HOST PLANT EFFECTS ON THE BIOLOGY, BEHAVIOR AND ECOLOGY OF BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (STÅL) (HEMIPTERA: PENTATOMIDAE)

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Host plant effects on the biology, behavior and ecology of brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)

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ABSTRACT

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a polyphagous, invasive, and highly significant pest of mid-Atlantic fruit orchards. Its capacity to invade orchards season-long is believed to be facilitated by the availability of wild host plants near orchards and the high dispersal capacity of adults and nymphs. Interactions between *H. halys* and its hosts were investigated via laboratory and field experiments. Rearing *H. halys* nymphs in the laboratory on several of its known wild and tree fruit hosts used alone and in combination showed that peach was much more suitable for nymphal development and survival than was apple, tree of heaven, or catalpa and that nymphal performance was enhanced on mixed host diets. Nymphs reared on different host plant diets yielded adults that differed in protein, sugar or lipid content, offering new insights into plant diet effects on *H. halys* nutrition. The majority of tree species identified from unmanaged woodlands next to apple orchards in Virginia and West Virginia were known hosts of *H. halys*. Passive trunk traps were developed to capture *H. halys* nymphs walking up and down tree trunks and used to measure their temporal patterns of movement onto and off of wild and tree fruit hosts at the orchard-woodland interface via weekly assessments during two consecutive seasons. More upward- than downward-walking nymphs were captured, which may be partially explained by the positively phototactic and negatively geotactic behavior of *H. halys* nymphs demonstrated in laboratory studies. Most nymphs captured while walking up and down trunks were second instars, although the data also reflected temporal changes in the distribution of instars and temporal variation in the numbers of nymphs captured. A field study to characterize injuries to apple and peach fruit at harvest from feeding by young or older nymphs early or late in the season revealed that feeding by young nymphs early in the season resulted in least injury to both crops. In combination, these studies greatly improve our understanding of the biology and ecology of *H. halys* in eastern fruit orchards and provide relevant data that will inform the development of ecologically-based management programs for it.

DEDICATION

To my family, friends and mentors who helped me
stand on the shoulders of giants

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CHAPTER 1: LITERATURE REVIEW AND RESEARCH JUSTIFICATION

Nomenclature. The brown marmorated stink bug (Hemiptera: Pentatomidae) was previously named *Pentatoma halys* Stål, *Poecilomes mistus* Uhler, *Dalpada brevis* Walker and *Dalpada remota* Walker (as cited in Hoebeke and Carter 2003). It has been confused with the Indian species, *H. picus* (Fabricius), and in Asia has been commonly referred to as *H. mista* or *H. brevis* (Rider et al. 2002, Lee et al. 2013a). Josifov and Kerzhner (1978) determined that only one species of *Halyomorpha* was present in eastern China, Japan and Korea, which was subsequently named *H. halys* by Rider et al. (2002).

Origins and Distribution. *Halyomorpha haly*s (Stål) is native to Japan, South Korea, China and Taiwan (Lee et al. 2013a), where it is considered an agricultural and a residential nuisance pest. It was detected in Allentown, PA in the mid-1990's (Hoebeke and Carter 2003) and this population was subsequently found to have originated from a single introduction from Beijing, China (Xu et al. 2014). In the early and mid-2000s, there were anecdotal detections and published reports (Hamilton 2009, Nielsen et al. 2013, Wallner et al. 2014) of overwintering *H. halys* populations in the mid-Atlantic region of the USA. In the latter half of that decade, tree fruit growers in some parts of that region reported increasing levels of stink bug injury to fruit at harvest, although this was not widely ascribed to *H. halys* at that time. During the unusually hot and dry summer that began in June, 2010 (www.ncdc.noaa.gov), a widespread outbreak of *H. halys* in much of the mid-Atlantic became apparent by July, resulting in severe economic injury and losses of orchard crops at harvest (Leskey et al. 2012a).

Beginning in fall 2010, *H. halys* was detected in many other US States. Currently in North America, established populations or detections have been reported from 41 states, the District of Columbia and two Canadian provinces (see http://www.StopBMSB.org), ranging from Ontario to Florida east of the Mississippi River, Idaho to Arizona east of the Rocky Mountains, and from California to Washington on the Pacific coast. Of these, 16 states have reported only detections, 9 have reported that *H. halys* has become a nuisance pest, 8 have indicated that it is both an agricultural and a nuisance pest, and 8 consider it to be a severe agricultural and nuisance pest, including most of the mid-Atlantic states. Internationally, established populations have also been reported from Canada (Gariepy et al. 2014), Italy (Cesari et al. 2015), Hungary (Vetek et al. 2014), Switzerland (Wermelinger et al. 2008), and Greece (Milonas and Partsinevelos 2014), and while it has been intercepted at ports of entry in New Zealand (Harris 2010), no established populations have been reported to date.

Zhu et al. (2012) used global climate comparisons and ecological niche modeling to examine the invasion biology of *H. halys* and to predict its geographic distribution and showed that northeastern North America, northern Europe, and some parts of Australia and New Zealand are at high risk. Recent evidence suggests that successful establishment of *H. halys* may be associated with the effects of temperature and photoperiod interactions on female reproductive maturity (Nielsen and Walgenbach, unpublished). On a regional scale, a study on the invasion dynamics of *H. halys* in New Jersey using black light traps from 2004 to 2011 has shown that its establishment and spread are influenced by landscape factors (Wallner et al. 2014). Its initial establishment was associated mainly with urban/residential developments and railroads while subsequent expansions (2-3 years after establishment) were associated with urban, forest and wetland habitats.

Morphological Descriptions and Life History. *Halyomorpha halys* egg masses (Fig. 1.1A) are most commonly found on the underside of leaves on tree hosts and contain an average of 28 eggs (Kawada and Kitamura 1983, Nielsen et al. 2008a). Hoebeke and Carter (2003) provided morphological descriptions for all immature stages of *H. halys*. Eggs are approximately 1-1.6 mm in diameter and the chorion is white with fine reticulations and minute spines. At 25°C, nymphal eclosion typically occurs at ~6 d following oviposition (Nielsen et al. 2008a). There are five nymphal instars. First instars (~2.4 mm long) (Fig. 1.1B) aggregate on and around the egg mass for several days, where they feed to obtain gut symbionts necessary for their survival (Taylor et al. 2014). Upon molting to the second instar (~3.7 mm long), nymphs disperse from the egg mass and begin foraging for and feeding on plant tissues. Third instars (~5.5 mm long) may be differentiated from second instars by the white band on the tibia of each leg (Fig. 1.1C) (Hoebeke and Carter 2003). Aside from their different size, third and fourth instars (~8.5 mm long) are similar in appearance (Fig. 1.1C), and fifth instars (~ 12 mm long) can be distinguished by their larger size and prominent wing pads (Fig. 1.1C) (Hoebeke and Carter 2003).

Adult *H. halys* are 12-17 mm long with a mottled grayish-brown and shield-shaped dorsum, white bands on the antennae and legs, and alternating black and white coloration on the margins of the abdominal dorsum (Fig. 1.1C) (Hoebeke and Carter 2003). The ventral surfaces are whitish with black or gray markings (Fig. 1.1D). Males are generally smaller than females and the sexes can be distinguished by the presence of claspers or genital plates on the last ventral abdominal segment in males and females, respectively (Fig. 1.1D).

Dispersal of adult *H. halys* from host plants to overwintering sites increases gradually during September, but is most pronounced between September 21 and about mid-October and then subsides markedly. Adults overwinter in dark, dry, and tight spaces in natural sites and human-made structures and are commonly observed in aggregations of several to many thousands of individuals. In the mid-Atlantic Region, Lee et al. (2014a) found overwintering aggregations under flaky bark and within decomposing wood on standing dead trees that were most often oak (*Quercus* spp.) or locust (*Robinia* spp.). Overwintering adult females are reproductively immature and unmated (Nielsen and Hamilton 2009).

Emergence of adults from overwintering sites may begin in April, but peaks between mid-May and early June (Bergh and Leskey, unpublished). The early emergence peaks of appear to be associated with brief periods of warm weather, while the later, larger, and more prolonged peak of emergence appeared to be associated primarily with photoperiod (Bergh and Leskey, unpublished). Upon emergence, adults forage among and feed on plant hosts to acquire nutrients for reproduction (Nielsen, unpublished). In Asia, female reproductive maturity and ovarian development occurred when the photophase was >13 h (reviewed in Lee et al. 2013a).

Research in the eastern USA has indicated that *H. halys* is univoltine in New Jersey and Pennsylvania (Nielsen et al. 2008a) and bivoltine in West Virginia, Virginia, and North Carolina (Bakken et al. 2015, Leskey et al. 2012b). In West Virginia, oviposition by overwintered females begins between late May and mid-June and F1 adults were present by mid-July (Leskey et al. 2012b). F2 eggs were observed between mid- and late July. Completion of development of this generation to the adult stage occurs by mid-September and it is considered highly likely that the spring and summer generations of nymphs and adults overlap both temporally and spatially.

Pest Status and Host Plants in the Mid-Atlantic Region. Since its widespread outbreak in the mid-Atlantic region in 2010, *H. halys* has been a significant pest in many agricultural and horticultural crop systems, including fruit orchards, vegetables, row crops, small fruit, grapes, and ornamentals (Leskey et al. 2012a). Indeed, the estimated loss to the value of the apple crop in 2010 was $37 million (American/Western Fruit Grower 2011) and some Maryland growers experienced up to 100% loss of their peach crop that year (Leskey et al. 2012a). In the intervening years, data from *H. halys* monitoring tools and tactics have shown that its annual populations in the mid-Atlantic region have varied considerably in size. It is thought that abiotic factors, such as the effects of extreme winter temperatures on overwintering adult mortality (Cira et al. 2015) and/or cool spring and summer conditions, may be largely responsible for this variation.

Like other phytophagous pentatomids, *H. halys* injures crops via injecting enzymes which digest the tissue surrounding the feeding site, enabling them to imbibe the liquefied tissue (McPherson and McPherson 2000, Peiffer and Felton 2014). Feeding injury on tree fruit is expressed as discoloration, depressions, or deformations on the surface and discrete areas of internal necrosis (Joseph et al. 2015, Leskey et al. 2012c) (see Chapter 5). When injury occurs early in peach development, fruit abscission may occur (Nielsen and Hamilton 2009). *H. halys* also feeds on and injures orchard crops with hard, thick shells, such as hazelnuts (Hedstrom et al. 2014).

The main factors underlying the great economic risks from *H. halys* to numerous crops are; 1) its highly polyphagous nature, 2) the extended period over which it can pose a threat annually, 3) its strong capacity to disperse among hosts and habitats, and 4) unlike native pentatomids, the fact that both nymphs and adults can feed on and injure crops. Rice et al. (2014) noted that *H. halys* has been reported to utilize >100 plant species as reproductive and/or feeding hosts, including many cultivated plants as well as wild hosts growing in managed and unmanaged landscapes. Consequently, *H. halys* is considered a pest on a landscape scale and is therefore fundamentally different from other less polyphagous agricultural pests that utilize fewer hosts and are constrained by host suitability or acceptability. Although it can feed on both reproductive and vegetative structures of plants, such as stem and leaves (Hoebeke and Carter 2003, Martinson et al. 2013), a survey of ornamental trees in Maryland nurseries by Martinson et al. (2015) found highest populations of *H. halys* nymphs and adults associated with species bearing mature fruit, suggesting a preference for fruiting hosts. An extensive survey in urban, rural and forested areas in the eastern US by Bakken et al. (2015) revealed that among the 78 shrub and tree species examined, *H. halys* egg masses, nymphs, and adults were found on 34 species, while only nymphs and adults were found on 41 species and none were found on 3 species. Some of the species on which all life stages were found included tree of heaven (*Ailanthus altissima* Mill. Swingle), catalpa (*Catalpa speciosa* Warder), paulownia (*Paulownia tomentosa* Thunb.), and cherry (*Prunus* sp.), all of which grow in unmanaged woodlands surrounding agricultural and horticultural fields in the mid-Atlantic region.

The numerous potential hosts of *H. halys* in these unmanaged woodlands likely serve as an important reservoir for this pest, increasing the risk of its season-long invasion into nearby crops. Indeed, several studies have documented its prolonged annual threat to orchard crops (Nielsen and Hamilton 2009, Leskey et al. 2012c, Joseph et al. 2015). As well, studies in Asia have shown that survival of *H. halys* nymphs and the fitness of resultant adults was enhanced when nymphs developed on a diet of multiple hosts (Funayama 2006) and that some economically important hosts (e.g. apple) were suboptimal for nymphal development (Funayama 2004). These findings suggest that *H. halys* nymphs may require nutrients from different host plants for optimal survivorship and development, possibly explaining to some extent its highly polyphagous nature and considerable dispersal capacity.

There is strong evidence for the high dispersal capacity of both *H. halys* nymphs and adults. Flight mill studies demonstrated that foraging adults flew longer distances, particularly in late spring, compared with overwintering adults (Lee and Leskey 2015). In general, foraging adults were capable of flying up to 117 km in 22 hr, while overwintering adults flew up to 34 km (Lee and Leskey 2015) with average distances between 1-2 km (Wiman et al. 2014, Lee and Leskey 2015). Adult flight behavior was affected by wind speed and temperature; more adults took flight under still-air and higher ambient temperature conditions (Lee and Leskey 2015). In the laboratory, *H. halys* nymphs walked up to 41 m in 1 hr and up to 8 m in 15 min on smooth horizontal and vertical surfaces, respectively (Lee et al. 2014b). In the field, marked nymphs walked 20 m to pheromone-baited traps deployed in a mowed grass field within 4-5 h (Lee et al. 2014b). As with adult flight behavior, nymphal walking capacity was also affected by temperature; nymphs walked longer distances at temperatures >25°C.

Lastly, economic risks to the agricultural sector from *H. halys* are exacerbated by the fact that both nymphs and adults can feed on and injure crops. Studies have characterized nymphal feeding injury on soybean (Owens et al. 2013, Koch and Rich 2015), cotton (Kamminga et al. 2014), blueberry (Wiman et al. 2015), and vegetables (Kuhar et al. 2012a, 2015) but the contributions of each life stage to orchard crop injury has not been compared or contrasted systematically.

Monitoring *H. halys*. Khrimian et al. (2014) identified the *H. halys* aggregation pheromone, a 3.5: 1 mixture of (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol, and Weber et al. (2014) showed that methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT) used in combination with the pheromone synergized captures in traps. Monitoring *H. halys* presence and relative abundance in orchard systems is currently focused primarily on the use of black pyramid traps baited with the pheromone-synergist combination (Leskey et al. 2012b,c, Leskey et al. 2015a). This tool has yielded season-long captures of both nymphs and adults (Leskey et al. 2015a), revealed that *H. halys* population density peaks between late August and mid-September (Weber et al. 2014, Leskey et al. 2015a), and has been used to develop *H. halys* management thresholds in apple orchards (Leskey and Short, unpublished). Black-light traps have been used to monitor *H. halys* populations and its distribution in New Jersey (Nielsen et al. 2013) and black pyramid traps augmented with light sources (Leskey et al. 2015b) also have been evaluated. Direct sampling procedures (i.e. sweep net, limb jarring, visual surveys) have been less effective in fruit orchards (Leskey et al. 2012c). Ongoing efforts are focusing on refining the *H. halys* trap (Morrison et al. 2015a) and lure formulation, toward a standardized monitoring tool that will be most practical and readily adopted by growers.

Managing *H. halys* in Tree Fruit Orchards. Following the *H. halys* outbreak in 2010 and in the absence of alternative management tactics or strategies, the initial response of mid-Atlantic tree fruit growers to the threat from this pest was to increase their reliance on insecticide-based control. Leskey et al. (2012c) reported that many growers increased their frequency of insecticide applications up to four-fold in 2011. Prior to 2010, one laboratory study compared the effectiveness of different insecticides against *H. halys* nymphs and adults (Nielsen et al. 2008b). After the 2010 outbreak, efforts to find effective chemistries against *H. halys* were heightened, using techniques such as treated glass surfaces (Leskey et al. 2012d, Lee et al. 2013b), bean-dip bioassays (Kuhar et al. 2012b) and topical applications (Krawczyk, unpublished). Most of the currently-registered insecticides for use in fruit orchards that were shown to be effective against *H. halys* are in the pyrethroid, neonicotinoid, and carbamate classes. Leskey et al. (2014)

conducted trials examining the efficacy and residual effectiveness of insecticides in peach and apple orchards and showed that the residual activity of most products decreased within 3 d of application. As well, they showed higher mortality of adults that had emerged from overwintering sites than of summer generation adults. Nielsen et al. (2008b) and Kuhar et al. (2012) reported that nymphs were more susceptible to insecticides than adults.

Many of the most effective products for *H. halys* management have broad-spectrum effects on arthropods and their increased use in the post-bloom period in fruit orchards has disrupted established IPM programs significantly. In apple orchards, outbreaks of secondary pests such as European red mite, San Jose scale and woolly apple aphid became increasingly common. Due to these negative repercussions from *H. halys* management programs, more ecologically and environmentally sustainable tactics have been explored, including organic insecticides (Lee et al. 2014c), microbial insecticides (Gouli et al. 2012, Parker et al. 2015), and the potential role of native natural enemies in *H. halys* biocontrol.

Numerous egg parasitoids have been shown to attack *H. halys* in its native Asian range, including species in the genera *Trissolcus*, *Anastatus* and *Telenomus,* which have been reported to cause maximum parasitism rates of 63-80 %, 77 %, and 85 %, respectively (reviewed in Lee et al. 2013a). Among these, *Trissolcus japonicus* (Ashmead) (=*halyomorphae* Yang) is considered the most effective, being the predominant species with >10 generations per year and accounting for 50% average annual parasitism rate in Chinese fruit orchards (Yang et al. 2009). In the US, numerous native parasitoids (e.g. *Trissolcus* spp., *Telenomus podisi*, *Anastatus reduvii, Trichopoda pennipes*) and predators (e.g. *Bicyrtis quadrifasciatus*, *Astata unicolor*) were found to attack *H. halys* (Biddinger et al. 2012, Rice et al. 2014), although their capacity to suppress its populations below economic levels has been limited. For example, overall egg parasitism rates reported from different agriculture systems have ranged from <1% to 11% (Rice et al. 2014). The detection of *T. japonicus* from sentinel *H. halys* egg masses deployed at Beltsville, MD in 2014 represented an important potential long-term solution to biological control of *H. halys* in the US (Talamas et al. 2015). Expanded surveys using sentinel egg masses in 2015 resulted in additional detections in Maryland, the District of Columbia, Virginia, and Washington.

Basic research on *H. halys* biology has yielded important insights into its genomics (Loannidis et al. 2014, Sparks et al. 2014), the relevance of bacterial endosymbionts to its development (Taylor et al. 2014), and the salivary proteins involved in the formation of its feeding sheath (Peiffer and Felton 2014). Coupled with the development of an RNA interference tool (Mittapelly and Michel, unpublished), these novel findings may inform the development of other novel management approaches for this invasive pest.

***Halyomorpha halys* as a Perimeter-Driven, Arboreal Pest.** *Halyomorpha halys* is not known to reside permanently in any cropping system; rather it is a perimeter-driven pest. Annual invasion is by individuals that move into crops from host plants growing at or near the perimeter of cultivated land. The timing of such movements into crops may be associated with specific stages of crop development, such as in corn or soybeans (Venugopal and Dively 2015), or as is considered the case in orchards, may occur on an on-going basis for much of the fruiting period. In Asia, *H. halys* is considered primarily an arboreal pest (Lee et al. 2013a) and the many potential wild tree hosts growing adjacent to vulnerable crops likely contribute significantly to the development and size of local populations and to the risk of crop invasion and economic injury. In the US, more crop injury and higher bug populations have been reported at the edges of cultivated fields adjacent to unmanaged woodlands than in the interior of fields (Leskey et al. 2012c, Venugopal et al. 2015). In apple orchard blocks, more fruit on trees on border rows adjacent to woodlands incurred feeding injury from *H. halys* than fruit in the block interior and more fruit from the upper canopy were injured than fruit from the mid- or lower canopy (Joseph et al. 2014). Pheromone-baited trap transects that extended from woodlots into apple orchards revealed highest captures of adults and nymphs at the orchard and woodland edges (Bergh and Leskey, unpublished data). Thus, perimeter-based management tactics against *H. halys* have become an important research area and efforts to evaluate the effectiveness of targeted sprays only to orchard border rows (Blaauw et al. 2015) or only to pheromone-baited trees at intervals along the orchard border, known as 'attract-and-kill' (Morrison et al. 2015b), are ongoing. In support of these efforts, it is therefore imperative to gain a better understanding of the ecology and behavior of *H. halys* at the orchard-woodland interface and the factors that influence its populations and pest status.

Justification for Research

Host plants have broad effects on a range of biological parameters of herbivorous insects, including but not limited to development, survivorship, reproduction, behavior, and ecology

(Tsai and Wang 1996, Hunter and McNeil 1997, Awmack and Leather 2002, Mirmohammadi et al. 2009). For polyphagous pentatomid pests of economically important crops, understanding how host plants affect these biological parameters is directly relevant to obtaining pertinent information about their basic biology, which may, in turn, inform the development of ecologically- or behaviorally-based management options. In particular, research on host plant effects on species that utilize both cultivated and wild hosts has important implications for understanding their movement in the landscape, host use patterns, and the susceptibility of economic crops to attack (Velasco and Walter 1993, Velasco et al. 1995, Panizzi 1997, Panizzi 2000, Silva and Oliveira 2010, Panizzi and Parra 2012). This is especially relevant to recently invasive species, for which there are often key knowledge gaps regarding their biology and ecology in the invaded range, as is the case for *H. halys* in the mid-Atlantic USA.

The establishment of *H. halys* in mid-Atlantic orchards and increasing populations in other fruit production regions in North America and abroad has become a major concern for orchardists. Its highly polyphagous nature, great propensity for dispersal, and the availability of wild hosts near or adjacent to orchards facilitate its ability to invade orchards season-long. There remain some important knowledge gaps before a comprehensive picture of the basic biology and ecology of *H. halys* can be developed, toward the potential for improved management capabilities. For example, we lack an understanding of the relative suitability of individual wild and cultivated host plants at the orchard-woodland interface for *H. halys* development and survivorship. Similarly, the effect of mixed diets of these hosts on *H. halys* development and survival has not been examined. Such information is directly relevant to a deeper understanding of the highly dispersive nature of *H. halys* adults and nymphs. In Chapter 2, the results of experiments examining the relative suitability of vegetative and reproductive tissues from single and mixed diets of selected wild and tree fruit hosts on *H. halys* development, survival and fitness are reported. In an effort to quantify the effects of host plant diet on *H. halys* nutrition, novel information on the nutrient content of adults that developed from nymphs reared on the different diet treatments is provided in Appendix I.

The walking dispersal of *H. halys* nymphs among different potential tree hosts growing near one another at the orchard-woodland interface has not been studied. Such information can enhance our understanding of nymphal dispersal trends during the season, and may help to predict the timing of its invasion of orchards and periods of increased pest pressure which are relevant to developing temporally and spatially precise pest management programs. To monitor and quantify this movement in the field, Chapter 3 reports experiments that evaluated and compared several traps designed to intercept nymphs walking up and down tree trunks. Chapter 4 presents the results of a census of the tree species growing in unmanaged woodlands surrounding orchards in Virginia and West Virginia and a two-year field study in which the superior traps for monitoring nymphal movement onto and off of trees were used to measure temporal and directional patterns of nymphal movement on selected fruit and wild tree hosts.

Last, although injury to apples and peaches from feeding by adult *H. halys* has been well characterized, the effects of nymphal feeding have not. The relative vulnerability of apples and peaches to injury from feeding by different stages of *H. halys* at different points in the growing season may have important implications for its management, particularly with respect to the need for and timing of intervention. In Chapter 5, injury to apple and peach fruit at harvest from feeding early and later in the season by early instars, late instars, and adults was characterized, quantified and compared.

Research Objectives

Objective 1. Determine the effect of single and mixed diets of selected fruit and wild tree hosts on the development and survivorship of *H. halys* nymphs and the nutrient content of resultant adults

Objective 2. Evaluate and compare trunk traps for monitoring the upward and downward movement of *H. halys* nymphs on host trees

Objective 3. Census the tree species growing in unmanaged woodlands adjacent to orchards in West Virginia and Virginia

Objective 4. Monitor the temporal and directional patterns of movement of *H. halys* nymphs on selected fruit and wild tree hosts at the orchard-woodland interface

Objective 5. Characterize and quantify the injury to apples and peaches at harvest from early- and late-season feeding by different *H. halys* instars

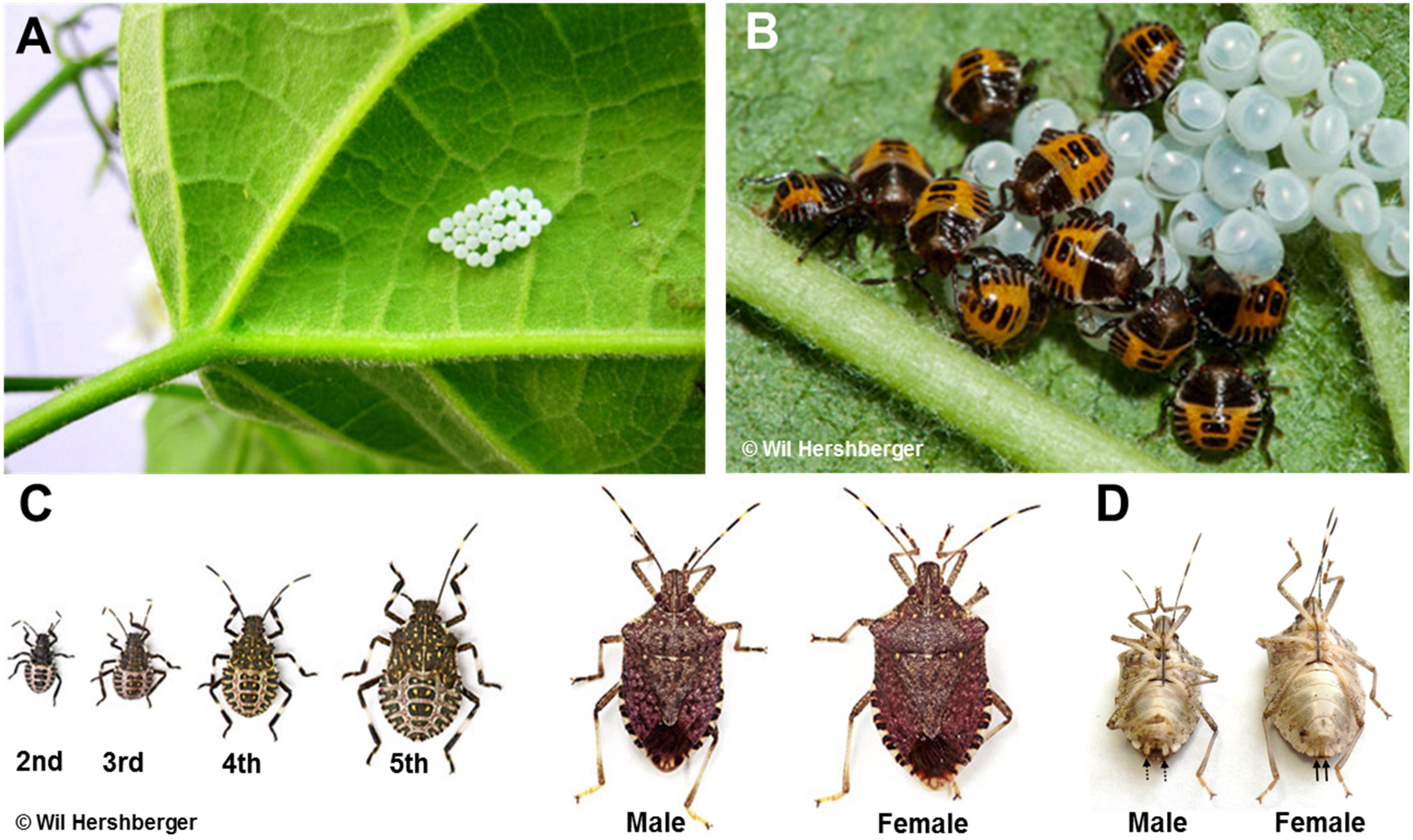

Figure 1.1. Developmental stages of *H. halys*; (A) Egg mass on the underside of a catalpa leaf, (B) First instars clustered around a mass of newly-hatched eggs, (C) Four nymphal instars and adults of *H. halys,* (D) Ventral view of *H. halys* adults. Dashed arrows point to the claspers on the male abdomen and solid arrows indicate the genital plates on the female abdomen. Images B and C used with permission from Will Hershberger.

CHAPTER 2: HOST PLANT EFFECTS ON *HALYOMORPHA HALYS* NYMPHAL DEVELOPMENT AND SURVIVORSHIP

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Acebes-Doria, A. L., T. C. Leskey, and C. J. Bergh. 2016. Host plant effects on *Halyomorpha halys* (Hemiptera: Pentatomidae) nymphal development and survivorship.

Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a highly polyphagous, invasive species and an important pest of orchard crops in the US. In the mid-Atlantic region, wild hosts of *H. halys* are common in woodlands that often border orchards and *H. halys* movement from them into orchards poses ongoing management issues. To improve our understanding of host plant effects on *H. halys* populations at the orchard-woodland interface, nymphal survivorship, developmental duration, and adult fitness (size and fresh weight) on apple (*Malus domestica* Borkh.), peach (*Prunus persica* (L.) Batsch), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), and northern catalpa (*Catalpa speciosa* (Warder)) were examined in laboratory studies. Specifically, I investigated nymphal performance on the foliage and fruiting structures of those hosts and on single versus mixed host diets, as well as the effects of host phenology on their suitability. Nymphal performance was poor on a diet of foliage alone, regardless of host. When fruiting structures were combined with foliage, peach was highly suitable for nymphal development and survivorship, while apple, tree of heaven and catalpa were less so, although nymphal survival on tree of heaven was much greater later in the season than earlier. Mixed host diets yielded increased nymphal survivorship and decreased developmental duration compared with diets of suboptimal single hosts. Adult size and weight were generally greater when they developed from nymphs reared on mixed diets. The implications of my results to the dispersal behavior, establishment and management of *H. halys* are discussed.

Key words: host plant suitability, brown marmorated stink bug, mixed diet, polyphagy

Introduction

Plant species vary widely in their suitability as food for polyphagous insect herbivores (Scriber 1984) and can affect insect development and survival differently via their chemical (e.g. nutritional quality, allelochemicals) or physical (e.g. trichomes, tissue hardness) characteristics, which may vary with plant age and phenology (Bernays and Chapman 1994). Polyphagous insect herbivores may complete development on a single host but their fitness is generally enhanced when they feed and develop on multiple plant species (Bernays et al. 1994, Hägele and Rowell-Rahier 1999, Miura and Ohsaki 2004). For example, the polyphagous hemipteran, *Bemisia tabaci* (Gennadius), exhibited higher survivorship and increased fecundity on a mixed diet of cotton, cucumber, tomato, cabbage and kidney beans than on any of those plants alone (Zhang et al. 2014). Earlier studies found that fitness advantages of mixed diets were associated with nutritional complementarity and/or the dilution of allelochemicals (Bernays et al. 1994, Hägele and Rowell-Rahier 1999).

Research on the relative suitability of plant species to polyphagous insect pests that utilize both cultivated and wild hosts has important implications for understanding aspects of pest biology and ecology, such as their movement in the landscape, host use patterns, and population dynamics, as well as the susceptibility of economic crops to attack (Panizzi 1997). Such studies have yielded beneficial information about basic pest biology, informed the development of ecologically-based pest management options (Panizzi and Parra 2012), and are especially relevant to recently invasive economic pests, about which there are often important knowledge gaps regarding their biology and ecology in the invaded range. The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a classic example of this.

Halyomorpha halys is an invasive pest from Asia that did not become a major pest until the late 2000's (Leskey et al. 2012a), following its initial detection in Allentown, PA about a decade earlier (Hoebeke and Carter 2003). Since its widespread outbreak in the mid-Atlantic region of the US in 2010, *H. halys* has caused significant economic damage to various fruit, vegetable and field crops (Rice et al. 2014). Its effects in mid-Atlantic fruit orchards have been especially pronounced via reductions in fruit yield and quality and changed pest management practices (Leskey et al. 2012b) that have resulted in secondary pest outbreaks. *Halyomorpha halys* is reported to utilize well over 100 plant species as feeding or reproductive hosts (Rice et al. 2014), many of which are deciduous trees that grow in forested areas that often border commercial orchards in this region (Bakken et al. 2015). The abundance of wild hosts in these woodlands, the development of large *H. halys* populations on some, and the high dispersal capacity of *H. halys* adults and nymphs (Lee et al. 2014, Wiman et al. 2014, Lee and Leskey 2015) combine to create pest pressure in commercial orchards through most or all of the fruiting period (Joseph et al. 2015). Moreover, Funayama (2006) showed that fitness of *H. halys* nymphs was positively affected when they developed on a mixed diet.

