A FLORIDA PERSPECTIVE ON HOST PREFERENCE, EARLY DETECTION, AND IDENTIFICATION OF THE BROWN MARMORATED STINK BUG, *Halyomorpha halys* (Stål)

By

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The brown marmorated stink bug (*Halyomorpha halys* (Stål)) is an invasive agricultural and urban pest in the United States with over 300 recorded host plants. Since its first detection in the late 1990s, this species has become established in at least twelve states and the distribution continues to expand. Although it is not known to be established in Florida, increasing our understanding of the pest potential for *H. halys* in Florida may allow for early detection and provide informed, economical management recommendations. To determine host specificity and potential impact of *H. halys*, eight plant species commonly found in Florida's agronomic crops and landscapes were tested using choice and no-choice tests. The results suggested that *H. halys* preferred to feed on woody hosts, such as ligustrum and hibiscus, as opposed to *Citrus* spp. and avocado. This research will be continued using a variety of other plant species in order to yield more conclusive results.

A Florida-specific stink bug field guide and invasive species workshop trainings targeted to Florida State Park Service personnel were developed to increase identification and monitoring efforts of *H. halys*, and ultimately assist with management

efforts if and when it establishes. The efficacy of the stink bug field guide was assessed through an identification activity, and only 18% of participants correctly identified *H. halys*. Pre- and post-surveys used to assess the efficacy of the workshop trainings illustrated an overall positive impact. Future training sessions will target Florida Master Gardeners, nurserymen, small farm producers, and public gardens personnel.

CHAPTER 1
IMPORTANCE OF INVASIVE SPECIES AND THE BROWN MARMORATED STINK BUG IN FLORIDA

Introduction

An invasive pest is defined as a plant, animal or pathogen species that is not native to the ecosystem that causes or is likely to cause economic or environmental harm or harm to human, animal, or plant health (ISAC 2006). Invasions by non-native species in the United States cost an estimated $137 billion annually. Arthropod species account for $20 billion of this total due to environmental damages, lost agricultural productivity, and increased health problems (Pimentel et al. 2010). The introduction and establishment of an invasive species often leads to expensive eradication and control efforts and can severely impact the trade industry in addition to the direct environmental and agricultural damage caused by pest species.

Because invasive species are commonly introduced through accidental human involvement or immigration, it is no surprise that Florida is considered a high risk state for invasive species due to multiple pathways of introduction, suitable climate conditions, and a large agriculture industry. With 12 international airports and 14 deepwater seaports, Florida is a major destination for global trade especially for agricultural commodities (Silagyi 2005). In fact, Miami International Airport is the epicenter of United States cut flower, and fruit and vegetable imports, receiving 88% and 55% of shipments respectively. Only a small percentage of imported plant materials are sampled and inspected at ports of entry by the Department of Homeland Security's (DHS) Customs and Border Protection and U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) for exotic plant pests, diseases, pathogens, and noxious weeds (USDA-APHIS-PPQ 2012).

Although safeguarding systems have been established and implemented by state and federal agencies to prevent, detect, eradicate, and mitigate damage of invasive pests, an average of two new non-native arthropod species are currently detected in Florida per month. The Florida Cooperative Agricultural Pest Survey (CAPS) program also facilitates the prevention of pest introduction through import inspections at high-risk interstate interdiction stations. In 2012, the Florida CAPS Interdiction Survey team collaborated with FDACS Agricultural Law Enforcement and detected 116 exotic pest species (Russel 2012). Of the 116 species, an important invasive species commonly known as the brown marmorated stink bug (*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)) was intercepted at least three times. Additional interceptions of this invasive species were from tourist vehicles travelling to Florida from states infested with this pest (Halbert and Hodges 2011).

Significance of *Halyomorpha halys*. *Halyomorpha halys*, the brown marmorated stink bug, is an invasive pentatomid species found in North America. This highly polyphagous species has over 300 host plants recorded (Nielsen et al. 2008a), including host plants of agricultural importance such as vegetable crops, woody shrubs, and fruit trees. Initially, *H. halys* was not known to be a significant agricultural pest in the United States. Since 2006, *H. halys* has become a major pest of fruit trees and soybean in the Northeastern and Mid-Atlantic U.S. Nielsen and Hamilton (2009a) recorded 25 – 80 % damage by *H. halys* to apples and pears in Pennsylvania and New Jersey. In the early season, *H. halys* may cause catfacing, dimpling, or concave surface damages. If *H. halys* feeds on the fruit in mid to late season, necrotic areas are seen where the

proboscis was inserted. As the distribution of *H. halys* has expanded and numbers increased, it has become a pest of economic concern in the United States.

Halyomorpha halys is native to East Asia and is found in China, Japan, Korea, Myanmar, Taiwan, and Vietnam (Yu and Zhang 2007; Yang et al. 2009). Although the means of introduction are not certain, *H. halys* is thought to have been accidentally brought into the United States by hitchhiking on imported cargo from China, Japan, or Korea (Hoebeke and Carter 2003). In 2001, *H. halys* was detected in Allentown, Pennsylvania and identified by Karen M. Bernhard, an extension entomologist with the Lehigh County Cooperative Extension office. Although this was the first report of this species in the Western hemisphere, *H. halys* is believed to have immigrated to the United States as early as 1996. Since 2001, *H. halys* has become established in the following states: llinois, Maryland, Michigan, Minnesota, New Jersey, North Carolina, Ohio, Oregon, Pennsylvania, Tennessee, Virginia, and Washington (NAPIS 2013). *Halyomorpha halys* has also been detected or intercepted, but is not known to be established, in the following states: Colorado, Connecticut, Florida, Georgia, Indiana, Kentucky, Maine, Massachusetts, New Hampshire, Rhode Island, South Carolina, Vermont, West Virginia, and Wisconsin (Halbert, personal communication; NAPIS 2013). *Halyomorpha halys* has also spread to Europe. In 2007, *H. halys* was reported in Zurich, Switzerland. Wermelinger et al. (2008) described five separate occurrences of *H. halys* in Switzerland – three reports of single individuals, one report of egg masses, and one report of infestation. At this time, it is unknown how the species was introduced to Zurich.

Literature Review of the Brown Marmorated Stink Bug

Life History

As with most pentatomid species, *Halyomorpha halys* (Stål) completes five nymphal instars before reaching the adult stage. At an optimal temperature of 25^{o}C, egg ecolsion occurs after six days from oviposition (Nielsen et al. 2008a). First-instar nymphs burst through a black triangular structure (egg burster) and exit through a circular operculum surrounded by micropylar processes at the top of the egg (Yang et al. 2009). Nymphs tend to aggregate around the egg mass until ecdysis, or molting, from first to second instar occurs. Second-instar nymphs disperse and begin feeding on leaves, stems, and occasionally the fruit of host plants. Developmental time at an optimal temperature of 25^{o}C for first-, second-, third-, fourth-, and fifth-instar nymphs is 5, 10, 7, 7, and 11 respectively with a total developmental time (egg incubation to adult) of 50 days (Nielsen et al. 2008a). Although development can occur between 17 and 33^{o}C, temperature significantly effects developmental time and mortality.

Adult *H. halys* emerge mid-March to April and are active until October in Pennsylvania (Nielsen and Hamilton 2009b). Females must complete reproductive maturation before copulation can occur. Adult females will oviposit between June and September in the northeast. Egg masses are deposited in clusters on the underside of leaves with an average of 28 eggs per mass (Nielsen et al. 2009b; Leskey et al. 2012b; Medal et al. 2012). The eggs are small (about 1mm diameter), pale green to white in color, and spherically shaped (Gyeltshen et al. 2011).

From mid-September to early spring in its current U.S. distribution, adults reach a reproductive diapause and exhibit overwintering behavior. Males release pheromones for aggregation purposes, although the pheromone has not been identified, and large

numbers of aggregated adults will retreat to nearby buildings and structures (Yu and Zhang 2007). In contrast, other native stink bugs (i.e. *Euschistus* spp., *Brochyamena* spp. and *Parabrochyamena* spp.) diapause in leaf litter of wooded areas. Since effective methods for control or long-term management are not available, homeowners are being overwhelmed with the quantity of *H. halys* invading their homes (Jacobs 2009; Gyeltshen et al. 2011; Halbert and Hodges 2011).

Halyomorpha halys has been known to produce one or two generations per year, both in northern China and mid-Atlantic regions of the United States (Nielsen et al. 2008a; Nielsen and Hamilton 2009b). However, *H. halys* has produced up to six generations per year in southern regions of China (Hoffman 1931) and a similar trend may occur in southern states such as Florida. According to a potential geographic distribution map produced using ecological niche modeling, the northern regions of Florida are highly suitable for *H. halys* establishment while central and southern regions of Florida may be less suitable for establishment (Zhu et al. 2012). Ecological niche modeling identifies environmentally suitable areas for invasive species by comparing climatic conditions in their native range to areas where the species have or could have been introduced. Zhu et al. (2012) considered six bioclimatic variables (e.g. temperature, precipitation, sunshine, and elevation) to produce the potential geographic distribution of *H. halys*. Although ecological niche modeling is useful for detecting potential areas of invasion and generates opportunities for exclusion, it does not consider biotic interactions (i.e., predators, parasitoids, pathogens, etc.) or dispersal ability of a species. For that reason, all areas of predicted suitability as suggested by ecological niche modeling may not be suitable for establishment.

Natural Enemies

There are many natural enemies of *H. halys* with some being more effective than others. Predators and parasitoids of *H. halys* may attack the eggs, nymphs, or adults and can include parasitic microhymenoptera and flies, ants, earwigs, lacewings, assassin bugs, other stink bugs, spiders, and birds.

Six egg parasitoids of *H. halys* have been documented in China, each with varying rates of parasitism. These are: *Trissolcus flavipes* Thomon, *Telenomus mitsukurii* (Ashmead), *Anastatus* spp., *Acroclosoides* spp., *Telenomus podisi* Ashmead, and *Trissolcus halyomorphae* Yang (Yu and Zhang 2007; Yang et al. 2009). Of the six species mentioned, *T. halyomorphae* has the highest parasitism rate and seems to be the most effective egg parasitoid for biological control (Yang et al. 2009).

Control Methods

Nielsen et al. 2008b tested the toxicity of nine insecticides on *H. halys* in the laboratory: one organophosphate (phosmet); three neonicotinoids (dinotefuran, acetamiprid, and thiomethoxam); and five pyrethroids (β-cyfluthrin, cyfluthrin, fenpropathrin, bifenthrin, and λ-cyhalothrin). Pyrethroids, particularly bifenthrin and λ-cyhalothrin, and neonicotinoids, dinotefuran and thiomethoxam, were the most effective insecticides against *H. halys* populations (Nielsen et al. 2008b). However, the insecticides were only tested in a laboratory setting and field results have not yet been reported.

Although insecticides may provide minor relief in and around homes, they have not been successful at controlling populations of *H. halys* for long-term pest management (Jacobs 2009). Instead, natural enemies have been the focus for potential biological control. In the U.S., no known naturally occurring specialist-predators of *H.*

halys currently exist. In China, *Trissolcus halyomorphae* is a native solitary egg parasitoid of *H. halys* with a high parasitism rate of 20 – 70% (Yang et al. 2009). In northern regions of China, *T. halyomorphae* can respond rapidly to increases in numbers of *H. halys* because *T. halyomorphae* can produce up to ten generations per year while *H. halys* produces one or two generations per year.

It is unknown whether this species would be an effective biological control agent or negatively impact native stink bugs in the United States. The Agricultural Research Service (ARS) program of the United States Department of Agriculture (USDA) is collaborating with several states in a research project to determine the suitability of *T. halyomorphae* as a biological control agent of *H. halys*. Testing of *Trissolcus halyomorphae* is currently taking place in Delaware, Michigan, Mississippi, Oregon, and Florida (Smith, personal communication). In Florida, the Bureau of Methods Development and Biological Control at the Florida Department of Agriculture and Consumer Services Division of Plant Industry (FDACS DPI) is currently researching the host preference of *T. halyomorphae* by exposing the parasitoid to native and established stink bugs of Florida. If the results of the research are favorable, *T. halyomorphae* may be considered for release in the United States to control *H. halys*.

Host Plants

Halyomorpha halys is a polyphagous plant feeder with a wide range of host plants. In its native habitat, *H. halys* reportedly feeds on at least 45 different host plants (Table 1-1). These include fruit trees such as apples (*Malus* spp.), oranges (*Citrus* spp.), pears (*Pyrus* spp.), and plums (*Prunus domestica* L.) (Yu and Zhang 2007). Vegetables and other crops serve as hosts as well. For example, *H. halys* has been

known to feed on cotton (*Gossypium hirsutum* L.), soybeans [*Glycine max* (L.)], and wheat (*Triticum aestivum* L.) (Yu and Zhang, 2007) (Table 1-1).

