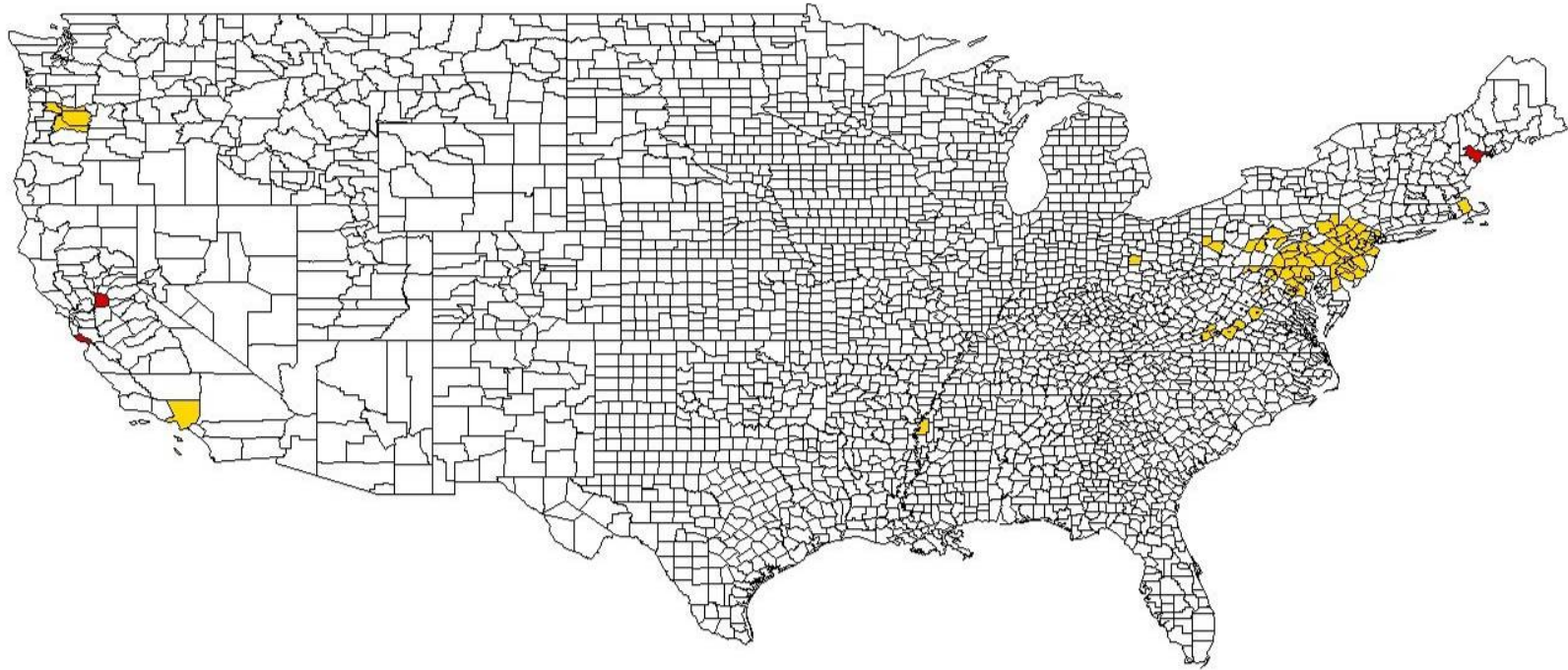
CHAPTER 3

OCCURRENCE AND DAMAGE TO TREE FRUIT BY THE INVASIVE SPECIES
HALYOMORPHA HALYS **(HEMIPTERA: PENTATOMIDAE)**

Abstract: *Halyomorpha halys* is an introduced stink bug species from Asia that has
spread throughout the Mid-Atlantic States. In its native range, it is a pest of tree fruits,
including apples and pears. Cage experiments with *H. halys* adults placed on apple and
peach during the critical growing stages demonstrate that it can cause damage during
mid- and late- season fruit development and that feeding preference occurs on the
proximal two-thirds of the fruit. Mid and late-season damage was apparent at harvest
while early-season damage to both apples and peaches resulted in fruit abortion. Farms
located in Pennsylvania and New Jersey were sampled weekly in 2006-2007 to detect the
occurrence of *H. halys* in pear and apple. Apple does not appear to be suitable as a
developmental host, although adults were found on apple in high densities. Pears were
utilized by adult and nymphs with peak populations occurrring in early July and mid-
August, the time when mid and late-season damage occurs. Stink bug damage ranged
from 25 to 90% of fruit per tree at both farms each year and can be attributable to *H.
halys* since it was present at both locations at significantly higher densities than all other
pentatomids combined. The data presented here documents the potential for *H. halys* to
cause damage in orchards throughout the Mid-Atlantic United States and demonstrates
the need for determination of action thresholds.

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a recently introduced

stink bug believed to have been introduced from Asia into Allentown, PA in 1996

(Hoebeke and Carter 2003). Since then it has spread throughout the Mid-Atlantic States,

with isolated populations occurring in Mississippi, Ohio, Oregon, and California. In its

native range, *H. halys* is considered to be an occasional pest of tree fruit and soybeans

(*Glycine max* (L.) Merr.) (Hoffman 1931, Kobayashi et al. 1972, Funayama 2002). The

introduction of *H. halys* in the United States has raised concerns because of its

polyphagous feeding habits and preference for tree fruit and soybean. In the United

States, stink bugs are recognized as increasingly important pests in many crops, due to

the reduction or replacement of broad-spectrum insecticides that have historically

managed populations (Todd et al. 1994, Riley et al. 1997, McPherson and McPherson

2000, Brown 2003, Leskey and Hogmire 2005).

Feeding damage from stink bugs can occur at early, mid and late-season stages of

fruit development. Stink bug damage to apples [*Malus domestica* Borlch. (Rosales:

Rosaceae)] can be confused with cork spot or bitter pit, physiological disorders caused by

calcium deficiencies (Brown 2003). Cork spot causes discrete brown discolored spots on

the exterior of the fruit that may be sunken and are accompanied by diffuse brown

necrotic tissue underneath. This tissue may be separate from the skin and lacks a stylet

puncture (Brown 2003). Early season damage occurring immediately following bloom or

when fruit is still small results in aborted or cat-faced fruit (Rings 1957). Feeding

damage that occurs at any of these critical growing periods causes unmarketable fruit

(Rings 1957). During mid-season fruit development tissue cells have high mitotic

activity and feeding results in damage similar to late-season damage but appears more depressed around the feeding site and the fruit may be gummy (Mundinger and Chapman 1932). Late-season damage differs from cork spot or bitter pit by the presence of a stylet puncture, by being contiguous with the fruit skin, damage at the skin surface is circular in appearance, and corking (necrotic tissue) is uniform (Brown 2003). In apples, the necrotic tissue is often brown; where as in peaches [*Prunus persica* (L.) (Rosales: Rosaceae)], cherry [*Prunus spp.* (Rosales: Rosaceae)], and pear [*Pyrus spp.* L. (Rosales: Rosaceae)] corking appears white to brown in color.

Halyomorpha halys is a pest of tree fruit in Japan and South Korea, particularly of persimmons, apple, and pear (Chung et al. 1995, Choi et al. 2000, Funayama 2002). It is the dominant stink bug pest in South Korean non-astringent persimmon [*Diospyros kaki* L. (Ericales: Ebenaceae)] and Yuzu [*Citrus junos* Siebold (Sapindales: Rutgaceae) with population peaks occurring in mid-August (Chung et al. 1995, Choi et al. 2000). Apple may serve as an early season host for overwintering *H. halys* adults which inflict significant damage in early and mid-season apple varieties in Japan (Funayama 2002, 2004). Damage in pears can occur throughout the growing season (Fujiie 1984). Prior to the introduction of *H. halys* in Northeastern states such as New Jersey and Pennsylvania, stink bugs have primarily been a pest in peaches and pears (Brown 2003). With the introduction of a new pentatomid species we wanted to determine *H. halys'* potential as an agricultural pest, particularly in tree fruit. The work presented here will discuss the occurrence and seasonality of *H. halys* in apple and pear orchards and provide feeding damage estimates.

Materials and Methods

<u>Cage Study</u>

To determine if *H. halys* feeding could cause damage during critical fruit growth

stages, nylon mesh exclusion cages (29cm height x 20cm width) were placed on the

terminal ends of flowering or fruit bearing branches for peach and apple (Red Delicious,

2006 and 2007) at the Rutgers Fruit and Ornamental Research Extension facility in

Cream Ridge, NJ. No insecticide applications were applied to the treatment blocks. In

the peaches, regular fungicide applications were applied as needed to protect the trees

according to the Rutgers University Fruit Management Guidelines (NJAES 2006, 2007).

During the critical growing periods evaluated, 15 cages were tied with twine and two

adult *H. halys* were placed inside for a 48h period at the three critical growing stages. At

the end of each 48h period, the insects were removed and the cage re-secured and left on

until harvest. In peaches these stages are shuck-split, pit hardening, and swell. To

determine pit hardening and swell, fruit was cut and measured weekly. In apples the

critical growing stages were classified as petal fall, mid-season, and late-season. Fruit

was also measured weekly with a Vernier caliper (BioQuip, Rancho Dominguez, CA) at

the shoulder for apples and the fruit center diameter for peaches. Exclusion cages

remained on the fruit until harvest. Five exclusion cages were placed on the branches

containing fruit, at each of the critical growing periods, without insects to determine the

effect of the cages on the fruit. At harvest (Peach – 14 August 2007; Apple - 21

September 2006, 21 September 2007), cages were removed and fruit harvested, fruit was

photographed to document feeding damage. Fruit was peeled and cut to identify feeding

and the location of feeding, and damage descriptions were recorded. Fruit in control

cages were treated identically, and any feeding damage was recorded. Feeding location

was recorded as shoulder, middle, and calyx by dividing the fruit into three sections and

recording the number of feeding sites within each region. The number of fruit that were

aborted or pre-maturely dropped was also recorded.

Seasonality and Damage

Two commercial orchards (Lichtenwalner Farms, Macungie, PA and Peaceful

Valley Farms, Pittstown, NJ) were sampled weekly from petal fall until harvest for stink

bug seasonal dynamics and density. These locations were selected to represent high (PA)

and low population densities (NJ) of *H. halys*. At each location, 20 pear [10 pear *Pyrus*

spp. (Rosaceae: Rosales), 10 Asian pear (*P. pryifolia* (Burm.) Nak.], and 20 apple [*Malus*

domestica (Rosaceae: Rosales)] trees were selected for sampling. Peaches were not

present at both locations and were not sampled. Each tree was sampled by limb-tapping

(also known as 'beating method') from 1.5m – 3.0m in height around the circumference

of the tree (Southwood and Henderson 2000). Each tree limb was tapped sharply three

times with a rubber bat, using a canvas beat sheet (71cm x 71cm, BioQuip, Rancho

Dominguez, CA) to collect dislodged insects. Dislodged pentatomid species were

identified to species and life stage. Regular pesticide spray regimes were maintained for

other fruit pests according the Rutgers University Fruit Management Guidelines (2006,

2007). A 110 volt blacklight trap (Gempler's, Madison, WI) was run at each location

from 1 May to 1 October and collected bi-weekly to assess population density and flight

activity of stink bugs. All stink bug species were identified and recorded from the

blacklight traps.

At harvest (3 September 2006, 4 September 2007), 25 fruit per tree were haphazardly selected from 10 randomized trees per location and type. Fruit was cut and the number of damaged fruit per tree was recorded. Damage that occurred in the calyx region was most likely caused by early season *Lygus spp.* populations and was not included in damage assessment.