Investigation of the effects of different wild and cultivated host plants on *H. halys* development and survivorship in its invaded range should further our understanding of their relative contributions to local *H. halys* population densities and the risk to economic crops and may aid the development of ecologically-based pest management tactics against this important pest. Here, I report laboratory experiments that examined the effects of selected wild and tree fruit hosts in VA on the survivorship and developmental duration of *H. halys* nymphs and aspects of adult fitness (size and fresh weight). Specifically, the suitability of apple (*Malus domestica* Borkh.), peach (*Prunus persica* (L.) Batsch), tree of heaven (*Ailanthus altissima* (Mill.) Swingle), and northern catalpa (*Catalpa speciosa* (Warder)) was examined, focusing on vegetative and reproductive structures, the effects of single versus mixed host diets, and changes in host suitability during the growing season.

Materials and Methods

Insects. Adult male and female *H. halys* collected from natural overwintering aggregations in northern Virginia in April 2012 and February 2013 were placed in black, plastic bags with crumpled newspaper and held in a dark room at 4°C at Virginia Tech's Alson H. Smith, Jr. Agricultural Research and Extension Center (AHS AREC), Winchester, VA. In mid-April of each year, ~30 male and ~30 female adults collected during the same year were placed in each of several 30.48 cm^3, screened cages (BioQuip Products, Inc., Rancho Dominguez, CA) in a laboratory room at ~25°C, ~70% RH, and a photoperiod of 16L: 8D provided by overhead banks of 34 watt fluorescent lights (Ace Hardware Corp., Oak Brook, Illinois). For the experiment that began in August 2013, fifth instars and adult *H. halys* were collected from the field in July and reared under the same conditions. Cages were provisioned regularly with popcorn kernels, barley, buckwheat, soybeans, dried figs, dry roasted, unsalted peanuts, sundried tomatoes and water. Oviposition substrates in each cage included paper towel on the cage floor and three to four freshly excised compound leaves of tree of heaven in a water-filled vase. Egg masses produced between late May and early June and in early August were used in the early- and late-season experiments, respectively. Female *H. halys* most often deposit eggs in clutches of 28 (Nielsen et al. 2008). Egg masses (≤ 1 d old) were removed *in situ*, held in groups of five in 100 mm x 15 mm Petri dishes (Thermo Fisher Scientific Inc., Pittsburgh, PA) in the same room, and monitored daily for hatch. Those that hatched within a 5-d period and had ~28 first instars were assigned to the diet treatments. First instar *H. halys* aggregate around and on the empty egg mass prior to dispersing (Taylor et al. 2014) and thus were easily transferred to cages as cohorts from each egg mass.

Host Plant Sources. ‘Smoothee Golden’ apple and ‘Redhaven’ peach trees growing at the AHS AREC were the sources of cultivated host plant material. These trees were treated with fungicides, but not insecticides, during the growing season. Northern catalpa (hereafter referred to as catalpa) and tree of heaven growing on or near the AHS AREC property were selected as the wild hosts, based primarily on their inclusion in the host list for *H. halys* (see StopBMSB.org) but also on reports (Bakken et al. 2015) and anecdotal observations of large populations of *H. halys* nymphs and adults on both species and the results of a census showing that tree of heaven was the most common deciduous tree species growing at the edge of woodlands adjacent to orchards in this region (Acebes-Doria, unpublished data). Like *H. halys*, tree of heaven is an invasive species from Asia (Kowarik and Säumel 2007) and catalpa is native to eastern North America (see http://dendro.cnre.vt.edu).

Freshly excised foliage and reproductive structures (flowers, fruit or seed pods) of apples, peaches, tree of heaven and catalpa were offered to nymphs. The reproductive structures used reflected the stage of development of each host plant in the field at the time each experiment was conducted. From early- to mid- June, immature apple and peach fruit (3-4 per replicate) and foliage, tree of heaven flowers and foliage, and catalpa flowers and foliage were used. The volume of tree of heaven and catalpa flowers offered was comparable to the total volume of the apple and peach fruit offered. Later in the season, 1-2 larger apple and peach fruit and foliage were offered. At that time, three catalpa seed pods (10.16 – 12.70 cm long) and foliage and tree of heaven samaras and foliage were offered. The volume of seedpods and samaras offered was approximately the same as the other treatments.

The plant materials were washed thoroughly under running tap water to remove contaminants (e.g. fungicide residues, other arthropods, etc.). The foliage offered to nymphs included three terminal twigs of apples and peaches with 6-8 leaves (20.3 – 25.4 cm long), two apical branches of catalpa with 2-3 leaves (15.24 – 20.32 cm long) and three compound leaves of tree of heaven (25.4 – 30.5 cm long). All shoots offered, including those with flowers, samaras or catalpa pods, were inserted into 2-4 holes (8 mm diameter) in the plastic lid of plastic containers (8 cm height, 115 diam) containing water, while peach and apple p were placed on the cage floor. All plant tissues were replaced 2-3 times per week.

Single Host Diets. A completely randomized design with two factors, host plant species and plant tissue (foliage versus foliage plus reproductive structures), was used in an experiment between late May and early August, 2012. Cohorts of first instars, each with ~28 individuals, were assigned individually to seven replicates of each diet treatment. Each cohort of first instars on the egg mass was placed on or near the plant material inside a 30.5 cm^3 cage (BioQuip Products, Inc, Rancho Dominguez, CA) with *ad libitum* access to the food source and water. Water was provided in a plastic container with a cotton wick inserted through the lid. Cages were inspected daily for the presence of exuviae, which indicated a molt between instars, and the instars were identified following Hoebeke and Carter (2003). As well, the number of live and dead nymphs and live adults were recorded. Stage-specific survivorship and nymphal developmental rate were recorded for each diet treatment.

Single versus Mixed Host Diets Early and Late in the Growing Season. The onset of these experiments coincided with two key points in the seasonal phenology of *H. halys* populations in the mid-Atlantic region. Peak emergence of *H. halys* adults from overwintering sites occurs between ~mid-May and early June (Bergh and Leskey, unpublished data) and F_1 generation adults are estimated to be reproducing in early to mid-August based on degree-day accumulations (Leskey et al. 2012c, Bakken et al. 2015). Experiments initiated in late May (early season) and mid-August, 2013 (late season) included the following treatments in a completely randomized design with host plant as the factor; 1) apple, 2) peach, 3) tree of heaven, 4) catalpa, 5) apple plus tree of heaven, 6) apple plus tree of heaven plus peach and, 7) apple plus tree of heaven plus peach plus catalpa. Host plants in the mixed diet treatments were provided all at once. Hereafter, we refer to the diet treatments that consisted of three or four hosts as the 3-host and 4-host diet, respectively. All treatments included foliage and fruiting structures and each treatment was replicated 4 times.

As in the previous experiment, cohorts of ~28 *H. halys* first instars on the egg mass were placed individually in 30.5 cm^3 rearing cages provisioned *ad libitum* with the diet treatment and water. Cages were checked daily to monitor the development and survivorship of nymphs from each egg mass. Stage-specific survivorship and nymphal developmental duration were recorded for nymphs from each cohort. Within 48 hr after molting to the adult stage, the fresh weight of all adults produced was measured (nearest 0.1 mg) using a digital scale (AB54-S Mettler Toledo, Columbus, OH) and their protonum width was measured (nearest 0.01 mm) with a digital caliper (ROK International Industry Co., Ltd., Guangdong, China). Wild adult *H. halys* (n = 30) collected from tree of heaven trees at the AHS AREC in early September 2013 also were weighed and measured for comparison with adults obtained from the late-season laboratory experiments.

Data Analyses. All analyses were conducted using JMP® Pro version 11 (SAS Institute Inc., Cary, NC) and outcomes were considered significant at $P < 0.05$. Data that did not satisfy the assumptions of parametric tests were transformed using arcsine$\sqrt{x}$ for percentage data and log(x+1) for nymphal developmental duration and the pronotal width and fresh weight. Two-way ANOVA was used to compare nymphal stage-specific survivorship and developmental duration among the diets that included foliage alone and foliage plus reproductive structures. One-way ANOVA was used to analyze the survivorship and developmental duration among the diets during the early- and late- season experiments. Student's t-test was used to compare nymphal survivorship between the early- and late-season experiments for each diet. One-way ANOVA was used to compare the effect of host plant diet on the fresh weight and pronotal width of the females and males that developed to the adult stage during the early- and late-season experiments, including measurements from the field-collected adult males and females, which were compared only with the adults reared from the late-season experiment. Student's t-test was used to compare the fresh weight and pronotal width, based on pooled data across all diet treatments. Multiple mean comparisons used Tukey's post hoc HSD.

Results

Single Host Diets. In this experiment, 94.45 ± 1.39% of the eggs from each mass hatched. Since first instar *H. halys* do not feed on plant tissue, their survivorship was not significantly affected by diet treatment (Table 2.1). There were significant effects of host plant and the inclusion of reproductive structures on the survivorship of second through fifth instars (Table 2.1). In general, the percentage of nymphs that survived each of these instars was much higher on diets that combined foliage and reproductive structures than on foliage alone, although this effect was much less pronounced on tree of heaven, on which the fewest number of nymphs survived the second instar.

Since the developmental duration of first instars was not significantly affected by diet treatment ($F_{7, 48} = 0.30$, $P = 0.95$), the duration between the second instar and adult eclosion was compared. Moreover, since diets of foliage alone yielded very few adults, developmental duration was analyzed only for treatments that included fruiting structures. Among those, the developmental duration varied significantly among the treatments (Table 2.1) and was shortest on tree of heaven and significantly shorter on peach than on apple or catalpa, which did not differ from each other.

Single versus Mixed Host Diets Early and Late in the Growing Season: Survivorship. In this experiment, 93.61 ± 1.25 % of eggs from each mass hatched. As in the previous experiment, diet treatment had no effect on the survivorship of first instars during the early or late season experiments (Table 2.2). In the early-season study, there were numerical, but not statistically significant diet effects on second instar survivorship, with the lowest survivorship on apple. In the late season study, apple also resulted in second instar survivorship that was significantly or numerically lower than on the other diets, which did not differ.

The early and late season experiments yielded significant diet effects on survivorship during the third through fifth instars (Table 2.2). In the early season study, highest nymphal survivorship through the fifth instar was on peach, apple plus tree of heaven, and the 3- and 4-host diets; the lowest survivorship was on apple, tree of heaven and catalpa. In the late season study, apple and catalpa again yielded the lowest survivorship through the fifth instar; whereas, the other diets yielded > 69% survivorship. In both experiments, there was a pronounced decrease in survivorship on catalpa between the fourth and fifth instars that was not observed on the other diets.

Between the early and late season experiments, nymphal survival to the adult stage increased from 20% to 72% on tree of heaven ($t = 6.32$, df = 6, $P = 0.001$); survivorship to the adult stage on the other diets did not differ significantly between early and late season studies (apple: $t = 2.34$, df = 6, $P = 0.06$, peach: $t = -2.19$, df = 6, $P = 0.07$, catalpa: $t = 0.66$, df = 6, $P = 0.54$, apple plus tree of heaven: $t = 0.79$, df = 6, $P = 0.46$, 3-host diet: $t = -1.06$, df = 6, $P = 0.33$ and 4-host diet: $t = 0.09$, df = 6, $P = 0.93$).

Single versus Mixed Host Diets Early and Late in the Growing Season: Developmental duration and adult size and fresh weight. There was no effect of diet on the developmental duration of first instars (Table 2.3). Significant diet treatment effects on the developmental duration of second, fourth, and fifth instars were recorded during the early season experiment, while in the late season study, there were significant effects on second, third, and fifth instars. In the early season, total developmental period was significantly shorter on peach and the 3- and 4-host diets than on apple or catalpa, with intermediate durations on the others. The late season study yielded similar results, with significantly shorter total developmental duration on peach, tree of heaven, and the 4-host diet than on apple or catalpa, with intermediate periods among the other diets.

Based on pooled data across all diet treatments, mean adult pronotal width was significantly greater for females (7.95 ± 0.02 SE mm) than males (7.17 ± 0.02 SE mm) ($t = -26.18$, df = 878, $P < 0.0001$) and females were significantly heavier (109.37 ± 1.10 SE mg) than males (87.48 ± 0.86 SE mg) ($t = -15.61$, df = 878, $P < 0.0001$). Diets that yielded <3 adult males or females were excluded from statistical comparisons. In both the early and late season experiments, the fresh weight and pronotal width of both sexes varied significantly among diets (Table 2.4). In the early season, tree of heaven and apple plus tree of heaven yielded females that were heaviest and had the largest pronotal width, while apple yielded the lightest and smallest females. The heaviest and largest males also were recorded from tree of heaven and apple plus tree of heaven, while the lightest and smallest males were from catalpa and apple. In the late season study, the heaviest females and males were recorded from peach, although the 3- and 4-host diets also produced relatively heavy individuals of both sexes (Table 2.4). As in the early season experiment, females and males from apple and catalpa were the lightest. The 3- and 4-host diets produced females with the largest pronotal width and smallest females were from catalpa and apple, while male pronotal width was largest from the 4-host diet and smallest from apple. Field-collected females and males had statistically or numerically comparable fresh weights and pronotal widths to those from the 3- and 4- host diet treatments during the late season experiment (Table 2.4).

Discussion

The survivorship and developmental duration of laboratory-reared *H. halys* nymphs were significantly affected by host plant tissue, host plant species, host phenology, and diet mixing. Like other pentatomid species, such as the southern green stink bug, *Nezara viridula* (L.) and the neotropical brown stink bug, *Euchistus heros* (Fabr.) (Panizzi 2000), *H. halys* nymphs have been known to feed on non-reproductive parts of plants such as the stem (Martinson et al. 2013) and foliage (Hoebeke and Carter 2003). For all plants examined, we found that diets consisting only of stems and foliage were unsuitable, and that most nymphs did not survive the second instar on them.

Martinson et al. (2015) showed that the presence of fruit on ornamental trees in a nursery strongly influenced the abundance of *H. halys* nymphs and adults and that fruit removal had a profound negative effect on *H. halys* counts. Complementing their results, we found that combining foliage and fruiting structures of peach dramatically increased *H. halys* nymphal survivorship and reduced their developmental duration compared with foliage alone. For apple and catalpa, we also showed that fruiting structures and foliage only led to marginal improvements in nymphal survivorship both early and late in the season. Tree of heaven foliage plus samaras produced the same result early in the season, but nymphal survivorship on that diet increased in the late season study.

With regard to the cultivated plant species offered as single host diets that combined foliage and fruit, the relatively poor suitability of apple for *H. halys* nymphs concurs with Funayama (2002), who reported that nymphs reared on apple had low survivorship and developed poorly. In my study, I consistently found that nymphal survivorship was higher and their developmental duration was shorter on peach than on apple, indicating a clear difference in the suitability of these two economically-important hosts for nymphal development. The high suitability of peach for nymphal development and survival conforms to field observations that peach can support large *H. halys* populations from late May through harvest (Nielsen and Hamilton 2009) and often incurs higher levels of injury early in the growing season than apple

(Leskey et al. 2012b, Joseph et al. 2015). While apples can sustain substantial injury from *H. halys* (Nielsen and Hamilton 2009, Leskey et al. 2012b, Joseph et al. 2015), this may be associated more with transient visits and feeding bouts by adults and nymphs than by resident populations; Morrison et al. (2015) used harmonic radar to show that tagged *H. halys* adults remained on apple trees for only 3.5h.

A survey of 78 species of native and invasive trees and herbaceous shrubs in urban, rural and forested areas in the eastern US by Bakken et al. (2015) revealed that catalpa and tree of heaven were among the species that consistently yielded comparatively high counts of *H. halys* adults and nymphs. In China, tree of heaven is considered a preferred host of *H. halys* (reviewed in Lee et al. 2013). A possible explanation for the relatively poor performance of *H. halys* nymphs on catalpa and tree of heaven, discussed above, is that members of the Simaroubaceae (e.g. tree of heaven) and Bignoniaceae (e.g, catalpa) produce secondary metabolites with insecticidal properties (Tsao et al. 2002, De Feo et al. 2009, Castillo and Rossini 2010). The pronounced reduction in survivorship between the fourth and fifth instars of nymphs reared on catalpa may have been associated with the cumulative effects of these allelochemicals during their development. As well, the marked seasonal difference in the suitability of tree of heaven for nymphal survival may have been due to seasonal variation in allelochemical concentration and/or increasing nutritional value of the maturing samaras.

Diet mixing was clearly beneficial to the survivorship of *H. halys* nymphs. Funayama (2006) reported similar results when carrots were added to a peanut and soybean diet for *H. halys* nymphs; indeed, mixed diets for rearing *H. halys* have been universally adopted (Medal et al. 2012, Leskey and Hamilton 2014). In the early season study in 2013, the diet that combined apple and tree of heaven, both of which were suboptimal as single hosts, yielded improved nymphal survivorship through the fifth instar, although this effect was not found for the same diet in the late season study, perhaps due to the apparently increased suitability of tree of heaven. Nymphs of the polyphagous grasshopper, *Parapodisma subastris* (Huang), reared on diets of two, four, and six suboptimal hosts also showed higher survivorship compared with those on a diet of a single suboptimal host (Miura and Ohsaki 2004). The survivorship of *P. subastris* nymphs reared on all mixed diets of suboptimal hosts was statistically equivalent to that on single diets of the superior hosts, as was the case for *H. halys* reared on mixed diets of suboptimal hosts versus those on peach alone.

Overall, total developmental durations from the second through fifth instars were considerably longer than reported from *H. halys* rearing studies under similar environmental conditions by Nielsen et al. (2008) and Medal et al. (2013). Both previous studies used a bean, peanut, corn, and carrot diet, resulting in 34-d and 37-d developmental durations from the second through fifth instars, respectively. The underlying reasons for the differences between the present and previous experiments are unknown, but may be associated with differences in the nutritional quality of the diets used.

Previous studies on *N. viridula* showed that adult size was positively correlated with longevity, female fecundity (McLain et al. 1990), and winter survival (Todd 1989). Moreover, studies on *N. viridula* (Kester and Smith 1984) and *B. tabaci* (Zhang et al. 2014) found that nymphs reared on mixed diets produced longer-lived adults and more fecund females. These results suggest that the larger and heavier adult *H. halys* from nymphs reared on suitable diets, whether of single or mixed hosts, may also have improved longevity and fecundity. The size and weight of adults from nymphs reared on mixed diets in the late-season study in 2014 did not differ significantly from field-collected adults in September, 2013, which had likely also developed from nymphs that had fed on a range of host plants. As well, Todd's (1989) finding of higher overwintering survivorship in larger than smaller *N. viridula* adults suggests that the bigger and heavier *H. halys* adults from nymphs reared on mixed diets or peach later in the season also may be better able to overwinter successfully.

Further investigation of the physiological mechanisms underlying the differences in the relative suitability of the different host plants used in this study and the benefits of diet mixing on *H. halys* development and survival may further improve our understanding of its host-utilization at the orchard-woodland interface. Toward that end, I also quantified the nutrient content in the adults from this study that had developed from nymphs reared on the different diets, revealing new information about host plant effects on *H. halys* nutrition (Appendix A).

Despite the reports (Bakken et al. 2015) and observations of tree of heaven and catalpa supporting large populations of *H. halys* in the eastern US, my results suggest that *H. halys* nymphs may need to disperse from these trees during their development to find and feed on other plants. In the laboratory, Lee et al. (2014) demonstrated that *H. halys* nymphs can walk up to 41 m in 1 hr and up to 8 m in 15 min on smooth horizontal and vertical surfaces, respectively. In a follow-up field experiment using pheromone-baited traps near a woodlot, Lee et al. (2014) found that marked nymphs walked over 20 m on a mowed grassy lawn within 4-5 h. Acebes-Doria et al. (2016; Chapter 3) captured *H. halys* nymphs in traps designed to monitor their upward and downward movement on tree trunks. Results from tree of heaven revealed that second through fifth instars were captured walking up and down tree trunks, but that more nymphs were captured while walking up than down, leading them to speculate that nymphs may also disperse from the tree canopy by dropping. Subsequent field studies using trunk traps (Chapter 4) examined seasonal patterns of the upward and downward walking dispersal of *H. halys* nymphs on cultivated and wild tree hosts at the orchard-woodland interface.

The relative suitability of available hosts may affect the extent to which *H. halys* nymphs disperse among hosts at the orchard-woodland interface. My data suggest that nymphs from eggs laid on apple, catalpa, or tree of heaven early in the season would be more likely to disperse to other hosts than those on peaches. Host phenology and the presence and maturity of fruiting structures appear to strongly influence seasonal patterns of host use by adults and nymphs (Martinson et al. 2015). Moreover, Bakken et al. (2015) reported that among the 78 plant species surveyed, *H. halys* egg masses, nymphs, and adults were found on 34 species, including tree of heaven and catalpa, while only nymphs and adults were found on 41 species and none were detected on 3 species. These findings further support the likelihood that *H. halys* nymphs and adults disperse among available plants, that some species serve as its feeding and reproductive hosts and that others are only feeding hosts or are unsuitable.

At the orchard-woodland interface, many known wild and tree fruit hosts of *H. halys* are commonly found growing close to one another (Chapter 4) and well within the dispersal distance of which nymphs are capable (Lee et al. 2014). Consequently, additional and very relevant questions that follow from the data reported here include, 1) how long do nymphs remain on a particular host, 2) do nymphs that disperse from one host species move to the same or different species, 3) do nymphs disperse from wild hosts into orchards, and if so, 4) how far into the orchard do nymphs move? Moreover, the potential geographic distribution of *H. halys* based on niche modeling indicates that much of the eastern USA and portions of its Pacific coastal regions could be successfully colonized by this invasive species (Zhu et al. 2012). My results indicate that within a particular ecosystem, available host plants also could have a major impact on the survivorship and population growth of *H. halys*, particularly if they are suboptimal for nymphal development and survivorship. The apparent need for diet mixing due to suboptimal hosts could limit *H. halys* establishment and build-up in areas that lack suitable host diversity. Indeed, some suboptimal hosts may be considered a 'dead end' for nymphal survivorship and development if alternate hosts that provide additional nutritional benefits are not available nearby.

In summary, *H. halys* nymphs are commonly found on wild hosts adjacent to fruit orchards and have the capacity to disperse between the two habitats (Lee et al. 2014). Indeed, transects of pheromone-baited traps that extended from woodlots into apple orchards revealed highest captures of adults and nymphs in traps at the orchard and woodland edges (Bergh, unpublished data). Additionally, field studies using trunk traps deployed on apple and peach trees and known tree hosts of *H. halys* at the orchard-woodland interface revealed movement of *H. halys* nymphs both up and down the trees during much of the growing season (Chapter 4). As well, more *H. halys* injury has been recorded from apples in trees at orchard edges adjacent to woodlands than from orchard interior trees (Leskey et al. 2012b, Joseph et al. 2014). These findings and my demonstration of the effects of host plant species and diet mixing on nymphal performance suggest the likelihood that *H. halys* nymphs move between these two habitats during much of the growing season and that effective management programs targeting *H. halys* in orchards next to woodlands might be perimeter-driven. Restricting applications of the most efficacious insecticides against *H. halys* to the orchard perimeter, whether via border sprays (Blaauw et al. 2014) or sprays to pheromone-baited trees at intervals along the borders ("attract-and-kill") (Morrison et al. 2015), should translate to fewer secondary pest problems throughout the orchard and facilitate a return to the more ecologically and economically sustainable programs that were widely practiced before *H. halys* became an issue.

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Table 2.1. Stage-specific survivorship and developmental duration of *H. halys* nymphs reared on single host plant diets consisting of foliage or foliage plus reproductive structures, 2012

Diet treatment (N)^a	Mean ± SE percent survivorship^b					Mean ± SE duration (days) from second instar to adult eclosion (N)^c
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	
Foliage alone						
Apple (195)	100.0 ± 0.0a	0.0 ± 0.0d	0.0 ± 0.0c	0.00 ± 0.00d	0.0 ± 0.0c	
Peach (188)	97.4 ± 1.3a	1.7 ± 1.2d	0.6 ± 0.6c	0.55 ± 0.55d	0.6 ± 0.6c	87.0 (1)
Catalpa (199)	99.5 ± 1.3a	7.9 ± 4.1cd	7.3 ± 4.1bc	5.26 ± 0.55cd	0.0 ± 0.0c	
Tree of heaven (199)	98.9 ± 0.7a	9.0 ± 2.6bcd	8.0 ± 2.3bc	7.43 ± 2.99bcd	5.8 ± 2.0bc	76.4 ± 4.4 (11)
Foliage plus fruit						
Apple (186)	97.5 ± 1.7a	29.3 ± 8.9bc	27.5 ± 8.2b	26.70 ± 8.26b	16.3 ± 5.4b	77.1 ± 5.7a (28)
Peach (187)	100.0 ± 0.0a	86.5 ± 2.8a	83.7 ± 4.9a	79.65 ± 6.50a	72.5 ± 8.1a	52.9 ± 2.4b (135)
Catalpa (192)	97.0 ± 1.4a	33.2 ± 8.8b	21.9 ± 5.4b	15.11 ± 2.87bc	7.1 ± 3.4bc	72.9 ± 3.5a (11)
Tree of heaven (196)	100.00 ± 0.0a	13.3 ± 7.7bcd	11.9 ± 6.8bc	11.93 ± 6.81bcd	11.4 ± 6.9bc	36.5 ± 2.4c (20)
						One-Way ANOVA
Two-way ANOVA	$F_{7, 48} = 1.79$ $P = 0.112$	$F_{7, 48} = 22.66$ $P < 0.0001$	$F_{7, 48} = 25.49$ $P < 0.0001$	$F_{7, 48} = 24.66$ $P < 0.0001$	$F_{7, 48} = 25.75$ $P < 0.0001$	$F_{3, 19} = 23.15$ $P < 0.0001$

[a] Total number of first instars at the outset of the experiment

[b] Stage-specific survivorship was computed as the number of surviving individuals in a specific stage / initial no. of individuals x 100.

[c] Total number of nymphs that developed to the adult stage. The developmental duration of the plant feeding stages of nymphs (second through fifth instars) was compared only among diets that included foliage plus fruit.

Means with the same letters under the same column are not significantly different (Tukey's HSD, $\alpha = 0.05$).

Table 2.2. Stage-specific survivorship of *H. halys* nymphs reared on single and mixed host plant diets in experiments beginning in May (early season) and mid-August (late season), 2013

Diet treatment	(N)[a]	Mean ± SE percent survivorship[b]				
Early season		First instar	Second instar	Third instar	Fourth instar	Fifth instar
Apple	(120)	92.9 ± 6.0a	34.7 ± 23.6a	15.0 ± 8.9c	14.4 ± 8.4b	8.2 ± 5.2b
Peach	(115)	98.2 ± 1.0a	91.4 ± 5.4a	88.8 ± 5.8a	87.9 ± 5.3a	86.2 ± 6.0a
Catalpa	(103)	99.0 ± 1.0a	91.7 ± 3.3a	81.5 ± 2.8a	74.2 ± 5.7a	9.8 ± 1.9b
ToH[c]	(109)	99.1 ± 0.9a	60.7 ± 22.7a	26.3 ± 4.6bc	22.0 ± 3.4b	20.2 ± 2.3b
Apple + ToH[c]	(110)	100.0 ± 0.0a	73.5 ± 11.2a	70.7 ± 10.3ab	70.1 ± 10.3a	68.3 ± 10.4a
3-host[d]	(113)	100.0 ± 0.0a	95.6 ± 0.9a	94.7 ± 1.8a	92.1 ± 1.7a	89.4 ± 2.0a
4-host[e]	(114)	97.1 ± 2.88a	89.7 ± 9.1a	86.0 ± 9.5a	82.9 ± 10.5a	80.8 ± 11.5a
One-way ANOVA		$F_{6, 21} = 0.89$ $P = 0.522$	$F_{6, 21} = 2.02$ $P = 0.108$	$F_{6, 21} = 14.23$ $P < 0.0001$	$F_{6, 21} = 13.87$ $P < 0.0001$	$F_{6, 21} = 21.7$ $P < 0.0001$
Late season		First instar	Second instar	Third instar	Fourth instar	Fifth instar
Apple	(112)	99.1 ± 0.9a	53.1 ± 11.7b	48.3 ± 14.0b	39.5 ± 9.5b	26.26 ± 9.6bc
Peach	(104)	99.2 ± 0.8a	90.3 ± 1.8a	87.7 ± 0.9ab	82.3 ± 2.2a	69.74 ± 4.6ab
Catalpa	(82)	97.3 ± 0.9a	77.0 ± 3.5ab	67.0 ± 4.0ab	48.0 ± 2.8ab	13.64 ± 5.6c
ToH[c]	(105)	99.1 ± 0.9a	89.7 ± 7.9a	87.9 ± 7.5a	83.3 ± 7.2a	72.04 ± 7.9ab
Apple + ToH[c]	(106)	99.1 ± 0.9a	96.4 ± 2.1a	92.9 ± 3.2a	87.2 ± 4.3a	77.78 ± 6.3a
3-host[d]	(100)	97.1 ± 1.9a	83.3 ± 11.8ab	75.6 ± 16.8ab	72.8 ± 18.5ab	70.48 ± 17.7ab

4-host[e]	(104)	97.8 ± 1.3a	95.4 ± 1.8a	90.7 ± 1.1ab	83.8 ± 2.4a	81.81 ± 3.2a
One-way ANOVA		$F_{6,21} = 0.61$ $P = 0.717$	$F_{6,21} = 4.41$ $P = 0.005$	$F_{6,21} = 3.45$ $P = 0.016$	$F_{6,21} = 4.68$ $P = 0.004$	$F_{6,21} = 8.44$ $P < 0.0001$

[a] Total number of first instars at the outset of the experiment

[b] Stage-specific survivorship was computed as the number of surviving individuals in a specific stage / initial no. of individuals x 100.

[c] ToH denotes tree of heaven

[d] The 3-host diet consisted of the foliage and fruiting structures of apple, ToH, and peach

[e] The 4-host diet consisted of the foliage and fruiting structures of apple, ToH, peach, and catalpa

Means with the same letters under the same column and in the same experiment are not significantly different (Tukey's HSD, α = 0.05).

Table 2.3. Stage-specific stadia and developmental duration (mean days ± SEM) of *H. halys* nymphs on single and mixed host plant diets during the early and late season experiments, 2013

Diet treatment	(N)[a]	Mean ± SE developmental duration (days)					Mean ± SE duration (days) from second instar to adult eclosion (N)[b]
Early season		First instar	Second instar	Third instar	Fourth instar	Fifth instar	
Apple	(120)	5.3 ± 0.5a	18.0 ± 0.1a	20.1 ± 1.3a	33.5 ± 3.8a	44.7 ± 1.5ab	91.8 ± 2.6a (10)
Peach	(115)	5.3 ± 0.3a	13.3 ± 0.5bc	13.1 ± 0.7a	17.6 ± 1.1b	24.7 ± 2.1bc	53.5 ± 2.7c (99)
Catalpa	(103)	5.2 ± 0.3a	13.1 ± 1.3c	18.9 ± 6.1a	28.5 ± 3.7ab	51.3 ± 8.7a	77.5 ± 8.7ab (10)
ToH[c]	(109)	5.3 ± 0.2a	16.1 ± 0.9ab	20.1 ± 2.8a	22.9 ± 3.3ab	19.0 ± 3.6c	58.7 ± 5.0bc (22)
Apple + ToH[c]	(110)	5.2 ± 0.3a	17.0 ± 0.9a	19.0 ± 1.7a	23.5 ± 1.5ab	23.3 ± 1.7c	62.6 ± 3.8abc (75)
3-host[d]	(113)	5.2 ± 0.3a	13.2 ± 0.6c	14.3 ± 1.0a	18.6 ± 1.6b	19.3 ± 0.7c	49.2 ± 2.8c (101)
4-host[e]	(114)	5.2 ± 0.4a	13.0 ± 1.1c	14.0 ± 1.2a	19.8 ± 2.2ab	23.5 ± 1.6c	52.9 ± 2.6c (96)
One-way ANOVA		$F_{6, 21} = 0.03$ $P = 0.999$	$F_{6, 19} = 4.04$ $P = 0.009$	$F_{6, 19} = 1.45$ $P = 0.246$	$F_{6, 19} = 3.92$ $P = 0.010$	$F_{6, 19} = 10.69$ $P < 0.0001$	$F_{6, 19} = 7.43$ $P = 0.0003$
Late season		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Mean ± SE duration (days) from second instar to adult eclosion (N)[b]
Apple	(112)	4.8 ± 0.7a	22.4 ± 1.1ab	22.7 ± 0.7a	30.9 ± 2.1a	53.9 ± 4.3ab	105.2 ± 3.5a (32)
Peach	(104)	5.9 ± 0.5a	16.9 ± 1.1ab	17.3 ± 0.3ab	20.7 ± 1.3a	28.0 ± 2.1c	63.6 ± 2.9c (68)
Catalpa	(82)	5.7 ± 0.3a	22.3 ± 0.6ab	22.8 ± 1.9a	28.6 ± 3.7a	61.7 ± 6.5a	100.8 ± 4.5ab (12)
ToH[c]	(105)	5.3 ± 0.3a	15.1 ± 2.6b	14.2 ± 2.4b	18.8 ± 4.0a	32.2 ± 6.5c	62.1 ± 11.9c (82)

Apple + ToH[c]	(106)	5.2 ± 0.2a	18.6 ± 2.0ab	18.0 ± 2.5ab	25.6 ± 2.8a	41.3 ± 3.7abc	71.6 ± 4.1bc	(85)
3-host[d]	(100)	5.6 ± 0.3a	23.2 ± 2.8a	19.4 ± 1.2ab	25.6 ± 2.0a	34.4 ± 4.0bc	68.0 ± 2.0bc	(73)
4-host[d]	(104)	5.8 ± 0.3a	18.6 ± 0.7ab	19.7 ± 0.2ab	25.2 ± 2.5a	31.2 ± 2.8c	65.6 ± 1.5c	(84)
One-way ANOVA		$F_{6,\,21} = 0.95$ $P = 0.482$	$F_{6,\,21} = 3.23$ $P = 0.021$	$F_{6,\,21} = 3.86$ $P = 0.009$	$F_{6,\,21} = 2.32$ $P = 0.071$	$F_{6,\,20} = 5.87$ $P = 0.001$	$F_{6,\,20} = 7.23$ $P = 0.0003$	

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[a] Total number of first instars at the outset of the experiment

[b] Total number of nymphs that developed to the adult stage. Second through fifth instars are the plant-feeding stages of juvenile *H. halys*

[c] ToH denotes tree of heaven

[d] The 3-host diet consisted of the foliage and fruiting structures of apple, ToH, and peach

[e] The 4-host diet consisted of the foliage and fruiting structures of apple, ToH, peach, and catalpa

Means with the same letters under the same column and in the same experiment are not significantly different (Tukey's HSD, α = 0.05).