Populations of *H. halys* have been reported on the following hosts in the U.S. and China: apple, bean, celosia, corn, grape, hibiscus, lilac, pear, princess tree, soybean, and sunflower (Table 1-1). In Pennsylvania, host plants with the largest amount of *H. halys* activity consist of highbush cranberry (*Viburnum opulus* L. var. *americananum* Aiton), Siberian pea shrub (*Caragana arborescens* Lamarck), and princess tree [*Paulownia tomentosa* (Thunberg)] (Nielsen et al. 2008a).

Since its detection in Switzerland in 2007, infestations of nymphs and egg masses of *H. halys* have been documented on eight host plants including asparagus (*Asparagus* spp.), blue bean shrub (*Decaisnea fargesii* Franchet), Japanese angelica tree [*Aralia elata* (Miquel)] Japanese stewartia (*Stewartia pseudocamellia* Maximowicz), and nasturtium (*Tropaeolum majus* L.) (Wermelinger et al. 2008). Two hosts of *H. halys* in common with the U.S. and Switzerland include butterfly bush and maple (Hoebeke and Carter 2003; Wermelinger et al. 2008).

Much of the literature has reported host plants of *H. halys* through observations based on presence or feeding on these plants. However, some sources have not thoroughly documented the ability of *H. halys* to survive and reproduce on a given host. In fact, several documented hosts may be alternative hosts when preferred hosts are not available. Further research on the host preferences of *H. halys* will distinguish the known hosts from alternative hosts.

Monitoring Methods

Monitoring and identification of both commonly occurring and exotic species can be an important component of a successful integrated pest management (IPM)

program. Implementing pest monitoring as a part of IPM programs may allow for early detection of exotic species and improved overall pest management and/or eradication efforts. Various methods have been used to monitor populations of *H. halys* including visual pyramid traps, pheromone-baited pyramid traps, beat sheet sampling, sweep net sampling, and light traps.

Visual pyramid traps have commonly been used to monitor for several stink bug species, such as *Chinavia hilare* (Say), *Euschistus* spp., *Nezara viridula* (Linnaeus), and *Oebalus pugnax* (Fabricius) (Leskey and Hogmire 2005; Rashid et al. 2006; Kamminga et al. 2012). Generally, a yellow base pyramid trap design is used to trap stink bugs with or without a pheromone bait due to its attractiveness across species (Leskey and Hogmire 2005; Mizell 2008; Kamminga et al. 2012). However, significantly greater numbers of *H. halys* nymphs and adults were captured with black pyramid traps than any other colored traps with visual stimuli of green, yellow, clear, or white (Leskey et al. 2012b). The most effective results were obtained when the traps were baited with 450 mg of methyl (2E, 4E, 6Z)-decatrienoate.

Pyramid traps baited with the aggregation pheromone of *Plautia stali* Scott (Hemiptera: Pentatomidae), methyl-(2E,4E,6Z)-decatrienoate have been reported to yield more *H. halys* individuals than any other type of monitoring method (Khrimian et al. 2008; Leskey et al. 2012a; Leskey et al. 2012b). This pheromone attracts both male and female adults as well as nymphs, and the number of individuals captured is dependent on increasing amounts of methyl-(2E,4E,6Z)-decatrienoate (Leskey et al. 2012a). However, this lure is only reliable during late-season population peaks from July to the

first frost (Nielsen et al. 2011). Although it is currently being investigated, the identification of the true aggregration pheromone for *H. halys* is unknown.

Active sampling techniques to monitor for *H. halys* include beat sheet sampling and sweep net sampling. Beat sheet sampling has been used to collect adults and nymphs from fruit trees and woody shrubs (Nielsen et al. 2008b; Nielsen and Hamilton 2009a; Nielsen and Hamilton 2009b; Leskey et al. 2012), while sweep net sampling is more commonly used for field crops such as soybean (Nielsen et al. 2011). These methods were less effective than pyramid traps in the early crop season (Nielsen et al. 2011). Higher capture rates of nymphs and adults were obtained with these methods late in the growing season.

Black light traps are commonly used in the field to monitor the distribution and abundance of *H. halys*, primarily because these traps are effective, easily positioned in the field, and fairly inexpensive. Like many other pentatomid species, *H. halys* is more active at dusk and in the evening. Although these traps are non-specific, they are a highly reliable and effective monitoring tool for early season and low density populations of *H. halys* (Nielsen et al. 2013) unlike pheromone or pyramid traps.

When used independently, each collection technique has its own limitations during different seasons and times of day as well as varying levels of cost and benefits. However, applying multiple detection methods would be a highly effective approach for monitoring *H. halys* populations.

Identification

Adult *H. halys* have a dark brown marbled color on the dorsal side, a pale color on the ventral side, and are typically 12-17mm long. Alternating light and dark bands occur along the lateral edges of the abdomen. The shoulders of the pronotum are

rounded and smooth. The most distinguishing feature is the light and dark banding on the fourth and fifth segments of the antennae. The same banding is often present on the legs as well. Synonyms of *H. halys* in the literature include *H. picus* (F.), *H. brevis* (Walker), and *H. mista* (Walker) (Hoebeke and Carter 2003).

Many other Hemipteran species may be mistaken for *H. halys*. Species of similar appearance in the United States include the brown stink bug [*Euschistus servus servus* (Say)], bark stink bugs (*Brochymena* spp. and *Parabrochymena* spp.), spined soldier bug (*Podisus maculiventris* Say), *Holcstethus* spp., and late instar nymphs of leaf-footed bugs [*Leptoglossus phyllopus* (L.)] (Hoebeke and Carter 2003; Sargent et al. 2010; Halbert and Hodges 2011). The brown stink bug (*E. servus*) may be confused with *H. halys* because it has similar coloring and alternate light and dark banding on the edges of the abdomen. The two species may be distinguished by the pale marks on the edge of the abdomen – *H. halys* has triangular markings and the markings on *E. servus* are more quadrate. *Brochymena* and *Parabrochymena* species may also have similar coloring and banding; however, these bugs have denticulate anterolateral margins on the pronotum. In addition, the shoulders of the pronotum and the membrane of the forewing has dark markings outlining reticulate veins, whereas the anterolateral margins on *H. halys* are smooth and the veins are pale with a few darker spots. *Podisus maculiventris* has spinose shoulders on the pronotum, and both *P. maculiventris* and *Holcostethus* spp. are smaller in size compared to *H. halys*.

Brown Marmorated Stink Bug Extension Education

In 2011, Florida reached a record number of 87.3 million visitors and an estimated value of $67 billion for the tourism industry making this state the top travel destination in the world (“Florida Quick Facts,” 2013). As previously mentioned, *H. halys*

has been intercepted multiple times in Florida at agricultural interdiction stations and on vehicles traveling from infested states. Although established populations have not been detected, *H. halys* populations are likely to be introduced to Florida via tourist vehicles or luggage.

Efforts have been made to educate the Florida county extension agents about *H. halys* through state and UF-IFAS outreach and extension materials. Even with significant efforts, limited Florida State Park personnel educational activities have occurred for this species. Florida State Parks receive an average of over 20 million visitors traveling from Florida, other U.S. states, and international countries annually. As many reported hosts for *H. halys* include woody shrubs and trees, the potential establishment of undetected populations in Florida State Parks is a significant possibility. Delivery of effective extension education and monitoring tools to Florida State Park personnel is important for early detection of *H. halys* in Florida.

Research Objectives

Over the past ten years, published research on *H. halys* has increased significantly and a majority of current research is focused on monitoring and control methods as established populations become crop pests in the United States. Major gaps in the understanding of host preferences for this invasive pest exist. As the distribution increases, it is important to research the potential behaviors and impacts of *H. halys* on host plants unique or of significant economic importance to Florida's agriculture prior to establishment. Increasing our understanding of the pest potential for *H. halys* in Florida will assist in providing informed and economical management recommendations to extension clientele. In addition, communicating this knowledge to state employees, extension agents, and the general public may allow for early detection

of *H. halys* and create general awareness about the invasive pest. My research objectives include:

1. Determining host specificity of *H. halys* through host range and host preference tests on economically important host plants significant to Florida's agriculture and landscape.

2. Assessment of effective invasive species workshop training delivery to Florida State Park Service personnel.

3. Development and assessment of a Florida-specific stink bug identification card deck to be used by the general public.

Table 1-1. Reported hosts of *Halyomorpha halys*.

Common Name	Scientific Name	Location of Host Status	Detection Method	Reference
Angelica-tree, Japanese	*Aralia elata* (Miquel) Seemann	Switzerland	Heavy infestation of nymphs.	Wermelinger et al. 2008
Apple	*Malus* spp.	China	Literature	Yu and Zhang 2007; Sargent et al. 2010
Apple	*Malus domestica* Linnaeus	China		Hoebeke and Carter 2003
Apple, paradise	*Malus pumila* Mill.	China	Literature	Yu and Zhang 2007
Apricot	*Prunus armeniaca* (L.)	China	Literature	Yu and Zhang 2007
Apricot, Japanese	*Prunus mume* Siebold and Zuccarini	China		Hoebeke and Carter 2003
Arborvitae, oriental	*Platycladus orientalis* (L.)	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Argyi wormwood	*Artemisia argyi* H.Lév and Vaniot	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Asparagus	*Asparagus* spp.	Switzerland	Two egg masses collected; observed nymphs feeding.	Wermelinger et al. 2008
Basswood	Tilia spp.	United States		Hoebeke and Carter 2003
Bean	*Phaseolus* spp.	China; United States	Literature	Yu and Zhang 2007; Sargent et al. 2010
Beet	*Beta vulgaris* L.	China	Literature	Yu and Zhang 2007
Black nightshade	*Solanum nigrum* L.	China	Nymphs or adults observed on plant.	Hoebeke and Carter 2003; Yu and Zhang 2007
Bushkiller	*Cayratia japonica* Thunberg	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Butterfly-bush	*Buddleia spp.*	United States		Hoebeke and Carter 2003; Sargent et al. 2010
Butterfly-bush	*Buddleia davidii Franchet*	Switzerland	Slight infestation of nymphs; three nymphs collected from gardens and reared into adult stage.	Wermelinger et al. 2008

Table 1-1. Continued.

Common Name	Scientific Name	Location of Host Status	Detection Method	Reference
Camphor tree	*Cinnamomum camphora (L.)*	China	Literature	Yu and Zhang 2007
Catalpa	*Catalpa* spp.	United States		Hoebeke and Carter 2003; Sargent et al. 2010
Celosia	*Celosia argentea* L.	China; United States		Hoebeke and Carter 2003; Sargent et al. 2010
Chaste tree	*Vitex negundo* L.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Cherry	*Prunus avium* L.	China		Hoebeke and Carter 2003; Sargent et al. 2010
Cherry, Black	*Prunus serotina* Ehrhart	United States		Sargent et al. 2010
Cherry, Chinese sour	*Prunus pseudocerasus* L.	China	Literature	Yu and Zhang 2007
Cherry, Yoshino	*Prunus x. yedoensis* Matsumura	United States		Sargent et al. 2010
Corn	*Zea mays* L.	China; United States	Nymphs or adults observed on plant.	Yu and Zhang 2007; Sargent et al. 2010
Cotton, upland	*Gossypium hirsutum* L.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Eastern Redbud	*Cercis canadensis* L.	United States		Sargent et al. 2010
Elm	*Ulmus* spp.	United States		Sargent et al. 2010
Elm, Siberian	*Ulmus pumila* L.	China	Literature	Yu and Zhang 2007
Fig	*Ficus carica* L.	China	Literature	Hoebeke and Carter 2003; Yu and Zhang 2007; Sargent et al. 2010
Grape	*Vitis vinifera* L.	China; United States	Literature	Yu and Zhang 2007; Sargent et al. 2010
Hawthorne	*Crataegus pinnatifida* Bunge	China	Literature	Yu and Zhang 2007
Hibiscus	*Hibiscus rosa-sinensis L.*	China; United States		Hoebeke and Carter 2003; Sargent et al. 2010

Table 1-1. Continued.

Common Name	Scientific Name	Location of Host Status	Detection Method	Reference
Hollyhock	*Althaea rosea* L.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Honeysuckle	*Lonicera* spp.	United States		Hoebeke and Carter 2003; Sargent et al. 2010
Hop, Japanese	*Humulus scandens* (Lour.) Merr.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Jujube	*Ziziphus jujuba* (L.)	China	Literature	Yu and Zhang 2007
Lilac	*Syringa* spp.	China; United States	Literature	Yu and Zhang 2007; Sargent et al. 2010
Locust, black	*Robinia pseudoacacia* L.	China	Literature	Yu and Zhang 2007
Locust, honey	*Gleditsia triacanthos* L.	United States		Sargent et al. 2010
Maple	*Acer* spp.	United States		Hoebeke and Carter 2003; Sargent et al. 2010
Maple, sycamore	*Acer pseudoplatanus* L.	Switzerland	One nymph observed feeding on maple seed.	Wermelinger et al. 2008
Mulberry	*Morus* spp.	China		Hoebeke and Carter 2003; Sargent et al. 2010
Mum	*Chrysanthemum morifolium* (Ramat.) Kitam.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Nasturtium	*Tropaeolum majus* L.	Switzerland	Heavy infestation of nymphs.	Wermelinger et al. 2008
Oak	*Quercus* spp.	United States		Sargent et al. 2010
Orange, mandarine	*Citrus reticulata* Blanco	China	Literature	Yu and Zhang 2007
Orchid	*Brassia* spp.	China	Literature	Yu and Zhang 2007
Parasol tree, Chinese	*Firmiana platanifolia (L.)*	China	Literature	Yu and Zhang 2007

Table 1-1. Continued.

