Biology and pest status of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards and raspberry plantings

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Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive insect from Asia that has recently become a major pest of agricultural crops and a nuisance to home and business owners in the Mid-Atlantic USA. Since 2010, *H. halys* has been reported in many vineyards in Virginia, but the pest significance in this crop is unknown. Sampling was conducted in four commercial vineyards across Virginia in 2012 and 2013 to study the seasonal phenology and pest status of *H. halys* in vineyards. Adults moved into vineyards as early as May and laid eggs usually on the undersurface of leaves, but occasionally on the berry or the rachis. Grapevines were an early season reproductive host for *H. halys*. A vineyard adjacent to a sub-urban area with homes and buildings in proximity had an early season peak of *H. halys* as compared to vineyards adjacent to woods. However, populations declined sharply in late season due to the possible movement of bugs to more preferable host plants such as soybean and corn. In contrast, *H. halys* was recorded throughout the grape-growing period in a vineyard that was surrounded by forests. Significantly more *H. halys* were recorded from border than interior section of vineyards. A degree-day model suggested that there were enough degree-days to complete a generation of *H. halys* in Virginia vineyards. *H. halys* caused direct injury to the grape berries at veraison and pre-harvest berries. Injury expression in the veraison and pre-harvest berry can be described as an appearance of a small necrotic spot at the site of the stylets insertion. The spot gradually increased in size and the berries became deformed. *H. halys* is an economic pest of raspberry, causing direct injury to the berries. Sampling of stink bugs in raspberry plantings in southwestern Virginia showed that the *Euschistus* species were the most abundant stink bugs in 2008, 2009, 2011 and 2012. However, *H. halys* became the most abundant in 2013.

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Classification

Order: Hemiptera

Family: Pentatomidae

Subfamily: Pentatominae

Tribe: Pentatomini

Genus: *Halyomorpha*

Species: *H. halys* (Stål)

Fig. 1.1. Adult *Halyomorpha halys*

Halyomorpha halys (Fig. 1.1) was described for the first time as *Pentatoma halys* by Stål in 1854 (Hoebeke and Carter 2003). In some studies, it has been synonymously named as *H. picus*, *H. mista*, *H. mysta* and *H. brevis*. Altogether 37 species under the genus *Halyomorpha* have been described, of which 16 are reported from Africa, eight from Indian regions and 13 from eastern Asia. In 1978, Josifov and Kerzhner justified that there is only one species under the genus *Halyomorpha* in the eastern Asia, and synonymized the species as *H. halys* (Hoebeke and Carter 2003).

Introduction

Stink bugs (Hemiptera: Pentatomidae) are pests of agricultural crops throughout the world. Some species are crop specific while some have a very wide host range and attack numerous crops (McPherson and McPherson 2000). *Halyomorpha halys* (Stål), commonly known as brown marmorated stink bug, was recently introduced in the USA (Hoebeke and Carter 2003). It is a highly polyphagous insect and attacks important crops including grapes (Leskey et al 2012). In addition, *H. halys* is considered a nuisance pest when it enters houses to overwinter, often in large numbers. Since its introduction into Pennsylvania in the late 1990s, *H. halys* has spread dramatically and established in the Mid-Atlantic States of the USA (Leskey et al. 2012). The wide host range, high reproductive capacity, adaptability to different climatic conditions and lack of natural enemies are possible reasons for its spread and establishment in the Mid-Atlantic States (Hamilton, 2009, Leskey et al. 2012). The rapid spread of *H. halys* has been aided by its propensity to "hitchhike" in vehicles and other conveyances (Hamilton 2009). *H. halys* has surpassed native stink bugs in abundance, and has become the dominant stink bug species in different cropping systems in some regions (Nielsen 2011). An invasive species is always a concern as it competes with native species for food, space and other resources that could result in species displacement, ultimately posing a threat to ecosystem and biodiversity (Elton 2000). On grapes, *H. halys* causes direct injury by feeding on the grape berries. High densities of *H. halys* at harvest are an additional threat when bugs are present in harvested clusters and crushed during juice extraction. A noticeable odor called "stink bug taint" can be perceived, but it may fade away with the fermentation and aging process (Fiola 2013). However, a preliminary study using Pinot Noir indicated that there was detection and rejection of wine due to the presence of trans-2 decenal emitted from *H. halys* for ten months (Tomasino et al. 2013). In addition, stink bugs and their odor can be a significant nuisance in wine tasting rooms, particularly in the fall when bugs aggregate on and in manmade structures.

Invasion history and spread in North America

With the rapid increase in agricultural trade among countries and continents, introductions of exotic species are increasing at a rapid pace. *H. halys* may end up being one of the most significant invasive pests in the USA because it feeds on multiple hosts and has both agricultural and urban significance. The earliest confirmed sighting of *H. halys* in the USA was from Allentown, Pennsylvania in 1996, and the first confirmation in the USA was made by E. Richard Hoebeke, Curator of the Cornell University Insect Collection in 2002 (Hoebeke and Carter 2003). Genetic studies by Xu et al. (2013) revealed that the population of *H. halys* in the USA is native to the Beijing area in China. *H. halys* is native to east Asian countries, such as China (excluding Xingjian and Qinghai), Japan, South Korea, Vietnam and Myanmar (Wang and Liu 2005). As it resembles some indigenous species in the genera *Brochymena* and *Euschistus*, it was initially misidentified, and not given serious attention during its initial establishment phase in Pennsylvania. As of 2013, *H. halys* had become established throughout the northeastern USA and is found in 40 states of the USA. It was reported from Oregon and California since 2004 and 2005 respectively (Belisle 2012). It has also been reported from Ontario, Canada since 2010 (Hueppelsheuser 2013) and Europe (Zurich, Switzerland) since 2007 (Wermelinger et al. 2008). *H. halys* was first reported in Virginia in 2004 and by 2010, it was established in most locations in the northern part of the state (Day et al. 2011).

Zhu et al. (2012) used ecological niche modeling (ENM) to determine suitable climatic regions for *H. halys* across the globe and concluded that locations at latitudes between 30 ° to 50 ° are at the highest risk. Northern Europe, northeastern North America, southern Australia, the north island of New Zealand, Angola in Africa and Uruguay in South America fall within this range of latitudes. *H. halys* is established in the Mid-Atlantic States of the USA, which were described as a suitable habitat by ENM modeling. Bernon (2004) mentioned that the currently established locations of the green stink bug, *Chinavia hilaris* (Say), are the southern part of Canada, Pacific coast, northern and southwestern USA, and predicted that the final distribution of *H. halys* will be similar to that of *C. hilaris.*

Host plants, injury and impact in the USA

Halyomorpha halys is a highly polyphagous pest that attacks important crops (Hoffmann 1931, Hoebeke and Carter 2003). It is a landscape scale pest that switches host plants in search of nutritious food throughout the growing season (Funayama 2004). The preference and severity of damage to crops varies in different geographical locations. Hoffman (1931) mentioned that the adults and nymphs of *H. halys* feed on stems, flowers and pods of lima beans, and lima bean is one of the preferred host plants. Yingnan (1988) reported that pear is the most affected fruit in eastern China. In Japan, it is an economic pest of tree fruits, and is a pest of crops and vegetables only during outbreaks (Funayama 2004). The fruit crops that *H. halys* feeds on include apple, peach, pear, cherry, blackberry, raspberry, plum, pomegranate, common jujube, citrus, persimmon, mulberry, hawthorn, apricot, kiwifruit, and strawberry (Yang et al. 2009). Since the female *H. halys* that feeds on apple produces fewer than the average number of eggs, apples are not considered its primary host in Japan; however, apples are good source of food for population development (Funayama 2004). In the USA, more than 300 host plants have been reported for *H. halys* including the economically important plants such as soybean, corn, pome and stone fruits, ornamentals and vegetables (Bernon 2004, Hoebeke and Carter 2003, Kuhar et al. 2012). *H. halys* has also been reported to feed on coniferous cones and is considered a pest in cedar and cypress seed farms; the mature seeds in cones are suitable for the development of *H. halys* nymphs (Funayama 2004, Kiritani 2006). In the USA, injury to cedar and cypress has not yet been documented. *H. halys* is a recently described pest in grape in the USA (Khrimian et al. 2007, Hamilton 2009, Leskey et al. 2012, Pfeiffer et al. 2012). In its native range, grape is listed only as a host of *H. halys* (Ohira 2003), and its pest significance is unclear.

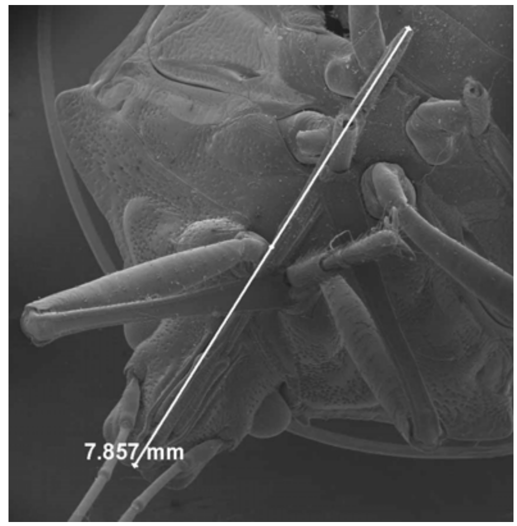

Fig. 1.2. ESEM image of *Halyomorpha halys* showing mouth parts and the measurement of stylets

Stink bugs have piercing and sucking mouthparts. The length of stylets measured with Environmental Scanning Electronic Microscope (ESEM) was 7.8 mm (Fig. 1.2). The stylets are placed in the space between the legs when at rest. The piercing and sucking mouthparts consists of labrum, labium, maxillary and mandibular stylets. The labium is observed as a long segmented beak attached to the head. It is a hollow structure that encloses the stylets. It does not take part in injection or sucking juice, but provides a solid foundation for the stylets. The mandible and maxilla are modified into a long fascicle bundle and are anterior to the labium. The elongated structures present on both sides are part of labium. The proboscis and the labium consist of the salivary canal through which saliva is injected into the host tissue and the cell sap is extracted from food canal. After feeding, a salivary sheath can be observed in the plant tissues after staining with acid fuchsin (Bowling 1979).

In its native range, *H. halys* is a vector of a disease called *Paulownia* witches broom [*Paulownia tomentosa* (Thunberg)] (Hoebeke and Carter 2003). After infection with the *Phytoplasma*, the growth and vigor of *Paulownia* trees are greatly reduced and can result in premature death of the tree (Hiruki 1997). In the USA, *P. tomentosa* is considered an invasive tree from Asia. *H. halys* can also transmit a plant pathogenic yeast, *Eremothecium coryli* (Peglion) Kurtzman, to fruit tissue and fruiting tissue of vegetables, soybean, and cotton (Brust and Rane 2011). Due to the high mobility of *H. halys* and its prolonged feeding activity in different varieties of grapes, *H. halys* may vector diseases in vineyards or facilitate infection by wound-invading pathogens like *Botrytis*.

Seasonal biology in the USA

Halyomorpha halys adults overwinter in large aggregations in man-made structures, inside rock crevices and dead logs (Lee et al. 2013). Adult pentatomids have high fat content, reduced body mass and under-developed oocytes as they undergo reproductive diapause (Borges et al. 1998). With the increase in temperature and day length in spring, adults start moving out of overwintering sites in search of suitable hosts for feeding (Funayama 2004). Adults can disperse at least 2 km in distance (Zhang et al. 1993, Lee et al. 2012). *H. halys* undergoes incomplete metamorphosis; eggs hatch into nymphs that develop through five instars to the adult stages. Egg masses are found from early June throughout the summer and are generally laid on the underside of leaves (Nielsen and Hamilton 2009). Freshly laid eggs are pale green in color, and gradually become white. Fengjie and Zhifang (1997) reported that a single mating is sufficient to fertilize multiple egg masses and a single female can lay up to five egg masses and a total of 140 eggs. First instar nymphs of stink bugs stay around the egg mass and acquire microbial symbionts (Kikuchi et al. 2007).

In the native range and the Mid-Atlantic USA, one or two generations are found (Nielson et al. 2009, Leskey et al. 2012), while there may be more generations in the southern USA (Bernon

2004). *H. halys* was detected in Florida in 2009 (Halbert and Hodges 2011), and the number of generations has not yet been reported.

Biological control

Stink bug eggs are parasitized by various wasp species belonging to the families Platygastridae, Encyrtidae and Eupelmidae. The platygastrid genera *Telenomus* and *Trissolcus* contain the majority of stink bug egg parasitoid species recorded (Esselbaugh 1948). A successful example of the management of a stink bug by classical biological control is the use of *Trissolcus basalis* (Wollaston) and the tachinid, *Trichopoda pennipes pilipes* (F.) for controlling southern green stink bug, *Nezara viridula* (L.) in Hawaii during mid-1960s. The common parasitoids of stink bugs in field crops and vegetables in southeastern Virginia are *Telenomus podisi* (Ashmead), *T. basalis*, *T. edessae* (Fouts), and *T. euschisti* (Ashmead) (Koppel et al. 2009). *T. podisi* and *T. basalis* are the major parasitoids of native stink bugs in the U. S. (Yeargan 1979, Ehler 2002). A solitary endoparasitioid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is described as a primary natural enemy of *H. halys* in China, with a parasitism rate of 70% (Yang et al. 2009). The female parasitoids prefer to oviposit in the freshly laid eggs of the host (Hokyo et al 1966, Bruni et al. 2000). *T. japonicus* has its shortest development period at 30 °C (86 °F), but its longevity decreases with increasing temperatures (Yang et al. 2009). The average summer temperature in Virginia is approximately 30 °C, which is likely to be appropriate for the development of *T. japonicus*. *T. japonicus* also parasitizes eggs of other species of stink bug. *T. mitsukurii,* a solitary endoparasitoid, also parasitize the eggs of *H. halys* and individuals of *T. mitsukurii* emerging from *H. halys* are larger due to the larger egg size of *H. halys* (Arakawa et al. 2004). *T. mitsukurii* is a primary parasitoid of *N. viridula* in Japan (Kiritani 2006).