Table 2.4. Mean ± SE fresh weights (mg) and pronotal widths (mm) of ≤ 2-day-old *H. halys* adults reared on different diets during the early- and late-season experiments, and adults collected from the field in early September, 2013

Diet treatment	Females			Males		
Early season	N	Weight (mg)	Pronotal Width (mm)	N	Weight (mg)	Pronotal Width (mm)
Apple	3	70.53 ± 7.76d	6.98 ± 0.34d	7	67.44 ± 5.91c	6.62 ± 0.10c
Peach	51	111.84 ± 2.50c	7.72 ± 0.04c	48	96.87 ± 1.66ab	7.11 ± 0.04b
Catalpa	2	97.00 ± 22.20*	7.67 ± 0.53*	8	59.44 ± 4.10c	6.60 ± 0.11c
ToH[a]	7	138.04 ± 4.41a	8.50 ± 0.09a	15	107.47 ± 2.66a	7.55 ± 0.05a
Apple + ToH[a]	33	132.38 ± 3.25a	8.39 ± 0.06a	42	102.80 ± 1.70a	7.47 ± 0.04a
3-host[b]	55	121.11 ± 2.02bc	8.09 ± 0.04b	46	93.21 ± 1.96b	7.20 ± 0.05b
4-host[c]	50	118.04 ± 2.27c	8.04 ± 0.04b	46	96.62 ± 2.07ab	7.27 ± 0.04b
One-way ANOVA		$F_{5,\,193} = 13.32$ $P < 0.0001$	$F_{5,\,193} = 28.23$ $P < 0.0001$		$F_{6,\,205} = 28.84$ $P < 0.0001$	$F_{6,\,205} = 20.93$ $P < 0.0001$

	Females			Males		
Late season	N	Weight (mg)	Pronotal Width (mm)	N	Weight (mg)	Pronotal Width (mm)
Apple	15	77.20 ± 2.27de	7.14 ± 0.10d	17	62.32 ± 2.30d	6.48 ± 0.09d
Peach	42	112.07 ± 2.90a	7.93 ± 0.06bc	26	85.96 ± 2.50ab	7.12 ± 0.04bc

Catalpa	10	67.85 ± 4.28e	6.92 ± 0.11d	2	66.45 ± 1.25*	6.64 ± 0.12*
ToH[a]	43	93.43 ± 3.37cd	7.75 ± 0.08c	39	75.50 ± 2.22bc	7.16 ± 0.08abc
Apple + ToH[a]	41	98.61 ± 3.79bc	7.79 ± 0.06c	44	73.93 ± 2.05cd	6.96 ± 0.06c
3-host[b]	31	104.46 ± 2.67abc	8.11 ± 0.06ab	42	83.62 ± 1.54b	7.19 ± 0.04ab
4-host[c]	48	106.85 ± 2.22ab	8.19 ± 0.04a	36	83.43 ± 1.99bc	7.37 ± 0.04ab
Field-collected (Sept. 2013)	15	121.90 ± 8.23a	8.30 ± 0.13a	15	98.72 ± 8.35a	7.47 ± 0.11a
One-Way ANOVA		$F_{7,\,237} = 13.08$ $P < 0.0001$	$F_{7,\,237} = 26.41$ $P < 0.0001$		$F_{6,\,212} = 11.83$ $P < 0.0001$	$F_{6,\,212} = 17.32$ $P < 0.0001$

[a] ToH denotes tree of heaven

[b] The 3-host diet consisted of the foliage and fruiting structures of apple, ToH, and peach

[c] The 4-host diet consisted of the foliage and fruiting structures of apple, ToH, peach, and catalpa

*Treatment excluded from analyses due to sample size

Means with the same letters under the same column and in the same category are not significantly different (Tukey's HSD, α = 0.05).

CHAPTER 3: DEVELOPMENT AND COMPARISON OF TRUNK TRAPS TO MONITOR MOVEMENT OF *HALYOMORPHA HALYS* NYMPHS ON HOST TREES

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Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) has recently become a major orchard pest in the mid-Atlantic, USA. Large *H. halys* populations can develop on wild tree hosts adjacent to orchards, posing an ongoing threat to fruit. Adults and nymphs feed on tree fruit, causing economic injury. Understanding the seasonal patterns of nymphal host use among trees at the orchard-woodland interface may aid the development of integrated pest management strategies for this pest. In laboratory and field experiments, modified versions of published trap designs – 'Circle', 'Hanula', 'M&M' (after Moeed & Meads) traps – were compared for their effectiveness for capturing *H. halys* nymphs walking up and down tree trunks. In the laboratory, second instars were released at the top and bottom of tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), logs and captures were recorded after 24 h. Circle and M&M traps, respectively, were most effective for capturing nymphs walking up and down. In the field, traps were deployed on tree of heaven trees next to apple orchards and captures were recorded weekly from 24 July to 11 September 2013. As in the laboratory, Circle and M&M traps captured the greatest number of upward- and downward-walking nymphs. Hanula traps were least effective in both experiments. In the field, 88% of total captures were of nymphs walking up trees. This was at least partially explained by behavioral assays in the laboratory demonstrating that nymphs exhibited negative geotaxis and positive phototaxis. Stage-specific trends in captures of instars walking up during field sampling were observed. These results suggest that trunk traps can be used to address important ecological questions about seasonal patterns of host use by *H. halys* nymphs.

Key words: geotaxis, phototaxis, walking behavior, Hemiptera, Pentatomidae, *Ailanthus altissima*

Introduction

Brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is invasive from Asia and has recently become a serious agricultural pest in the mid-Atlantic region of the USA (Leskey et al. 2012a, Rice et al. 2014). It has had an especially pronounced impact on the tree fruit industry, as it commonly feeds on deciduous tree species (Lee et al. 2013). *Halyomorpha halys* is highly polyphagous and reportedly uses >100 plant species as feeding or reproductive hosts, including wild trees in woodlands that often abut orchards in the mid-Atlantic region (Rice et al. 2014). Its season-long movement between woodlands and orchards has put continuous pressure on commercial orchards, resulting in increased insecticide use (Leskey et al. 2012b). The current necessity for aggressive insecticide-based management of *H. halys* is deemed ecologically and economically unsustainable; hence, the development of effective and sustainable management strategies based on an improved understanding of its behavior and ecology is paramount.

Studying the movement of insect pests is critical to monitoring and predicting their presence and potential impact throughout the season. The flight activity of alate insect pests has been studied more extensively (Muirhead-Thompson 1991, Mazzi and Dorn 2012) than has the walking dispersal of flightless agricultural pests. Lee et al. (2014) demonstrated that the dispersal capacity of *H. halys* nymphs by walking is considerable. In the laboratory, nymphs walked up to 41 m in 1 h and up to 8 m in 15 min on smooth horizontal and vertical surfaces, respectively. When attractive olfactory stimuli were deployed in a mowed grassy field near a woodlot, Lee et al. (2014) reported that responding nymphs walked over a distance of 20 m within 4-5 h. These findings suggest that *H. halys* nymphs can move readily among host plants growing near one another. Moreover, *H. halys* nymphs exhibited higher survivorship and faster development when reared on mixed diets of wild and cultivated tree hosts than on single diets of most of the same hosts (Acebes-Doria, unpublished data), concurring with Funayama (2006), and further suggesting the likelihood that nymphs move among hosts during the season.

In field studies, pitfall traps have been used to measure the abundance and movement of litter- and soil-dwelling arthropods (Duelli et al. 1990, Lalonde et al. 2012), but less commonly to monitor the movement of agricultural pests. The only well-studied agricultural pest in this regard is the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), as adults walk into agricultural fields from surrounding habitats (Boiteau et al. 2003). Traps to monitor the abundance and movement of arthropods walking on standing trees in forest ecosystems were used by Bar-Ness et al. (2012), Hanula and New (1996), Moeed and Meads (1983), and

Pinzón and Spence (2008), and in an agricultural system by Mulder et al. (1999).

Given that *H. halys* nymphs are commonly found on wild and cultivated tree hosts growing near one another, show optimal survivorship and development on a diet of mixed hosts, and can walk over considerable distances in the vertical and horizontal dimensions, I explored the utility of traps for capturing *H. halys* nymphs walking on the trunk of host trees. The traps were based on modifications of published designs used for other arthropod species in agricultural (Mulder et al. 1999) and forest systems (Moeed and Meads 1983, Hanula and Franzreb 1998). Here, the results of laboratory and field experiments that evaluated and compared the effectiveness of three traps designed to monitor *H. halys* nymphs moving up and down trees are reported and discussed in relation to the potential for using them to study seasonal movement patterns of *H. halys* nymphs on host trees at the orchard-woodland interface.

Materials and methods

Insects. Adult *H. halys* were collected from overwintering sites in northern Virginia (38°44'24.71"N 78°04'55.49"W) in February 2013 and held in a dark chamber at 4 °C inside a black garbage bag with crumpled newspaper. In late April 2013, the adults were removed from cold storage and held in groups of ~30 males and ~30 females in 30 × 30 × 30 cm plastic screened BugDorm™ cages (BioQuip Products Inc., Rancho Dominguez, CA) in a room at Virginia Tech's Alson H. Smith Jr. Agricultural Research and Extension Center (AHS AREC), Winchester, VA. Rearing room conditions were ~25 °C, 70% RH, and L16:D8 photoperiod, and the insects were provided a mixture of popcorn kernels, barley, buckwheat, soybeans, dried figs, unsalted dry-roasted peanuts, and sundried tomatoes in a Petri dish, and 2-5 peach or apple fruits. Water was provided in a plastic container with a cotton wick inserted through the lid. Folded paper towels on the cage floor and excised tree of heaven foliage in a plastic vase with water served as oviposition substrates and egg-laying began by the 3rd week of May. *Halyomorpha halys* typically deposit eggs in masses of 28 (Nielsen et al. 2008); newly-deposited egg masses were removed daily in situ and held in groups of five in covered polystyrene Petri dishes (10 × 1.5 cm; Thermo Fisher Scientific Inc., Pittsburgh, PA) with water-soaked cotton. First instars of *H. halys* aggregate around and on the empty egg mass until molting to the second instar (Taylor et al. 2014), then disperse and begin feeding on plant tissue. Egg masses were monitored regularly for eclosion of first instars and their molt to the second instar. Recently-molted second instars from each dish were transferred to a separate polyester, screened popup rearing cage (30.5 × 30.5 × 30.5 cm; BioQuip Products Inc.) containing fresh peach and apple fruit, the dry diet described previously, and water. Second instars at 2-4 days following their molt were used in laboratory studies conducted between 28 June and 10 July 2013.

Traps. Three trunk trap designs were modified from versions used in other studies. The 'Circle' trap (Fig. 3.1A) was invented by and named after a pecan grower who used it to monitor pecan weevil (Mulder et al. 1999), whereas the 'Hanula' trap (Fig. 3.1B) was developed to capture arthropods walking or crawling on tree boles (Hanula and New, 1996). Both of these traps were originally designed to capture insects walking up tree trunks, although for this study, complementary traps to capture downward-moving insects also were manufactured by inverting the funneling or guiding device and collection container (Figs. 3.1A,B). The third design was named the 'M&M' trap (Fig. 3.1C) after its inventors (Moeed and Meads, 1988), and was based on their design for sampling invertebrate fauna moving up and down forest trees in New Zealand. Hereafter, 'up' traps of each design refer to those intended to capture *H. halys* nymphs walking up tree trunks and 'down' traps refer to the inverted version of each, devised to intercept nymphs walking downward.

Although the materials and designs of the three traps differed, the common underlying principle was that insects walking on the trunk would be guided into a collection container via a material that tightly encircled the trunk. Selected surfaces on or in each trap were coated strategically with a fluoropolymer resin, PTFE-30 or fluon (BioQuip Products Inc.), a slippery material that served to prevent *H. halys* escape from the collection container or from walking over the funneling or guiding feature of the traps (Fig. 3.1). All down traps had four holes (6 mm diameter) on the bottom of the collection container that were covered with plastic screen (1.0 × 0.7 mm mesh size; Saint-Gobain Adfors America Inc., Grand Island, NY), allowing drainage of water. The collection container for each trap was held in place against the tree trunk by two 25.4-cm-long flexible cords with a hook at each end. After each trap was installed on tree trunks, any remaining gaps or spaces that could have affected their effectiveness were sealed using transparent High Tech Sealant (Ace Hardware Corp., Oak Brook, IL).

Circle traps were based on a funnel (Fig. 3.1A) intended to guide walking insects to the apex, where a second funnel led them into the collection container. The main funnel was constructed from a 63.5 × 45.7-cm piece of charcoal-colored, New York Wire™ Sunguard 85 plastic screen (Saint-Gobain Adfors America Inc.) with a mesh size of 1.0 x 0.70 mm. The long edge of the screen was folded to form the funnel and stapled to a wood lath (25.4 × 7.6 ×

5.1 cm). A second wood lath (33 cm long) was stapled to the screen directly opposite the shorter one and the two laths were aligned parallel to the trunk and separated by 5.1 cm long screws attached at both ends of each, creating a space between the two sides of the screen funnel that enabled entry by insects. The bigger aperture of the screen funnel was wrapped tightly around and attached to the trunk by staples along its edge. A piece of PVC pipe (7.6 cm long, 2.54 cm i.d.) was fastened to the smaller aperture of the screen funnel using a cable tie (20.3 cm). The interior surface of the pipe had been roughened using sandpaper to provide traction for insects walking on it and the pipe was glued to the entrance of a plastic funnel from a Dead-Inn™ trap container (AgBio Inc., Westminster, CO) that led to the inside of the collection container. The outer surface of the plastic funnel was coated with fluon (Fig. 3.1A), to prevent escape by insects that had entered the container.

The guiding portion of Hanula traps (Fig. 3.1B) consisted of a black plastic strip (4.45 cm wide, 0.75 mm thick; Master Mark Plastics®, Albany, MN) wrapped tightly around and stapled to the trunk along both edges, so that its guiding edge was perpendicular to the long axis of the trunk. The length of these strips was tailored to the circumference of individual trees to which they were attached. Most of the outer surface of the strip was coated with fluon, leaving a small section uncoated so that insects could walk either up or down on only that section. Insects encountering the edge were anticipated to follow it around the circumference of the trunk until reaching the uncoated section, beneath or above which was a ~400-ml plastic funnel (13.5 cm diameter at top opening) coated on strategic portions of its inner and outer surfaces with fluon (Fig. 3.1B). For the up traps, an area on the inner surface of the plastic funnels directly above the uncoated section of the strip was roughened for traction (Fig. 3.1B). A semi-circular notch cut from the upper lip of each funnel enabled the funnel to be fitted tightly and flush against the plastic strip. The bottom of the funnel inserted into a 2.4-cm-diameter hole in the lid of a plastic Rubbermaid collection container (17.8 × 17.8 × 8.6 cm, Easy Find Lids; Rubbermaid Inc., Atlanta, GA). For the up traps of the Hanula design, a 5 × 5-cm section was removed from the lid of each collection container directly above the funnel entrance and replaced by a piece of transparent plastic glued to the lid, enabling light transmission from above, as occurred in up traps of the other designs.

The guiding portion of M&M traps (Fig. 3.1C) was constructed of a strip of 8.9 cm wide and 0.75 mm thick black plastic (Master Mark Plastics®) wrapped diagonally around the trunk. Like the Hanula traps, the lengths of these strips also were individually tailored to fit the circumference of tree trunks. One edge was stapled to the trunk and the other edge was ~3.8 cm away from the trunk, forming a diagonal collar around the tree. In principle, these traps were designed to guide walking insects along the edge attached to the tree trunk to a funnel at the lowest or uppermost point, according to the trap orientation on the tree. An opening in the stapled edge led to a ~240-ml plastic funnel (8.6 cm diameter at top opening) (Hopkins Manufacturing Corp., Emporia, KS) that in turn led into the funnel of a Dead-Inn™ collection container that emptied inside the container. For the up traps, an area on the inner surface of the plastic funnel starting from the surface directly above the opening in the strip was roughened (Fig. 3.1C) to allow traction for nymphs walking on it. The inner surface of the plastic collar for down traps and the outer surface of the plastic collar for up traps, and portions of the interior and exterior surfaces of the trap funnels were coated with fluon (Fig. 3.1C) to prevent bugs from crossing to the opposite side.

Evaluation of Trap Efficiency

Capturing and retaining released nymphs in the laboratory. Separate experiments – evaluating and comparing trap efficiency for capturing *H. halys* nymphs, and assessing nymphal retention in the collection containers – were conducted in a walk-in growth chamber (4.4 × 2.7 × 2.1 m) maintained at 27.5 °C, ~65% RH., and L16:D8 photoperiod, provided by overhead florescent lighting. Tree of heaven logs (13.0 ± 0.5 cm diameter, 1.65 ± 0.03 m long) were cut from trees felled at the edge of woodlands at the AHS AREC. The logs were oriented vertically in the center of 15.1-l plastic nursery pots filled with soil to within ~5.4 cm of the rim. The top 5 cm of the inside of each bucket was coated with fluon to prevent released nymphs from escaping.

For the capture efficiency study, an up and a down trap of the same design was installed on individual logs. Traps of each design were installed on four logs; down traps were ~46 cm from the top of the log and up traps ~46 cm above the soil surface in the pot.

For each repetition, up and down traps of the three designs were evaluated simultaneously. Twenty second instars were released at the base (for up traps) or at the top (for down traps) of each log. Only one trap orientation was targeted for evaluation based on the release position of nymphs at each repetition (i.e. if nymphs were released at the base, only the up traps were being evaluated, and vice versa) (n = 2 logs per trap design). On each log, the number of nymphs that were captured by the trap being evaluated (i.e. up or down), by the opposite trap, and those in the release area, elsewhere on the log, or trap surface exterior, or/and missing were recorded at 24 h after release. The experiment was repeated 4× between 28 June and 3 July 2013, using different insects for each repetition. The position of logs with different trap designs was re-randomized within the chamber prior to each repetition.

For the retention study, 20 second instars were placed inside the collection container of up and down trap designs. For a given repetition, only the up or down trap of each trap design (n = 2 logs per design) was evaluated and each test was repeated on four separate days. The live and dead nymphs in each collection container were counted after 24 h.

Capturing nymphs in the field. A pair of up and down traps of each design was installed on each of four mature, female *A. altissima* trees growing next to orchards at the AHS AREC. Mean (± SE) diameter at breast height of the trees used was 17.4 ± 1.3 cm. Down traps were installed at 1-1.25 m above the tree base, whereas up traps were 0.8-1 m above the base. For these studies, a ~5.1-cm-long strip of Hercon Vaportape II (Hercon Environmental, Emigsville, PA) containing dimethyl-2,2-dichlorovinyl phosphate was suspended inside each collection container using a paper clip attached to a 5.1-cm-long corner brace that was screwed to the side or roof of the container. Use of these 'kill strips' in *H. halys* monitoring traps and their replacement at 2-week intervals, as was done in this study, is standard protocol (Joseph et al. 2013, Weber et al. 2014) and ensured the retention of captured nymphs. Captures of *H. halys* nymphs and adults in each trap were recorded weekly from 24 July to 11 September 2013, and the developmental stage of nymphs was determined following the morphological descriptions of Hoebeke and Carter (2003).

In response to lower captures of *H. halys* in down traps than in up traps during the first field evaluation (see Results) and based on the nymphal captures in pheromone-baited traps (Lee et al. 2014), a follow-up study examined whether captures in down traps could be increased by presenting an attractive olfactory stimulus near the tree base and, by extension, whether the lower captures in the first experiment were due to fewer nymphs walking down than up or to some other factor. To examine this, I exploited the fact that both *H. halys* adults and nymphs are attracted to the vicinity of lures containing its aggregation pheromone, a mixture of (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol (Khrimian et al. 2014) and the pheromone synergist methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT) (Weber et al. 2014), and that nymphs are known to respond quite quickly and over a distance of many meters to these stimuli (Lee et al. 2014). A 10-mg pheromone lure and a 66-mg MDT lure (AgBio Inc.) were attached to the top of a black pyramid trap (122 cm high, 51 cm wide at the base) (AgBio Inc.), the standard pheromone trap for *H. halys* (Leskey et al. 2012c, Joseph et al. 2013, Weber et al. 2014), positioned 3 m away from the base of trees on which a down trap was installed; each down trap design was installed on four trees. The lures were deployed for 2 days then removed for 2 days and this alternating deployment pattern was conducted over 24 consecutive days from 13 September until 7 October 2013, for a total of 12 days with and without lures. The *H. halys* nymphs captured in each down trap were counted after each 2-day sample interval.

Examination of Nymphal Phototaxis and Geotaxis. Behavioral experiments to examine possible cues affecting the direction of nymphal movement were conducted in the laboratory using second instars within 2-3 days following their molt. To examine their response to light, a wooden dowel (122 cm long, 1 cm diameter) was leveled horizontally between retort stands at 23 cm from each end. The dowel was 51 cm above a table surface and had a mark at the middle that served as the release point and marks at 30.5 cm on either side of the midpoint that served as response points. Light was provided by a 27-W Daylight 24^{TM} quad tube compact fluorescent bulb (Trademark Global Inc., Lorain, OH) atop a 1.4-m tall reading lamp with a flexible neck that enabled the light to be directed along the length of the dowel from either end. Using a small paintbrush, a single nymph was released at the midpoint and observed until it had walked to the left or right response point. The light source was alternated between the right and left ends of the dowel between consecutive observations, each of which used a different nymph. As a control, the same observations were conducted using a battery-operated LED headlamp with red filter at 150 lumens (Top Gun LED Headlamp; Primos Hunting, St. Flora, MS) worn by the observer.

To examine their geotactic response, the dowel was leveled vertically and held by a retort stand at 23 cm from the top. All observations were conducted using the red light headlamp. Single nymphs were released at the midpoint and observed until they had walked to the response point above or below. All experiments evaluating phototactic and geotactic responses were conducted at 23°C on a single day, with 30 replicates per treatment. Between uses for individual nymphs, the dowel was cleaned with wet tissue, air-dried, and re-leveled either horizontally or vertically.

Data Analyses. All analyses were conducted using JMP® Pro v. 11 (SAS Institute, Cary, NC). Percentage data from laboratory experiments that did not satisfy the assumptions of parametric tests were transformed using arcsine$\sqrt{x}$, whereas nymphal captures in the field were subjected to a log(x+1) transformation. One-way ANOVA was used to analyze the capture data within up and down orientations among trap designs from the laboratory studies. The difference between the numbers of nymphs captured walking up and down was compared for each trap design using Student's t-test. The percentage of nymphs not captured by the trap being evaluated during the trap efficiency experiment was compared among trap designs and within trap orientation using two-way ANOVA. The numbers of live and dead nymphs retained after 24 h were compared using Student's t-test. For the initial field experiment, two-way ANOVA was used to compare captures among trap designs and between trap orientations, and Student's t-test was used to compare captures of nymphs and adults in the field. For the follow-up field study, comparison of captures among down trap designs within the periods with and without lures used one-way ANOVA, whereas a Matched Pairs t-test (which accounted for the repeated measurement) was used to compare captures between the periods with and without lures for each trap design. Multiple mean comparisons among the trap designs used Tukey's post hoc test. Data from the experiments examining nymphal phototaxis and geotaxis were analyzed using a χ^2 two-tailed test, followed by a χ^2 one-tailed test when the null hypothesis of the two-tailed test was rejected.

Results

Evaluation of Trap Efficiency for Capturing and Retaining Released Nymphs in the Laboratory. The trap efficiency experiment revealed a significant effect of trap design on the number of second instars captured moving up ($F_{2,21}$ = 26.53, $P < 0.0001$) and down ($F_{2,21}$ = 23.58, $P < 0.0001$) tree of heaven logs (Fig. 3.2). Among the up traps, significantly more nymphs were captured in Circle and M&M traps than in Hanula traps and there was no significant difference between Circle and M&M traps (Fig. 3.2). Significantly more nymphs walking down were intercepted by M&M and Hanula traps than by the Circle trap, and captures in M&M and Hanula traps did not differ (Fig. 3.2). More upward- than downward-walking nymphs were captured in Circle traps (t = -15.93, df = 14, $P < 0.0001$), whereas there was no difference between captures in up and down traps of the Hanula (t = -0.88, df = 14, P = 0.40) or M&M (t = 1.97, df = 14, P = 0.07) trap designs.

Significantly more nymphs were captured in the opposing trap when down traps were evaluated (11.25 ± 2.28%) than when up traps were assessed (3.13 ± 1.03%); there were no significant differences in captures in opposing traps among the trap designs (Table 3.1). A few nymphs were found on the release area and elsewhere on the log or trap exterior surface, but there were no differences among up or down trap trials or among trap designs (Table 3.1). Significantly more nymphs were missing 24 h after release during the assessment of down traps (56.25 ± 4.65%) than of up traps (40.00 ± 6.22%) (Table 3.1). Additionally, there was a significant interaction between trap design and the orientation of assessed traps (i.e., release location of nymphs) in the percentage of missing nymphs (Table 3.1). Significantly more nymphs were missing 24 h after release when Hanula up traps were evaluated (nymphs released at the base of logs) and for down traps of the Circle and Hanula designs (nymphs released at the top) compared with the other designs and orientations (Table 3.1).

The nymphal retention study indicated that the percentage of nymphs remaining in the collection containers after 24 h did not differ among the three trap designs ($F_{2,33} = 0.82$, $P = 0.45$) or between trap orientations ($F_{1,34} = 2.46$, $P = 0.13$). The mean (± SE) percentage of nymphs remaining in the containers after 24 h was 92.22 ± 1.47% and significantly more live (57.08 ± 3.55%) than dead (35.10 ± 3.59%) nymphs were recovered ($t = 3.05$, df = 35, $P = 0.0043$).

Evaluation of Traps for Capturing Nymphs in the Field. Across all trap designs and orientations, significantly more nymphs (7.18 ± 1.33) than adults (0.28 ± 0.06) per trap were captured ($t = 10.68$, df = 382, $P < 0.0001$). As these traps were developed specifically for monitoring nymphs, adult captures were excluded from subsequent analyses. In total, 1378 nymphs were captured, 88% of which were in up traps, which was significantly more than in down traps (12%) ($F_{1,190} = 53.82$, $P < 0.0001$). There was a significant interaction between trap orientation and trap design in the number of nymphs captured ($F_{1,186} = 70$, $P < 0.0001$). Among the up traps, significantly more nymphs were captured in Circle than in M&M and Hanula traps, and significantly more in M&M than in Hanula traps (Fig. 3.3). Among the down traps, captures were significantly higher in M&M traps than in Hanula or Circle traps, captures in the latter two trap designs did not differ significantly (Fig. 3.3).

In the follow-up study that addressed whether captures in down traps were affected by presenting olfactory stimuli at the tree base, the presence or absence of lures did not significantly increase the number of nymphs captured in any of the trap designs (Circle, Hanula: $t = -1$, df = 3, $P = 0.39$; M&M: $t = 1.22$, df = 3, $P = 0.31$; Fig. 3.4), although there was a numerical effect on captures in M&M traps when lures were present. As in the previous laboratory and field experiments, there was a significant effect of down trap design on nymphal captures when lures were present ($F_{2,69} = 11.48$, $P < 0.0001$) or absent ($F_{2,69} = 4.61$, $P = 0.013$); M&M traps captured more downward-walking nymphs than the others in both scenarios (Fig. 3.4).

Examination of the distribution of instars trapped during each weekly sampling interval was based on data from the up version of Circle traps. Of the 859 nymphs captured in this trap between 24 July and 11 September, 0.7, 26.9, 10.9, 19.8, and 41.7% were in the first through fifth instars, respectively. Captures of second instars predominated between 24 July and 14 August; thereafter, increasing proportions of nymphs in later instars were captured (Fig. 3.5). There was not a similar trend in the distribution of instars moving down trees, possibly due to much lower captures.

Examination of Nymphal Phototaxis and Geotaxis. Nymphs responded rapidly to the cues presented by walking to one of the response points on horizontally- or vertically-oriented dowels in 20.40 ± 0.75 s. All nymphs exhibited positive phototaxis and moved in the direction of the light source regardless of whether it was positioned at the left or right end of the horizontal dowel (both $\chi^2 = 41.59$, $P < 0.0001$). In the absence of a light cue, there was an equal probability of nymphs walking to the left or right ($\chi^2 = 53.3$, $P = 0.72$). All nymphs released at the midpoint of a vertical dowel in the dark exhibited negative geotaxis by walking upward.

Discussion

As has been shown in previous studies with other arthropods (Moeed and Meads 1983, Hanula and New 1996, Mulder et al. 1999), my experiments have demonstrated that *H. halys* nymphs walking up and down the trunk of a known tree host can be intercepted using an appropriate trap. The strong concurrence between the results from laboratory and field studies in the relative effectiveness of the various traps for capturing upward- and downward-walking nymphs provides guidance for future studies addressing the movement of *H. halys* nymphs among its tree hosts.

The collection container types used among the trap designs did not influence their effectiveness in the laboratory study; most nymphs released in each container were recovered from the containers after 24 h and the majority of them were alive. In the field, the use of a killing agent in the container and its replacement at 2-week intervals undoubtedly enhanced the retention of captured nymphs (Joseph et al. 2013). Thus, differences in the relative effectiveness of the traps appear to have been influenced by features other than the collection container.

Among the three traps, the Circle up trap and the M&M down trap were most effective for capturing nymphs walking up and down trees, respectively, and it appears that the guiding surfaces on those traps were superior for steering nymphs toward and into the collection container compared to the surfaces on the Hanula trap. The conical shape of the screen funnel on the Circle trap and the diagonal collar on the M&M trap may have interfered less with the overall direction of nymphal movement than the abrupt and perpendicular barrier to upward or downward movement created by the guiding feature of the Hanula trap, which proved to be an inappropriate design for capturing *H. halys* nymphs.

Although the up and down forms of each trap were essentially inverted versions, the effectiveness of the two best designs (Circle and M&M) differed to varying degrees according to their orientation. In the laboratory and field, the up version of the Circle trap captured more upward-walking nymphs than the others, but its down version was consistently least effective. Conversely, the down form of the M&M trap was consistently most effective for capturing downward-moving nymphs, whereas its up version was only moderately effective.

