ABSTRACT

BAKKEN, AMANDA JO. The Biology and Ecology of Stink Bugs and Plum Curculio *Conotrachelus nenuphar* in North Carolina Tree Fruit Orchards. (Under the direction of James F. Walgenbach, Mark R. Abney, Clyde E. Sorenson, and Tracy C. Leskey.)

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is a highly polyphagous, invasive pest native to Asia that was first detected in the US in Allentown, PA, in 2001. It was first detected in NC in 2009, and has spread throughout the piedmont and mountain regions. Apples and peaches are two important agricultural crops in western NC that have experienced damage by BMSB in both its native Asia and in the mid-Atlantic region of the US. The objectives of this thesis as they pertain to BMSB were to 1) determine the range of host plants used by BMSB in non-managed habitats of NC and VA and 2) to assess the potential for damage by BMSB to apple at different phenological growth stages.

The first objective was investigated by conducting weekly visual sampling of stink bugs from 2011 to 2013 on different host plants in non-managed, wooded areas of VA and NC. Sampling was conducted in western areas of both states, as well as central and eastern areas of each state. Host plants on which BMSB were detected differed depending on the month sampled, but BMSB was found consistently on several plants, including *Paulownia sp*., *Ailanthus altissima*, and *Catalpa sp.* In all western locations and years, BMSB was found in greater numbers than any other species of stink bug, and data reflects that this insect has a possible two generations per year. Few BMSB were detected in eastern parts of VA and NC.

The second objective (BMSB to apple) was investigated by caging BMSB on seven different apple varieties for 48-hour feeding periods at three-week intervals throughout the growing season. The width and depth of punctures caused by BMSB feeding early in the season were higher than those inflicted later in the season. Apples appear to be most attractive, and sustained the greatest amount of damage, when presented as a mid season host. Damage to the middle of the apple was significantly greater in width and depth than width and depth of damage found on the shoulder and ventral portions of the fruit. Significantly more feeding damage was found on the middle portion of the fruit in comparison to shoulder and ventral portions.

A third objective was related to the biology of the plum curculio, *Conotrachelus nenuphar*, in North Carolina. While the plum curculio has been a documented pest of pome and stone fruit for over a hundred years, recently it has re-emerged as a serious pest in NC, largely as a result of decreased reliance of in broad-spectrum insecticides that normally controlled it after bloom. The objective of this study was to assess its phenology in western NC. Several different orchards at varying elevations were sampled in 2012 and 2013 in the southern Appalachians, using beat sampling and baited pyramid and circle traps. All female plum curculios caught were dissected and examined for ovarian development, and degree-day accumulations were used to plot curculio adult populations against temperature and time. Based on female dissections, reproductively mature females were detected during the expected emergence period of first generation adults at all locations, as well as later in the season when not expected, suggesting that the region contains both univoltine and multivoltine strains of plum curculio. The highest elevation sampled in North Carolina appeared to have fewer reproductively mature females compared with lower elevations per year. However, there was no evidence of a full second generation in any location.

The Biology and Ecology of Stink Bugs and Plum Curculio *Conotrachelus nenuphar* in North Carolina Tree Fruit Orchards

by
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BIOGRAPHY

Amanda Jo Bakken was born on June 22, 1988 and is the daughter of Keith and Gladys Bakken. She grew up outside of Crookston, Minnesota along with one sister, Katie (Bakken) Norby. Living in the country meant participating in many outdoor activities, and Amanda grew up spending copious amounts of time gardening, hiking, biking and running on the gravel roads and wooded paths around her home.

Amanda's love for nature was cultivated in the summers working for her parents taking care of the family's land, nurtured by science classes in school, and cemented by working for two summers in high school for The University of Minnesota Crookston as a field worker in the sugar beet and small grain test plots. After graduating from Crookston High School in 2006, Amanda continued her studies in science at The Concordia College in Moorhead Minnesota. She received her Bachelor of Arts degree in Biology, Chemistry and Psychology in 2010.

After taking a year off to travel, run, and work, Amanda applied to and was accepted into the Department of Entomology at North Carolina State University in 2011. Under the direction of Dr. James Walgenbach and Dr. Mark Abney she began work towards a Master of Science degree. Her research revolved around the biology and ecology of stink bugs and plum curculio in North Carolina tree fruit orchards.

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THE BIOLOGY AND ECOLOGY OF STINK BUGS AND PLUM CURCULIO (CONOTRACHELUS NENUPHAR: HERBST) IN NORTH CAROLINA TREE FRUIT ORCHARDS: AN INTRODUCTION

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive pest that was first detected in the US in Allentown, Pennsylvania in 2001 (Hoebeke and Carter, 2003). This insect is native to Japan, Korea, China, and Taiwan (Hoebeke and Carter, 2003), but recent reports have placed possible origins for the United States populations near Beijing, China (Xu et. al, 2014). It has since spread and has been detected in 41 states, although the mid-Atlantic states remain the region where it poses the greatest threat to agriculture (www.stopbmsb.org).

BMSB is a highly polyphagous pest with many reported host plants, including agricultural crops, fruits, and vegetables (Hoebeck and Carter 2003). It is known to overwinter in reproductive diapause in human structures such as houses and sheds, and non-human structures such as dead, standing trees (Lee et al, 2014), before exiting hibernation locations in the spring when it seeks suitable hosts for feeding and reproduction. BMSB is a known pest of tree fruits in Japan and South Korea, where apples can serve as an early-season host and suffer damage to fruit (Funayama 2002a, 2004). Economic losses from BMSB feeding was observed on apples, peaches, and pears in New Jersey and Pennsylvania in 2006 (Nielsen et al, 2008). By 2010 populations had increased dramatically, resulting in injury to many crops throughout the mid-Atlantic region. It is likely that as this pest continues to spread and establish in new regions of the United States, damage on these and other crops will increase.

Despite the polyphagous nature of BMSB, it is not known what plants this insect will gravitate towards in its new environment. In its native habitat, BMSB is found on a myriad of agricultural crops, including fruit trees, in particular apples and pears (Fujiie 1984, Funayama 2002a), while cedar and cypress are known to be main host plants in Japan (Oda et al, 1981). There is a paucity of information on host plants utilized in non-managed habitats in the United States, and there is a need to better understand its host range beyond crops of agricultural importance.

Within the mid-Atlantic region, individuals emerge from hibernation sites in April and May, well before most agricultural crops are available as a host source. Because this insect seeks hibernation sites away from agricultural crops, it is important to understand what native, or non-native wild host plants can serve as food sources. Wild host plants in non-managed habitats (i.e., wooded areas) are important hosts that the first generation likely relies on for transitional feeding between emerging from hibernation and moving into agricultural hosts. Wild host plants also likely serve as a source for infestation of crops throughout the season.

The first focus of this study was to identify which native plants BMSB could use as transitional hosts for reproduction and development. From 2011 to 2013, BMSB populations in non-managed wooded habitats were surveyed in different regions of North Carolina and Virginia to identify seasonal population dynamics of BMSB on potential host plants.

BMSB is potentially a full season pest of apple, with populations in Japan observed to fluctuate greatly during different stages of apple growth (Funayama 1996, 2002a, 2002b, 2004, 2008). It is likely that populations of brown marmorated stink bugs will also fluctuate greatly within mid-Atlantic apple orchards, depending on voltinism, host availability, overwintering sites, and proximity to alternative hosts.   Currently BMSB is considered an agricultural and nuisance problem in North Carolina, but populations are increasing and spreading rapidly throughout the main apple production regions in the mountains and Piedmont of North Carolina. Thus, the second purpose of this study was to assess the potential for BMSB to cause damage to apples at different phenological stages of apple growth.

The third focus of this research was on a different, but also increasingly important insect pest of North Carolina tree fruits, the plum curculio, *Conotrachelus nenuphar* (Herbst). The plum curculio, is a key pest of pome and stone fruit in eastern and central North America (Chapman, 1938; Racette et al. 1992; Hogmire, 1995; Vincent et. al 1999; Leskey 2008). Adult weevils feed on and oviposit into fruit, causing cat-facing damage, surface scarring, premature fruit drop, internal larval infestations, as well as potentially providing introduction sites for bacteria (Snapp 1930, Horton and Ellis 1989, Lan et al 2004). In the southeastern United States, where it is the most important fruit insect pest affecting peach and other stone fruits (Lan et al, 2004), plum curculio has traditionally been managed by broad spectrum insecticides applied shortly after petal fall (Chapman 1938, Reissig et al. 1998).

Plum curculio adults overwinter in ground litter and the top few centimeters of the soil on the edges of orchards and in hedgerows or woodlots adjoining orchards (Quaintance and Jenne 1912, Snapp 1930, Chapman 1938, Bobb 1949, Wylie 1954). Between bloom and petal fall, adults become active and move into orchards where they feed on petals and eventually, young fruit (Smith and Flessel 1968). After approximately two weeks of feeding and mating, females oviposit in fruit (Horton and Ellis 1989, Yonce et al. 1995). After hatching, larvae tunnel towards the center of the fruit while developing through four stadia. The larvae then exit the fruit, drop to the ground and pupate below the soil surface (Horton and Ellis 1989). After pupation, adults of the summer generation emerge, and migrate to overwintering sites to enter diapause if univoltine, or if multivoltine they feed and mate, with the females laying eggs in maturing fruit.

Plum curculio voltinism was first described by Quaintance and Jenne (1912) and later revised by Chapman (1938). The plum curculio occurs as one of two reproductively incompatible univoltine or multivoltine strains (Padula and Smith 1971). Every generation of the univoltine strain undergoes an obligatory diapause. The multivoltine strain undergoes facultative diapause that is controlled primarily by environmental conditions. These two strains are commonly referred to as the northern (univoltine) and southern (multivoltine) strain, because of their geographic distribution in North America. The line separating the two strains as reported by Chapman (1938) was in the mid-Atlantic region and ran along the Southern Appalachian Mountains. However, the discovery of some individuals in West Virginia with two periods of active oogenesis (Leskey 2008) indicates that a transition zone between northern and southern strains likely contains both strains.

The importance of the plum curculio as a pest of apple has increased in recent years due to the replacement of broad spectrum insecticides with reduced-risk products that have a more narrow range of pest activity (Agnello et al. 2009). Apple production in North Carolina is concentrated in the southern Appalachian Mountain region of western NC in a likely transitional zone between the northern and southern strains of plum curculio. There is a lack of knowledge about the biology and phenology of plum curculio in this region, which hampers the development of management programs for the pest. It is likely that in higher elevations of the Appalachian Mountains, univoltine plum curculios exist, whereas in lower elevations, multivoltine plum curculios exist. The third study described herein was conducted to improve our understanding of voltinism and phenology of plum curculio in the southern Appalachian Mountains.

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Chapter 1: Occurrence of brown marmorated stink bug *Halyomorpha halys* (Stål) on wild hosts in North Carolina and Virginia non-managed woodlands

Introduction

The brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål) is an invasive pest that was first detected in the United States in Allentown, Pennsylvania, in 2001 (Hoebeke and Carter 2003). This insect is native to Japan, Korea, China, and Taiwan (Hoebeke and Carter 2003), but recent reports have placed possible origins for United States populations near Beijing, China (Xu et al. 2014). It is now well established in the mid-Atlantic region of the United States, and has been reported throughout the US (www.stopbmsb.org), as well as Switzerland (Wermelinger et al. 2008), and Canada (Fogain and Graff. 2011). BMSB is a highly polyphagous pest, and is known to feed on many agricultural, horticultural, and ornamental plants, including tree fruit, vegetables, and soybeans, with much research focusing on apple, pear and peach (Fujiie 1985, Nielsen and Hamilton 2009a, Nielsen and Hamilton 2009b, Leskey et al. 2012). In its native habitat, BMSB is found on a myriad of agricultural crops, including fruit trees, with particular mention of apples, and pears (Fujiie 1984, Funayama 2002), while cedar and cypress are known to be main host plants in Japan (Oda et al. 1981).

The BMSB overwinters in the adult stage in aggregations in human structures, such as homes, barns, sheds, and RV's, as well as in forested areas, particularly standing dead trees (Lee et al. 2014). Within the mid-Atlantic region, individuals emerge from hibernation sites in April and May, well before most agricultural crops are suitable hosts. Non-managed forested areas are thought to be an important habitat for buildup of early season populations, as well as season-long reservoirs for dispersal of individuals to agricultural crops. Reported here are results of a three-year survey of BMSB occurrence on plants in non-managed habitats in North Carolina and Virginia. Currently, BMSB phenology in NC and VA is unknown. Reports from PA indicated that one generation per year exists in northern regions of the US (Nielsen and Hamilton 2009a), however, additional generations are expected in southern regions. BMSB phenology was depicted for each region.

Materials and Methods

Host Plant Survey: Trees and herbaceous shrubs of wooded habitats were sampled from 2011-2013 in western and eastern VA, and western and east-central NC. Each region contained of multiple sample sites, but data were pooled from sites within regions to better understand geographical trends. Sites in western Virginia (ridge and valley region) included locations in Montgomery and Roanoke Counties, and in eastern Virginia (coastal plain region) sampling was conducted in Accomack and Northampton counties. Two sites in western North Carolina (Blue Ridge Mountains) in Buncombe County were sampled each year from 2011-2013. In 2011, sampling was conducted in east-central NC at six sites in Edgecombe, Johnson, Lenoir, Orange, Wayne, and Wilson counties. In 2012, the east-central NC regions sampled included Chatham, Edgecombe, Johnson, Lenoir, Orange, and Wake counties. All locations contained both native and exotic trees and shrubs located in urban and rural areas. Plants chosen for sampling were based, in part, on published literature of BMSB preference (Hoebeke and Carter 2003, Bernon 2004, Nielsen and Hamilton 2009a), previous observations of BMSB presence in these areas, and plants commonly encountered in forested areas of NC and VA. In total, 45 different families of plants were sampled from 2011-2013. Predominant species sampled included *Paulownia tomentosa*, (Princess Tree/paulownia), *Prunus spp*. (cherry), *Morus rubra* (mulberry), *Mimosa spp*. (sensitive plant), *Albizia julibrissin* (mimosa) *Tilia Americana*, (American basswood), *Catalpa sp*. (catalpa), *Cladrastis kentukea*, (yellowwood), *Rubus sp*. (wild blackberry), and *Ailanthus altissima* (tree of heaven). In NC and VA, samples consisted of a two and three-minute search, respectively, of each host plant. Each tree or shrub was sampled by observing foliage and fruiting structures from the ground, paying special attention to the undersides of leaves where the brown marmorated stink bug is known to lay eggs (Nielsen and Hamilton 2009a). Sampling was conducted at one to two week intervals, and on each date multiple plants of the same species were sampled.

All pentatomids that were observed during visual searches were recorded in order to provide species complex information. If an egg mass or unknown stink bug specimen was found, it was transported to the laboratory and reared for identification. BMSB were recorded as adults, nymphs, or egg masses. Newly hatched egg masses in which individuals had not yet dispersed from eggs were recorded as egg masses.

Data Analysis: Stink bug data were expressed as number of egg masses, nymphs, and adults per two (NC) or three (VA) minute search on each host plant. Hence, even though some plant species were sampled more frequently than others, stink bug densities were always expressed as the mean number per two- or three- minute search per host. Data are presented by month, such that different sample dates in the same month were combined, and numbers were expressed as the mean number of stink bugs per host per two- or three-minute search. Data were also used to depict BMSB phenology in NC and VA. For this, the mean number of BMSB per sample across all plant species was calculated for each sample date and expressed as mean per date.

Results

Stink Bug Species Complex: The relative abundance of BMSB varied among sampling regions, with BMSB being the dominant species in the western regions of both states. BMSB accounted for 77%, 96%, and 87% of all stink bugs sampled in 2011, 2012, and 2013, respectively in western VA (Table 1.1). In western North Carolina, BMSB accounted for 51%, 82%, and 93% of all stink bugs in 2011, 2012, and 2013, respectively (Table 1.2). BMSB was observed less frequently in eastern sampling sites, accounting for 0.3% of all individuals in eastern VA in 2012 and as high as 47% in east-central NC area in 2012. In eastern VA and east-central NC, *Chinavia hilare* and *Euschistus servus* were commonly encountered in all years. These species were also present in western VA and western NC, but accounted for a lower proportion of all species due to the high BMSB densities. One other species regularity observed was the predatory rough stink bug, *Brochymena sp*. A total of 4,991 stink bugs were found across all years.

Western Virginia: BMSB were observed on 58 species of plants in western VA from 2011-2013. Species on which combined life stages of BMSB were commonly observed across all years include *Catalpa sp.* (catalpa), *Vitis sp.* (grape), *Paulownia sp.* (paulownia), *Ailanthus altissima* (tree of heaven), *Morus sp.* (mulberry) *Prunus virginiana*, (chokecherry), and *Celtis sp.* (hackberry). However, in individual years, other plants such as *Magnolia* *grandiflora* (southern magnolia), *Secale cereal* (rye), and *Armoracia rusticana* (horseradish) were observed to have large numbers on them in specific months (Tables 1.3, 1.3, and 1.4 respectively).