Researchers at the USDA-ARS Beneficial Insects Introduction Research Center, Newark, Delaware, are currently evaluating some *Trissolcus* spp. including *T. japonicus* and *T. mitsukurii,* for potential release against *H. halys*.

Fungal pathogens are also used to manage stink bugs. The FRM 515 strain of the fungal pathogen, *Metarhizium anisopliae,* is effective against different stink bugs including brown winged stink bug, *Plautia stali* (Scott) (Ihara et al. 2001) and polished green stink bug, *Glaucias subpunctatu* (Walker) (Ihara et al. 2008). However, *H. halys* adults have been shown to tolerate this strain of *M. anisopliae* (Ihara et al. 2008). Gouli et al. (2012) reported that a strain of *Beauveria bassiana* caused 80-100% mortality in 9-12 days against *H. halys*, but *M. anisopliae* had no promising effect.

Praying mantids, spiders, assassin bugs and *Brochymena* species are common predators of stink bugs. The first instars of *H. halys* were observed being attacked by lacewing larva in vineyards (Fig. 1.3). This is the first evidence that lacewing larvae can attack *H. halys* nymphs, and further study is needed to assess this species as a potential predator against *H. halys* nymphs.

Fig. 1.3. Lacewing larva attacking first instars of *Halyomorpha halys*

Chemical ecology

Fig. 1. 4. Brown coloration in skin caused by the defense chemicals of *Halyomorpha halys*

Halyomorpha halys releases a pungent smell upon disturbance from the glands located on the dorsal surface of the abdomen in nymphs and on the under surface of the thorax in adults. The foul smelling chemical, trans-2-decenal and trans-2-octenal, is produced as a defense and causes brown discoloration of the skin upon contact (Fig. 1.4). Isolating and synthesizing pheromones of insect pests can be a very useful approach in integrated pest management. Both the adults and nymphs of *H. halys* are cross-attracted to the aggregation pheromones of the brown-winged green stink bug, *Plautia stali* Scott (Tada et al. 2001a, b). Aldrich et al. (2007) verified that *H. halys* is attracted to the pheromone, methyl (E, E, Z) - 2, 4, 6- decatrienoate, and its related isomers. *H. halys* is more attracted to these pheromones in late season than early season. The aggregation pheromone of *H. halys* has been recently identified by USDA-ARS at Beltsville, Maryland and its performance being evaluated at various research stations in the Mid-Atlantic States.

Management in vineyards

Grapevines are perennial plants and a vineyard is usually managed as a monoculture. Disease and insect pest infestations are generally higher in monoculture compared to multiple cropping systems; therefore, implementing Integrated Pest Management (IPM) in monoculture is more difficult. IPM is a sustainable pest management approach in which pest populations are maintained below economic threshold levels in an economically justified manner with minimum environmental and health hazards (United Nations, Food and Agriculture Organization 2013). IPM strategies can reduce the yield loss caused by insects and prevent unnecessary input costs (Ragsdale et al. 2007). Cultural practices like crop rotation and crop fallowing are not possible in vineyards. The host plants like paulownia, catalpa and tree of heaven that grow near vineyards can be monitored and lures that attract *H. halys* can be used to trap the bugs. Sanitation can be maintained by removing overwintering harborages such as dead trees around vineyards and crop refuges. Harvesting ripe grapes as early as possible can be beneficial because the late presence of *H. halys* in the vineyard has been reported.

Chemical control

The organophosphate malathion, shows promising efficacy against *H. halys* but it is toxic to natural enemies (Abdelrahman 1973, Leskey et al. 2012a). Some pyrethroids and neonicotinoids show good activity (Nielsen et al. 2008, Leskey et al. 2012a) and have short pre-harvest interval. However, the use of pyrethroids can cause outbreaks of secondary pests like spider mites and mealybug species (a vector of grapevine leafroll virus). The neonicotinoid, dinotefuran is effective (Nielsen et al. 2008, Leskey et al. 2012a), but it may cause secondary pest outbreaks and pose a risk to pollinators. Secondary pests are not a problem under normal condition, but can occur if the activities, such as pesticide applications, eliminate populations of their natural enemies. Economic threshold levels for *H. halys* in row crops and fruit orchards and vineyards have not yet been well described; therefore there has been extensive use of chemical pesticides to control *H. halys*. It is very challenging to control an invasive pest in its new habitat in an ecologically sound and environmentally friendly way, partly because the natural enemies of the invasive species are not present. In response to higher *H. halys* populations during the harvest period, grape growers have dramatically increased their use of insecticides, including the use of broad-spectrum insecticides. Vacuuming inside the winery, sealing cracks in the windows and doors and screening on ventilation systems with mesh small enough to prevent an entry can help to reduce stink bugs inside a winery.

Research justification

Stink bugs have not been traditionally considered as economic pests of grapes, and control measures for them were rarely been applied in vineyards in the USA prior to the introduction of *H. halys*. *H. halys* has a much wider host range than native stink bugs, and grape is listed as one of its hosts in its native range (Ohira 2003). In recent years, high populations of *H. halys* have been reported in vineyards throughout the berry development stages. Although, it readily invades vineyards from surroundings, woodlands and structures, but the impact of this pest is not known. *H. halys* was recently described as a direct pest of grape in the Mid-Atlantic USA (Leskey et al. 2012a, Pfeiffer et al. 2012). Information on its basic biology and feeding injury in vineyard is very scarce. The timing of their invasion into vineyards is not known. Stink bugs showed a pronounced border effect in field crops (Tillman 2009, Owen 2012), but there is no information on the border effect of *H. halys* in vineyards. Besides causing direct injury to the berries, *H. halys* can hide inside the berry cluster during harvest and be crushed along with the berries. There have been complaints of "stink bug taint" in freshly prepared juice (Kelly 2012).

Halyomorpha halys has become well established in different locations across Virginia. Being a very opportunistic and highly mobile pest, the movement of *H. halys* into vineyards and its infestation of wine grapes has been of great concern to grape growers in northern Virginia. However, information on its seasonal impact to wine grapes is not known. The purpose of the study was to determine the seasonal phenology and injury level of *H. halys* in vineyards. *H. halys* is gradually establishing in different cropping systems. The impact of invasive *H. halys* on the native stink bug community in raspberry has been discussed.

The objectives of the study were:

1. To study the basic biology, phenology and population dynamics of *H. halys* in vineyards.
2. To examine the feeding injury of *H. halys* to grape berry developmental stages and varieties.
3. To study the impact of *H. halys* on the diversity of native stink bugs in raspberry plantings.

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Chapter 2. Seasonality and population dynamics of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in Virginia vineyards

Abstract

Four commercial vineyards in Virginia were sampled in 2012 and 2013 to study the basic biology, seasonality and population dynamics of *Halyomorpha halys* (Stål). At each vineyard, two blocks were selected. Weekly three-minute timed count visual sampling was performed in border and interior sections from late May until mid-September. Overwintering adult bugs were first detected in vineyards in May; however, the timing of first detection differed among vineyards. Egg masses were found primarily in June and July, and were usually found on the lower surface of grape leaves, although were occasionally on the upper leaf surface, on the berry or on the rachis. All developmental stages of *H. halys* were found in vineyards, suggesting that grape can serve as a reproductive host for *H. halys.* Substantial variation in *H. halys* densities was found among vineyards and throughout the growing season. The first instars were found on egg masses, and after molting, dispersed as they developed. The date on which the first egg mass was collected was considered as a biofix. Based on a degree-day model, there were enough degree-days for a completion of a generation in Virginia vineyards. Significantly higher numbers of *H. halys* were collected in border sections compared with interior sections. These results are discussed in relation to the potential pest status of *H. halys* in vineyards and implications for possible control strategies.

Keywords: *Halyomorpha halys*, distribution, phenology, vineyard, woods, border effect

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive insect pest from eastern Asia. Its history of invasion in the USA dates back to the late 1990s (Hoebeke and Carter 2003). Following its initial detection in Allentown, PA, it has subsequently spread to neighboring states, where it has become an important pest of numerous agronomic crops, fruits, vegetables, trees and shrubs (Leskey et al. 2012, Kuhar et al. 2013). Established populations of *H. halys* occur throughout much of Virginia, with especially large numbers having been recorded from northern and central areas of the state. Virginia is among the states known to be most heavily infested by this pest. In its native range, *H. halys* is an occasional pest of wild and cultivated grapes (Ohira 2003), although its pest status in commercial vineyards in the USA is not well understood. Invasive species can possibly expand their host range and can have a significant impact on crops that are not primary hosts in the native range. Prior to the *H. halys* invasion, stink bugs were not considered economic pests in vineyards, and pesticides targeting them were not often used. However, injury to the grape berry by stink bugs has been mentioned in the literature (Ohira 2003, Hamilton 2009, Leskey et al. 2012, Pfeiffer et al. 2012). Hamilton (2009) mentioned that *H. halys* caused direct injury to the grape berries by inserting the stylets into the fruit to extract fluid. Injury to the grape berries by the stink bug, *Euschistus conspersus* (Uhler), was described as a catfacing by Aldrich et al. (1995. Although, many species of stink bugs have been reported in vineyards (Jubb and Masteller 1977), there are no studies on their pest status or impact to the crop. *H. halys* may be a greater threat to wine grape production than native stink bugs because of its aggregated abundance and movement among its many possible host plants during the growing season. In addition, there have been reports on rachis feeding and subsequent abscission of the clusters by *H. halys* (Pfeiffer et al. 2012).

Phenology is the study of the seasonal growth of plants, animals or insects in relation to environmental factors, such as temperature, solar radiation and day length (Mullins et al. 1992). Understanding pest phenology and population dynamics can be very useful to predict their populations at different host plant growth stages, and can aid in the development of management strategies. *H. halys* in Virginia vineyards at harvest has created a great concern about injury to ripe or ripening berries and their presence in harvested clusters during juice extraction.

Halyomorpha halys is spatially distributed across various landscapes and the factors governing its movement among habitats and hosts are not well understood. In any given season, the movement of stink bugs into a vineyard can be affected by its latitude, geographical location, proximity to farm structures and surrounding vegetation. The stages of crop phenological development also play a role in colonization and abundance of stink bugs. For example, Smith et al. (2009) found that peak densities of stink bugs in soybean were observed during the full-pod (R7) developmental stages and Siebert et al. (2005) showed that the feeding preference of *Euschistus servus* (Say) varied among the developmental stages of cotton. Studies have also shown that stink bugs move from one crop to another in search of food (Jones and Sullivan 1982, Velasco and Walter 1992). Knowledge on pest distribution, host-plant relations and phenology is important to understand the pest status of an insect. Many stink bug species show border or edge effects. For example, Reeves et al. (2010) showed that the density of stink bugs and their injury was greatest at the border rows in cotton, and decreased as the distance from the border increased. Tillman et al. (2009) also observed pronounced border effect on the distribution of southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *E. servus* as they colonized cotton. Higher levels of stink bug injury in border rows can have economic consequences in soybeans; this was manifest as a delay in normal plant maturation (Owens 2012). Understanding whether *H. halys* exhibits border effects in vineyards could be very helpful toward the development of optimal management strategies for the application of pesticides at the right place and time; thereby conserving natural enemies. Therefore, border effects from *H. halys* were studied in vineyards.

Studies on *H. halys* in the USA have focused mainly on tree fruits, vegetables and agronomic crops. Different crops and fruits have been described as preferred hosts of *H. halys* in its native range in Asia (Watanabe et al. 1994a, b, Funayama 1996, Tada et al. 2001, Funayama 2004). Since the outbreak of *H. halys* in 2010 in Virginia, high populations of *H. halys* were reported at the harvest stage of grape, and there have been reports of “stink bug taint” in the juice. To understand the pest status of an insect, information on its seasonal pattern of abundance in the crop of concern, basic biology and injury level is important. The research presented here provides the basic biology and the seasonality of *H. halys* in Virginia vineyards.

Degree day accumulation

The factors governing insect growth and development are temperature, food availability and quality. The amount of heat required by an organism to complete its development is expressed in units called degree-days, which reflect insect development in response to daily temperatures. Degree-days are the accumulation of heat units within a range of predetermined upper and lower temperatures that support development. One degree day results when the average temperature within a 24 h period is one degree over the minimum threshold. The accumulation of degree-days is added over a period of time and used to predict insect development. The minimum temperature threshold for the development of *H. halys* is 14.17 °C and the total degree- days required to reach adults is 538 DD (Nielsen et al. 2008). Female adults require an additional 145 DD to lay eggs (Nielsen et al. 2008).

Materials and methods

Four commercial vineyards were selected across Virginia to study the seasonality and distribution pattern of *H. halys.* Three vineyards were located in northern Virginia and the fourth vineyard was in southwestern Virginia. The details of the vineyards sampled are provided in Table 2.1.

