BIOLOGICAL CONTROL OF *HALYOMORPHA HALYS* (STÅL) (HEMPITERA: PENTATOMIDAE): IDENTIFICATION AND EVALUATION

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ABSTRACT OF THE DISSERTATION

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive pest of American agriculture. The native range of *H. halys* includes China, Korea and Japan, where it is considered a sporadic pest of tree fruit and soybeans. *Halyomorpha halys* was first detected in the U.S. near Allentown, PA in the late 1990s and has subsequently spread to over 40 states and several Canadian provinces. Due to its highly polyphagous feeding habits, *H. halys* is considered a serious agricultural pest throughout the mid-Atlantic region known to damage tree fruit, berry crops, grapes, vegetables, field crops and ornamentals. Currently, *H. halys* is being managed by repeated applications of broad spectrum insecticides, derailing IPM practices in many crops. However, little is known about the effects of native natural enemies on the population dynamics of *H. halys.* The purpose of this doctoral dissertation was to identify natural enemies which effect *H. halys* in New Jersey agro-ecosystems and evaluate their effectiveness in laboratory, greenhouse and field settings. The effect of natural enemies was first studied on sentinel *H. halys* egg masses deployed at agricultural sites across southern New Jersey. After 48 h in the field, egg masses were assessed for signs of predation and incubated for 6 weeks to allow for parasitoid emergence and development. Utilization of *H. halys* eggs was generally low: 5.84% of eggs were consumed by predators while 1.43% of eggs were parasitized. A subset of sentinel *H. halys* egg masses were recorded with closed circuit security cameras to further identify those organisms effecting egg

masses in the field. Video recordings revealed 688 visits by organisms in 31 taxa. Muscoid flies were the most common visiting taxa but these visits did not include observable damage to the eggs. Orthopteran visitors consumed *H. halys* eggs on at least 3 occasions, and in two of these cases the Orthopteran consumed the egg mass entirely leaving no signs of eggs or predation. Sentinel egg masses do not provide information about the identity of predators of *H. halys* nymph stages so I developed a set of *H. halys*-specific molecular primers for use in gut content analysis. HhalysCO1Spec primers amplify an 89-bp region of the CO1 mtDNA gene and have been verified specific to *H. halys* by BLASTn query and by cross-amplification tests on non-target Pentatomidae present in the Eastern U.S. Timed digestion trials were used to determine the half-life of degradation for the sequence amplified by the HhalysCO1Spec primers in laboratory-fed *C. carnea* (Stephens) larvae. These laboratory-fed predators were also used to determine the DNA detectability half-life for the qPCR assay BMITS1 which amplifies a sequence of *H. halys* DNA of similar length to HhalysCO1Spec. Both primer sets successfully amplified target DNA from laboratory-fed predators, but further analysis revealed significant differences in the duration of DNA detectability between the two methods. The half-life of detectability for the BMITS1 assay (T_{50} = 48.87 h) was approximately 4 times longer than that of the HhalysCO1Spec method (T_{50} = 12.12 h). Due to the higher sensitivity of the BMITS1 assay, this amplification method was selected to screen field-collected predators for the presence of *H. halys* DNA. Throughout the summer months of 2014 through 2016, potential predators were collected from soy, peaches, peppers and sunflower plantings in southern New Jersey. These predators were assayed for *H. halys* DNA with the BMITS1 qPCR system. In total, 850 predators were collected and of these, 13.6% of samples assayed positive for *H. halys* DNA. Taxa with the highest proportion of positive assay results included Nabidae (29.4% ± 6.4%), Tettigoniidae (26.3% ± 7.2%), Acrididae (14.7% ± 6.2%), Dermaptera (12.8% ± 4.0%) and Coccinellidae (11.7% ± 1.6%). Although the sample size varied between crops, predators collected in sunflowers, peppers and raspberry displayed significantly higher rates of *H. halys* DNA detection than those collected in soybeans

and peaches. Despite the observed low rates of predation on *H. halys* egg masses, a greater diversity of predator taxa were found to contain *H. halys* DNA indicating that predators can consume other stages of *H. halys.* To identify generalist predator taxa which consume *H. halys* nymphs, I conducted no-choice predator feeding trials in laboratory-based microcosms. Field collected predators were exposed to 1) one *H. halys* egg mass, 2) 20-30 1st instar *H. halys* nymphs or 3) five *H. halys* 2nd instar nymphs. Prey were deposited on a sunflower seedling within a plastic predation arenas, while predator-protected control prey of identical stage and age were kept in cups within the arena. After 48 h of exposure, nymph survivorship was assessed while predation on eggs was measured by assessing hatch rate. Predation was determined statistically by comparing the survivorship of treatment prey which were exposed to predators to that of protected prey. Egg predation occurred from predators in the following taxa: Acrididae, *Coccinella septempunctata* (L.), *Podisus maculiventris* (Say), and Tettigoniidae. Predators in the families Nabidae and Reduviidae caused significant reduction in the survivorship of 1st instar nymphs while Nabidae and *P. maculiventris* nymphs reduced the survivorship of 2nd instar nymphs. Several taxa of predator showed stage-specific differences in their consumption of *H. halys* immatures, with Acrididae and Tettigoniidae preying upon eggs but not nymphs, while Hemipteran predators of the taxa Nabidae, Reduviidae and Pentatomidae attacked nymphs but not eggs. Based on the aforementioned results, it is clear that *H. halys* is attacked by predators in laboratory and field settings. However, the utility of this predation as a means of preventing *H. halys* damage to agricultural crops is unclear. It is also unclear if observed rates of *H. halys* predation in laboratory settings would be affected by the presence of alternate prey. To study the effect of *H. halys* predation on plant yield, *H. halys* nymphs were exposed to predators on potted soybean plants in greenhouse mesocosms. *Aphis glycines* (Matsumura), an important aphid pest of soybean, were introduced into a subset of mesocosms as alternate prey. Two commercially available predators were introduced into the soybean mesocosms: *Hippodamia convergens* (Guerin-Meneville), a predator of *A. glycines*, and *P. maculiventris*, a predator of *H. halys*. After

21 days, prey abundance was assessed, as were metrics of plant health including vertical growth, lateral bud development and dry mass. Prey treatments significantly affected plant vertical growth, lateral bud development and final dry mass but in most cases, predator treatments did not significantly reduce the negative effects of herbivory. Plant health metrics were negatively affected by the presence of *A. glycines*, but these did not differ significantly from treatments which included both *A. glycines* and *H. halys*. *Halyomorpha halys* nymphal survival was unexpectedly higher in treatments which included *A. glycines* as alternate prey; the cause of this result is unknown. The results of this dissertation indicate that *H. halys* is affected by a relatively broad community of generalist predators in New Jersey agro-ecosystems. However, the abundance and efficacy of these predators varies widely by crop, growing season, and *H. halys* life stage. In greenhouse mesocosms, moderate predation on *H. halys* nymphs did not prevent measurable declines in soybean plant health, leading to the conclusion that natural control by endemic predators and parasitoids may impact annual *H. halys* populations but are insufficient to prevent economic injury.