<u>Statistical Analysis</u>

When needed, data was transformed using [square root(x+1)] and analyzed with a two-way GLM using Tukey's mean separation at $P \leq 0.05$ for damage treatment, tree species, and density. Caged damage was analyzed by critical growing period (damage treatment) for each year. Seasonality samples were analyzed as nymph, adult, and total. 'Nymph' represented all nymphal stages collected from a given host plant; 'total' represented the sum of all nymphal stages and adults. To compare the relative abundance of *H. halys* to other phytophagous pentatomid species, data for each species was summed over the sampling period to account for temporal differences in abundance. Mean total abundance was calculated for each species and analyzed using a one-way GLM and Tukey's mean separation at $P \leq 0.05$. Proportion of damaged fruit per tree was arcsin transformed and data was collapsed between sites. All analyses were done using SAS v. 9.1 (SAS Institute 2002-2003). Data is presented as untransformed means.

Results

<u>Caged Damage</u>

It was determined that *H halys* adults will feed upon developing apple and peach fruit at the three critical stages of development with damage appearing similar to that

described from other pentatomid species damage. In apple, there was significantly higher

levels of damage at harvest in the late-season fruit compared to the early season

treatments in 2006 (2006 – $F_{3, 66}$ = 15.75, $P \leq 0.001$; 2007 – $F_{3, 71}$ = 2.24, P=0.091) (Table

1). In 2006, mid-season damage was not significantly different from early-season

damage. However, the number of aborted fruit was significantly higher in the early-

season treatments in both years (2006 – $F_{3, 69}$ = 15.64, $P \leq 0.001$; 2007 – $F_{3, 29}$ = 4.26,

P=0.013). Maturing fruit had low levels of aborted fruit that was similar to the control

treatment. Much of the mid-season fruit was dimpled in appearance, consistent with

feeding by pentatomids. Late-season damage appeared with brown corking close to the

surface of the skin. Similar results were present in peach, with late-season damage being

significantly greater than occurrence of damage at other critical periods (2007 - $F_{3, 21}$ =

10.92, P=0.002). Early-season feeding trials had the highest levels of fruit abortion,

although it was not significantly different than the control treatment (2007 $F_{3, 34}$ = 16.15,

$P \leq 0.001$). Very low amounts of cat-facing damage were present in the peaches.

Pentatomids are not believed to feed in the calyx region of fruit; however damage was

present in the calyx region of fruit in apples (2006) and peaches (2007).

Seasonality

In 2006 the nymphal and overwintering adult populations in pear peaked in early

July (total mean=2.75±0.54 per tree) and again in late August/early September. The

second peak was due to the occurrence of first generation adults and late instar nymphs,

which can be the most damaging stages due to the time of occurrence and high densities.

In 2007, similar seasonality emerged occurred, although early season adult

(overwintering) and nymphal population (early instars) were more temporally separated.

Egg masses were found in Asian pear on 4 separate dates in 2007 (July 2, Aug. 2, 13, 23, 2007). Despite this, occurrence of nymphs was rare on any of the tree fruit species. Population densities were significantly higher in pear than apple in both years (2006 – $F_{1, 314}$ = 16.19, $P \leq 0.0001$; 2007 – $F_{1, 709}$ = 4.52, P=0.03). In 2006, the population of *H. halys* in apple was too low to detect any seasonal patterns. No mid-season nymphal population peak occurred in either crop, which may indicate that pome fruits are not a preferred developmental host for *H. halys*. Comparison of the temporal dynamics of *H. halys* (as measured by the blacklight trap) in pome fruit to the flight activity of the adults showed an opposite relationship in occurrence (Fig. 3). Peak flight activity occurred in early August due to the eclosion of 1st generation adults, just prior to an increase in abundance in pears and apples indicating that 1st generation adults may be moving to new host plants at suitable phenological periods.

Halyomorpha halys was the predominant stink bug species present at Lichtenwalner Farms ($F_{1, 10}$ = 34.78, P=0.0002) with a mean seasonal abundance of 14.00 individuals per tree versus 1.18 for all other species combined. Pentatomid abundance at Peaceful Valley Farms in Pittstown, NJ 7 were too low to document temporal dynamics. In 2006 two adult *H. halys* and one other pentatomid species were found; in 2007, six *H. halys* were found and two other pentatomid species. At Lichtenwalner Farms, 1201 and 581 *H. halys* adults were collected from the blacklight trap in 2006 and 2007, respectively, compared to 5 and 10 individuals of other pentatomid species in the same years. A similar relationship between *H. halys* and all other pentatomid species was evident at Peaceful Valley Farms (Table 2).

Damage Assessment

Stink bug feeding damage to fruit assessed at harvest was above 26.7.0% at both locations (Fig. 2). In 2006In New Jersey, 45.2±5.0% of apple fruit per tree were damaged and 26.8±3.3% pear fruit per tree were damaged. In Pennsylvania, 73.1±4.0% of apple fruit per tree were damaged and 69.2±4.1% of pear fruit per tree had stink bug damage. The New Jersey farm had low numbers of *H. halys* in the beat samples and represented damage estimates that occur at low density. Damage was significantly higher at the Pennsylvania farm than in New Jersey in both apple ($F_{1, 39}$ = 19.57, $P \leq 0.0001$) and pear ($F_{1, 34}$ = 51.47, $P \leq 0.0001$)

Stink bug feeding damage to fruit assessed at harvest was above 25% per tree at both locations (Fig. 2). In 2006 at the New Jersey farm, 47.0±6.5% of apple fruit per treewere damaged and 24.6±4.5% pear fruit per tree were damaged. In Pennsylvania, 89.7±6.1% of the fruit per tree for apple and 72.5±7.0% of pears had stink bug damage. In 2007, in Pennsylvania 69.62±4.3 and 64.9±3.0% of fruit had stink bug damage in the apples and pears, respectively. The level of damage decreased slightly in 2007 at the Pennsylvania farm. In New Jersey, where lower numbers of *H. halys* were found in both the beat samples and blacklight trap, there were still 42.9±8.0 and 29.0±4.5% damaged fruit per tree in apple and pear, respectively in 2007. Damage was significantly higher in PA than in NJ for both apple and pear in 2006 and 2007 (Apple: 2006 - $F_{1, 12}$ = 21.03, $P \leq 0.001$; 2007 - $F_{1, 25}$ = 9.73, $P = 0.004$) (Pear: 2006 - $F_{1, 17}$ = 25.07, $P \leq 0.001$; 2007 - $F_{1, 15}$ = 37.96, $P \leq 0.001$).

Discussion

Halyomorpha halys caused damage both to apple and peaches at the critical growing stages, with the majority of feeding occurring late in the season. Beat and blacklight samples showed that *H. halys* is present during periods when mid-and late-season damage may occur. In Pennsylvania, *H. halys* exceeded one individual per tree per sampling date during these critical growing stages for both apple (2007) and pear (2006-2007). Although reproduction did occur in Asian pear (2007), nymphal populations were low on all tree fruit in both years indicating that they may not be optimal for developmental hosts (Funayama 2004). Instead, *H. halys* overwintering adults may utilize these hosts when emerging from diapause and then again late in the season, just prior to harvest when the 1st generation adults prepare to enter diapause. This type of host switching behavior based on plant phenology is common among stink bugs.

Binns and Nyrop (1992) state that temporal dynamics of a species must be considered when developing a sampling program to make pest management decisions. The data presented here are the first study documenting the seasonality and potential damage caused by *H. halys* in tree fruit and provides the initial step to develop a management program. While no action thresholds for any stink bug species exist in the Northeast for tree fruit, particularly in pome fruit, losses ranging from 27-69% are quite high. Since *H. halys* was the dominant stink bug species found, the majority of this damage is likely attributable to high densities of *H. halys*, demonstrating the need for the development of action thresholds and control measures.

Beat sampling is a preferred sampling method for stink bugs in a variety of agricultural commodities, including tree fruit. However, stink bug populations can be

difficult to detect because of nocturnal activity and hiding behaviors. For this reason, the blacklight trap set up at each farm gave an indication of not only flight activity but also provided an additional population estimate. Blacklight traps were capable of detecting overwintering adults as they disperse into the orchards and 1st generation adults that are moving to the orchards. Adult *H. halys* overwinters primarily in man-made structures (Watanabe et al. 1994) and likely utilize developing pome fruits early in the season as a nutrient source during reproductive maturation (Funayama 2004). Our data shows that once *H. halys* populations are found in blacklight samples, beat sampling in the orchard should begin. Despite very low numbers found in this study during beat sampling at the New Jersey farm, high levels of stink bug damage to fruit still were present. This is likely attributable to behaviors typical of stink bugs including hiding behavior and nocturnal activity. The 1st generation late instar nymphs and adults are inflicting the majority of damage in Pennsylvania and New Jersey in August and September during the late-season critical growing period.

The temporal dynamics show a population peak in late August/early September, which is a couple of weeks prior to harvest, depending on the variety. A primary concern among growers is how to reduce damage that occurs just prior to harvest, after most insecticide use is prohibited due to the pre-harvest interval period. Pyramid traps baited with aggregation pheromone have been effective again native stink bug species in orchards at reducing their populations (Leskey and Hogmire 2005). *Halyomorpha halys* is attracted to these yellow pyramid traps baited with methyl (*E, E, Z*) 2,4,6-decatrieonate, especially during late August/early September (Nielsen in preparation).

These traps have great potential in an orchard setting as a mass-trapping tool 1st generation adult *H. halys* and should be investigated further.

Based on the data presented here, it is anticipated that *H. halys* will become a pest in late-season apples and pears in the Mid-Atlantic region but not in early-season peaches. Most peaches in this region are harvested prior to the occurrence of late instars and 1st generation adults. However, as *H. halys* disperses into Southern peach growing regions, it may become bivoltine (Nielsen et al. 2008) and as a result be present during the susceptible stages of peach development. More survey work needs done to determine how wide-spread populations of *H. halys* are in the Eastern United States and if similar damage levels are occurring in other areas. Growers in Western Maryland have reported large populations of *H. halys* and describe a large increase in stink bug damage in apples in recent years (J. Fiola personal communication). We believe the majority of this damage is caused by *H. halys*, and that it has become a significant agricultural pest since its introduction and control efforts need to be evaluated.