Consistent differences in the relative effectiveness of the various trap designs and orientations in laboratory and field experiments may have been related to effects of one or more design features on nymphal behavior and also to the basic behavior of nymphs. With respect to trap design, nymphs moving down the logs in laboratory experiments often walked over the mesh funnel of Circle traps to the outer surface, whereas upward-walking nymphs were rarely seen to behave in this manner. If *H. halys* nymphs are positively phototactic, differences in directional light cues coming from the apex of up and down versions of the M&M and Circle traps may have influenced their propensity to follow the guiding feature of traps to the apex, resulting in higher captures in up than down traps of these designs. Higher captures in the down version of the M&M trap than in the corresponding version of the Circle trap also may have been affected by the slippery coating on the inside of the M&M plastic guide preventing nymphs from crossing it.

More upward- than downward-walking *H. halys* nymphs were captured in the laboratory and field. Despite observations and reports (Bakken et al. 2015) that *H. halys* egg masses and high nymphal populations are common on tree of heaven foliage and female reproductive structures, down traps installed on tree of heaven trees in the field captured only 12% of all nymphs recovered. Furthermore, presentation of pheromone plus pheromone synergist lures near the base of trees did not appreciably increase captures in down traps. In the only other field study that used both up and down traps on the trunk of host trees, Moeed and Meads (1983) reported a similar result from New Zealand; only 20% of 22,696 invertebrates captured over 19 months on four tree species were recovered from down traps.

In combination, the effects of positive phototaxis on the direction of nymphal *H. halys* movement and their strongly negative geotactic behavior may explain much of the difference in captures between up and down traps. In addition, as nymphs are presumed to move onto new tree hosts only via walking up the trunk, lower captures in down traps suggests that they use other behaviors to disperse from trees. Some smaller hemipterans can disperse via phoresy (Magsig-Castillo et al. 2010), on air currents (da Silva-Torres et al. 2013), or by adhering to falling leaves (Daane et al. 2006). It is possible that some *H. halys* nymphs may simply drop from host trees. Indeed, Moeed and Meads (1983) speculated that the disparate captures they recorded between up and down traps may have been due to invertebrates dropping from the canopy rather than walking down the trunk. Dropping may benefit walking insects by requiring less time and energy and by reducing the risk of predation. This behavioral explanation matches my observation that many nymphs released at the top of tree of heaven logs fell down and were captured walking up on the same log and that nymphs were commonly missing from those logs.

Dispersal of pentatomid pests from host plants is known to be affected by host quality and the presence of more suitable hosts nearby, as was documented for the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euchistus servus* (Say), which fed initially on peanuts and then dispersed to cotton when bolls were available (Tillman et al. 2009). In woody ornamentals, the host-utilization pattern of *H. halys* appeared to be influenced by seasonal changes in resource availability; nymphal counts were highest when fruit and seeds were present (Martinson et al. 2015). Moreover, survivorship of *H. halys* nymphs feeding on a single diet of less suitable hosts was highest during the second instar but decreased in later instars (Acebes-Doria, unpublished data). Overall survivorship improved significantly when nymphs were reared on a diet of two or more hosts, including pairings of hosts that were relatively unsuitable as single diets. My field data from Circle up traps suggested that the traps were sensitive to temporal changes in the distribution of instars. Only a few first instars were captured, conforming with observations that they do not disperse from the egg mass until after acquiring symbionts from it and molting to the second instar (Taylor et al. 2014). The preponderance of second instars captured through mid-August may reflect nymphal movement from other hosts onto tree of heaven in search of fulfilling nutritional requirements. Later in the season, increasing proportions of later instars captured suggested that the progeny of the summer generation of adults were entering more advanced stages of development.

In summary, I have demonstrated that Circle and M&M traps can be used complementarily to monitor the upward and downward movements, respectively, of *H. halys* nymphs walking on host trees. Deploying these traps on fruit trees and nearby wild hosts to monitor captures season-long would enable comparisons of nymphal *H. halys* host selection and temporal changes in its host-use patterns at the orchard-woodland interface, thereby aiding the development of ecologically-based and temporally-precise management strategies for this destructive, invasive pest.

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Table 3.1. Location of second instars of *H. halys* (mean % ± SE) relative to each of three types and two orientations of trunk trap (n = 4), up (captured upward-walking nymphs) and down (captured downward-walking nymphs), 24 h after their release at the base or top of *A. altissima* logs.

Trap		Opposing non-target trap	Remained at the base of the log	Elsewhere[1] on the log	Missing[2]
Orientation	Design				
Up	Circle	1.3 ± 1.3	0.6 ± 0.6	1.3 ± 0.8	16.3 ± 5.1c
	M&M	4.4 ± 2.0	3.8 ± 3.8	0	34.4 ± 7.1bc
	Hanula	3.8 ± 2.1	5.0 ± 3.7	6.9 ± 6.9	69.4 ± 9.8a
Down	Circle	18.1 ± 4.1	1.3 ± 0.8	7.5 ± 2.7	71.3 ± 6.5a
	M&M	10.0 ± 4.5	1.3 ± 1.3	1.3 ± 1.3	38.1 ± 5.9bc
	Hanula	5.6 ± 1.5	0	2.5 ± 1.3	59.4 ± 7.4ab

Two-way ANOVA	Factor	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
	Trap design	2,42	0.44	0.65	0.10	0.90	2.04	0.14	8.34	0.001
	Trap orientation	1,42	11.47	0.002	1.22	0.28	1.78	0.20	7.79	0.008
	Design*orientation	2,42	3.16	0.05	1.30	0.28	1.08	0.35	11.54	<0.0001

[1] In or on the guiding device, outside the collection container, and in between the two traps.

[2] Nymphs that were not captured by the traps under evaluation and not found elsewhere on the log.

Means within the same orientation followed by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$).

The percentages of nymphs captured in the target traps are presented in Figure 3.2

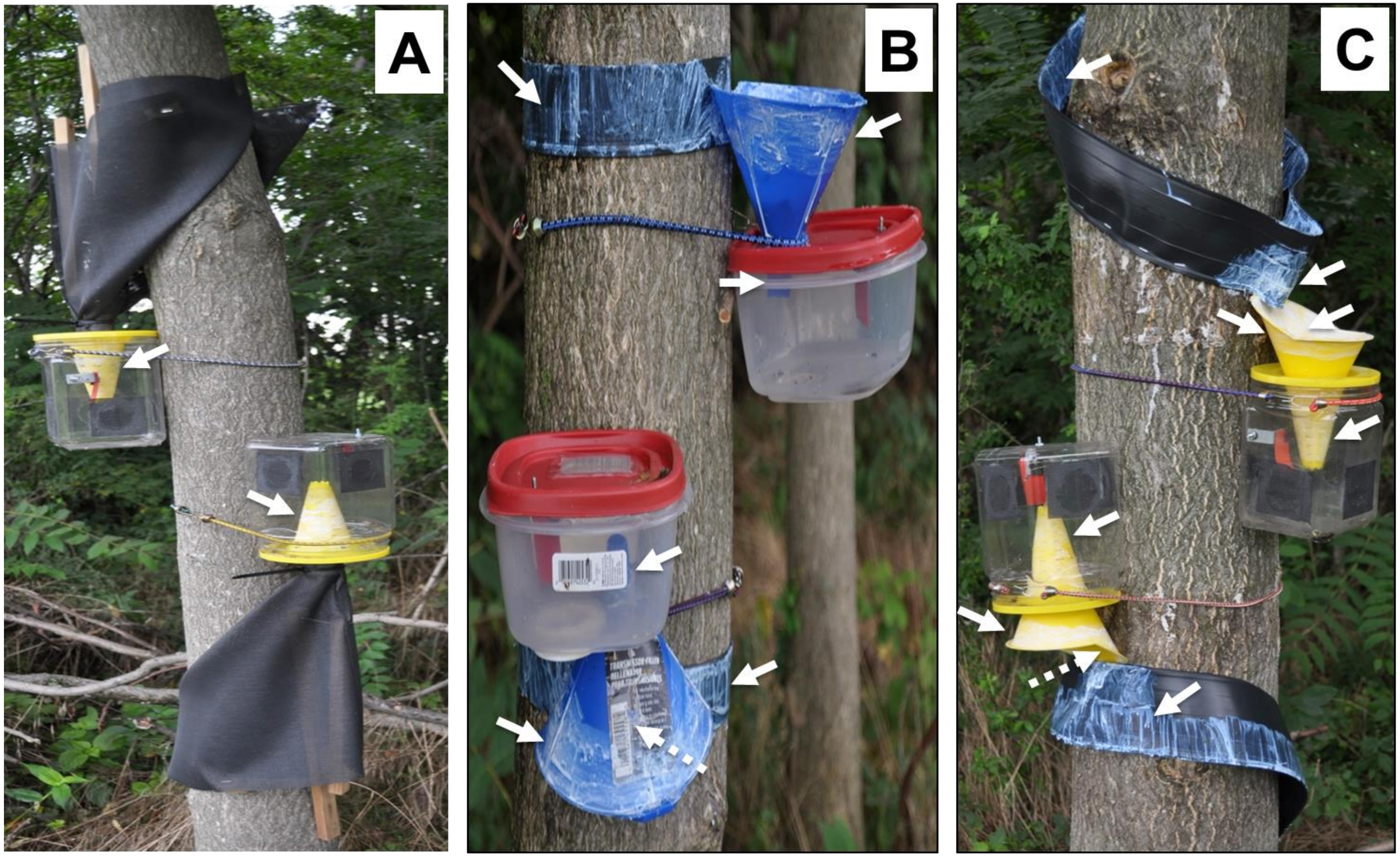

Figure 3.1. (A) Circle, (B) Hanula, and (C) M&M (Moeed & Meads) traps used to capture *H. halys* nymphs walking on *A. altissima* logs and trees in laboratory and field studies, respectively, at Winchester, VA. Solid arrows indicate surfaces coated with fluon and the broken arrows indicate the roughened area in the interior of the funnel.

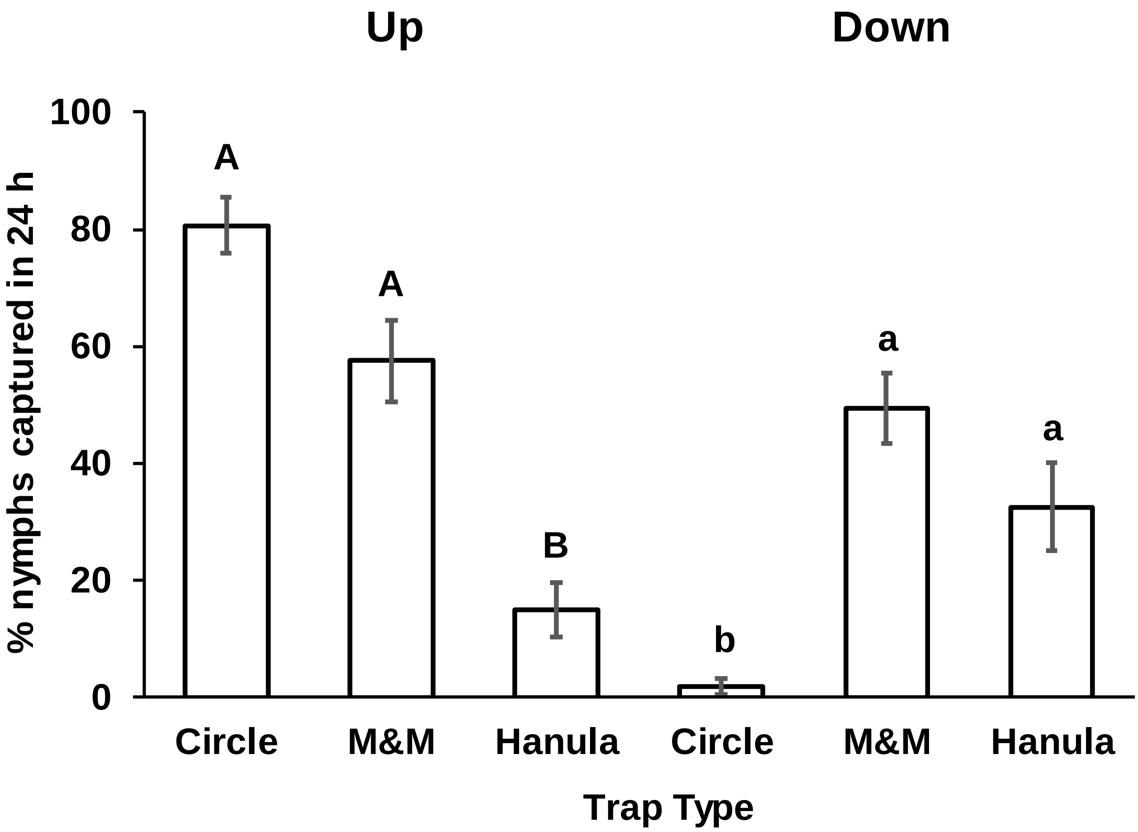

Figure 3.2. **Mean (± SE) percentage of second instar *H. halys* captured in three types of trunk trap (see Fig. 3.1) and two orientations, up (captured upward-walking nymphs) and down (captured downward-walking nymphs) at 24 h after their release at the bottom or top of *A. altissima* logs in a growth chamber (n = 4 per replicate per trap design and orientation). The experiment was repeated 4x. Bars within an orientation capped with the same letters are not significantly different (Tukey's HSD, α = 0.05).**

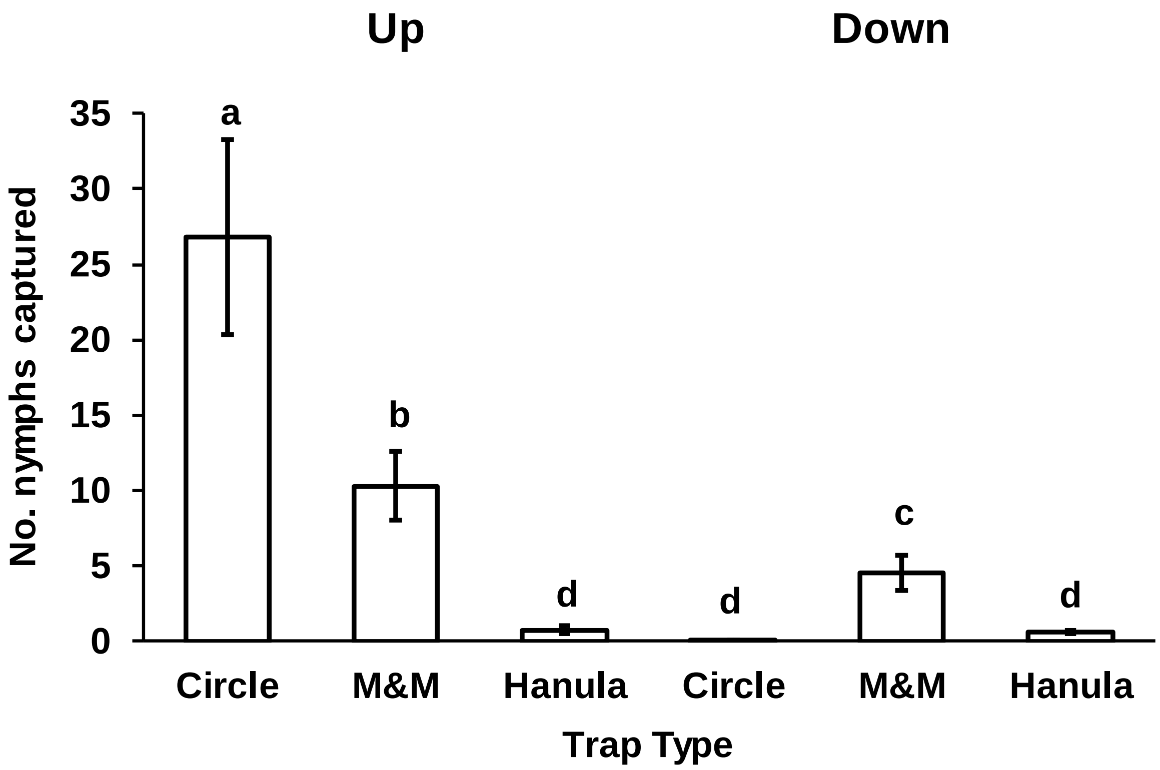

Figure 3.3. Mean (± SE) weekly captures of *H. halys* nymphs in three types of trunk trap (n = 4) and two orientations, up (captured upward-walking nymphs) and down (captured downward-walking nymphs) on *A. altissima* trees near Winchester, VA between 24 July and 11 September 2013. Bars within an orientation capped with the same letters are not significantly different (Tukey's HSD, α = 0.05).

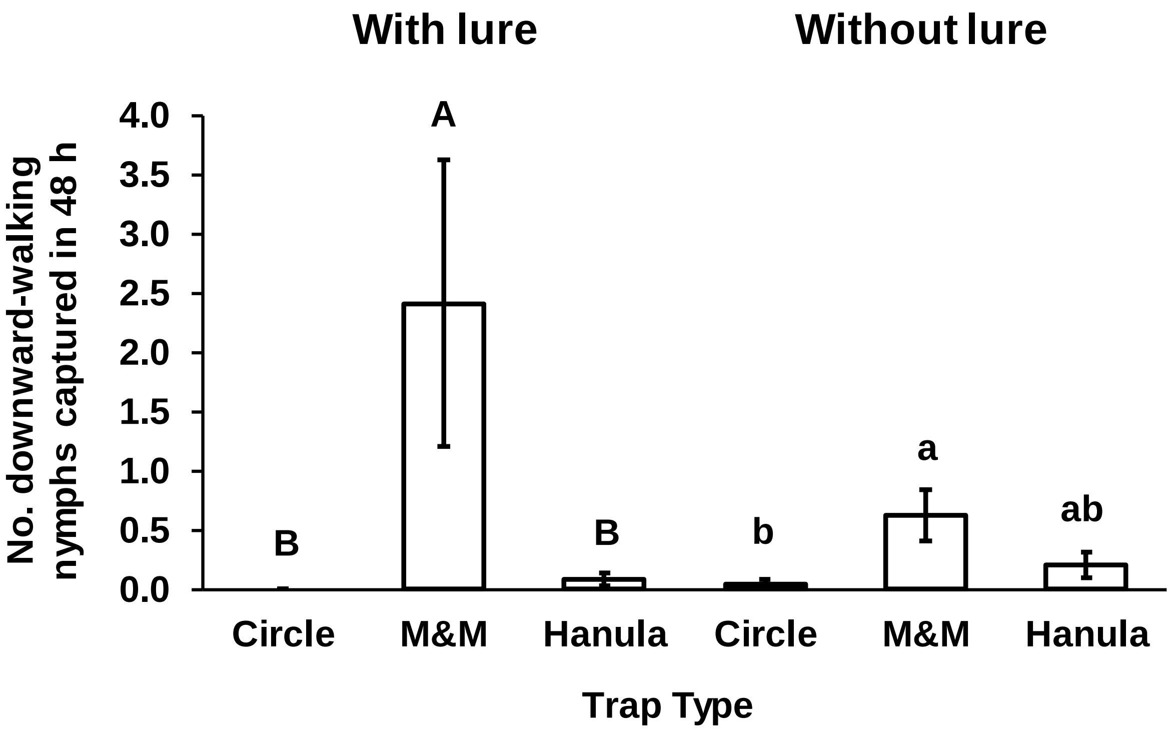

Figure 3.4. Mean (± SE) captures of downward-walking *H. halys* nymphs in three types of trunk traps (n = 4) on *A. altissima* trees with and without aggregation pheromone lures placed 3 m away from the tree base. There were six alternating 48-h periods with and without lures between 13 September and 7 October 2013. Bars within treatment (with vs. without lure) capped with the same letters are not significantly different (Tukey's HSD, α = 0.05).

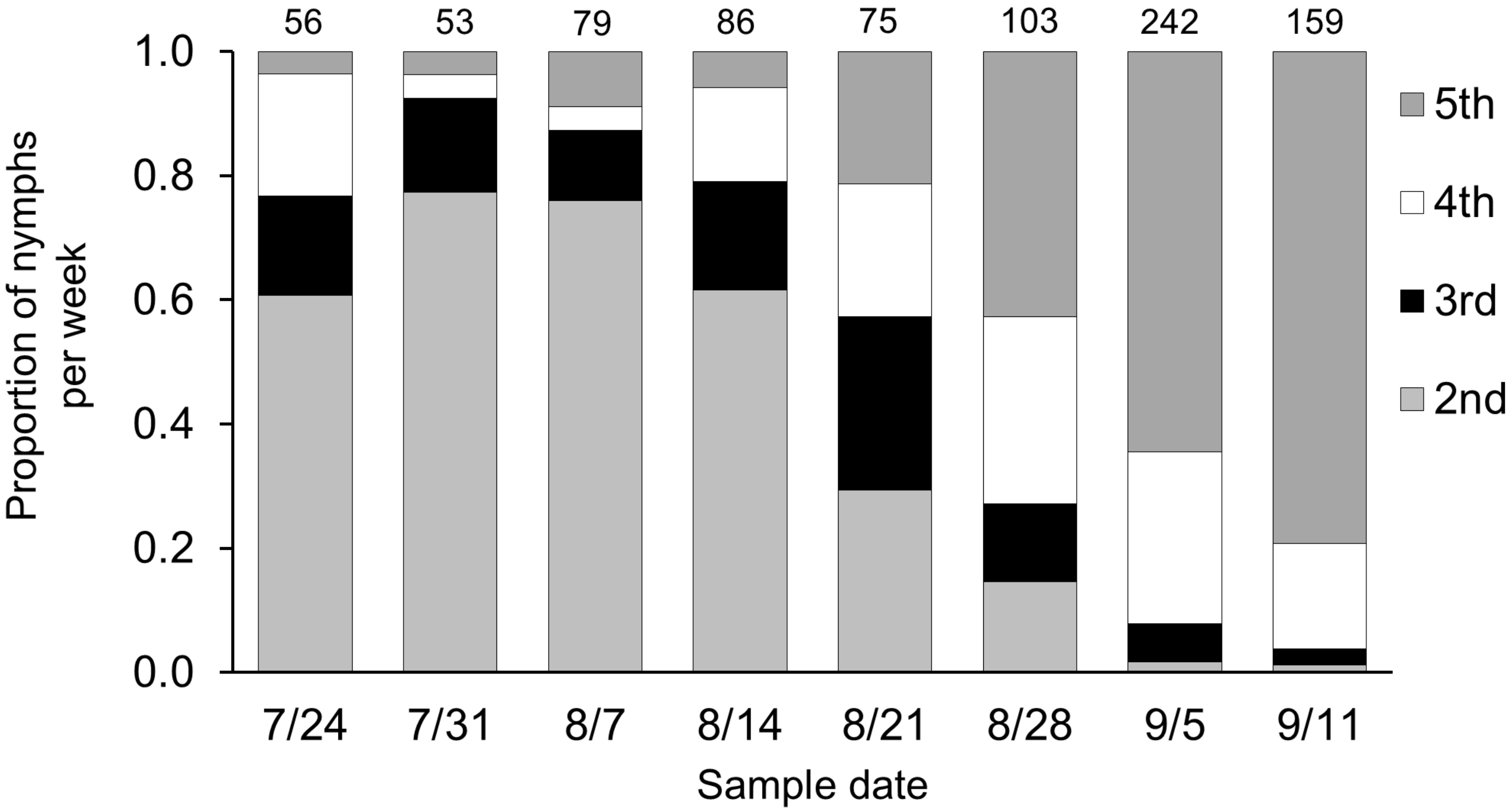

Figure 3.5. Weekly distribution of *H. halys* instars captured in Circle traps (n = 4) while walking up the trunk of *A. altissima* trees near Winchester, VA, between 24 July and 11 September 2013. Numbers capping the bars indicate total captures per week, excluding the six first instars captured.

CHAPTER 4: TEMPORAL AND DIRECTIONAL PATTERNS OF *HALYOMORPHA HALYS* NYMPHAL MOVEMENT ON THE TRUNK OF SELECTED WILD AND FRUIT TREE HOSTS

Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive and highly polyphagous pest in mid-Atlantic tree fruit orchards. Both nymphs and adults feed on and injure fruit. *Halyomorpha halys* nymphs have a substantial dispersal capacity by walking and their developmental rate and survivorship are enhanced by feeding on multiple hosts. To broaden our understanding of nymphal *H. halys* use of and movement among its potential host trees at the orchard-woodland interface, I conducted a census of the tree species in unmanaged woodlands adjacent to one experimental and 14 commercial apple orchards in Virginia and West Virginia and monitored their directional and temporal patterns of movement on selected tree fruit and wild tree hosts at the orchard-woodland interface. Many of its wild hosts (e.g. tree of heaven, hackberry, black cherry) were common and abundant in the woodlands bordering orchards. Passive "up" and "down" traps designed to capture *H. halys* nymphs walking onto and off of tees, respectively, were installed on the trunk of apple and peach trees in orchard border rows next to woods and on wild tree hosts at the woods edge at three apple and two peach sites in 2014 and 2015. Captures of each instar were recorded weekly from mid-May to mid-October, 2014 and mid-May to late September in 2015 and compared among host trees across both seasons. In both years, more nymphs were intercepted while walking up than down and captures of upward and downward walking nymphs varied significantly among the hosts. Although all of the dispersive nymphal instars were captured and captures reflected seasonal changes in instar distribution, most captures were of second instars. Results are discussed in relation to host and seasonal effects on the movements of *H. halys* nymphs at the orchard-woodland interface, and their management implications.

Keywords: orchard, brown marmorated stink bug, apple, peach, tree of heaven, tree survey

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), is an invasive stink bug from Asia that was detected in the United States in the mid-1990s (Hoebeke and Carter 2003). It is highly polyphagous and reported to utilize > 100 plant species, including many agricultural and horticultural crops (Rice et al. 2014). In 2010, its populations reached critical levels in the mid-Atlantic region, resulting in considerable economic losses to many crops, particularly tree fruit (Leskey et al. 2012a). In response to the threat posed by *H. halys*, initial efforts were focused on identifying effective insecticides for it. Many of the most effective products (Lee et al. 2013a) have broad spectrum activity against arthropods, and their increased use by growers in the post-bloom period led to increased outbreaks of secondary pests (Leskey et al. 2012b). In order to return to more sustainable orchard pest management, research goals are now focused on developing ecologically-based management programs for *H. halys*, necessitating a broader understanding of its behavior and ecology at the orchard-woodland interface.

In tree fruit orchards, *H. halys* is considered to be primarily a perimeter-driven threat; high populations have been recorded at the edge of woodlands adjacent to orchards and at the orchard edge next to these woodlands (Bergh and Leskey, unpublished). Moreover, higher levels of fruit injury have been recorded from orchard border rows next to woods than from orchard interior rows (Leskey et al. 2012b, Joseph et al. 2014). Its capacity to infest orchard crops over an extended period during the season may be facilitated by the availability of numerous wild hosts growing in these wooded areas and its high propensity to disperse among plants and habitats (Lee et al. 2014, Wiman et al. 2014, Lee and Leskey 2015). A comprehensive survey of plant species in the Eastern US showed that many were utilized by *H. halys* for feeding and/or reproduction (Bakken et al. 2015). However, the species composition and relative abundance of these hosts in unmanaged woodlands adjacent to commercial fruit orchards is unknown; thus documenting this may improve our ability to assess the localized threat from *H. halys* and the risk of its invasion of orchards.

Unlike native stink bug pests of tree fruit, of which only the adults are thought to injure fruit, both *H. halys* nymphs and adults can feed on and injure fruit (Chapter 5), highlighting the importance of studying the movement of these highly dispersive nymphs among their host plants. *Halyomorpha halys* nymphal survivorship and developmental rate are enhanced when they feed on multiple plant hosts (Funayama 2006, Chapter 2), which may at least partially explain its highly dispersive behavior. Lee et al. (2014) demonstrated that nymphs walked 20 m within 4-5 h on a grassy field in response to pheromone lures. In the laboratory, nymphs walked up to 41 m in 1 hr and up to 8 m in 15 min on smooth horizontal and vertical surfaces, respectively (Lee et al. 2014). As well, nymphs have been observed walking up and down tree trunks, leading Acebes-Doria et al. (2016) to develop and compare traps to monitor this upward and downward movement, based on designs used by other researchers to monitor the movement of pecan weevil (Coleoptera: Curculionidae) on pecan trees (Mulder et al. 1999) and to survey arthropod communities in a New Zealand forest (Moeed and Meads 1983). Different trap designs were most effective for capturing nymphs walking up or down (Acebes-Doria et al. 2016) and have tremendous utility to address ecologically-important questions about the directional and temporal movement patterns of *H. halys* nymphs on cultivated and wild trees at the orchard-woodland interface. Such ecological studies can enhance our understanding of nymphal dispersal trends during the season and may help to predict timing of invasion into orchards and periods of increased pest pressure, both vital to developing temporally and spatially precise pest management programs. Thus, the objectives of this study were to quantify the relative abundance and species composition of potential tree hosts of *H. halys* growing in unmanaged wooded areas adjacent to commercial fruit orchards in Virginia and West Virginia and to monitor the seasonal patterns of *H. halys* nymphal movement at the orchard-woodland interface, using passive traps installed on tree trunks.

Materials and Methods

Tree Census. One experimental and 14 commercial apple orchards in Virginia and West Virginia were selected, all of which had an unmanaged woodland ($\leq$ 25 m) along at least one border (Table 4.1). Three 10 m^2 areas at the woods edge and in the interior were surveyed. The interior sections were $\geq$ 40 meters inside the woodlot from the edge. The edge and interior sampling areas were separated by $\geq$ 10 m. All trees and shrubs $\geq$ 1 m tall within each sampling area were identified to species using dendrology factsheets from Virginia Tech's Department of Forest Resources and Environmental Conservation (http://dendro.cnre.vt.edu/dendrology) and counted. The abundance of each tree species relative to the total number of trees identified was calculated for the interior and exterior sections of the woodlands.

Nymph Traps. The passive traps used were described and compared by Acebes-Doria et al. (2016). The “up” trap was designed to intercept upward-walking nymphs while the “down” trap was intended to capture nymphs walking down tree trunks (Fig. 4.1). Briefly, the up trap was a modified version of the “Circle" trap originally used to monitor pecan weevil (Mulder et al. 1999), and constructed of a mesh funnel leading to a piece of PVC pipe that, in turn, led to the inside of a plastic collection jar. The down trap was loosely based on the design used by Moeed and Meads (1983) to survey the invertebrate communities on forest trees in New Zealand. The guiding portion of the down traps was a black plastic collar wrapped diagonally around the trunk, with one edge stapled to the trunk. An opening in the stapled edge led to a plastic funnel that, in turn, led into a plastic collection jar. To allow drainage of water, the down traps had small, screen-covered holes in the bottom of the collection jar. Selected surfaces on or in both traps were coated with a fluoropolymer resin, PTFE-30 or fluon (BioQuip Products Inc.), a slippery material that served to prevent *H. halys* escape from the collection container and/or from walking over the funneling or guiding feature of the traps (Fig. 4.1). The diameter of the materials wrapped around the trunk (i.e. mesh funnel or of the collar) was tailored to fit the circumference of the trunk of individual trees selected for trapping. The average trunk diameter where traps were installed was 38.01 ± 0.90 cm and ranged from 19.24 to 92.17 cm across the different host trees used. A strip of Vaportape II (Hercon Environmental, Emigsville, PA) containing dimethyl-2,2-dichlorovinyl phosphate was suspended inside the collection jar of each trap and served to retain the *H. halys* captured. These were replaced at 2-week intervals, based on established protocols (Joseph et al. 2013, Weber et al. 2014).

Field Sites. Three commercial apple orchards near Winchester, VA were selected, based on the fact that they contained fruit for processing and were not treated with insecticides targeting *H. halys* and that they had at least one edge adjacent to an unmanaged woodland. The two experimental peach orchards used were located at the Alson H. Smith AREC (AHS AREC) in Winchester, VA and the USDA Appalachian Fruit Research Station (AFRS) in Kearneysville, WV. These orchards also were next to wooded areas and were not treated with insecticides for *H. halys*. All fruit in the experimental peach trees were removed on 7 August 2014 and 5 August 2015 to simulate peach harvest in commercial orchards.

At each site, traps were deployed on fruit trees in the border row of the orchard and on wild trees at the edge of the adjacent woodlot. The mean (±SE) distance (m) between the orchard and woodlot edges was 23.30 ± 3.33 and ranged from 6.8 m to 80.6 m. At each site, 5 pairs of apple or peach trees along the orchard border were selected for trapping. Trees within each pair were separated by 9.63 ± 0.97 m, and the distance between pairs of trees was 39.95 ± 6.82 m. One up trap and one down trap was deployed on each pair of trees. Along the woods edge at each site, 5 pairs of female Tree of Heaven were selected. At the location of each pair of Tree of Heaven, a pair of another known wild tree host of *H. halys* was also selected, the species of which varied among locations at each site, based on the composition of the forest at each point along the woods edge (Table 4.2). Wild trees within each pair were separated by 3.25 ± 0.83 m, and the distance between pairs of trees was 24.04 ± 4.53 m. One up trap and one down trap was deployed on each pair of Tree of Heaven and on the second pair of host trees at each location. There was a total of 150 traps (75 up and 75 down) installed on trees at five sites (Table 4.2). Traps were deployed on the same trees in both years of the study.