Very few egg masses were detected, but those that were detected were most commonly found in June, July, and August with no apparent host preference observed for oviposition. Egg masses were observed on 24 different species of plants during these months across all years combined. Nymphs were most often found in July and August. In July 2011, plants with the largest amount of nymphs were *Chenopodium album* (lamb's quarter), rye, and grape. Lamb's quarter and rye, however, were single occurrences and not a common host for this month. Paulownia and tree of heaven also had higher numbers of nymphs recorded, and likely better represent nymphal activity for this month (Table 1.3). In August, nymphs were detected on *Albizia julibrissin* (mimosa) and paulownia. In 2012, nymphs were most abundant on catalpa and *Tilia sp.* (basswood) in July, and on paulownia, redbud, mulberry and mimosa in August (Table 1.4). In 2013, large numbers of nymphs were found on paulownia, catalpa and tree of heaven in July, and hackberry, *Acer rubrum* (red maple), catalpa, and tree of heaven in August (Table 1.5).

The number of adults was largest in August and September. Adults were found on a diversity of plants, but those with the greatest frequency were catalpa, tree of heaven, and paulownia. In individual years, large numbers of adults were detected on *Lagerstroemia sp.* (crepe myrtle) in August 2011 (Table 1.3), and on *Datura stramonium* (jimsonweed) in September 2012 (Table 1.4). In 2013, adults were not frequently found in September with occurrences on only five species of plants.

Western North Carolina: BMSB was found on 38 species of plants in western NC from 2011-2013. Species on which combined life stages of BMSB were most commonly observed across all three years included include tree of heaven, paulownia, *Solidago sp.* (goldenrod), *Juglans nigra* (walnut), catalpa, *Cladrastis kentukea* (yellowwood), *Prunus sp*. (cherry), and grape (Tables 1.6, 1.7 and 1.8). A large number of nymphs were observed on *Celastrus orbiculatus* (oriental bittersweet), in July 2013, but this was the only instance of this occurrence (Table 1.8).

Very few egg masses were detected, but those that were detected were most often found on paulownia and tree of heaven. Nymphs were most often found in July and August on catalpa, grape, yellowwood, paulownia, and cherry (Tables 1.6-1.8). Adults were most commonly found in August and September in all three years on catalpa, yellowwood, paulownia, *Platanus occidentalis* (sycamore), and redbud (Tables 1.6-1.8). In September 2012, adults did not appear to have a specific host preference (Table 1.7), but were fairly evenly dispersed among 18 different plant species.

East-Central North Carolina and eastern Virginia: BMSB were found on four different species of plants in east-central NC in 2011, and on 18 different species of plants in central NC in 2012. In 2012, combined life stages of BMSB were most commonly found on catalpa, yellowwood, *Sassafras sp.* (sassafras), *Ligustrum sp*. (privet), and wild grape. It is noteworthy that although BMSB occurred at much lower densities in eastern vs. western NC, they were detected on the same plant species in both regions. In eastern VA (Table 1.11)

BMSB were found on just three species of plants.

Phenology: Overwintering BMSB adults are known to emerge in southwestern VA and western NC in April and May, and first egg masses were detected in late May to early June. In western VA, peaks of nymphs in July were likely the first generation, and the peak in mid August (followed by an increase in adult numbers in late August and early September) was likely a second generation (Fig. 1.1). In all years, overwintering adult populations were observed in low densities in May (2012, 2013) and June (2011), followed by low activity until mid-July (2013) or mid-August (all years) through early September. Periods of adult activity in July likely represent the first generation, and peaks in August through early September likely represent the second generation. Peaks in nymphal activity precede the second generation of adults. The decline in adult activity in early September is representative of adults dispersing to overwintering sites.

Due to low populations, there were no discernable phenological trends observed in western NC in 2011 (Fig. 1.2). Peaks of nymphs in July and early August were likely the first generation, and the peak in late August to early September (followed by an increase in adult numbers) was likely a second generation. In 2012, overwintering adult populations were observed at low densities in May and June, which was followed by two distinct peaks of adult activity in July and from mid-August through September, likely representing first and second generations. These two peaks were preceded by a relatively small peak of nymphs in mid June, and large numbers of nymphs in July. Weather conditions in 2013 were unusually wet and cool, and phenological trends suggested only a single generation.

Following the typically low numbers of overwintered adults observed in May and June, there was one extended period of adult activity from mid-July through mid-September. This adult peak was preceded by a single nymphal peak in mid July. The relatively rapid decline of adult numbers in mid to late September in all years was representative of adults dispersing to overwintering sites.

Discussion

Non-managed woodlands are an integral part of the population dynamics of BMSB in that they serve as an important early-season habitat for population build-up and movement into agricultural crops, as well as serving as a reservoir for dispersal into crops during the growing season. This study is the first to report BMSB hosts in non-managed habitats in the mid-Atlantic region, specifically in NC and VA.

BMSB populations were much higher in western NC and western VA compared to eastern regions of either state. Relative to western NC and VA, very few BMSB were found in eastern and central NC and eastern VA. Brown marmorated stink bug has occurred in VA for over a decade and was first detected near Roanoke in 2004 (T. Kuhar, personal communication) while BMSB was first detected in NC in Winston-Salem in 2009 (M. Bertone, NCSU). It is likely that BMSB has been transported multiple times throughout eastern Virginia and North Carolina in vehicles, shipping containers, etc. Possible reasons that populations are lower in eastern locations include, absence of certain hosts, asynchrony between host and BMSB phenology, or potentially unfavorable climatic conditions. Catalpa, redbud, and yellowwood, plants on which BMSB were found in eastern and central NC sites, overlapped with their western counterparts. BMSB populations in eastern VA were very low relative to western VA; however, BMSB was found on mimosa and wild grape/grape in both eastern and western locations. It is possible additional plants are needed for BMSB development, and these additional plants do not exist in eastern and central locations, or exist in such low numbers that BMSB does not have sufficient access to them for feeding and development.

In western NC, BMSB was most commonly found on tree of heaven, catalpa, yellowwood, paulownia, cherry, walnut, redbud, and grape. Several of these species, such as tree of heaven, catalpa, and paulownia have been reported in previous studies (Hoebeke and Carter 2003, Bernon 2004, Nielsen and Hamilton 2009a), as have plants in the genera *Prunus* and *Rubus* (cherry, raspberry) (Bernon 2004). BMSB was found consistently on three plant species, catalpa, tree of heaven, and paulownia. Tree of heaven and paulownia are aggressive invasive species of Asian origin (paulownia to China-Hoebeke and Carter 2003, tree of heaven to China-Miller 2003). Both species were observed with one or more BMSB life stages throughout the season. This study is the first to report BMSB on yellowwood, on which BMSB of all life stages were observed in both 2012 and 2013. Yellowwood is a rare tree that is native to the SE U.S. and is found principally on the limestone cliffs of Kentucky, Tennessee and North Carolina (Keeler 1900).

The relationship between BMSB and paulownia has been noted in the literature, because BMSB is known to transmit Witches' Broom Phytoplasma to paulownia trees in China (Jin et al. 1981 as reported in Bernon 2004). BMSB have tested negative for this phytoplasma in Pennsylvania (Bernon 2004), however, this close relationship may be a contributing factor to increases in BMSB in the mid-Atlantic region.

The visual sample data reported here is difficult to interpret, in regards to how BMSB was utilizing plants, because this insect is highly mobile in both nymphal and adult life stages and capable of moving among hosts. Additionally, our sampling methods did not note if BMSB were feeding, resting, moving, etc. It is possible that a bug was not utilizing the plant on which it was detected. In fact, multiple hosts are thought to be required for BMSB development. Funayama (2004) measured BMSB nutritional levels and found that although BMSB immigrate to orchards to feed on fruits, the nutritional level of, and number of eggs deposited by, adults fed apple were reduced compared to those fed peanuts and soybeans. This suggests that multiple hosts are needed, and seasonal fluctuations in BMSB populations within a host could be attributed to BMSB seeking additional hosts. The consistency with which BMSB was found on certain hosts, such as tree of heaven, catalpa, yellowwood, paulownia, cherry, walnut, and grape, suggests that these plants are important hosts of BMSB in NC and VA, with BMSB also occurring consistently on southern magnolia, mulberry and hackberry in VA.

With the exception of 2013 in western NC, which was characterized by an unusually cool and wet season, BMSB appeared to complete two generations per season in southwestern VA and western NC; only one was observed in western NC in 2013. This is in contrast to populations in PA, where they were reported to be univoltine (Nielsen and Hamilton 2009a). Peak abundance occurred from late June through early September, with all life stages present during this time. Both temperature and day length are known to affect voltinism. In Japan, the critical photoperiod that stimulated reproductive diapause in BMSB was approximately 14 hours (Fujiie 1985). Although the latitudes between Japan (where the study was conducted), Beijing, China (where the American population is speculated to have originated), and Asheville, NC are all different (140.1°, 116.38°, and 82.56° respectively), by late July, day length in Asheville, NC was less than 14 hours, and few egg masses were found in August. It is likely that temperature and perhaps other unknown factors contribute to adults seeking overwintering sites because adult activity was still recorded after day length dropped below 14 hours.

The data presented here demonstrates that a variety of host plants in wooded habitats serve as hosts of BMSB throughout the year. This information will be useful in helping to detect BMSB in new areas by focusing searches for individuals on certain plants, particularly catalpa, cherry, grape, paulownia, tree of heaven, and yellowwood. Understanding the relative importance of these different plants in the population dynamics of BMSB may also aid in developing strategies for managing this pest before it moves to agricultural crops, and helps to explain the distribution of BMSB in new areas.

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Table 1.1. Total number of adult stink bugs collected from all host plants in Virginia.

Stink Bug Species	Western 2011	Western 2012	Eastern 2012	Western 2013	Eastern 2013
Halyomorpha halys	96	583	3	311	27
Chinavia hilare	0	6	596	13	128
Euschistus servus	6	3	463	14	176
Brochymena sp.	10	10	1	13	0
Oebalus pugnax	0	0	6	5	1
Thyanta spp.	0	0	11	0	12
Euschistus tristigmus	12	4	0	2	0
Other	1	0	0	1	0
Total	125	606	1080	359	344

Table 1.2. Total number of adult stink bugs collected from all host plants in North Carolina.

Stink Bug Species	Western 2011	East-Central 2011	Western 2012	East-Central 2012	Western 2013
Halyomorpha halys	280	15	475	112	943
Chinavia hilare	99	29	38	33	25
Euschistus servus	123	21	12	20	15
Brochymena sp.	9	17	45	24	11
Thyanta accera	0	8	0	0	0
Euschistus tristigmus	0	11	0	45	0
Other	37	6	6	2	16
Total	548	107	576	236	1010

Table 1.3. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 3-minute sample on various woodland plant species. Western, VA. 2011

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer rubrum* (red maple)	0.1	0.1	0	0	0	2	0	0	1	-	-	-	0.1	0.1	3
Altingiaceae, *Liquidambar stryraciflua* (sweetgum)	-	-	-	-	-	-	0	1	0	-	-	-	0	1	0
Annonaceae, *Asimina sp*. (paw paw)	0	0.7	0	0.1	0.7	0.2	0	0.7	0.1	0	0	0.3	0.1	2.1	0.6
Apiaceae, *Anthriscus sp*. (wild parsley)	0	0.5	3.5	-	-	-	-	-	-	-	-	-	0	0.5	3.5
Asteraceae, *Arctium sp*. (burdock)	-	-	-	0	3	1	-	-	-	-	-	-	0	3	1
Bignoniaceae, *Catalpa sp*. (catalpa)	0.4	0.5	2	0.1	2.9	1.2	0	3.4	9.4	0.3	0	38.5	0.8	6.8	51.1
Campsis sp. (trumpet vine)	-	-	-	-	-	-	0	2	1	-	-	-	0	2	1
Cannabaceae, *Celtis sp*. (hackberry)	0	0.1	0.1	0.1	0.4	0.1	0.1	0.1	0	0	0.3	0.8	0.2	0.9	1
Caprifoliaceae, *Diervilla sp.* (honeysuckle)	0	1	1	0	0	0	-	-	-	-	-	-	0	1	1
Chenopodiaceae, *Chenopodium album* (lamb's quarter)	-	-	-	0	25	0	-	-	-	-	-	-	0	25	0
Cornaceae, *Cornus sp*. (dogwood)	0.3	0	0	0	4	0	0	0.5	0.3	0	0	1	0.3	4.5	1.3

Table 1.3. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Elaeagnaceae, *Elaeagnus umbellate* (autumn olive)	-	-	-	-	-	-	0	5	1	-	-	-	0	5	1
Ericaceae, *Vaccinium sp.* (blueberry)	0	0	1.6	0	0.8	0	-	-	-	-	-	-	0	0.8	1.6
Fabaceae, *Albizia julibrissin* (mimosa)	0	1.6	1.3	0.4	3.3	2	0.1	9.8	1.3	0	0.4	2.1	0.5	15.1	6.7
Cercis canadensis (redbud)	0.5	0	1.5	0.1	1.9	0.1	0	0.3	0.5	0	0	4.7	0.6	2.2	6.8
Pueraria sp. (kudzu)	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0
Robinia pseudoacacia (black locust)	0.2	0	0.1	0	0	0.3	0	0.2	0	0	0	1	0.2	0.2	1.4
Fagaceae, *Quercus alba* (white oak)	0.3	0	0	-	-	-	0	0.7	0.3	-	-	-	0.3	0.7	0.3
Quercus prinus (chestnut oak)	0	0	0.3				0.3	0	0				0.3	0	0.3
Hamamelidaceae, *Hamamelis sp.* (witch hazel)	-	-	-	0	1	0.3	0	0	0	-	-	-	0	1	0.3
Juglandaceae, *Carya sp*. (hickory)	0	0.1	0.5	-	-	-	0	0	0	-	-	-	0	0.1	0.5
Juglans sp. (walnut)	0.3	0.9	0.9	0.3	3.3	0.3	0.3	2.3	0.3	0	0	1.5	0.9	6.5	3
Lythraceae, *Lagerstroemia sp.*(crepe myrtle)	-	-	-	-	-	-	0	8	7.2	1.5	1.5	8	1.5	9.5	15.2

Table 1.3. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Magnoliaceae, *Liriodendron tulipifera* (tulip poplar)	0	0	0	0.3	0	0	0	0	0	0	0	3	0.3	0	3
Magnolia grandiflora (southern magnolia)	4	38	6	0	12	0	0	9	7	-	-	-	4	59	13
Moraceae, *Morus sp.* (mulberry)	0.4	1.6	1.3	0.8	0.9	0.1	0.4	2.4	1.2	0	0.3	1.3	1.6	5.2	3.9
Oleaceae, *Fraxinus sp*. (ash)				0	0	2	-	-	-	-	-	-	0	0	2
Forsythia sp. (forsythia)	-	-	-	0	7.3	0.3	0	0.8	0.5	-	-	-	0	8.1	0.8
Paulowniaceae, *Paulownia sp*. (paulownia)	1.2	8.5	0.4	0.1	10.8	0	0.2	15.3	4.3	0	0	6	1.5	34.6	10.7
Phytolaccaceae, *Phytolacca americana* (pokeweed)	0	0	0	0	1.2	0	0.1	2	1.6	0	0	1.3	0.1	3.2	2.9
Poaceae, *Secale cereal* (rye)	-	-	-	0	58.3	1.7	-	-	-	-	-	-	0	58.3	1.7
Rosaceae, *Amelanchier sp.* (serviceberry)	-	-	-	1	0.5	0	0.2	0.4	0	-	-	-	1.2	0.9	0
Malus sp. (crab apple)	-	-	-	-	-	-	0	1	1	-	-	-	0	1	1
Prunus sp. (cherry)	0	0	0.2	0	1.2	0	0.1	3.5	0.5	0	0	0.3	0.1	4.7	1
Rubus sp. (raspberry)	-	-	-	-	-	-	0.5	0.5	0	-	-	-	0.5	0.5	0
Rubus villosus (blackberry)	-	-	-	0	3.5	0.3	0	0	0.3	-	-	-	0	3.5	0.6
Rubiaceae, *Cephalanthus occidentalis* (buttonbush)	-	-	-	-	-	-	0	2	2	-	-	-	0	2	2

Table 1.3. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Sapindaceae, *Acer negundo* (boxelder)	0	0.1	0.1	0	0.3	0.1	0	0.1	0	0	0	2.5	0	0.5	2.7
Sapindaceae, *Aesculus glabra* (buckeye)	0	0	0	0	1	2	-	-	-	-	-	-	0	1	2
Scrophulariaceae, *Buddleja davidii* (butterfly bush)	-	-	-	-	-	-	-	-	-	0	10	1	0	10	1
Scrophulariaceae, *Verbascum thapsus* (common mullein)	0	0	0.8	-	-	-	-	-	-	-	-	-	0	0	0.8
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0.3	1.1	1.3	0.5	10	0.5	0.2	4.3	0.8	0	0.2	6.9	1	15.6	9.5
Ulmaceae, *Ulmus sp.* (elm)	0	0	0.3							-	-	-	0	0	0.3
Vitaceae, *Vitis sp*. (grape)	0.2	0	0	0	54.3	0.3	-	-	-	-	-	-	0.2	54.3	0.3
Vitis sp. (wild grape)	0	0	0	0	0.3	0	0	0.6	0.9	0	0	1.8	0	0.9	0.9

Table 1.4. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 3-minute sample on various woodland plant species. Western, VA. 2012