Common Name	Scientific Name	Location of Host Status	Detection Method	Reference
Paulownia	*Paulownia tomentosa* (Thunberg) Steudel	United States		Hoebeke and Carter 2003
Paulownia tree	*Paulownia* spp.	China	Literature	Hoebeke and Carter 2003; Yu and Zhang 2007
Peach	*Prunus persica* Batsch	China, United States		Hoebeke and Carter 2003; Sargent et al. 2010
Pear	*Pyrus* spp.	China	Literature	Yu and Zhang 2007; Sargent et al. 2010
Pear	*Pyrus pyrifolia* Nakai	China		Hoebeke and Carter 2003
Pear, Asian	*Pyrus serotina* L.	United States		Sargent et al. 2010
Persimmon, Japanese	*Diospyros kaki* L.	China	Literature	Hoebeke and Carter 2003; Yu and Zhang 2007; Sargent et al. 2010
Plum	*Prunus domestica* L.	China	Literature	Yu and Zhang 2007
Plum	*Prunus* spp.	China	Literature	Yu and Zhang 2007
Pomegranate	*Punica granatum* L.	China	Literature	Yu and Zhang 2007
Raspberry	*Rubus* spp.	United States		Sargent et al. 2010
Rose, Japanese	*Rosa rugosa* Thunberg	United States		Sargent et al. 2010
Serviceberry	*Amenlanchier* spp.	United States		Hoebeke and Carter 2003
Serviceberry, juneberry	*Amelanchier lamarckii* F. G. Schroed	Switzerland	Slight infestation of nymphs.	Wermelinger et al. 2008
Shadbush	*Amelanchier* spp.	United States		Hoebeke and Carter 2003
Shrub, blue bean	*Decaisnea fargesii* Franchet	Switzerland	Excessive infestation of nymphs.	Wermelinger et al. 2008
Soybean	*Glycine max* (L.)	China, United States	Literature	Hoebeke and Carter 2003; Yu and Zhang 2007; Sargent et al. 2010
Spider flower	*Cleome* spp.	United States		Sargent et al. 2010
Spinach, climbing	*Basella rubra* L.	China		Hoebeke and Carter 2003
Spindle tree, Japanese	*Euonymus japonicas* Thunberg	China	Nymphs or adults observed on plant.	Yu and Zhang 2007

Table 1-1. Continued.

Common Name	Scientific Name	Location of Host Status	Detection Method	Reference
Staghorn sumac	*Rhus typhina* L.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Stewartia, Japanese	*Stewartia pseudocamellia* Maximowicz	Switzerland	Excessive infestation of nymphs.	Wermelinger et al. 2008
Sunflower	*Helianthus annuus* L.	China, United States	Nymphs or adults observed on plant.	Yu and Zhang 2007; Sargent et al. 2010
Sycamore	*Platanus* spp.	United States		Sargent et al. 2010
Tea plant	*Camellia sinensis* (L.)	China	Literature	Yu and Zhang 2007
Tea-oil camellia	*Camellia oleifera* Abel	China	Literature	Yu and Zhang 2007
Tobacco, Japanese	*Nicotiana alata* Link and Otto	China	Literature	Yu and Zhang 2007
Tulip tree	*Liriodendron tulipifera* L.	United States		Sargent et al. 2010
Walnut	*Juglans* spp.	United States		Hoebeke and Carter 2003
Weeping scholar tree	*Sophora japonica* L.	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Wheat	*Triticum aestivum* L.	China	Literature	Yu and Zhang 2007
White poplar, Chinese	*Populus tomentosa* Carriere	China	Literature	Yu and Zhang 2007
Wisteria, Chinese	*Wisteria sinensis* (Sims)	China	Nymphs or adults observed on plant.	Yu and Zhang 2007
Wolfberry, Chinese	*Lycium barbarum* L.	China	Literature	Yu and Zhang 2007
Yellow wood	*Cladrastis kentukea* (Dumont de Courset) Rudd	United States		Sargent et al. 2010
Zelkova	*Zelkova serrata* (Thunberg) Makino	United States		Sargent et al. 2010

CHAPTER 2
LABORATORY HOST PREFERENCE OF THE BROWN MARMORATED STINK BUG (*HALYOMORPHA HALYS* (STÅL)) ON PLANT SPECIES RELATIVE TO FLORIDA

Introduction

Halyomorpha halys (Stål), commonly known as the brown marmorated stink bug (BMSB), is an invasive pentatomid species in the United States. Native to Asia, this species was first identified in Allentown, Pennsylvania in 2001. Subsequent to initial detection, *H. halys* has been detected in 39 states and agricultural problems have been reported in states as far south as North Carolina and Tennessee (Leskey and Hamilton 2012). With over 300 host plants recorded (Nielsen et al. 2008a), *H. halys* is considered to be highly polyphagous species and a major pest of vegetable crops, woody shrubs, and fruit trees in the Northeastern and Mid-Atlantic states. Crop damage reports of 25 – 80% have been reported for apples and pears in Pennsylvania and New Jersey. Host plant preference of *H. halys* when presented with host plants important to Florida's agriculture is unclear from the currently available literature.

Florida's agriculture industry reaches an estimated value of $7 billion per year and is the nation's top producer of grapefruit (*Citrus paradisi* Macfadyen) and orange (*Citrus sinensis* (Linnaeus) Osbeck) (NASS 2012). Although *H. halys* has a reproductive diapause in the winter months, the subtropical and tropical climate conditions in central and south Florida may result in a shortened or abbreviated diapause period and allow for the occurrence of more generations per year. In southern China, up to six generations per year of *H. halys* have been reported (Hoffman 1931). Successful establishment of *H. halys* in Florida could have significant economic and ecological impacts for previously reported hosts, such as oranges, as well as other crops with unknown host potential, such as grapefruit and strawberry. Host specificity tests

relevant to a few Florida agronomic crops and common landscape ornamental plants for *H. halys* may provide improved information regarding host preference and the overall impact this pest could have in the state.

Host specificity experiments test the relative degree of likeliness that an organism would be used as a host by another organism (Van Driesche and Murray 2004). This type of testing could be used to determine the host preference and host range of *H. halys* on economically important crops in Florida. Host range and host preference are generally assessed by a combination of three tests: no-choice, choice, and open field (Capinera 1985; Van Driesche and Murray 2004; Withers and Mansfield 2005). As these testing schemes have developed from year to year, the combination of no-choice and choice tests is most commonly used. Choice tests measure the preference or rank of multiple hosts usually through oviposition or feeding preferences while no-choice tests measure the suitability of a host for development of an organism. In choice tests, two or more test species are placed in a cage with one or more specimens of the agent for a fixed period of time under standard laboratory conditions. In no-choice tests, a single test species is placed in a cage with one or more specimens of the agent for a fixed period of time under standard laboratory conditions.

Our research will focus on the host specificity of *H. halys* using economically important crops including navel orange (*Citrus* x *sinensis* (Linnaeus) Osbeck [*maxima x reticulata]*), strawberry (*Fragaria* spp. Linnaeus), ruby red grapefruit (*Citrus* x *paradisi* MacFadden [*maxima x sinensis*]), avocado (*Persea Americana* Miller), and key lime (*Citrus* x *floridana* (Ingram and Moore) Mabberley) with an overall estimated value of at least $1.86 billion (NASS 2012). Other plant species falling into the category of nursery

and greenhouse products, an industry with on overall value of $1.7 billion (NASS 2012), include the following: elaeagnus (*Elaeagnus angustifolia* Linnaeus), hibiscus (*Hibiscus lunariifolius* Willdenow), and ligustrum (*Ligustrum* spp. Linnaeus). At least three interceptions of *H. halys* have occured at agricultural interdiction stations (Russel 2012) and on tourist vehicles travelling to Florida from infested states (Halbert and Hodges 2011). Nevertheless, established populations have not been detected in Florida. Elaegnus, hibiscus, and ligustrum are commonly found in the Florida landscape or along major highways, and could be possible reservoirs for *H. halys* to establish before migrating to agricultural crops.

Materials and Methods

Laboratory Colony

Adult specimens and egg masses of *H. halys* were obtained from laboratory colonies at the Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS-DPI) Florida Biological Control Laboratory; United States Department of Agriculture-Agricultural Research Service’s (USDA-ARS) Beneficial Insects Introduction Research Unit; and North Carolina State University. *Halyomorpha halys* was reared in the quarantine laboratory at the University of Florida Entomology and Nematology Department in Gainesville, FL. The adult colony was maintained on apples, carrots, green beans, okra and water at room temperature and humidity (27^{o}C and 60%RH, respectively) with a 16L:8D photoperiod (Nieslen et al. 2008b). Food and water were replaced three times per week. Eggs were collected from adult cages three times per week using a wet acrylic paintbrush. Egg masses were placed on damp Kimwipes® and positioned in separate cages (round clear plastic containers, 6” diameter x 1” depth); eggs were misted each day with deionized water.

Upon emergence from the eggs, first instar nymphs remained in the same cage and were misted daily with water. After molting, second instar nymphs were removed and placed in larger cages (round clear plastic containers; 10” diameter x 3.5” depth) where they remained until molting into adults. The second to fifth instar nymphs were maintained on carrots, green beans, okra and water under room temperature and humidity in a 16L:8D photoperiod.

Plant Sources

The following plants were purchased in January 2013: eight one-gallon pots of key lime, navel orange, and ruby red grapefruit purchased from Howey in the Hills, Florida; eight three-gallon pots of elaeagnus and ligustrum and eight one-gallon pots of strawberry and hibiscus purchased from Gainesville, Florida; and eight one-gallon pots of avocado purchased from Homestead, FL. The plant supply was replenished in May 2013and consisted of eight one-gallon pots of key lime, navel orange, and ruby red grapefruit purchased from Howey in the Hills, Florida; eight one-gallon pots of elaeagnus, hibiscus, ligustrum, and strawberry purchased from Gainesville, Florida; and eight one-gallon pots of avocado purchased from Homestead, FL.

The plants remained in the original pots and were kept in an enclosed greenhouse and watered as needed then moved into the quarantine laboratory for the choice test or no-choice test trials. When in the quarantine laboratory and not in use, each plant was held in a separate cage (Live Monarch™ Large Castle, 13” x 13” x 24”) to avoid cross contamination with other plants. A saucer (clear plastic, 8”) was kept under the pot for water and the plants were watered as needed.

No-choice Tests

To determine the potential for early instar nymph development and to test the host range of *H. halys*, eight plant species were tested using no-choice tests. An artificial plant, constructed with artificial branches, a one gallon black pot, green STYROFOAM™ sheets, and potting soil, was also tested as a negative control. These tests were completed in three week intervals for a total of three replicates from May 2013 – July 2013. To reduce experience-based bias on a plant species, newly emerged second instar nymphs were chosen for the no-choice experiments as this is the first life stage of *H. halys* to feed on plant material.

Newly laid egg masses were reared in separate cages (round clear plastic containers, 6”diameter x 1”depth) with water until second instars emerged. Fifteen second instar nymphs were chosen at random and placed on the top leaf of a test plant using a damp paintbrush. Each test plant species was placed in separate mesh cages (Live Monarch™ Large Castle, 13” x 13” x 24”) with a saucer (clear plastic, 8”) under the pot for water. The specimens were allowed to mature for three weeks.

A total of four observers were used to record the recovery and mortality of all nymphs. Each observer was allotted ten minutes per trial to collect and record data on a standardized datasheet. To minimize observer error, the observations were repeated three times per trial. The data recorded included the nymphal stage of alive and dead specimens as well as molts recovered. Feeding injury and damage to the plant were additional observations recorded.

Choice Tests

The host preference of *H. halys* and ranking of host material were tested using an incomplete block design choice test. Two of the eight plant species were presented

simultaneously to four adult specimens of *H. halys*, two males and two females. All possible combinations of the eight plant species and negative control (Table 2-1) were analyzed in 24 hour intervals and each combination was replicated three times for a total of 108 trials from January 2013 to July 2013.

In preparation for each trial, two males and two females were randomly chosen from the laboratory colony at 8:00PM the day prior. Each specimen was marked with a small dot of Testors® enamel paint on the center of the pronotum. The following colors of paint were used to identify each insect's sex and trial number: purple indicated first trial females; white indicated second trial females; green indicated third trial females; yellow indicated first trial males; red indicated second trial males; and light blue indicated third trial males. In an effort to reduce experience-based bias on a plant species, each individual was used in no more than three trials. After marking, the specimens were starved for twelve hours and placed in a small cage (round clear plastic container, 6"diameter x 2.75"depth) equipped with paper towels and water until the trial began the following day.