Table 2.1. Description of the sampling blocks in four Virginia vineyards used for sampling *Halyomorpha halys* in 2012 and 2013

	V1B1	V1B2	V2B1	V2B2	V3B1	V3B2	V4B1	V4B2
Location	Rappahannock	Rappahannock	Rappahannock	Rappahannock	Orange	Orange	Montgomery	Montgomery
Elevation (m)	215	215	234	234	113	113	385	385
Variety	Chardonnay Viognier	SB, CS	Nebbiolo, PT	SB Chardonnay CS, PV	CS, Viognier	Nebbiolo CS	Chardonnay Viognier	VB
Block size (ha)	0.5	0.75	0.25	1	3	3	0.5	1
No. of insecticide sprays	4	4	1	1	1	NA	NA	NA
Surroundings	Forest Pasture	Forest Pasture	Forest Pasture School Houses	Forest Pasture School Houses	Sub-urban Soybean field	Sub-urban Soybean field	Forest Pasture	Forest Pasture
Proximity to forest (m)	10	600	100	100	10000	10000	10	10

V1: vineyard 1, V2: vineyard 2, V3: vineyard 3, V4: Vineyard 4, B1: Block 1, B2: Block 2, SB: Seyval Blanc, CB: Cabernet Sauvignon, PV: Petit Verdot, VB: Vidal Blanc

Sampling method

In each vineyard, two blocks were selected for sampling. A vineyard block corresponded to the continuous rows of vines grown and contained specific variety, soil and rootstock or management system. The first five rows facing the woods or the winery were considered as border section and the rows beyond the fifth row were considered as the interior section. A panel is a space between two-trellis posts, which is approximately 5 m in length. At least two panels towards the edge of interior rows were excluded from sampling. Visual counts over three-minute intervals were performed weekly from 30 May to 12 September in 2012 and from 31 May to 12 September in 2013. Three samples were taken at the border section and three in the interior section. The number of *H. halys* egg masses, nymphs, and adults were recorded. Special attention was given to the lower surface of leaves, where *H. halys* usually laid egg masses. Egg masses with first instars clustered around them were recorded as an egg mass. For hatched egg masses, the number of nymphs around the mass was recorded to determine the percentage hatch of field-collected eggs. Only the first instar nymphs were taken into account because the second instars disperse away from the egg mass.

Vineyard 1 was located 50 km from Virginia Tech's Alson H. Smith Jr. Agricultural Research and Extension Center at which average daily maximum and minimum temperatures were recorded using thermometer (ET106 Weather Station, Campbell Scientific). Accumulated degree-days were calculated in 2012 and 2013. Biofix was the date on which the first egg mass was collected; the accumulation of degree-days began after biofix. Daily accumulation was derived by averaging the maximal and minimal temperature in °C, and subtracting the minimum developmental temperature threshold.

Statistical analysis

To compare the counts between border section and interior section, the number of nymphs (excluding first instars) and adults recorded from the respective sections of each vineyard were pooled for each weekly sample. The total counts were transformed as [square root (x+1)] and analyzed using repeated measure Multivariate Analysis of Variance (MANOVA). The vineyard location effect (border versus interior) was the main factor and time (week) was the repeated measure factor. Repeated measures MANOVA was preferred over repeated measures analysis of variance (ANOVA) because sampling time points were not evenly spaced in a given week (Norman and Streiner 2007).

With the repeated measures MANOVA, within subject and between subjects analysis were performed. Between subjects analysis compared the mean abundance of *H. halys* between border and interior sections. Within subjects examined the trend in *H. halys* abundance in vineyards across time. In the repeated measures MANOVA, Mauchly's Test of Sphericity was taken into consideration based on correlations in response variable across time and the equal variances. If the Sphericity test was significant, the adjusted of *F*- test values were reported, but if found not significant, adjusted epsilon values were reported.

Results

Seasonal phenology in Vineyard 1

The seasonal phenology of *H. halys* in vineyard 1 was similar in 2012 and 2013, although total counts were higher in 2012 than 2013 (Figs. 2.5 and 2.6). Adults were first detected on 30 May and 14 June in 2012 and 2013 respectively. No egg masses were found in August or September in either year. Nymphal counts peaked in the last week of July, which were the progeny of the overwintering adult population. In both years, adult counts peaked in July and again in the second week of September, with the late peak coinciding with grape harvest in both years (Figs. 2.1 and 2.2). These peaks were possibly due to the mass movement of *H. halys* into vineyards from host plants in surrounding habitats. Pesticides were sprayed in the vineyard in 2012 after the population was noticed. The population recovered even after pesticide applications. The adult population again peaked in the second week of September, coinciding with the harvest of grapes. There was greater fluctuation in adult counts as they are mobile and can fly from one row to next.

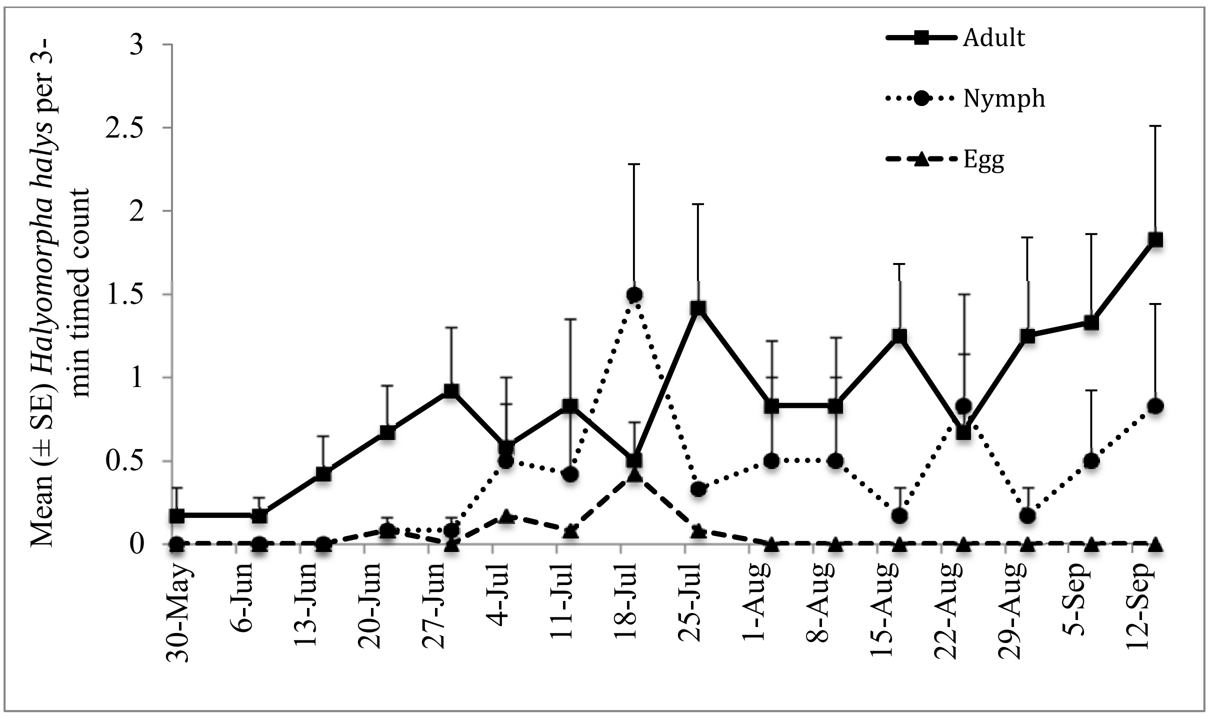

Fig. 2.1. Mean (± SE) abundance of eggs, nymphs and adults *Halyomorpha halys* in Vineyard 1, Huntly, VA (Rappahannock County) in 2012

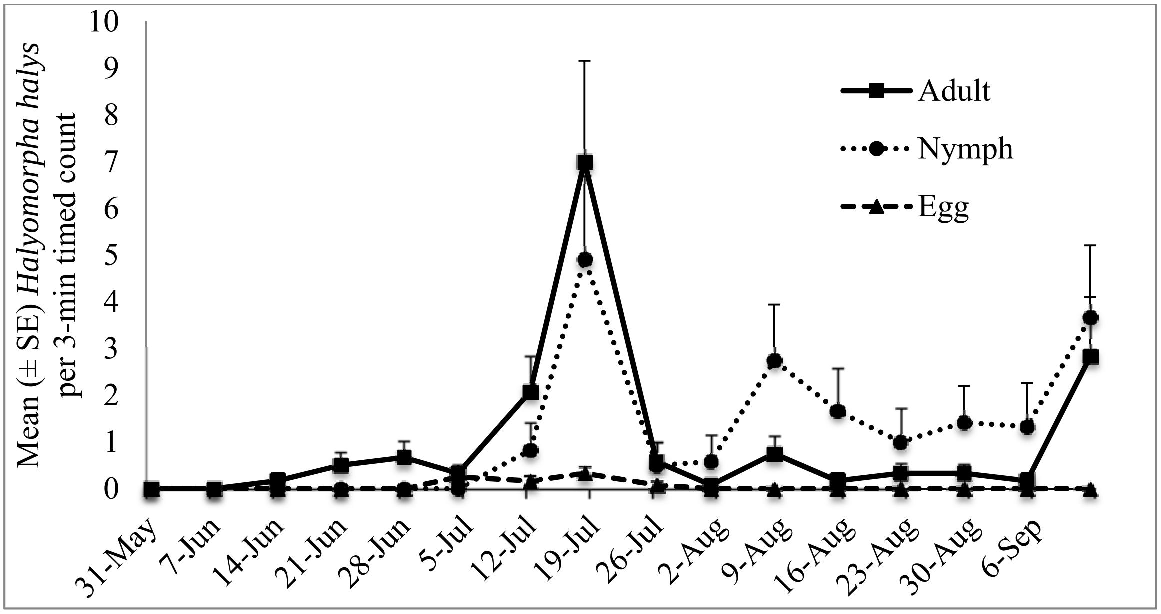

Fig. 2.2. Mean (± SE) abundance of eggs, nymphs and adults *Halyomorpha halys* in Vineyard 1, Huntly, VA (Rappahannock County) in 2013

In 2012 and 2013, the first egg masses were collected on 4 July and 12 July, respectively (Figs. 2.3 and 2.4). No egg masses were collected in August or September. Approximately 538 DD are required to complete a generation (Nielsen et al 2009). Thus, at this vineyard, enough degree days were accumulated after the first week of September to observe the second generation eggs deposited. However, no egg masses were found at that time. Therefore, there are enough degree-days for only a generation to complete in Virginia vineyards.

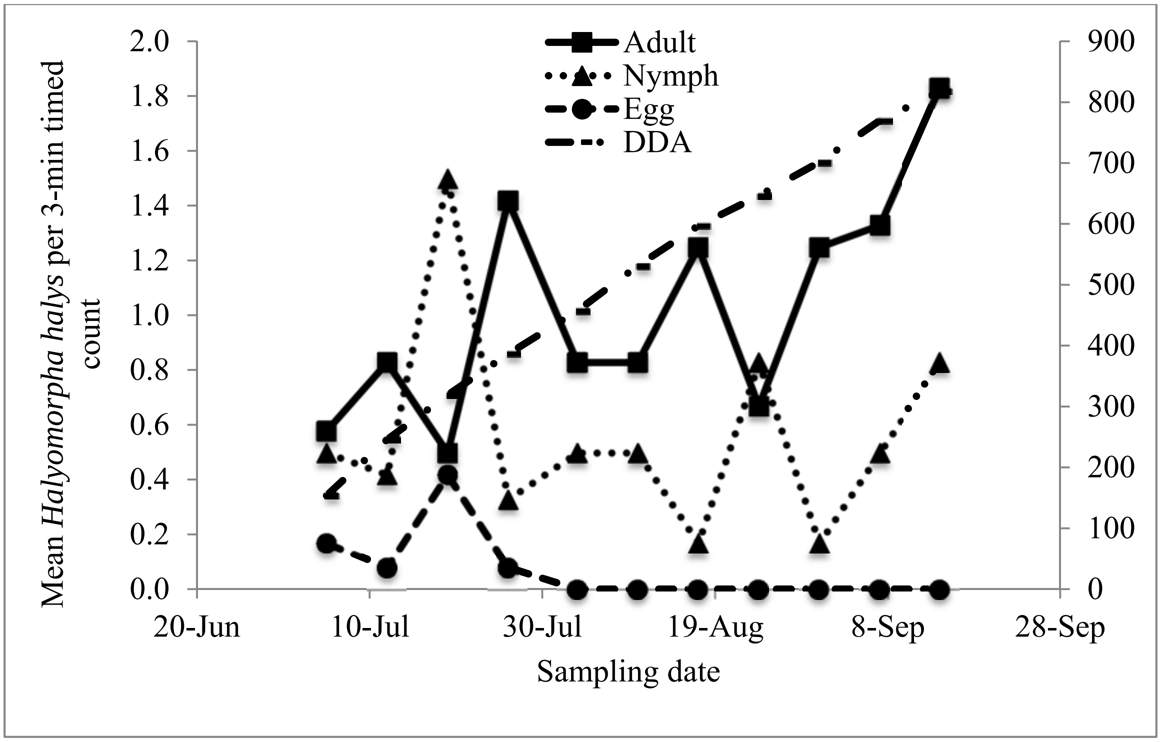

Fig. 2.3. Mean *Halyomorpha halys* counts and degree-days accumulation in vineyard 1, Huntly, VA (Rappahannock County) in 2012