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer rubrum* (red maple)	0.5	0	0	0	0	0	0	0	0	-	-	-	0.5	0	0
Annonaceae, *Asimina sp.* (paw paw)	0	0	0.3	0	0	0	0	0.1	0.1	0	0	0.8	0	0.1	1.2
Bignoniaceae, *Catalpa sp.* (catalpa)	1.1	2.3	0.9	0.8	3.8	0.6	0.01	0.7	0.2	0	0.3	4.3	1.9	7.1	0.6
Brassicaceae, *Armoracia rusticana* (horseradish)	0	0	15	-	-	-	-	-	-	-	-	-	0	0	15
Cannabaceae, *Celtis occidentalis* (hackberry)	0.1	0.2	0.9	0	0	0.1	0	0.5	0.5	0	0.1	0.4	0.1	0.8	1.9
Cornaceae, *Cornus sp.* (dogwood)	0.2	0	0.4	0	0	0	0.3	0.3	0.5	0	0	0.8	0.3	0.3	1.7
Elaeagnaceae, *Elaeagnus umbellate* (autumn olive)	-	-	-	-	-	-	0	6	0	-	-	-	0	6	0
Fabaceae, *Cercis canadensis* (redbud)	0	0	1	-	-	-	0.3	8.3	1.3	-	-	-	0.3	8.3	2.3
Mimosa pudica (mimosa)	0.1	0.1	0.2	0.1	0.1	0.2	0.1	6.4	0.6	0.1	0.5	0.8	0.4	7.1	1.8
Robinia pseudoacacia (black locust)	-	-	-	0.1	1.3	0	0.3	0.8	0.3	0	0	0	0.4	2.1	0.3

Table 1.4. Continued

Family, genus species (common)	May/June			September			August			Total			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Fagaceae, *Castanea dentate* (chestnut)	-	-	-	-	-	-	0.7	0	0.7	0	0	0	0.7	0	0.7
Quercus prinus (chestnut oak)	-	-	-	0	0	0.3				-	-	-	0	0	0.3
Ficeae, *Ficus sp*. (figs)	0	0	3.5	-	-	-	-	-	-	-	-	-	0	0	3.5
Juglandaceae, *Juglans nigra* (black walnut)	0	0	0.2	-	-	-	-	-	-	-	-	-	0	0	0.2
Juglans sp. (walnut)				-	-	-	-	-	-	0	0	2			2
Lauraceae, *Sassafras sp.* (sassafrass)	0	0	0.7	0	0	0	0	1	0	0	0	0	0	1	0.7
Magnoliaceae, *Magnolia grandiflora* (southern magnolia)	0	0	4	-	-	-	-	-	-	-	-	-	0	0	4
Malvaceae, *Tilia sp*. (basswood)	-	-	-	0	4	0	-	-	-	-	-	-	0	4	0
Moraceae, *Morus sp.* (mulberry)	0.1	0	2.1	0.2	0	0.4	1.2	6.6	0	0.4	0.4	0.8	2.3	7	4.9
Oleaceae, *Syringa sp.* (lilac)	0	0	3.5	-	-	-	-	-	-	-	-	-	0	0	3.5
Paeoniaceae, *Paeonia sp*. (peony)	0	0	5.3	-	-	-	-	-	-	-	-	-	0	0	5.3
Paulowniaceae, *Paulownia sp*. (paulownia)	0	0	2.4	0	0.5	0	0	47.5	7	-	-	-	0	48	9.4
Phytolaccaceae, *Phytolacca americana* (pokeweed)	0	0.3	0	0	0.3	0	0	1.3	0.3	0	0.3	1.3	0	2.2	1.6

Table 1.4. Continued

Family, genus species (common)	May/June			September			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Rosaceae, *Prunus sp*. (cherry)	0.1	0	1.1	0	0	0.5	0.1	0.4	1.3	0	0	0	0.2	0.4	2.9
Rubus sp. (blackberry)	-	-	-	-	-	-	0	1.5	0	-	-	-	0	1.5	0
Sapindaceae, *Acer negundo* (boxelder)	0.1	0.2	0.4	0.1	0	0.3	0.1	5.6	1.6	0	0.1	0.5	0.3	5.9	2.8
Aesculus glabra (buckeye)	0	0	0	-	-	-	0	1	0	0	0	0	0	1	0
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0.2	1.0	0.3	0.3	2.9	0.9	0.2	6.1	1.8	0.03	0.1	1.4	1	10.1	4.4
Solanaceae, *Datura stramonium* (jimsonweed)	-	-	-	-	-	-	-	-	-	0	0.7	3.7	0	0.7	3.7
Vitaceae, *Vitis sp.* (wildgrape)	0	0	0.3	0	0	0	0	0.5	0.4	0	0	0.2	0	0.5	0.9

Table 1.5. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 3-minute sample on various woodland plant species. Western, VA. 2013

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer rubrum* (red maple)	-	-	-	0.3	0	0	1	19.4	1	-	-	-	1.3	19.4	1
Annonaceae, *Asimina triloba* (paw paw)	0	0	0	0	0	1	-	-	-	0	0	0	0	0	1
Bignoniaceae, *Catalpa sp*. (catalpa)	0	3.2	0.8	0.3	14.3	1	0	9.7	4.5	-	-	-	0.3	27.2	6.3
Cannabaceae, *Celtis sp.* (hackberry)	0	0	0	-	-	-	0	22	3	-	-	-	0	22	3
Ericaceae, *Vaccinium corymbosum* (blueberry)	0.5	5.5	0	-	-	-	0	7	0	0	2	0	0.5	14.5	0
Fabaceae, *Albizia julibrissin* (mimosa)	0.4	3.4	0	0.1	5.1	0.3	0.2	4.1	0.3	0	0	0	0.7	12.6	0.6
Pueraria sp. (kudzu)	-	-	-	-	-	-	1	0	0	-	-	-	1	0	0
Robinia pseudoacacia (black locust)	-	-	-	-	-	-	-	-	-	0	0	1	0	0	1
Juglandaceae, *Juglans nigra* (black walnut)	0	0	0	0	11	0	0	3.7	0	0	0	1	0	14.7	1
Moraceae, *Morus sp*. (mulberry)	0	1.8	1.9	0	3	4.7	0	4.3	1.8	-	-	-	0	9.1	8.4
Paulowniaceae, *Paulownia sp.* (paulownia)	1	105	5.5	0.5	25.5	3	-	-	-	-	-	-	1.5	131	8.5

Table 1.5. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Phytolaccaceae, *Phytolacca americana* (pokeberry)	-	-	-	0	1	0	0	1	0	-	-	-	0	2	0
Rosaceae, *Prunus sp.* (cherry)	-	-	-	0	2	0	0	2	0	0	0	2	0	4	2
Prunus virginiana (chokecherry)	0	2.5	12.5	0	13	0	0	2	0	-	-	-	0	17.5	12.5
Rubus sp. (raspberry)	0	0	0	0	0	0.5	0.3	0	0.3	0	0	1	0.3	0	1.8
Sapindaceae, *Acer negundo* (boxelder)	-	-	-	0	2	0	0	1	0.5	-	-	-	0	3	0.5
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0.3	2.3	4.3	0.1	10.3	2.3	0	9.6	4.5	0	13.4	2.2	0.4	35.6	13.3
Vitaceae, *Vitis sp*. (grape)	0.3	0	0.3	0	0.5	0	0	0.4	0.2	0	1.7	0	0.3	2.6	0.5

Table 1.6. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 2-minute sample on various woodland plant species. Western, NC. 2011

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer sp.* (maple)	-	-	-	0	0	1	0	0	0	-	-	-	0	0	0
Asteraceae, *Solidago sp.* (goldenrod)	-	-	-	-	-	-	-	-	-	0	0	5	0	0	5
Bignoniaceae, *Catalpa sp.* (catalpa)	-	-	-	0	2.2	0.2	0	0.4	1	0	0.5	0.6	0	3.1	1.8
Cornaceae, *Cornus sp.* (dogwood)	0	0	0	0	0	0	0	0	1.3	0	0	3	0	0	4.3
Fabaceae, *Robinia sp.* (locust)				0	0.5	0	0	1	1	-	-	-	0	1.5	1
Juglandaceae, *Juglans nigra* (black walnut)	-	-	-	0	0	0	0	0	5	-	-	-	0	0	5
Paulowniaceae, *Paulownia sp.* (paulownia)	0	0	0	0.2	0.8	0.4	2.1	0	1.8	0	0.2	0.8	2.3	1	3
Rosaceae, *Prunus sp.* (cherry)	0	0	0	0	0	0.3	0	0	1.3	0	0	0	0	0	1.6
Rubus sp. (blackberry)	0	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0.1
Salicaceae, *Salix sp.* (willow)	-	-	-	-	-	-	-	-	-	0	0	1	0	0	1
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0	0	0	0.2	1.1	0	0	0.3	1.9	0	1	2.2	0.2	2.4	4.1
Vitaceae, *Vitis sp.* (grape)	0	0	0	0	0.2	0	0	0.6	1.1	-	-	-	0	0.8	1.1

Table 1.7. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 2-minute sample on various woodland plant species. Western, NC. 2012

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer sp* .(maple)	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0.5	0
Anacardiaceae, *Rhus sp.* (sumac)	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0.5	0
Bignoniaceae, *Catalpa sp.* (catalpa)	0.04	1.4	0.9	0.1	1.1	0.8	0	0.9	1.8	0	0.1	1.2	0.14	3.5	4.7
Celastraceae, *Celastrus orbiculatus* (oriental bittersweet)	-	-	-	0	1.5	0	-	-	-	-	-	-	0	1.5	0
Cornaceae, *Cornus sp*. (dogwood)	-	-	-	0	0	0	0	0	0	0	0	0.2	0	0	0.2
Fabaceae, *Cercis canadensis* (redbud)	-	-	-	0	0	0	0	0	0.3	0	0	0.6	0	0	0.9
Cladrastis sp. (yellowwood)	0	0	0	0	16	43	0	1.1	2.6	0	0	0.3	0	16.8	46
Robinia sp. (locust)	0	0.1	0	0	0.1	0	0.03	1.5	0.4	0	0.1	0.1	0.03	1.8	0.5
Fagaceae, *Castanea mollissima* (Chinese chestnut)	-	-	-	-	-	-	0	1	0	0	0	0	0	1	0
Quercus sp. (Oak)	0	0	0	0	0	0	0	0	0.1	0	0	0.5	0	0	0.1
Juglandaceae, *Juglans nigra* (black walnut)	0	0.1	0	0	0.5	0	0	1.1	0.4	0	0	0.5	0	1.7	0.9
Lamiaceae, *Mentha sp.* (mint)	-	-	-	0	5	0	0	0	0	-	-	-	0	5	0
Lauraceae, *Sassafras sp.* (sassafras)	0	0	1	-	-	-	-	-	-	-	-	-	0	0	1

Table 1.7. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Magnoliaceae, *Liriodendron sp*. (tulip poplar)	0	0	0	0	0.1	0	0	0.1	0.1	0	0.1	0.2	0	0.3	0.3
Magnolia sp. (magnolia)	-	-	-	0	0	0	0	0	0	0	0	0.3	0	0	0.3
Oleaceae, *Fraxinus sp.* (ash)	-	-	-	0	0	0	-	-	-	0	0	0.3	0	0	0.3
Ligustrum sp. (privet)	-	-	-	-	-	-	-	-	-	0	0	0.5	0	0	0.5
Paulowniaceae, *Paulownia sp.* (paulownia)	0	0.3	1.3	0	4.6	0.3	0	2.9	1.1	0	0	1.2	0	7.8	3.9
Phytolaccaceae, *Phytolacca sp*. (pokeberry)	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
Platanaceae, *Platanus occidentalis* (sycamore)	-	-	-	0	0	1	0	0.2	1.8	0	0	0.8	0	0.2	3.6
Rosaceae, *Prunus sp.* (cherry)	0	0.2	0	0	2	0.1	0	10.2	1.4	0	0	0	0	12	1.5
Prunus sp. (plum)	-	-	-	-	-	-	0	1	0	-	-	-	0	1	0
Rosa multiflora (multiflora rose)	0	0	1.3	0	0.8	0	-	-	-	-	-	-	0	0.8	1.3
Rubus sp. (blackberry)	0	0	0	0	0.2	0	0	0	0	0	0.5	0.8	0	0.7	0.8
Sapindaceae, *Acer negundo* (boxelder)	0	0	0	0	0	0.3	0	0.3	0.8	0	0	0	0	0.3	1.1
Aesculus glabra (buckeye)	0	0	0	0	0.5	0.5	0	0.2	0.6	0	0	1	0	0.7	2.1
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0.03	0.1	0.13	0	0.8	0.02	0	1.7	0.8	0	0	0.2	0.03	2.6	1.2
Ulmaceae, *Ulmus sp*. (elm)	-	-	-	-	-	-	-	-	-	0	0	1	0	0	1
Vitaceae, *Vitis sp*. (grape)	0	0	0	0	2	0	0	2.2	0.9	0	0.1	0.1	0	4.3	1

Table 1.8. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 2-minute sample on various woodland plant species. Western, NC. 2013

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, *Acer platanoides* (Norway maple)	-	-	-	0	1	0	0	0.2	0	-	-	-	0	1.2	0
Acer sp. (maple)	0	0	0	0	0	0	0.1	0	0	0	0	1.8	0.1	0	1.8
Anacardiaceae, *Rhus sp.* (sumac)	0	0	0	0	1	0	-	-	-	-	-	-	0	1	0
Asteraceae, *Eutrochium sp.* (joe pye weed)	-	-	-	-	-	-	0	0.3	0	-	-	-	0	0.3	0
Solidago sp. (goldenrod)	-	-	-	-	-	-	0	0	1	-	-	-	0	0	1
Betulaceae, *Betula sp*. (birch)	-	-	-				0	1	0	-	-	-	0	1	0
Bignoniaceae, *Catalpa sp.* (catalpa)	0.2	0	0.5	0.7	1	0	0	3.4	0.4	0	0.5	0.6	0.9	4.9	1.5
Caprifoliaceae, *Lonicera sp.* (honeysuckle)	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
Celastraceae, *Celastrus orbiculatus* (oriental bittersweet)	-	-	-	0	18	0	0	2	0	0	0	2	0	20	2
Cornaceae, *Cornus sp.* (dogwood)	0.1	0.1	0.1	0.1	0.5	0	0	0	0.1	0	0	0	0.2	0.6	0.2
Fabaceae, *Cercis canadensis* (redbud)	0	0	0.3	0.7	0.7	0	0	1	0.8	0	0	14	0.7	1.7	15.1
Cladrastis kentukea (yellowwood)	0.2	0.3	0.2	0.1	5.9	0.4	0	1.9	0.6	0	0	1.2	0.3	8.1	2.4
Robinia sp. (locust)	0	0	0.02	0	0.1	0	0.03	1	0.6	0	0	0.5	0.03	1.1	1.12

Table 1.8. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Fagaceae, *Castanea mollissima* (Chinese chestnut)	0	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0.1
Quercus sp. (oak)	0	0	0	0	0.04	0	0	0	0	0	0	0.3	0	0.04	0.3
Juglandaceae, *Carya sp* (hickory)	0.04	0	0.1	0.1	0.1	0	0	0	0.2	0	0	0.2	0.14	0.1	0.5
Juglans sp. (walnut)	0	1.1	0.3	0	2.5	0	0	4.2	1.4	0	0.5	2.5	0	8.3	4.2
Lamiaceae, *Mentha sp.* (mint)	-	-	-	0	0.3	0	0	0.5	0	0	0	0	0	0.8	0
Magnoliaceae, *Liriodendron sp.* (tulip poplar)	0.1	0	0.04	0.04	0.1	0	0	0.1	0.1	0	0.2	2.5	0.14	0.4	2.64
Oleaceae, *Fraxinus sp.* (ash)	0	0	0	0	0	0	0.1	0.7	0.7	0	0	2	0.1	2.7	0.8
Ligustrum sp. (privet)	0	0	0	-	-	-	-	-	-	0	0	1	0	0	1
Paulowniaceae, *Paulownia sp.* (paulownia)	0.3	2.3	0.6	0.4	3.4	0.3	0.2	5.6	3.5	0	0.1	16.4	0.9	11.4	20.8
Platanaceae, *Platanus occidentalis* (sycamore)	0	0	0	0	0.3	0	0	5.3	4.3	0	0	0.5	0	5.6	4.8
Rosaceae, *Prunus sp.* (cherry)	0.1	0	0.4	0	0.8	0.2	0	9.0	2.3	0	2.2	1.8	0.1	12	4.7
Rosa sp. (multiflora rose)	0	0	0	0	0	0	0	1	0	-	-	-	0	1	0
Rubus sp. (blackberry)	0	0	0.2	0.1	0.6	0.3	0	6.7	1.4	0	1.3	0.2	0.1	8.6	2.1
Sapindaceae, *Acer negundo* (boxelder)	0	0.2	0.4	0.2	0.9	0	0	6.3	1.6	0	0.3	2.5	0.2	7.7	4.5