Between 7:45AM and 8:30AM (M1), the specimens were released in the bottom center of a mesh cage (Live Monarch™ JUMBO Castle, 24" x 24" x 36") equidistant from both test plants. Once released, the specimens were observed for 30 minutes. Three additional thirty-minute observations were made starting between 1:45PM and 2:30PM (A), 7:45PM and 8:30PM (E), and 7:45AM and 8:00AM (M2) the following day, for a total of four observations per trial and an overall total of 432 observations. After each observation, the specimens remained undisturbed in the mesh cage with the two test plants until the trial concluded.

A total of five observers were used to record the specimen behaviors. To minimize observer error, a standardized question-based data sheet was used to uniformly record observations. The recorded behaviors included the following: location of each specimen, feeding activity and feeding location, duration of feeding activity. Additional behaviors, including mating and oviposition, were also recorded.

Statistics

No-choice Tests

The mean number of second instars, third instars, and exuviae was determined using Microsoft Excel 2010. Due to the small sample size and low recovery of individuals, additional summary statistics were not performed.

Choice Tests

For the choice tests, summary statistics for the number of specimens present on each plant and the number of specimens feeding were prepared using the binomial distribution in PROC GLIMMIX (General Linear Model for Mixture Distributions), accounting for non-normality and non-homogenous variances, and Least Squares Means in SAS 9.3 (SAS Institute Inc., 2002 – 2010, Cary, NC, USA). The differences between each plant species for the number of specimens present and the number of specimens feeding was analyzed using Tukey-Kramer Least Squares Means Adjustment for multiple comparisons (SAS Institute Inc., 2002 – 2010, Cary, NC, USA), allowing for adjusted p-values for all pairwise comparisons. The correlation between the specimen's presence on a test plant and documented feeding on a test plant and the correlation between the specimen's presence on a test plant and percentage of time spent feeding on a test plant were analyzed using multivariate correlations, pairwise correlations, and non-parametric Spearman's ρ in SAS 9.3 (SAS Institute Inc., 2002 –

2010, Cary, NC, USA). For determining the difference between the number of specimens feeding by observational time of day, the Poisson distribution in PROC GLIMMIX was used along with Tukey-Kramer Least Squares Means Adjustment for multiple comparisons.

Results

No-choice Tests

On average, more second- instar nymphs were able to survive on hibiscus, ligustrum and strawberry in the no-choice tests (Table 2-2). The second-instar nymphs were also able to molt into the third instar in at least one replication when tested with hibiscus, ligustrum, and elaeagnus.

Choice Tests

The average number of times adult specimens were present on a test plant (F=10.73; df=8, 200; P=0.0001) and feeding on a test plant (F=3.58; df=8, 147; P=0.0008) in the choice tests were significant between test plant species. Adult specimens were present more often on elaeagnus, ligustrum, and orange, and differed significantly from all other plant species (Figure 2-1). However, adults fed more on ligustrum than any other plant and fed significantly more on ligustrum than avocado and the negative control (Figure 2-1). The Spearmans ρ for the correlation between adult specimens present on a test plant and adult specimens feeding on a test plant was 0.4227 (P<0.0001). Although, the Spearmans ρ for the correlation between adult specimens present on a test plant and percentage of adult specimens present and feeding on a test plant was 0.1389 (P=0.0817).

The average number of times adult specimens were feeding on a test plant (F=6.05; df=3, 311.4; P=0.0005) in the choice tests was also significant between

observational times of day. More adult specimens were feeding during the evening observations, and differed significantly from both morning observations (Figure 2-2).

Discussion

Survivorship of multiple generations is important when determining the potential host range of an insect. The survivorship of *H. halys* early instar nymphs were tested in no-choice tests. More second-instar nymphs survived on ligustrum and strawberry, and significantly more on hibiscus. In addition, at least one nymph was successfully able to molt into the third instar when on hibiscus, ligustrum, and elaeagnus. Therefore, hibiscus and ligustrum could be considered host plants of *H. halys*. However, additional replications are needed to confirm these findings. Also, less than half of the individuals were recovered in no-choice tests with avocado, elaeagnus, grapefruit, key lime, orange, and negative control. It is unclear if the individuals escaped from the cages or were lost in the soil. Furthermore, the current methodology may not be truly indicative of the actual survivorship due to the low recovery rate of individuals. The methods of the no-choice tests may need to be modified for a higher recovery rate of individuals. Future modifications may include utilizing a positive control to test against the unknown hosts for a better understanding of the effects in the no-choice experiments. Additional modifications may include a smaller arena for a higher recovery rate of individuals, as well as sufficient humidifiers and thermostats for constant control over the environmental conditions.

When given a choice, adult specimens were present more often on elaeagnus, ligustrum, and orange in comparison to all other test plant species. However, the number of adult specimens feeding on avocado was lower than elaeagnus, grapefruit, hibiscus, key lime, orange, and strawberry and significantly lower than ligustrum. The

discrepancy between the significant presence of *H. halys* on avocado compared to the low feeding occurrence supports the Spearmans ρ (ρ=0.1389; P=0.0817) for the correlation between adults present on a test plant and percentage of adults feeding on a test plant. Even though the correlation is significant at the α=0.1 level, the Spearmans ρ value shows a very weak relationship between percentage of adults feeding on a plant, and adult specimens present on a plant. Overall, these results suggest that adult presence on a plant may be more indicative of roosting behavior rather than feeding. Furthermore, the adult specimens were observed probing on the negative control in five trials for an average feeding time of 5 minutes and 41 seconds. These results suggest that *H. halys* may taste a plant before committing to feeding. Consequently, the documented host lists of *H. halys* in older literature may inaccurately report occurrences of *H. halys* on a plant species as hosts if the rate and length of feeding activity was not properly documented.

Experiments in a laboratory setting control abiotic and biotic factors, and possibly eliminate external nuisance factors that could affect the results of the data. Because outside factors are eliminated or reduced in a laboratory setting, host ranges may be more definitely determined. Nonetheless, it is important to consider host experience and host deprivation when designing choice and no-choice tests (Withers and Mansfield 2005). For example, it is best to use naïve specimens that are unfamiliar with the test species and avoid rearing specimens on similar plants in order to decrease bias. Due to limited numbers in the laboratory colony, some adult specimens in each trial of the choice tests may have been used in previous trials. Thus, some error may have occurred due to bias in previously experience plant species. Host deprivation may also

generate false positive if specimens are deprived of food for too long or false negative responses if specimens are fed shortly before the experiment begins. Depriving each specimen in the choice tests of food for 12 hours may or may not have caused error or false positive results. Other sources of error may include insect confinement, causing the insects to feed on a test species due to starvation or feed on a test species that would not have been selected in the field (Capinera 1985; Van Driesche and Murray 2004).

More adults fed during the afternoon and evening observations, and the evening observations differed significantly from both morning observations. The increased feeding activity in the afternoon and evening correspond with the increased flight activity of *H. halys* observed at dusk and evening hours (Nielsen et al. 2013). Increased feeding and flight activity throughout the evening may indicate that black lights could be a lower cost, yet highly effective method of monitoring for *H. halys* in Florida in comparison to visual pyramid (black or yellow) or pheromone-baited pyramid traps. Black light traps could be placed along the edges of agricultural crops near forested areas as well as agricultural interdiction stations and major highways.

Halyomorpha halys most frequently fed upon ligustrum in the choice tests, and more second instar nymphs were able to survive on ligustrum. Ligustrum is in the plant family Oleaceae, a botanical family including evergreen and deciduous species, which includes related plants such as lilacs (*Syringa* spp.) and jasmines (*Jasminum* spp.). Following ligustrum, *H. halys* most frequently fed on hibiscus (Malvales: Malvaceae), and second instar nymphs were also able to survive on hibiscus. The plant family Malvaceae includes woody ornamentals and, most importantly, cotton (*Gossypium*

spp.). The preference of *H. halys* for woody hosts in this study is consistent with the fruit tree damage reported in the Mid-Atlantic States. Future host specificity tests and monitoring efforts on Florida-specific plants should include the Oleaceae, Malvaceae, and other related families most commonly grown in greenhouses and nurseries. Although reported in the literature as a host (Yu and Zhang 2007), our research suggests that *Citrus* spp. (Sapindales: Rutaceae) may not be preferred compared to other woody hosts, particularly those found in the order Rosales. Further replications are needed to confirm our findings.

Table 2-1. All combinations of plant species tested in choice tests of adult *Halyomorpha halys*.

Number	Combinations
1	avocado x hibiscus
2	avocado x key lime
3	avocado x ligustrum
4	avocado x orange
5	avocado x grapefruit
6	avocado x strawberry
7	avocado x elaeagnus
8	hibiscus x key lime
9	hibiscus x ligustrum
10	hibiscus x orange
11	hibiscus x grapefruit
12	hibiscus x strawberry
13	hibiscus x elaeagnus
14	key lime x ligustrum
15	key lime x orange
16	key lime x grapefruit
17	key lime x strawberry
18	key lime x elaeagnus
19	ligustrum x orange
20	ligustrum x grapefruit
21	ligustrum x strawberry
22	ligustrum x elaeagnus
23	orange x grapefruit
24	orange x strawberry
25	orange x elaeagnus
26	grapefruit x strawberry
27	grapefruit x elaeagnus
28	strawberry x elaeagnus
29	negative x avocado
30	negative x hibiscus
31	negative x key lime
32	negative x ligustrum
33	negative x orange
34	negative x grapefruit
35	negative x strawberry
36	negative x elaeagnus

Table 2-2. Mean number of *Halyomorpha halys* individuals recovered in no-choice tests.

Plant Species	2nd Instars Recovered	3rd Instars Recovered	Exuviae Recovered
Avocado	5.667	0	0
Elaeagnus	4.667	0.333	0
Grapefruit	4	0	0
Hibisucs	11	2	1.667
Key lime	1.667	0	0
Ligustrum	8.333	5	4
Orange	6	0	0
Strawberry	8.667	0	0
Negative Control	4.667	0	0

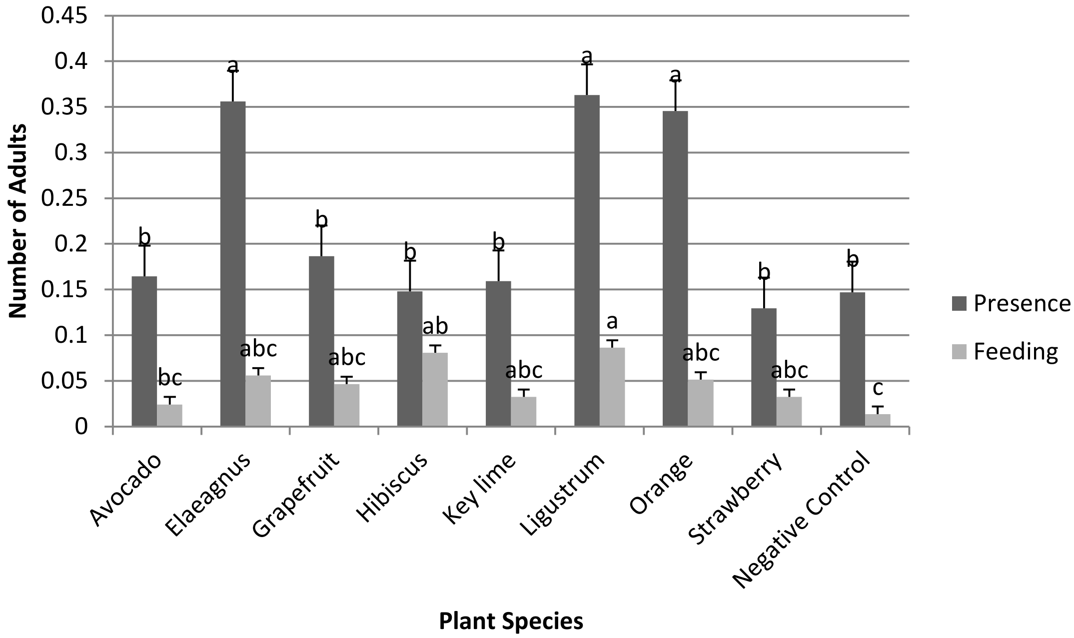

Figure 2-1. Mean (standard error of the mean, SEM) number of adult *Halyomorpha halys* present or feeding on test plant species in choice tests. Means with the same letter are not significantly different (Tukey-Kramer, P=0.05).