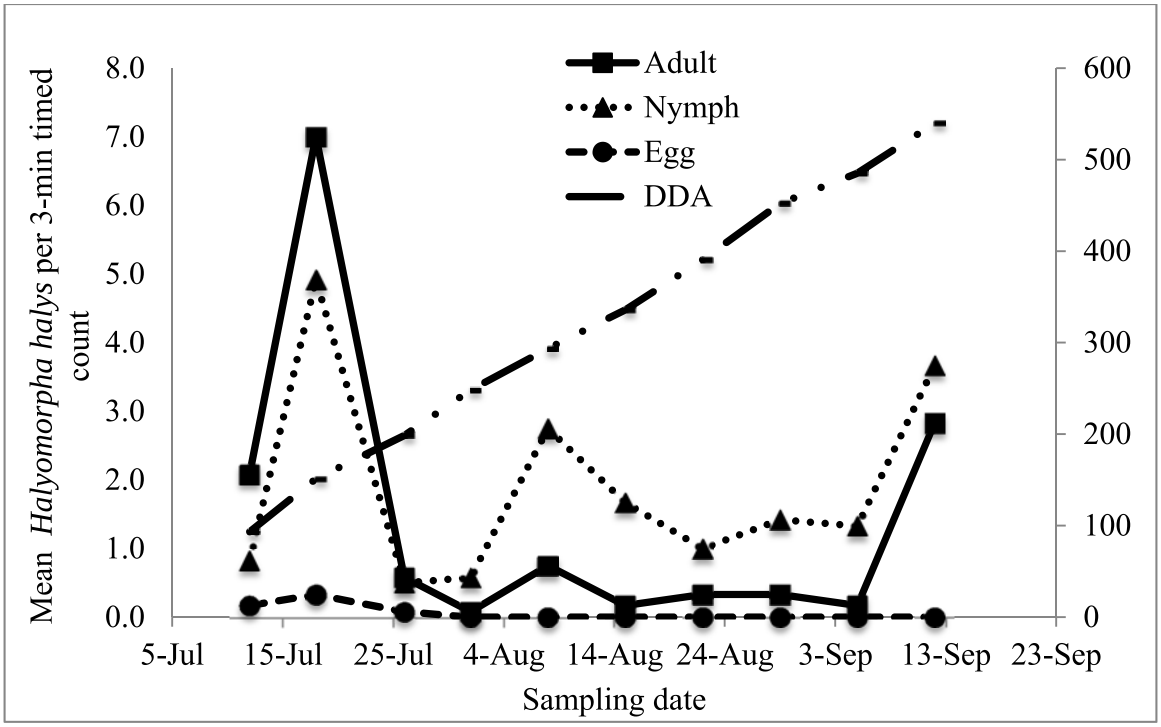

Fig. 2.4. Mean *Halyomorpha halys* counts and degree days accumulation in vineyard 1, Huntly, VA (Rappahannock County) in 2013

Seasonal phenology in Vineyard 2

Vineyard 2 had a similar landscape to vineyard 1, and was in the same vicinity. The vineyard was near a forest with a few pastures nearby. Trends in *H. halys* counts were similar between the two locations. In 2012, the adult population of *H. halys* peaked in the last week of July and again in the last week of August (Fig. 2.5). Adult counts were low in the late season in 2013 (Fig. 2.6). The nymphal counts were greater in the late season. A few egg masses were collected in June and July, but were not found in August and September.

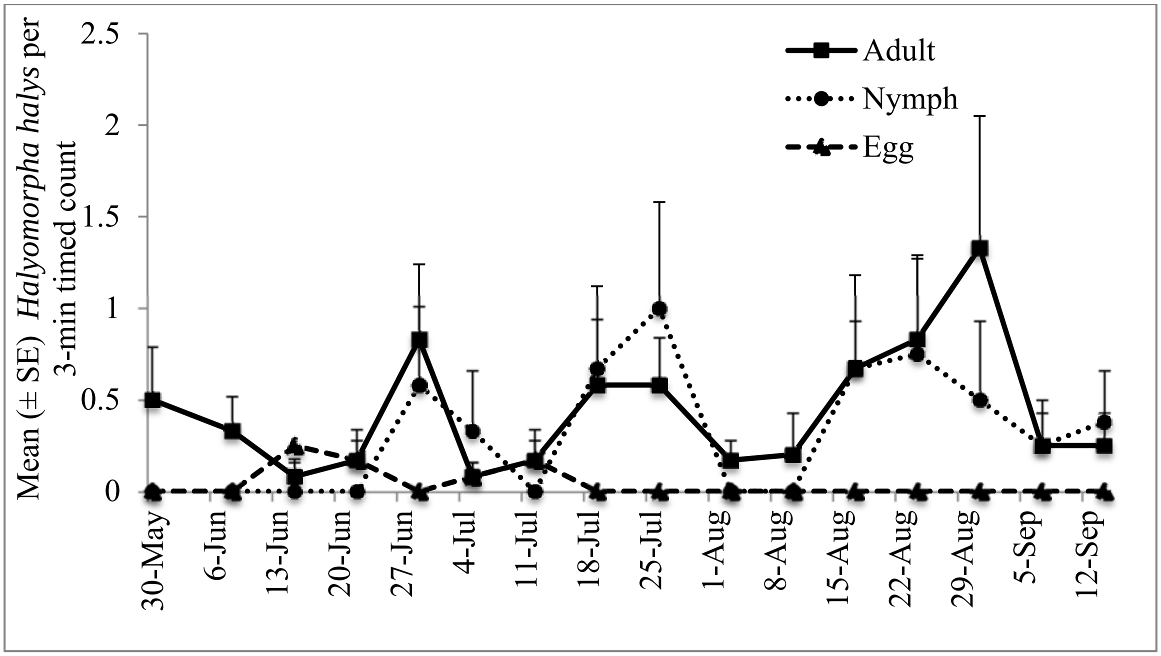

Fig. 2.5. Mean (± SE) abundance of eggs, nymphs and adults *Halyomorpha halys* in Vineyard 2, Washington, VA (Rappahannock County) in 2012

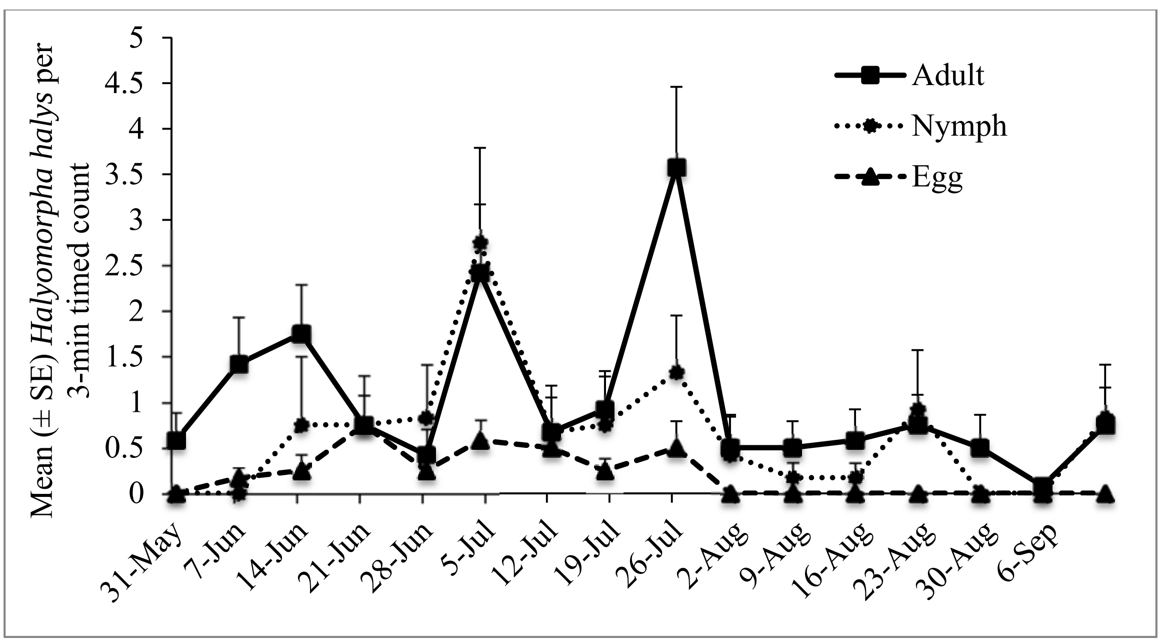

Fig. 2.6. Mean (± SE) abundance of eggs, nymphs and adults *Halyomorpha halys* in Vineyard 2, Washington, VA (Rappahannock County) in 2013

Seasonal phenology in vineyard 3

As compared to the other vineyards sampled, the adult, nymphal and egg counts of *H. halys* peaked in the early season. Adults were first detected on 30 May and 7 June in 2012 and 2013 respectively. They peaked in the last week of June, decreased gradually and were not collected in late season. Nymphs were found in June and July and declined sharply thereafter. Egg masses were mostly collected in June and July, and a few in the first week of August (Figs. 2.7 and 2.8)

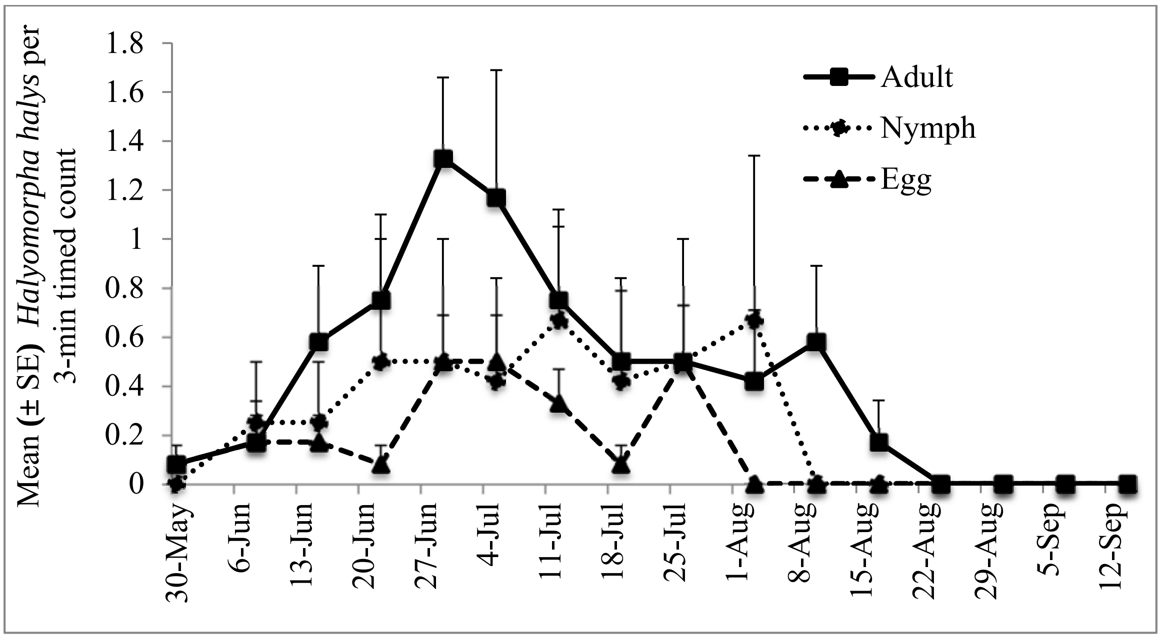

Fig. 2.7. Mean (± SE) abundance of eggs, nymphs and adults *Halyomorpha halys* in Vineyard 3, Orange, VA (Orange County) in 2012

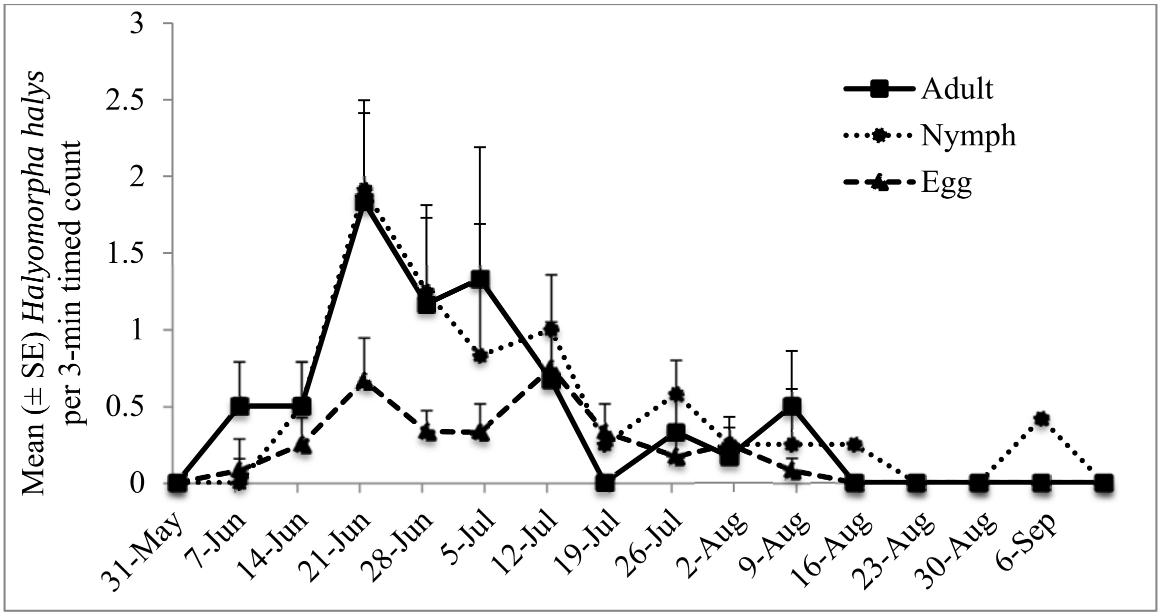

Fig. 2.8. Mean (± SE) abundance of eggs, nymphs, and adults *Halyomorpha halys* in Vineyard 1, Orange, VA (Orange County) in 2013

Seasonality of *H. halys* in Virginia in Vineyard 4

During two years of sampling in vineyard 4, only ten and six adults of *H. halys* were collected in 2012 and 2013 respectively. Therefore, the data were excluded from the analysis of border effect. The landscape of vineyard 4 was similar to that of vineyards 1 and 2. This revealed that *H. halys* were present in southwestern Virginia.