Table 1.8. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aesculus sp. (buckeye)	0	0.7	1.0	0	1.6	0	0	0.7	1.5	0	0.1	4.1	0	3.1	6.6
Simaroubaceae, *Ailanthus altissima* (tree of heaven)	0.1	0.1	0.3	0.1	1.5	0.6	0	12.3	0.8	0.1	0.9	1.2	0.3	14.8	2.9
Vitaceae, *Vitis sp.* (grape)	0	0.5	0.1	0	0.6	0.1	0	5.7	1.1	0	0.6	1.2	0	7.4	2.5
Unknown, Unknown (unknown ornamental)	0	0	0	0.5	2	0	-	-	-	-	-	-	0.5	2	0

Table 1.9. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 2-minute sample on various woodland plant species. East-central, NC. 2011

	May/June			July			August			September			Total		
Family, genus, species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Bignoniaceae, *Catalpa sp.* (catalpa)	0	0	0	0	0	0	0	0.1	0.1	-	-	-	0	0.1	0.1
Fabaceae, *Cercis Canadensis* (redbud)	-	-	-	-	-	-	0	5.3	4	0	0	0	0	5.3	4
Mimosa pudica (mimosa)	-	-	-	-	-	-	-	-	-	0	0	0.2	0	0	0.2
Oleaceae, *Ligustrum sp.* (privet)	-	-	-	-	-	-	-	-	-	0	0	0.1	0	0	0.1

Table 1.10. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 2-minute sample on various woodland plant species. East-central, NC. 2012

	May/June			July			August			September			Total		
Family, genus species (common)	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Aceraceae, Acer rubrum (red maple)	1	0	0	0	0	0	-	-	-	-	-	-	1	0	0
Aceraceae, *Acer sp.* (maple)	-	-	-	0	0.3	0.3	-	-	-	-	-	-	0	0.3	0.3
Anacardiaceae, *Rhus sp.* (sumac)	0	0	0	1	1.9	0	0	0	0	-	-	-	1	1.9	0
Aquifoliales, *Ilex sp.* (holly)	-	-	-	0	0.3	0	0	0	0	-	-	-	0	0.3	0
Araliaceae, *Aralia spinosa,* (devil's walkingstick)	-	-	-	-	-	-	-	-	-	0	0	1	0	0	1
Asteraceae, *Arctium sp.* (burdock)	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0
Bignoniaceae, *Catalpa sp.* (catalpa)	0	29	0	0	0.4	0.4	-	-	-	-	-	-	0	29.4	0.4
Fabaceae, *Cercis canadensis* (redbud)	0	0.1	0	0	0	0	0.1	1.4	0.3	0	0	0.5	0.1	1.5	0.8
Cladrastis kentukea (yellowwood)	-	-	-	0	8	0	0	1	2	0	0	0	0	9	2
Mimosa sp. (mimosa)	0	0	0	0	1	0	-	-	-	-	-	-	0	1	0
Lauraceae, *Sassafras sp.* (sassafras)	0.3	2.3	0	0	0.6	2.1	-	-	-	-	-	-	0.3	2.9	2.1
Moraceae, *Morus sp.* (mulberry)	0	1	0	0	0	0	-	-	-	-	-	-	0	1	0
Oleaceae, *Chionanthus sp.* (fringetree)	-	-	-	-	-	-	0	0	0.5	0	0	0.3	0	0	0.8

Table 1.10. Continued

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
*Ligustrum sp. (*privet)	0	0	0	0.04	0.2	0.4	0	0	0	0	0.3	2.9	0.04	0.5	3.3
Rosaceae, *Prunus serotina* (black cherry)	0	0.1	0.05	0	0.3	0	0	0	0	-	-	-	0	0.4	0.05
Simaroubaceae, *Ailanthus sp.* (ailanthus)	0	0	0	0	0.06	0	-	-	-	-	-	-	0	0.06	0
Vitaceae, *Vitis sp.* (wild grape)	0	0.3	0.1	0	1.4	1.4	-	-	-	-	-	-	0	1.7	1.5
Unknown, Unknown, (weeds)	0	0	1	-	-	-	-	-	-	-	-	-	0	0	1

Table 1.11. Number of *Halyomorpha halys* egg masses (E), nymphs (N) and adults (A) per 3-minute sample on various woodland plant species. Eastern, VA. 2013

Family, genus species (common)	May/June			July			August			September			Total		
	E	N	A	E	N	A	E	N	A	E	N	A	E	N	A
Bignoniaceae, *Campsis radicans* (trumpeter vine)	-	-	-	-	-	-	0	0.2	0.1	0	0	0.1	0	0.2	0.2
Fabaceae, *Mimosa pudica* (mimosa)	-	-	-	-	-	-	0	0.2	0.1	0	0.1	0.1	0	0.3	0.2
Vitaceae, *Vitis sp.* (wild grape)	-	-	-	0	0.1	0	0	0	0	0	0	0	0	0.1	0

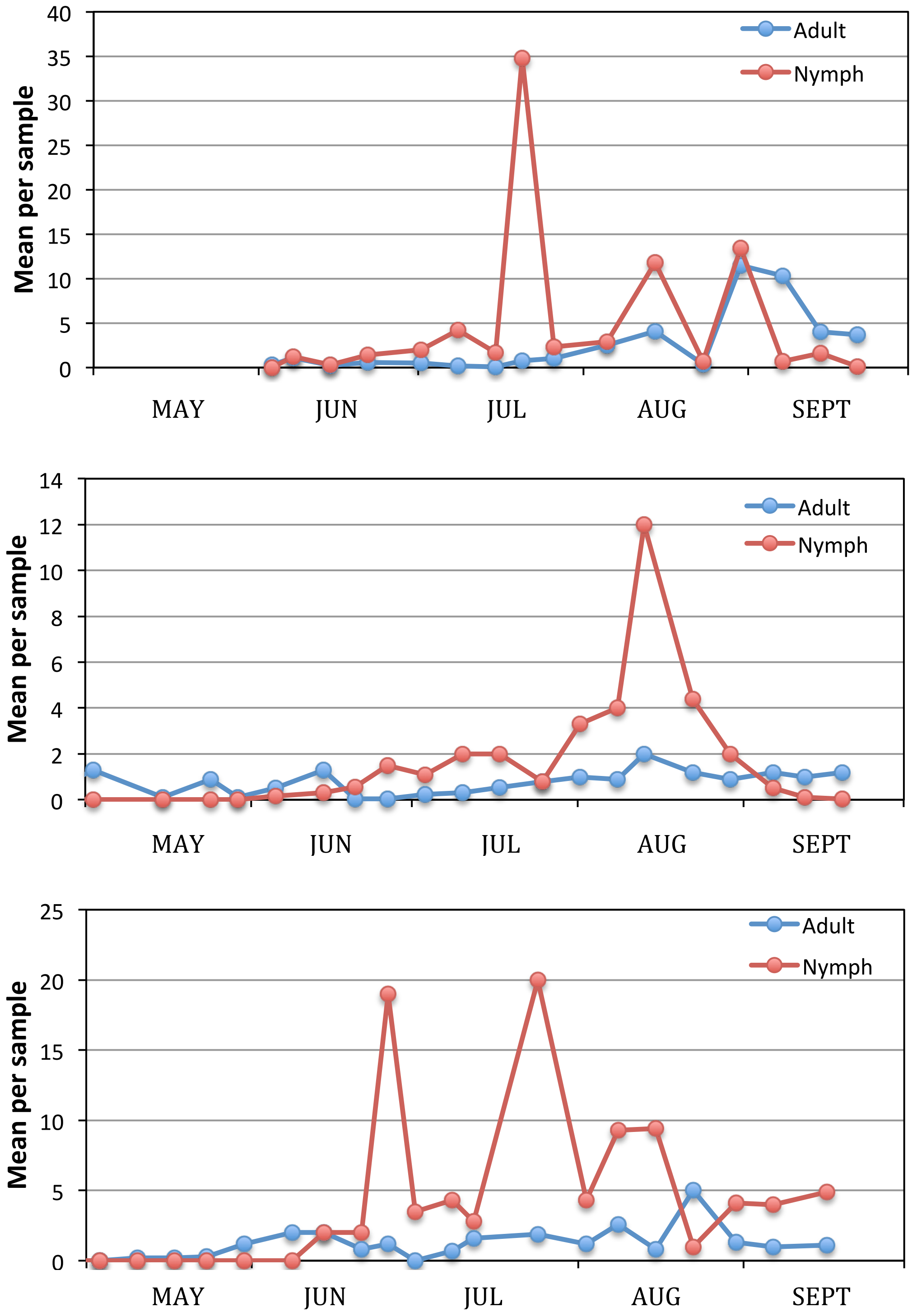

Figure 1.1. Seasonal phenology of BMSB in non-managed woodland habitats in western Virginia, 2011 (top), 2012 (middle), 2013 (bottom).

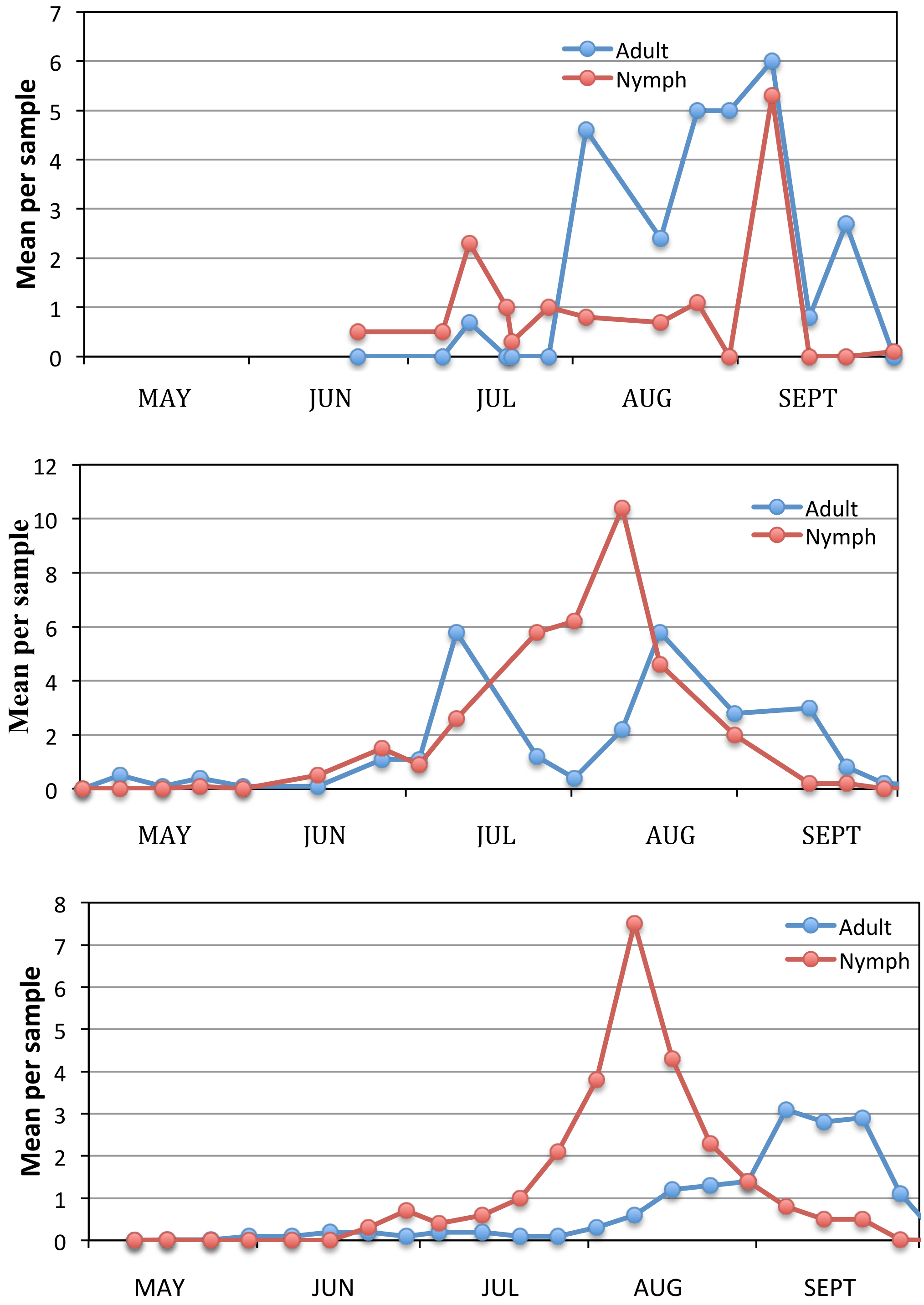

Figure 1.2. Seasonal phenology of BMSB in non-managed woodland habitats in western North Carolina, 2011 (top), 2012 (middle), 2013 (bottom)

Chapter 2: *Halyomorpha halys* (Stål) feeding damage to seven apple varieties at different phenological stages

Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive pest native to Japan, China, and Korea (Hoebeke and Carter 2003) that was first detected in the United States in Allentown, PA, in 2001. It has since been detected in 41 states, although the Mid-Atlantic States remain the region where it poses the greatest threat to agriculture (www.stopbmsb.org).

The BMSB is a highly polyphagous pest with many reported host plants, including agricultural crops, fruits, and vegetables (Hoebeck and Carter 2003). It is known to overwinter in human structures such as houses and sheds, and non-human structures such as dead, standing trees (Lee et al. 2014) in reproductive diapause before exiting hibernation locations in the spring, when it seeks suitable hosts for feeding and reproduction. The BMSB is a known pest of tree fruits in Japan and South Korea, where apples can serve as an early-season host and suffer damage to fruit (Funayama 2002a, 2004). Economic losses from BMSB feeding were observed on apples, peaches, and pears in New Jersey and Pennsylvania in 2006 (Nielsen et al. 2008), and by 2010 populations had increased dramatically, resulting in injury to many crops throughout the mid-Atlantic region. It is likely that as this pest continues to spread and establish in new regions of the United States, this damage will increase.

Several different species of native stink bugs, including *Euschistus servus* (Say), *Euschistus tristigmus* (Say), *Acrosternum hilare* (Say), *Brochymena quadrapustulatus* (Fabricius), and *Euschistus variolaris* (Beauvois) cause similar types of damage to apples, most prevalently in the mid- to late season (Brown (2003). Stink bug feeding damage to apples is similar to calcium-deficiency related problems commonly referred to as cork spot and bitter pit. Cork spot is characterized by the appearance of brown, discolored spots on the exterior of fruit that resemble sunken dimples with brown necrotic tissue beneath the apple skin (Brown 2003). Differences between stink bug feeding damage and cork spot include; 1) stink bug feeding results in corking immediately below the fruit skin whereas cork spot damage may have healthy flesh between the skin and necrotic tissue, 2) stink bug damage is usually uniform in shape, while cork spot is irregular, and 3) surface depressions due to stink bug feeding damage are generally circular with a gradual slope into the feeding depression, and depressions are often clustered and accompanied by a puncture mark where the stink bug inserted its stylet. Cork spot is characterized by a more abrupt surface depression, no stylet puncture, and is found more randomly distributed across the apple (Faust and Shear 1968, Brown 2003). Bitter pit, another calcium deficiency disorder, differs from stink bug damage in that it most often occurs during storage, has the appearance of small, shallow, irregularly shaped black depressions with limited corking beneath the flesh; stink bug damage does not develop or progress after harvest (Faust and Shear 1968, Brown 2003). Additional damage that may result from stink bug feeding includes invasion of saprophytic bacteria via stink bug puncture wounds and increased levels of aborted fruit (Zhang et al. 2007).

The BMSB is potentially a full season pest of apple in NC. Previous studies have examined stink bug damage to only a limited number of apple growth stages and varieties (Brown 2003, Nielsen and Hamilton 2009). The purpose of this study was to compare damage inflicted by BMSB to a wide variety of apple varieties and phenological growth stages representative of apple production in North Carolina.

Materials and Methods

To determine if stink bug feeding damage varies throughout the growing season, stink bugs were caged with apple fruit at three-week intervals from June through October. Nylon mesh exclusion cages (63.5cm circumference, 68.6cm height, for an 20.3cm diameter) were fitted around cylindrical deer fencing (Blue Hawk 213cm x 30.5m black plastic/polyresin) cut to a 48.3cm circumference with a 15.2cm wooden dowel strung across the interior (Madison Mill, 1cm diameter x 122cm poplar) of the cylinder. The cages were placed on the terminal ends of fruit bearing branches when the fruit was approximately one inch in diameter. Branches were thinned to one apple before attaching the cage. The nylon mesh was secured on the ends with twist ties.

Seven different apple varieties [*Malus domestica* Borlch. (Rosales: Rosaceae)], ranging from early to late maturing varieties were used for this study: Ginger Gold, Gala, Golden Delicious, Jonathan, Delicious, Rome and Pink Lady. Apples were located in a commercial orchard in Fruitland, NC. Adult stink bugs were caged onto fruit at three or more distinct growth stages, depending on variety and harvest date. Before caging stink bugs onto apples, individuals were starved for 12 hours, as it has been shown that *H. halys* shows positive sitotaxis to food post starvation (Xin et al. 2007). For each introduction period on each apple variety, two adult *H. halys* were placed inside each of 10 cages. Adults were removed from cages after 48 hours. Fruit remained caged the remainder of the year. For each variety, 10 fruit were caged and not infested with stink bugs and served as the control.