Figure 1: Seasonality of *H. halys* adults and nymphs in A) Pear 2006 B) Pear 2007 C) Apple 2007 in Macungie, PA. The 2006 population in apples was too low to describe here.
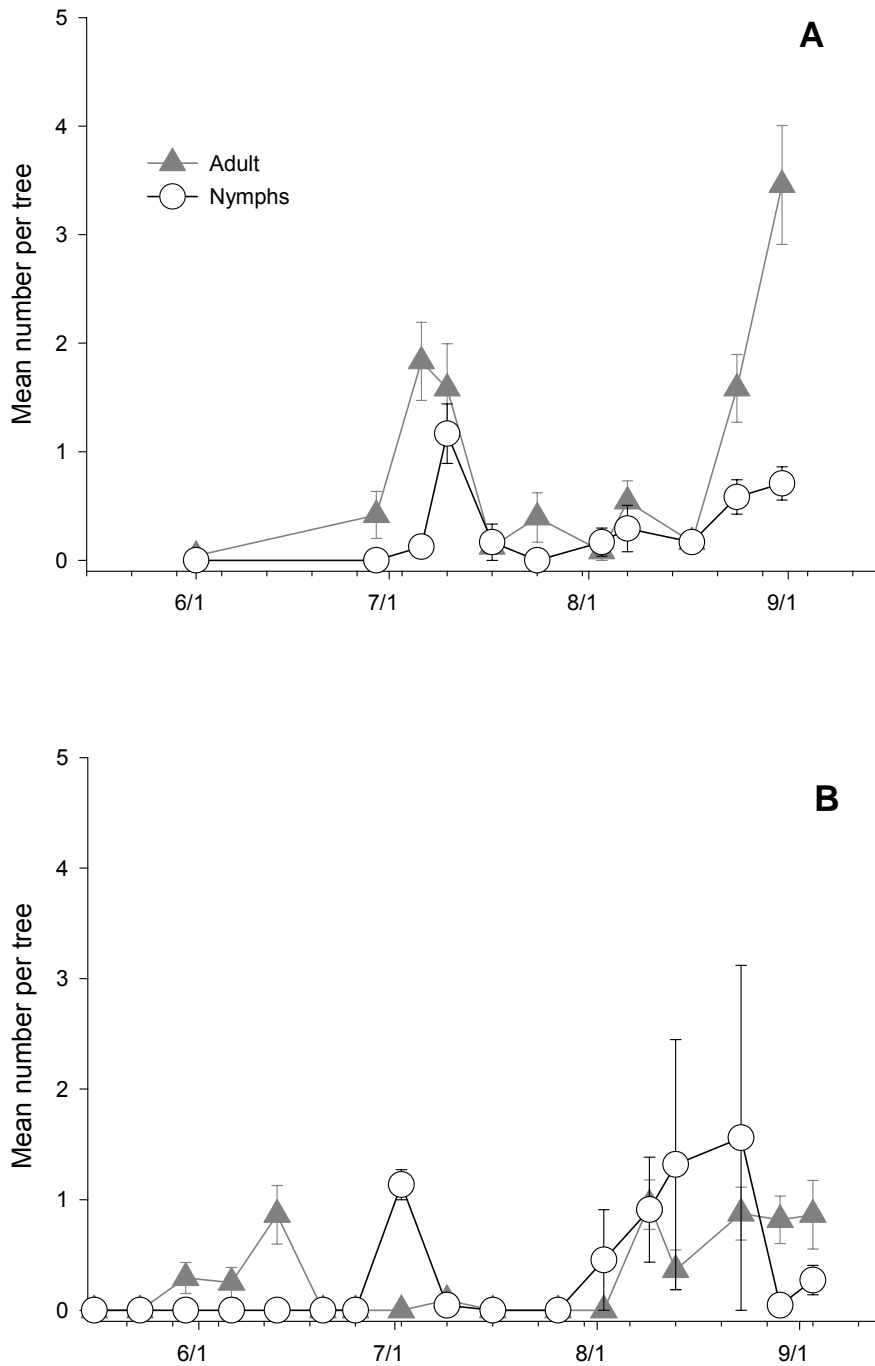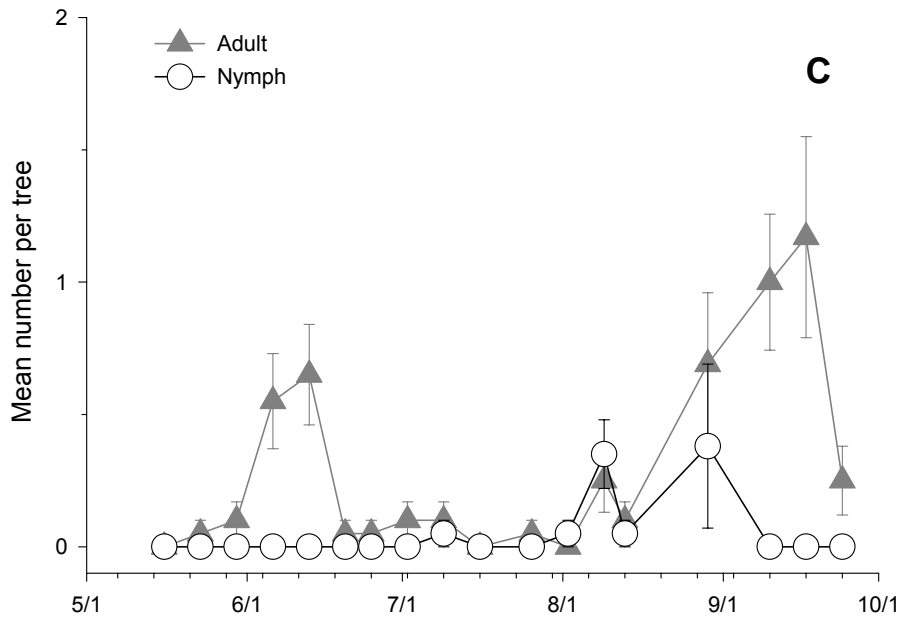
Figure 2: Proportion of stink bug damaged fruit in apple and pear. In both fruit types, there was significantly higher stink bug damage at the Pennsylvania location (Tukey's, $P \leq 0.001$).
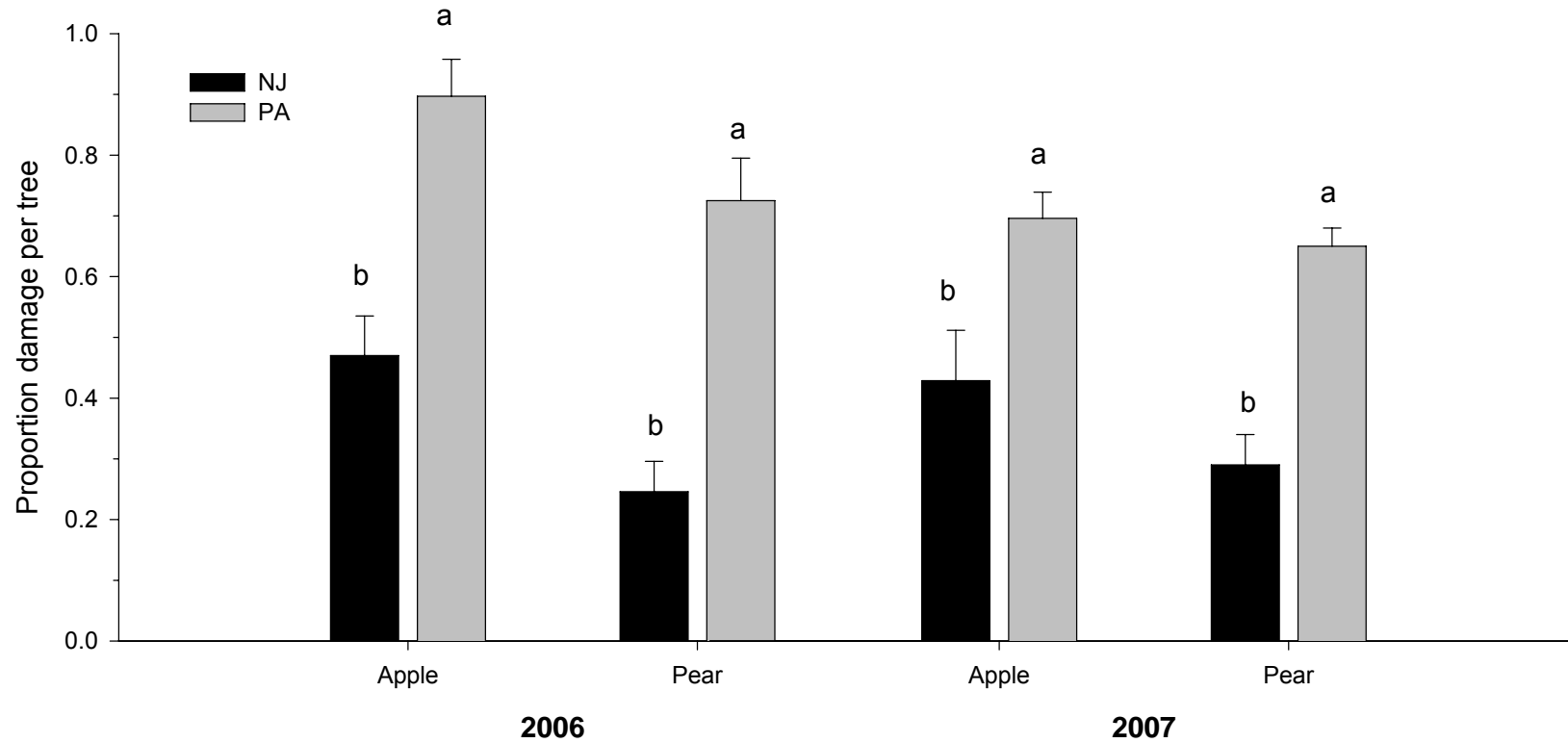
Figure 3: Temporal dynamics (Mean ± SE) of *H. halys* in apple and pear orchards from beat sampling and flight activity of adults as indicated by blacklight trap in Macungie, PA.
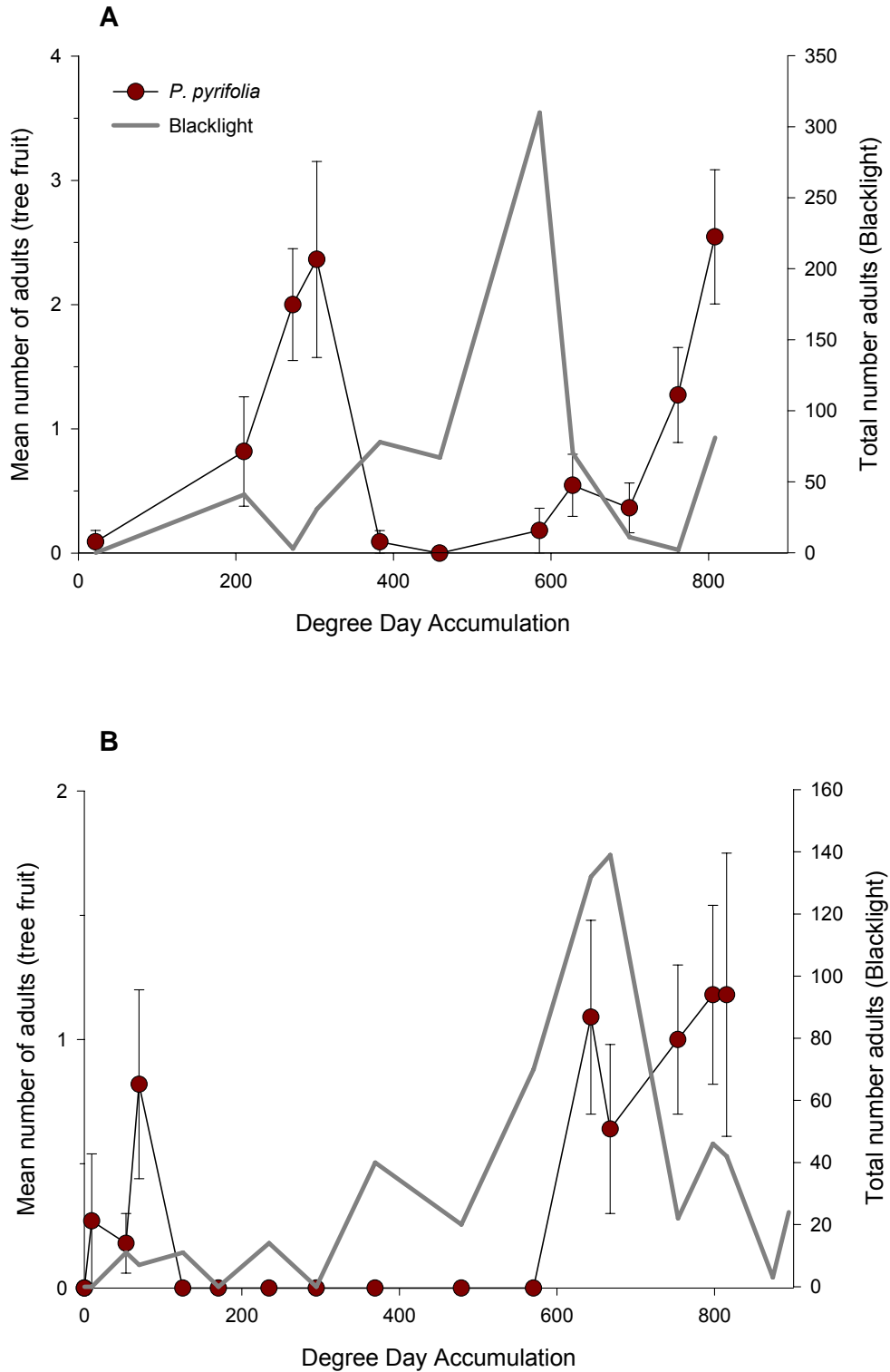
Table 1: Mean (±SE) of *H. halys* feeding sites after 48h exposure, shown by location on fruit and growing period.