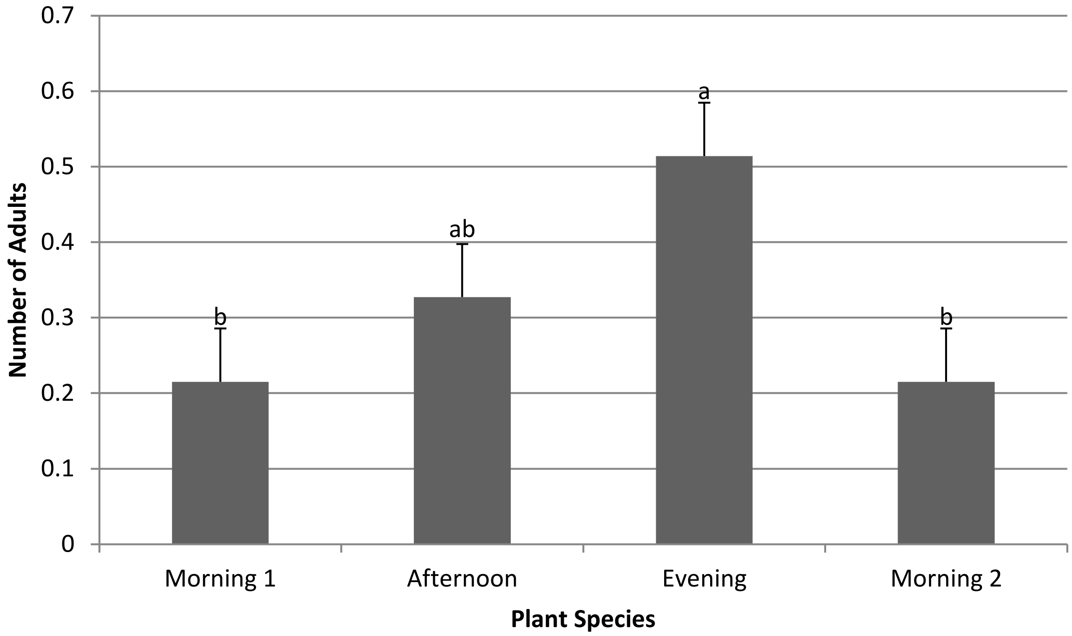

Figure 2-2. Mean (standard error of the mean, SEM) number of adult *Halyomorpha halys* feeding on any test plant at different times of day. Means with the same letter are not significantly different (Tukey-Kramer, P=0.05).

CHAPTER 3
EVALUATION OF THE INVASIVE SPECIES WORKSHOP PROGRAM FOR FLORIDA PARK SERVICE PERSONNEL

Introduction

Invasive species are described as plants, animals, or pathogens that are not native to an ecosystem and cause or are likely to cause economic or environmental harm or harm to human, animal, or plant health (ISAC 2006). The introduction and establishment of an invasive species may result in increased eradication and control costs as well as negatively impact trade and the environment. In the United States, invasions of non-native species cost an estimated $137 billion annually, and $20 billion of these costs are due to environmental damages, lost agricultural productivity, and increased health problems caused by arthropods species (Pimentel et al. 2010).

Preventing the introduction of exotic invasive species is the best defense against the destructive effects of invasions. Preventing invasive species introductions may not always be possible, especially in a state such as Florida. Florida has 12 international airports and 14 deepwater seaports where plant materials are received. However, only a small percentage of imported plant materials are sampled and inspected at ports of entry by the Department of Homeland Security’s (DHS) Customs and Border Protection (CBP) and the U.S. Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS) for exotic plant pests, diseases, pathogens, and noxious weeds (USDA-APHIS-PPQ 2012). Because there are multiple pathways for species introduction and suitable climate conditions, Florida is considered a high risk state for invasive species introductions.

Early detection and rapid response (EDRR) systems have been developed as a second line of defense to prevent permanent establishment of invasive species through

monitoring and control methods (National Invasive Species Council 2003). In the United States, EDRR systems have been adopted on the national level by the Federal Interagency Committee for the Management of Noxious and Exotic Weeds, National Invasive Species Council, and the United States Forest Service (FICMNEW 2003; NISC 2003; USFS 2006). Florida agencies, such as the Florida Invasive Species Partnership and Florida Cooperative Invasive Species Management Areas, have also implemented enforced EDRR programs (FISP 2013). All EDRR systems consist of three actions for reducing impacts of invasive species: early detection, rapid assessment, and rapid response (NISC 2003). For the purposes of this research, we will concentrate on early detection efforts.

Early detection of invasive species through monitoring and identification efforts is not only an essential component for successful EDRR systems, but also for integrated pest management (IPM) programs (NISC 2003; USEPA 2012). The Florida Cooperative Agricultural Pest Survey (CAPS) Program is responsible for conducting pest surveys in high-risk areas for species on special watch lists (FDACS-DPI 2008). The current and ongoing survey program managed by the Florida CAPS Program may target individual pest species such as red palm mite (*Raoiella indica* Hirst), light brown apple moth [*Epiphyas postvittana* (Walker)], and rice cutworm [Spodoptera litura (Fabricius)] or may concentrate on specific high risk areas and/or commodities such as crop surveys, imported warehouses, and solid wood packing materials. Due to limited resource, the Florida CAPS Program cannot monitor for invasive species throughout the entire state of Florida. Involving organizations or volunteers in collaborative monitoring efforts, referred to as passive detection networks, is important for expanding early detection

efforts. By monitoring for invasive species while conducting other activities in their daily lives, passive detection networks may expand the area covered and monitor for invasive species not occurring on special watch lists (i.e., brown marmorated stink bug [Halyomorpha halys (Stål)], red palm weevil [*Rhynchophorus ferrugineus* (Olivier)], Mexican bromeliad weevil [*Metamasius callizona* (Chevrolat)], etc.).

Coordinating public outreach and educational extension events is an effective way to distribute information about the importance of invasive species and the benefits of EDRR systems. Professionals and volunteers should be sufficiently trained in detection, collection, and reporting methods for suspected invasive species. Our research will focus on the development and delivery of Florida-specific invasive species education materials to be used in a series of workshop trainings directed toward Florida State Park Service personnel.

Materials and Methods

Target Audience

The Invasive Species Workshop training sessions and corresponding educational extension materials were tailored for Florida State Park Service personnel (e.g. biologists, park rangers, and volunteers), as well as affiliated groups with a general interest in invasive species detection and management. A total of three training sessions were held at separate Florida State Park district offices in the following locations: northwest region (District 1), northeast (District 2), and central (District 3).

Workshop Presentations

Each workshop consisted of PowerPoint presentations developed through the collaborative efforts of the University of Florida (UF) Biosecurity Research & Extension Lab, UF Agricultural Education and Communication Department, USDA-APHIS-PPQ,

the Florida Department of Agriculture and Consumer Services-Division of Plant Industry, and the Florida Cooperative Agricultural Pest Survey Program on the following topics: importance of invasive species; federal and state agencies affiliated with invasive species, trapping and monitoring methods for target pests; sampling and reporting methods for commonly occurring and exotic species. An additional six species-specific presentations were delivered at each workshop and included presentations on the following species: the brown marmorated stink bug , cactus moth [*Cactoblastis cactorum* (Berg)], emerald ash borer (*Agrilus planipennis* Fairmaire), bean plataspid [*Megacopta cribraria* (Fabricius)], Mexican bromeliad weevil, pine bark beetles (*Ips* spp. and *Dendoctronus* spp.), redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff), and red palm weevil. Species were selected based on responses from district biologists and for likelihood of establishment in each region.

In addition to the PowerPoint presentations, the participants engaged in a hands-on activity. The hands-on activity was comprised of six stations with mounted specimens of invasive species discussed at each workshop, as well as native species that are commonly confused with the invasive species. Hand lenses and microscopes were provided when necessary.

Overall, the order of presentations and activities were uniformly delivered across workshops. Each workshop lasted five hours and included a scheduled thirty-minute lunch break.

Evaluation Instruments

Prior to participating in any educational tests, participants were required to complete and sign an informed consent form. This voluntary agreement is an essential process for any research conducted at the University of Florida utilizing human

participants, and provides participants with a description of the planned procedure to record data (UF IRB-02). All data were collected in such a manner so that the participants remained anonymous and disclosure of participant responses did not place the participant at risk.

The workshop trainings were evaluated using a pre-test and post-test (Figures 3-1 – 3-3). The purpose of the pre-test was to collect demographic information and determine any previous experiences with invasive species workshops or programs. The post-test consisted of 43 questions and was designed using a four-level model to assess participant reaction, learning, change in behavior, and desired results (Kirkpatrick 1996). Questions #1 – 36 were modeled after Rockwell and Kohn (1989) post-then-pre evaluation design to measure the participant's self-perceived change in behavior and reactions. The remaining seven questions assessed participant learning through a set of multiple choice questions.

The desired result of this workshop series was to inspire participants to become actively involved in early detection of invasive species through monitoring, collecting, and reporting of suspected species. To measure the efficacy of the workshops, all participants were given three pest survey forms and encouraged to survey for any of the invasive species discussed at the training sessions. Completed pest survey forms were mailed to and analyzed by Lyle Buss at the University of Florida Insect Identification Lab.

All documents used in this research were approved by the Behavioral/NonMedical Institutional Review Board (IRB02).

Statistics

The change in "before" and "after" workshop responses for questions #1 – 36 on the post-test was summarized with PROC UNIVARIATE in SAS 9.3 (SAS Institute Inc., 2002 – 2010, Cary, NC, USA). The responses for questions #1 – 36 from the post-test were assigned numerical values: strongly disagree (SD) = 1; disagree (D) = 2; uncertain (U) = 3; agree (A) = 4; and strongly disagree (SA) = 5. The groups of participants from different workshops, educational backgrounds, and previous experience with invasive species workshops or educational programs were compared in PROC GLM in SAS 9.3 (SAS Institute Inc., 2002 – 2010, Cary, NC, USA) and analyzed using Tukey-Kramer Least Squares Means Adjustment for multiple comparisons (SAS Institute Inc., 2002 – 2010, Cary, NC, USA) to calculate mean responses for each question and test for significant differences in means (ANOVA) between groups of each category.

Results

Demographics

There were 56 participants, representing the three Florida State Park districts. The demographic profile of the participants indicated that 52% (*n*=29) were male. The majority of the participants (98%, n=54) were white, while 2% (n=1) were Hispanic and one participant this section blank. The mean age was 42 ± 13 years old.

Pre-test and Post-test Evaluation

There was a positive overall change from the workshop for questions #1 – 36 on the post-test (Figure 3-4). There were no significant differences in responses from participants that had (55%) or had not (45%) previously attended workshops on invasive species, or from participants that had (5%) or had not (95%) previously attended a First Detector, Sentinel Plant Network, or Protect US program. However, there were

significant differences in responses between levels of education for questions 1, 5, 10, 11, 26, 29, 33, 34, and 36 (Figure 3-5). Participants that completed trade, technical or vocational training (21%) tended to have a higher change in response than participants that completed some post-doctoral work (5%) or had a post-doctoral degree (40%). There were also significant differences in responses between groups that had or had not previously attended workshops or seminars on invasive species for questions 3, 25, 26, 34, 35, and 36 (Figure 3-6). Participants that had not previously attended workshops or seminars on invasive species (45%) tended to have a higher change in response.

Post-workshop Pest Surveys

Pest survey forms were collected from the beginning of the workshop series in February 2013 to July 2013. A total of seven pest survey forms were completed by workshop attendees, returned, and analyzed by Lyle Buss at the University of Florida Insect Identification Lab. Two participants collected and correctly identified the bean plataspid [*Megacopta cribraria* (Fabricius)], and two new county records of the bean plataspid in Florida have been recorded as a result. One participant collected cactus pads and correctly identified cactus moth [*Cactoblastis cactorum* (Berg)] damage. The remaining four participants did not find invasive species when surveying for pests in their state parks.

Discussion

The Invasive Species Workshop Series had a positive overall impact based on the responses to question on the post-test (Figure 3-4). In some areas, the workshop had a more positive impact on participants with an intermediate level of education (Figure 3-5) and participants that had not previously attended workshops or seminars focused on invasive species (Figure 3-6). Although Florida State Park Service

personnel have limited training in entomological pests, it may be beneficial to include more technical information in the presentations for those participants with higher levels of education and those participants that have attended similar workshops.

Some invasive species discussed, such as the brown marmorated stink bug (*H. halys*) and the bean plataspid (*M. cribraria*), are considered agricultural pests and may or may not be of ecological concern in Florida State Parks. However, these species may become well establish in forested areas before migrating to agricultural crops at which point the species would be difficult to manage. It is important to have collaborative efforts from forestry and agriculture to mitigate the effects of invasive species on economic and ecological interests. As demonstrated by the post-workshop pest survey forms, reaching out to Florida State Park personnel may help with early detection of invasive species and set new county records for invasive species.

Additional training sessions will target different audiences, including Florida Master Gardeners, nurserymen, small farm producers, and public gardens personnel. The Invasive Species Workshop Series training sessions are scheduled to be completed by July 2014. As the training sessions conclude, a follow-up survey will be conducted with previous attendees for additional feedback. In the future, collaboration with the Florida State Park Service, and even the National Park Service, through workshops and other educational extension materials to increase the probability of detecting invasive species.