Introduction periods began 19 June, 2013, approximately five weeks post petal fall, which occurred 7 May, 2013 for Delicious. Each subsequent introduction period took place three weeks after the preceding introduction, so that each block for each introduction period was exposed to stink bug feeding only once. All varieties and their respective introduction periods are shown in Table 2.1. Apples from all introduction periods were harvested at the normal harvest period for each variety, which occurred about one week after the last introduction period. The number of mature and aborted fruit was recorded for each introduction period for each variety. Fruit was stored at 35-40°F for one to two weeks at which time damage was assessed. During processing, apples were measured for size using a Vernier caliper for both height and width of fruit. The location of feeding damage on fruit was separated into one of three zones: shoulder (stem), middle, and ventral (calyx). Within each region, feeding sites were examined in two ways; first an exterior measurement was taken of the diameter of the feeding depression, and second, fruit were bisected to measure the depth of damage. The remaining apple was peeled and cut to ensure that no feeding marks went unnoticed.

Statistical Analysis: Data were analyzed to assess the dependence of puncture damage metrics on introduction period and puncture location using GLM procedure of the SAS system (SAS 2011). Puncture width, puncture depth, and number of puncture wounds were analyzed individually as dependent variables with introduction period as a predictor variable. Puncture width and puncture depth were also analyzed individually with puncture location (shoulder, middle, and ventral) as a predictor variable. Interaction effects were not a valid calculation, because unequal introduction periods resulted in a high percentage of missing data. Means were separated using least squares differences t-tests. Data from the last introduction period (7) was omitted because there was no damage.

Results

Brown marmorated stink bug adults fed on developing and mature apple fruits during the first six introduction periods evaluated, but no damage was found in the seventh, final introduction period on Pink Lady (introduced 21-23 October, 2013). Damage was similar to that described by Brown (2003) in that the outer apple flesh displayed gradual dimpling with white to brown corky flesh lying directly beneath the surface of the skin. Puncture marks made from the stink bug inserting its stylet sheath were not always observable; however, damage was assumed to be a result of stink bug feeding, because damage did not resemble that of calcium-related deficiencies.

Fruit abortion was low in all treatments and was not statistically different (p=0.2174). The overall percentage of aborted fruit across all cultivars and introduction periods was 5.7% compared to 8.6% for all control fruit.

Puncture wounds. The number of puncture wounds varied significantly among introduction period (df=5, 287; F=17.70, P<0.001) and variety (df=6, 287; F=10.29; P<0.001), but not by location on the fruit (df=2, 287; F=2.20; P=0.113). The number of puncture wounds was highest during the second introduction on 10 July (3.4 punctures per fruit), followed by the third (2.6 punctures) and fourth (2.2 punctures) on 31 July and 19 August, respectively (Table 2.2). Punctures per fruit were lowest during the first, fifth and sixth introduction periods, varying between 0.4 and 0.8 punctures per fruit. No punctures were observed on Pink Lady during the last introduction period on 21 October. Injury to the different varieties varied from 1.4 punctures per fruit on Ginger Gold to a high of 3.1 on Jonathan. With the exception of injury to Jonathan, damage was generally higher on later-maturing cultivars (Rome and Pink Lady) compared to earlier maturing varieties.

Width and depth of injury by period and variety: The width and depth of injury were significantly influenced by the effects of introduction period, location of puncture on the fruit, and apple variety (Table 2.2). Illustrated in box plots are the results of the how the width and depth of puncture wounds varied across introduction period (Fig 2.1, and 2.2). The earlier the injury occurred, the greater the puncture width and depth when compared to later introduction periods. The estimated width declined by 0.578 mm per introduction period (Fig 2.1, F=51.17, p<.0001, α=.05), and the average puncture depth declined by 0.455 mm per introduction period (Fig 2.2, F=44.30, p<.0001, α=.05). Greatest mean width was recorded on Rome (Fig 2.3), while greatest mean depth was recorded on Jonathan (Fig 2.4). Smallest mean width and depth were recorded in Delicious (Fig 2.3, 2.4).

Width and depth of injury occurring on different locations on the fruit:

Feeding wound width on the middle portion of the fruit was significantly greater than that on the shoulder and ventral portions, but there was no significant difference between the width of damage wounds on the shoulder and ventral portions (Fig. 2.5). Puncture depth on the middle portion of the fruit was significantly greater than that on the shoulder and ventral portions, but there was no significant difference between shoulder and ventral puncture depth (Fig 2.6).

Discussion

Based on the results of Nielsen and Hamilton (2009), it was anticipated that early introduction periods would have more aborted fruit than later introduction periods. However, there were no significant difference in the number of aborted fruit among introduction periods in our study. Differences in amount of aborted fruit reported in Nielsen and Hamilton (2009) may have been a result of the variety, or in our study the introduction periods may have been late enough to avoid June drop.

Past studies have shown that stink bug damage is more common later than earlier in the season (Brown 2003, Nielsen and Hamilton 2009), and we expected higher levels of damage during the later introduction periods. Nielsen and Hamilton (2009), did not report the date of their introduction periods; however, harvest was completed by 21 September. In our study, temperature and daylight may have influenced feeding behavior for introduction periods 5, 6, and 7. The critical photoperiod that stimulates reproductive diapause in the brown marmorated stink bug has been reported to be approximately 14 hours (Fujiie 1985). Introduction periods 5, 6, and 7 (9 September, 30 September, and 21 October) all occurred when day length was less than 14 hours. Differences between Nielsen and Hamilton (2009) and our results - i.e., reduced feeding damage for periods 5 and 6, and absence of feeding damage in introduction period 7 – could be due to 1) BMSB were in diapause and not feeding, or 2) cool temperatures and a one week interval between BMSB introduction to the cages and harvest was not long enough for damage to develop.

Early introduction periods may have allowed stink bug damage to develop longer than that of later introduction periods, providing more time for enzymes injected with the insect’s saliva to dissolve components of the cell walls and intercellular matrix (Brown 2003, Miles 1958, Miles 1959). Presumably the earlier in the season that damage is inflicted, the longer digestive enzymes have to break down host material, ultimately resulting in the corking that is characteristic of stink bug feeding damage. Brown and Short (2010) reported that the key factor contributing to variation in stink bug injury appearance was the stage of fruit maturity at the time of injury. They also noted that damage first manifests itself as a small puncture mark, which then became discolored, and progressed as a depression before becoming discolored (Brown and Short 2010). It follows, therefore, that early season introduction periods had a longer time for damage to manifest itself. Brown and Short (2010) further reported that most stink bug feeding damage required at least two weeks and as long as one month to complete its progression. However, Faust and Shear (1968) and Brown (2003) reported that stink bug damage did not develop after harvest. If indeed stink bug damage does not progress post harvest, late-season feeding may be less significant than that inflicted earlier in the season, at least on late-maturing varieties. Further research is needed to more closely examine the importance of late season BMSB feeding damage.

Differences in the width and depth of stink bug feeding injury on different portions of the fruit may be related to how apple fruit grows. Goffinet et al. (1995) studied the development of four tissue regions of ‘Delicious’ apples for five stages of development from bloom to harvest. They reported that cell volume is greatest in the proximal lobe (reported as ‘shoulder’ in our study), followed by equatorial cortex (‘middle’), equatorial pith (core of the apple), and distal lobe (‘ventral’). Based on how fruit grows, one might expect damage to be more severe on the shoulder; however, damage in our studies did not follow this trend, since the width and depth of damage was greatest on the middle portion of the fruit. The differences between final cell volumes for shoulder and middle portions were very similar (Goffinet et al. 1995). It is possible that feeding damage that resulted from multiple punctures adjacent to one another could have been recorded as a singular damage mark. Goffinet et al. (1995) only studied one variety (Delicious), and it is possible that different varieties grow differently. Additional research is needed on both cell volume of more apple varieties, and on damage per location on fruit.

In summary, this study has shown that both the frequency and severity of damage was affected by the period of the year and variety. Damage caused later in the season was less severe than that during early or mid-season introductions. Further research on the impact of late season feeding injury will help clarify these issues.

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Table 2.1. Brown marmorated stink bug feeding introduction periods on seven varieties of apple. Fruitland, NC. 2013.

Variety	6/19-21 (1)	7/10-12 (2)	7/31-8/2 (3)	8/19-21 (4)	9/9-11 (5)	9/30-10/2 (6)	10/21-23 (7)
Ginger Gold	X	X	X				
Gala	X	X	X	X			
Golden Delicious	X	X	X	X	X		
Jonathan	X	X	X	X	X		
Delicious	X	X	X	X	X		
Rome	X	X	X	X	X	X	
Pink Lady	X	X	X	X	X	X	X

Table 2.2. ANOVA results of the effects of introduction period, injury location, and variety on the width and depth of BMSB injury on apples.

Injury	Factor	*F*	DF	*P*-value
Width	Introduction Period	66.46	1	<.0001
	Location on Fruit	55.69	2	<.0001
	Variety	36.11	6	<.0001
Depth	Introduction Period	48.87	1	<.0001
	Location on Fruit	38.53	2	<.0001
	Variety	25.08	6	<.0001

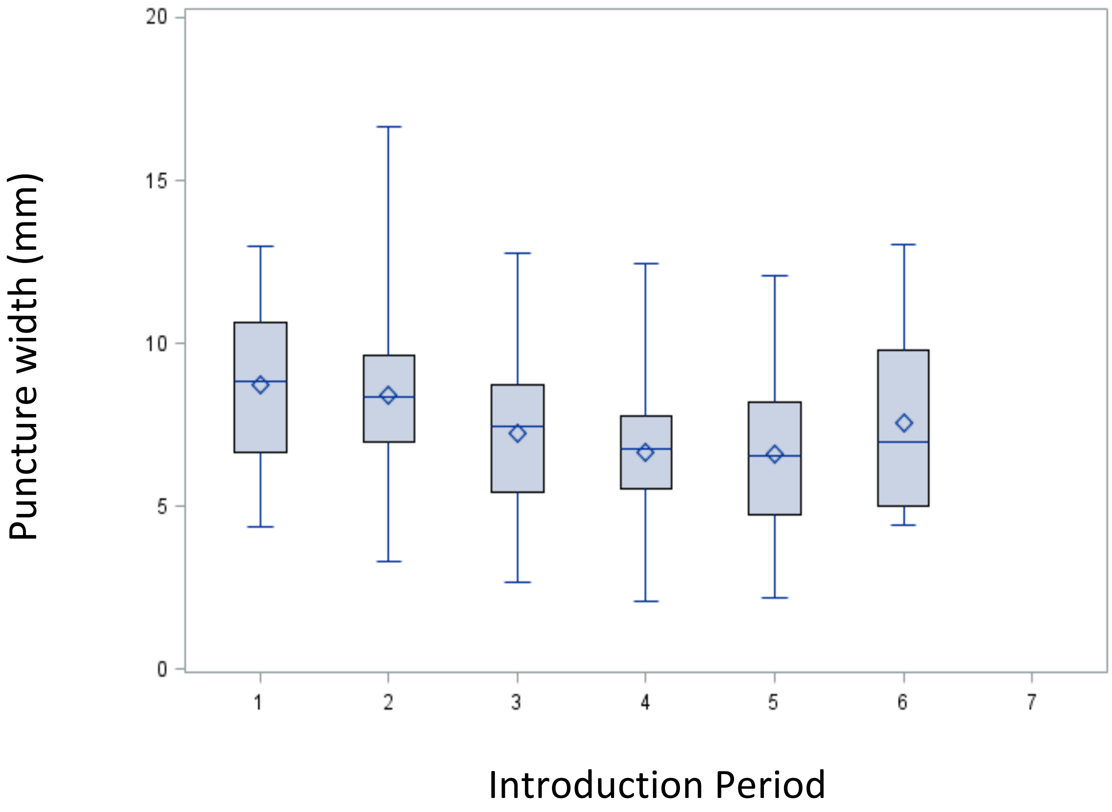

Figure 2.1. Width of *H. halys* feeding injury to apple inflicted at different introduction periods.

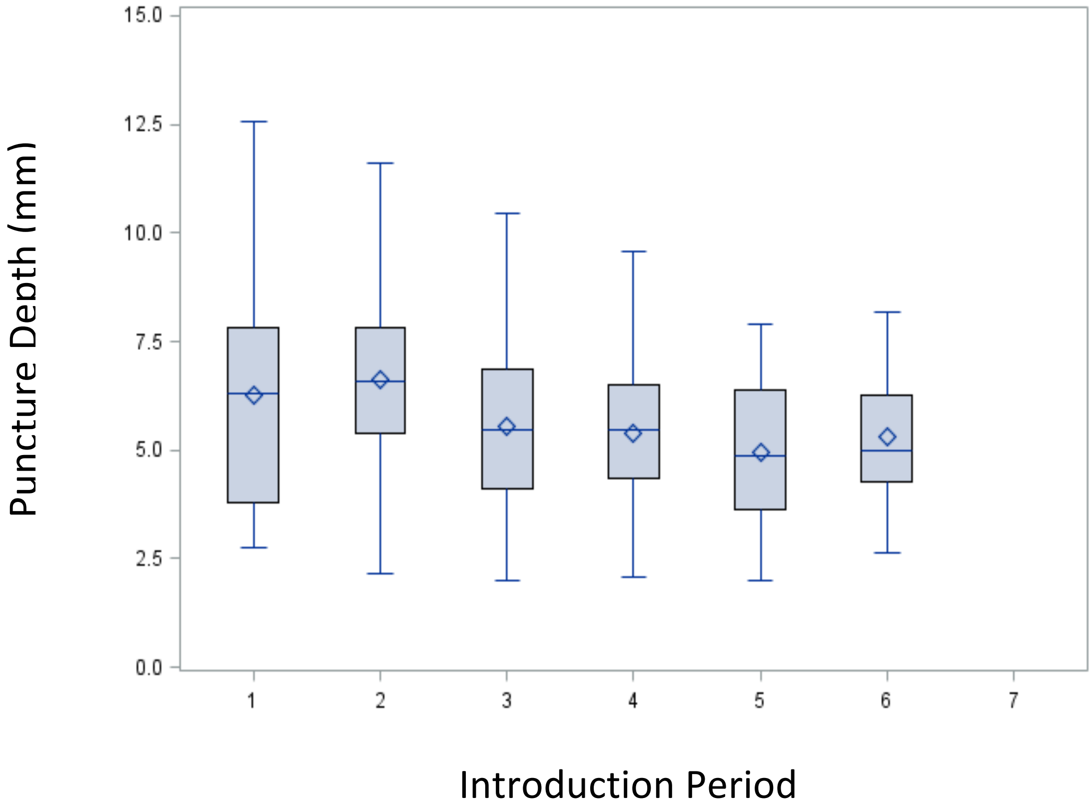

Figure 2.2. Depth of *H. halys* feeding injury to apples inflicted at different introduction periods.

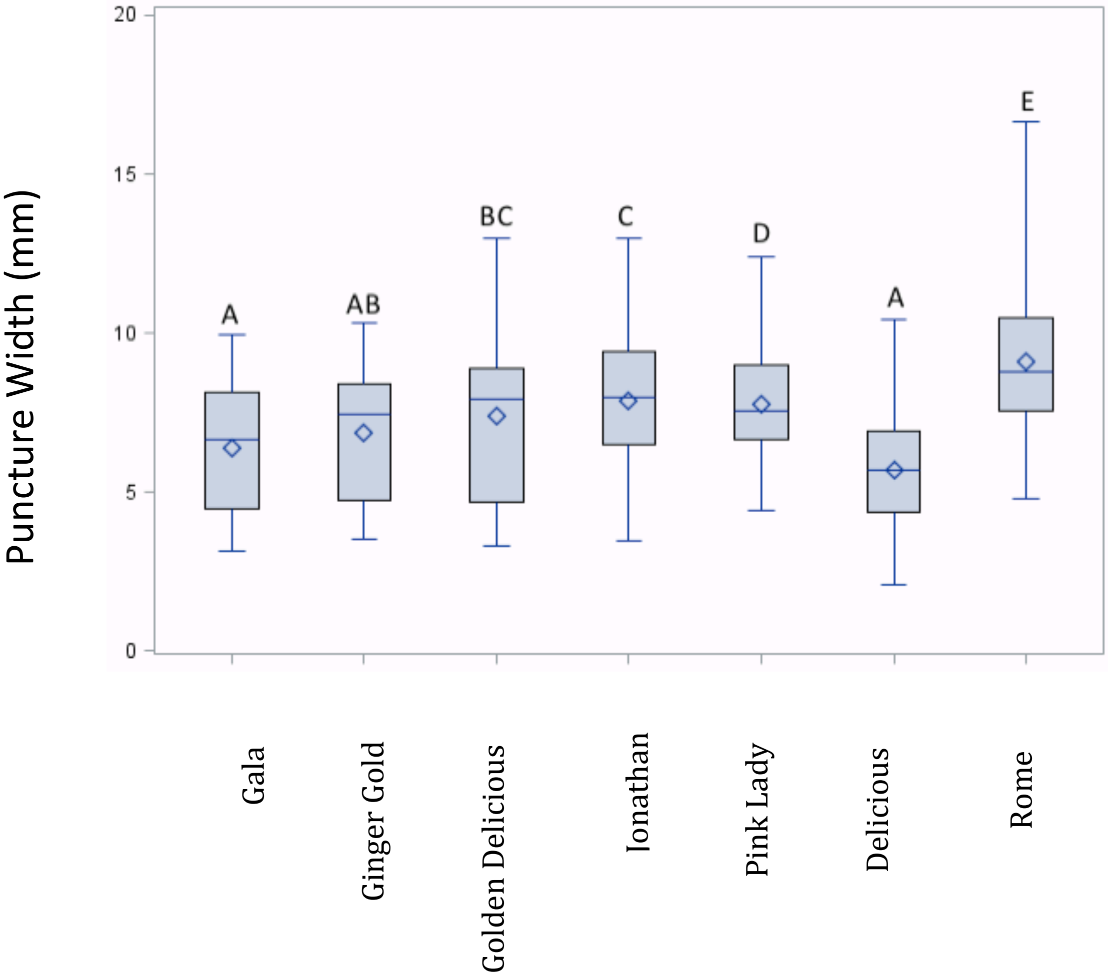

Figure 2.3. Width of *H. halys* feeding injury to different varieties of apple.