Border effect study of *H. halys* in vineyards

Between subjects results of repeated measure MANOVA showed that there was a significant border effect in 2012 ($F = 6.48$, df = 1, 9, $P < 0.05$) (Fig. 2.9) and 2013 ($F = 13.87$, df = 1, 9, $P < 0.05$) (Fig. 2.10). Within subject repeated measures MANOVA showed that there were border effects regardless of the time in 2012 (Univ G-G Epsilon = 1.62, df = 2.86, 25.77, $P > 0.05$) and 2013 (Univ G-G Epsilon = 1.01, df = 3.91, 39.15, $P > 0.05$).

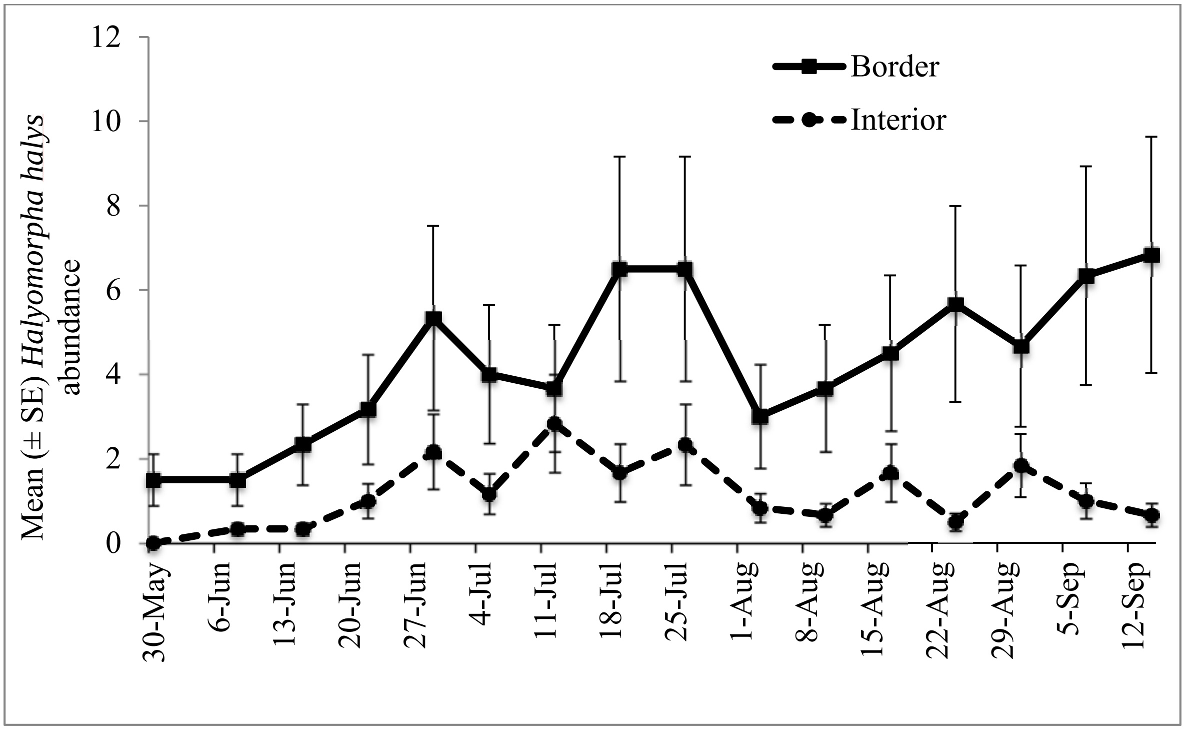

Fig. 2.9. Mean (± SE) number of *Halyomorpha halys* in border and interior sections of three vineyards in Virginia 2012

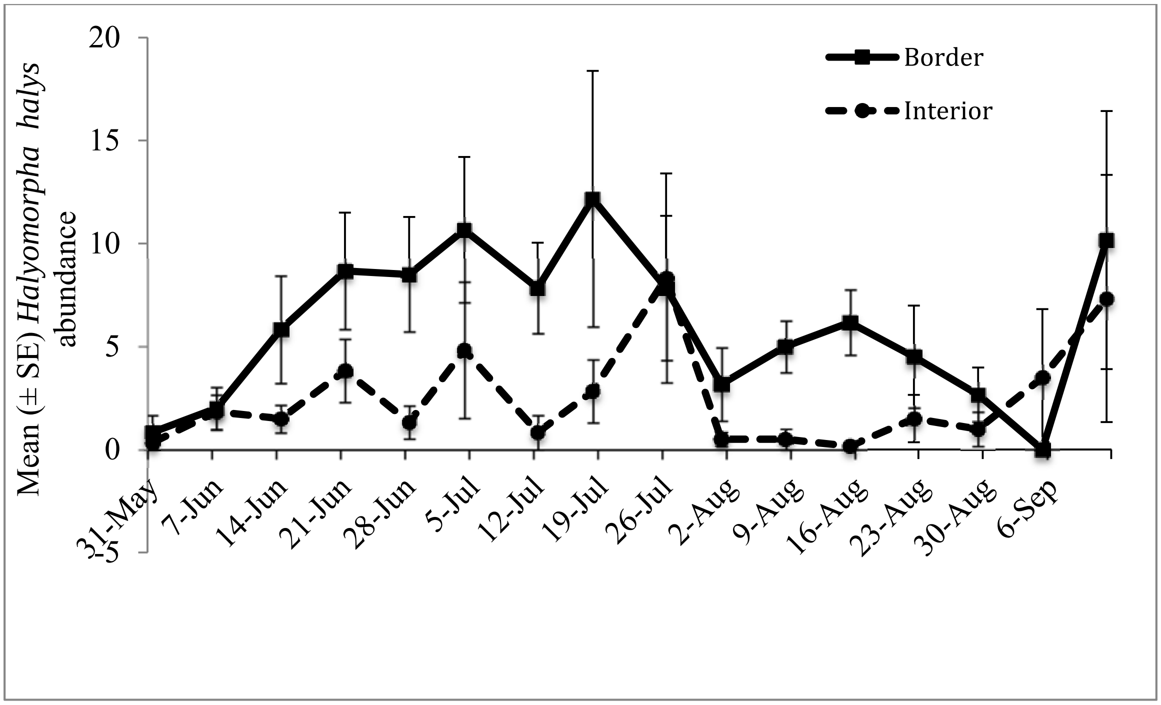

Fig. 2.10. Mean (± SE) number of *Halyomorpha halys* in border and interior sections of three vineyards in Virginia in 2013

Observation on *Halyomorpha halys* egg masses

In total, 223 egg masses were recorded. Egg masses were typically laid on the lower surface of grape leaves (Fig. 2.11. A), but were also found on the surface of grape berries (Fig. 2.11 B) and on the rachis (Fig. 2.11 C). The mean number of eggs per mass was 26.72 ± 0.25 (range = 14 - 32). The hatchability of field-collected egg was 84.07 ± 0.94 % (N = 111).

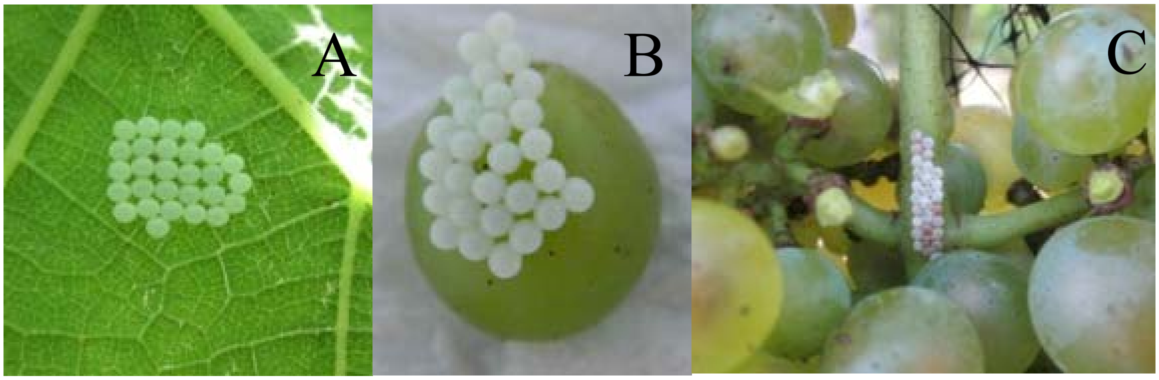

Fig. 2.11. Egg mass of *Halyomorpha halys* on grape leaf (A), berry (B), and rachis (C)

Discussion

These studies have shown that grape is a suitable season host of *H. halys.* Bugs overwintering in buildings and other structures may move into the vineyard to feed and oviposit with the rise in temperature and increase in the daylight in spring. Jones and Sullivan (1981) showed that increasing temperature stimulated spring emergence of stink bugs but the time of emergence varied; for example, in South Carolina the green stink bug, *Chinavia hilaris* (Say) emerged as early as April and *E. servus* (Say) emerged from late March. The princess tree, *Paulownia tomentosa* (Thumb.) is a suitable host of *H. halys* (Funayama 2004) and was found in proximity to the vineyards. All *H. halys* life stages were found in vineyards. *H. halys* began egg laying in vineyards as early as mid-May. However, the timing of egg laying varied among vineyards and may have been affected by vineyard location and different host plants nearby. Vineyards close to a suburban area had earlier invasions compared to those surrounded by woods. A large population of *H. halys* can move into vineyards at any time if it is established in that location. The large number of hatched egg masses collected in the vineyards suggested that grapevines were a good reproductive host for *H. halys*. Nymphs appeared to develop well on grapevines. These developed adults have great mobility (Nielsen and Hamilton 2009). *H. halys* are highly mobile and very good fliers, so they may move out of vineyards if more preferable host plants are nearby. For two consecutive years, soybean was cultivated adjacent to one of the vineyards sampled and *H. halys* were not found in the vineyard, but were observed in high populations towards border rows in soybean (S.B., personal observation). This could possibly be due to the greater preference of soybean versus grape. The pod formation stage of soybean coincided with the veraison and harvest stages of grape. Grape as an early season reproductive host and soybean as a late season feeding host can make the management of the invasive pest more difficult in these cropping systems.

The seasonal abundance of *H. halys* in different vineyards varied greatly during the season, or from vineyard to vineyard. Pesticide applications and other management inputs occurred in the vineyards sampled. The population of *H. halys* recovered even after pesticide applications in some vineyards. The distribution of *H. halys* inside a vineyard can be categorized in two ways; aggregating in certain pockets and dispersing in a small number throughout the vineyard. Stink bug species can cause significant injury even at a low population densities. Jackai and Daoust (1986) showed that *Euschistus* species are capable of causing injury to pods and seeds of cowpeas at low populations. A pronounced border effect was observed in vineyards; *H. halys* were recorded more frequently in border rows than interior rows.

My study also revealed that there were enough degree-days for *H. halys* to complete a generation in Virginia vineyards. Egg masses were mainly collected in the first and second week of July. Enough degree-days had accumulated by the second week of August in 2012 and the second week of September in 2013 in a vineyard in northern Virginia, but no egg masses were collected at that time. Niva and Takeda (2003) determined that short day length and decreased temperature caused female *H. halys* to prepare for diapause. Borges et al. (1998) reported that adult pentatomids store high fat, reduce their body mass, and have under-developed oocytes before entering diapause. Therefore, my degree-days data and the physiology of *H. halys* strongly suggested one generation of this bug in Virginia vineyards. *H. halys* is univoltine in Pennsylvania and New Jersey (Nielsen et al. 2008), but can have multiple generations in warmer climates (Hoffmann 1931).

My study on *H. halys* phenology is also important for biological control using parasitoids. The oviposition period varied among vineyards. Egg parasitoids generally prefer a freshly laid egg mass (Hokyo et al. 1966, Bruni et al. 2000). Therefore, the study of border effect and phenology is valuable information for releasing effective parasitoids of *H. halys*.

The result on the seasonal occurrence and border effect showed that vineyards can be invaded multiple times by *H. halys* during a season. Infestation in the late season has also been reported and is a great concern because they can easily infest the winery. Insects are in search of a nutritious food source in the late season for storing fat as an energy reserve. Grape, late season apples and soybean are potential food sources for *H. halys* before they go to overwintering sites. Pome fruit is a nutritious food source for reproductive development in early season and storing fat for diapause in the late season for *H. halys*. However, *H. halys* that feeds on apples produce fewer eggs (Funayama 2004).