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The list of people to whom I owe thanks for their assistance and support throughout my graduate studies is longer than this dissertation, but here I will try to express the profound sense of gratitude to those who helped me finish this PhD. First, I would like to thank my family for tolerating my absence from their lives for 4 years. I often had to make hard choices and miss attending countless birthdays, holidays, and weddings while I was in New Jersey, but my family tolerated my nonappearances and my whining through all of it. I was similarly absent from the lives of my close friends who made me feel welcome whenever I returned to the Midwest for a visit, and who have opened their hearts and arms to me now that I've returned. Knowing that I had a group of lovable misfits waiting for me whenever I finished was a much needed light at the end of a long dark tunnel. The group of people who had the shortest end of the bargain was my coworkers, including my advisor, Dr. Anne Nielsen, our technician, Ann Rucker, and supportive, kind and friendly post-docs like Dr. Brett "Ace" Blaauw, Dr. Monique "Snake Eyes" Rivera and Dr. Joe "Maverick" Kaser. I am not an easy person to work with in all situations but these brave souls tolerated my messy desk, my disregard for professional mores and my pathological forgetfulness. I literally would not have finished without their patience, advice, support and friendship (or Ann's snack stash). Additionally, my collaborator Rafael Valentine, and my committee members, Dr. George Hamilton, Dr. Dina Fonseca, Dr. Peter Morin and Dr. Paula Shrewsbury provided me with invaluable assistance, expertise, editing and mental stability. I was also fortunate to work with two excellent undergraduate research assistants, Jessica Blanchard and Meghin Rollins. These brave young scientists provided organization, hard work and endurance in dealing with me and my experiments. Finally, the greatest thanks of all is owed to my partner in all things, Chelsea Reynolds. The East Coast would have been a frightening nightmare-scape without her calm, steady support and her seemingly endless supply of patience. The amount of gratitude I owe her cannot be adequate expressed by any combination of words I currently possess, but I hope to repay that debt as she earns a doctorate of her own.

DEDICATION

The only sacrifices that have ever mattered are those made of ourselves, to ourselves. Thus, I dedicate all of my sacrifices over last four years and the whole of this dissertation to myself. I sacrificed much to achieve my dream, but now I hope to spend the remainder of my days helping others achieve theirs.

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Other chapters have not yet been submitted for publication.

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Chapter 1: Introduction and Literature Review

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive Pentatomid pest of New Jersey agriculture. First confirmed in the U.S. in 2001 (Hoebeke and Carter 2003), *H. halys* has spread to over 40 states, with states in the Mid-Atlantic region reporting the highest populations (Leskey, Hamilton, et al. 2012). In its native range of East Asia, *H. halys* is considered an occasional pest of tree fruits and soybeans (Hoffman 1931, Kobayashi et al. 1973, Umeya 1976, Funayama 2004). Although it was first detected on the East Coast, *H. halys* has spread rapidly into the Pacific Northwest, Midwest, and South Eastern U.S. (Swanson 2012, Bakken et al. 2015).

Many agricultural crops in the U.S. can be utilized by *H. halys* as host plants, leading to the classification of this pest as a "severe economic risk" by the U.S. Department of Agriculture's Plant Protection and Quarantine program (Holtz and Kamminga 2010). Economic damage from *H. halys* was first recorded in 2006 and subsequent losses have increased drastically (Nielsen and Hamilton 2009b, Leskey, Hamilton, et al. 2012). Feeding damage from *H. halys* can cause economic damage in tree fruit, vegetables, cane berries, row crops, and ornamentals (Nielsen and Hamilton 2009a, Leskey, Hamilton, et al. 2012, Basnet et al. 2014, Rice et al. 2016, "StopBMSB.org" 2017). Damage caused by *H. halys* feeding in Mid-Atlantic apples caused an estimated $37 million in 2009 alone, and has led to the disruption of IPM programs and wide-spread increases in insecticide use (Leskey, Hamilton, et al. 2012, Leskey, Short, et al. 2012). Large-scale increases in insecticide applications are known to detrimentally effect natural enemies and the pest suppression services they provide (Ruberson et al. 1998) often resulting in secondary pest outbreaks. Moreover, the degree to which *H. halys* populations are suppressed by natural enemies is unclear (need REF).

Invasive species, like *H. halys*, may proliferate in invasive ranges due to the absence of effective co-evolved natural enemies (Elton 1958, Keane and Crawley 2002, Pyšek and

Richardson 2010). Attempts to control invasive pests often focus on classical biological control, the importation of specialist natural enemies from the pest's native range (Caltagirone 1981), but this is not always possible (see Howarth 1991, Barratt et al. 2010). Generalist natural enemies in the invaded range may help guard against the establishment and spread of invaders by decreasing initial propagule size and limiting population growth (Case 1990, 1991, Reusch 1998, Symondson et al. 2002, Pyšek and Richardson 2010). Generalists may also help suppress established populations of invasive species through prey switching: predator preference changes to favor the most abundant prey (Jaworski et al. 2013). Many invasive species, including *H. halys*, experience higher reproductive rates than native species in their invaded ranges (Jaworski et al. 2013). In this way, invasive species may displace native competitors and eventually come to dominate the local ecosystem (Jaworski et al. 2013, Basnet et al. 2014). However, generalist predators are known to preferentially consume more abundant prey items, causing increased predation on abundant invasive pests and reduced predation on scarce native species (Symondson et al. 2002, Venzon et al. 2002). Native generalists may also initially reject invasive prey due to novel anti-predator adaptations or predator/parasitoid naiveté. For these natural enemies, prey switching can only occur after a period of learning and/or adaptation (King et al. 2006, Carlsson et al. 2009). The relationship between exotic prey and native natural enemies is complex and often difficult to predict, thus studying enemy-prey interactions is a key component of understanding the life-history and population dynamics of novel exotic species and to their successful management.

Determining the natural enemies of an invasive pest in its native range is a key step in developing an ecological-informed pest management strategy. In Asia, *H. halys* is attacked by a number of natural enemies including several species of parasitoid wasps, a Tachinid fly parasitoid (*Bogosia spp.*), *Arma chinensis* (Hemiptera: Pentatomidae) and *Orius spp.* (Hemiptera: Anthocoridae) (Kawada and Kitamura 1992, Arakawa and Namura 2002, Arakawa et al. 2004, Qiu 2010). Parasitoids appear to be the most effective natural enemies for suppressing *H. halys* in

Asia (Lee et al. 2013). Among these, the egg parasitoid *Trissolcus japonicus* (= *T. halyomorphae*) (Ashmead) (Hymenoptera: Scelionidae) is a Pentatomid specialist which can cause up to 70% annual parasitism in *H. halys* (Yang et al. 2009, Talamas et al. 2013). Throughout much of its native range, *H. halys* is only considered a sporadic agricultural pest (Lee et al. 2013), possibly due to the impacts of effective co-evolved natural enemies.

Although the identity and efficacy of *H. halys* natural enemies has only begun to be explored in its invaded range, the effects of parasitoids and predators on native Pentatomidae have been well studied. For many native species like *Euschistus servus* (Say) (Hemiptera: Pentatomidae), the effects of natural enemies vary widely. Koppel et al. (2009) found that predation on *E. servus* sentinel egg masses ranged from 0 – 2.4% in field crops while earlier work reported considerably higher predation rates (25% – 40%) (Yeargan 1979, Koppel et al. 2009). Overall, parasitism rates on *Euschistus spp.* ranged from 40% – 50%, while that of *P. maculiventris* (Say) (Hemiptera: Pentatomidae) ranged from 11.2% – 31.3% (Yeargan 1979, Orr et al. 1986, Koppel et al. 2009). For many endemic Pentatomidae, mortality from parasitoids is higher than predation mortality (McPherson and McPherson 2000). However, *Chinavia hilaris* (= *Acrosternum hilare*) (Say) (Hemiptera: Pentatomidae), an exotic Pentatomid with global distribution, does not experience high rates of parasitism in the U.S. (0% – 15.7%) but is predated upon at rates similar to that of native Pentatomidae (Yeargan 1979, Orr et al. 1986). Although the exact mechanism for low *C. hilaris* parasitism is unknown, this evidence indicates that local parasitoids may be poorly adapted to attacking non-native hosts.