Directions: Please answer the questions below.

What is your gender? Male ☐ Female ☐

What is your age: _______

In which Florida county do you live? _________________________

What is the highest level of education you have completed? (Circle one.)

a. Some high school
b. High school graduate
c. Some college
d. Trade, technical or vocational training
e. College graduate
f. Some post-graduate work
g. Post-graduate degree

Which race best describes you? (Circle those that apply.)

a. White
b. Black or African American
c. American Indian or Alaska Native
d. Asian
e. Native Hawaiian or Other Pacific Islander

Do you identify your origin as Hispanic, Latino, or Spanish? Yes ☐ No ☐

Have you ever previously attended an invasive species workshop or seminar? Yes ☐ No ☐

If so, where? ____

Have you ever attended a First Detector, Sentinel Plant Network, or Protect US program? Yes ☐ No ☐

If so, where? ____

Have you ever interacted with the United States Department of Agriculture, the Cooperative Agricultural Pest Survey, or the National Plant Diagnostic Network? Yes ☐ No ☐

If so, where? ____

During the last year, approximately how many times have you: (Circle one.)

Sought advice from your local extension service?	0	1 – 5	6 – 10	> 10
Received plant materials from outside Florida?	0	1 – 5	6 – 10	> 10
Received plant materials from outside the U.S.?	0	1 – 5	6 – 10	> 10

How would you describe yourself? (Circle one.)

a. Independent gardener
b. Volunteer at public gardens
c. Staff at public gardens
d. Volunteer at state park
e. Staff at state park
f. Professional grower
g. Other ______________________________

Approximately how many years have you been engaged in the Florida State Park Service? _________

Figure 3-1. Pre-test for the Invasive Species Workshop Series.

Directions: There are 2 columns for each statement below. The first column asks you to select your level of agreement with the statement **AFTER** completing the workshop. The second column asks you to select your level of agreement with the statement **BEFORE** the workshop.

Level of agreement responses are as follows: **SD = strongly disagree** **D = disagree** **U = uncertain** **A = agree** **SA = strongly agree** Please check one box for each column, for each statement.	**NOW** that you have completed the workshop — please check the box that best describes your level of agreement with each statement.	Thinking back, **BEFORE** you were in the workshop — please check the box that best describes your level of agreement with each statement.

	Statement	SD	D	U	A	SA	SD	D	U	A	SA
1	I understand how new pests establish in Florida.										
2	I know the different pathways by which new pests could enter Florida.										
3	Cultivated landscapes contribute to the establishment of new pests in Florida.										
4	Human transport of plant materials contributes to establishment of new pests in Florida.										
Regarding new pests, I know the role of:											
5	…the United States Department of Agriculture.										
6	…the Florida Dept. of Agriculture and Consumer Services.										
7	…the Cooperative Agricultural Pest Survey.										
8	…land-grant universities, such as University of Florida.										
9	…the National Plant Diagnostic Network.										
I would trust ______________________ to be considerate of my property concerns when responding to a report of a pest on my plant/crop/land.											
10	…the United States Department of Agriculture…										
11	..the Florida Dept. of Agriculture and Consumer Services..										
12	…the Cooperative Agricultural Pest Survey…										
13	…land-grant universities, such as University of Florida…										
14	…the National Plant Diagnostic Network…										
15	Generally speaking, I have a lot of faith in governmental agencies (e.g., Florida Department of Agriculture and Consumer Services).										
16	I know how to identify new pest species based on **what they look like**.										
17	I know how to identify new pest species by the **damage they cause** to plants.										
18	I know how to search for new pest species.										
19	I know how to submit samples of new pest species.										
20	I know how to contact federal and state agencies about new pest species.										
21	Early detection sampling will **not** require more time and energy than I have available.										
22	Early detection sampling will **not** be too complicated a process for me.										
23	Early detection sampling will **not** involve too many instructions and requirements.										
24	I am likely to **search** for new pest species.										
25	I am likely to **report** a new pest species I find to the appropriate agency.										

Figure 3-2. Front page of the post-test for the Invasive Species Workshop Series.

	Statement	AFTER WORKSHOP					BEFORE WORKSHOP				
		SD	**D**	**U**	**A**	**SA**	**SD**	**D**	**U**	**A**	**SA**
26	I can benefit from early detection of new pest species.										
27	Early detection of new pests and pathogens is important.										
28	Early detection is a good way to avoid establishment of new plant pests.										
29	Avoiding establishment of new plant pests is important.										
26	Early detection is a good way to minimize economic loss.										
27	Minimizing economic loss is important.										
28	Early detection is a good way to preserve biodiversity.										
29	Preserving biodiversity is important.										
30	Early detection is a good way to prevent plant loss.										
31	Preventing plant loss is important.										
32	As a result of this workshop, I want to learn more about new pest species.										

Directions: Please circle the correct answer.

33. If I sample and find a suspected invasive pest, I should:
    a. Kill it.
    b. Stop sampling and immediately report it
    c. Collect it and finish sampling, and then submit the sample and report it.

34. If I find a suspected invasive insect pest, I will:
    a. Do nothing.
    b. Kill it.
    c. Collect the specimen and mail it to the appropriate agency.

35. If I sample and **do not find** any suspected invasive pests, this most likely means:
    a. I am not doing it right.
    b. There are none, and I have wasted my time.
    c. There are none, and this is informative and useful.

36. If I sample and **do not find** any suspected invasive pests, I will:
    a. Do nothing.
    b. Report date, location, host plant, and results to the appropriate agency.

37. The pest discussed today that I feel **least** confident that I can find and identify is: ________________________.

38. What did you gain from this workshop? (Check Yes or No.)

	Yes	No
a. Answers to my questions		
b. Resource materials I can use		
c. Knowledge about new pest species		
d. Knowledge about early detection of new pest species		
e. New skills that will help me in my job		
f. Ideas I can try immediately		
g. Names of other people to contact		
h. Nothing new		
i. Other:		

39. How much of the content of this workshop did you already know? (Check one.)
    a. ___ None
    b. ___ A little
    c. ___ Quite a bit
    d. ___ Most of it

Figure 3-3. Back page of post-test for the Invasive Species Workshop Series.

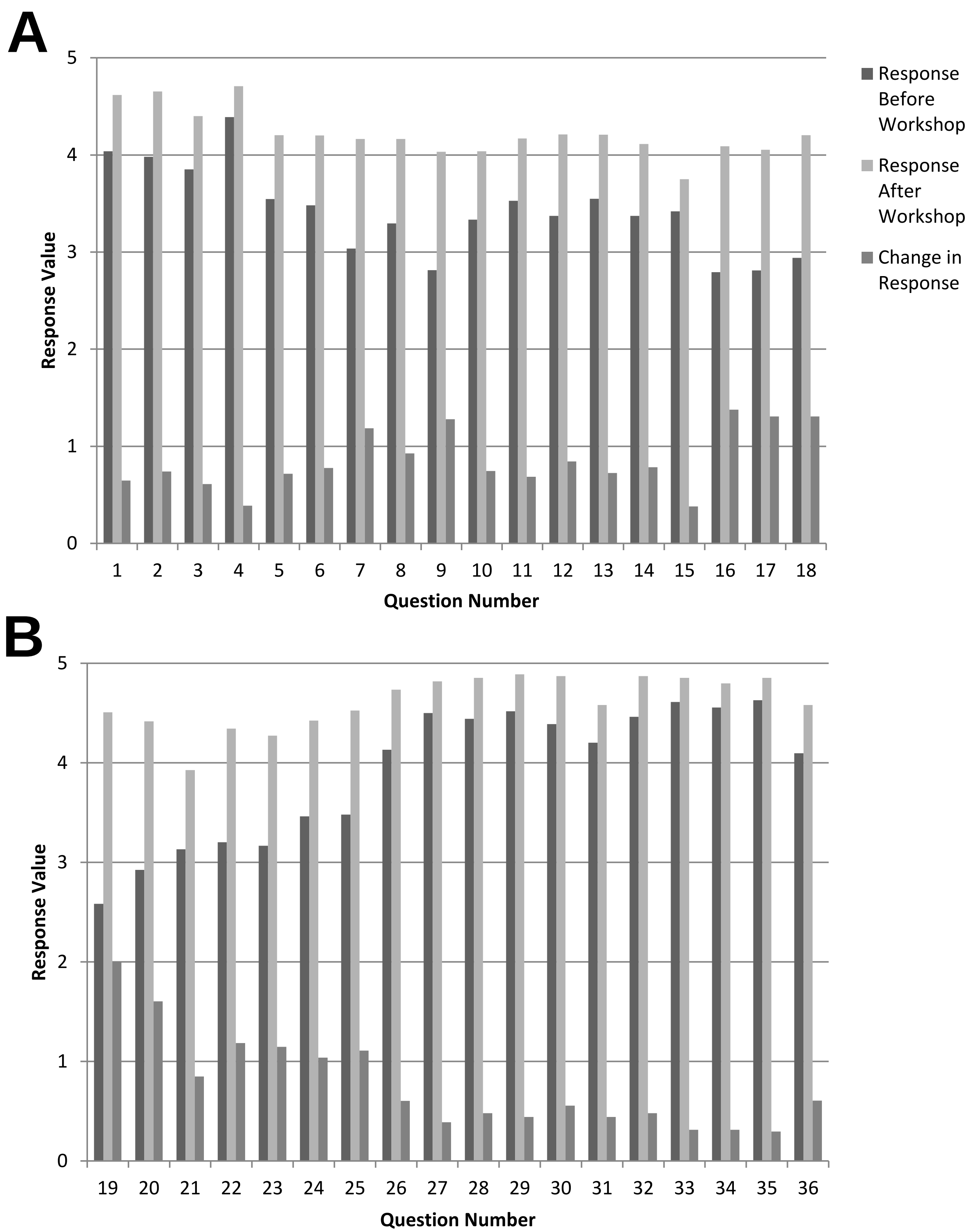

Figure 3-4. Overall mean question responses from post-test. A) Mean question responses for question numbers 1 – 18. B) Mean question responses for question numbers 19 – 36. The responses were assigned numerical values: strongly disagree (SD) = 1; disagree (D) = 2; uncertain (U) = 3; agree (A) = 4; and strongly disagree (SA) = 5.

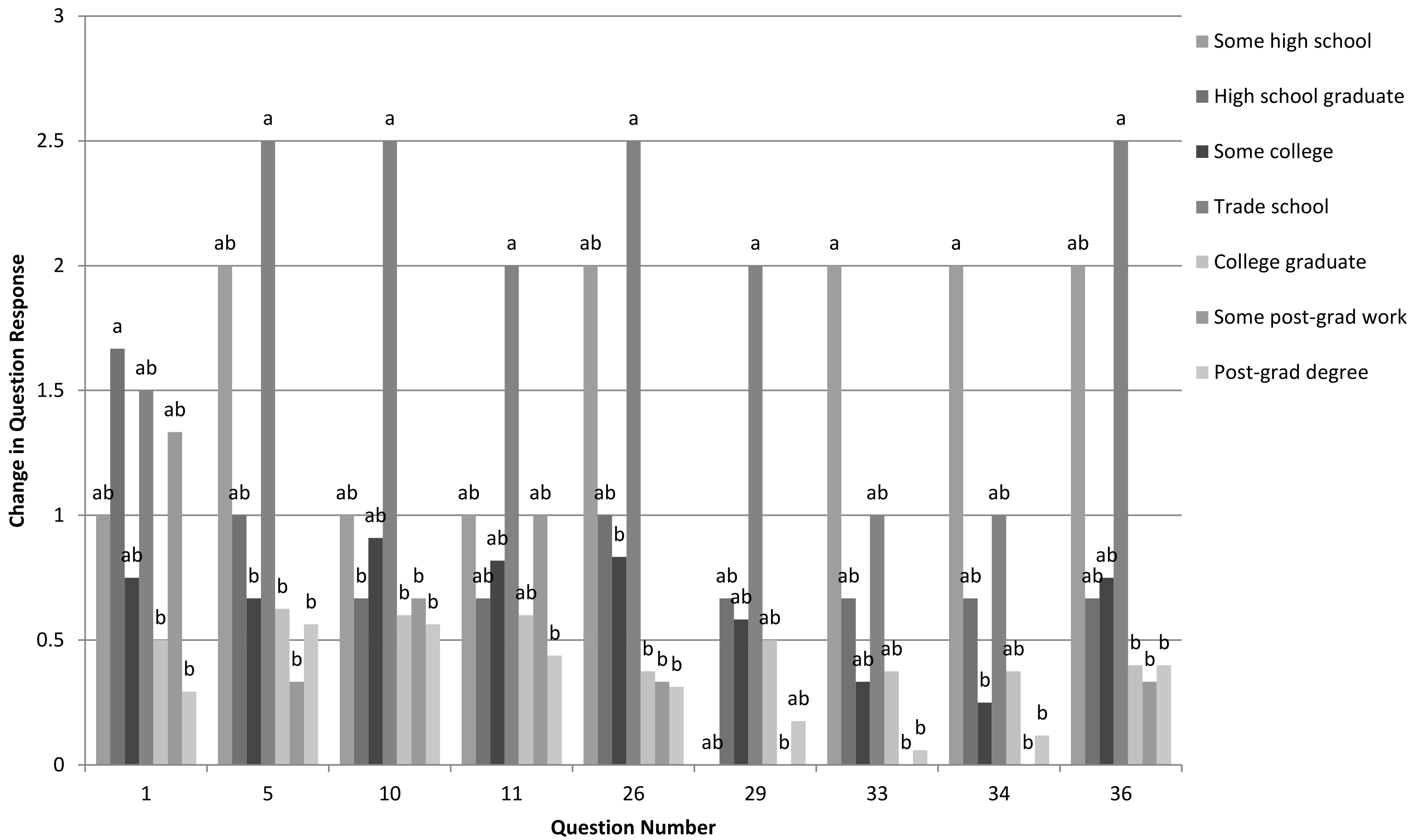

Figure 3-5. Differences in mean change of question responses from post-test between participant levels of education. Means with the same letter are not significantly different (Fisher's exact test, P=0.05).