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Chapter 3. Feeding injury and preference of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) to wine grape

Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), an invasive insect from Asia, is a pest of numerous crops, including grapes. Cage experiments to study the injury level of *H. halys* to grape varieties and grape berry developmental stages were conducted in Virginia in 2011, 2012 and 2013. Seyval Blanc and Cabernet Sauvignon at the pea-sized, veraison and pre-harvest developmental stages were used in 2012 and 2013. Four adult *H. halys* were placed inside a nylon bag, and hung around five grape clusters in both studied varieties when the berries were in pea-sized, veraison and pre-harvest stage for a week. After 7 days, the bugs were removed from the cages but the cages remained there for 7 more days and the number of berries in each cluster showing feeding punctures was recorded. The number of punctures in Seyval Blanc berries was significantly higher than Cabernet Sauvignon berries. Injuries were detected in all the berry developmental stages examined. Significantly more punctures were observed in pre-harvest fruit than in veraison or pea-sized or control berries in 2012. However in 2013, significantly more punctures were recorded from pre-harvest or veraison stage fruit than in pea-sized berries or control berries. Injury to Seyval Blanc was more conspicuous than injury to Cabernet Sauvignon. These results have documented the potential of *H. halys* to feed on and injure grape berries at different developmental stages and have cause different injury level to different varieties of grape.

Keywords: *Halyomorpha halys*, grape, variety, developmental stage, preference, injury level

Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an introduced stink bug from eastern Asia that has recently become established in the Mid-Atlantic region of the USA (Hoebeke and Carter 2003), and spread to other parts of the country. Stink bugs cause direct injury to crops by inserting their stylets into plant parts for feeding. During stylet insertion, mechanical pressure is created by which saliva-dissolving enzymes reach the cell wall and intercellular matrix and cell sap is sucked (Miles 1958 1959). *H. halys* has potential to cause feeding injury in tree fruits throughout the growing season, but the damage can be more severe in mid-season, when there is rapid increase in the size of fruits (Nielsen and Hamilton 2009). Stink bug feeding on immature apples causes dimples or depressed areas (Solymar 1999), which degrades the market value of the fruit. In peaches, feeding results in catfacing injury (Ring 1958), which is a small depressed and damaged area on the surface of fruit. Internal injury is only visible after cutting or biting into the fruit. The storage of such fruits causes a significant post-harvest loss. The internal injury caused by stink bugs in apple can be confused with a physiological disorder called "cork spot" that results from calcium deficiencies (Brown 2003). In apple, the necrotic tissue is often brown whereas it is white to brown in peach, cherry and pear. Infestation in peaches was as high as 99.4 % in China (Fengjie and Zhifang 1997). Yang et al. (2009) showed that *H. halys* feeds on the young fruit of peach, pear, and apple. *H. halys* has been reported as an occasional pest of grape in its native range (Ohira 2003), but has become a pest of grapes in the USA (Hamilton 2009, Leskey et al. 2012, Pfeiffer et al. 2012). *H. halys* can cause direct injury to grape berries (Hamilton 2009) and can feed on the grape rachis and cause subsequent abscission of the clusters (Pfeiffer et al. 2012).

Although native stink bugs are not considered important pests in vineyards, *H. halys* is recognized as a potential grape pest, and several incidences of vineyard infestation have been reported (Leskey et al. 2012). Stink bugs can cause direct injury to grapes by piercing and feeding on the berries; however, the expression of injury and the relative susceptibility of different varieties are not understood. The experiments reported here assessed the injury and relative susceptibility of one red and one white grape variety to *H. halys* feeding at different berry developmental stages.

Materials and methods

Insect source

Adult male and female *H. halys* were collected from soybean or corn fields and starved for 24 hours in the laboratory at the ambient laboratory temperature before using them in experiments.

Cage study in 2011

An experiment was performed in 2011 at Virginia Tech's Kentland Farm, near Blacksburg, VA to assess the injury to grape berries by *H. halys*. The vineyard plot was situated on an elevated site above the New River in southwestern Virginia (37.20 °N, 80.59 °W, 615.7 m elev.). The plot was adjacent to woods on one side and to different crops on the other sides. The varieties in the vineyard included 'Reliance', 'Vidal Blanc', 'Chambourcin', 'Himrod' and 'Mars Seedless'. 'Reliance' and 'Vidal Blanc'. A sample of 20 grape clusters was selected from each variety after fruit set and caged with nylon mesh of size 25.4 x 50.8 cm (1467 A, Sock enclosure, Bioquip, Rancho Dominguez, CA). At the pea-sized, veraison and pre-harvest stages, five grape clusters were selected randomly and four adult *H. halys* were introduced to each cage. The bugs were removed from the cages after 7 days but the cages remained. The five remaining cages were considered as control.

Cage studies in 2012 and 2013

Cage studies were conducted at a commercial vineyard in Rappahannock county, VA (38.69° N, 78.16 ° W, 234 m elevation) using the same cages described above. 'Cabernet Sauvignon' (red grape) and 'Seyval Blanc' (white grape) were used. The pea-sized stage refers to the green berries that are at the size of a pea. At the veraison stage, there is a characteristic change in the coloration of the berry. Pre-harvest stage refers to the ripe berries that are ready to harvest. In 2011, developmental stages of grape berry used were pea-sized, veraison and pre-harvest stage. During each developmental stage of grape berry, five grape clusters were selected in both the varieties. The berries in each cluster were checked thoroughly and removed if found diseased or had prior injury. In addition, if the berries were found too close to each other, a few berries were removed from the clusters to create space so that the punctures could be clearly visible. From the beginning of the pea-sized stage, five grape clusters were selected as control and were caged without bugs. The clusters were marked with red tape and used as control in other developmental stages of berry as well. Four adult *H. halys* were introduced to each cage for 7 days. The cages were checked every 2 days and the bugs were replaced if found dead. The berries in each cluster were checked thoroughly and removed if diseased or had any kind of pervious injury. After a week, the bugs were removed from the cages, but the cages remained for 7 days to allow the punctures to be more visible. The cages were removed and the number of punctures in each cluster was recorded. The berries were photographed to document the expression of feeding injury.

Statistical analysis

Data collected in 2012 and 2013 were transformed as function log (x+10) to ensure normality and analyzed as two-way ANOVA (JMP 10, SAS Institute, Inc. Cary, NC).

Results

Cage study in 2011

Most of the cages were blown away during a storm on 20 August 2012 and data were not collected. However, the progression of injury in the 'Vidal Blanc' berries was photographed and documented. The berries showed progression of injury as, a small necrotic spot appeared at the feeding site (Fig. 3.2 A), which gradually increased in size (Fig. 3.2 B), and ultimately the berry became intensively deformed (Fig. 3.2 C).

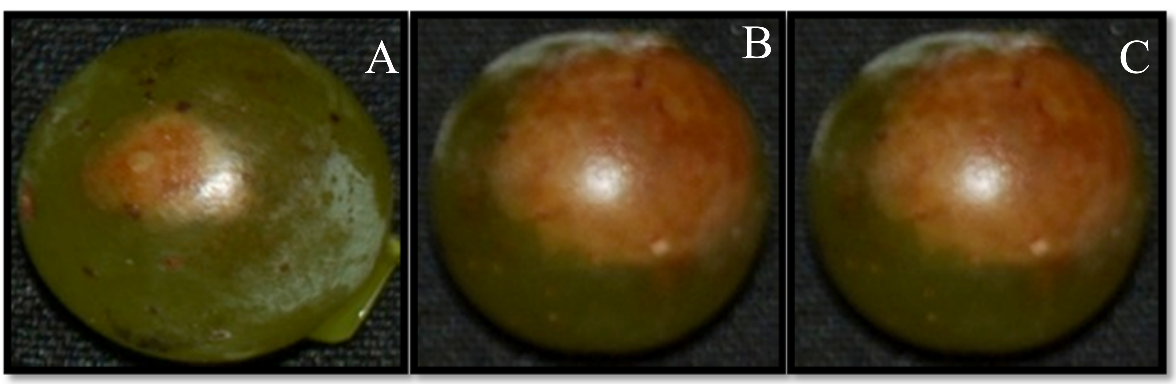

Fig. 3.1. Progression of injury in ripening 'Vidal Blanc' grape berries caused by *Halyomorpha halys*; (A) a small necrotic spot around the feeding site (B) the necrotic spot gradually increased, (C) the berry got intensively deformed

Cage study in 2012 and 2013

Significantly more punctures were observed in berries of 'Seyval Blanc' than of 'Cabernet Sauvignon' in 2012 ($F = 7.57$, df = 1, 35, $P < 0.05$) and 2013 ($F = 7.64$, df = 1, 35, $P < 0.05$) (Fig. 3.2).

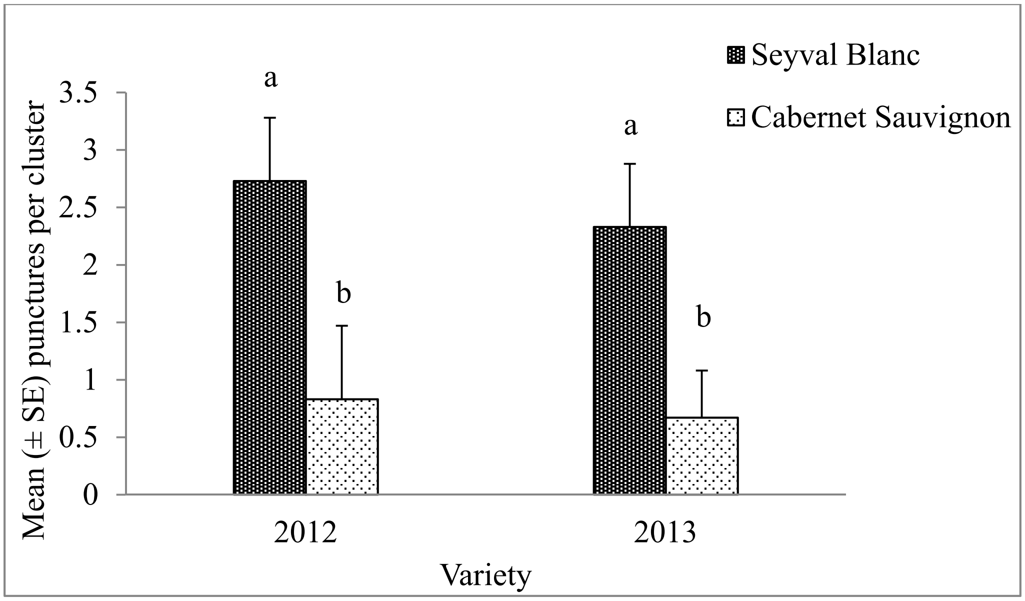

Fig. 3.2. Mean (± SE) number of punctures in Seyval Blanc and Cabernet Sauvignon grapes in 2012 and 2013. Means with different letters were significantly different (Student's; $P < 0.05$).

The number of punctures significantly differed in different grape berry developmental stages in 2012 ($F = 10.03$, df = 3, 35, $P < 0.05$) (Fig. 3.3) and 2013 ($F = 7.64$, df = 3, 35, $P = P < 0.05$) (Fig. 3.4).

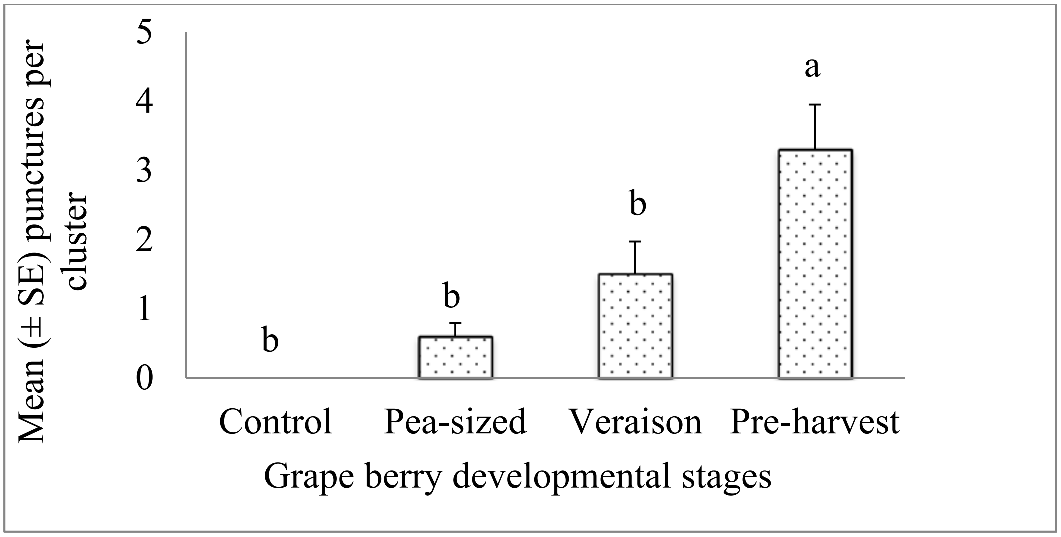

Fig. 3.3. Mean (± SE) number of punctures per cluster in different developmental stages of grape in 2012. Means with different letters were significantly different (Tukey-Kramer test; $P < 0.05$).