The proliferation of *H. halys* in the U.S. may be explained by the ineffectiveness of native natural enemies (Elton 1958). Several studies have tested this hypothesis by identifying the native natural enemies associated with *H. halys* and quantifying their effects on mortality. Sentinel egg masses have revealed relatively low overall utilization of *H. halys* eggs by natural enemies in agricultural settings (Cornelius et al. 2016, Herlihy et al. 2016, Ogburn et al. 2016). Predation affected 4.4 – 12.7% of sentinel eggs, while the rate of successful parasitoid emergence

from *H. halys* sentinel egg masses was only 0.5 – 4.1% (Cornelius et al. 2016, Herlihy et al. 2016, Ogburn et al. 2016). *Halyomorpha halys* eggs are accepted as hosts by the parasitoid *T. podisi* at rates similar to native Pentatomidae, but successful development and emergence of *T. podisi* on *H. halys* eggs is rare (Abram et al. 2014). In laboratory bio-assays, predation of *H. halys* eggs was found to be highly variable between predator taxa (Morrison et al. 2016). The taxa which attacked *H. halys* eggs most frequently were Carabidae, Tettigoniidae, and Salticid spiders, with chewing predators consuming significantly more eggs than sucking predators (Morrison et al. 2016). Adult *H. halys* are known to be affected by *Trichopoda pennipes* (Fab.) (Diptera: Tachinidae) (an ectoparasitoid), bats and web building spiders, but the agricultural significance of these interactions has not been determined (Rice et al. 2014, Valentin et al. 2016, Maslo et al. 2017, Morrison et al. 2017). Based on the results to date, it appears that *H. halys* eggs are not frequently utilized by native predators. However, the identity of predators affecting the egg stage cannot be determined by sentinel egg masses alone and the impact of predators and parasitoids on *H. halys* nymphs has yet to be determined.

Trissolcus japonicus is a specialist parasitoid of *H. halys* in east Asia (Yang et al. 2009). In their native range, *T. japonicus* regularly and effectively parasitizes *H. halys* eggs with annual parasitism rates often exceeding 60% (Yang et al. 2009). *Trissolcus japonicus* was originally imported into the U.S. in 2007 under quarantine for screening as a classical biological control organism (Milnes et al. 2016). In 2014, an adventive population of *T. japonicus* was collected from *H. halys* sentinel egg masses deployed in Maryland (Herlihy et al. 2016). After this, subsequent discoveries of *T. japonicus* were made in other mid-Atlantic and Pacific northwest locations (Milnes et al. 2016). These populations are genetically distinct from those in quarantine, indicating unrelated and independent introductions rather than accidental escape (Milnes et al. 2016). Given its effectiveness on *H. halys* in Asia, the accidental introduction and spread of *T. japonicus* in the U.S. presented a new and potentially effective agent of natural population suppression. However, the biology and ecology of *T. japonicus* indicate that it may not be a

"magic bullet" for preventing *H. halys* pest pressure. *Trissolcus japonicus* shows a strong preference for woody habitats, and thus far has only been detected in the U.S. from sentinel egg masses deployed outside of agricultural fields (Talamas et al. 2015, Herlihy et al. 2016, Milnes et al. 2016). Research conducted on the quarantine population of *T. japonicus* revealed frequent parasitism of *P. maculiventris*, a native predatory Pentatomid which attacks dozens of crop pests including *H. halys* (McPherson 1980, Morrison et al. 2016). However, quarantined and wild populations of *T. japonicus* are genetically distinct and may have differing host and/or habitat preferences (Milnes et al. 2016). Current understanding of *T. japonicus* behavioral ecology in the U.S. is limited, but these early results indicate that this species may not be an effective agent of *H. halys* natural control. Furthermore, unintended introductions of exotic species rarely occur without environmental and economic impacts (Howarth 1991, Barratt et al. 2010, Liu and Piper 2016), so the spread of extant *T. japonicus* populations should be controlled until its ecology in the U.S. is better understood.

Halyomorpha halys is not the only invasive stink bug pest affecting U.S. agriculture. *Nezara viridula* is a Pentatomid agricultural pest with world-wide distribution (Todd 1989). Unlike *H. halys*, *N. viridula* has been present in the U.S. for over 100 years and is well established within American agroecosystems (Drake 1920, Todd 1989). As such, the natural enemy ecology of *N. viridula* in the U.S. may provide insight into the future of the *H. halys* invasion. *Trissolcus basalis* (Woll.), a specialized *N. viridula* parasitoid, exists throughout parts of its host's invaded range and has been released as a classical biological control agent against *N. viridula* in Hawaii and Australia (Caltagirone 1981, Todd 1989). However parasitism of *N. viridula* is highly variable and is often exceeded by the effects of predators (Stam et al. 1987, Correa-Ferreira and Moscardi 1996, Tillman 2010). In soybean plantings, successful parasitism occurs in less than 1% of *N. viridula* eggs while over 50% of eggs are consumed by predators (Stam et al. 1987). *Nezara viridula* nymphs are also subject to predation from a number of generalist predators including Geocoridae, Nabidae and Araneae (Ragsdale et al. 1981, Stam et

al. 1987). Tillman (2010) found similar rates of predation on *N. viridula* and *E. servus* eggs, although the latter is native to the eastern U.S. Despite the shared evolutionary history between *T. basalis* and its host, non-specific generalists may have a greater impact on *N. viridula* population dynamics in some cropping systems (Stam et al. 1987).

Similarly, the discovery of *T. japonicus* in the U.S. may not lead to major decreases in *H. halys* populations if *T. japonicus* does not efficiently attack its host in agricultural settings; some evidence exists to support this (Herlihy et al. 2016). In light of ineffective native generalist parasitoids and exotic parasitoids which are only effective in non-agricultural landscapes, assemblages of generalist predators may still be the most effective source of *H. halys* natural enemy mortality (Cornelius et al. 2016, Herlihy et al. 2016). Over time, interactions between *H. halys* and local generalists are expected to increase in magnitude as predators adapt to their novel prey (Jaworski et al. 2013).