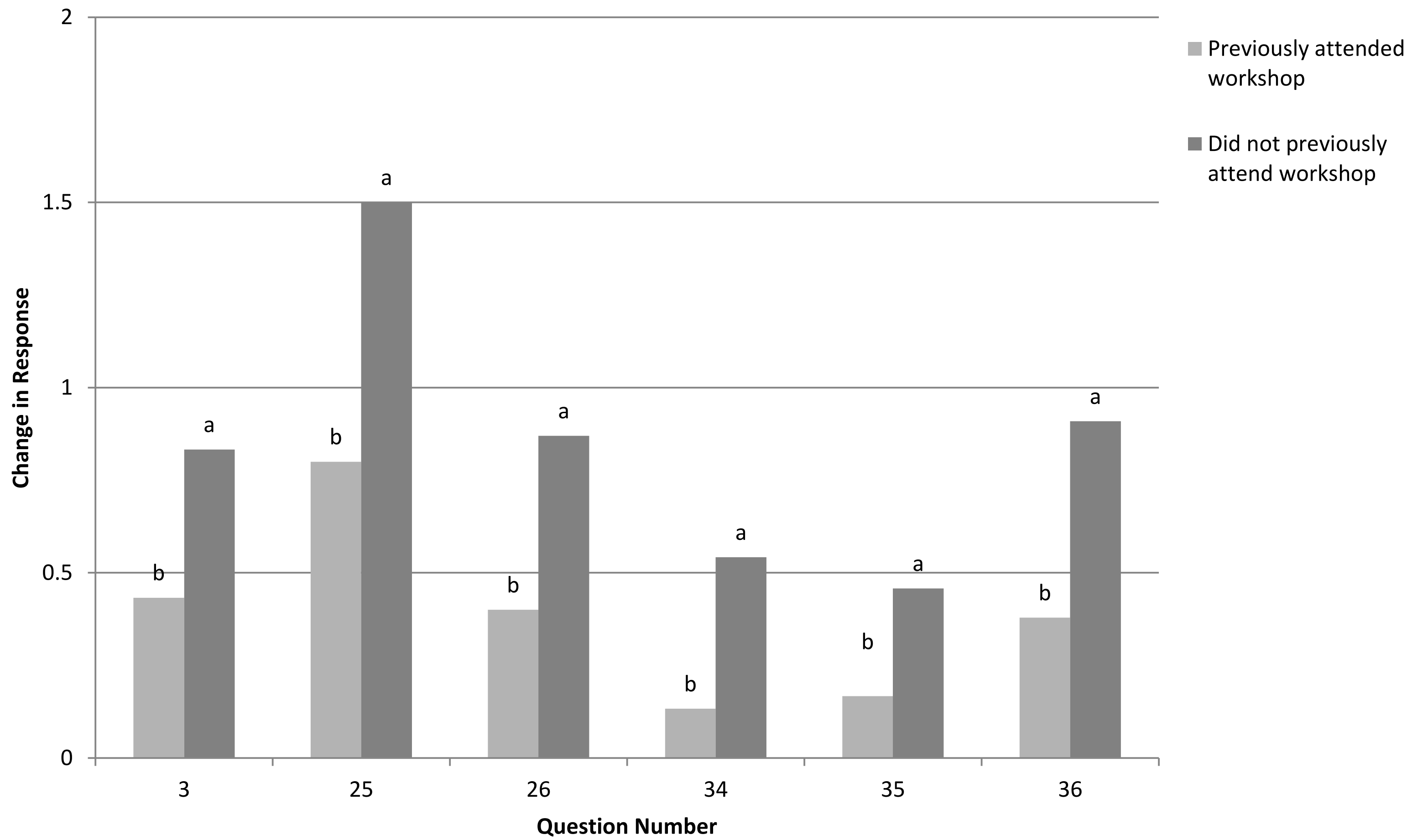

Figure 3-6. Differences in mean change of question responses from post-test between participant experience with invasive species workshops. Means with the same letter are not significantly different (Fisher's exact test, P=0.05).

CHAPTER 4
ASSESSMENT OF IDENTIFYING STINK BUGS AND SIMILAR TRUE BUGS OF FLORIDA FIELD GUIDE

Introduction

Field guides are a type of reference designed to aid in the identification of plants and animals in their natural environment, and are generally small enough to be carried in the field. Although more technical field guides exist, most field guides are accessible to the general public and include pictures and descriptions of multiple life stages to assist in distinguishing between similar groups or species. There are many field guides available for general insect identification, i.e., Peterson Field Guide to Insects (Borror and White 1998), National Audobon Society Field Guide to North American Insects and Spiders (Milne et al. 1980), and Kaufman Field Guide to the Insects of North America (Eaton and Kaufman 2007). However, there are only a handful of field guides exclusively for stink bug identification. The most common field guides for stink bug identification include Field Guide to Stink Bugs of Agricultural Importance in the Upper Southern Region and Mid-Atlantic States (Kamminga et al. 2009) and Stink Bugs of the Midwest (Jesse et al. 2011).

Stink bugs, as well as many other true bug species, are commonly found in crops, gardens, and landscapes throughout the state of Florida. Infestations of herbivorous stink bugs, such as the southern green stink bug [*Nezara viridula* (Linnaeus)], may significantly damage plants. However, predacious stink bugs are beneficial in controlling plant pests. For instance, the spined soldier bug [*Podisus maculiventris* (Say)] preys on larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), corn earworm [*Helicoverpa zea* (Boddie)], fall armyworm [*Spodoptera frugiperda* (J.E. Smith)], Mexican bean beetle (*Epilachna varivestis*

Mulsant), and several more economically important pests (De Clercq 2008). Proper identification of stink bugs and true bugs is essential before determining appropriate pest management options.

Until recently, there has not been a Florida-specific field guide for stink bug identification. Due to multiple pathways of introduction, suitable climate conditions, and a large-scale agricultural production, Florida is considered a high risk state for invasive species. Invasive stink bug species of particular agricultural concern include the brown marmorated stink bug [*Halyomorpha halys* (Stål)] and the bagrada bug [*Bagrada hilaris* (Burmeister)]. Monitoring for these species may be difficult because there are species native to Florida with similar markings and coloration. For this reason, a field guide titled Identifying Stink Bugs and Similar True Bug Species in Florida was developed as a reference for identifying Florida-specific stink bug and related true bug species.

My research will focus on assessing the utility of Identifying Stink Bugs and Similar True Bug Species in Florida through an identification activity of commonly encountered stink bug and true bug species in Florida.

Materials and Methods

Development of Field Guide

Development of Identifying Stink Bugs and Similar True Bugs in Florida occurred from January 2012 until May 2013. This field guide includes photographs and information on 25 species with 68 colored pages. Species selected for this field guide are frequently encountered in Florida with the exception of two invasive species of economic concern that are not currently established in Florida, the brown marmorated stink bug and the bagrada bug.

Labeled diagrams of the dorsal and ventral surfaces of a stink bug were incorporated in the field guide, including separate diagrams for the ventral surfaces of herbivorous and predaceous species with distinguishable characteristics highlighted. At the top of each species page, the species name and common name (if applicable) were provided along with an indication of “pest”, “harmless”, or “beneficial” status. The eggs, nymphs, adults, and common hosts were described for each species with corresponding images for each life stage and an “actual size” reference. Florida distribution maps were also included when the information was available for a species.

Target Audience

The target audience for this study was Florida Master Gardeners, which is made up of citizens of Florida with a common interest in providing research-based information about gardening to educate Floridians (UF Master Gardener Program 2012). Master Gardeners receive some general training in entomology with an emphasis on insects of horticultural significance. The following county extension offices in northwest, northeast, and central Florida with active Master Gardener Programs were chosen to participate in the assessment for Identifying Stink Bugs and Similar True Bugs of Florida: Alachua, Bay, Citrus, Duval, Flagler, Nassau, Orange, Seminole, St. Lucie, and Washington. A maximum of 20 Florida Master Gardeners from each county were invited to participate in the assessment.

Evaluation Instruments

Prior to participating in the assessment, participants were required to carefully read and sign an informed consent form. This voluntary agreement is an essential part of any research conducted by the University of Florida utilizing human participants, and provides participants with a description of the planned procedure to record data (UF

IRB-02). All data must be collected in such a manner so that the participants remain anonymous and disclosure of participant responses does not place the participant at risk.

For evaluation of the field guide, participants were given a pre-survey (Figure 4-2) before and a post-survey (Figure 4-4) immediately after the identification activity (Figure 4-3). The pre-survey was designed to collect demographic information and determine any previous experiences with insect identification. The post-survey was modeled after the four-level approach for evaluations (Kirkpatrick 1996). The front page consisted of the identification activity to assess participant learning. On the back page of the post-survey, there were an additional five questions to assess participant reaction and change in behavior as a result of using the field guide (Kirkpatrick 1996).

The informed consent form, pre-survey, and post-survey for this study were approved by the University of Florida Institutional Review Board (Protocol #2013-U-0596, 2013).

Identification Activity

The following specimens were left unmarked, placed in a covered box, and respectively numbered 1 – 8: bean plataspid [*Megacopta cribraria* (Fabricius)]; milkweed bug [*Oncopeltus fasciatus* (Dallas)]; wheel bug [*Arilus cristatus* (Linnaeus)]; rice stink bug [*Oebalus pugnax* (Fabricius)]; harlequin bug [*Murgantia histrionica* (Hahn)]; leaffooted bug [*Leptoglossus phyllopus* (Linnaeus)]; *Euschistus quadrator* (Rolston); and brown marmorated stink bug (Table 4-1). Each county extension office received a separate collection box of unmarked specimens. Using only the field guide as a resource, the participants were given thirty minutes to identify all unmarked

specimens and classify each specimen as an herbivore or a predator. Hand lenses were also provided for easy viewing of the specimens.

Delivery of Materials and Assessments

Materials sent to each county extension office included one collection box of unmarked specimens, twenty informed consent forms, twenty individually marked pre-test and post-tests, and twenty Ziploc bags with a copy of Identifying Stink Bugs and Similar True Bugs of Florida, a hand lens, and a pen for each participant. All pre-tests and post-tests were placed in manila envelopes to conceal the questions and responses from each participant.

All materials were mailed to each county extension office, with the exception of Alachua and Nassau County, and assessments were completed between June 2013 and July 2013. The county extension agents were given standardized instructions on how to deliver the assessment. Upon completion, the county extension agents were instructed to mail back the informed consent forms along with the pre-tests and post-tests. In regard to Alachua and Nassau County, we arranged to personally deliver the assessments to the Master Gardeners.

Statistics

For the identification activity, the frequency of correct responses for species identification and classification (e.g. herbivore or predator) were analyzed using frequency and contingency tables produced by PROC FREQ in SAS 9.3 (SAS Institute Inc., 2002 – 2010, Cary, NC, USA). Fisher's exact test was used to test for significant differences of responses between the following categorical variables: participants from different county extension offices; participants with different educational backgrounds; participants with different background of entomological training; and previous

experience with identification resources (i.e. Distance Diagnostic and Identification System, National Plant Diagnostic Network, Protect U.S., etc.).

Results

Demographics

With an overall total of 75 participants, 32% (*n*=24) were male and 68% (*n*=51) were female. The majority of the participants (91%, *n*=68) were white, while 3% (*n*=2) were Asian, 1% (*n*=1) were African American, 1% (*n*=1) were American Indian or Alaska native, and 4% (*n*=3) did not respond. The mean age was 64 ± 9 years old.