In 2014, the trees selected for trapping were prepared by clearing the vegetation from around their base and by removing any vines growing on them. The funneling portions of all traps were installed between 14 April and 14 May, but installation of the collections jars was on 14 May. After the 2014 season, the collection jar from all traps and the plastic funnel from down traps were removed, while the mesh funnel of the up traps and the plastic strip of the down traps were left in the field. Since vegetation around the base of these trees was periodically removed during the 2014 season, no site preparation was necessary in 2015, when the collection jars were installed on 13 May. The mean (±SE) height (cm) at which the up traps (i.e. collection jar) were installed on fruit and wild trees was 51.04 ± 1.81 and 87.18 ± 1.55, respectively. The mean (±SE) height (cm) at which the down traps were installed on fruit and wild trees was 30.02 ± 1.38 and 83.92 ± 0.89, respectively. From 14 May – 16 October, 2014 and 13 May – 30 September, 2015, the adult and nymph *H. halys* captured in each trap were removed and counted weekly. Nymphal instars were identified following Hoebeke and Carter (2003). Captures in traps that were destroyed by animals or compromised by unfavorable environmental conditions (e.g. broken collection jar, torn mesh, detached plastic collar) were not recorded, although this affected only ~1 % of all the traps across both years of study.

Data Analyses. Since total captures varied substantially between years, seasonal trends in nymphal captures between the seasons were compared using weekly proportions of total captures. Percentages of the different nymphal instars captured weekly was computed for each year.

All analyses were conducted using JMP® Pro version 11 (SAS Institute Inc., Cary, NC, 2007) and outcomes were considered significant at $P < 0.05$. Data that did not satisfy the assumptions of parametric tests were transformed using arcsine-square root for proportion data and log(x+1) for count data. Comparisons of the mean weekly proportion of total captures between years and the difference between the overall upward and downward nymphal captures were conducted using Student's t-test. A repeated measures ANOVA using date as the repeated variable was used to compare the upward and downward nymphal total weekly captures throughout the trapping period for each year. One-way ANOVA was used to compare mean weekly captures in up and down traps among the different tree species for each year, using only data from weeks when nymphs were captured, which included 18 June – 1 October, 2014 and 18 June – 30 September, 2015. Tree species that only had one replicate were excluded from the analyses, and included black walnut (*Juglans nigra* L.), boxelder (*Acer negundo* L.), mulberry (*Morus rubra* L.), sweet cherry (*Prunus avium* L.) and green ash (*Fraxinus pennsylvanica* Marsh.). Significant differences among treatment means were compared using Tukey's HSD.

Nymphal captures were examined in relation to the seasonal phenology of *H. halys* using degree-day accumulations based on the sine-wave method (Baskerville and Emin 1969) and the lower developmental threshold (14.17°C) established by Nielsen et al. (2008). Since ovarian development of overwintering female *H. halys* begins at $\geq$14-hr photoperiod (reviewed in Lee et al. 2013b), biofix for initiation degree-day accumulations was the date on which photophase reached 14-hr, occurring on 5 May, 2014 and 6 May, 2015. It was assumed that oviposition started on 15 May for both years. Estimated onset of emergence of first generation adults was based on the degree-day requirement for egg-adult development of *H. halys* which was at 538 DD (Nielsen et al. 2008) and 570 DD (Leskey et al. 2012c). Since the two estimations were different, I used both to present a range of the period during which F_1 adults were estimated to be present.

Results

Tree census. There were 47 species of trees from 19 families recorded from sampling at the border and in the interior of unmanaged woodlands adjacent to apple orchards (Table 4.3). The five most common species at the edge of the wooded areas adjacent to orchards were Tree of Heaven, common hackberry, sassafras, spicebush and black locust, while in the interior, common hackberry, green ash, spicebush, black cherry and eastern redbud were the five most common species encountered. Pooling the data from the border and interior sampling areas, the five most abundant species included Tree of Heaven (17%), common hackberry (11%), sassafras (7%), spicebush (6%), and green ash (6%).

Nymphal trapping. In 2014 and 2015**,** no nymphs were captured between mid-May and mid-June and the first nymphal capture occurred during the same week, in mid-June. Total nymphal captures were higher in 2014 (n = 729) than in 2015 (n = 278). Based on pooled captures from both up and down traps across all sites and tree species, 90% and 84% of all captures were of nymphs in 2014 and 2015, respectively, with the remainder being adults. Despite large difference in total nymphal captures between years, the mean weekly proportion of total captures were statistically equal in 2014 and 2015 ($t = -0.19$, df = 41, $P = 0.85$) and the two seasonal trends were remarkably similar (Fig. 4.2). Weekly data pooled across all sites showed that significantly more upward- than downward-walking nymphs were captured in 2014 ($t = -2.75$, df = 42, $P = 0.01$) and 2015 ($t = -3.29$, df = 38, $P = 0.0021$).

Data pooled across all sites showed that nymphal captures in both up and down traps differed significantly throughout the season in 2014 ($F_{21, 2058} = 2.31$, $P = 0.0006$; Fig. 4.3) and 2015 ($F_{19, 2299} = 3.34$, $P < 0.0001$; Fig. 4.4). Based on degree-day accumulations, the onset of F_1 adult emergence was estimated to have occurred between 22 - 26 July, 2014 and 18 - 21 July, 2015. Nymphs captured before those dates were assumed to be predominantly the F_1 progeny of overwintered adults. Nymphs captured during the peak between late July and early August were assumed to be a combination of F_1 and F_2 nymphs, while those captured from mid-August onward were assumed to be mostly the F_2 progeny of summer generation adults (Figs. 4.3 and 4.4).

In 2014, 54.6% of the nymphs captured were second instars. First, third, fourth, and fifth instars comprised 0.20%, 20.1%, 12.1%, and 13% of captures, respectively. Similarly, 57.5% of the nymphs captured in 2015 were in the second instar. First, third, fourth, and fifth instars comprised 0.40%, 18.5%, 7.7%, and 15.9% of captures, respectively. The temporal distribution of nymphal instars captured varied in both years (Figs. 4.5 and 4.6). In 2014 and 2015, second and third instars predominated from mid-June through late August, while fourth and fifth instars were most abundant from early September onward. In both years, only fifth instars were captured at the end of the sampling period.

In 2014, mean weekly captures of nymphs in up and down traps differed significantly among the seven tree species from which sample sizes enabled analysis (Up: $F_{6, 1097} = 7.45$, $P < 0.0001$; Down: $F_{6, 1109} = 5.63$, $P < 0.0001$; Fig. 4.7). Highest captures of upward-walking nymphs were recorded from tree of heaven and black cherry while lowest captures were from apple, peach, and hackberry. Mean captures of nymphs walking up black locust and sassafras were intermediate. Captures in down traps in 2014 were significantly highest on peach and did not vary among the rest of the tree hosts. Interestingly, about 60% of the nymphs captured in the down traps on peach trees occurred after all the fruit were removed on 7 August.

In 2014, there were instances of higher numbers of nymphs in some traps and some weeks. For example, more nymphs were captured walking up on tree of heaven than on apple and peach from 18 June to 14 August with highest captures on 6 August (Fig. 4.8). On 20 August more nymphs were captured walking up on peach than on tree of heaven and apple. Throughout the season, nymphs captured walking up on apple and peach were comparable. Among the other hosts, highest captures of nymphs walking up on black cherry, hackberry, sassafras and black locust were observed on 9 July, 6 August, 6 August, and 14 August, respectively (Fig. 4.8). An apparent increase in the downward movement of nymphs was observed on peach on 14 August while the peak downward movement of nymphs on tree of heaven was detected on 6 August (Fig. 4.8). Downward movement of nymphs on other hosts remained low throughout the season.

In 2015, nymphs captured in up traps significantly differed among the seven tree species from which sample sizes enabled analysis while captures in down traps were statistically equivalent among the tree hosts (Up: $F_{6, 1094} = 4.22$, $P = 0.0003$; Down: $F_{6, 1105} = 0.61$, $P = 0.72$; Fig. 4.9). Highest captures in up traps were observed on tree of heaven and were significantly higher than captures on apple and peach. Upward nymphal captures on black cherry, black locust, hackberry and sassafras were intermediate and were statistically comparable to tree of heaven and the two cultivated hosts.

In 2015, season-long captures of nymphs walking up on apple and peach were relatively comparable while upward nymphal captures on tree of heaven were higher across the season with the highest peak on 5 August with noticeable early-season and late-season peaks (Fig. 4.10). Among the other hosts, peak nymphal captures on up traps on black locust, hackberry, black cherry and sassafras were detected on 8 July, 15 July, 29 July and 29 July, respectively. As in 2014, a pronounced peak in the downward movement of nymphs on tree of heaven was observed on 5 August, while down trap captures remained relatively low on other hosts (Fig. 4.10).

Discussion

Baseline information of the identity and relative abundance of tree species growing in woodlands adjacent to apple orchards in Virginia and West Virginia had provided additional context about the biotic factors that may influence *H. halys* populations and pest pressure in orchards within this region, where its populations have been highest and most damaging since its initial outbreak in 2010. Data from passive trunk traps installed on fruit tree and wild tree hosts at the orchard-woodland interface confirmed that *H. halys* move onto and off of host trees and revealed notable annual consistencies in their temporal and directional patterns of movement across two seasons.

Of the 47 tree species recorded from unmanaged woodlands next to orchards, 37 have been reported as hosts for *H. halys* for feeding and/or reproduction (Bakken et al. 2015, StopBMSB.org). Among the most common species, tree of heaven, hackberry, sassafras and green ash, all life stages of *H. halys* were documented by Bakken et al. (2015). Tree of heaven was the most common species at the woodland edge and was consistently among the hosts with highest *H. halys* populations reported by Bakken et al. (2015). Given that tree of heaven is a noxious, weedy, and invasive species, and that it is a preferred host of the recently-discovered invasive spotted lanternfly, *Lycorma delicatula* (White), growers may consider removing it from the landscape near their orchards to mitigate it potential effects on local populations. I note that since the data from this survey represented only 15 sites in two mid-Atlantic states, they likely do not reflect the tree community in other regions where *H. halys* populations have established (see StopBMSB.org).

Using the traps that were most effective for intercepting nymphs walking up and down tree hosts, respectively (Acebes-Doria et al. 2016), I showed that, 1) seasonal trends in the captures of *H. halys* nymphs were consistent in 2014 and 2015, 2) nymphal captures reflected both the seasonal phenology of *H. halys* in this region and seasonal changes in nymphal distribution, 3) highest captures were of second instars, 4) more nymphs were captured walking up than down, and 5) the directional movement of nymphs varied significantly on selected orchard and wild trees at the orchard-woodland interface across the season.

I was able to monitor the directional and temporal patterns of nymphal movement on specific cultivated and wild host trees. Nymphs actively moved off of and onto different host trees via their main trunk. Relative to 2014, total nymphal captures showed a 62% decrease in 2015, which was undoubtedly associated and consistent with numerous reports at the 2015 BMSB Working Group Meeting of lower *H. halys* populations in 2015, following an unusually cold winter. However, despite these differences in the raw data, the proportions of weekly captures were very similar between years, indicating consistent seasonal trends (Fig. 4.1). For example, it is noteworthy that first nymphal captures and peak captures were on the same weeks each year.

Studies in West Virginia and Virginia have indicated that *H. halys* is bivoltine (Bakken et al. 2015, Leskey et al. 2012c). Adult *H. halys* emergence from overwintering sites begins in April and peaks between mid-May and early June (Bergh and Leskey, unpublished). Upon emergence, adults presumably feed on host plants before they reproduce (Nielsen, unpublished), and oviposition begins between late May and mid-June. Plant-feeding by nymphs does not occur until the second instar. F_1 adults complete development by mid-July and the F_2 generation starts between mid-July and late July Leskey et al. (2012c). Based on degree-day accumulations and the established developmental threshold for *H. halys* (Nielsen et al. 2008, Leskey et al. 2012c), seasonal trends in nymphal captures and the distribution of instars captured provide further support for bivoltine populations of *H. halys* in northern Virginia and West Virginia and conform to the results of phenological studies by Leskey et al. (2012c). As well, capture trends in conjunction with the degree-day data suggested that the nymphal generations overlap in mid-season (i.e. late July to early-August) when highest captures were recorded.

Seasonal changes in the distribution of nymphal instars captured may have important management implications for the potential risk of injury to tree fruit. As discussed in Chapter 5, injury to apple and peach varied significantly between feeding by young and old *H. halys* nymphs and differed depending on the period during which feeding occurred. Feeding on tree fruit (i.e. apple and peach) by older nymphs later in the season was qualitatively and quantitatively more detrimental than feeding by young nymphs early in the season and feeding by older nymphs caused injury that was equivalent to that from adults. These findings and the nymphal trapping results implied that including older nymphs in management thresholds and heightened control measures later in the season may be important considerations in designing management programs against *H. halys* in orchard systems.

Similar to the results reported by Acebes-Doria et al. (2016), captures of second instars were highest. There are three plausible reasons for this, which are not necessarily mutually exclusive. First, as mentioned above, nymphs disperse from the egg mass and begin feeding on host plants in the second instar, hence increased dispersal activity and captures of this instar may be expected. Second, it may be that second instars are more abundant than older instars possibly because as a consequence of their dispersal many of them die before they molt to the third instar (e.g. loss of nutrient reserves, increased chance of predation, feeding on suboptimal hosts). Third, it may also be possible that later instars do not disperse to the same degree and remain on individual hosts throughout their developmental period.

In 2014 and 2015, respectively, 77% and 86% of nymphal captures were in up traps. These results conform well to those of Acebes-Doria et al. (2016), who found that 88% of all nymphs captured were in up traps. As well, Moeed and Meads (1983), installed trunk traps on trees in A New Zealand forest and reported that 80% of the 22,696 invertebrates sampled were found in up traps. Acebes-Doria et al. (2016) showed that second instar *H. halys* nymphs are positively phototactic and negatively geotactic, which may partially explain the consistently higher captures in up traps. In addition, Moeed and Meads (1983) suggested that arthropod dispersal from host trees may be mainly via dropping rather than by walking down the trunk. Numerous anecdotal observations have indicated that *H. halys* adults and nymphs drop from host plants when disturbed, suggesting that this behavior also may be associated with nymphal dispersal from hosts and may further explain the higher captures in up traps. I attempted to determine whether nymphs disperse from trees via dropping by placing sets of 4 sticky cardboard traps (36.83 cm^2 each) beneath of canopy of six female tree of heaven for 7-d intervals over 3 wk in 2014, but was unable to document such behavior, possibly due to the small trapping surface area and to the ability of nymphs (especially older nymphs) to extricate themselves from the sticky material as demonstrated in laboratory studies.

This study revealed differences in the upward and downward movement of nymphs among different host trees. Notably, total captures of nymphs walking up trunks of wild hosts such as tree of heaven, black cherry and black locust were higher than on peach and apple while the captures in down traps were highest on peach. As well, there were periods during which upward or downward nymphal movement were noticeably higher on certain hosts. For example, upward captures on tree of heaven were particularly high during the first week of August, when tree of heaven samaras were mature. Moreover, highest captures on peach were recorded from down traps after fruit were removed from the trees, paralleling the results of Martinson et al. (2015) who showed that *H. halys* nymphal and adult abundance decreased significantly when fruit from ornamental trees was removed completely. Martinson et al. (2015) also surveyed different ornamental trees known to be hosts of *H. halys* and found highest populations associated with trees bearing mature fruit. In combination with their results, the considerable dispersal capacity of nymphs (Lee et al. 2014) and the apparent variation in upward and downward movement on different hosts demonstrated in my studies suggest that nymphs may have an underlying ‘need’ to utilize different hosts during the season. This may be partly explained by the fact that nymphal development and survivorship of *H. halys* are significantly enhanced when they feed on multiple diets (Funayama 2006, Chapter 2). Hence, the orchard-woodland interface may serve as an ideal environment for *H. halys* to thrive, given an assured food source (i.e. tree fruit) and the substantial diversity of wild hosts growing at the edge of the nearby woodlands. Not surprisingly, *H. halys* injury to apples was higher on trees in border rows next to woods than on those in the orchard interior blocks (Leskey et al. 2012b, Joseph et al. 2014). Indeed, our results further reinforce the need to develop perimeter-based, integrated management strategies such as “Crop Perimeter Restructuring” (Blaauw et al. 2015) and “Attract and Kill” at orchard borders (Morrison et al. 2015) to manage this perimeter driven threat.

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Table 4.1. GPS coordinates for census site for species identification and abundance of trees growing in unmanaged woodlands adjacent to one experimental and 14 commercial apple orchards.

Site	Coordinates	
West Virginia		
Site 1	39°24'01.67"N	77°53'24.34"W
Site 2	39°26'28.63"N	78°02'28.02"W
Northern Virginia		
Site 3	39°06'40.76"N	78°17'17.23"W
Site 4	39°06'57.72"N	78°14'50.96"W
Site 5	39°04'35.57"N	78°18'19.06"W
Site 6	39°03'46.13"N	78°19'41.87"W
Site 7	39°08'42.74"N	78°15'26.06"W
Site 8	39°13'53.00"N	78°09'21.31"W
Site 9	39°12'01.98"N	78°12'27.77"W
Site 10	38°41'31.34"N	78°43'08.74"W
Site 11	38°41'41.51"N	78°41'22.15"W
Site 12	38°41'06.26"N	78°42'38.85"W
Central Virginia		
Site 13	37°50'35.60"N	79°01'03.53"W
Site 14	37°57'33.38"N	78°44'33.70"W
Site 15	37°45'58.03"N	79°02'36.46"W

Table 4.2. Orchard sites at which *H. halys* nymphs were trapped in 2014 and 2015.

Orchard Location	Fruit Tree	Main wild host	Other Wild Hosts
Virginia Tech Research Station, Winchester, VA	Peach (5,5)	Tree of heaven (5,5)	Black cherry (3,3) Black locust (1,1) Hackberry (1,1)
USDA Fruit Research Station, Kearneysville, WV	Peach (5,5)	Tree of heaven (5,5)	Black cherry (1,1) Boxelder (1,1) Hackberry (1,1) Mulberry (1,1) Sassafras (1,1)
Solenberger Orchard 1 Winchester, VA	Apple (5,5)	Tree of heaven (5,5)	Black cherry (2,2) Black locust (2,2) Sassafras (1,1)
Solenberger Orchard 2 Winchester, VA	Apple (5,5)	Tree of heaven (5,5)	Green ash (1,1) Sweet cherry (1,1) Black Walnut (1,1) Hackberry (2,2)
Solenberger Orchard 3 Winchester, VA	Apple (5,5)	Tree of heaven (5,5)	Black cherry (1,1) Black locust (1,1) Hackberry (1,1) Sassafras (2,2)

Numbers in parentheses indicate the number of trees with up and down traps.

Table 4.3. Number and percent relative abundance of the tree species identified at the border and the interior of unmanaged woodlands surrounding one experimental and 14 commercial apple orchards in Virginia and West Virginia.

Family	Scientific Name	Common Name	Exterior		Interior		*H. halys* host
			Count	%	Count	%	
Adoxaceae	*Viburnum prunifolium* L.	Blackhaw	43	2.83%	21	2.49%	Yes[a]
	Viburnum acerifolium L.	Mapleleaf viburnum	10	0.66%	0	0.00%	No
Anacardiaceae	*Rhus glabra* L.	Smooth sumac	6	0.39%	4	0.47%	Yes[b]
	Toxicodendron pubescens Mill.	Poison-oak	3	0.20%	0	0.00%	No
Betulaceae	*Betula lenta* L.	Sweet birch	18	1.18%	13	1.54%	No
	Carpinus caroliniana Walter	Hornbeam	1	0.07%	5	0.59%	No
	Ostrya virginiana (Mill.) K. Koch	Hop hornbeam	12	0.79%	4	0.47%	No
Cannabaceae	*Celtis occidentalis* L.	**Common hackberry**	**174**	**11.43%**	**96**	**11.37%**	No
Cornaceae	*Cornus florida* L.	Flowering dogwood	11	0.72%	23	2.73%	Yes[a,b]
	Nyssa sylvatica Marshall	Blackgum	8	0.53%	14	1.66%	Yes[a]
Elaeagnaceae	*Elaeagnus umbellata* Thunb.	Autumn olive	6	0.39%	3	0.36%	Yes[a,b]
Fagaceae	*Quercus alba* L.	White oak	24	1.58%	26	3.08%	Yes[a,b]
	Quercus velutina Lam.	Black oak	21	1.38%	24	2.84%	No
	Quercus montana Willd.	Chestnut oak	15	0.99%	13	1.54%	Yes[b]
	Quercus rubra L.	Northern red oak	8	0.53%	4	0.47%	Yes[a]
Juglandaceae	*Carya tomentosa* (Lam.) Nutt.	Mockernut hickory	35	2.30%	22	2.61%	Yes[b*]
	Carya glabra (Mill.) Sweet	Pignut hickory	34	2.23%	22	2.61%	Yes[b*]

	Carya cordiformis (Wangenh.) L. Koch	Bitternut hickory	60	3.94%	8	0.95%	Yes[b*]
	Carya ovata (Mill.) K. Koch	Shagbark hickory	9	0.59%	2	0.24%	Yes[a,b*]
	Juglans nigra L.	Black walnut	12	0.79%	2	0.24%	Yes[a,b]
Lauraceae	*Lindera benzoin* (L.) Blume	**Spicebush**	**77**	**5.06%**	**69**	**8.18%**	No
	Sassafras albidum (Nutt.) Nees	**Sassafras**	**128**	**8.41%**	34	4.03%	Yes[a,b]
Leguminosae	*Cercis canadensis* L.	**Eastern redbud**	8	0.53%	**55**	**6.52%**	Yes[a,b]
	Gleditsia triacanthos L.	Honeylocust	6	0.39%	8	0.95%	Yes[a]
	Robinia pseudoacacia L.	**Black locust**	**70**	**4.60%**	1	0.12%	Yes[a,b]
Magnoliaceae	*Liriodendron tulipifera* L.	Tulip poplar	37	2.43%	28	3.32%	Yes[a,b]
	Magnolia acuminate (L.) L.	Cucumber tree	0	0.00%	3	0.36%	No
Malvaceae	*Tilia americana* L.	American basswood	14	0.92%	0	0.00%	Yes[a,b]
Moraceae	*Morus rubra* L.	Red mulberry	18	1.18%	11	1.30%	Yes[b*]
	Morus spp.	Mulberry	0	0.00%	5	0.59%	Yes[b*]
Oleaceae	*Fraxinus pennsylvanica* Marshall	**Green ash**	54	3.55%	**79**	**9.36%**	Yes[a,b*]
	Fraxinus americana L.	White ash	39	2.56%	40	4.74%	Yes[a,b*]
Pinaceae	*Pinus strobus* L.	Eastern white pine	0	0.00%	9	1.07%	No
	Tsuga Canadensis (L.) Carrière	Eastern hemlock	0	0.00%	1	0.12%	Yes[a]
	Pinus spp.	Pine	1	0.07%	0	0.00%	No
Platanaceae	*Platanus occidentalis* L.	American sycamore	4	0.26%	1	0.12%	Yes[a,b]
Rosaceae	*Prunus serotina* Ehrh.	**Black cherry**	60	3.94%	**63**	**7.46%**	Yes[a,b]
	Prunus avium (L.) L.	Sweet cherry	16	1.05%	13	1.54%	Yes[a]
	Crataegus spp.	Hawthorn	3	0.20%	0	0.00%	Yes[a*]
Sapindaceae	*Acer platanoides* L.	Norway maple	14	0.92%	21	2.49%	Yes[a]

	Acer negundo L.	Boxelder	5	0.33%	6	0.71%	Yes[a]
	Acer rubrum L.	Red maple	9	0.59%	1	0.12%	Yes[a,b]
	Acer spp.	Maple	0	0.00%	5	0.59%	Yes[b*]
Scrophulariaceae	*Paulownia tomentosa* Steud	Paulownia	5	0.33%	0	0.00%	Yes[a,b]
Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	**Tree of heaven**	**374**	**24.57%**	32	3.79%	Yes[a,b]
Ulmaceae	*Ulmus rubra* Muhl.	Slippery elm	55	3.61%	48	5.69%	Yes[a*,b*]
	Ulmus americana L.	American elm	15	0.99%	5	0.59%	Yes[a,b*]

% Percent relative abundance

Species in bold font in each column are the five most abundant species in each sampled area.

[a] Stopbmsb.org

[b] Bakken et al. (2015)

* Unspecified species

Figure 4.1. Up and down traps used, respectively, to capture *H. halys* nymphs walking up and down trunks of selected wild and fruit tree hosts at the orchard-woodland interface at three apple orchard and two peach orchard sites in Virginia and West Virginia.

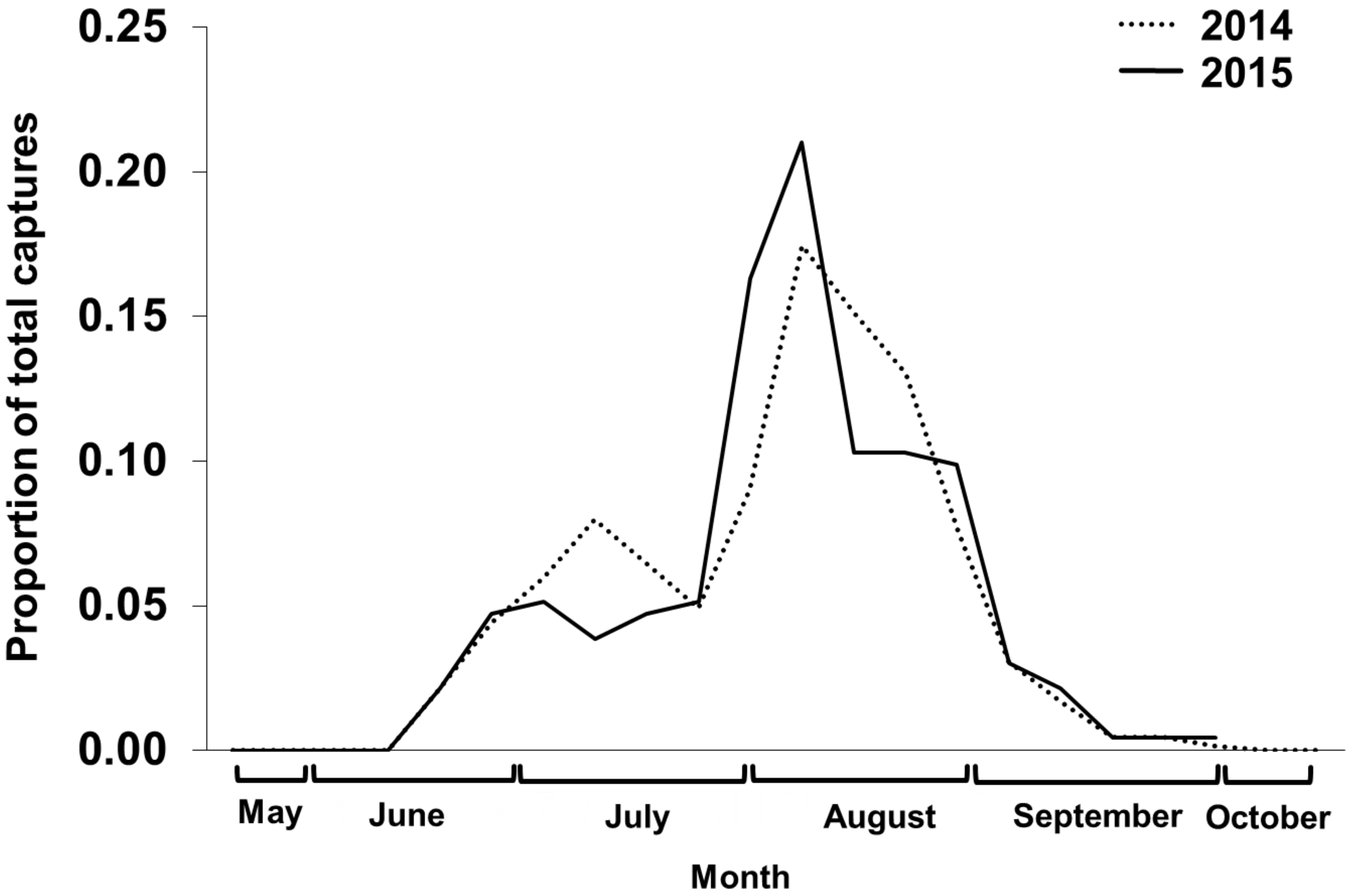

Figure 4.2. Weekly proportion of total seasonal captures of *H. halys* nymphs across all sites and tree species, 2014 and 2015.

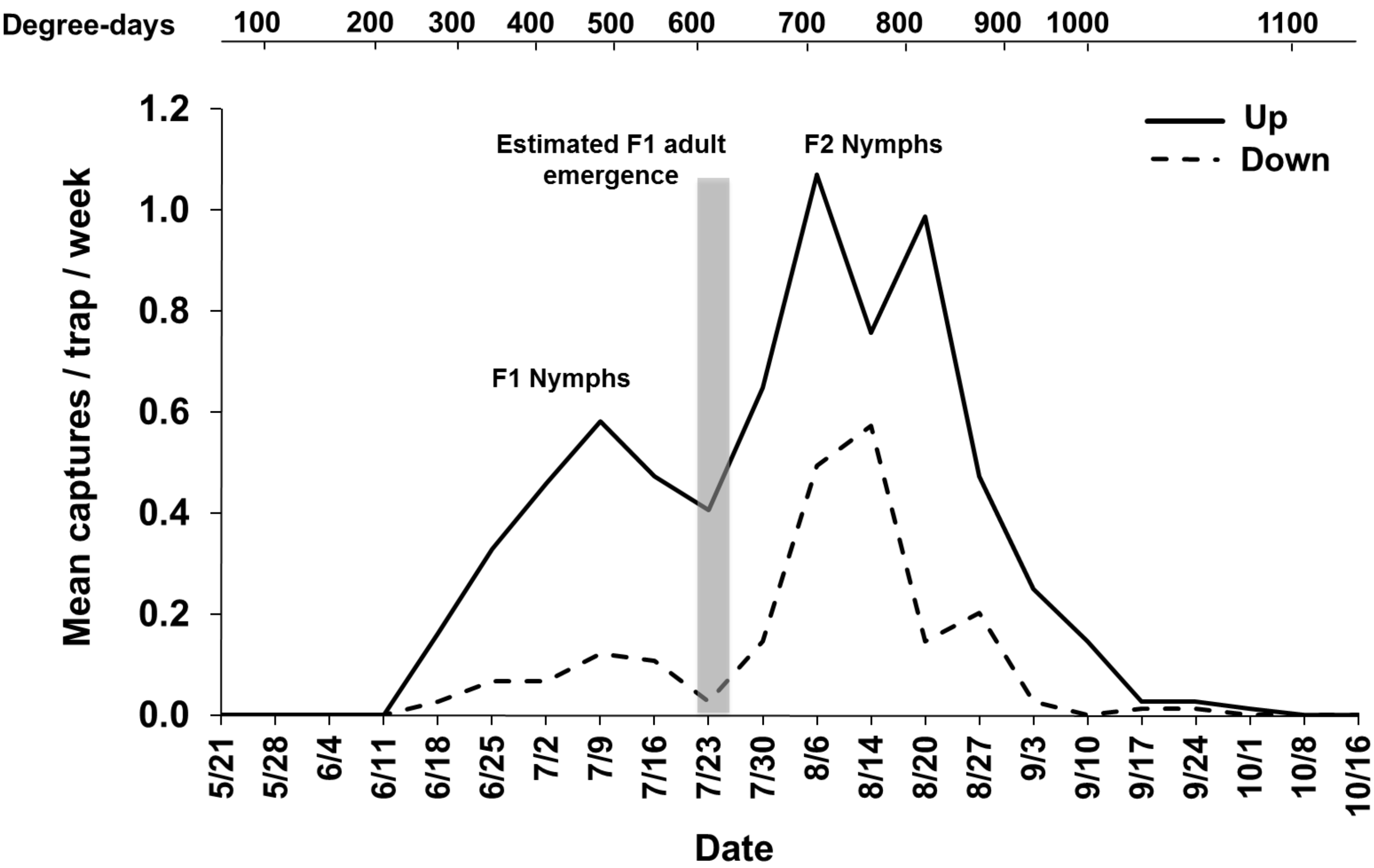

Figure 4.3. Mean weekly captures of *H. halys* nymphs in up (N = 75) and down (N = 75) trunk traps on selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia, 2014. Degree-day accumulations and estimations of *H. halys* phenology are provided. Grey bar indicates the estimated period when F_1 adults were first present.