Directly studying the trophic links in an arthropod predator/prey system is often challenging due to the small size and concealed/nocturnal feeding behaviors of some of these organisms (Sheppard and Harwood 2005). Molecular gut content analysis (GCA) of predators is a method of studying arthropod predator-prey systems without disruptive or artificial manipulative experiments (Hoogendoorn and Heimpel 2001, Sheppard and Harwood 2005, King et al. 2008, Eitzinger et al. 2013). Molecular GCA employs prey-specific oligonucleotide primers to assay predators from laboratory or field experiments for the presence of prey DNA within their digestive tracts (Sheppard and Harwood 2005, King et al. 2008). Molecular techniques have been used to study community ecology in a number of ways including the quantification of specific predator-prey relationships (Harwood & Obrycki 2005; Zhang et al. 2007; Kobayashi et al. 2011), the study of intra-guild predation (e.g. Gagnon et al., 2011; Harwood et al., 2007; Sheppard et al., 2005; Yang et al., 2016) and the identification of effective generalist predators for the management of pests (e.g. Chen et al., 2000; Furlong et al., 2014; Greenstone et al., 2010). Unlike visual observations, molecular GCA allows for the quantification of predation events between

small, concealed and/or nocturnal organisms and, unlike sentinel prey studies, can be used to study predation across all prey life stages (Sheppard and Harwood 2005). However, the rate at which prey DNA is detected during GCA is affected by a multitude of factors which must be understood before assay results can be interpreted.

Prey DNA begins to decay shortly after predation as a result of digestive processes and is only detectable by molecular techniques for a limited time (Greenstone et al. 2007, 2014). The duration of DNA detectability is further affected by a variety of factors including predator life stage, nutritional status, number/stage of prey consumed, and temperature during digestion (Hoogendoorn and Heimpel 2001, Naranjo and Hagler 2001, Greenstone et al. 2007, Harwood et al. 2009). The duration of DNA detectability also varies between predatory taxa, as target DNA sequences persist longer in slow digesting species (Greenstone et al. 2014). Comparing the results of GCA assays between predator taxa therefore requires an understanding of the relative digestion speed of the taxa in question, often determined through laboratory digestion time studies (Greenstone et al. 2010, 2014).

Molecular GCA can be accomplished through a number of genetic techniques but experimental methodology can considerably affect the duration of prey DNA detectability. For example, DNA detectability is affected by various methods of DNA preservation including submersion in ethanol and freezing (Weber and Lundgren 2009). Prey DNA detectability can also be affected by the method of amplification used to assay predators. Quantitative PCR (qPCR) techniques improve the sensitivity of conventional PCR by employing fluorescent dyes and/or probes to detect small quantities of target DNA (Gomez-Polo et al. 2015). This technique was first used in GCA to detect traces of fish DNA from the feces of sea lions (Deagle et al. 2006) and has since been used for exploring a variety of arthropod food webs (e.g. Lundgren and Ellsbury, 2009; Lundgren and Weber, 2010; Troedsson et al., 2009; Valentin et al., 2016; Weber and Lundgren, 2009; Zhang et al., 2007). To understand the relative sensitivity of quantitative techniques, several studies have assayed field collected predators with both conventional PCR

and qPCR methods. In each case, the qPCR method was able to detect target DNA in samples which assayed negative by conventional PCR (Zhang et al. 2007, Gomez-Polo et al. 2015, 2016). These studies provide relative evidence of higher sensitivity in qPCR methods, but direct comparison of the maximum limits of DNA detectability between conventional and quantitative approaches has not been completed. Similarly, a qPCR method for amplifying *H. halys* DNA has been developed (Valentin et al. 2016), but the sensitivity of this method has not been compared to that of conventional PCR primers.

Research Objectives

In light of the economic threat posed by *H. halys*, the goal of this doctoral dissertation was to determine the identity of native predators which attack *H. halys* and quantify their effects. Specifically, the objectives of this work were to:

1. Quantify natural enemy utilization of *H. halys* sentinel egg masses in New Jersey, and identify visitors to egg masses with video recordings.

   Rationale and Background: As an invasive species, *H. halys* exists in the U.S. without closely evolved natural enemies. The identity of predators and parasitoids affecting *H. halys* in New Jersey agro-ecosystems has not been determined, nor has the effect of natural control on *H. halys* abundance. In order to understand the population dynamics of *H. halys* in its invaded range, we need to identify the natural enemies attacking *H. halys* and quantify their effects. The research described in this chapter was conducted as part of two multi-state collaborative projects studying the natural enemies of *H. halys* across the U.S. (Ogburn et al. 2016, Poley et al. *unpublished*). Within this chapter, I present the results of these projects as they pertain to New Jersey agro-ecosystems, and compare local findings to nation-wide trends.

2. Develop a set of *H. halys*-specific conventional PCR primers for use in molecular GCA, and compare the sensitivity of this method to that of a qPCR approach.

   Rationale and Background: Valentin *et al.* (2016) developed a novel species-specific qPCR assay for detecting *H. halys* DNA in the guano of insectivorous bats. This method, BMITS1, utilizes *H. halys*-specific primers and a TaqMan fluorescent probe (Valentin et al. 2016). The use of TaqMan probes theoretically increases assay specificity due to its requirement of a complete sequence match between target region and probe (Tyagi and Kramer 1996). Despite this, a quantitative comparison of the sensitivity of TaqMan assays and those that rely on conventional PCRs has yet to be completed. We created a set of *H. halys*-specific primers for use in conventional PCR systems (HhalysCO1Spec) and compared the duration of *H. halys* DNA detectability of these primers to that of the BMITS1 qPCR method.

3. Screen field-collected predators for *H. halys* DNA using molecular GCA.

   Rationale and Background: Multiple studies have used sentinel egg masses to quantify the rate of natural enemy utilization on *H. halys* eggs in the field (Jones et al. 2014, Cornelius et al. 2016, Herlihy et al. 2016, Morrison et al. 2016, Ogburn et al. 2016). However, studying the predation of *H. halys* nymphs in the field is more difficult due to their high mobility (Stam et al. 1987, Leskey and Lee 2014). Laboratory no-choice feeding trials have been conducted to assess predator preference for *H. halys* nymphs (Pote and Nielsen 2017) but understanding the effects of *H. halys* predation across all life stages is needed. We used prey-specific primers to assay predators collected from three New Jersey agroecosystems for the presence of *H. halys* DNA.

4. Determine the effect of native predators on eggs and early nymphal instars of *H. halys* in laboratory no-choice predation assays.

Rationale and Background: Our understanding of predator impacts on *H. halys* to date has focused on one sessile life stage and likely does not accurately represent life-long natural enemy effects. Evidence from other Pentatomidae suggests the communities of predators affecting eggs and nymphs may be mutually exclusive (Ragsdale et al. 1981). Behavioral differences exist between sessile *H. halys* eggs, aggregated 1st instar nymphs, and mobile 2nd instar nymphs (Nielsen and Hamilton 2009a), which may affect predator preferences. To address these issues, I conducted laboratory no-choice feeding trials to identify predators which accept *H. halys* eggs, 1st and 2nd instar nymphs as prey. At the time of writing, the work presented in this chapter has been accepted for publication in Biological Control and is currently *in press*.

5. Determine the realized rates of predation on *H. halys* nymphs on soybean plants in greenhouse mesocosms in the presence of alternate prey, and quantify the effects of predators and prey on metrics of soybean growth and development.

   Rationale and Background: Results from the previous chapter showed that multiple generalist predator species will attack *H. halys* nymphs in laboratory microcosm experiments. However, in that study, *H. halys* prey items were presented in an artificially manipulated setting in the absence of alternate prey. Many of the predators which attacked *H. halys* nymphs in the laboratory are relatively abundant in soybean fields (Pote, *unpublished*, O'Neil, 1988), a known *H. halys* host plant (Owens et al. 2013). However, it is unclear if these predators will attack *H. halys* on soybean or if predation experienced by *H. halys* on soy can cause tri-trophic effects resulting in increased plant health and development. I studied predation of *H. halys* nymphs by *P. maculiventris* and *Hippodamia convergens* (Guerin-Meneville) on soybean in the presence of alternate prey, *Aphis glycines* (Matsumura) in greenhouse cage mesocosms.