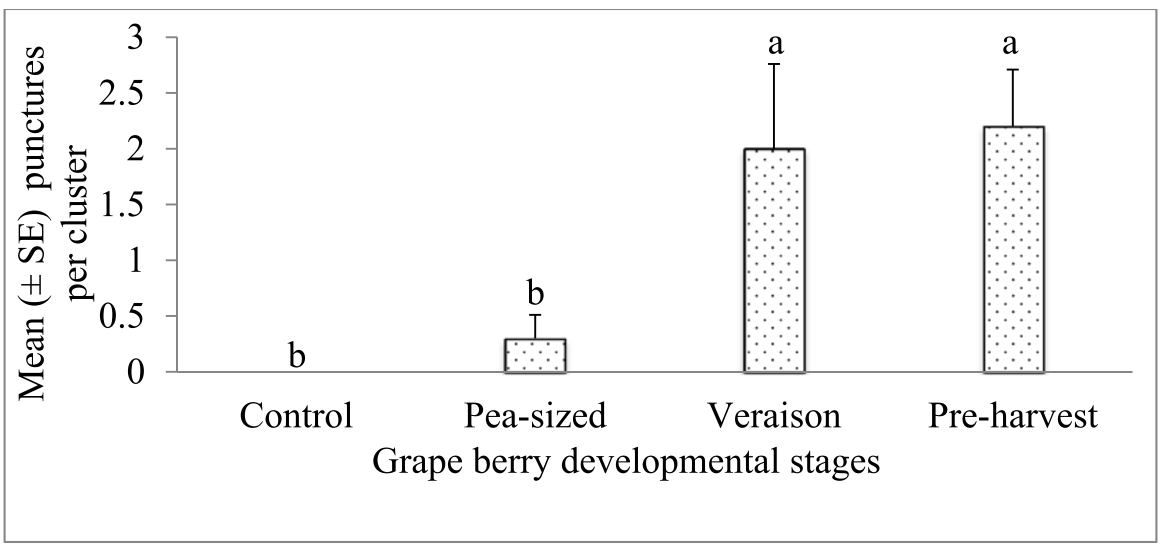

Fig. 3.4. Mean (± SE) number of punctures per cluster in different developmental stages of grape in 2012 and 2013. Means with different letters were significantly different (Tukey-Kramer test; $P < 0.05$).

Discussion

This study is the first documentation of *H. halys* feeding injury expression in grape berries. Injury expression differed across three developmental stages of grapes. Injury to pea-sized stage berries can be described as catfacing injury. Aldrich et al. (1995) mentioned that the stink bug, *E. conspersus,* also caused catfacing in grapes. At the pea-sized stage, berries do not accumulate sugar, and the feeding site may be less vulnerable to pathogenic activity due to the lack of sugar that triggers pathogens. Feeding on immature berries caused a sunken area at the feeding site. The area gradually changed into brownish or blackish. In contrast, feeding on the ripening berries caused a necrotic spot to appear around the feeding site that gradually increased in size. As the grape berry ripens, it starts accumulating sugar and the progression of injury may be facilitated by the infection of microorganisms. Such deformed berries may be discarded for wine and table purposes.

In addition, this study also showed that *H. halys* has different levels of varietal susceptibility and preferences to different developmental stages of grape. The injury was more pronounced in veraison stage and harvest stage compared to the pea-sized stage. Besides the fruiting structures, stink bugs also feed on the stems and leaves of various plants (McPherson and McPherson 2010). *H. halys* was observed feeding on the flowering structures of grape. Abortion of young grape clusters due to the feeding of *H. halys* on the rachis in young clusters has been reported, but further study is needed to address the severity of this type of injury (Pfeiffer et al. 2012).

The punctures in 'Seyval Blanc' were more clearly visible than in red grape berries. The skin thickness and sugar content of grape berries may also affect on the preferences of *H. halys*.

Prior to the introduction of *H. halys*, there were reports of stink bug presence in vineyards. Esselbaugh (1948) reported that the green stink bug, *Chinavia* (*Acrosternum) hilaris* (Say), the rough stink bug, *Brochymena quadripustulata* (F.), and the onespotted stink bug, *Euschistus variolarius* (Palisot de Beauvois), fed on grapes. Jubb and Masteller (1977) mentioned that five pentatomid species were found in vineyards in western Pennsylvania and that *Cosmopepla bimaculata* (Thomas), *C. hilare*, *B. quadripustulata*, and *E. variolarius* cause direct injury to the berries. Stink bugs are capable of causing injury to the crops even in a low population (Jackai and Daoust 1986). *H. halys* can have an economic impact even at low population densities. The research presented here showed that *H. halys* has potential to cause significant economic problems in Virginia vineyards.

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Chapter 4. The stink bug (Hemiptera: Pentatomidae) community in primocane-bearing raspberries in southwestern Virginia

Abstract

Raspberries are widely grown in Virginia and stink bugs have become a significant pest of this crop in recent years. To understand which species are attacking the crop, we sampled an established primocane-bearing raspberry plantings near Blacksburg, Virginia in 2008-2009 and in 2011-2013. Sixteen species of stink bugs were found on the raspberries. The brown stink bug, *Euschistus servus* (Say), was the predominant species in 2008, 2009, 2011 and 2012. The invasive species, *Halyomorpha halys* (Stål), was not found on the raspberries in 2008 or 2009, but was detected in 2011 and 2012, and was the most abundant stink bug species in these plantings in 2013. Our results showed that the Shannon-Weaver diversity index and Shannon's equitability in 2012 and 2013 were higher than in 2008 and 2009. This increase in diversity and equitability revealed that *H. halys* may be displacing *E. servus* populations in Virginia raspberry plantings. Similar trends have occurred on other crops in the Mid-Atlantic USA, where *H. halys* has become well established. Stink bugs were found on plants from mid-July to September, which corresponded to the presence of fruit. Both nymphal and adult stink bugs fed on the raspberry fruit. Most of the stink bugs found were adults and no egg masses were collected from raspberry plants. Thus, there is no evidence that stink bugs commonly utilize raspberry as a reproductive host.

Keywords: Stink bug, raspberry, *H. halys*, diversity indices, invasive species

Introduction

The raspberry industry has flourished in the eastern United States in the recent years, and there is a great demand for this fruit in Virginia (Stiles et al. 2009). Phytophagous stink bugs are important pests of raspberry (Kieffer et al. 1983). Stink bugs include both phytophagous and predacious species, and several of the phytophagous stink bugs are considered to be serious agricultural pests (McPherson and McPherson 2000). In the mid-Atlantic USA, including Virginia, the most common predacious stink bugs are the spined soldier bug, *Podisus maculiventris* (Say) and the twospotted stink bug, *Perillus bioculatus* (F.), which attack cutworms and other caterpillars, as well as Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Westich and Hough-Goldstein 2001).

Phytophagous stink bugs species are much more diverse and abundant than predacious species. Most species prefer to feed on seeds and immature fruits of a wide array of plants (Panizzi and Corrêa-Ferreira 1997), and also may feed on stems, petioles, foliage and flowers (McPherson et al. 1994). Hoffman (1971) reported 26 genera of stink bugs in Virginia. Historically, two important stink bug pest species in the state have been the brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Chinavia* (*Acrosternum*) *hilaris* (Say) (Hoffman 1971, Kamminga et al. 2009a, b, Koppel et al. 2009, Day et. al 2011). Both of these species are polyphagous and attack a wide range of crops, including soybean, cotton, corn, fruiting vegetables, and tree fruit (McPherson and McPherson 2000). The harlequin bug, *Murgantia histrionica* (Hahn), is also a significant pest in Virginia, but feeds predominately on brassicaceous vegetables (Wallingford et al. 2011). The species complex of the stink bugs varies greatly with respect to geographical location and cropping system (Hoffman 1971, McPherson and McPherson 2000, Kamminga et al. 2009c, Temple et al. 2013). Very little is known about the stink bug species present on or their pest significance to raspberries. The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), that was first established around Allentown, Pennsylvania in the late 1990s (Hoebeke and Carter 2003), has become established in Virginia (Day et al. 2011). However, its abundance in raspberry plantings and impact on native stink bug species has not been studied. Herein, we report the results of a multi-year survey of stink bugs on an established primocane-bearing raspberry plantings near Blacksburg, Virginia.

Materials and methods

Study site

Research was conducted in primocane- bearing raspberry plots that were planted in 2000 at the Virginia Tech Kentland Agricultural Research Farm, near Blacksburg, VA. The plots were situated on an elevated site in southwestern Virginia (37° 12.417' N, 80° 35.513' W, 616 m elev.). The raspberry plantings comprised of area 0.32 ha, and was consisted of six raised-bed rows and with a blackberry plot at one end. The raspberry cultivars included 'Anne', 'Autumn Bliss', 'Autumn Britten', 'Caroline', 'Dinkum', 'Fall Gold', 'Heritage', 'Himbo Top', 'Josephine', 'Nova' and 'Prelude' and included both early and late bearing varieties. Each cultivar plot was 6.2 m long and 1.0 m wide with 3.0 m between each row. The raspberry and blackberry plot was bordered by an apple orchard to the west, woods to the north and pasture to the east and south.

Stink bug species composition

In each year, stink bugs were sampled by gently shaking the plants over a 71 cm^2 beat sheet (Bioquip, Rancho Dominguez, CA). In 2008 and 2009, sampling was conducted in eight randomly selected 6.2 m^2 cultivar plots. In 2008, stink bugs were collected on 6, 16, 21, 31

August and 14 and 19 September. In 2009, stink bugs were collected on 7 and 21 July and 5, 10 and 17 August. In 2011, 2012 and 2013, we decreased the sampling time interval and area. Sampling was done in 1.4 m^2 sections of 24 randomly selected raspberry plots. In 2011, stink bugs were sampled on 27 and 30 July, 5, 19, 23, and 26 August, and 2, 9, 16 and 26 September. In 2012 and 2013, raspberry plants were sampled from 30 June to 14 September on a weekly basis. Since the area sampled and the total number of samples collected in each year was different, we calculated the mean abundance of stink bugs per m^2. Raspberry plantings were also surveyed for stink bug egg masses in 2011-2013. The stink bugs found on each date were collected into a vial containing alcohol and taken to the laboratory for identification using the taxonomic key in McPherson and McPherson (2000).

Statistical analysis

Student *t*-tests ($\alpha = 0.05$) were used to compare the mean number of adults and nymphs using JMP 10 (SAS Institute, Inc. Cary, NC).

Diversity index is a procedure used to measure species diversity, based on the species composition and richness in a community. Equitability measures the evenness of the species in a community. The diversity index and the equitability of stink bugs species for each year were calculated using the Shannon-Weaver diversity index and Shannon's equitability. These methods are the most commonly used techniques to measure species diversity and evenness in ecology (Spellerberg and Fedor, 2003).

Results and Discussion

In 2008, 2009, 2011-2013, a total of 16 species of stink bugs were found (Table 4.1). Only *E. servus, C. hilaris*, *Cosmopepla lintneriana* (Kirkaldy) and the dusky stink bug, *E. tristigmus*

(Say) were found in all years. *Euschistus servus* is listed as the most common stink bug in agricultural crops in Virginia (Hoffman 1971, Kamminga et al. 2009b, Koppel et al., 2009). The survey showed that *E. servus* constituted one third of the overall stink bug composition before the introduction of *H. halys*. *H. halys* was not detected in 2008 or 2009, but was found in low numbers in 2011 and 2012, and became the most abundant stink bug species in 2013. *Chinavia hilaris* is a common significant pest of fruits, vegetables and field crops in the Mid-Atlantic States, including Virginia (McPherson and McPherson 2000). Counts of *C. lintneriana* varied annually. *C. lintneriana* was described as an insect of minor impact in agriculture by Kamminga et al. (2009b) and not mentioned by McPherson and McPherson (2000). Some factors for *C. lintneriana* being considered a less economically important species of stink bug are its relatively small size, early season activity, and high abundance only in some years. Three species of stink bugs, *H. halys*, *Podisus maculiventris* (Say) (a predator), and *M. histrionica* were collected in 2011, 2012, and 2013, but not in 2008 or 2009. The harlequin bug, *M. histrionica,* is a pest of crucifers (Wallingford et al. 2011). Although it had not been reported previously in raspberry plantings, a few individuals were detected from our sampling. Cruciferous plants were grown within 1 km of the sampling location, and *M. histrionica* may have originated from those plantings.

Table 4. 1. Species composition (%) of stink bugs in raspberry plantings in southwestern Virginia in 2008, 2009, 2011-2013.

Species	Common Name	2008	2009	2011	2012	2013
Halyomorpha halys	Brown marmorated stink bug	0.0	0.0	22.3	12.8	49.3
Euschistus servus	Brown stink bug	30.8	58.3	50.0	27.5	18.3
Cosmopepla lintneriana	Twicestabbed stink bug	3.8	37.5	14.6	32.1	10.6
Euschistus tristigmus	Dusky stink bug	56.1	1.3	1.5	5.3	5.3
Chinavia hilaris	Green stink bug	2.5	0.7	3.4	18.9	9.3
Murgantia histrionica	Harlequin bug	0.0	0.0	3.4	3.4	5.3
Podisus maculiventris	Spined soldier bug	0.0	0.0	4.9	0.0	0.0
Euschistus variolaris	Onespotted stink bug	3.4	0.0	0.0	0.0	0.0
Thyanta custator accerra	Redshouldered stink bug	2.2	0.0	0.0	0.0	0.0
Banasa euchlora	Juniper stink bug	0.4	0.0	0.0	0.0	0.0
Thyanata calceata	NA	0.2	0.0	0.0	0.0	0.0
Coenus delius	NA	0.4	0.0	0.0	0.0	0.0
Basana calva	NA	0.0	0.5	0.0	0.0	0.0
Dendrocoris humeralis	NA	0.0	0.5	0.0	0.0	0.0
Brochymena quadripustulata	Rough stink bug	0.0	0.5	0.0	0.0	1.9
Hymenarcys nervosa	NA	0.0	0.5	0.0	0.0	0.0

The Shannon-Weaver diversity index of the stink bug species was higher in 2012 or 2013 than in 2008 or 2009. There was a gradual increase in Shannon's equitability through 2012 after which it dropped slightly in 2013 (Table 4.2). The species in the community were more evenly distributed as the *H. halys* population increased. The sharp decline of *E. servus* and the increase in *H. halys* lead to the increase in evenness in the population. Therefore, the invasive *H. halys* has made a prominent impact on the native stink bug community in raspberry.