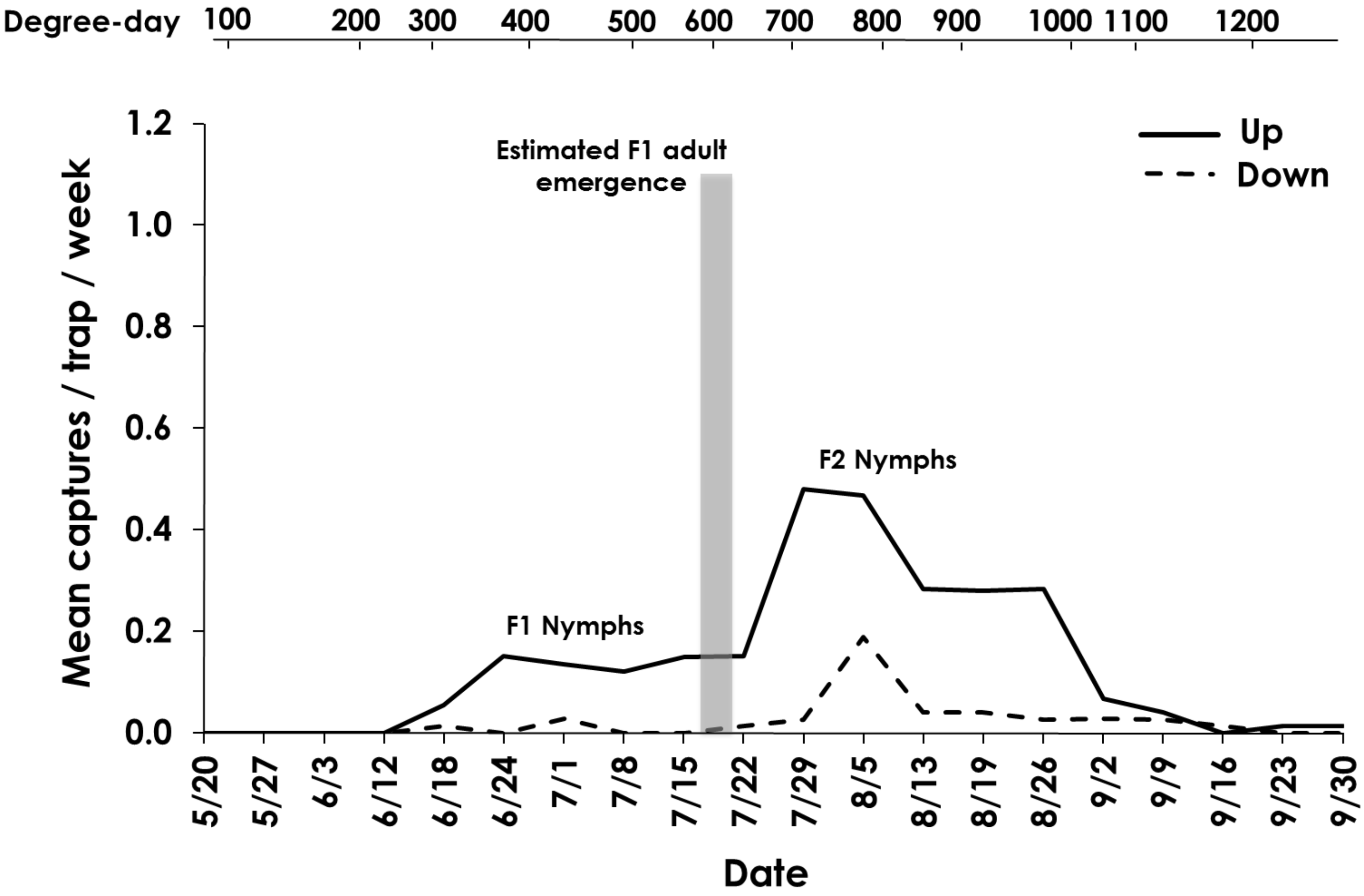

Figure 4.4. Mean weekly captures of *H. halys* nymphs in up (N = 75) and down (N = 75) trunk traps on selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia, 2015. Degree-day accumulations and estimations of *H. halys* phenology are provided. Grey bar indicates the estimated period when F_1 adults were first present.

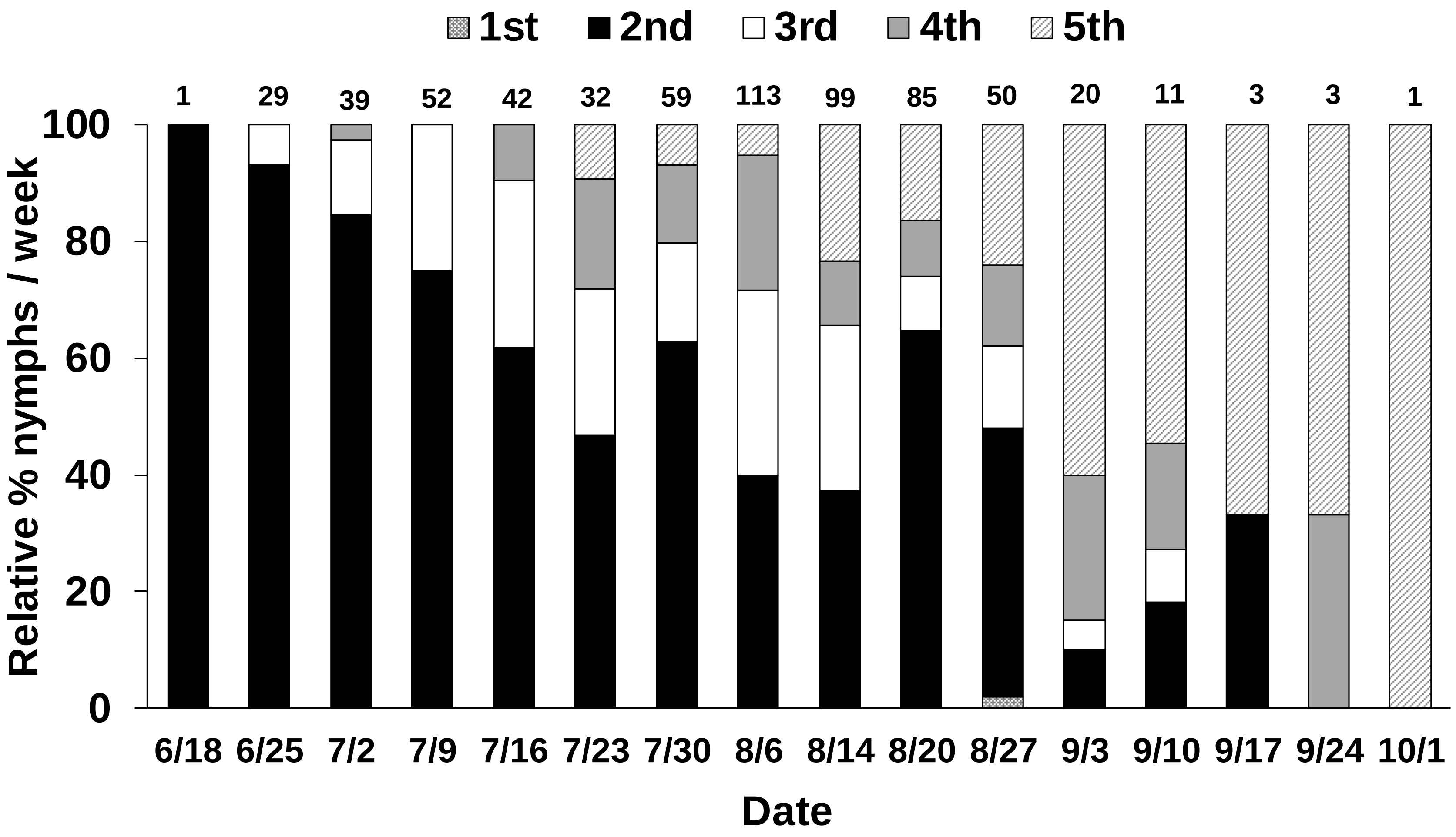

Figure 4.5. Weekly distribution of *H. halys* nymphal instars captured in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 1 October, 2014. Numbers atop bars indicate total weekly captures. No nymphs were captured prior to the week of 18 June.

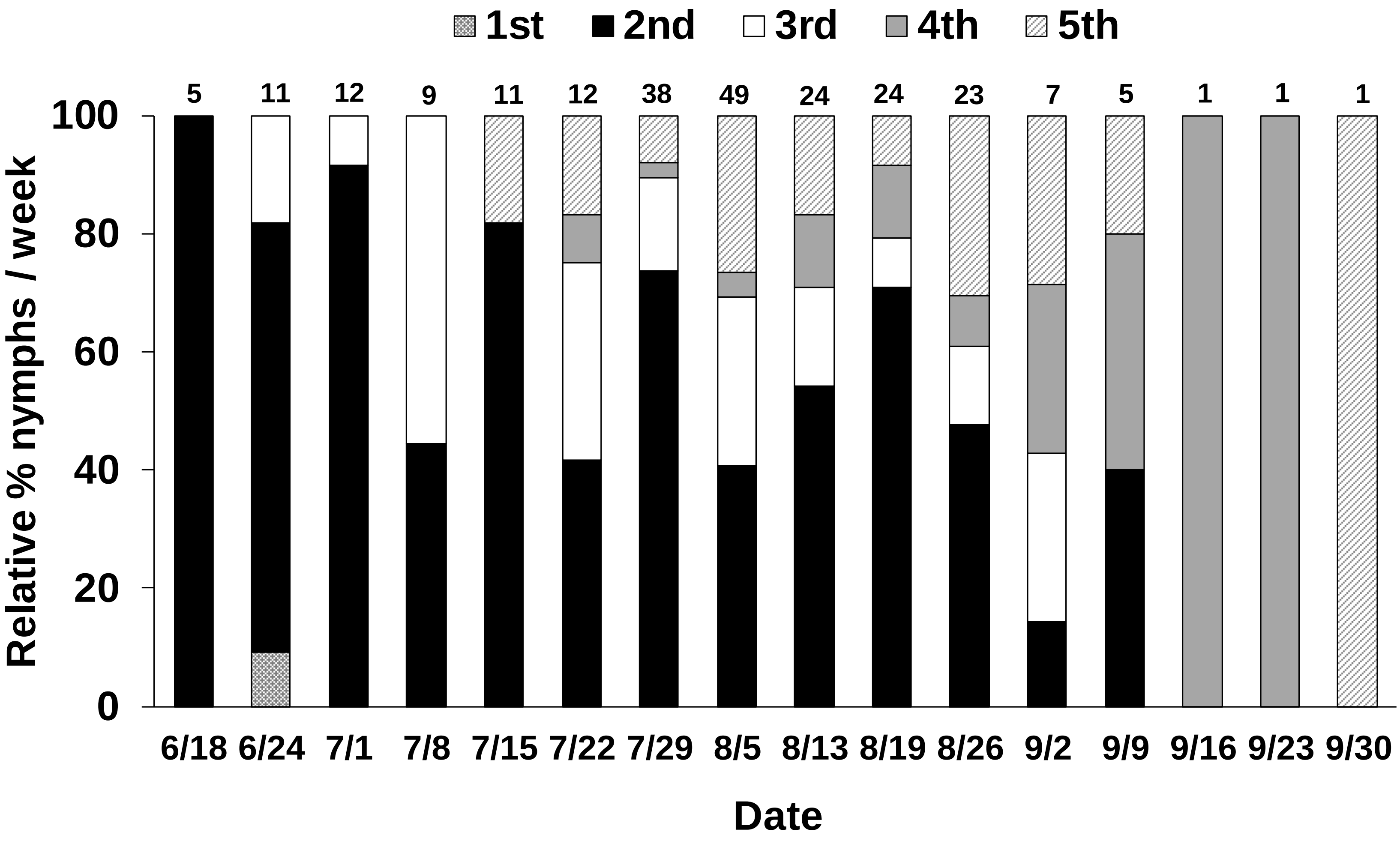

Figure 4.6. Weekly distribution of *H. halys* nymphal instars captured in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 30 September, 2015. Numbers atop bars indicate total weekly captures. No nymphs were captured prior to the week of 18 June.

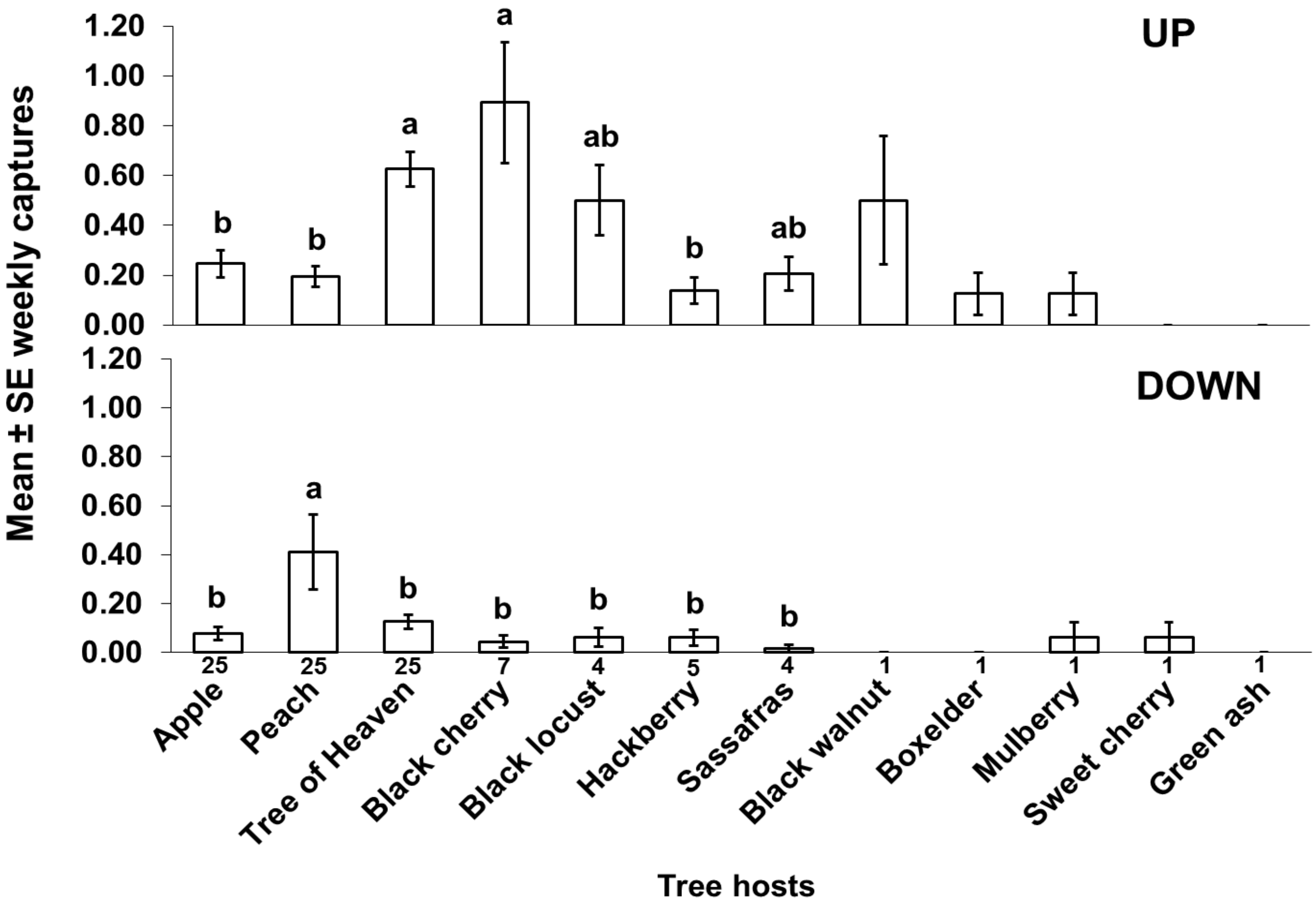

Figure 4.7. Captures of *H. halys* nymphs in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 1 October, 2014. Numbers next to tree names indicate the number of replications for each species. Hosts with n = 1 were excluded from the analyses.

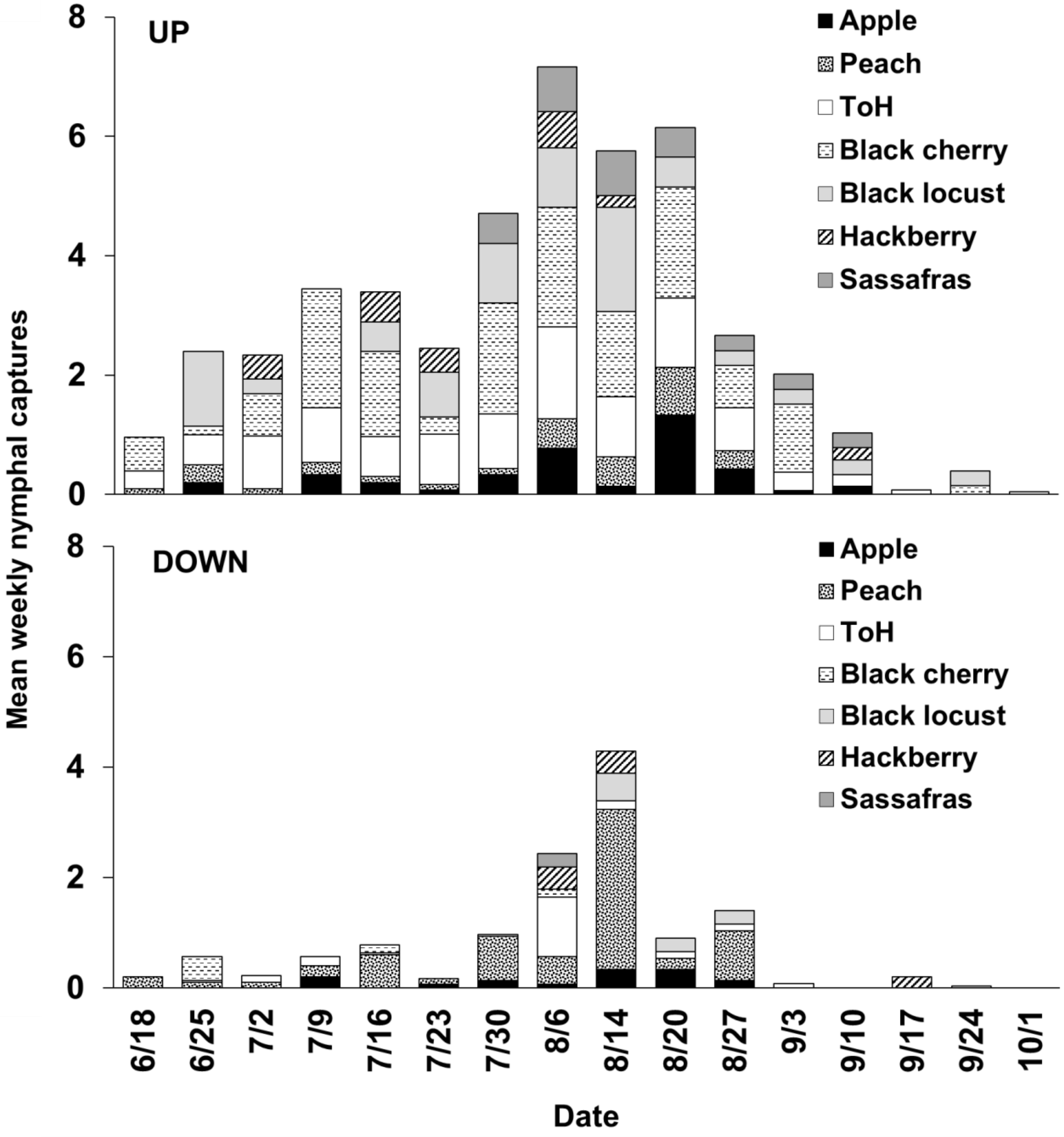

Figure 4.8. Mean number of *H. halys* nymphs captured weekly in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 1 October, 2014.

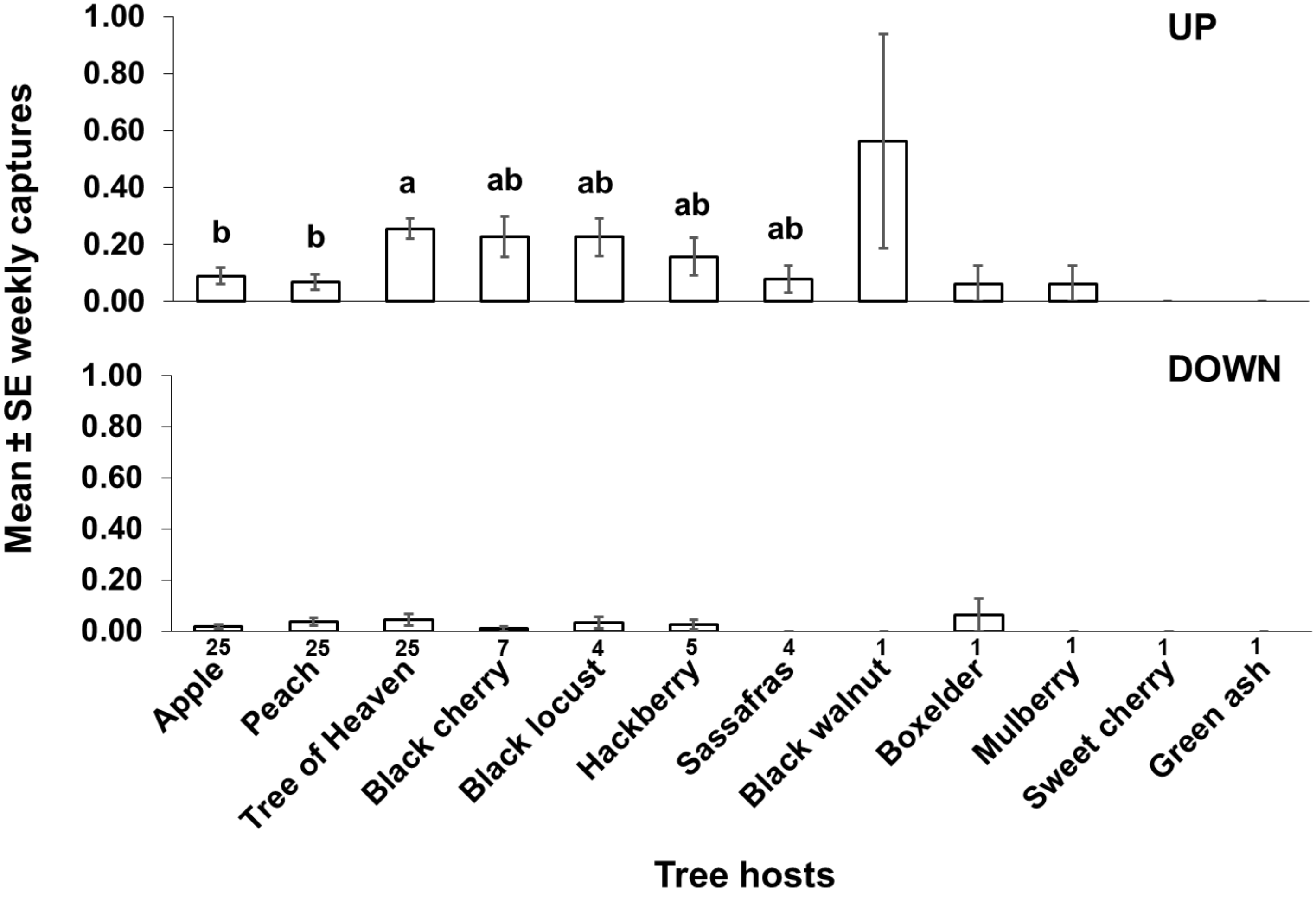

Figure 4.9. Captures of *H. halys* nymphs in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 30 September, 2015. Numbers next to tree names indicate the number of replications for each species. Hosts with n = 1 were excluded from the analyses.

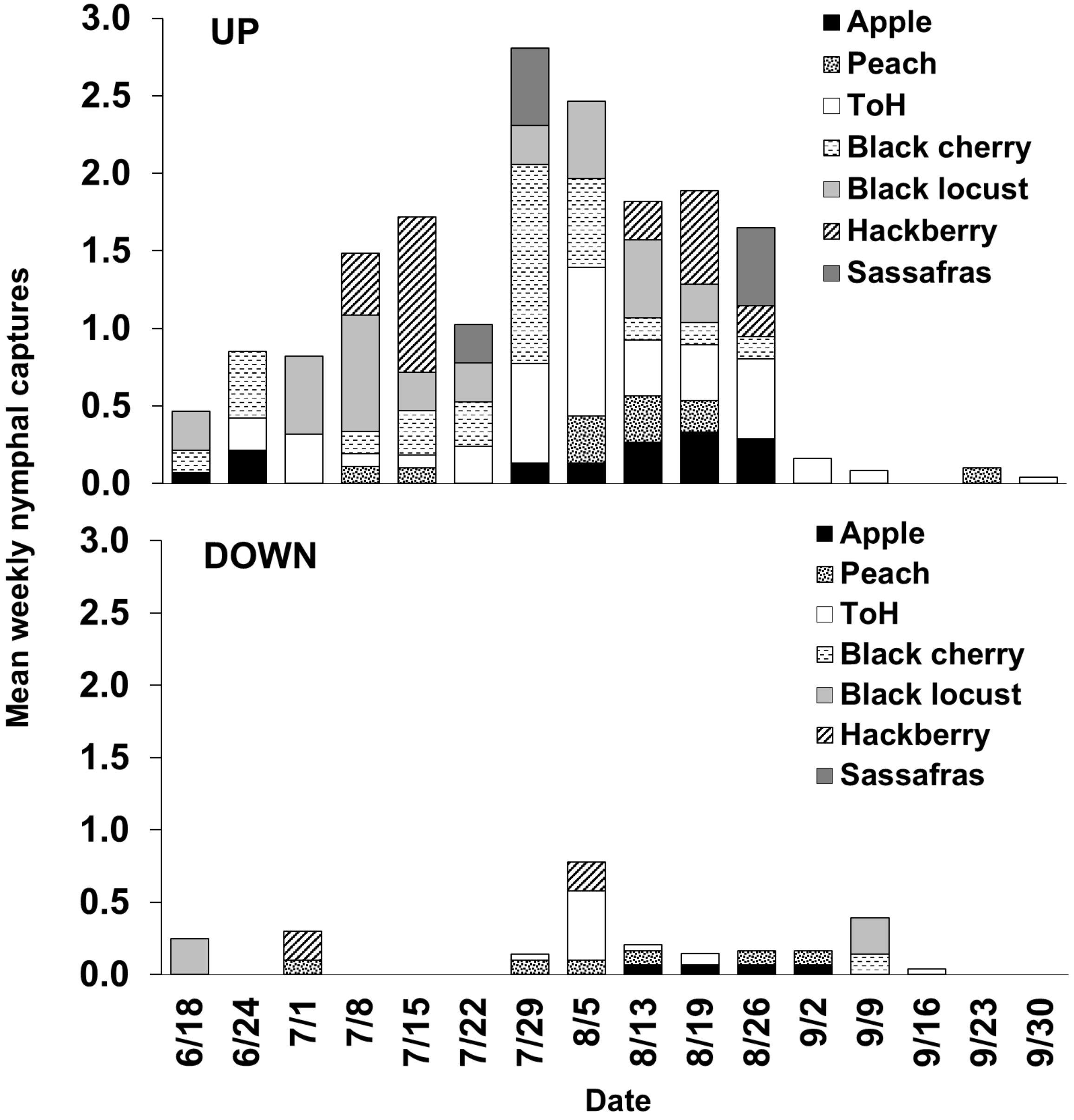

Figure 4.10. Mean number of *H. halys* nymphs captured weekly in up (N = 75) and down (N = 75) traps on the trunk of selected wild and fruit tree hosts at the orchard-woodland interface at apple and peach orchard sites in Virginia and West Virginia from 18 June to 30 September, 2015.

CHAPTER 5: INJURY TO APPLES AND PEACHES AT HARVEST FROM FEEDING BY *HALYOMORPHA HALYS* NYMPHS EARLY AND LATE IN THE SEASON

Abstract

Halyomorpha halys adults and nymphs feed on tree fruits. Feeding injury from adults has been characterized but the injury from nymphs has not been examined systematically. Since the four plant-feeding instars of *H. halys* (second through fifth) differ substantially in size, it is plausible that the effects of their feeding on fruit injury and injury expression may differ among them. I compared feeding injury at harvest from young nymphs (second plus third instars), older nymphs (fourth plus fifth instars), and adults that were caged on 'Smoothee Golden' apples and 'Redhaven' peaches in early June (peach and apple), late July (peach), and late August (apple). Individual apples and peaches were caged at fruit set and assigned to the following treatments (n=28/treatment): 1) control (no *H. halys*), 2) young nymphs or 3) adults early in the season, and 4) young nymphs, 5) older nymphs or 6) adults later in the season. Fruit in each treatment were exposed to 3-4 young nymphs, two older nymphs or 1-2 adults placed in the cages for 96 h and evaluated for external and internal feeding injury within 36 h after harvest. No injury was recorded from unexposed peaches or apples. The percentage of injured fruit and number of injuries per fruit varied significantly among the exposed treatments. Early-season feeding by young nymphs yielded the least injury to peaches and apples. In apples, the highest percentage of injured fruit and number of injuries per fruit were caused by late-season feeding by adults. In peaches, early-season adult feeding produced the highest percentage of injured fruit and injuries per fruit. More internal than external injury was recorded on peach and no such difference was observed on apple. The implications of these findings on *H. halys* management in fruit orchards are discussed.

Keywords: tree fruit, brown marmorated stink bug, *Malus domestica*, *Prunus persica*

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive species that has become an important orchard pest in the mid-Atlantic region of the US. Following its widespread outbreak in this region in 2010, *H. halys* feeding injury resulted in losses to the mid-Atlantic apple crop valued at $37 million and a complete loss of peaches in some Maryland orchards (Leskey et al. 2012a). Aside from the fact that *H. halys* populations have not yet shown indications of being adequately suppressed by native parasitoids and predators, several basic aspects of its biology and behavior contribute to its status as a key orchard pest. First, it is highly polyphagous (Rice et al. 2014) and is present throughout most of fruiting period of orchard crops (Leskey et al. 2015). Many of its host plants are common at the forest edge adjacent to orchards in the mid-Atlantic region (Acebes-Doria, unpublished data) and can support the development of large, localized populations that can pose a season-long risk to nearby orchards. As well, the ability of *H. halys* to invade orchards season-long is facilitated by the strong flight dispersal capacity of adults (Wiman et al. 2014, Lee and Leskey 2015) and the ability of nymphs to walk considerable distances in search of food plants (Lee et al. 2014). Finally, unlike the native stink bug pests of tree fruits, both nymphs and adults of *H. halys* can feed on and injure fruit.

While the injury symptoms from feeding by adult *H. halys* on apples and peaches have been well characterized (Nielsen and Hamilton 2009, Leskey et al. 2012b, Joseph et al. 2015), those associated with feeding by nymphs have not. *Halyomorpha halys* has five nymphal instars, of which the second through fifth feed on plants. These developmental stages vary considerably in size (Hoebeke and Carter 2003) and may therefore differ in the extent to which they cause economic injury to tree fruit and/or the nature of injury expression. As well, there is a portion of each growing season when *H. halys* captures in pheromone-baited traps consist predominantly of nymphs (Leskey et al. 2015) and thus it is important to understand the relative risk of injury from feeding by the different nymphal instars to apples and peaches at points during the season and to what extent nymphal feeding contributes to economic injury at harvest.

Here, I characterized, quantified and compared the injury to apple and peach fruit at harvest from feeding early and later in the season by young instar nymphs, older instar nymphs, and adults.

Material and Methods

Insects and Study Site. Male and female *H. halys* adults collected from an overwintering population inside a dwelling in Stephens City, VA in April 2015 were held in mixed-sex groups of 50-60 in 30.48 cm^3 screened cages (BioQuip Products, Inc., Rancho Dominguez, CA) in a laboratory room at ~25°C, ~70% RH, and 16L:8D photoperiod. Cages were provisioned regularly with a diet of popcorn kernels, barley, buckwheat, soybeans, dried figs, dry roasted, unsalted peanuts and sundried tomatoes in a Petri dish and a fresh apple in a separate dish. Water was provided in a small plastic container with a cotton wick inserted through the lid. Folded paper towels on the cage floor and three to four freshly excised, compound leaves of tree of heaven in a water-filled vase were provided as oviposition substrates. Starting in mid-May, egg masses in the cages were collected at 2-d intervals and held in Petri dishes in the rearing room described above. After the eggs had hatched, a piece of wet cotton placed in each dish provided moisture for the nymphs. Upon molting to second instar, nymphs were transferred to 30.48cm^3 screened cages provisioned with the same diet described above. The second and third instar nymphs from these laboratory colonies were used in the early-season field experiment. As well, subsets of the field-collected overwintered adult that were reared in the laboratory since April were used in the early-season field experiments. For the late season experiments, a combination of laboratory-reared and field-collected summer generation nymphs and adults were used. The field-collected *H. halys* were mainly from tree of heaven and catalpa growing near Winchester, VA and were held under laboratory conditions described above for $\geq$ 24 h prior to use in the experiments.