	Location	2006				2007			
		Early	Mid	Late	Control	Early	Mid	Late	Control
Apple	Total	2.33±0.90b	3.17±0.63b	5.53±0.87a	0.65±0.21c	0.00±0.00a	1.35±0.37a	1.72±0.87a	0.72±0.27a
	Damage[a]	c							
	Aborted Fruit[b]	1.21±0.21a	0.23±0.09b	0.33±0.14b	0.15±0.09b	0.92±0.33a	0.14±0.10b	0.00±0.00b	0.21±0.15ab
Peach	Total	-	-	-		0.50±0.50b	0.69±0.34b	4.92±1.06a	0.00±0.00b
	Damage[a]								
	Aborted Fruit[b]	-	-	-		2.29±0.29a	0.36±0.13b	0.19±0.10b	1.78±0.55a

[a] Mean number of feeding locations (sum of all sites) per fruit. Means within a row for each year followed by a different letter are significantly different ($P \leq 0.05$);
[b] Mean (±SE) of aborted fruit per replicate. Means within a row for each year followed by a different letter are significantly different ($P \leq 0.05$);

Table 2: Pentatomid species composition and total abundance in Macungie, PA and Pittstown, NJ from blacklight traps.

Pentatomid species	2006		2007	
	PA	NJ	PA	NJ
	Blacklight	Blacklight	Blacklight	Blacklight
Halyomorpha halys	1201	27	581	60
Acrosternum hilare	1	0	3	0
Euschistus servus	0	0	0	2
E. tristigmus	0	0	0	0
E. variolarius	1	0	3	3
Thyanta spp.	3	3	1	3
Banasa spp.	1	0	3	0

CHAPTER 4

COMPARISON OF TRAPS BAITED WITH METHYL (*E,E,Z*)-2,4,6-DECATRIENOATE FOR MONITORING POPULATIONS OF THE INVASIVE *HALYOMORPHA HALYS* (STÅL) (HEMIPTERA: PENTATOMIDAE)

Abstract: Improved monitoring tools are needed for phytophagous stink bugs as they become increasingly important pests in many crop systems. Four different trap designs baited with methyl (*E,E,Z*) 2,4,6-decatrienoate were evaluated for their attractiveness to a recently introduced stink bugs species, *Halyomorpha halys*. The large pyramid trap consistently captured the most adults in both soybean and ornamental plots. In 2006 and 2007, two sizes of yellow pyramid trap (small and large) were compared against the industry standard sweep net samples in soybean. The large pyramid trap caught significantly more *H. halys* in both years (2006: $F_{2, 10} = 13.19$, *P*=0.002; 2007: $F_{2, 10} = 18.99$, *P*≤0.001). Sweep net samples indicated that *H. halys* is present beginning at the critical R4 phenological stage of soybean development and in Pennsylvania, *H. halys* is significantly more abundant than native pentatomid species in soybean ($F_{2, 71} = 11.55$, *P*≤0.0001).

Introduction

Identifying monitoring methods to detect the occurrence and estimate density of insect crop pests, such as stink bugs, is an essential aspect of developing an ecologically sound integrated pest management program (IPM) (Metcalf and Luckmann 1994). The Food Quality Protection Act of 1996 restricts or eliminates the use of organophosphate and carbamate insecticides and pushes the emphasis towards management programs that utilizing ecologically sound management options. Replacement or reduction of broad-spectrum insecticides has, in part, allowed stink bugs to become dominant or to buildup populations in crops before dispersing to alternate hosts where they can inflict damage (McPherson and McPherson 2000, Snodgrass et al. 2005). In particular, herbivorous stink bugs are becoming of significantly greater concern in cotton (*Gossypium spp.*) and tree fruit (Riley et al. 1997, McPherson and McPherson 2000, Leskey and Hogmire 2005). Complimenting this has been an increase in soybean (*Glycine max* (L.) Merr.) production acreage in the United States since the 1960's and increased acreage of transgenic *Bt*-cotton (Todd et al. 1994). Damage induced by stink bug feeding in soybean includes deformed seeds, delayed maturity, reductions in yield and oil (Daugherty et al. 1964, McPherson 1996, Boethel et al. 2000, McPherson and McPherson 2000). Damage and control efforts aimed at stink bugs, have also increased, primarily in the southern states. In Georgia alone, stink bug damage and control costs in 1996 exceeded $3 million from feeding by *Nezara viridula* L., *Acrosternum hilare* (Say), and *Euschistus servus* (Say) (Riley et al. 1997).

A recently introduced stink bug species, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Hoebeke and Carter 2003), could become an important member of the

stink bug complex in the Eastern United States if its distribution continues to expand. In New Jersey and Pennsylvania, *H. halys* has caused economic losses in apples and pears (ALN in preparation), is present in high densities in soybean, and overwintering populations create a significant homeowner nuisance (Hamilton et al. 2008, Nielsen et al. 2008). Prior to the introduction of *Halyomorpha halys*, in the Northeastern states such as New Jersey and Pennsylvania, stink bugs have not been serious pest in soybeans (J. Ingerson-Mahar, personal communication).

In Asia, *H. halys* is a known pest of legumes (including soybeans) and tree fruit (Kobayashi et al. 1972, Funayama 1996, Choi et al. 2000, Toyama et al. 2006). Populations established in the Mid-Atlantic States have increased in density and are being found at higher levels than native species (ALN unpublished). *Halyomorpha halys* males and females are highly attracted to methyl (*E,E,Z*)-2,4,6-decatrienoate, the aggregation pheromone of a sympatric Asian species, *Plautia stali* Scott (Hemiptera: Pentatomidae) (Khrimian 2005, Khrimian et al. 2008). An aggregation pheromone has not been identified for *H. halys*, but for purposes of this paper, we will refer to methyl (*E,E,Z*)-2,4,6-decatrienoate as an aggregation "pheromone" for *H. halys* (Khrimian et al. 2008). This pheromone has been used in Asia for monitoring populations (Lee et al. 2002, Adachi et al. 2007); however, no studies have been done in soybean on the use of a pheromone trap for *H. halys* in either Asia or the United States. Additionally, the population dynamics of *H. halys*, especially in relation to pentatomid species native to the United States, is unknown.

The use of trapping methods, such as pheromone trapping is an attractive sampling or monitoring tools for stink bugs because of difficulty encountered during

sampling and has been successful in a variety of cropping systems (Leskey and Hogmire 2005, Cullen and Zalom 2006). The cryptic coloration, nocturnal feeding, aggregated dispersion and behavior of dropping off plants or hiding when disturbed may result in the underestimation of population levels (Shearer and Jones 1996, McPherson and McPherson 2000, Krupke et al. 2006). Trapping stink bugs can be complicated due to intraspecific communication occurs through pheromones as well as vibrational signals (Millar et al. 2002). Color is also known to attract certain insect species, specifically yellow is interpreted by herbivorous insects as an indicator of plant health and can have a positive impact/attraction to motile insects (Prokopy and Owens 1983). Trap designs that incorporate both visual and chemical cues have successfully trapped stink bugs and other insects (Mizell and Tedders 1995, Leskey and Hogmire 2005).

We set out to compare the effectiveness of different trap designs baited with methyl (*E,E,Z*)-2,4,6-decatrienoate for capturing *H. halys* in various ornamental host-plant species and fields of soybean (Study 1). Using the most efficient trap design from Study 1, we then compared these traps to conventional sampling methods used in soybean while determining if *H. halys* was present during susceptible stages of soybean growth (Study 2).

Methods

<u>Aggregation Lures</u>

The pheromone compound methyl (*E,E,Z*)-2,4,6-decatrienoate used in this study was synthesized by USDA-ARS in Beltsville, MD following the procedures outlined by Khrimian (2005). Rubber septa lures were impregnated with 2.5mg of methyl (*E,E,Z*)-

2,4,6-decatrienoate. In all trials, lures were replaced every two weeks.

Photoisomerization does not affect attractiveness of *H. halys* and thus the lures used in

both studies were not shielded from ultraviolet exposure (Khrimian et al. 2008).

Initial Trap Comparison (Study 1)

Four different types of traps baited with methyl (*E,E,Z*)-2,4,6-decatrienoate were

evaluated from mid-September to mid-October in ornamental plantings and soybean in

Allentown, PA at the Rodale Working Tree Group and Lichtenwalner Farms. This time

period reflects the movement of adult *H. halys* to overwintering locations. Sampling was

terminated at frost or harvest. *Halyomorpha halys* adults aggregate in large numbers

during diapause and it was hypothesized that attraction to the pheromone would be high

during this period. We evaluated the Smart[©], "apple", "football" (Sterling International,

Inc., Spokane, WA), and a large pyramid trap (Mizell and Tedders 1995) for their

effectiveness at attracting and containing *H. halys* (Fig. 1). The "apple" trap was a small

round trap (5.5" diameter) made to be placed on a limb of a tree, the "football" trap was a

football-shaped trap (11.0" height) meant to be hung in the tree canopy while touching

either foliage or bark. Both of these traps were double-cone traps made of clear plastic.

The pyramid trap was a single-cone pretzel jar trap placed on top of a tall bright yellow

cone (donated by R. Mizell) in which the insects crawled up the cone into the jar (Mizell

and Tedders 1995). The Smart[©] trap was a single-cone translucent green plastic bullet-

shaped trap with a LED light encircling the top. Similar versions of all four traps are

available commercially.

Each year the study was conducted at two sites: the Rodale Working Tree Center

("Rodale"), an arboretum that houses many native and exotic ornamental trees and shrubs

in Allentown, PA, and at a soybean field at Lichtenwalner Farms, Macungie, PA. Traps were arranged in a randomized complete block design and replicated three times at each site. At the Rodale site, each replicate consisted of a different ornamental plant. The host plants differed in 2004 from 2005 and 2006. In 2004, English pear, (*Pyrus sp.*), Russian olive (*Elaeagnus angustifolia* L.), and Siberian Pea Shrub (*Caragana arborescens* Lam.) were evaluated. In 2005 and 2006, Russian olive and English pear were replaced by Blackhaw Viburnum (*Viburnum prunifolium* L.) and Asian pear (*Pyrus sp.*). The distance between replicates in the ornamentals exceeded 100m and traps were hung 10m apart at a height of 1.5m in the canopy. At Lichtenwalner Farms, traps were evaluated in 2004 and 2005 in soybean [Asgrow 3602, 30" row spacing, planting date May 30th, both years] and were placed 10m apart and hung at either 0.5m or the height of the soybean canopy. In soybean, each replicate was separated by 10m. In 2004, the experiment was terminated on 8 October 2004, three days before the soybeans were harvested. All traps at both locations were collected twice per week and individual stink bugs were identified to species and sexed or identified to life-stage in the laboratory.