Identification Activity

Over 70% of participants correctly classified each specimen as herbivore or predator with the lowest percentage of correct responses for *E. quadrator* (73%) and *H. halys* (78%) (Figure 4-1). For specimen identification, over 70% of participants correctly identified the following species: *M. cribraria* (88%), *O. fasciatus* (92%), *A. cristatus* (88%), and *M. histrionica* (89%). Fewer than 70% of participants correctly identified *O. pugnax* (67%), *L. phyllopus* (65%), *E. quadrator* (33%), and *H. halys* (18%), and the percentage of correct responses for *E. quadrator* and *H. halys* was significantly lower.

Post-test Responses

After completing the identification activity, the participants were asked 1.) if they would refer to the field guide as an identification resource for stink bugs and true bugs in the future, 2.) if they were confident in distinguishing herbivores from predators when using the field guide as a reference, and 3.) which specimen they felt least confident identifying. A high percentage of participants strongly agreed (63%) or agreed (29%) that they would use the field guide as an identification resource. When using the field guide, the majority of participants agreed (55%) or strongly agreed (25%) that they felt

confident distinguishing herbivores from predators. The specimens participants felt least confident identifying were *O. pugnax* (31%) and *E. quadrator* (31%), followed by *H. halys* (11%), *M. cribraria* (6%), *O. fasciatus* (6%), and *L. phyllopus* (5%).

Discussion

Participants were able to successfully distinguish herbivores from predators when referring to the field guide. However, only four out of eight specimens had a percentage of correct responses above 70%. *Halyomorpha halys* and *E. quadrator* had the lowest percentage of correct responses, with 18% and 33% of correct responses respectively. Most participants misidentified these species as *E. servus* (Say), *E. tristigmus* (Say), or *Podisus maculiventris* Say. Both *H. halys* and *E. quadrator* have a similar brown marbled coloration with alternating light and dark colored banding along the outer edges of the abdomen. This suggests that stink bug or true bug species with the same coloration, particularly a brown marbled color, are more difficult to identify. In future revisions of the field guide, it would be beneficial to add photographic keys for species with similar appearances and highlight distinguishable characteristics within the images.

Table 4-1. Correct responses in the identification activity for the assessment of Identifying Stink Bugs and Similar True Bugs of Florida.

Specimen number	Specimen identification	Specimen classification (herbivore or predator)
1	*Megacopta cribraria* (Fabricius)	Herbivore
2	*Oncopeltus fasciatus* (Dallas)	Herbivore
3	*Arilus cristatus* (Linnaeus)	Predator
4	*Oebalus pugnax* (Fabricius)	Herbivore
5	*Murgantia histrionica* (Hahn)	Herbivore
6	*Leptoglossus phyllopus* (Linnaeus)	Herbivore
7	*Euschistus quadrator* (Rolston)	Herbivore
8	*Halyomorpha halys* (Stål)	Herbivore

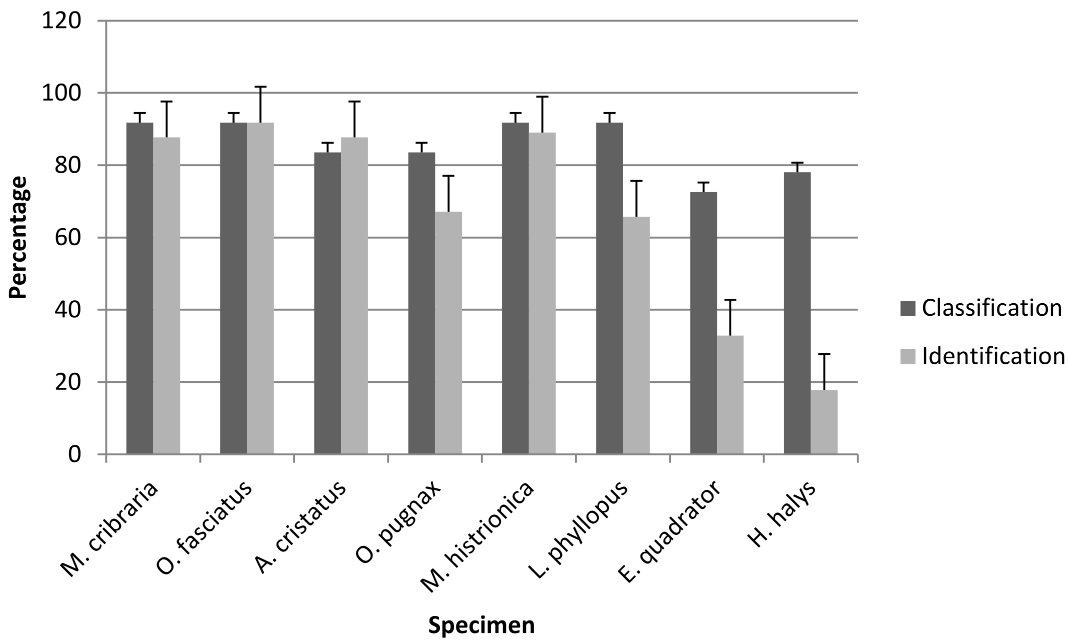

Figure 4-1. Overall percentage of correct responses from the identification activity used for assessing Identifying Stink Bugs and Similar True Bugs of Florida. Participants classified each specimen as herbivore or predator, as well as identify the species of each specimen.

Pre-Survey Form: *Identifying Stink Bugs and Similar True Bugs of Florida* Field Guide Assessment

Directions: Please answer the questions below.

What is your gender? Male ☐ Female ☐

What is your age: ________

Which Florida county do you live in? ________________________

What is the highest level of education you have completed? (Circle one.)
- a. Some high school
- b. High school graduate
- c. Some college
- d. Trade, technical or vocational training
- e. College graduate
- f. Some post-graduate work
- g. Post-graduate degree

Which race best describes you? (Circle those that apply.)
- a. White
- b. Black or African American
- c. American Indian or Alaska Native
- d. Asian
- e. Native Hawaiian or Other Pacific Islander

Do you identify your origin as Hispanic, Latino, or Spanish? Yes ☐ No ☐

Approximately how often do you consult your local county extension agent about insect or plant pathogen identification?
- a. 1 – 3 times per week
- b. 1 – 3 times per month
- c. 1 – 3 times per year
- d. Never

Do you have any background in entomology education or training? Yes ☐ No ☐

If so, please specify: ____

____

Have you ever utilized insect identification resources with the Distance Diagnostic and Identification System, National Plant Diagnostic Network, or Protect U.S.? Yes ☐ No ☐

If so, which resources have you used? ________________________________

____

How would you describe yourself? (Circle one.)
- a. Independent gardener
- b. Volunteer at public gardens
- c. Staff at public gardens
- d. Professional grower
- e. Other ________________________

Approximately how many years have you been engaged in the Florida Master Gardener Program? ________________

Figure 4-2. Pre-test for assessment of Identifying Stink Bugs and Similar True Bugs of Florida.

Field Guide Assessment for *Identifying Stink Bugs and Similar True Bugs of Florida*

IDENTIFICATION ACTIVITY

Directions: Using the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide, you will identify eight unmarked, pinned specimens within a 30-minute period. First, refer to pages 6 and 7 of the field guide to determine if each specimen is an herbivore or a predator. Then, use the field guide to determine the identification of each specimen and record the common name or species name next to "Identification". Hand lenses will be provided for your convenience.

Specimen #1

Herbivore or Predator? ________________

Identification: ________________________

Specimen #2

Herbivore or Predator? ________________

Identification: ________________________

Specimen #3

Herbivore or Predator? ________________

Identification: ________________________

Specimen #4

Herbivore or Predator? ________________

Identification: ________________________

Specimen #5

Herbivore or Predator? ________________

Identification: ________________________

Specimen #6

Herbivore or Predator? ________________

Identification: ________________________

Specimen #7

Herbivore or Predator? ________________

Identification: ________________________

Specimen #8

Herbivore or Predator? ________________

Identification: ________________________

CONTINUE ON BACK

Figure 4-3. Identification activity for the assessment of Identifying Stink Bugs and Similar True Bugs of Florida.

Field Guide Assessment for *Identifying Stink Bugs and Similar True Bugs of Florida*

FIELD GUIDE ASSESSMENT

Directions: After completing the identification activity, please answer the following questions about the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide.

1. If I sample and find a stink bug or true bug, I will refer to the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide as an identification resource.
   a. Strong Agree
   b. Agree
   c. Disagree
   d. Strong Disagree
   e. Uncertain

2. When referring to the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide, I know how to distinguish herbivores from predators.
   a. Strong Agree
   b. Agree
   c. Disagree
   d. Strong Disagree
   e. Uncertain

3. Of the eight specimens, I feel **least** confident in identifying:
   a. Specimen #1
   b. Specimen #2
   c. Specimen #3
   d. Specimen #4
   e. Specimen #5
   f. Specimen #6
   g. Specimen #7
   h. Specimen #8

4. What feature of the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide do you find most useful? ____
____

5. What changes can be made to improve the *Identifying Stink Bugs and Similar True Bugs of Florida* field guide? ____
____

Figure 4-4. Post-test for the assessment of Identifying Stink Bugs and Similar True Bugs of Florida.

CHAPTER 5
IMPLICATIONS AND FUTURE DIRECTIONS FOR *H. halys* RESEARCH

The introduction and establishment of invasive species often leads to expensive eradication and control efforts, direct environmental and agricultural damage, and can severely impact the trade industry. Once an invasive species becomes established, eradication may not be possible and effective control methods can be difficult to achieve. When possible, prevention of invasive species introductions or early detection of new populations is the best defense against the destructive effects of invasions.

Halyomorpha halys is an invasive agricultural and urban pest in the United States. Since its detection in the late 1990s, *H. halys* has become established in at least twelve states and the distribution continues to expand. Most research in the past decade pertaining to *H. halys* has been focused on the development of monitoring and control methods. However, little research concentrates on the host preferences of *H. halys* and the potential impacts it may have on agricultural commodities for uninfested states. If *H. halys* were to establish as far south as Florida, the economic and ecological impact of *H. halys* could be significant.

The preference of *H. halys* for woody hosts is consistent throughout the literature, the fruit tree damage reported in the Mid-Atlantic States, and this research. Monitoring and early detections programs for *H. halys* in the southeastern United States, as well as other states without established populations, should set pyramid or black light traps around the perimeter of woody agricultural crops, along highways with woody vegetation, or in areas with an abundance of woody landscape plants. Although *Citrus reticulata* is reported as a host in native regions of China (Yu and Zhang 2007), our research suggests that *Citrus* spp. may not be preferred and may serve as alternate

hosts. The University of Florida Biosecurity Research and Extension Lab will continue additional replications of the choice and no-choice tests and include a larger variety of plant species for a better understanding of the host specificity of *H. halys*. New no-choice methodology will also be tested. Following additional replications of choice tests, new choice test methodology may also tested.

Early detection of *H. halys* is essential for mitigating the ecological and economic effects of this invasive species. Partnerships with federal and state government agencies, researchers, agriculture extension clientele, and the general public increases the likelihood of detecting *H. halys* and other invasive species. These collaborative efforts may include workshop trainings, E-learning modules, and other educational extension materials. The purpose of the Invasive Species Workshop Series was to bring diverse groups together for a common cause of early detection and invasive species awareness. The Invasive Species Workshop Series will include additional training sessions and target a variety of different audiences, including Florida Master Gardeners, nurserymen, small farm producers, and public gardens personnel. The training sessions are scheduled to be completed by July 2014. In addition, the brown marmorated stink bug presentation will be developed into an E-learning module and be available through the National Plant Diagnostic Network and Protect U.S. websites.

The field guide, Identifying Stink Bugs and Other True Bugs, was developed for general public use. The field guide assessment indicated that stink bug and true bug species with similar coloration may be easily misidentified. Only 18% of participants correctly identified *H. halys*, which was commonly misidentified as *E. servus* (Say), *E. tristigmus* (Say), or *Podisus maculiventris* Say. Future revisions of the field guide will

include photographic keys for species with similar coloration and highlight distinguishable characteristics within the images. In order to provide improved public accessibility, an electronic version of the field guide will be available once all revisions have been made. Additional trainings for Florida Master Gardeners and the general public may be useful to better explain how to use the field guide for identifying true bug specimens.

Halyomorpha halys research continues to have a significant impact on monitoring strategies and control techniques for this species. Although it seems that the introduction of this invasive species to Florida is inevitable, the host specificity research and extension efforts should continue in hopes that the destructive effects of this species are reduced.

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BIOGRAPHICAL SKETCH

Ashley Poplin is from Daytona Beach, Florida and graduated from Keystone Heights High School in May 2007. After graduation she attended University of Central Florida and majored in forensic science with a minor in chemistry and a certificate in crime scene investigation. During her senior year at UCF, Ashley was inspired by a lecture in her forensic archeology class and gained an interest in entomology.

Ashley was offered a research assistantship under the direction of Dr. Amanda Hodges at the University of Florida beginning August 2011. As part of her assistantship, she collaborated with multiple government agencies to develop extension education materials. The appeal of developing these materials motivated her to incorporate extension education as part of her research to complement her project on the brown marmorated stink bug. Ashley's career goals are to become a crime scene investigator and continue her education in forensic entomology.