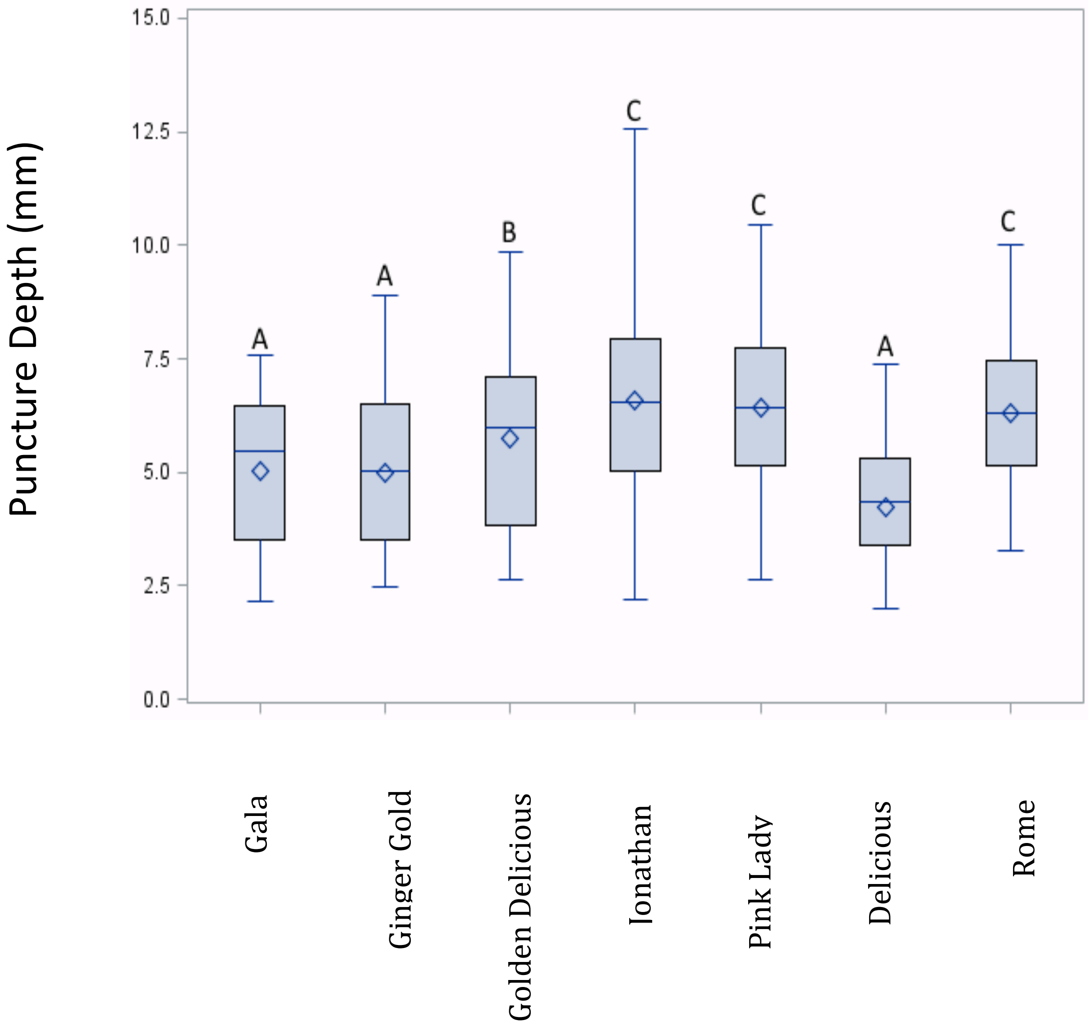

Figure 2.4. Depth of *H. halys* feeding injury to different varieties of apple.

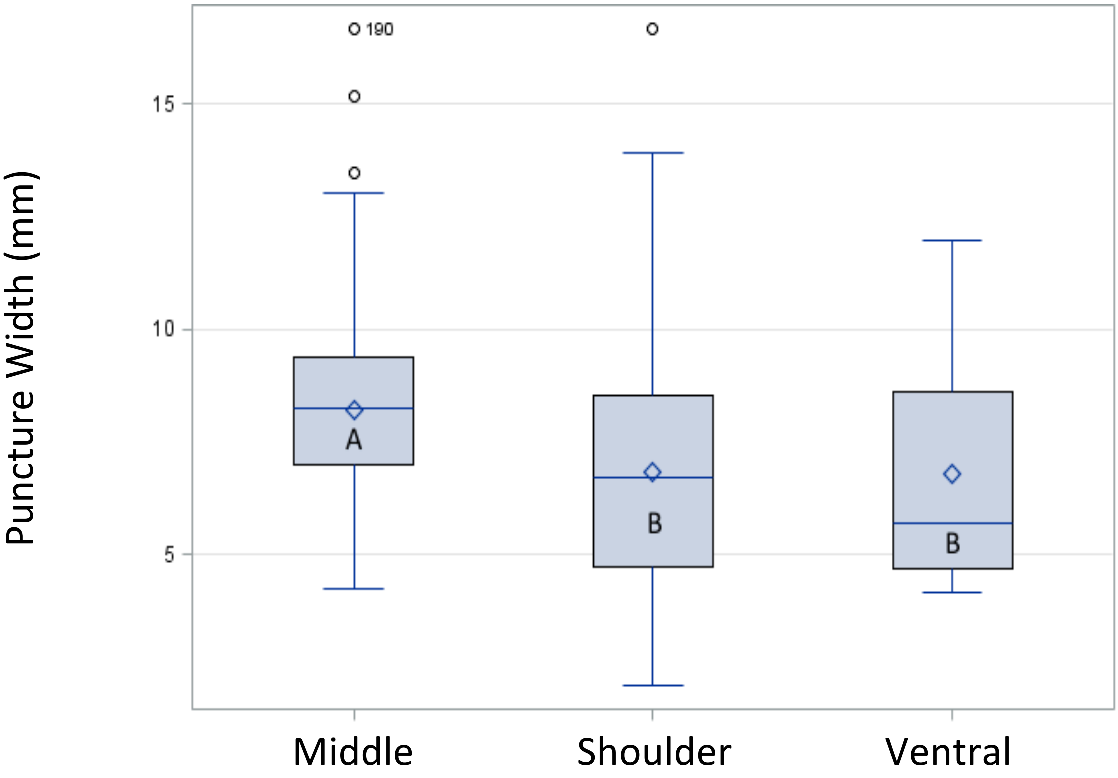

Figure 2.5. Width of *H. halys* feeding injury on different portions of apple fruit (t=1.96, α=.05).

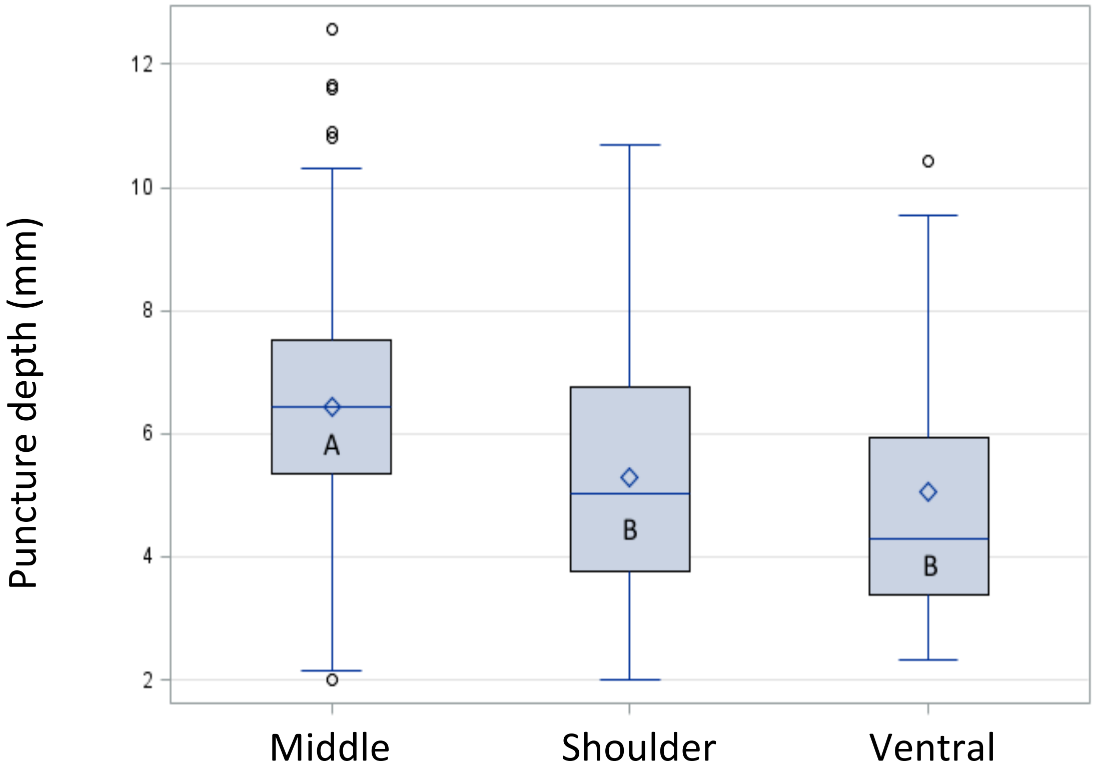

Figure 2.6. Depth of *H. halys* feeding injury to different portions of apple fruit (t=1.96, α=.05).

Table 2.3. Mean (±SEM) number of feeding injuries per fruit to seven apple varieties exposed to BMSB at different introduction periods.

	Introduction period						
Variety	1 (6/19)	2 (7/10)	3 (7/31)	4 (8/19)	5 (9/1)	6 (9/30)	Mean per[1] variety
Ginger Gold	0.4 (±0.3)	1.5 (±0.3)	2.2 (±0.4)	-	-	-	1.4 (±0.2)a
Gala	0.5 (±0.3)	1.3 (±0.5)	2.0 (±0.3)	0.9 (±0.5)	-	-	1.2 (±0.2)a
Golden Delicious	0.6 (±0.2)	1.6 (±0.2)	2.3 (±0.5)	1.4 (±0.5)	0.9 (±0.4)	-	1.4 (±0.2)ab
Jonathan	1.2 (±0.3)	6.8 (±1.5)	4.2 (±1.0)	2.4 (±0.4)	1.0 (±0.2)	-	3.1 (±0.4)d
Red Delicious	0.0 (±0.0)	1.0 (±0.6)	3.2 (±1.2)	2.6 (±0.3)	0.8 (±0.4)	-	1.6 (±0.3)bc
Rome	0.2 (±0.2)	6.7 (±2.1)	2.8 (±1.0)	1.9 (±0.5)	0.8 (±0.4)	0.8 (±0.3)	2.2 (±0.4)cd
Pink Lady	0.3 (±0.3)	4.8 (±0.8)	1.7 (±0.7)	4.1 (±1.2)	0.6 (±0.2)	0.7 (±0.3)	2.0 (±0.3)cd
Mean per introduction period[1]	0.4 (±0.2)ab	3.4 (±0.2)d	2.6 (±0.2)c	2.2 (±0.2)b	0.8 (±0.2)a	0.7 (±0.2)a	-

[1] Means injuries per variety, and per introduction period followed by the same letter are not significantly different by t-test (p<0.05) for LS means.

Chapter 3: Plum curculio, *Conotrachelus nenuphar* (Herbst), phenology and ovarian development in the southern Appalachians

Introduction

The plum curculio, *Conotrachelus nenuphar* (Herbst), is a key pest of pome and stone fruit in eastern and central North America (Chapman 1938, Racette et al. 1992, Hogmire 1995, Vincent et al. 1999, Leskey 2008). Adult weevils feed on and oviposit into fruit, causing cat-facing damage and premature fruit drop, as well as potentially providing introduction sites for bacteria (Snapp 1930, Horton and Ellis 1989, Lan et al. 2004). In the southeastern United States, where it is the most important fruit insect pest affecting peach and other stone fruits (Lan et al. 2004), plum curculio has traditionally been managed by broad spectrum insecticides applied shortly after petal fall (Chapman 1938, Reissig et al. 1998).

Plum curculio adults overwinter in ground litter and the top few centimeters of the soil on the edges of orchards and in hedgerows or woodlots adjoining orchards (Quaintance and Jenne 1912, Snapp 1930, Chapman 1938, Bobb 1949, Wylie 1954). Between bloom and petal fall, adults become active and move into orchards where they feed on petals and eventually, young fruit (Smith and Flessel 1968). After approximately two weeks of feeding and mating, females oviposit in fruit (Horton and Ellis 1989, Yonce et al. 1995). After hatching, larvae tunnel towards the center of the fruit while developing through four stadia. The larvae then exit the fruit, drop to the ground and pupate below the soil surface (Horton and Ellis 1989). After pupation, adults of the summer generation emerge, and migrate to overwintering sites to enter diapause if univoltine, or if multivoltine, feed and mate, with the females laying eggs in maturing fruit.

Plum curculio voltinism was first described by Quaintance and Jenne (1912) and later revised by Chapman (1938). The plum curculio occurs in one of two reproductively incompatible univoltine or multivoltine strains (Padula and Smith 1971). Every generation of the univoltine strain undergoes an obligatory diapause. The multivoltine strain undergoes facultative diapause that is controlled primarily by environmental conditions. These two strains are commonly referred to as the northern (univoltine) and southern (multivoltine) strains, because of their geographic distribution in North America. The line separating the two strains as reported by Chapman (1938) was in the mid-Atlantic region and ran along the Southern Appalachian Mountains. However, the discovery of some individuals in West Virginia with two periods of active oogenesis (Leskey 2008) indicates that a transition zone between northern and southern strains likely contains both strains.

The importance of the plum curculio as a pest of apple has increased in recent years due to the replacement of broad spectrum insecticides with reduced-risk products that have a more narrow range of pest activity (Agnello et al. 2009). Apple production in North Carolina is concentrated in the southern Appalachian Mountain region of western NC in a likely transition zone of northern and southern strains of plum curculio. There is a lack of knowledge about the biology and phenology of plum curculio in this region, which hampers the development of management programs for the pest. The study described herein was conducted to improve our understanding of voltinism and phenology of plum curculio in the southern Appalachian Mountains.

Materials and Methods

Sampling Locations. Two and four orchards were chosen in 2012 and 2013, respectively, as study sites from which to monitor plum curculio phenology, ovarian development and fruit damage. These locations were selected to represent the range of elevations and temperatures where apples are commercially grown in NC. To ensure the presence of plum curculio, only abandoned or minimally managed orchards were selected. Orchards were also selected based on elevation within the apple growing regions of western North Carolina and northwestern South Carolina. An orchard at Altapass, NC, the highest elevation at 875 m., was sampled in both 2012 and 2013. A research orchard at the Mountain Horticultural Crops Research Station (MHCRS), Mills River, NC, was an intermediate elevation at 661 m., and was also sampled in 2012 and 2013. Two low elevation orchards were sample in 2013; an apple orchard in Lincoln County, NC (elevation 335 m.), and a peach orchard in Greenville County, SC (elevation 305 m.).

A Watchdog M100 Data Logger (Spectrum Technologies) was placed at each orchard when sampling began to record daily maximum and minimum temperatures for calculating cumulative degree-day accumulations. Degree-days were calculated using the sine-wave method (Baskerville and Emin 1969); accumulation began when the first adult plum curculio was captured in the spring. Lan et al. (2004) determined that the thermal time requirement from oviposition to emergence of summer generation adults was 658 DD.