Trees in a 1.1-ha experimental block of 7-yr-old ‘Smoothee Golden’ apples and a 0.2-ha experimental block of 14-yr-old ‘Redhaven’ peaches at Virginia Tech’s Alson H. Smith Jr., Agricultural Research and Extension Center (AHS AREC), Winchester, VA were used. The trees were treated with a routine disease management program, but no insecticides were applied to them during the study.

Inclusion Cage Experiments. Cylindrical, open-ended cages (17.8 cm diam, 17.8 cm long) were constructed from semi-rigid, black plastic mesh (13 × 3 mm openings) covered by a 5-gal paint strainer (Ace Hardware Corp, Oak Brook, IL) with one open end. A wood dowel (0.7 cm diam, 18 cm long) placed perpendicularly in the center of cage was secured at both ends to the cage wall. In early May, soon after fruit set, the cages were used to enclose one or two peach and apple fruit growing near branch tips; in mid-May the number of fruit in each cage was reduced to one. Only fruit that showed no evidence of injury from any insect pest were selected. Plastic twist ties were used to attach the dowel to the branch, to secure one edge of the plastic cage to the branch so the fruit was not touching the cage wall, and to close the opening of the mesh sleeve around the branch.

In total, 168 apples on 28 trees (6 fruit/tree) and 168 peaches on 14 trees (12 fruit/tree) were caged. Pre-determined numbers of insects from the laboratory culture were transferred to small lidded plastic cups and released in each cage. To assess the effects of *H. halys* feeding either early or later in the fruiting period of each crop, there were two separate 96-hr exposure periods. For both apples and peaches, the early season exposure occurred between 6 and 10 June. Since fourth and fifth instar nymphs would not be expected in the field at the time of the early season experiment (see Chapter 4), the following treatments were used; 1) control (not exposed to *H. halys*), 2) four second and third instar nymphs, and 3) two adults, with 28 replicates each. The late season exposure duration also was 96 hr, but the exposure dates differed between the crops, based on differences in projected harvest dates and fruit maturity; peaches and apples were exposed from 20-24 July and 21-25 August, respectively. Since all nymphal instars would be expected in field populations of *H. halys* at the time of the late season exposure (see Chapter 4), the treatments included, 1) control (not exposed to *H. halys*), 2) three second and third instars, 3) two fourth and fifth instars, and 4) 1-2 adults, each with 28 replicates. At the end of each 96-hr exposure period, the number of live and dead bugs in each cage was recorded and the insects were removed from the cages. Of all cages to which bugs were introduced, only 2.5% contained no live individuals at the end of the exposure period and since those bugs would have been alive and presumably have fed on the fruit within the 96-hr exposure, the exposed fruit were included in the evaluation. The caged peaches and apples were harvested on 31 July and 11 September, respectively. Those that had dropped from the branch prior to harvest were excluded from the evaluation, which represented only 4.8 % of both apples and peaches. Fruit were stored at 4°C and evaluated for injury within 36 hr of harvest. The average daily temperature and rainfall during the exposure periods were recorded using the WatchDog weather station (Model 900ET, Spectrum Technologies, Inc., Plainfield, IL) installed at the AHS AREC.

Injury Evaluation. Criteria used to evaluate injury to the apples and peaches followed Joseph et al. (2015). For apples, external injury was recorded as the number of ‘punctures’ and/or ‘dimpling’ (Figure 5.1A) and surface discoloration with or without distinct depressions (Figs. 5.2A and 5.3A, respectively). Internal injury to apples was recorded as the number of areas of necrotic tissue after peeling the skin and slicing each fruit to the core in all quadrants (Figs. 5.1C, 5.2B and 5.3B). For peaches, external injury was recorded as the number of areas of gummosis (Fig. 5.4) and the number of surface depressions or deformations (Figs. 5.5A and 5.6A). Internal injury was recorded as the number of discrete areas of necrotic tissue after fruit were peeled and sliced to the core in all quadrants (Figs. 5.4B, 5.4C, 5.5B and 5.6B). To adjust the injury recorded based on differences in the number of insects per cage, the total number of injury sites on each fruit was divided by the number of bugs to which that fruit had been initially exposed.

Data Analyses. Analyses were conducted using JMP® Pro version 11 (SAS Institute Inc., Cary, NC, 2007) and outcomes were considered significant at $P < 0.05$. The number of injured apples and peaches were compared among treatments using nominal logistic regression. When the likelihood ratio chi-square test indicated a significant overall treatment effect, the difference between two treatments was examined using the odds ratio. The number of each injury type per fruit and mean daily rainfall data were transformed using log(x+1) to satisfy the assumptions of parametric tests. Differences in the number of external injuries and the number of internal injuries per fruit among treatments in each experiment were analyzed using One-Way ANOVA. For external injuries, each injury type was analyzed separately. One-Way ANOVA was also used to compare the differences on the mean daily temperatures and rainfall among the exposure periods. When a significant treatment effect was found in the ANOVA, means were compared using Tukey’s HSD test. Differences between the number of surface depressions and internal injuries per fruit were compared using the pairwise t-test.

Results

Of all nymphs and adults released into cages, 86% were recovered alive after the 96-hr exposure period and 97.5% of cages contained ≥1 live individual. For both the early and late exposure periods, control fruit showed no evidence of injury (Tables 5.1 and 5.3), indicating that the cages excluded *H. halys* and other pests effectively. Mean (± SE) daily temperatures recorded during the exposure periods on 6–10 June, 20-24 July and 21-25 August were 21.1 ± 0.6°C, 24.0 ± 0.9°C, and 22.3 ± 0.5°C, respectively, which differed significantly ($F_{2,12} = 4.60$, $P = 0.03$). The mean daily temperature on 20-24 July was significantly higher than on 6-10 June and 21-25

August, which did not differ. Mean (± SE) daily rainfall during the respective exposure periods was 0.05 cm, 0.06 cm, and 0.0 cm, and did not differ significantly ($F_{2,12} = 0.56$, $P = 0.58$).

Apples. The percentage of apples showing external and internal injury at harvest differed significantly among the treatments (Table 5.1). The percentage of apples with punctures/dimpling was significantly highest on fruit exposed to adults early in the season. Exposure to older nymphs and adults later in the season resulted in the highest percentages of apples with discolored areas with or without distinct depressions (Table 5.1). Feeding by young nymphs later in the season also resulted in substantial surface injury that was significantly greater than earlier feeding by young nymphs and adults. The highest percentage of fruit that incurred internal injury were those exposed to adults and older nymphs late in the season, followed by those exposed to adults early in the season and young nymphs later in the season.

Table 5.2 shows the severity of injury (i.e. number of injuries per fruit) to apples at harvest. Exposure to adults early in the season resulted in significantly more punctures/dimpling than all other treatments, which did not differ. Fruit exposed to adults late in the season showed the greatest numbers of discolored depressions (Table 5.2), followed by those exposed to older nymphs during the same period. Exposure to young nymphs and adults early in the season caused the fewest discolored depressions, and were comparable to those from exposure to young nymphs later in the season. Similarly, the numbers of internal injury sites were significantly highest from exposure to adults late in the season. There were numerical but not significant differences in the number of internal injuries from exposure to older nymphs later in the season and adults earlier, with numerically or statistically fewest from young nymphs during both exposure periods.

Peaches. The percentage of peaches that incurred external and internal injury varied significantly among treatments (Table 5.3). A high percentage of fruit exposed to adults early in the season expressed gummosis, and this injury was not otherwise detected. Significantly more fruit with surface depressions were recorded from fruit exposed to adults early in the season, followed by adults late in the season. Exposure to both young and older nymphs late in the season resulted in intermediate percentages of peaches with surface depressions that were not statistically different. The lowest percentage of fruit with this injury was recorded from fruit exposed to young nymphs early in the season. The percentages of fruit showing internal necrosis at harvest were significantly highest from adult exposures early and late in the season, followed by those exposed to older nymphs in late season. Young nymphs caused significantly more fruit to show this injury from exposures during the late season than the early season, but both exposure periods resulted in a significantly lower percentage of injured fruit than the other treatments.

The number of internal and external injuries per peach at harvest differed significantly among the treatments (Table 5.4). Feeding by adults early in the season resulted in the greatest numbers of surface depressions ($P < 0.05$), followed by adults late in the season with all other treatments having significantly less and statistically equal numbers of depressions. Internal injuries were significantly more abundant on fruit exposed to adults early and late in the season, followed by those exposed to older nymphs late in the season. Feeding by young nymphs early and late in the season resulted in the least internal injury that did not differ.

Comparisons between the number of surface depressions (external injury) and internal injury pooled across all treatments showed that peaches expressed more internal than external injury per fruit ($t = 6.98$, df = 159, $P < 0.0001$); whereas, there was no difference between these two measures of injury in apples ($t = 1.41$. df = 159, $P = 0.1594$).

Discussion

This study demonstrated that injury to apples and peaches from feeding by *H. halys* differed according to the life stage to which fruit were exposed and to the time at which feeding occurred. The types of injury recorded were similar to those described previously (Nielsen and Hamilton 2009, Leskey et al. 2012b, Joseph et al. 2015). However, data from field studies by Leskey et al. (2012b) and Joseph et al. (2015) reasonably assumed that injury was from feeding by *H. halys*, based on recent grower experiences and on much higher populations of *H. halys* than native stink bugs species in orchards, but did not differentiate between injury caused by adults and nymphs. In a similar cage study by Nielsen and Hamilton (2009), apples and peaches were exposed only to adult *H. halys*. Here, we have systematically characterized the differences between adult and nymphal feeding injury and showed that injury to apples and peaches was quantitatively and qualitatively different. Exposure to second and third instar nymphs early in the season consistently resulted in lowest levels of external and internal injury to apples and peaches. However, with the exception of gummosis in peaches, young nymphs did cause both external and internal injury to both crops. Later in the season, when the fruit were near full maturation, second and third instar nymphs caused significantly or numerically more external and internal injury to both crops than they did early in the season. Most injury assessments following exposures late in the season revealed that older nymphs caused significantly or numerically more injury than younger nymphs. With the exception of punctures/dimpling from adult feeding on apples early in the season, more apples exposed to adults late in the season were injured and showed more injury than those exposed to adults early in the season. Also, punctures alone would not likely be recorded as economic injury at harvest if not associated with other surface injury. Feeding by adults and older nymphs late in the season caused similar percentages of apples to show external and internal injury at harvest, although the severity of injury was higher for adults than older nymphs. In peaches, high percentages of fruit showed surface depressions and internal necrosis following exposure to adults early or late in the season, with similar levels of injury severity between the exposure periods. During the late season exposure, adults caused significantly or numerically more injured fruit than older nymphs and significantly greater numbers of injuries per fruit.

Early season injuries on apples were expressed mainly as punctures/dimpling and as less apparent internal injury. In some fruit, necrotic areas were evident deeper in the flesh (Fig. 5.1). It may be helpful for growers to monitor stink bug injury by not only peeling the skin of fruit that have evident external injury but also to slice the fruit to check for deeper internal damage. The less pronounced expression of injury on apples early in the season may be attributed to the rapid rate of cell division at that time, which may reduce injury expression. Late-season injury on apple by older nymphs and adults was expressed as discolored surface depressions and as internal tissue necrosis which was mostly close to the surface (Figs. 5.2 and 5.3). These findings suggest that apples are more susceptible to economic injury at harvest from *H. halys* feeding late in the season than earlier. Although apple growers in the mid-Atlantic region who produce fruit for processing are tolerant of external injury and of some minor internal injury from *H. halys*, the quality of finished products could still be negatively affected by heavily damaged internal tissues. As well, both external and internal fruit defects are problematic for growers that produce fruit for fresh market.

On peaches, gummosis was an indicator of external feeding injury from *H. halys* early in the season and was only recorded only from fruit exposed to adults during that period (Fig. 5.4), concurring with previous findings that gummosis was associated with early-season stink bug feeding injury and was often be accompanied by surface discoloration or depressions and internal necrosis (Joseph et al. 2015). Early-season injury on peach was more pronounced than late-season injury, with obvious surface discoloration and deformations and deeper brownish internal necrosis (Fig. 5.4), whereas injuries from nymphs and adults later in the season were expressed mainly as shallow surface depressions and superficial whitish necrosis (Figs. 5.5 and 5.6). Since peach is considered a high value crop and produced mainly for the fresh market product in the mid-Atlantic region, these results imply that attack by *H. halys* either early or late in the season can negatively affect the marketability of this crop.

The lack of apparent fruit injury at harvest from feeding by young nymphs early in the season suggests that early-season injury to peach (Leskey et al. 2012b) is mainly caused by overwintered adults. This conforms to observations that overwintered adults start moving into orchards in mid-April (Leskey et al. 2015). Leskey et al. (2014) found that overwintered adults were more susceptible to insecticide applications than summer generation adults, suggesting the practicality of early-season insecticide applications on peach. On the other hand, the application of stronger broad spectrum insecticides may be more useful later in the season, when populations are mostly composed of older nymphs and adults than young nymphs. Additionally, since early-season injury to apple from both nymphs and adults was less than that incurred late in the season, there may be a period early in the season when *H. halys* management in apple orchards may not be as necessary as later in the season, when populations of older nymphs and adults are present.

Efforts are ongoing to establish management thresholds for *H. halys* in orchard systems. My results suggest that the inclusion of fourth and fifth instars in the development of thresholds may be important to consider since their feeding injury is qualitatively and quantitatively comparable to that from adults. This may be particularly useful later in the season, when these older nymphs are more abundant and attracted to the semiochemical lures used to monitor *H. halys* populations in the field (Weber et al. 2014, Leskey et al. 2015).

Moreover, *H, halys* is generally considered a perimeter-driven threat; both nymphs and adults are capable of dispersing from wild hosts into orchards and are present in orchards in substantial numbers, particularly in the border areas adjacent to woodlands (Bergh and Leskey, unpublished) where highest injury from *H. halys* has been recorded (Leskey et al. 2012b, Joseph et al. 2014). In Chapter 4, I showed that nymphs actively disperse among fruit and wild tree host along the orchard-woodland interface, suggesting that perimeter-based management tactics such as border sprays (Blaauw et al. 2015) and "attract and kill" strategies at orchard borders (Morrison et al. 2015) may be promising management tactics for this pest.

Temperature has been known to affect the feeding activity of *H. halys* adults. Optimal activity was observed between 16°C and 17°C and no feeding occurred above 26.5°C - 29.5°C (Wiman et al. 2014). As well, feeding injury to blueberries by late-instar nymphs and adults was higher in Oregon than in New Jersey and attributed to optimal temperatures in Oregon (Wiman et al. 2015). In my study, daily low and high temperatures during the exposure periods ranged from 11°C to 33°C, with mean daily temperatures between 19°C and 25°C implying that *H. halys* could feed during these periods, albeit not at optimal levels. The highest mean daily temperature was recorded during the late-season exposure of peach fruit suggesting that feeding activity during this period may not have been as high as during the other two exposure periods. This may explain the higher number of external injuries per peach from adult feeding early in the season compared to later. Although the injury severity on apple differed significantly between the early- and late-season exposures, the mean temperatures during those exposure periods were the same. Thus, it is not possible to draw unequivocal conclusions about the effects of temperature on feeding and fruit injury, especially since the phenological stage of the fruit during exposure also may have contributed.

In conclusion, *H. halys* nymphs caused injury to tree fruit and the extent to which the injuries were expressed varied significantly depending on the developmental stage of the nymphs and the period during which feeding occurred. My results confirmed previous findings regarding the stage of fruit development during which apple and peach fruit were more vulnerable to feeding injury by *H. halys* adults and provide useful information that has implications for developing temporally and spatially precise management programs against *H. halys* in apple and peach orchards.

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Table 5.1. Percentage of caged 'Smoothee Golden' apples injured at harvest following exposure to *H. halys* nymphs or adults for 96 h early and late in the season.

Treatment	No. of fruit evaluated	Mean % apples with external injury		Mean % apples with internal injury
		Punctures/ Dimpling[1]	Discolored depressions[2]	
Control	23	0.00 b	0.00 d	0.00 d
<u>Early season</u>				
Young nymphs (2nd/3rd instars)	26	0.00 b	15.38 c	23.08 c
Adults	28	85.71 a	25.00 c	71.43 b
<u>Late season</u>				
Young nymphs (2nd/3rd instars)	28	3.57 b	64.29 b	60.71 b
Older nymphs (4th/5th instars)	28	0.00 b	92.86 a	75.00 ab
Adults	27	3.70 b	88.89 a	92.59 a
Statistics		$\chi^2 = 101.86$ $P < 0.0001$	$\chi^2 = 98.22$ $P < 0.0001$	$\chi^2 = 74.91$ $P < 0.0001$

Means within columns followed by the same letter are not significantly different based on the χ^2 value from the odds ratio comparison at $P < 0.05$.

[1] Punctures and/or dimpling without discoloration (Figure 5.1A)

[2] Surface discoloration with or without distinct depressions (Figs 5.2A and 5.3A, respectively)

Table 5.2. Mean (± SE) number of injuries per fruit on caged 'Smoothee Golden' apples at harvest following exposure to *H. halys* nymphs or adults for 96 h early and late in the season.

Treatment	No. of fruit evaluated	Mean ± SE no. external injury*		Mean ± SE no. internal injury
		Punctures/ Dimpling[1]	Discolored depressions[2]	
Control	23	0.00 b	0.00 d	0.00 d
Early season				
Young nymphs (2nd/3rd instars)	26	0.00 b	0.04 ± 0.02 d	0.07 ± 0.03 d
Adults	28	2.80 ± 0.42 a	0.18 ± 0.06 cd	0.95 ± 0.17 bc
Late season				
Young nymphs (2nd/3rd instars)	28	0.01 ± 0.01 b	0.60 ± 0.12 c	0.45 ± 0.11 cd
Older nymphs (4th/5th instars)	28	0.00 b	2.30 ± 0.51 b	2.07 ± 0.57 b
Adults	27	0.02 ± 0.02 b	5.56 ± 0.68 a	5.72 ± 0.67 a
Statistics		$F_{5,154} = 75.04$ $P < 0.0001$	$F_{5,154} = 57.06$ $P < 0.0001$	$F_{5,154} = 44.72$ $P < 0.0001$

*No. of injuries per fruit divided by the corresponding no. of stink bugs released in each cage.

Means with columns followed by the same letter are not significantly different at α = 0.05 using Tukey's HSD.

[1] Punctures and/or dimpling without discoloration (Figure 5.1A)

[2] Surface discoloration with or without distinct depressions (Figs 5.2A and 5.3A, respectively)

Table 5.3. Percentage of caged 'Redhaven' peaches injured at harvest following exposure to *H. halys* nymphs or adults for 96 h early and late in the season.

Treatment	No. of fruit evaluated	Mean % peaches with external injury		Mean % peaches with internal injury
		Gummosis	Surface depressions	
Control	26	0.00 b	0.00 d	0.00 d
<u>Early season</u>				
Young nymphs (2nd/3rd instars)	25	0.00 b	4.00 d	8.00 d
Adults	25	72.00 a	100.00 a	100.00 a
<u>Late season</u>				
Young nymphs (2nd/3rd instars)	28	0.00 b	46.43 c	39.29 c
Older nymphs (4th/5th instars)	28	0.00 b	39.29 c	82.14 b
Adults	28	0.00 b	75.00 b	96.43 ab
Statistics		$\chi^2 = 82.90$ $P < 0.0001$	$\chi^2 = 103.70$ $P < 0.0001$	$\chi^2 = 74.91$ $P < 0.0001$

Means within columns followed by with the same letter are not significantly different based on the χ^2 value from the odds ratio comparison at $P < 0.05$.

Table 5.4. Mean (± SE) no. of injuries per fruit on caged 'Redhaven' peaches at harvest following exposure to *H. halys* nymphs and adults for 96 h early and late in the season.

Treatment	No. of fruit evaluated	Mean ± SE no. external injury*		Mean ± SE no. internal injury
		Gummosis	Surface depressions	
Control	26	0.00 b	0.00 c	0.00 c
<u>Early season</u>				
Young nymphs (2nd/3rd instars)	25	0.00 b	0.02 ± 0.02 c	0.03 ± 0.02 c
Adults	25	1.5 ± 0.32 a	4.00 ± 0.41 a	6.88 ± 0.51 a
<u>Late season</u>				
Young nymphs (2nd/3rd instars)	28	0.00 b	0.39 ± 0.15 c	0.54 ± 0.15 c
Older nymphs (4th/5th instars)	28	0.00 b	0.54 ± 0.14 c	2.18 ± 0.34 b
Adults	28	0.00 b	3.11 ± 0.66 b	7.93 ± 0.99 a
Statistics		$F_{5,154} = 41.51$ $P < 0.0001$	$F_{5,154} = 45.77$ $P < 0.0001$	$F_{5,154} = 102.32$ $P < 0.0001$

*No. of injuries per fruit divided by the corresponding no. of stink bugs released in each cage.

Means within columns followed by the same letter are not significantly different at α = 0.05 using Tukey's HSD.

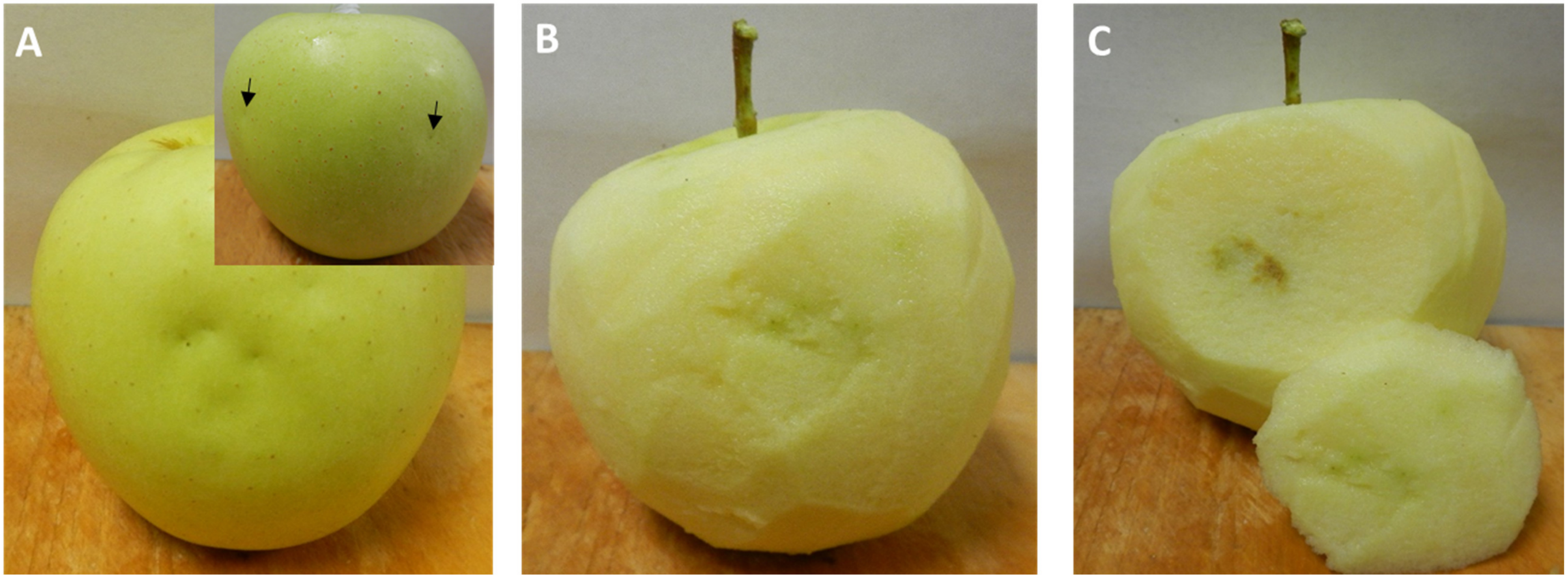

Figure 5.1. Early-season feeding injury from *H. halys* adults on 'Smoothee Golden' apples; (A) External injury: punctures (inset) and 'dimpling' (B) Internal injury (less apparent) below the surface, (C) Evidence of deeper internal necrosis.

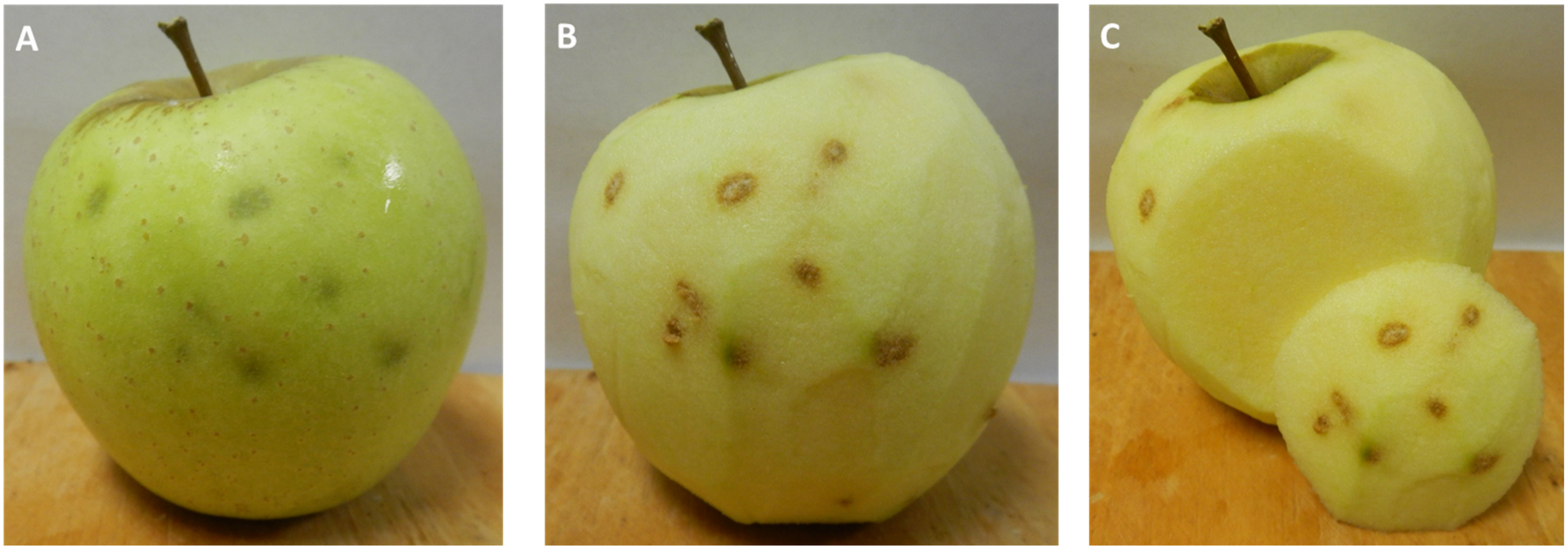

Figure 5.2. Late-season feeding injury from third and fourth instar *H. halys* nymphs on 'Smoothee Golden' apples; (A) External injury: surface discoloration with distinct depressions, (B) Internal injury (brown necrosis) below the surface, (C) No evidence of deeper internal necrosis.

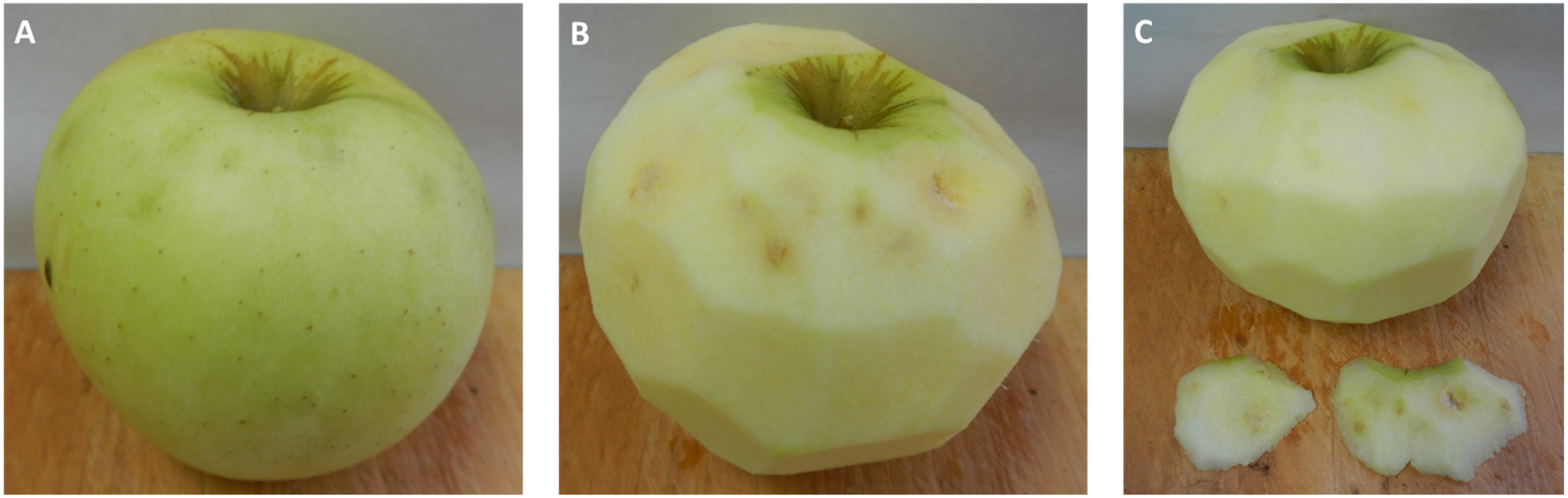

Figure 5.3. Late-season feeding injury from *H. halys* adults on 'Smoothee Golden' apples; (A) External injury: surface discoloration without distinct depressions, (B) Internal necrosis just below the surface, (C) No evidence of deeper internal necrosis.

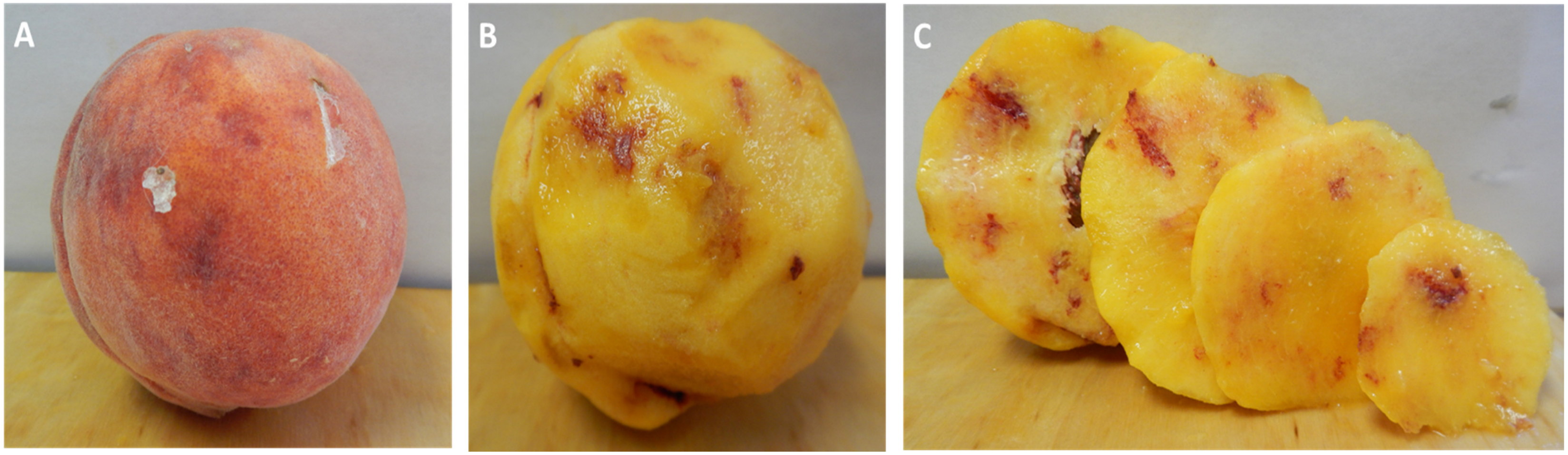

Figure 5.4. Early-season feeding injury from *H. halys* adults on 'Redhaven' peaches; (A) External injury: gummosis and surface depressions (B) Internal injury (brown necrosis) below the surface, (C) Deeper internal necrosis.