Sampling Method Evaluation in Soybean (Study 2)

To compare the effectiveness of pheromone trapping versus traditional sweep net sampling methods for stink bugs in soybean, we compared two sizes of the yellow pyramid trap baited with a rubber septa impregnated with 2.5mg of methyl (*E,E,Z*)-2,4,6-decatrienoate to sweep net captures in 2006 and 2007. Sweep net samples are a common sampling method in soybean for stink bugs, and were used as the control treatment in our study. Sweep net samples were taken over a 10m row distance, equal to approximately 20 sweeps, using a 38cm diameter sweep net (BioQuip, Rancho Domingueq, CA). A plot

at the Rutgers University Snyder Farm Research Station in Pittstown, NJ [DKB 31-51 (2006), DKB 36-52 (2007), planting date May 24th, both years] and a plot located at Lichtenwalner Farm in Macungie, PA [Asgrow 3602 (2006), Seedway 50 (2007), planting date May 30th both years] were selected. Both plots were no-till soybean with 30" row spacing and maintained without fertilizer or insecticide treatments. The plot in Pennsylvania had high populations of *H. halys* for multiple years and was bordered by sweet corn plantings on at least one side. The New Jersey plot had only grass borders. Both locations had a hedgerow containing various plants including multiflora rose (*Rosa multiflora* Tunb.), black locust (*Robinia pseudoacacia* L.), honeysuckle (*Lonicera spp.*), and wild grape (*Vitis vulipina* L.) that were ~15m from the field edge. *Halyomorpha halys* was first found at the New Jersey location in 2005 and represented a "low" population density of *H. halys* in soybean. The plots were set up in a randomized complete block design with rows as the blocking factor. Each replicate consisted of a large pyramid trap, small pyramid trap and a sweep net sample, each placed 10m apart. Replicates were placed 10m apart and sampling began 10m from the field edge. Samples were taken beginning at the V3 stage of soybean growth. Sweep net samples commenced at R1 stage, previous samples were done with visual counts. All samples were collected biweekly until frost and the phenological stage of soybeans was recorded weekly.

The pyramid traps were constructed by ALN and GCH using bright yellow sheets of plastic, 6mm thick (Laird Plastics, Dayton OH) cut to 32"L by 12"W (Small) (Leskey and Hogmire 2005) and 56"L by 22"W (Large) (Mizell and Tedders 1995). Trap tops were constructed out of 1 gallon PET clear plastic containers (9.75"h x 5.5"w, United States Plastic Corps., Lima, OH) where the bottom was replaced with an inverted wire

mesh cone, previously designed for boll weevil pheromone traps (Great Lakes IPM, Vestaburg, MI). Eight 1.5" holes were drilled in two columns around the clear plastic container and covered with flexible black plastic mesh for ventilation. The pheromone lure was hung from the removable lid by a metal hook with a metal binder clip to secure the lure.

One sweep net sample (~20 sweeps) was taken in each replicate, ranging from a distance of 10m to 70m from the border edge during each sampling period. These samples were used as a control treatment and also served to monitor the population levels of all stink bug species present in the field. Phytophagous pentatomid captures in the sweep net samples were identified to species and life stage. Blacklight traps were also run at each farm.

Statistical Analysis

Bi-weekly trap capture data from Study 1 were transformed using [log(x+1)]. All trap data were analyzed with a two-way GLM and Tukey's mean separation at $P \leq 0.05$. Trap captures were analyzed as nymph, adult, and total. 'Nymph' represented all immature stages captured in the trap in a given sample; 'total' represented the sum of nymphs and adults.

Data for the sampling methodology study in soybean were summed over the entire season to account for low trap captures early in the season before the plants were attractive to stink bugs. Means and standard error were calculated for seasonal dynamics. A one-way GLM was used to analyze seasonal means for stink bug abundance in sweep net captures. All analyses were done using SAS v. 9.1 (SAS Institute 2002-2003).

Results

<u>Initial Trap Comparison (Study 1)</u>

Traps were compared over a three year period at the Rodale site. All four trap designs were attractive to *H. halys* nymphs and adults although catches were lower for nymphs, likely due to the time of year sampling occurred (Table 1). The pyramid trap consistently caught high numbers of *H. halys* in both ornamentals and soybean. In the ornamentals, a significant treatment effect on nymph capture (2004 - $F_{3, 6}$ = 9.95, *P*=0.0096; 2005 - $F_{3, 6}$ = 3.79, *P*=0.0776; 2006 - $F_{3, 6}$ = 3.92, *P*=0.0729) was seen in 2004. For adult capture, this effect was seen between trap designs only in 2005 (2004 - $F_{3, 6}$ = 4.77, *P*=0.0497; 2005 - $F_{3, 6}$ = 10.63, *P*=0.0081; 2006 - $F_{3, 6}$ = 3.57, *P*=0.5256). The pyramid trap caught the highest mean number of nymphs in all years, 3.7 (±0.7), 5.3 (±2.3), and 32.7 (±15.7), respectively. In 2004 and 2006, the pyramid trap also caught the most adult *H. halys* (133.0±77.6 and 197.0±106.4, respectively) but in 2005 the football trap caught more adults (260.3±119.8) than the pyramid trap (Table 1). The apple trap also caught high numbers of individuals but trap capture was lower than both the pyramid and football traps and was very low for the nymphs. Least effective was the Smart[©] trap, due to the lack of an appropriate method of retaining *H. halys*. In 2005, the Smart[©] trap caught significantly fewer adult *H. halys* than the other trap designs. Modifications to the Smart[©] trap to improve retention may increase the trap's ability to capture *H. halys*.

In the soybean trials, the pyramid trap caught significantly higher mean number of nymphs in 2004 (2004 - $F_{3, 6}$ = 16.60, *P*=0.003; 2005 - $F_{3, 6}$ = 1.0, *P*=0.455). Adult

captures for the pyramid trap were significantly higher in both 2004 and 2005 (2004 - $F_{3, 6}$ = 4.85, P=0.048; 2005 - $F_{3, 6}$ = 6.33, P=0.027) (Table 1).

At both sites, peak trap captures occurred during the third week of September, followed by a decline with decreasing nightly temperatures. In the ornamentals there was a significant effect of replicate (host plant species) each year for the adult population (2004 – $F_{2, 6}$ = 31.05, $P \leq 0.001$; 2005 – $F_{2, 6}$ = 10.17, P=0.012; 2006 – $F_{2, 6}$ = 7.67, P=0.022) which is likely attributable to differences in abundance on individual host plant species.

Sampling Method Evaluation in Soybean (Study 2)

Seasonality and phenology: Soybean plots in New Jersey and Pennsylvania were selected to represent a low and high population, respectively. The established population in Pennsylvania provided information about the sampling methods utilized and seasonality of *H. halys* in soybean. Intermittent catches of *H. halys* in the pheromone traps were first observed in early June (Fig. 2). Populations began to build beginning at the R3 soybean phenological stage, with peak populations in late July coinciding with the R5 and R6 stages. *Halyomorpha halys* egg masses and successive instars were found both years indicating that soybean supported reproductive development of *H. halys*. Populations also appeared to be highly aggregated along field and neighboring crop borders.

Halyomorpha halys had not been detected in the soybeans in New Jersey prior to this study, and was first found at our study farm in 2005. In New Jersey, *H. halys* was not captured in the sweep net samples in 2006 or 2007. However, in 2007, a hail storm

with 160km/h winds on August 17th destroyed the soybean crop and many of the traps, just as populations were starting to build, leaving an incomplete data set for that year.

Blacklight traps indicated a difference in attractiveness than the pheromone traps. Peaks in capture of adults were seen late July, following a decline in trap captures a peak in sweep net captures was observed.

Trap comparison: In Study 1, the large pyramid trap caught the most *H. halys* in the soybean trial. To determine the ideal monitoring method in soybean, we compared the large pyramid trap to a smaller version as well as to sweep net samples. In Pennsylvania the large yellow pyramid trap caught significantly higher mean numbers of *H. halys* (except for nymphs in 2006) throughout the season compared to the other sampling methods in 2006 (nymphs – $F_{2, 10} = 0.96$, $P=0.415$; adults - $F_{2, 10} = 14.87$, $P=0.001$, total - $F_{2, 10} = 13.19$, $P=0.002$) and 2007 (nymphs – $F_{2, 10} = 7.32$, $P=0.011$; adults - $F_{2, 10} = 14.68$, $P=0.011$, total - $F_{2, 10} = 18.99$, $P\leq0.001$) (Table 2). The pyramid trap was attractive to second to fifth instars as well as adults of both sexes. The small pyramid trap and sweep net (control) monitoring methods were not significantly different from each other and both were successful at detecting the presence of *H. halys* but at significantly lower densities, than the pyramid trap. In New Jersey, due to the low population density present and the early termination of the study in 2007, the large pyramid trap did capture a few *H. halys* individuals but these totals were not significantly different than the other methods (2006: nymphs – $F_{2, 10} = 1.00$, $P=0.402$; adults - $F_{2, 10} = 5.00$, $P=0.030$, total - $F_{2, 10} = 3.05$, $P=0.092$; 2007: nymphs – $F_{2, 10} = 2.50$, $P=0.132$; adults - $F_{2, 10} = 1.00$, $P=0402$, total - $F_{2, 10} = 2.14$, $P=0.168$) (Table 2).

In 2007, the Pennsylvania plot was bordered by field corn on two sides. The rows directly bordering the corn had significantly higher mean number of *H. halys* present ($F_{5, 10} = 6.23$, *P*=0.0071). Seasonality, particularly prior to the R5 stage, also influenced the sampling methods, with both pyramid trap sizes capturing *H. halys* adults and nymphs before detection in sweep samples. In fact, individuals were collected 60m into the field before they were present in sweep net samples. Despite early season differences, there was a weak but significant correlation between the large pyramid trap and sweep net samples (R^2=0.40, *P*=0.0009) in Pennsylvania.

Relative Abundance

Native pentatomid species were detected in both sizes of pheromone traps as well as the sweep net samples. The following species were found: *Euschistus servus*, *E. tristigmus* (Say), *E. variolarius* (Palisot de Beauvois), *Acrosternum hilare*, and *Thyanta accera* McAtee (Hemiptera: Pentatomidae). Significantly higher mean number of *H. halys* were found in sweep net samples than either *E. variolarius* or *E. servus* ($F_{2, 71} = 11.55$, *P*≤0.0001) in Pennsylvania (Fig. 3). The other species were found at much lower densities, which was consistent with blacklight captures.

Discussion

While all trap designs attracted *H. halys* (Study 1), the large pyramid trap caught higher numbers of individuals in both ornamentals and soybean. The football trap, which has been used by Khrimian et al. (2008), was also effective at collecting and retaining *H. halys* but capture varied depending on host plant for adults. We believe the yellow base of the pyramid trap improved visual identification and movement into the trap.

Comparison of sampling methods in soybean by utilizing two different size pyramid traps allowed us to demonstrate the potential of using these traps to monitor populations of *H. halys* in soybean. The use of pheromone traps for stink bug monitoring has historically had mixed success because the individuals communicate using both chemical and vibrational cues (Borges et al. 1998, Cullen and Zalom 2005). Long-range attraction occurs as a response to the chemical volatile but an absence of short-range cues can limit trap captures because the insects may not physically enter it (Millar et al. 2002). Combining both visual and chemical cues may have contributed to the effectiveness of the pyramid trap design since stink bugs have been observed to walk up a plant in the presence of pheromone (Millar et al. 2002), the yellow pyramid trap encourages this behavior as the insects walk up the cone and are unable to escape. Our results are consistent with those of Leskey and Hogmire (2005) that found the pyramid traps to be successful at capturing stink bugs in tree fruit. The open space surrounding the traps in soybean may also have contributed to attraction of *H. halys*.