Sampling Methods: Plum curculio is notoriously difficult to monitor, thus multiple sampling methods were used at each site to enhance the potential for detecting adults. In early-to-mid April, two different traps were deployed and two different mechanisms of beat sampling were used in each orchard. Sampling methods included 1) baited pyramid traps (Tedders and Wood 1994, Mulder et al. 1997), 2) baited circle traps (or also called screen traps) (Mulder et al. 1997, Akotsen-Mensah et al. 2010) (Great Lakes IPM, Vestaburg, MI), 3) tree tapping of baited trees, and 4) tree tapping of non-baited trees. Baited pyramid traps are considered most effective when placed directly next to the tree under the canopy (Prokopy and Wright 1998); however, canopies in these orchards did not allow for optimal placement and pyramid traps were placed next to trees, just outside of the canopy, and in-between border row trees and adjacent woods. Because plum curculio adults overwinter on the edges of orchards and in woodlots adjoining orchards, and in the spring immigrate into orchards and congregate on border rows (Quaintance and Jenne 1912, Snapp 1930, Chapman 1938, Bobb 1949, Wylie 1954), sample trees were selected based on their proximity to the edge of orchards adjacent to bordering woods and shrubs. Three to five replicates of each sample type were used at each site depending on orchard size and number of trees bordering woods and shrubs. Baits consisted of grandisoic acid (Chemtica Internacional S.A., Westminster, CO) and benzeldehyde (Sigma-Aldrich, Milwaukee, Wisconsin) mixed in a 9:1 ratio with trichlorobenzene (Fisher Scientific, Suwanee, Georgia) and dispensed in UV-protected low-density polyethylene containers with lids (Thermo Fisher Scientific, Millville, NJ). Grandisoic acid is a male-produced aggregation pheromone attractive to both sexes of plum curculio (Eller and Bartelt 1996); this component of the bait was replaced at one-month intervals. Benzeldehyde is a plant-based volatile that is attractive to plum curculio (Leskey et al. 2001); it was deployed at the beginning of the season and replaced in July. Both baits were used because of improved trap performance when used in combination (Akotsen-

Mensah et al. 2010). Baits were deployed in baited treatments at the time plots were established in 2013, but grandisoic acid lures and benzeldehyde dispensers were not deployed until late May in 2012.

Traps were checked and tree taps were performed a minimum of once per week at each location from early April to late September, depending on apple harvest date and the number of plum curculio caught. Tree tapping was performed by holding a beat cloth beneath tree branches greater than one inch in diameter while simultaneously tapping the branches in the canopy and collecting the fallen insects. This beat sampling required moving around the tree for five samples per tree. Orchards were sampled at different times throughout the day; however, an individual location was sampled at approximately the same time of day each week. Weevils caught in traps or via tree tapping were placed in vials containing 70% ethanol, and then later separated by sex. Females were dissected to monitor ovarian development.

All individuals captured throughout the season were sexed according to characteristics described by Thomson (1932). All females were then dissected using a modified version of Smith and Salkeld's (1964) description of the stages of ovarian development. This modified version included three classifications of ovarian maturity (Leskey 2008): 1) sexually immature females containing no signs of development and lacking oocytes in the vitellarium; 2) presence of developing oocytes in the vitellarium indicating that female was becoming sexually mature; and 3) sexual maturation; the female was capable of oviposition due to mature eggs in the calyx.

Fruit in each orchard was monitored on each sample date for the presence of feeding damage and oviposition marks. Fifty fruit per tree were selected at random on four to ten trees, and the number with feeding or oviposition scars was recorded. Variation in the number of trees sampled was due to orchard size and amount of trees producing fruit.

Except for the application of a fungicide at the MHCRS in both 2012 and 2013, no systemic or foliar pesticides were applied to orchards during this study. Routine orchard floor maintenance was performed during this study by mowing the understory at weekly intervals to aid in trap maintenance and data collection.

Results

Sampling Methods. Table 3.1 displays the mean (± SEM) number of season total plum curculios recorded using various sampling methods. Data from 2012 includes only captures from traps and tree baiting samples after baits were deployed (18 May, 2012). Total captures were greatest at MHCRS for both 2012 and 2013. With the exception of Altapass in 2012, the highest mean captures were in baited beat trees, and baits did not appear to enhance numbers of plum curculios caught.

Altapass Location: Altapass was the highest elevation orchard at 875 m. Despite being a minimally managed orchard, plum curculio populations were low, with a total of only 17 adults in 2012 (Fig. 3.1), and 38 adults in 2013 (Fig. 3.2). Adult activity was detected from early May to mid-September in 2012 with one reproductively active female sampled in late May, and seven between 5 July and 2 August. Shown in Fig. 3.1A are degree-day accumulations from the date of the first adult captured on 3 May. Approximately 658 DD are required for development from egg to adult (Lan et al. 2004), which indicates that those reproductive females sampled in late July and early August could be reproductively active f_1

females. Fruit damage increased from approximately 7% in mid April to >35% in mid May, and then declined the remainder of the year, until sampling ceased at the end of August when damage was approximately 8%.

The period of adult activity in 2013, from late April through mid August, was similar to 2012, despite the fact that considerably higher numbers of females were captured in 2013 (14 in 2012, 29 in 2013). In contrast to 2012, the majority of reproductively active females were observed between mid May and late June, followed by low captures for the remainder of the season with only two reproductively active females observed in early August. Based on the degree-day accumulations, all reproductively mature females in May and June were likely from the overwintering generation, and those females captured in early August were probably first generation adults. A late spring in 2013 resulted in damage being later than in 2012; in 2013, initial fruit damage was not detected until mid May, and peak early season damage was observed in mid June at ~9%. Damage then declined and increased again to ~15% in mid August.

MHCRS Location: The MHCRS elevation of 661 m. was representative of the majority of commercial apple orchards in NC. Plum curculio populations were very high in this non-sprayed orchard, with a total of 65 and 437 adults sampled in 2012 and 2013, respectively. Adult activity was detected from 27 March to 16 October 2012, and the first reproductively active female was sampled on 27 March. Shown in Figure 3.3 are degree-day accumulations from the date of the first adult captured on 27 March. Based on temperature requirements for development, those reproductive females sampled in mid-June and July could be reproductively active f_1 females. The phenology of fruit damage in 2012 was not consistent with adult populations sampled. Fruit damage increased from approximately 3% in the first half of April to almost 50% by the second half of June, then gradually declined for the remainder of the year until assessment ceased at the end of September, when damage averaged ~8%.

The period of adult activity in 2013, from 18 April to 26 September was similar to 2012, despite the fact that considerably higher numbers were captured in 2013 (65 in 2012, 437 in 2013). As in 2012, highest captures in 2013 occurred in May rather than April. In contrast to 2012, in 2013 reproductively mature females were detected from April through mid-August, compared to March through late July in 2012. The degree-day scale in Figure 3.4A highlights the possibility of f_1 reproductively active females, as the 658 DDs required to complete a generation occurred by early July. Fruit damage phenology differed from 2012; damage increased from just over 20% in early May to >90% by the end of July. Damage remained over 70% for the remainder of the sampling season.

Lincoln County Location: This orchard was representative of apple production in the lower elevation foothill production region of North Carolina, with an elevation of 335 m. The period of primary adult activity was 17 April-24 September, with a total of 160 collected over the season. The first reproductively active female was sampled on 17 April. Degree-days accumulation of 880 between 17 April (first adult) and when stage three ovaries were observed after a period of none (the first on 23 July) indicate that these individuals could be reproductively mature f_1 females. The phenology of fruit damage at this location was not consistent with adult populations sampled (Fig. 3.5). Fruit damage increased from approximately seven percent in the beginning of May, to 45% in early June. Damage then decreased until late July, when a spike in damage (~60%) was recorded. Damage decreased from ~60% to ~20% and remained at this level until assessments ceased.

South Carolina Location: This orchard was representative of peach production in the lower elevation foothill production region of South Carolina, with an elevation 305 m. Adult activity occurred from 16 April- 24 September, with a total of 206 collected. The first reproductively active female was sampled on 22 April. Using 16 April as biofix, 607 DD accumulated by 19 June and 712 DD by 26 June. Hence, those reproductive females sampled in mid-June to late August may have been reproductively active f_1 females. Unfortunately, fruit damage at this location was could not be assessed after 19 June due to a high degree of rot.

Discussion

Trap Captures: There were difficulties obtaining plum curculio lures in 2012, and trees and pyramid traps were not baited until the week of 18 May. In previous studies, pyramid traps baited with benzaldehyde and/or grandisoic acid were significantly more attractive to plum curculio (Akotsen-Mensah et al. 2010) in the early season (Piñero et al. 2001). The lack of pheromone early in the season for the high elevation location in 2012 may provide a partial explanation for why this site resulted in the lowest capture rates for all sample sites. Collections of plum curculio adults were low in 2012 with all sampling techniques. In 2013, however, highest mean collection numbers were found with baited tree tapping. In contrast to past studies, our results suggest that baited trees and traps do not increase the attractiveness to plum curculio adults.

Low captures in pyramid traps may have been due to traps not being optimally placed for plum curculio captures. Prokopy and Wright (1998) reported that pyramid traps adjacent to apple trees captured significantly more adults than those placed between trees, between apple trees and adjacent woods, and between apple trees and an adjacent field. Because of the dense canopy of our orchards, most traps were placed just outside the canopy. Lafleur et al. (2007) suggested that the plum curculio, like other organisms, seeks to avoid predation. Thus, positioning of the pyramid trap away from the tree trunk and it's protective canopy could have exacerbated the plum curculio's thanatosis behavior, causing the insect to drop to the ground prior to reaching the lures positioned in the top of the trap.

Trap captures were also low in circle traps. Johnson et al. (2002) reported that screen traps may be used on trees >38 cm in circumference, and that when used on trees of this circumference, trap captures between pyramid and circle traps were similar. All trees used in this study met screen trap criteria. Although circle traps caught more weevils than pyramid traps, in our study, both trapping mechanisms appear to be less effective than beat sampling.

A contributing factor to overall higher trap and beat sampling captures in 2013 than 2012 may have been greater rainfall in 2013. Lamothe et al. (2008) demonstrated that an increase in humidity or rainfall is associated with an increase in the number of plum curculios captured. At MHCRS in 2012, a total of 43.8 cm of rain fell between 1 April and 31 August, compared to 2013, when 123.7 cm of rain fell in the same period and location. Past studies have also shown that at full bloom in apple orchards, plum curculios moved into tree canopies when humidity was high, then returned to the ground when humidity was low (Racette et al. 1991, 1992), indicating that plum curculio activity is associated with rainfall (Lanthrop 1949). This, in conjunction with early season trap and tree baiting, could have influenced capture amounts in this study.

This study is the first description of the phenology of plum curculio in western North Carolina. This area is considered a transition zone between univoltine and multivoltine strains (Chapman 1938). Similar to results in West Virginia (Leskey and Wright 2004, Leskey 2008), two general periods of active oogenesis were detected, with overwintered females reaching sexual maturity in late April-early May, and f_1 females reaching sexual maturity late June-early August. The results reported here suggest that the proportion of individuals that were multivoltine, as seen in the presence of developing and mature ovaries in July and August, is small. In 2012 for both locations reported here, 65.2% of ovaries showed no signs of development (stage 1), 13% were developing (stage 2), and 21.9% were mature (stage 3). These numbers were similar to all locations combined in 2013, with 71.4% females containing stage 1 ovaries, 22.8% developing (stage 2), and 5.9% mature (stage 3).

It is likely that a partial generation exists at these three locations, as dates of active oogenesis differ from dates further south (Akotsen-Mensah et al. 2010), such as in Alabama where the second generation of plum curculios begins emerging in late May and early June. These findings do not directly contradict the only published map that illustrates the distribution of univoltine and multivoltine plum curculio, but rather strengthen the legitimacy of the statement that western North Carolina along the Appalachian Mountains is a transition zone in voltinism. Zhang et al. (2008), using partial sequences of mitrochondrial cytochrome oxidase I (mtCOI) gene, revealed that univoltine and multivoltine strains of plum curculio were phylogenetically distinct, with differences in the strains of *Wolbachia* bacteria present in each plum curculio strain. Their approach could be used to further clarify the relative abundance of univoltine and multivoltine strains in different zones of the transition area.

In summary, this study demonstrated the possibility of a partial second generation of plum curculio existing at the three lower elevations, MHCRS, Lincoln NC and Greenville, SC, in the Southern Appalachians. Highest insect captures occurred via baited tree tapping in late April and early May for higher and middle elevations, and in July in lower elevations. When left unmanaged, the plum curculio can cause anywhere from 15% to +90% damage to crops. Future studies should include additional sample sites to further clarify the distribution and relative abundance of univoltine and multivoltine strains in this region.

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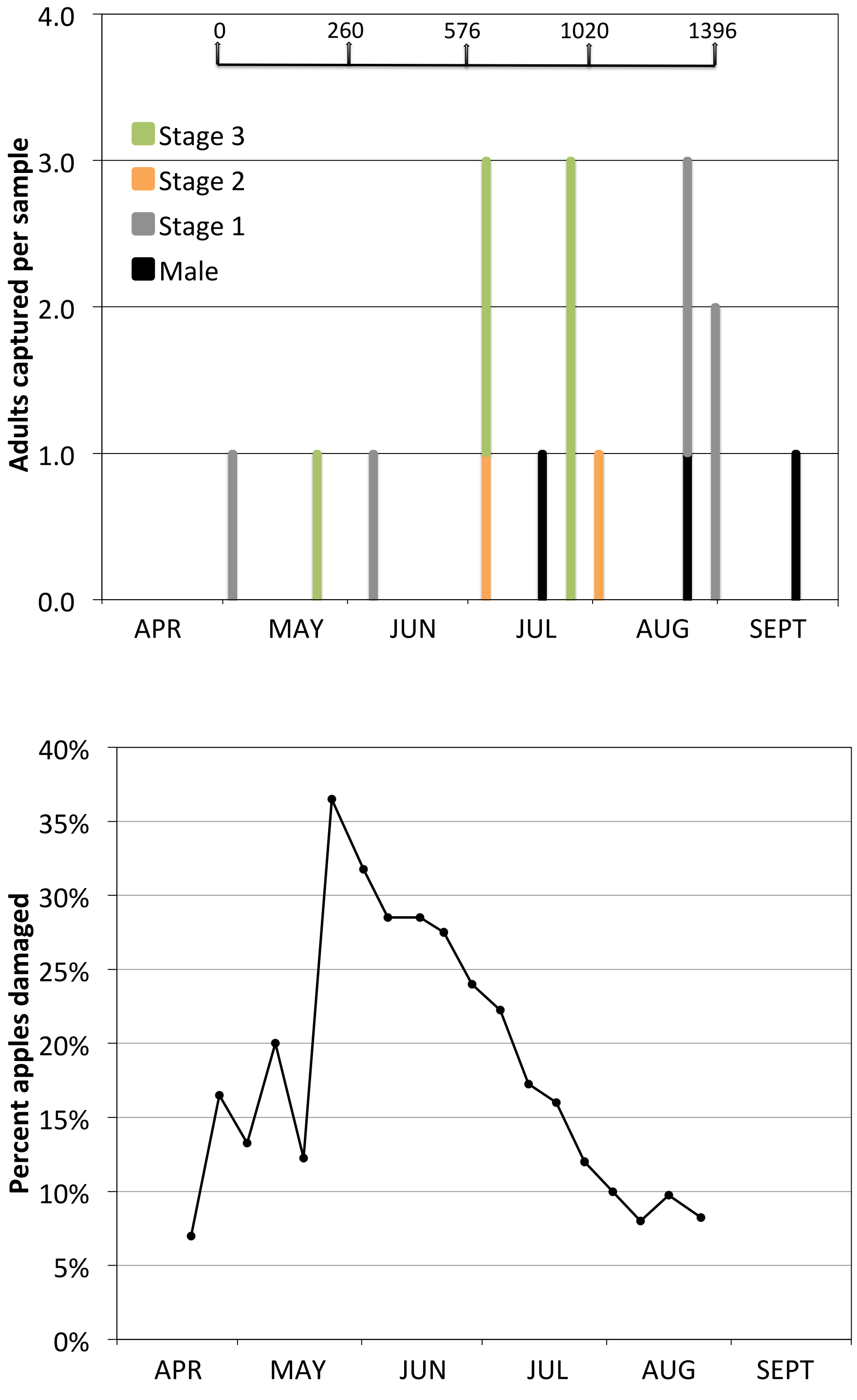


Figure. 3.1 Total number plum curculio adults captured separated by sex and stage of ovarian development of females (top figure), and percentage of fruit damaged (bottom figure). Altapass orchard, 2012.