The species richness of stink bugs and the predominant species varies with crops and the geographical area. Kamminga et al. (2009a) reported 11 economically important stink bug species in the upper southern and the Mid-Atlantic USA, including *C. hilaris*, *E. quadrator* (Rolston), *E. servus*, the dusky stink bug, *E. tristigmus* (Say), *H. halys*, the harlequin bug, *M. histrionica*, southern green stink bug, *N. viridula* (L.), rice stink bug, *Oebalus pugnax*, the redbanded stink bug, *Piezodorus guildinii* (Westwood), redshouldered stink bug, *Thyanta accerra* (McAttee), and *Thyanta custator* (F.). In southern Arkansas, Smith et al. (2009) reported five species of stink bugs in soybean, of which *N. viridula* was the dominant one. In Louisiana soybeans, the redbanded stink bug, *P. guildinii* has risen to a greater dominance in that cropping system since its introduction, although without data on community diversity before and after, it is difficult to determine its impact on the rest of the community. Four different species of stink bugs were found in rice fields in Florida, with *Oebalus pugnax* (F.) being the predominant species (Jones and Cherry 1986). Brennan et al. (2013) reported six species of stink bugs on blackberries in Florida and *E. quadrator* was the major one. In Brazil, at least 54 stink bug species have been recorded in soybean (Panizzi and Slansky Jr 1985), but only a few are economically important. Compared with other crops, the number of stink bug species found on raspberries in the present was relatively high. The higher species richness in the study was possibly due to multiple crops being grown on the research farm, including raspberry, apple, pear, plum and cherry that are common feeding hosts of many stink bugs. Both raspberry and raspberry are the hosts of stink bugs (Kieffer et al. 1983).

Table 4.2. Shannon-Weaver diversity index and Shannon's equitability of the stink bug species in raspberry plantings in southwestern Virginia in 2008, 2009, 2011, 2012 and 2013

Year	Shannon-Weaver diversity index (*H*)	Shannon's equitability (E_H)
2008	1.336	0.5802
2009	1.35	0.6492
2011	1.18	0.66
2012	1.522	0.8494
2013	1.503	0.772

Halyomorpha halys has become well-established in Virginia (Leskey et al. 2012b). After the severe outbreak of this pest in 2010 in Virginia, it has become the dominant stink bug species in many cropping systems with possible displacement of native stink bugs. The establishment of an invasive species is always a threat to the native ecosystem and biodiversity because there is competition for space and food. This displacement of native species can disrupt ecological communities and have an adverse impact on the ecosystem (Elton 2000, Mack et al. 2000). Invasive species often arrive without natural enemies. If an invasive species establishes in a new area, it can have adverse ecological and economic effects (Paini et al. 2008). For establishment, an invasive species should have superior competitive ability compared to the native species (Paini and Roberts 2005). Some of the factors enabling *H. halys* to become established in a new habitat are lack of natural enemies, high abundance, wide host range, climatic factors, and potential to spread quickly.

Seasonality of stink bugs in raspberry

In the early part of the cropping cycle of primocane-bearing raspberries, the Japanese beetle, *Popillia japonica* (Newman), and green June beetle, *Cotinus nitida* (L.), were observed with stink bugs feeding on the berries. Stink bugs were found throughout the sampling period. The population of both adults and nymphs peaked early in the season and decreased gradually in the latter months in 2011 and 2012, but increased in 2013 due to the higher late season activity of *H. halys* (Fig. 4.1). Adults were significantly more abundant than nymphs (t = -6.07; df = 12.1; P = 0.0001) (Fig. 4. 2).

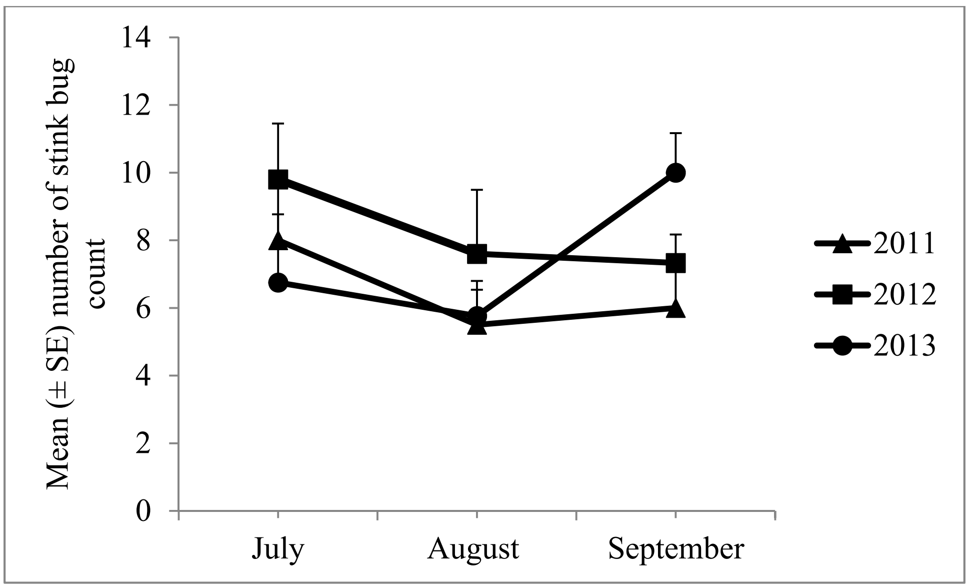

Fig. 4.1. Seasonal occurrence of stink bugs in southwestern Virginia collected in raspberry plantings in 2011, 2012 and 2013

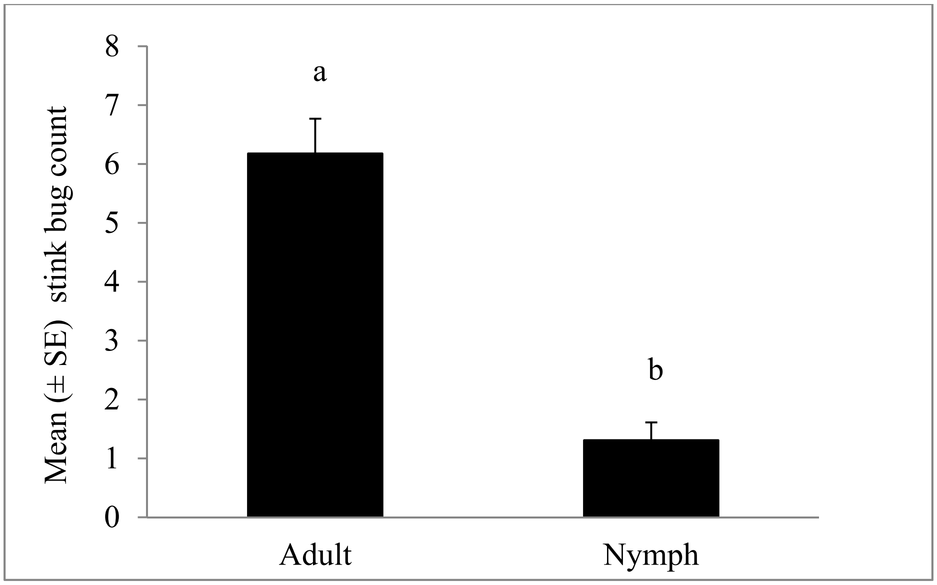

Fig. 4. 2. Adult and nymphal count (mean ± SE) of stink bugs collected in raspberry plantings at Kentland Farm (Montgomery, VA). Bars with different letters were significantly different (Student's, $P \leq 0.05$)

Use of raspberry as a host by stink bugs

Both adult and nymphal stink bugs were observed feeding on ripening and ripe berries. Maxey (2011) reported that stink bugs fed on the berries by inserting their stylets between the drupelets. Coombs (2000) reported that the green stink bug from Australia, *Plautia affinis* (Dallas), feeds on green, ripening and ripe raspberry. Information on raspberry plantings as a reproductive host is limited. Roy et al. (1999) pointed out that the presence of trichomes on the stem, leaf and petiole of raspberry may hinder oviposition. Presence of a significantly higher population of adults than nymphs and the absence of egg masses on raspberry leaves strongly suggested that raspberry is not a common reproductive host. Other plants that grow within the raspberry plots or surrounding vegetation may be overwintering sites, and adults and nymphs may move to, and develop on raspberry fruits. Stink bug nymphs are highly mobile and can move to preferred host plants (Nielsen and Hamilton 2009). The raspberry plot sampled was bordered by apple and peach orchards and the movement of stink bug across these crops was possible. Pome fruits provide nutrients for reproductive development in early season and fat to be stored for diapause in the late season. However, Funayama (2004) found that *H. halys* that fed only on apples produce fewer eggs.

In the area of introduction (Pennsylvania and New Jersey), *H. halys* has become the predominant species of stink bug and its phenology closely follows soybean development (Nielsen et al. 2008). Laboratory studies have shown that *H. halys* requires 538 DD (50 °F base temperature) to complete development from egg to adult (Nielsen et al. 2008) and climate data suggest that *H. halys* can have multiple generations per year in the warmer parts of the United States (Nielsen et al. 2008). As *H. halys* expands its range and becomes established in the other regions of the United States, it will likely become a significant pest of soybean and other crops. These results could easily be applied to other field crops, such as cotton, corn and peanuts. The results suggested that in a 3-years period (2011- 2013), the invasion of *H. halys* into a habitat substantially changed pentatomid species dominance in raspberries. The total impact of *H. halys* to commercial raspberries still needs to be determined by assessing damage and developing economic and treatment thresholds. This study on stink bug species composition in raspberry plantings helps to identify the stink bugs of economic importance and guide future management strategies for stink bugs.

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Chapter 5. Conclusions

The studies presented here showed that *H. halys* has the potential to cause economic impacts in vineyards. Grape served as a reproductive host for *H. halys* in the early season and was a suitable feeding host in the late season. The seasonal occurrence of *H. halys* in vineyards varied greatly among vineyards in Virginia, which may have been due to the availability of other host plants or overwintering sites nearby. Based on our sampling in four commercial vineyards, *H. halys* moved into vineyards as early as May and egg masses were found mainly in June. The study was conducted in commercial vineyards in which routine insect management practices as well as the pesticides targeting *H. halys* were performed. Populations of *H. halys* recovered multiple times in vineyards even after pesticide applications. This appeared to depend largely on the abundance of *H. halys* in nearby crops and the proximity of the vineyards to woods. In a vineyard adjacent to a soybean field, *H. halys* were observed along the border rows in soybean, but not in the adjacent vineyard blocks, suggesting that soybean may be a more suitable host than grape.

Halyomorpha halys fed on grape berries when the berries started to accumulate sugar at the early veraison stage. The injury was more conspicuous at the pre-harvest and veraison stages than at the pea-sized stage. If the berry was fed upon at the pea-sized stage, the feeding site gradually became sunken, and visible brownish or black marks, which could be described as catfacing injury in grape. However, if the berries were in the veraison or harvest stages, necrosis around the feeding site that slowly increased in size (due to the possible microbial infection) was observed.

A multi-year sampling program for stink bugs in raspberry plantings in southwestern Virginia revealed that the *H. halys* has become the most abundant stink bug species in raspberry. Prior to the establishment of *H. halys*, the native brown stink bug, *E. servus* constituted about 30-40% of the overall stink bug species composition. As it becomes broadly established in the southern USA, the number of annual generations of *H. halys* may prove to increase relative to the Mid-Atlantic regions, where one or two generations are reported (Nielsen et al. 2013). Consequently, *H. halys* has the potential to become the dominating stink bug in cotton and other crops. Based on our observational studies, both adult and nymphal *H. halys* fed upon and caused injury to ripening and ripe raspberries.

This study showed that *H. halys* abundance inside vineyard is greatly affected by the surroundings. Farm landscape adjacent to a vineyard, and the distance from woods and urban settings can affect *H. halys* abundance inside vineyards. Understanding the effect of adjacent habitat on the abundance of *H. halys* can be important in tracking the movement of *H. halys* into vineyard and possible control strategies. Stink bugs have been shown to transmit certain plant pathogens and fruit rotting micro-organisms (Hoebeke and Carter 2003, Medrano et al. 2009, Brust and Rane 2011), and more research is needed on *H. halys* as a possible vector of grape diseases in vineyards, and the susceptibility of the grape berries to pathogens after being punctured by *H. halys*. In addition, the varietal preference test of grape berry can provide useful information. In a soybean field that was adjacent to vineyard (Fig. 5.1), a higher population of *H. halys* was observed in border than interior rows. However, *H. halys* were not found in the adjacent vineyard. Studies on the preference of *H. halys* to these crops could determine the potential of soybean as a trap crop.

Fig. 5.1. A vineyard adjacent to soybean field

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