Many native species of pentatomids were observed in the traps in both studies. This effect was increased in soybean (Study 2), possibly due to shared food resources. The action of methyl (*E,E,Z*) 2,4,6-decatrienoate or other aggregation pheromones as an attraction to other stink bug species is not uncommon and has been suggested to act as a kairomone (Aldrich et al. 2007). *Thyanta spp*. produce methyl (*E,Z,Z*) 2,4,6 decatrienoate as a sex pheromone and were commonly found in the pheromone traps. Field observations of late-summer *H. halys* aggregations often have *A. hilare* present as well suggesting that this pheromone may be used by multiple stink bug species for host finding and identifying overwintering locations. One of us (ALN) also has observed

mating in the pheromone traps suggesting that attraction to the aggregation pheromone may serve dual purposes.

Pheromone traps have been used to monitor insipient populations of an invasive species, suggesting long-range attraction of the chemical, and have been deployed for mass-trapping (Burkholder and Ma 1985, Reardon 1991, Katsoyannos et al. 1999, Brockerhoff et al. 2006, El-Sayed et al. 2006). For *H. halys* we have had minimal success at utilizing aggregation traps for *H. halys* as a monitoring tool to detect new populations as the species disperses (ALN unpublished), which suggests that long-distance attraction to methyl (*E,E,Z*)-2,4,6-decatrienoate may be limited and that its application is restricted to within-farm monitoring of existing populations. Blacklight traps on the other hand, have proven to be a much more successful monitoring method for *H. halys* at low population densities (ALN in preparation). Temporal differences in blacklight and pheromone catches may indicate that the blacklight traps are highly attractive to first generation *H. halys* as they move to food resources (Tada et al. 2001a).

We were successful at employing the pyramid traps as a season-long monitoring program in soybean for *H. halys*. The monitoring program was particularly successful at population peaks, when the first generation nymphs and adults are abundant and coincides with the susceptible stages of soybean development. Our results support the use of pyramid traps, baited with the aggregation compound to monitor the presence of *H. halys* and shows potential for detecting dispersal of *H. halys* populations into agricultural fields. The use of pheromone traps for this purpose has previously been suggested for other pentatomid species but population density estimates continue to be done using sweep net or beat samples (McPherson and McPherson 2000). Our study not

only demonstrated long-term attraction to baited traps but also found a significant

correlation between the large pyramid trap catches and sweep net estimations. This

suggests that large baited pyramid traps can be utilized further for *H. halys* populations,

possibly as part of an IPM program or as mass-trapping and could be applied to other

crops such as cotton if *H. halys* becomes a pest in the South. Sweep net samples have not

been efficient in crops such as cotton, where stink bugs are increasing as pests and the

implementation of pheromone traps for monitoring or control efforts, like the pyramid

trap evaluated here, may be very useful (Borges et al. 1998, Bundy and McPherson

2000). Additional work is necessary to confirm whether the large pyramid aggregation

trap can be used in place of sweep net samples as this relationship has not been found for

Euschistus spp. in processing tomatoes (Cullen and Zalom 2005). Although more

expensive, the pheromone traps are efficient monitoring tools that can be less destructive

to the crop. Large numbers of individuals were not observed in the surrounding plants, a

problems that has been identified with other stink bug pheromone traps, which is most

likely due to the design of the yellow pyramid itself (Millar et al. 2002).

No economic thresholds have yet been determined for *H. halys* in soybean.

However, populations of *H. halys* in the Pennsylvania soybean field (sweep net samples)

were within the economic thresholds for *Nezara viridula* (L.), *E. servus* and *A. hilare*

(Todd and Herzog 1980, Deighan et al. 1985). In Pennsylvania, *H. halys* is the

predominant species of stink bug and is univoltine. Degree-day data supports that *H.*

halys can have multiple generations per year in the southern United States (Nielsen et al.

2008). If populations become established in the southern United States and are able to

reach similar population densities, it may become a significant concern in soybean and other crops.

Acknowledgements

Fig 1: Trap designs employed for capturing *H. halys*: a) football b) Smart© c) apple d) large pyramid

Figure 2: Seasonality of sampling methods for *H. halys* in soybean in Allentown, PA 2006-2007.
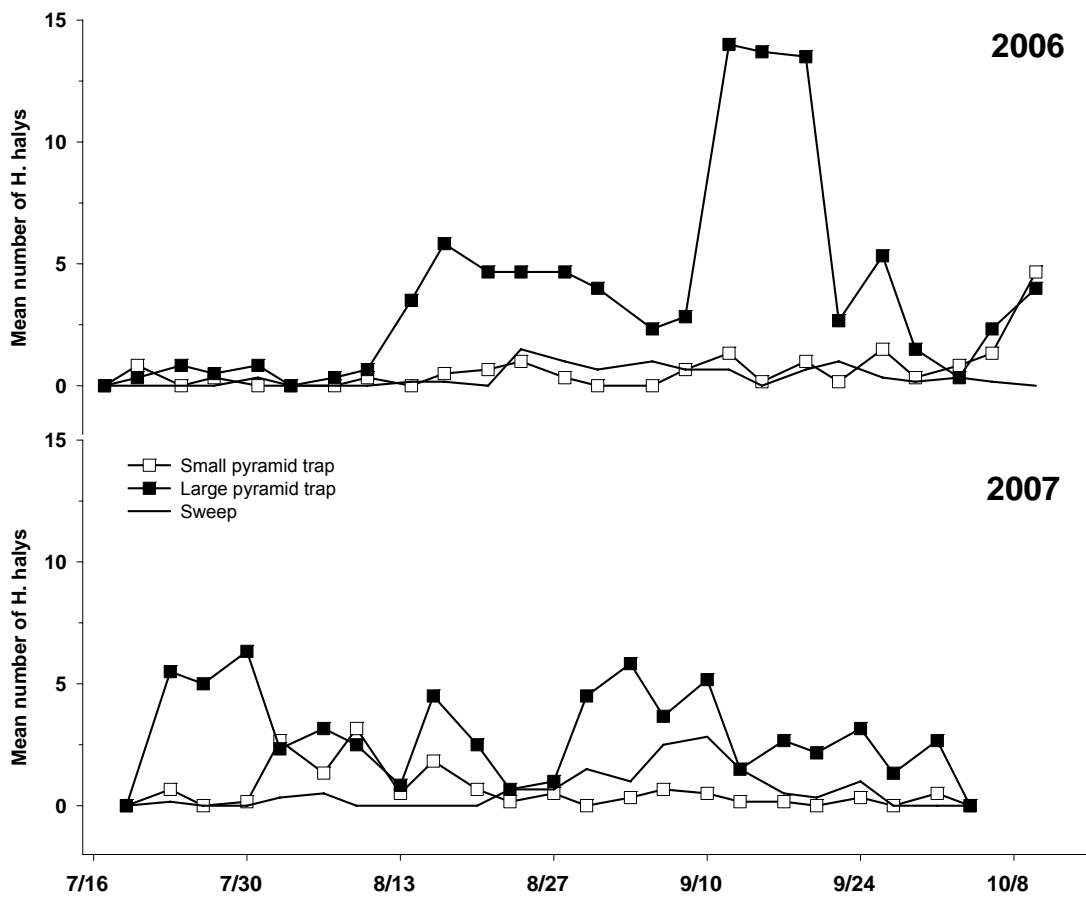
Figure 3: Mean (±SE) numbers of *H. halys, E. variolarius, and E. servus* collected in 10m sweep net samples.
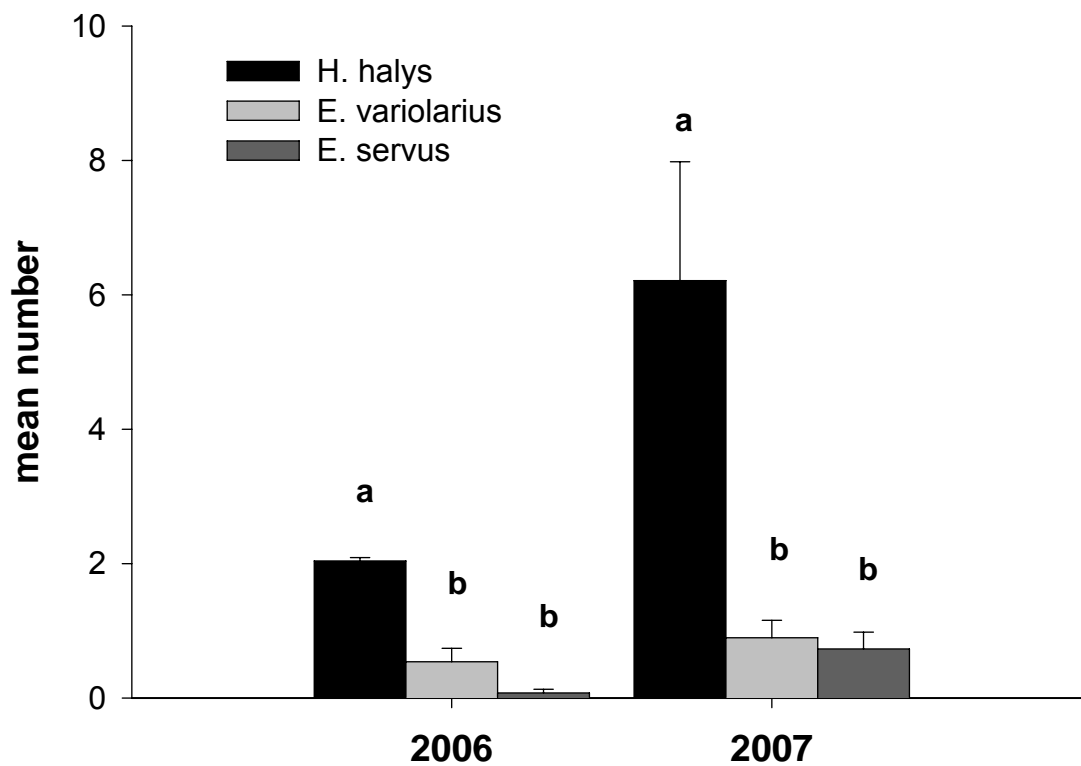
Table 1: Mean (±SE) trap captures for nymphs and adult *H. halys* in design comparison study

Host	Trap	2004[a]		2005[a]		2006[a]	
		Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
Ornamental	football	1.3±0.7b	44.0±28.2a	0.7±0.3a	260.3±119.8a	16.3±13.3a	141.7±92.4a
	Smart	2.7±0.9ab	38.3±23.5a	0.7±0.3a	50.0±12.5b	9.3±4.2a	51.3±11.7a
	apple	0.3±0.3b	87.3±50.7a	2.0±2.0a	164.3±41.2a	5.3±5.3a	115.0±81.2a
	pyramid	3.7±0.7a	133.0±77.6a	5.3±2.3a	177.3±63.4a	32.7±15.7a	197.0±106.4a
Soybean	football	0.3±0.3b	5.3±4.8ab	0.3±0.3a	4.7±4.7b	--	--
	smart	0.7±0.3b	2.7±1.7ab	0.3±0.3a	2.0±1.5b	--	--
	apple	0.0±0.0b	0.0±0.0b	0.3±0.3a	5.7±3.8ab	--	--
	pyramid	2.3±0.3a	13.7±3.2a	1.0±0.6a	32.0±14.1ab	--	--

[a] Means shown are untransformed. For each site, means within columns followed by the same letter are not significantly different for transformed means (Tukey's, $P \leq 0.05$).

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Table 2: Mean (±SE) abundance of *H. halys* in soybean as indicated by various sampling methods.