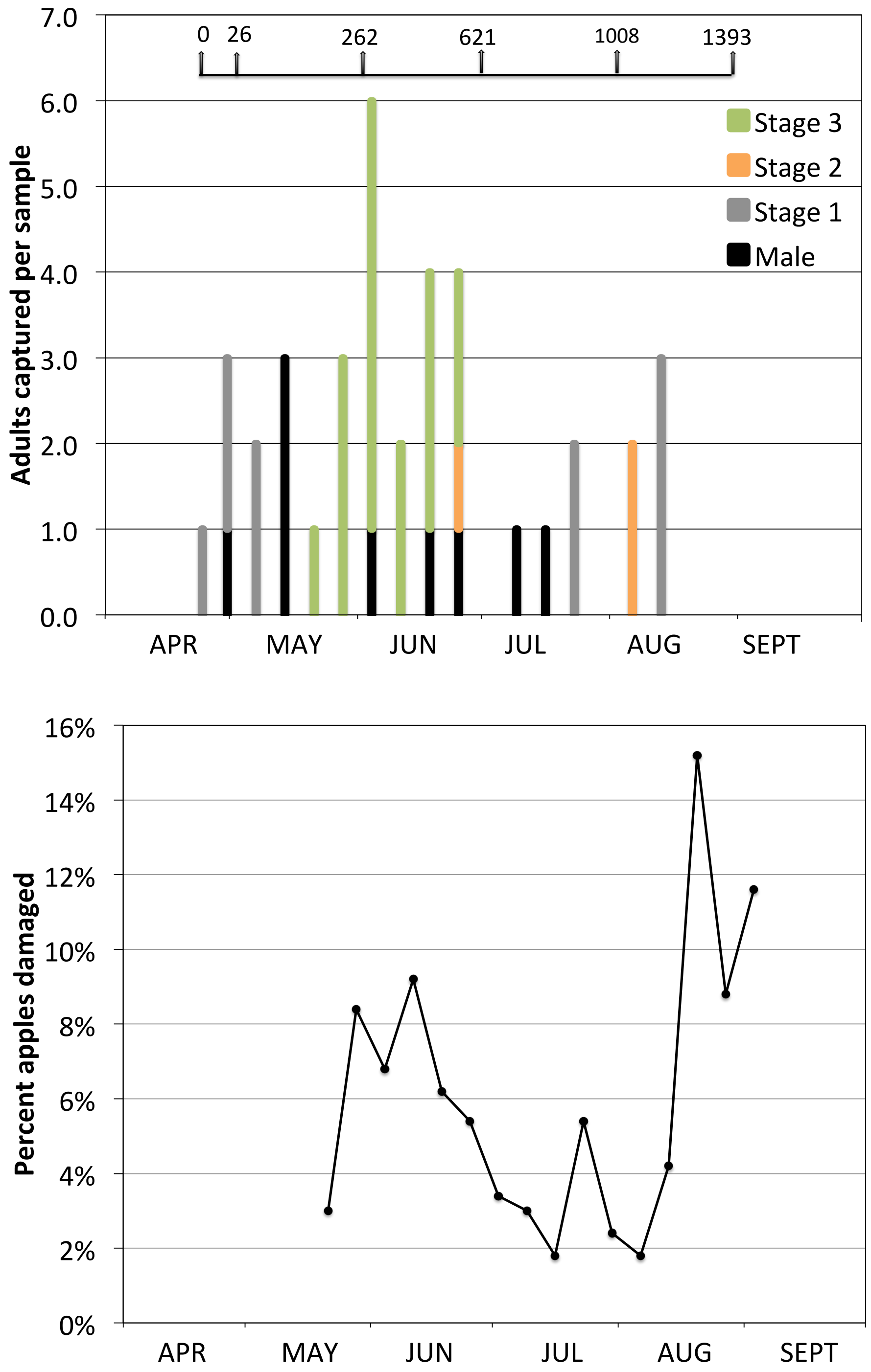


Figure. 3.2 Total number plum curculio adults captured separated by sex and stage of ovarian development of females (top figure), and percentage of fruit damaged (bottom figure). Altapass orchard, 2013.

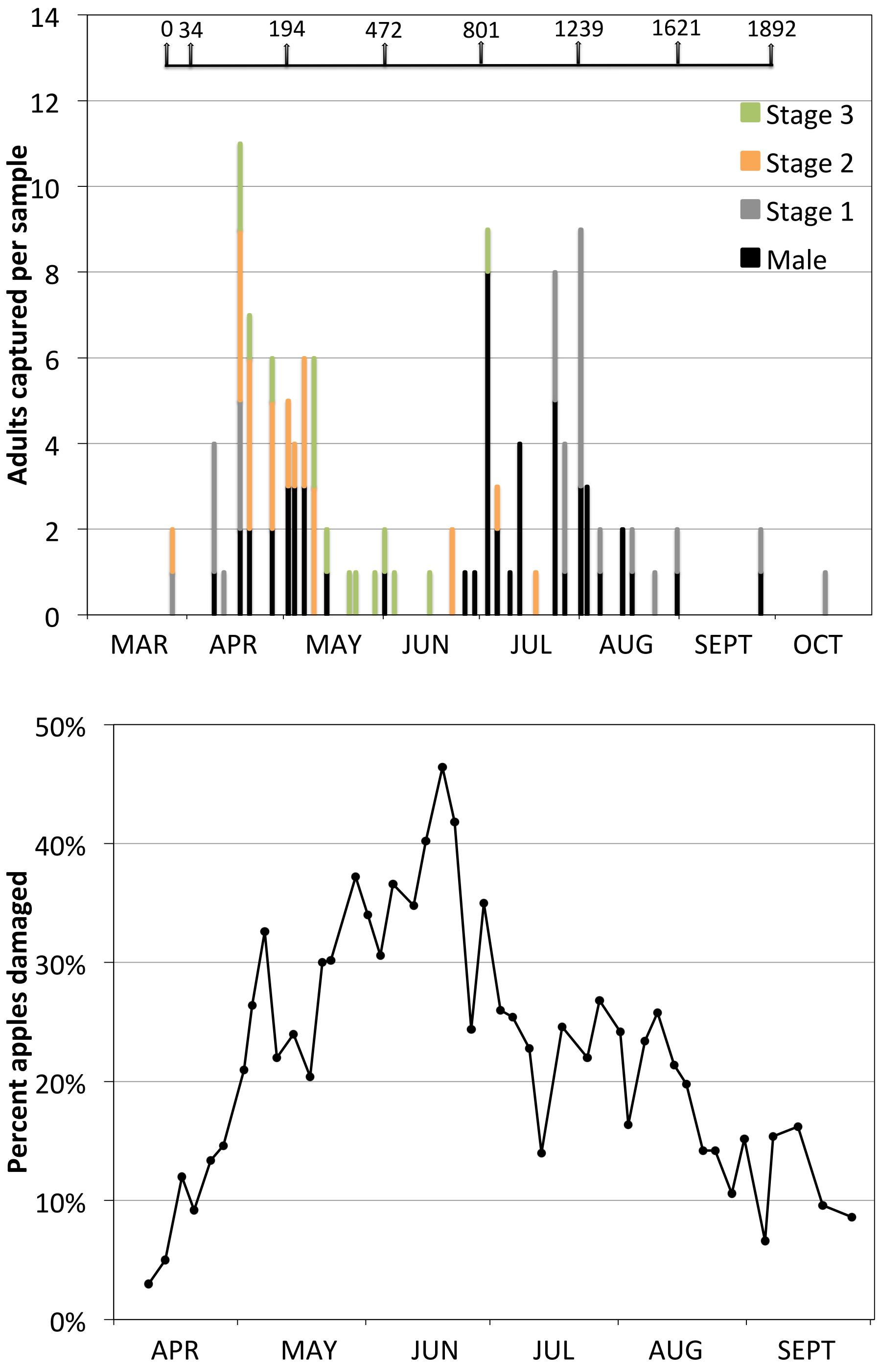
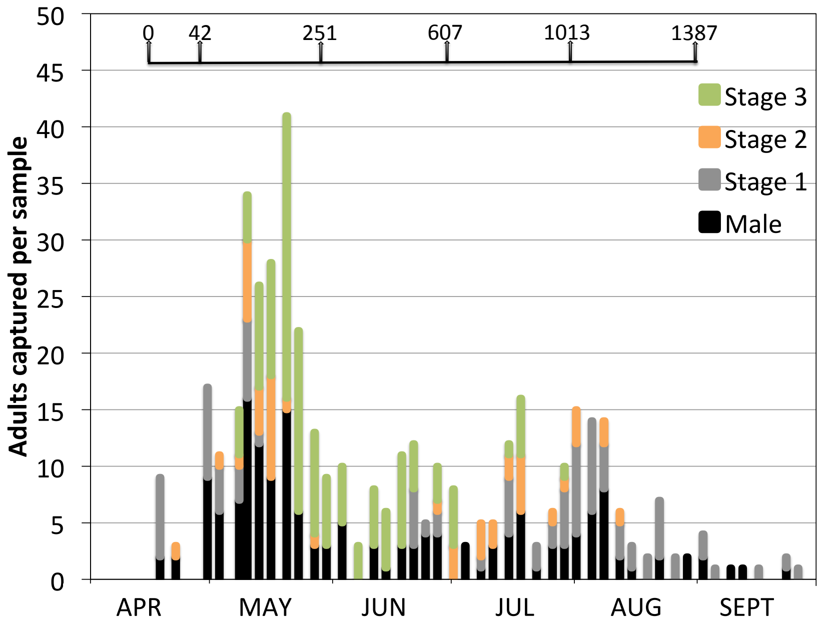


Figure. 3.3 Total number plum curculio adults captured separated by sex and stage of ovarian development of females (top figure), and percentage of fruit damaged (bottom figure). MHCRS orchard, 2012.



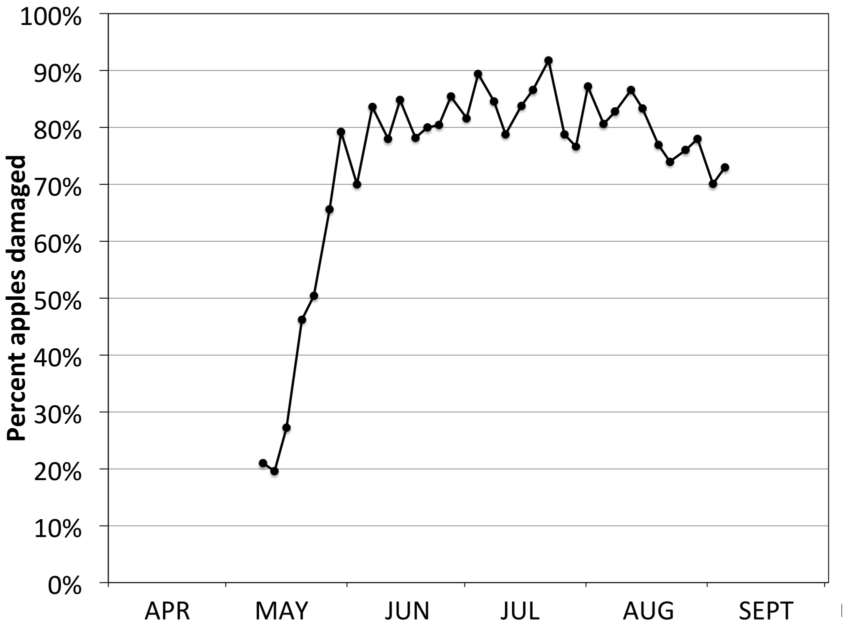


Figure. 3.4 Total number plum curculio adults captured separated by sex and stage of ovarian development of females (top figure), and percentage of fruit damaged (bottom figure). MHCRS orchard, 2013.

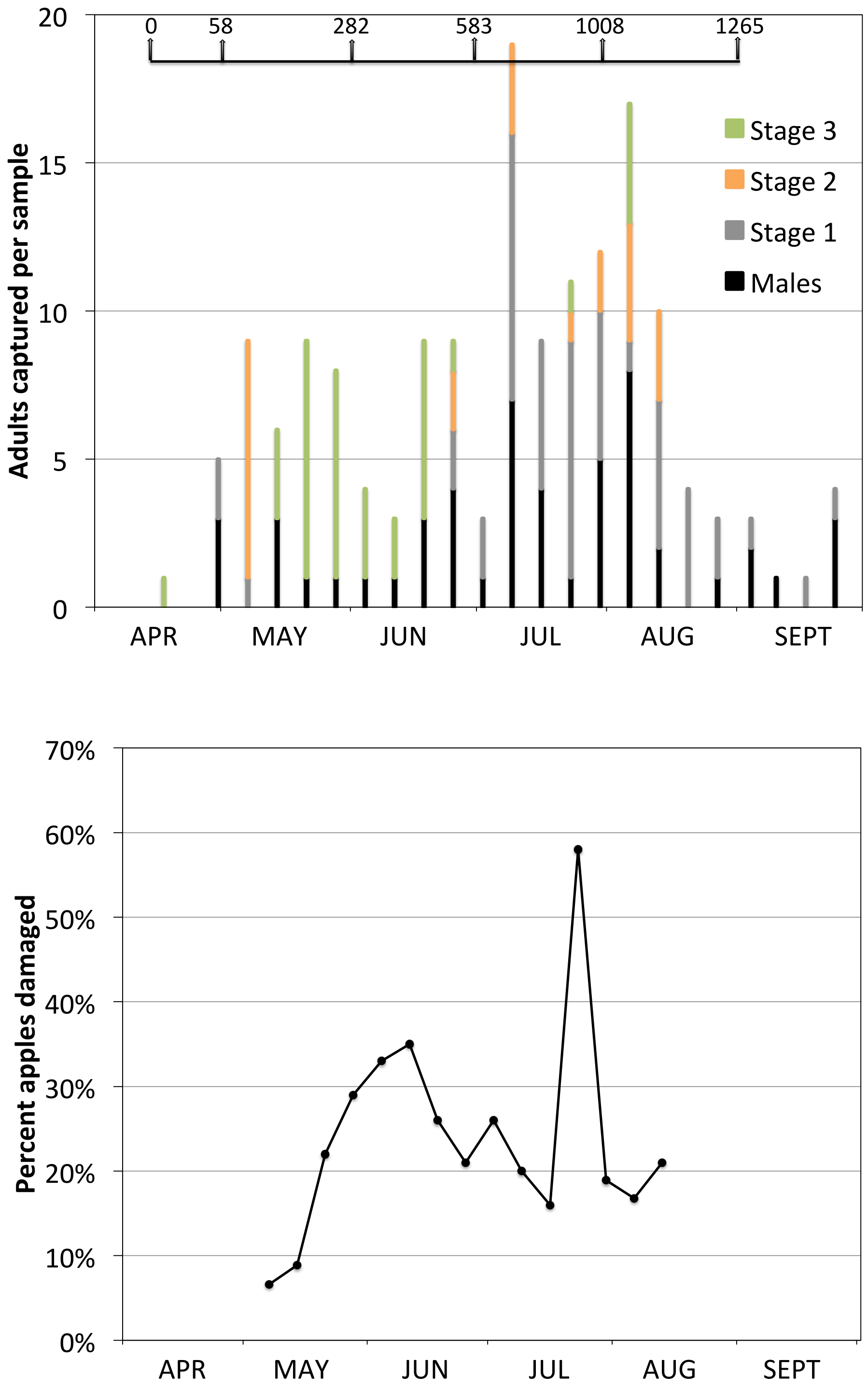


Figure. 3.5 Total number plum curculio adults captured separated by sex and stage of ovarian development of females (top figure), and percentage of fruit damaged (bottom figure). Lincoln Co. orchard, 2013.

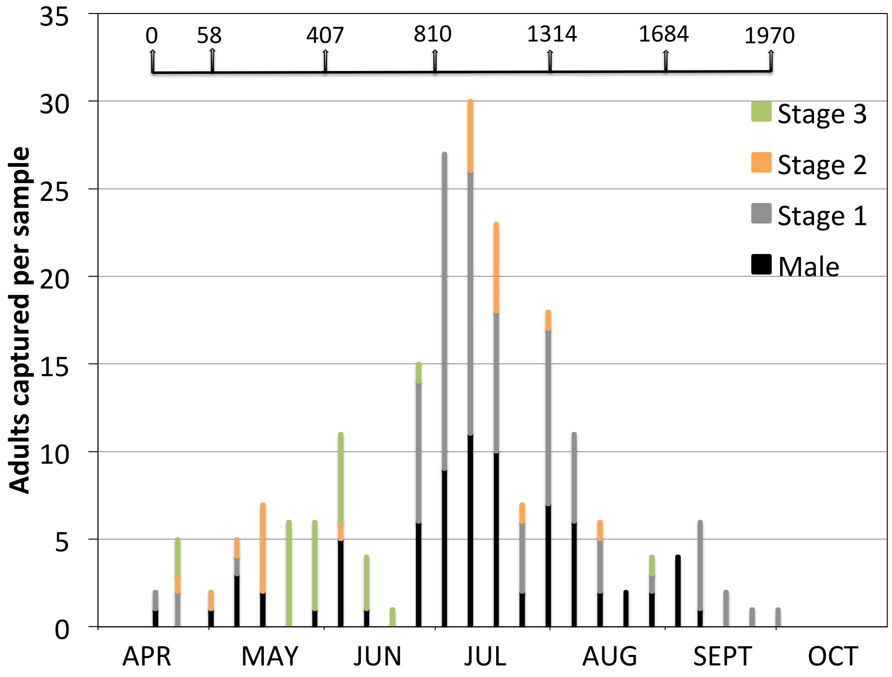


Figure. 3.6 Total number plum curculio adults captured separated by sex and stage of ovarian development of females. Greensville SC, 2013.

Table 3.1. Mean (± SEM) number of season-total plum curculios recorded using various sampling methods.

	2012		2013			
Sampling method	Altapass	MHCRS	Altapass	MHCRS	Lincoln	SC
Baited beat	1.5 (0.5)	4.4 (1.5)	5.0 (1.1)	45.8 (10.1)	8.0 (1.4)	30.7 (10.4)
Unbaited beat	0.3 (0.3)	2.6 (0.9)	2.5 (0.6)	38.3 (10.3)	7.7 (1.6)	25.0 (7.1)
Baited Circle trap	1.5 (1.5)	1.5 (1.5)	1.5 (0.6)	6.0 (5.4)	4.4 (1.0)	11.0 (1.5)
Unbaited Circle trap	0.5 (0.5)	2.0 (0)	-	-	-	-
Baited pyramid trap	0.5 (0.5)	0.5 (0.5)	0.5 (0.3)	2.3 (0.8)	3.0 (0.9)	2.3 (0.9)
Unbaited pyramid trap	2.0 (0)	0	-	-	-	-
Unbaited beat (peach)	—	1.8 (1.0)	-	8.2 (1.0)	-	-
Total	6.3	12.8	9.5	100.6	23.1	69

[1] Baited traps included both grandosic acid and benzaldyhede.