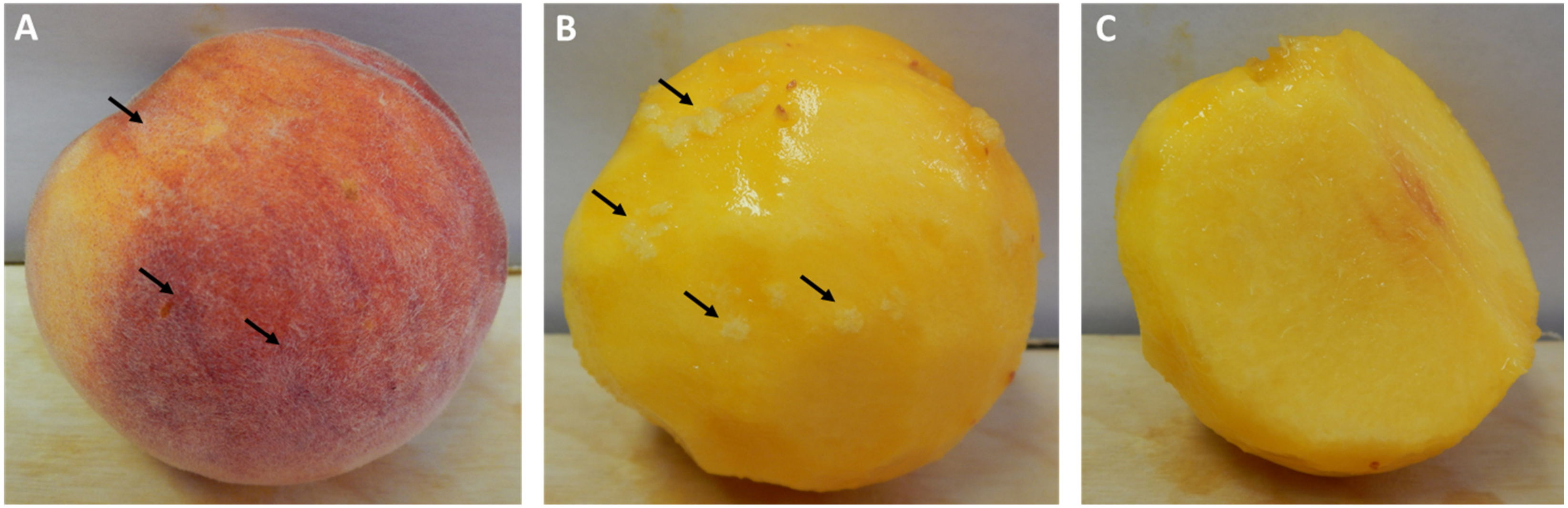

Figure 5.5. Late-season feeding injury from third and fourth instar *H. halys* nymphs on 'Redhaven' peaches; (A) External injury: shallow depressions, (B) Internal injury (white necrosis) below the surface, (C) No evidence of deeper internal necrosis.

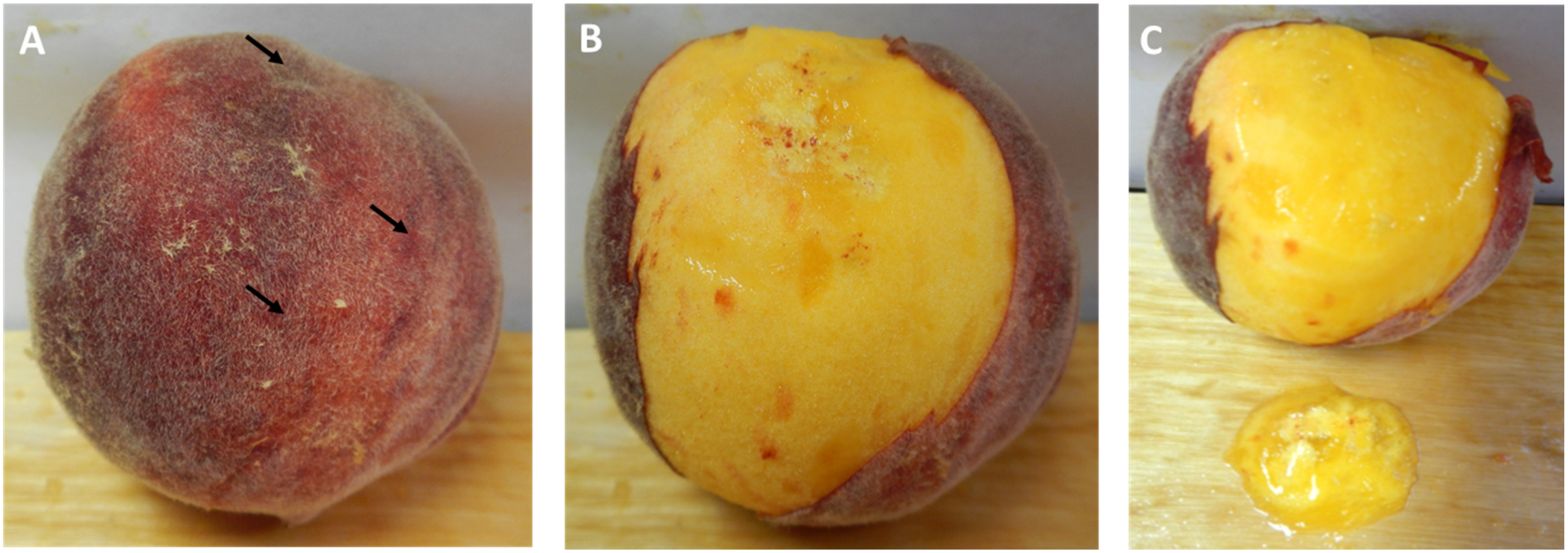

Figure 5.6. Late-season feeding injury from *H. halys* adults on ‘Redhaven’ peaches; (A) External injury: shallow depressions, (B) Internal injury (white necrosis) below the surface, (C) No evidence of deeper internal necrosis.

CHAPTER 6: SUMMARY, IMPLICATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

This research has revealed novel and important insights into host plant effects on *H. halys* biology, behavior, and ecology at the orchard-woodland interface and the effects of feeding by *H. halys* nymphs on injury and injury expression in two important orchard crops. In combination, these results improve our understanding of the pest status of *H. halys* in mid-Atlantic fruit orchards and may aid in the development of sustainable and ecologically-based orchard management programs against it, as discussed below.

Results Summary and Implications

Although *H. hays* is known to feed on a wide range of plant species, my data have suggested strongly that its tree hosts (a considerable number of which comprised the flora at the edge of woodlands next to orchards; Chapter 4) likely vary considerably in their suitability for nymphal development, survivorship, and adult fitness (size and weight). Despite having evaluated the suitability of only apple and peach (the two main tree fruit crops in this region) and just two of its many known wild hosts, I showed that key measures of nymphal performance were very different among them (Chapter 2). While all of the plants examined were unsuitable when only their foliage was offered to nymphs, I also showed that combining foliage with the fruiting structure of each host improved nymphal survivorship markedly, suggesting that the growth of field populations of *H. halys* populations is influenced by host phenology and perhaps by the sex of dioecious tree species (e.g. tree of heaven).

Peach was a highly suitable host, implying that it is very vulnerable to infestation and can support large *H. halys* populations. In contrast, apple was a suboptimal host, confirming previous results from Asia and implying that if other, more suitable hosts are nearby, apple would be less preferred by *H. halys* and less susceptible to attack. Of the two wild hosts examined, tree of heaven was the most common species recorded at the edge of woodlots adjacent to orchards in Virginia and West Virginia. Despite its reputation as an important host of *H. halys*, tree of heaven as a single diet was not suitable for nymphal development and survivorship, although its suitability appeared to increase later in the season. I did not detect catalpa from the woodlands surveyed, but it too is considered a host of *H. halys* and was essentially unsuitable for nymphal development. However, my conclusive evidence that the performance of *H. halys* nymphs was enhanced when they developed on mixed host diets (exemplified by their increased survivorship on a combination of two sub-optimal hosts, apple and tree of heaven) suggests that each of the species examined can support *H. halys* populations in the field if other hosts are available in some reasonable proximity.

My analysis of the nutrient contents of *H. halys* adults that developed from nymphs reared on different host plants and host plant combinations were comparative and not intended to provide unequivocal conclusions about plant diet effects on adult fitness or behavior, but these data provide useful baseline information for any future studies concerning the nutritional ecology of *H. halys*, and suggested that the levels sugars, proteins and lipids in adults appeared to be influenced by nymphal plant diet and the stage of plant phenology.

The demonstrated benefits of diet mixing on nymphal survivorship and developmental duration and on certain adult fitness parameters suggested that nymphs likely choose or need to move among host plants in the field during their development. My comparison of passive trunk traps for capturing nymphs walking on tree hosts and demonstration of their utility for this purpose (Chapter 3) was instrumental to addressing basic ecological questions about their movement among hosts. Certainly, *H. halys* nymphs appear to have ample opportunity to readily access and utilize a range of potential plant species at the orchard-woodland interface. My survey of woodlands adjacent to fifteen apple orchards in Virginia and West Virginia (Chapter 4) revealed a mix of 47 different tree species, 37 of which are known hosts of *H. halys*. This information may prove useful to future efforts to assess the risk of *H. halys* establishment and pest pressure near commercial orchards. Additionally, the abundance of tree of heaven next to orchards in Virginia and West Virginia and observations by many investigators, including me, of large *H. halys* populations on female tree of heaven suggests that removal of these trees might mitigate local pest pressure to some degree.

Installation of trunk traps on selected fruit and wild tree species at the orchard-woodland interface confirmed that *H. halys* nymphs move off of and onto trees throughout the season (Chapter 4) and by inference, that this dispersal is associated with diet optimization by feeding on more than one host during their development. Nymphal capture data from traps installed on the same trees over two consecutive seasons showed remarkably consistent temporal trends, despite substantial differences in total captures between the two seasons that appeared to reflect the lower *H. halys* adult populations recorded across this region in 2015 than in 2014. Second instars were captured most frequently, suggesting that, 1) they are the most dispersive stage, and/or 2) they are more abundant than older instars (possibly due to increased mortality during dispersal resulting in low numbers developing to the next instar), and/or 3) later instars remain on individual hosts throughout their developmental period. However, all instars were captured at points during the season and captures appeared to reflect the seasonal changes in the nymphal distribution, with highest captures of older nymphs later in the season. Trunk traps captured many more upward- than downward-walking nymphs, suggesting the possibility of a limitation in the effectiveness of the down trap design and/or that nymphs also leave host trees using other behaviors, such as dropping. Short duration peaks of nymphal captures in down traps on peach tree following the removal of fruit in both years conformed interestingly to my findings about the importance of fruiting structures for nymphal development and to a previous study showing that *H. halys* populations significantly declined after fruit in ornamental trees were removed.

It remains to be determined whether or to what extent nymphal *H. halys* move from wild hosts to nearby fruit trees during their development, but demonstration of their strong dispersal capacity by other investigators suggests that they are likely capable of traversing the space between the forest edge and adjacent orchard trees (typically ≤ 23 m). My assessments of nymphal *H. halys* feeding injury to apple and peach fruit revealed that the nature and extent of injury were related to the developmental stage of the nymphs and to the stage of fruit development when feeding occurred. Less injury was associated with feeding by second and third instars than from third and fourth instars, which was often similar to that from adults. This new information may further inform the development of management thresholds for *H. halys* in orchards, using pheromone traps. My findings suggest that including older nymphs in trap-based management thresholds may warrant consideration, given the injury they cause to tree fruit and their high abundance later in the season (Chapter 4).

Suggestions for Future Research

While results from the peach and apple host suitability study are informative, other economically important stone fruit and European and Asian pears are grown in the mid-Atlantic region and even more commonly in other regions where *H. halys* is established or in the process of establishing and spreading. Consequently, evaluation of the suitability of other orchard crops for *H. halys* nymphal development and survival relative to that of apple and peach may yield important comparisons and contrasts. Moreover, many other common wild hosts, such as hackberry, black cherry and sassafras, growing adjacent to orchards should also be investigated, toward a more comprehensive understanding of the hosts that contribute importantly to local *H. halys* populations. The methodology established by my research could guide such future studies.

An intriguing concept that arose from the results of my nymphal development and dispersal studies was that of "dead-end hosts", based on extremely low nymphal survivorship on some hosts, their apparent need for a mixed host diet, and on their apparent dispersal among hosts through the season. In combination, my data suggest that if *H. halys* nymphs hatch from eggs deposited on a suboptimal host and do not have access to other hosts nearby, it is highly likely that these nymphs will not survive beyond the second instar. Based on abiotic climatic factors, *H. halys* has been predicted to establish in several geographic regions in the US, but the abundance and diversity of its host plants in newly-invaded areas also may affect its establishment. Where some of its hosts (e.g. corn and soybeans) are grown in monoculture, especially those that are suboptimal for nymphal development, the likelihood of *H. halys* establishment may be low. The concept of dead-end hosts may have significant implications for the invasion biology and pest status of *H. haly*s in certain areas and habitats, and warrants further investigation.

My tree survey suggested that the species diversity and abundance of known hosts of *H. halys* in unmanaged woodlands next to orchards should not limit the capacity of nymphs to access different host trees during their development in this region. However, given that the survey encompassed only sites in Virginia and West Virginia, the results may not pertain to other areas experiencing increasing *H. halys* populations (e.g. Pacific Northwest), suggesting that similar surveys in these newly-invaded areas may help to assess the risk of *H. halys* establishment in them.

Since only the effects of nymphal diet on adult size and weight were investigated, further examination of diet effects on other important adult fitness parameters, such as longevity, female fecundity, and overwintering survivorship would be informative. Additionally, an examination and comparison of diet effects (e.g. optimal versus suboptimal hosts/mixed diets) on the dispersal capacity of nymphs and adults may also enhance our understanding of their host-use patterns and dispersal capacity.

My 2-year nymphal trapping study provided new information on the movement of nymphs onto and off of different host trees during the growing season, but was not designed to address questions about their patterns of host use or feeding duration on particular hosts. This may be accomplished via mark-release-recapture or harmonic radar tagging of late instars released on certain hosts (e.g. cultivated and wild hosts at the orchard-woodland interface) and documenting their location at intervals following release. Relevant research questions that might be addressed using this approach include; 1) how long do nymphs remain on a particular host, 2) do nymphs that disperse from one host species move to the same or different species, 3) do nymphs disperse from wild hosts into orchards, and 4) if so, how far into the orchard do nymphs move? Similar behavioral studies could also be done with adults to supplement our understanding of their dispersal behavior.

Complete interpretation of results from the nymphal trapping study were affected by our lack of understanding about whether and to what extent nymphs disperse from host trees via dropping. Much lower captures in down than up traps suggested this possibility. Therefore, examination of this question may prove to be very informative and provide a more comprehensive picture of nymphal dispersal behavior and movement to and from host trees during the season.

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APPENDIX A

NUTRIENT CONTENT ANALYSIS OF *HALYOMORPHA HALYS* ADULTS REARED FROM NYMPHS THAT HAD DEVELOPED ON SINGLE AND MIXED DIETS OF SELECTED FRUIT AND WILD TREE HOSTS

Objective

To quantify the nutrient contents of *H. halys* adults that developed from nymphs reared on single and mixed diets of apple, peach, Tree of Heaven, and catalpa

Materials and Methods

Adult female and male (< 48-h-old) *H. halys* adults that completed development on each of the single versus mixed host diet treatments used in the experiments of Chapter 2 were stored at -80°C. Since the different diet treatments yielded widely varying numbers of adults, all adults from some treatments were used for nutrient content analyses, while a random subset was used for others (n = 2 - 15). Adult *H. halys* (n = 30) collected in early September, 2013 from Tree of Heaven at the AHS AREC were used for comparison with the adults reared during the late-season experiments in the same year.

Standard curve experiments using bovine serum albumin (Sigma-Aldrich, St. Louis, MO) for protein, vegetable oil (Fisher Science Education, Nazareth, PA) for lipid, and D(+)-glucose anhydrous (Acros Organics, Antwerpen, Belgium) for sugar were conducted to check for linearity within a range of concentrations and to gauge the precision of the procedures for each assay. For all assays, only linear equations from the standard curves that produced R^2 values of >0.95 were used. Due to the large number of insect samples analyzed during each assay, the standard curve aliquots were not simultaneously analyzed with the insect samples; rather, to ensure standardized assay results, they were conducted separately when new assay reagents and buffer solutions were prepared.

Insects were placed individually in 2-mL microcentrifuge tubes (Fisherbrand™, Waltham, MA) with 1 mL sodium phosphate (pH 7.8) and crushed using a plastic pestle (8.5 cm long, 0.5 cm diameter). Sodium phosphate buffer, prepared by mixing 400 mL 0.1 M dibasic sodium phosphate (Fisher Scientific, Waltham, MA) and 250 mL 0.1 M monobasic sodium phosphate (Fisher Scientific, Waltham, MA), was added to each tube. The solutions were centrifuged for 20 minutes at 3000 rpm at 4°C. A 200 µL aliquot of supernatant from each homogenate solution was used for lipid and sugar assays while 333 µL was used for protein analysis.

The supernatant aliquot for lipid and sugar analyses was mixed with 800 µL of a 1:1 chloroform:methanol solution and centrifuged at 5000 rpm for 5 minutes at 4°C. The supernatant was transferred to a new 2-mL microcentrifuge tube with 300 µL distilled water and centrifuged at 3000 rpm for 5 minutes at 4°C. The top fraction of the mixture with water and ethanol was used for sugar analysis and the bottom portion with chloroform was used for lipid analysis. The tubes containing insect homogenates were held in crushed ice throughout the process. A maximum of 16 insect samples were homogenized and analyzed simultaneously during a given assay. Total sugar and lipid content of individual adults was quantified following the procedures described by Van Handel and Day (1988), while measurement of total proteins was loosely based on the procedure described by Smith et al. (1985). Analysis of all nutrients, including proteins, was based on three replicates of the homogenate from each insect and three replicates of sodium phosphate solution as controls.

For sugars, the reagent anthrone was stored at 4°C and prepared by mixing 750 mg anthrone powder (Acros Organics, Antwerpen, Belgium) with 385 mL 95-98% sulfuric acid and 150 mL distilled water. For analysis, 50 µL of insect homogenate preparation (top portion) or the control solution was mixed with 300 µL anthrone reagent in 500 µL PCR tubes (Axygen®, Tewksbury, MA) and the solutions were heated for 17 minutes at 100°C. Once they had cooled and a greenish-blue color became prominent in samples with detectable amounts of sugars, 200 µL of solution from each tube was pipetted into individual wells of a flat bottom 96-well microplate (Fisherbrand™, Waltham, MA). Optical densities for all nutrients were determined using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA) and nutrient concentrations (mg/ml) were obtained from the linear equations from the standard curve for each nutrient. For sugars, the optical density was determined at 625 nm and 30°C.

Total lipids were quantified using the reagent, vanillin, which was stored in the dark and prepared by mixing 600 mg of vanillin powder (Acros Organics, Antwerpen, Belgium) in 100 mL hot distilled water and 400 ml 85% phosphoric acid. For analysis, 50 µL of insect homogenate preparation (bottom portion) or control solution was mixed with 100 µL of 95-98% sulfuric acid in 500 µL PCR tubes (Axygen®, Tewksbury, MA), heated for 10 minutes at 100°C and then mixed with 300 µL vanillin reagent. When the solutions with measureable amounts of lipid turned pink, 200 µL of solution from each tube was transferred to individual wells of the microplate and the optical density was determined at 625 nm and 30°C.

Measurement of total proteins used the reagent, bicinchoninic acid assay (BCA) solution (Thermo Scientific™ Pierce™ BCA™, Waltham, MA), which was mixed with 4% cupric sulfate (40 ml dH_2O and 1.6 g Fisher Scientific cupric sulfate anhydrous) during each assay. Since the protein concentration of individual adult *H. halys* exceeded the spectrophotometer capacity, we created a 9x dilution of the homogenate from each insect by adding sodium phosphate solution. A 20 µL aliquot of diluted homogenate was pipetted into each well of the microplate and mixed with 180 µL of the BCA-cupric sulfate solution. The solutions were incubated for 30 minutes at 37°C, then held at room temperature for 5 min. Optical densities were measured at 560 nm and 30°C and estimated concentrations were corrected for the 9x dilution factor.

Data Analyses. All analyses were conducted using JMP® Pro version 11 (SAS Institute Inc., Cary, NC, 2007) and outcomes were considered significant at $P < 0.05$. Data that did not satisfy the assumptions of parametric tests were transformed using log(x+1) transformation. Diet treatments with low sample sizes (≤2) for either sexes were excluded from the analyses. Sugar, lipid and protein content of adult male and female *H. halys*, including field-collected adults, were compared separately among the host plant diets using one-way ANOVA. Student's t-test was used to compare the sugar, lipid, and protein levels between male and female adult stink bugs during the early- and late-season experiments for each diet treatment. Multiple mean comparisons used Tukey's post hoc HSD.

Results

Sugar. There was a significant effect of diet treatment on the sugar content of laboratory-reared adult female and male *H. halys* generated from the early and late season experiments (Table A.1). In the early season, highest concentrations in females were from peach, apple plus Tree of Heaven, and the 3- and 4-host diets (Table A.1) and lowest from Tree of Heaven alone. Sugar content in males was highest from peach and the 3-host diet and lowest from Tree of Heaven and catalpa. In the late season experiment, sugar concentrations were significantly greatest in females and males from peach. The sugar content in laboratory-reared females increased significantly between the early and late season studies from peach ($t = 5.89$, df = 28, $P < 0.0001$) and Tree of Heaven ($t = 5.33$, df = 20, $P < 0.0001$), but were equivalent from apple plus Tree of Heaven ($t =$

0.59, df = 28, $P = 0.56$), and the 3-host ($t = -0.53$, df = 28, $P = 0.60$), and 4-host diets ($t = -0.76$, df = 28, $P = 0.45$). In laboratory-reared males, sugar content was significantly greater in the late season from apple ($t = 4.36$, df = 21, $P = 0.0003$), peach ($t = 2.53$, df = 28, $P = 0.02$) and Tree of Heaven ($t = 6.09$, df = 26, $P < 0.0001$), with no difference on apple plus Tree of Heaven ($t = 0.58$, df = 28, $P = 0.57$). Conversely, the sugar content of laboratory-reared males from the 3-host ($t = -2.90$, df = 28, $P = 0.007$) and 4-host diets ($t = -2.72$, df = 28, $P = 0.01$) was significantly greater early in the early season than later. The sugar concentration in field-collected adult males and females was statistically or numerically lower than of the adults reared from the late-season laboratory experiments.

Lipid. Lipid levels in laboratory-reared female and male *H. halys* were significantly affected by diet treatment in the early season study, but not in the late season (Table A.1). In the early season, they were highest in females reared from nymphs on Tree of Heaven and lowest on peach and the 3-host and 4-host diets, while in males they were highest on apple plus Tree of Heaven and lowest on apple, peach and catalpa. Lipid levels in laboratory-reared females were significantly higher early in the season than later on Tree of Heaven ($t = -3.07$, df = 20, $P = 0.006$), apple plus Tree of Heaven ($t = -2.19$, df = 28, $P = 0.04$) and the 4-host diet ($t = -2.12$, df = 28, $P = 0.04$) but did not differ between the early and late experiments on peach ($t = 1.23$, df = 28, $P = 0.23$) or the 3-host diet ($t = -1.88$, df = 28, $P = 0.07$). Lipid content in laboratory-reared males was significantly higher early than later in the season on Tree of Heaven ($t = -2.63$, df = 28, $P = 0.01$), apple plus Tree of Heaven ($t = -2.90$, df = 28, $P = 0.01$), and the 3-host ($t = -3.49$, df = 28, $P = 0.001$) and 4-host diets ($t = -4.29$, df = 28, $P = 0.0002$), but there were no differences on apple ($t = 2.08$, df = 28, $P = 0.05$) or peach ($t = -0.98$, df = 28, $P = 0.34$). Lipid contents in field-collected female and male adults did not differ significantly from those of the laboratory-reared adults from the late-season experiment.

Protein. Protein levels in laboratory-reared females were statistically equivalent among the diet treatments early in the season but varied significantly in the later study (Table A.1), when highest levels were from females on the 3-host diet and apple and lowest from those on catalpa and Tree of Heaven. There were significant diet treatment effects on protein content in laboratory-reared males during the early and late season experiments (Table A.1). Early in the season, lowest protein content was recorded from catalpa, with no differences among the other diets. Later, highest protein levels were from laboratory-reared males from the 3-host diet and the lowest were from males on Tree of Heaven and apple plus Tree of Heaven, while levels on apple, peach and 4-host diet were intermediate. Protein levels in laboratory-reared females from peach ($t = 3.08$, df = 28, $P = 0.005$), the 3-host ($t = 3.52$, df = 28, $P = 0.002$), and the 4-host diets ($t = 3.17$, df = 28, $P = 0.004$) increased between the early and late season experiments, but did not differ in females from Tree of Heaven ($t = -1.15$, df = 20, $P = 0.26$) and apple plus Tree of Heaven ($t = -0.99$, df = 28, $P = 0.33$). Laboratory-reared males from the 3-host ($t = 4.54$, df = 28, $P < 0.0001$) and 4-host diets ($t = 2.43$, df = 28, $P = 0.02$) showed a higher protein content later in the season, while those from apple ($t = 1.17$, df = 28, $P = 0.26$), peach ($t = 0.73$, df = 28, $P = 0.47$), Tree of Heaven ($t = 0.42$, df = 28, $P = 0.68$), and apple plus Tree of Heaven ($t = -1.30$, df = 28, $P = 0.21$) did not differ between the early and late season experiments. Protein content of field-collected females was statistically lower than of the females reared from apple and the 3-host diet later in the season while protein levels of field-collected males were statistically less than of males reared from 3-host diet in the late-season laboratory experiment.

Discussion

This study was conducted to complement the results from Chapter 2 and to quantify host plant diet effects on *H. haly* nutrient contents. Due to the varying suitability of the host plants examined for *H. halys* nymphal development and survival, it was reasonable to speculate that the differences may have been associated with differences in the nutritional value of the diets. For example, macronutrients such as sugars, lipids and proteins have profound effects on herbivore development and survivorship (Scriber and Slansky Jr 1981, Panizzi and Parra 2012). I revealed some differences in the nutrient content of adults reared from the plant diets tested and that, in some instances, they were affected by plant phenology (i.e. early- vs late-season experiments). As well, nutritional contents differed between males and females.

A similar study conducted in Oregon (Skillman et al., unpublished data) examined seasonal fluctuations in the sugar, lipid, and glycogen contents of field-collected *H. halys* adults. Although no unequivocal conclusions about diet effects on the nutritional ecology of *H. halys* can be made from the data presented here, in combination with results from the aforementioned study, they provide novel and important baseline information that may underpin any future studies of *H. halys* nutrition.

In summary, sugar levels were consistently variable among the diet treatments regardless of the period in the season; while differences, or lack thereof, in the lipid and protein levels among the diet treatments appeared to be influenced by plant phenology. Sugar was generally highest in adults from the more suitable diets (i.e. peach and mixed diets early in the season and on peach later in the season). Sugar contents of adult males from the apple diet increased significantly later in the season, which appeared to be correlated with the numerical increase in nymphal survivorship on apple later in the season. It also appeared that sugar levels were related to the increased suitability of Tree of Heaven later in the season. Lipid levels in adults reared from Tree of Heaven were lower early in the season while their protein contents did not vary between early and late season. I hypothesize that lipids may not be a major determinant of *H. halys* nymphal development since the highest lipid levels were measured from adults reared from Tree of Heaven, which was a sub-optimal host for nymphs. As well, protein levels in adults did not appear to be related to any specific trends in nymphal survivorship. In particular, the protein content of adult males and females from nymphs reared on apple and peach was equally high, although the suitability of these hosts varied greatly. These results suggest that among the macronutrients measured, sugars were most closely associated with host plant suitability for *H. halys* nymphal development and survival to the adult stage. In addition, the variation I observed among macronutrients measured for single and mixed diets early and late in the season coupled with the documented differences in developmental rate and survivorship on these diets (see Chapter 2) indicate that the potential for *H. halys* to obtain a mixed diet in nature could be critical to its success.

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Table A.1. Sugar, lipid and protein contents of < 48-hr-old adult female and male *H. halys* from nymphs reared on single and mixed plant diets during early and late season experiments.

Early season	**Females**				**Males**			
Diet treatments	N	Sugar (mg/mL)	Lipid (mg/mL)	Protein (mg/mL)	N	Sugar (mg/mL)	Lipid (mg/mL)	Protein (mg/mL)
Apple	2	0.07 ± 0.04*	0.71 ± 0.03*	4.32 ± 2.77*	7	0.07 ± 0.01bc	0.51 ± 0.03d	10.16 ± 1.82a
Peach	15	0.19 ± 0.02a	0.85 ± 0.04bc	11.01 ± 0.79a	15	0.18 ± 0.02a	0.77 ± 0.07cd	10.24 ± 0.65a
Catalpa	2	0.08 ± 0.03*	0.81 ± 0.25*	7.85 ± 4.76*	8	0.03 ± 0.01c	0.60 ± 0.03cd	3.11 ± 0.40b
ToH[a]	7	0.03 ± 0.01b	1.96 ± 0.54a	11.22 ± 0.61a	14	0.04 ± 0.01c	1.16 ± 0.12ab	9.11 ± 0.43a
Apple + ToH[a]	15	0.18 ± 0.03a	1.50 ± 0.22ab	11.47 ± 0.75a	15	0.12 ± 0.03ab	1.29 ± 0.14a	9.88 ± 0.53a
3-host[b]	15	0.18 ± 0.02a	0.83 ± 0.06c	10.02 ± 0.69a	15	0.19 ± 0.01a	1.00 ± 0.07abc	8.59 ± 0.59a
4-host[c]	15	0.19 ± 0.04a	0.89 ± 0.08bc	9.84 ± 0.71a	15	0.16 ± 0.02ab	0.89 ± 0.05bcd	9.00 ± 0.42a
One-way		$F_{4, 62} = 2.86$	$F_{4, 62} = 6.35$	$F_{4, 62} = 0.99$		$F_{6, 80} = 11.85$	$F_{6, 82} = 7.70$	$F_{6, 82} = 10.05$
ANOVA		$P = 0.031$	$P = 0.0002$	$P = 0.42$		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Late season	**Females**				**Males**			
Diet treatments	N	Sugar (mg/mL)	Lipid (mg/mL)	Protein (mg/mL)	N	Sugar (mg/mL)	Lipid (mg/mL)	Protein (mg/mL)
Apple	14	0.24 ± 0.01b	0.94 ± 0.07a	16.23 ± 1.01ab	16	0.17 ± 0.01b	0.69 ± 0.06a	11.87 ± 0.84ab
Peach	15	0.40 ± 0.03a	0.95 ± 0.07a	14.47 ± 0.80abc	15	0.25 ± 0.02a	0.68 ± 0.06a	10.94 ± 0.71ab
Catalpa	10	0.23 ± 0.04bc	0.79 ± 0.05a	8.75 ± 0.91c	2	0.15 ± 0.03*	0.87 ± 0.03*	7.14 ± 0.61*
ToH[a]	15	0.15 ± 0.01bc	0.95 ± 0.10a	9.69 ± 0.85c	15	0.16 ± 0.02bc	0.92 ± 0.10a	9.58 ± 1.03b
Apple + ToH[a]	15	0.20 ± 0.02bc	0.98 ± 0.21a	10.46 ± 0.69bc	15	0.14 ± 0.02bcd	0.84 ± 0.07a	8.76 ± 0.68b

3-host[b]	15	0.16 ± 0.01bc	0.64 ± 0.09a	17.30 ± 2.99a	15	0.13 ± 0.01bcd	0.63 ± 0.08a	13.96 ± 1.03a
4-host[c]	15	0.15 ± 0.02bc	0.66 ± 0.10a	14.39 ± 1.24abc	15	0.10 ± 0.01cd	0.56 ± 0.06a	12.03 ± 1.18ab
Field-collected (Sept. 2013)	15	0.13 ± 0.02c	0.80 ± 0.12a	9.64 ± 0.98c	15	0.08 ± 0.01d	1.22 ± 0.39a	9.15 ± 1.17b
One-Way		$F_{7, 106} = 17.18$	$F_{7, 106} = 1.49$	$F_{7, 106} = 5.40$		$F_{6, 98} = 12.37$	$F_{6, 99} = 1.93$	$F_{6, 99} = 3.72$
ANOVA		$P < 0.0001$	$P = 0.18$	$P < 0.0001$		$P < 0.0001$	$P = 0.083$	$P = 0.002$

Nutrient concentrations are expressed as mg/mL where 1 adult stink bug was homogenized in 1 mL sodium phosphate.

[a] ToH denotes Tree of Heaven

[b] The 3-host diet consisted of the foliage and fruiting structures of apple, ToH, and peach

[c] The 4-host diet consisted of the foliage and fruiting structures of apple, ToH, peach, and catalpa

*Treatment excluded from analyses due to sample size

Means with the same letters under the same column and in the same category are not significantly different (Tukey's HSD, α = 0.05).