Site	Treatment	2006			2007		
		Immature[a]	Adult[a]	Total[a]	Immature[a]	Adult[a]	Total[a]
Pennsylvania	Small Pyramid	8.83±2.64	8.17±2.83b	17.00±4.81b	12.83±6.51b	1.67±0.56b	14.50±7.00b
	Large Pyramid	10.33±4.63	69.83±17.97a	80.17±21.78a	31.50±9.97a	30.83±8.09a	62.33±15.31a
	Sweep Net	5.00±3.44	3.83±1.17b	8.83±4.52b	11.50±6.50b	2.00±1.00b	13.50±7.36b
New Jersey	Small Pyramid	0.00±0.00ab	0.00±0.00	0.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Large Pyramid	0.33±0.33a	0.50±0.22	0.83±0.48	0.33±0.21a	0.17±0.17a	0.50±0.34a
	Sweep Net	0.00±0.00b	0.00±0.00	0.00±0.00	0.00±0.00b	0.00±0.00b	0.00±0.00b

[a] Results shown as untransformed means. For each site, means within columns followed by the same letter are not significantly different for transformed means (Tukey's, $P \leq 0.05$).

CHAPTER 5

TOXICITY OF INSECTICIDES TO *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) USING GLASS-VIAL BIOASSAYS

Abstract:

A scintillation glass-vial bioassay was used to test technical grade insecticides against the non-native stink bug, *Halyomorpha halys* (Stål). *Halyomorpha halys* is emerging as an important pest in the Mid-Atlantic States, especially in tree fruits and as a homeowner nuisance during the winter. Pyrethroid insecticides, especially bifenthrin, caused mortality against *H. halys* at low doses with LC_{50} values of $0.03 – 0.49$ (µg a.i./cm^2)(mg body mass^{-1}). Three nicotinoids were tested against adults with LC_{50} values ranging between $0.05 – 2.64$ (µg a.i./cm^2)(mg body mass^{-1}). Phosmet had LC_{50} values that were up to 3.6-fold higher than other classes of insecticides tested. Fifth instar *H. halys* were evaluated against selected chemicals and were generally susceptible at lower rates than the adults. Due to significant differences in weight, males and females were individually weighed, tested, and analyzed separately. Sex-related differences in susceptibility were found in the responses to thiomethoxam with males being less susceptible despite having a smaller body mass.

Introduction

Stink bugs are becoming increasingly abundant pests of a variety of crops, including soybean, cotton, and tree fruits (Leskey and Hogmire 2005, Snodgrass et al. 2005). In the eastern United States, the stink bug complex in tree fruits consists primarily of *Euschistus servus* (Say), *Euschistus tristigmus* (Say), and *Acrosternum hilare* (Say) (Hemiptera: Pentatomidae). Their feeding damage varies depending on the crop phenology when attacked. In peaches, early season feeding by stink bugs produces deformed or 'catfaced' fruit (Mundinger and Chapman 1932, Rings 1957), while feeding late in the development of fruit causes discolored necrotic tissue and may lead to dimpling and discoloration on or near the fruit surface. Similar late season damage occurs in apples and pears although it is easily mistaken for physiological disorders (Brown 2003). In soybeans, feeding by stink bugs during the R-IV and R-V stages of pod formation may result in deformed seeds and reduced oil yield. Regardless of the crop, stink bug feeding can introduce pathogens at the site of stylet insertion (McPherson and McPherson 2000).

Stink bugs have historically been managed using organophosphorus insecticides, however, changes in insecticide chemistries and U.S. Environmental Protection Agency decisions that limit or prohibit the use of this class of insecticides has led researchers to investigate other management options. A recently introduced species, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Hoebeke and Carter 2003), is the newest member of the stink bug pest complex in the Mid-Atlantic states, causing economic losses in apples and pears in New Jersey and Pennsylvania (A.L.N., unpublished data).

Populations have also become established in Oregon (2005), California (2006), Ohio (2007) and Mississippi (2007).

Halyomorpha halys is also a considerable homeowner nuisance, increasing the scope of interest in its insecticide susceptibility. As photoperiod and temperatures decline in September and October, adult *H. halys* move to overwintering sites. Unlike many stink bug species, the preferred overwintering location of *H. halys* is in man-made structures, such as houses (Watanabe et al. 1994, Hamilton et al. 2008). Large numbers of stink bugs congregating on the exterior or interior of houses have caused concern in residential areas and raised demands for finding management solutions for this pest. The chemicals used to control *H. halys* in agriculture may also be effective for control in and around homes, emphasizing the need to investigate reduced-risk insecticides. Overwintering populations in Japan were reduced with pyrethroid insecticide applications of permethrin, phenothrin, and cyphenothrin (Wantanabe et al. 1994). Previous success with pyrethroids against *H. halys* and changes in insecticide uses in the United States led us to test primarily this class of insecticide using a scintillation glass-vial bioassay. In addition, we evaluated one organophosphorus and three neo-nicotinoid insecticides. The glass-vial bioassay is a quick tool to determine insecticide susceptibility in the laboratory and has been successfully used to evaluate susceptibility of various pentatomid species to pyrethroid and organophosphorous insecticides (Willrich et al. 2003, Snodgrass et al. 2005). It is necessary to establish baseline mortality data for *H. halys* so that its susceptibility to insecticides can be monitored as it expands its geographic range. This is the first study on insecticide susceptibility for the United States population of *H. halys*

and one of the few laboratory investigations evaluating stink bugs against technical grade neo-nicotinoid insecticides.

Materials and Methods

Insect Source

Adult and immature *H. halys* were collected from July – October in 2005 and 2006 from the Rodale Working Tree Farm in Allentown, PA. The site is an organic arboretum containing native and exotic ornamental trees and shrubs that are maintained by the Rodale Corporation and has had a large population of *H. halys* since at least 2000. Ornamental trees and shrubs were sampled using a beating sheet ($71cm^2$, BioQuip Products Inc., Rancho Dominguez, CA) to dislodge specimens. Collected insects were held in BugDorm2 rearing cages (60 x 60 x 60cm, Megaview Science Education Services Co., Ltd. Taichung, Taiwan) and provided green beans and peanuts for a minimum of 24h before treatment.

Bioassay

A scintillation glass-vial bioassay was chosen to evaluate residual insecticidal activity on *H. halys*. This method allows for rapid dosing of large numbers of insects while using small amounts of product (Snodgrass 1996, Willrich et al. 2003, Snodgrass et al. 2005). Three classes of insecticides were evaluated; organophosphorus [phosmet (Gowan Company, LLC)], pyrethroid [β-cyfluthrin, cyfluthrin (Bayer CropScience), fenpropathrin (Valent U.S.A. Corporation), bifenthrin (FMC Corporation), λ-cyhalothrin (Syngenta)] and neo-nicotinoid [acetamiprid (Cerexagi, Inc.), dinotefuran (Valent U.S.A. Corporation), thiomethoxam (Syngenta)]. For each insecticide, a known quantity of

technical grade active ingredient was dissolved in acetone (Fisher Scientific®, Atlanta, GA) and serially diluted to desired concentrations. A minimum of five graduated doses for each chemical were assayed on at least two different dates with 10 treated vials per dose per insect group (male, female, or 5th instar). A minimum of 90 male, female, and 5th instar *H. halys* individuals were tested per chemical, with over 4000 individuals tested. Each individual was weighed before testing in order to correct insecticide dose for mean body mass. The limited availability of 5th instars prevented them from being tested with every insecticide used against the adults.

The glass-vial bioassay was prepared by pipetting 0.5ml of insecticide and acetone solution into each 20ml scintillation vial (Wheaton Science Products, Millville, NJ). Vials treated with acetone alone served as controls. To ensure an even insecticide residue, vials were rolled on a modified hotdog roller with the heat unit disconnected (Helman Group Ltd., Oxnard, CA) in a fume hood until the acetone evaporated. Individual insects were weighed (Denver Instruments APX-203$^{©}$, Denver, CO), and then randomly placed into a treated scintillation vial which was then plugged with cotton. The vials were laid on their side under ambient laboratory conditions (~25°C) and supplemented with fluorescent lighting that provided a 16L:8D photoperiod. Mortality and morbidity was checked at 24 and 48h. Morbidity was defined as the inability of the insect to cling to the side of the vial or the insect being unable to right itself when inverted. The pyrethroid mode-of-action causes an initial knockdown from which it may recover, during which the insect appears moribund. If an individual scored as 'moribund' at 24h recovered at the 48h observation, it was reclassified as 'alive'. This revised 24h

data set was used for Probit analysis. Mortality and morbidity data were combined for analysis.

<u>Data Analysis</u>

Mean body mass of males, females, and 5th instars for each insecticide concentration and replicate were calculated using the PROC MEANS statement and compared using PROC GLM, with Tukey's mean separation (SAS v.9.1.3) for each chemical evaluated. Insecticide concentration was then corrected for mean body mass [$(\mu g\ a.i./cm^2)(mg\ body\ mass^{-1})$] and the insect response at 24h was analyzed using Probit analysis (PoloPlus v1.0, LeOra software) for males, females, and 5th instars. Higher doses were excluded from analysis if 100% mortality had already been reached. For each chemical, the male/female/immature LC_{50} ratio was tested for significance according to Robertson and Preisler (1992) to determine differences at $P\geq0.05$. This was achieved by calculating the 95% fiduciary limits (F.L.), if the limit of the tolerance ratio at the LC_{50} includes 1.0, then there is no significant difference between the sexes or stages (Robertson and Preisler 1992).

Results and Discussion

Many insects exhibit sexual dimorphism with one sex, usually the female, being larger. The implications this has for pest management are rarely taken into consideration (Shearer and Usmani 2001). *Halyomorpha halys* females are significantly heavier than their male counterparts. Mean (±SE) weight for males (0.09±0.001g, n=1614) and females (0.12±0.001g, n=1631) was significantly different ($F_{1,\ 3244} = 615.54$, $P\leq0.0001$). To exclude any effects due to differences in body mass on susceptibility to insecticides,

the insecticide concentration per unit area of treated vials was corrected for mean body mass (μg a.i./cm^2)(mg body mass^{-1}) in our study for each sex, chemical, replicate, and dose. Generally, males had higher LC$_{50}$ values than females for most chemicals tested (Table 1). This difference was only significant for thiomethoxam ($P \leq 0.05$) and may indicate sex-related differences in response to this chemical.

Halyomorpha halys adults had lower LC$_{50}$ values to the pyrethroid insecticides than to the other chemicals(Table 1). Knockdown and recovery was observed for all pyrethroids tested. Overall, the LC$_{50}$ value for the organophosphorus insecticide, phosmet, was higher than for all neo-nicotinoids and pyrethroids evaluated, with phosmet having LC$_{50}$ values up to 3.6-fold higher than for bifenthrin. The LC$_{50}$ values estimated for *H. halys* using pyrethroid insecticides ranged from 0.03 to 0.49 (μg a.i./cm^2)(mg body mass^{-1}) for adults supporting reports that *H. halys* is more susceptible to pyrethroid than to organophosphorus insecticides (Wantanabe et al. 1994). Of the five pyrethroid insecticides tested, bifenthrin was most toxic to *H. halys* with an LC$_{50}$ value of 0.03 (μg a.i./cm^2)(mg body mass^{-1}) for both males and females. Native species of pentatomids demonstrate similar responses to bifenthrin, λ-cyhalothrin, and cyfluthrin (Willrich et al. 2003, Snodgrass et al. 2005). Those studies ranked λ-cyhalothrin as having the lowest LC$_{50}$ values whereas in our study, λ-cyhalothrin was ranked as the second most toxic pyrethroid. Differences in toxicity between the chemicals may be slight however, and laboratory evaluations are needed to compare susceptibility of *H. halys* with native species.