References

(StopBMSB.org) Northeastern IPM Center. 2017. StopBMSB.org. (http://www.stopbmsb.org/where-is-bmsb/state-by-state/).

Abram, P. K., T. D. Gariepy, G. Boivin, and J. Brodeur. 2014. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol. Invasions. 16: 1387–1395.

Arakawa, R., M. Miura, and M. Fujita. 2004. Effects of host species on the body size, fecundity, and longevity of *Trissolcus mitsukurii* (Hymenoptera : Scelionidae), a solitary egg parasitoid of stink bugs. Appl. Entomol. Zool. 39: 177–181.

Arakawa, R., and Y. Namura. 2002. Effects of temperature on development of three *Trissolcus spp.* (Hymenoptera: Scelionidae), egg parasitoids of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). Entomol. Sci. 5: 215–218.

Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert, D. Reisig, T. P. Kuhar, and J. F. Walgenbach. 2015. Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. Environ. Entomol. 44: 1011–1021.

Barratt, B., F. Howarth, T. Withers, J. Kean, and G. S. Ridley. 2010. Progress in risk assessment for classical biological control. Biol. Control. 52: 245–254.

Basnet, S., L. M. Maxey, C. A. Laub, T. P. Kuhar, and D. G. Pfeiffer. 2014. Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in Southwestern Virginia. J. Entomol. Sci. 49: 304–312.

Caltagirone, E. 1981. Landmark examples in classical biological control. Ann. Rev. Entomol. 26: 213–232.

Case, T. J. 1990. Invasion resistance arises in strongly interacting species-rich model competition communities. Proc. Natl. Acad. Sci. 87: 5.

Case, T. J. 1991. Invasion resistence, species build-up and community collapse in metapopulation models with interespecies competition. Biol. J. Linn. Soc. 42: 239–266.

Chen, Y., K. L. Giles, M. E. Payton, and M. H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Mol. Ecol. 9: 1887–1898.

Cornelius, M. L., C. Dieckhoff, K. A. Hoelmer, R. T. Olsen, D. C. Weber, M. V. Herlihy, E. J. Talamas, B. T. Vinyard, and M. H. Greenstone. 2016. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control. 103: 11–20.

Correa-Ferreira, B. S., and F. Moscardi. 1996. Biological control of soybean stink bugs by inoculative releases of *Trissolcus basalis*. Entomol. Exp. Appl. 79: 1–7.

Deagle, B. E., J. P. Eveson, and S. N. Jarman. 2006. Quantification of damage in DNA recovered from highly degraded samples--a case study on DNA in faeces. Front. Zool. 3: 1–10.

Drake, C. 1920. The southern green stink-bug in Florida. Q. Bull. Florida State Plant Board. 4: 1–10.

Eitzinger, B., A. Micic, M. Körner, M. Traugott, and S. Scheu. 2013. Unveiling soil food web links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. Soil Biol. Biochem. 57: 943–945.

Elton, C. S. 1958. The ecology of invasions by animals and plants. J. Chem. Inf. Model. 53: 1689–1699.

Funayama, K. 2004. Importance of apple fruits as food for the brown-marmorated stink bug, *Halyomorpha halys* (Stal) (Heteroptera: Pentatomidae). Appl. Entomol. Zool. 39: 617–623.

Furlong, M. J., D. L. Rowley, R. Murtiningsih, and M. H. Greenstone. 2014. Combining ecological methods and molecular gut-content analysis to investigate predation of a Lepidopteran pest complex of Brassica crops. Entomol. Exp. Appl. 153: 128–141.

Gagnon, A. È., G. E. Heimpel, and J. Brodeur. 2011. The ubiquity of intraguild predation among predatory arthropods. PLoS One. 6: 1–7.

Gomez-Polo, P., O. Alomar, C. Castañé, T. Aznar-Fernández, J. G. Lundgren, J. Piñol, and N. Agustí. 2016. Understanding trophic interactions of *Orius spp.* (Hemiptera: Anthocoridae) in lettuce crops by molecular methods. Pest Manag. Sci. 72: 272–279.

Gomez-Polo, P., O. Alomar, C. Castañé, J. G. Lundgren, J. Piñol, and N. Agustí. 2015. Molecular assessment of predation by hoverflies (Diptera: Syrphidae) in Mediterranean lettuce crops. Pest Manag. Sci. 71: 1219–1227.

Greenstone, M. H., M. E. Payton, D. C. Weber, and A. M. Simmons. 2014. The detectability half-life in arthropod predator-prey research: what it is, why we need it, how to measure it, and how to use it. Mol. Ecol. 23: 3799–813.

Greenstone, M. H., D. L. Rowley, D. C. Weber, M. E. Payton, and D. J. Hawthorne. 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. Bull. Entomol. Res. 97: 201–209.

Greenstone, M. H., Z. Szendrei, M. E. Payton, D. L. Rowley, T. C. Coudron, and D. C. Weber. 2010. Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. Entomol. Exp. Appl. 136: 97–107.

Harwood, J. D., N. Desneux, H. J. S. Yoo, D. L. Rowley, M. H. Greenstone, J. J. Obrycki, and R. J. O'Neil. 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: A molecular approach. Mol. Ecol. 16: 4390–4400.

Harwood, J. D., and J. J. Obrycki. 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102: 335–350.

Harwood, J. D., H. J. S. Yoo, M. H. Greenstone, D. L. Rowley, and R. J. O'Neil. 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. Biol. Invasions. 11: 895–903.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One. 11: e0150275.

Hoebeke, E. R., and M. E. Carter. 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105: 225–237.

Hoffman, W. 1931. A pentatomid pest of growing beans in South China. Peking Nat. Hist. Bull.

Holtz, T., and K. Kamminga. 2010. Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in the United States. USDA APHIS-PPQ.

Hoogendoorn, M., and G. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol. Ecol. 10: 2059–2067.

Howarth, F. G. 1991. Environmental Impacts of Classical Biological Control. Annu. Rev. Entomol. 36: 485–509.

Jaworski, C. C., A. Bompard, L. Genies, E. Amiens-Desneux, and N. Desneux. 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. PLoS One. 8: 1–10.

Jones, A. L., D. E. Jennings, C. R. R. Hooks, and P. M. Shrewsbury. 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control. 78: 61–66.

Kawada, H., and C. Kitamura. 1992. The Tachinid Fly, *Bogosia sp* (Diptera: Tachinidae), as a Parasitoid of the Brown Marmorated Stink Bug, *Halyomorpha mista* Uhler (Heteroptera: Pentatomidae). Japanese J. Environ. Entomol. Zool. 4: 65–70.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17: 164–170.

King, R. a, D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17: 947–63.

Kobayashi, T., T. Hasegawa, and K. Kegasawa. 1973. Major insect pests of leguminous crops in Japan. Trop. Agric. Res. Ser. 6: 109–126.

Kobayashi, T., M. Takada, S. Takagi, A. Yoshioka, and I. Washitani. 2011. Spider predation on a mirid pest in Japanese rice fields. Basic Appl. Ecol. 12: 532–539.

Koppel, A. L., D. A. Herbert, T. P. Kuhar, and A. K. Kamminga. 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in Southeast Virginia. Environ. Entomol. 38: 375–379.

Lee, D.-H., B. D. Short, S. V Joseph, J. C. Bergh, and T. C. Leskey. 2013. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China,

Japan, and the Republic of Korea. Environ. Entomol. 42: 627–41.

Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. a. Hoelmer, D.-H. Lee, and S. E. Wright. 2012. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23: 218–226.

Leskey, T. C., and D. H. Lee. 2014. Dispersal capacity and behavior of nymphal stages of *Halyomorpha halys* (Hemiptera: Pentatomidae) evaluated under laboratory and field conditions. J. Insect Behav. 27: 639–651.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche (Stuttg). 2012: 1–14.

Liu, L., and B. Piper. 2016. Predicting the total economic impacts of invasive species: The case of *B. rubostriata* (red streaked leafhopper). Ecol. Econ. 128: 139–146.

Lundgren, J. G., M. E. Ellsbury, and D. A. Prischmann. 2009. Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. Ecol. Appl. 19: 2157–2166.

Lundgren, J. G., and D. C. Weber. 2010. Changes in digestive rate of a predatory beetle over its larval stage: Implications for dietary breadth. J. Insect Physiol. 56: 431–437.

Maslo, B., R. Valentin, K. Leu, K. Kerwin, G. C. Hamilton, A. Bevan, N. H. Fefferman, and D. M. Fonseca. 2017. Chirosurveillance: The use of native bats to detect invasive agricultural pests. PLoS One. 12: e0173321.

McPherson, J. 1980. A list of the prey species of *Podisus maculiventris* (Hemiptera: Pentatomidae). Gt. Lakes Entomol. 13: 17–24.

McPherson, J., and R. McPherson. 2000. Stink bugs of economic importance in America north of Mexico. CRC Press, Boca Raton.

Milnes, J., N. Wiman, E. Talamas, and J. Brunner. 2016. Discovery of an exotic egg parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Stål) in the Pacific Northwest. Proc. Entomol. Soc. Washingt. 188: 466–470.

Morrison, W. R., A. N. Bryant, B. Poling, N. F. Quinn, and T. C. Leskey. 2017. Predation of *Halyomorpha halys* (Hemiptera: Pentatomidae) from Web-Building Spiders Associated with Anthropogenic Dwellings. J. Insect Behav. 30: 70–85.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control. 97: 120–130.

Naranjo, S. E., and J. R. Hagler. 2001. Toward the quantification of predation with predator gut immunoassays: a new approach integrating functional response behavior. Biol. Control. 20: 175–189.

Nielsen, A. L., and G. Hamilton. 2009a. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 108: 608–616.

Nielsen, A. L., and G. C. Hamilton. 2009b. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102: 1133–1140.

Ogburn, E. C., R. Bessin, C. Dieckhoff, R. Dobson, M. Grieshop, K. A. Hoelmer, C. Mathews, J. Moore, A. L. Nielsen, K. Poley, J. M. Pote, M. Rogers, C. Welty, and J. F. Walgenbach. 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control. 101: 39–51.

Orr, D. B., J. S. Russin, D. J. Boethel, and W. A. Jones. 1986. Stink Bug (Hemiptera: Pentatomidae) Egg Parasitism in Louisiana Soybeans. Environ. Entomol. 15: 1250–1254.

Owens, D. R., D. A. Herbert, G. P. Dively, D. D. Reisig, and T. P. Kuhar. 2013. Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? J. Econ. Entomol. 106: 1317–1323.

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by generalist predators. Biol. Control. 114: 1-7.

Pyšek, P., and D. M. Richardson. 2010. Invasive species, environmental change and management, and health. Annu. Rev. Environ. Resour. 35: 25–55.

Qiu, L.-F. 2010. Natural enemy species of *Halyomorpha halys* and control effects of the parasitoids species in Beijing. North. Hortic. (http://en.cnki.com.cn/Article_en/CJFDTotal-BFYY201009072.htm).

Ragsdale, D. W., A. D. Larson, and L. D. Newsom. 1981. Quantitative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELISA. Environ. Entomol. 10: 402–405.

Reusch, T. B. H. 1998. Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in southern California, USA. Mar. Ecol. Prog. Ser. 170: 159–168.

Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Gariepy, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5: 1–13.

Rice, K. B., R. R. Troyer, K. M. Watrous, J. F. Tooker, and S. J. Fleischer. 2016. Landscape factors influencing stink bug injury in mid-Atlantic tomato fields. J. Econ. Entomol. 110: 94–100.

Ruberson, J., H. Nemoto, and Y. Hirose. 1998. Pesticides and conservation of natural enemies in pest management, pp. 207–220. *In* Barbosa, P. (ed.), Conserv. Biol. Control. Academic

Press.

Sheppard, S. K., J. Bell, K. D. Sunderland, J. Fenlon, D. Skervin, and W. O. C. Symondson. 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol. Ecol. 14: 4461–4468.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19: 751–762.

Stam, P. A., L. D. Newsom, and E. N. Lambremont. 1987. Predation and food as factors affecting survival of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in a soybean ecosystem. Environ. Entomol. 16: 1211–1216.

Swanson, D. 2012. An updated synopsis of the Pentatomoidea (Heteroptera) of Michigan. Gt. Lakes Entomol. 45: 263–311.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47: 561–94.

Talamas, E., M. Buffington, and K. Hoelmer. 2013. New synonymy of *Trissolcus halyomorphae* Yang. J. Hymenopt. Res. 33: 113–117.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. Buffington, M.-C. Bon, and D. C. Weber. 2015. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Tillman, P. 2010. Parasitism and predation of stink bug (Heteroptera: Pentatomidae) eggs in Georgia corn fields. Environ. Entomol. 39: 1184–1194.

Todd, J. W. 1989. Ecology and behavior of *Nezara viridula*. Annu. Rev. Entomol. 34: 273–292.

Troedsson, C., P. Simonelli, V. Nägele, J. C. Nejstgaard, and M. E. Frischer. 2009. Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). Mar. Biol. 156: 253–259.

Tyagi, S., and F. R. Kramer. 1996. Molecular beacons: Probes that fluoresce upon hybridization. Nat. Biotechnol. 14: 303–308.

Umeya, K. 1976. Outbreak of stink bugs in fruit trees. Plant Prot. 30: 133–141.

Valentin, R. E., B. Maslo, J. L. Lockwood, J. Pote, and D. M. Fonseca. 2016. Real-time PCR assay to detect brown marmorated stink bug, *Halyomorpha halys* (Stal), in environmental DNA. Pest Manag. Sci. 72: 1854–1861.

Venzon, M., A. Janssen, and M. W. Sabelis. 2002. Prey preference and reproductive success of the generalist predator *Orius laevigatus*. Oikos. 97: 116–124.

Weber, D. C., and J. G. Lundgren. 2009. Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. J. Insect Sci. 9: 41.

Yang, F., Q. Wang, D. Wang, B. Xu, J. Xu, Y. Lu, and J. D. Harwood. 2016. Intraguild predation among three common Coccinellids (Coleoptera: Coccinellidae) in China: detection using DNA-based gut-content analysis. Environ. Entomol. 42: 1–10.