Our study is one of the first laboratory evaluations of technical grade neo-nicotinoids against pentatomid species and demonstrates that this class may be a suitable

chemical option for stink bug control in the field. Acetamiprid, dinotefuran, and thiomethoxam all had lower LC_{50} values than phosmet, with adult LC_{50} values ranging between 0.05 to 2.64 (μg a.i./cm^2)(mg body mass^{-1}). Consistent with field-trials of formulated products against native pentatomid species, *H. halys* was not as susceptible to low doses of acetamiprid when compared to the other neo-nicotinoids tested (Willrich et al. 2002, Herbert et al. 2006, Tillman 2006).

Fifth instar *H. halys* were tested against six insecticides; dinotefuran, acetamiprid, thiomethoxam, β-cyfluthrin, fenpropathrin, and λ-cyhalothrin. Generally, immature *H. halys* were significantly more susceptible to the insecticides tested when compared with adult response ($P{\leq}0.05$). This is consistent with results for *A. hilare*, *Nezara Viridula (Linnaeus)*, and *E. servus* nymphs observed elsewhere (Willrich et al. 2003). Since toxicity differences that could have been due to variations in body mass were excluded (when the insecticide dose was corrected for mass), any significant differences in susceptibility may be due to absorption rates resulting from the softer-bodied nymphs or decreased levels of detoxification enzymes. Dinotefuran and λ-cyhalothrin, however, were equally toxic to the adults and 5th instars. For reasons that are unclear, fenpropathrin was significantly more toxic to female *H. halys* than to the nymphs. Chi-square comparisons revealed that the responses did not deviate from the expected results for either adult or immature *H. halys* ($P{\leq}0.05$).

No significant differences were found between LC_{50} ratios for males and females and the y-intercept and slopes were equal for all chemicals evaluated except for thiomethoxam. The results for thiomethoxam yielded different slopes and intercept values for males, females, and nymphs, as well as having significantly different LC_{50}

ratios. Susceptibility to thiomethoxam was greater for *H. halys* females than males when doses were corrected for body mass, indicating that body mass is not a factor in the response to this chemical. As sex-related differences were not apparent for uncorrected concentrations (data not shown), we hypothesize that increased tolerance in males may be due to differences in detoxifying enzymes or target-site insensitivity. Additional research is needed to test these hypotheses.

Halyomorpha halys has substantially enlarged both its range and the local population density since its introduction (Hamilton et al. 2008). In certain crops, *H. halys* has established itself as the predominant stink bug species and we are finding high levels of stink bug damage in commercial apple and pear orchards (A.L.N. unpublished data). As *H. halys'* range continues to expand, we predict that management of this species will need to be incorporated into control programs for other stink bug pests. Field-testing of Danitol (fenpropathrin), Baythroid (β-cyfluthrin), Assail (acetamiprid), Imidan (phosmet) and Warrior (λ-cyhalothrin) against catfacing insects, including stink bugs, in New Jersey peaches showed that only Danitol significantly reduced late-season catfacing damage (Nielsen et al. 2007). However, the use of pyrethroids in an orchard system can disrupt IPM programs by causing a resurgence of phytophagous mites and scale insects post application (Croft 1990). As insecticides that do not disrupt IPM programs are especially desirable, some neo-nicotinoid insecticides may provide *H. halys* control with minimal impacts to biological control agents. Further field testing and research is required to evaluate field efficacy of these chemicals for *H. halys* management.

Acknowledgements

Table 1: Toxicity of selected insecticides to *Halyomorpha halys* from residual 24h exposure.

Insecticide	Sex or Stage	N	Slope ± SE	LC$_{50}$[a, b]	95% FL	χ^{2}[c]	df
Phosmet	Male	119	4.08 ± 0.98	12.24a	9.28 – 15.12	3.83	4
	Female	110	4.85 ± 1.45	10.23a	6.96 – 12.95	0.75	4
Dinotefuran	Male	110	1.62 ± 0.31	0.14a	0.02 – 0.50	3.80	3
	Female	100	1.60 ± 0.50	0.08a	0.02 – 0.16	1.73	2
	5th instar	210	1.22 ± 0.17	0.15a	0.04 – 0.49	7.76	4
Acetamiprid	Male	160	1.13 ± 0.22	1.62a	0.17 – 3.95	9.25	6
	Female	140	1.20 ± 0.21	2.64a	0.80 – 5.52	8.21	6
	5th instar	109	1.73 ± 0.56	0.09b	0.01 – 0.20	1.50	3
Thiomethoxam	Male	185	1.02 ± 0.16	0.13a	0.02 – 0.55	7.23	4
	Female	185	1.43 ± 0.22	0.05b	0.03 – 0.08	4.00	4
	5th instar	124	1.22 ± 0.27	0.02c	0.01 – 0.03	2.73	3
β-cyfluthrin	Male	100	2.27 ± 0.50	0.49a	0.11 – 0.95	8.67	6
	Female	90	4.64 ± 1.36	0.41a	0.26 – 0.54	3.26	5
	5th instar	120	1.77 ± 0.36	0.03b	0.02 – 0.05	0.53	4
Cyfluthrin	Male	130	1.38 ± 0.44	0.18a	0.02 – 0.41	0.45	4
	Female	129	1.81 ± 0.44	0.06a	0.02 – 0.11	1.83	4
Fenpropathrin	Male	103	1.04 ± 0.21	0.12bc	0.06 – 0.28	2.75	4
	Female	114	1.06 ± 0.20	0.06c	0.03 – 0.12	2.30	4

	5th instar	160	1.03 ± 0.19	0.20ab	0.03 – 1.02	7.79	4
Bifenthrin	Male	110	1.81 ± 0.45	0.03a	0.01 – 0.05	1.72	3
	Female	110	1.81 ± 0.42	0.03a	0.02 – 0.06	1.28	3
λ – cyhalothrin	Male	119	1.26 ± 0.29	0.12a	0.04 – 0.25	1.59	2
	Female	114	1.40 ± 0.26	0.06a	0.03 – 0.11	2.22	3
	5th instar	188	1.41 ± 0.28	0.12a	0.05 – 0.22	0.51	4

[a]LC_{50} values are in (micrograms A.I./cm^2)(insect weight $^{-1}$).
[b]For each chemical, LC_{50} values within a column followed by the same letters are not significantly different (Roberston and Priesler (1992), $P>0.05$).
[c]All chi-square values fit the model at $P>0.05$

CONCLUSION

When *H. halys* was first discovered in the United States, there were conflicting

ideas regarding the potential impact it would have (Bernon 2004). However, stink bugs

are emerging as significant pests in a variety of agricultural crops including tree fruit,

soybean and cotton (Riley et al. 1997, Bundy and McPherson 2000, Leskey and Hogmire

2005). The addition of another species to this complex could elevate damage and

complicate management programs.

The data presented here demonstrates that *H. halys* has become established, and

has spread throughout the mid-Atlantic region of the United States. Isolated populations

have also been detected in Mississippi, Ohio, Oregon, and California. In the mid-Atlantic

region, *H. halys* is univoltine, with adults emerging from reproductive diapause in mid-

April; reproduction begins in early June. Nymphs develop throughout the summer,

requiring 537.7DD to complete development. During this time, multiple host plants, in

different plant families are utilized. Evidence was seen for potential host switching by

nymphs indicating that nymphal dispersal may occur at specific phenological stages of

the host plant. However, it is unknown whether this is a plastic response spurred by host

plant volatiles, a physiological behavior partially driven by nutritional requirements, or

an artifact of differential survival on dofferent host plants. Adults utilize the same host

resources, preferring *P. tomentosa* and *C. arborescens* in ornamental plantings.

Populations were also found in agricultural crops such as soybean, apples and pears.

In the agricultural commodities sampled, the occurrence of *H. halys* populations

coincides with critical periods of fruit or pod development. In apple and pear, *H. halys*

caused the greatest amount of damage during the mid- to late-season growth periods. During these periods feeding by stink bugs causes white to brown corking or necrotic tissue that is evident by discoloration and depressions on the fruit skin (Rings 1957). *Halyomorpha halys* was present in soybean beginning at the R3 stage, with the highest densities occuring during the R5 stage. Damage by stink bugs during this stage can cause deformed pods, reduced oil yield, and stunted growth (Daugherty et al. 1964, McPherson 1996, Boethel et al. 2000, McPherson and McPherson 2000).

To identify effective pheromone traps for *H. halys* different trap designs were evaluated. The large pyramid trap proved to be the most efficient trap design at capturing nymphal and adult *H. halys* in both ornamental and soybean crops. The large pyramid trap was also an effective monitoring tool for detecting *H. halys* movement into soybean from surrounding areas.

To begin the process of development a management program for *H. halys*, laboratory bioassays examining the toxicity of various insecticides were conducted. These assays showed that pyrethroid and neo-nicotinoid insecticides caused mortality at much lower rates than the organophosphate tested. Males were significantly less susceptible than females when exposed to thiomethoxam, despite having lower body mass. These data provide the baseline information needed to begin the development management strategies targeting *H. halys*; however, before any strategies can be employed, the effectiveness of these insecticides for controlling field populations of *H. halys* needs to be evaluated.

For a non-native species to be considered invasive, it must either cause ecological or economic losses (Lockwood et al. 2007). While we did not quantify economic losses,

the damage caused by *H. halys* in a commercially managed apple and pear orchards with low populations caused damage at levels greater than 25% damaged fruit per tree. In another commercially managed orchard with high population levels damage approached 89% damaged fruit per tree. In both cases, the damage levels observed were high enough to cause economical damage pointing to the need for the development of a management plan for *H. halys*. The results presented here also suggest that the addition of another stink bug species to the current complex could have serious implications.

Invasive species can negatively affect native species through competition. The effect of *H. halys* on other species was not evaluated but for all hosts sampled, *H. halys* was the dominant pentatomid species found. Blacklight traps are an additional monitoring tool that can supplement beat sheet or sweep net sampling. At both Lichtenwalner Farms (Macungie, PA) and Peaceful Valley Farms (Pittstown, NJ) samples collected in blacklight traps also indicated that *H. halys* were the most abundant stink bug species.

Further research needs to be done to determine if this is due to competition, changes in natural enemy behaviors, or low levels of native stink bug populations. More importantly, *H. halys* has caused significant damage to commercial orchards and is the dominant species present (in the areas sampled) in New Jersey and Pennsylvania. By applying developmental rate data together with the life-history and occurrence of *H. halys* in the commodities evaluated, a phenological model incorporating various monitoring techniques and degree-day (DD) requirements can be applied. This model can be futher developed to forecast population dynamics and develop control programs for *H. halys* (Cullen and Zalom 2005).

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Spring 2007, 2008   Guest Lecturer, Agricultural Entomology & Pest Management "Invasive Insects" and "Insect Classification"

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- Responsibilities include instructing undergraduate and graduate students in insect taxonomy; Created a digital specimen database using specimens from Rutgers University Museum, emphasizing characters specific to each character using a 3-D stereoscope.

08/03- 05/06   Teaching Assistant, Division of Life Sciences, Rutgers University
- General Biology 101 and 102, Genetics Recitation (Spring 2005), Principles of Biology 103 (Fall 2005)
- Responsibilities include lecturing and conducting 2 lab sections per week, creating and grading quizzes, grading homework and lab reports, instructing students

11/03 – 07/03   Regulatory and Litigation Paralegal, McDermott, Will & Emery, Washington, DC

EXTENION/OUTREACH:
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- Nielsen, A. and G. Hamilton "Monitoring for the Brown Marmorated Stink Bug" www.njaes.rutgers.edu/stinkbug