Yang, Z.-Q., Y.-X. Yao, L.-F. Qiu, and Z.-X. Li. 2009. A New Species of *Trissolcus* (Hymenoptera: Scelionidae) Parasitizing Eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with Comments on its Biology. Ann. Entomol. Soc. Am. 102: 39–47.

Yeargan, K. V. 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8: 715–719.

Zhang, G. F., Z. C. Lü, F. H. Wan, and G. L. Lövei. 2007. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Mol. Ecol. Notes. 7: 947–954.

Chapter 2: Using Sentinel Egg Masses and Videography to Identify Predators of *Halyomorpha halys* (Stål) in Organic Crops

Abstract:

Halyomorpha halys is an invasive polyphagous Pentatomid pest of American agriculture. As a recent invader, the identity and effectiveness of natural enemies which attack this pest were unknown. In this chapter, I describe the results of two studies which attempted to identify the natural enemies of *H. halys* eggs in New Jersey. During the 2013-2014 growing seasons, sentinel *H. halys* egg masses were deployed at farms in southern New Jersey and assessed for signs of predation and parasitism. In total, 12,644 egg were deployed and of these, 11,420 (90.3%) were successfully recovered. Overall utilization of eggs was relatively low; sucking predation affected 3.70% (± 0.59%) of eggs, chewing predation affected 2.14% (± 0.63%) of eggs and parasitoids successfully developed from 1.43% (± 0.36%) of eggs. Six species of parasitoids were recovered from *H. halys* eggs, however, egg dissections revealed unsuccessful and partial parasitoid development in some cases. Throughout both years of this study, a subset of sentinel egg masses were surveilled with closed circuit security cameras. These recordings were used to identify organisms visiting and feeding on *H. halys* egg masses throughout the study. The cameras recorded 688 visits, although only 14 resulted in observable damage to the eggs. The most frequent visitor to egg masses were Muscoid flies, none of which were observed directly feeding on eggs. Taxa observed causing damage to eggs were Anthocoridae, Acrididae, Elateridae, Geocoridae, Formicidae, Gryllidae, Miridae, Pentatomidae, Rhyparochromidae and Tettigoniidae. Regression analysis revealed no relationship between the duration of visits to *H. halys* egg masses and the successful hatch rate of those eggs. Predator diversity and diel periodicity were also analyzed using video footage. Although predator diversity did not vary significantly between sites, visitors to *H. halys* eggs were most common in the twilight hours near sunrise and sunset.

Introduction:

Invasive species are a major threat to the ecological and economic stability of our planet. Invasive pests are responsible for an estimated $1.5 trillion in damages annually, and may be associated with the decline of native species in invaded ranges (Gurevitch and Padilla, 2004; Liu and Piper, 2016; Pimentel et al., 2005). However, the success of potential invasive organisms is affected by characteristics of the invader and of the invaded ecosystem (Caley et al., 2008; Pyšek and Richardson, 2010; Roy et al., 2011). The ability of ecosystems to prevent the establishment of invasive species is dependent on abiotic properties of the local area (environmental resistance) (Byers, 2002) as well as properties of organisms within the ecosystem (biotic resistance) (Case, 1990; DeRivera et al., 2005; Lonsdale, 1999; Pimm, 1989). Several processes contribute to the biotic resistance of an ecosystem including community diversity (Case, 1991, 1990; Stachowicz et al., 1999), and natural enemy abundance (Baltz and Moyle, 2007; Herbold and Moyle, 1986; Keane and Crawley, 2002). Generalist predators can prevent the establishment of invasive species by consuming novel invaders prior to major population growth or by limiting the ability of invaders to fully exploit local habitats (Reusch, 1998; Symondson et al., 2002). A diverse and abundant community of native predators may provide long-term stability by disrupting the invasion process, but it is difficult to predict the ability of a predator community to resist a specific invader (Carlsson et al., 2009; Lonsdale, 1999; Pimm, 1989; Pyšek and Richardson, 2010). Identifying natural enemies of invasive pests is a key step in the development of ecologically based pest control strategies for invading arthropods.

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive species native to East Asia which is established throughout much of the U.S. and is now an important agricultural and homeowner pest (Hoebeke and Carter, 2003; Lee et al., 2013; Leskey et al., 2012). The *H. halys* invasion was first detected in Allentown, PA in 1996, and since then, this pest has spread to 43 states, and has been detected in Canada, Central Europe, and the former Soviet Union (Fogain and Graff, 2011; Gapon, 2016; Harris, 2010; Hoebeke and Carter, 2003; Maistrello et al., 2016;

"StopBMSB.org," 2017; Vétek et al., 2014; Wyniger et al., 2014). *Halyomorpha halys* spread from Pennsylvania to neighboring New Jersey where it was first detected in 1999, with peak populations occurring in 2010 (Hahn et al., 2016; Nielsen et al., 2013). *H. halys* has caused ecological disturbances in its invaded range by disrupting native stink bug populations, potentially altering the structure of herbivore communities in many agroecosystems (Basnet et al., 2014; Nielsen and Hamilton, 2009a). With over 200 known host-plants, *H. halys* is highly mobile and polyphagous, capable of causing economic damage in a wide variety of agricultural crops (Leskey et al., 2012; Nielsen and Hamilton, 2009b). In 2010 alone, *H. halys* caused significant economic injury to peach, Asian pear, cherry, tomato, corn, and soybeans and an estimated $37 million in damage to Mid-Atlantic apple production (Holtz and Kamminga, 2010; Leskey, 2010; Leskey et al., 2012).

Identifying and quantifying the effects of natural enemies is an important step in the development of biologically-based pest management programs for invasive species. Several studies have attempted to determine the identity of predators and parasites of *H. halys* in its invaded range. Two of these, Ogburn et al. (2016) and Poley et al. (*unpublished*), are multistate collaborative works from which the results of this chapter have been drawn. However, the results of additional research into the natural enemies of *H. halys* in the Eastern U.S. will now be presented. Sentinel egg masses have been used with mixed success to study the natural enemies of *H. halys* eggs in wooded and simulated suburban habitats (Cornelius et al., 2016). In this study, overall egg mass utilization was very low, with 3.8% (± 0.8%) egg mortality from parasitism and 4.4% (± 0.9%) mortality from predation; 1.4% of egg masses were also reported missing. Morrison et al. (2016) exposed *H. halys* eggs to an array of potential predators, and then photographed egg masses after feeding to document signs and symptoms of predation. The taxa which attacked *H. halys* eggs most frequently were Carabidae, Tettigoniidae, and Salticid spiders, with chewing predators consuming significantly more eggs than sucking predators (i.e. Hemipterans) (Morrison et al., 2016). Similarly, chewing predators caused significantly higher

predation to *H. halys* eggs in laboratory microcosm studies while sucking predators most frequently attacked nymphs (Pote and Nielsen 2017).

Native Chalcidoid wasps of the genera *Trissolcus*, *Telenomus*, and *Anastatus* will attack and develop on *H. halys* sentinel eggs with variable but generally low rates of success (Abram et al., 2014b; Cornelius et al., 2016; Jones et al., 2014). Jones et al. (2014) found that parasitism on naturally laid egg masses, especially those found in ornamental nurseries, were significantly higher than sentinel egg masses oviposited in colony and transported to the field. An exotic specialist parasitoid, *Trissolcus japonicus* (Ashmead), has recently been detected from *H. halys* egg masses in the U.S. for the first time (Talamas et al., 2015). The introduction of *T. japonicus* into the U.S. may eventually impact *H. halys* population dynamics but its current distribution is limited and evidence suggests that *T. japonicus* is more abundant in woody habitats than agricultural fields (Herlihy et al., 2016; Talamas et al., 2015). Until the distribution of *T. japonicus* stabilizes and its effects fully understood, native generalist predators of *H. halys* may represent the greatest potential for biological control of this pest (Symondson, 2002).

Investigating arthropod predation events in field settings can be difficult due to their small size and often concealed and/or nocturnal behavior, but several methods enable the study of these behaviors *in situ* (Grieshop et al., 2012; King et al., 2008; Symondson, 2002). Molecular technology can be used to identify prey remnants from predator digestive tracts in field settings (Sheppard and Harwood, 2005; Symondson, 2002) but these techniques require species-specific reagents and a degree of technical skill. Arthropod food webs can also be studied through the use of sentinel prey that rely on observations of sessile or tethered prey deployed in the field. These prey can be assessed for signs of predation after deployment which provides data on relative prey utilization rates but little or no information about predator identity (as in Ogburn et al. 2016). Sentinel prey can also be observed visually while in the field and although these observations can help identify predators as well as the diel patterns of predation, this method is relatively time and labor intensive prohibiting high replication (Costamagna and Landis, 2007).

In lieu of human observers, video recording equipment can be used to identify the predators of sentinel prey without the technical limitations of molecular tools or the labor requirements of direct observations (Grieshop et al., 2012).

Video recording equipment has been used to study insect behavior and ecology for over two decades (Hardie and Powell, 2002; Kindvall et al., 2000; Storer et al., 1999). Recent technological advancements in camera size, durability and cost now allow video recording to be used in a wider variety of experiments particularly in field settings (Grieshop et al., 2012). Modern video recording systems are relatively inexpensive, can be operated in isolated locations without access to AC power and can record for longer durations than previous generations of this technology. Grieshop et al. (2012) used video recording systems to study predation of insect pests in several agroecosystems including highbush blueberries and two bioenergy crops. They found significant differences between the predator community collected in pitfall traps and that observed on video recordings indicating a high degree of methodological dependency.

Our understanding of the identity and efficacy of generalist predators of *H. halys* remains incomplete. Thus the objectives of this study were to 1) quantify utilization of eggs by predators of *H. halys* eggs in New Jersey agroecosystems using sentinel egg masses, 2) compare natural enemy egg utilization among *H. halys* host-crops, and 3) identify visitors to *H. halys* eggs and quantify their effects through video recording of field-deployed sentinel egg masses.

Methods

We used *H. halys* sentinel egg masses and video recording equipment to study the identity and rates of predation on these eggs in organic cropping systems. This work was conducted during summer of 2013 and 2014 at three organic farms in southern and central New Jersey. In 2013, research sites included a 4 ha transitional organic plot at the Rutgers Agriculture Research and Extension Center (RAREC) in Bridgeton, NJ (39.518719, -75.205849), a university-operated multi-crop research facility, and a commercial partially organic multi-crop

farm in Princeton, NJ (40.331898, -74.726098). Due to management changes, the Princeton farm was replaced in 2014 with a certified organic multi-crop CSA in Monroe Township, NJ (39.703274, -75059854). In 2013, sentinel egg masses were deployed in peppers and apples at the Princeton farm. At RAREC, eggs were deployed in peppers and soybeans. In 2014, research was conducted in peppers and raspberries at both sites.

Sentinel Egg Masses. Healthy unfrozen *H. halys* egg masses were acquired from the New Jersey Department of Agriculture's Phillip Alampi Beneficial Insect Rearing Laboratory in Trenton, NJ. These egg masses were provided on a number of ovipositional substrates including leaves, paper towel, or cardboard. The number of eggs per mass was assessed prior to deployment and only those with >10 eggs per mass were used. In 2014, pre-deployment assessment was expanded to include an assessment of the number of stylet sheaths per egg mass. After the initial assessment, egg masses were pinned to the underside of a mid-canopy host plant leaf. At least six egg masses were deployed per crop per week, although the total number per week varied due to fluctuations in supplier availability. Egg masses were < 48 h old at the start of each deployment and remained in the field for 48 h. Deployments, were conducted from June through August in 2013 and May through August in 2014. Deployments alternated between sites each week.

After each deployment, egg masses were assessed for signs of predation with a dissecting stereomicroscope. Eggs with stylet sheaths protruding from them were considered consumed by sucking predators, while those which appeared crushed or shredded were considered consumed by chewing predators (see *Discussion*; Morrison et al. 2016). After assessment, egg masses were deposited in sealed Petri dishes (100mm x 15mm) and stored in an incubator (25° C, 40-60% RH, photoperiod 16 h light : 8 h dark) until nymph hatch. First instars were removed from the Petri dish after reaching the second instar, but egg masses were incubated for an additional 6 weeks to permit parasitoid development and emergence. Parasitism was considered "successful" if a fully formed parasitoid completely emerged from the host egg. Partial parasitoid emergence was considered "unsuccessful parasitism". In 2014, sentinel eggs were dissected after final parasitoid

emergence to check for partially developed but unhatched parasitoids. These un-emerged wasps were considered functionally equivalent to partially emerged parasitoids and so were recorded together as “unsuccessful parasitism.” Successfully emerged parasitoids were stored in 80% ethanol and identified by Dr. Christine Dieckhoff, at the USDA ARS Beneficial Insects Introduction Laboratory in Newark, DE.

Videography. To aid in predator identification, a subset of *H. halys* sentinel egg masses were filmed with closed circuit security cameras for the duration of the 48 h deployment. Video equipment was modified from Grieshop et al. (2012). Each egg mass was filmed with an indoor/outdoor surveillance camera equipped with 12 infrared Light Emitting Diodes (LEDs) for night viewing (model MT-MD202, DoCooler®, Shenzen, China). Camera units were powered with three 12-volt, 7 ah batteries (eComElectronics, Brooklyn, NY) and footage was stored to 32 GB SD cards (Sandisk, Milpitas, CA) with a single channel, high definition mini-DVR (model 700TVL, Zosi Tech, Hong Kong).

The DVR unit and batteries were stored inside a waterproof plastic toolbox (48.26 x 22.86 x 25.4 cm) to protect from the weather. The camera was mounted to a polyvinyl chloride (PVC) pipe attached directly to the toolbox, which was placed within the crop row. For egg masses in apple trees, cameras were affixed to sturdy fruit-bearing terminals with flexible wire. A square piece of plastic board, measuring 15 cm x 15 cm marked with a 2.5 cm grid was attached to the PVC pipe parallel to the camera approximately 15-20 cm away from the lens. To keep the eggs within the focal range of the cameras, egg masses were pinned through host plant leaves and into the plastic backdrop. Battery charge was checked after 24 h and replacement batteries supplied as needed.

In 2014, video footage revealed birds consuming egg masses in raspberries at the Monroe Township, NJ farm. Raspberry plants were then draped in a coarse mesh (2.5 cm grid) which was staked into the ground to prevent birds from entering. After installment of mesh barriers, all bird predation was prevented but arthropod visitation continued as before. Any egg masses affected by

vertebrate predation (as evidenced by video recordings) were omitted from all data analysis and statistical summary.

At the conclusion of each deployment, video data contained on SD cards were transferred to secure external hard drives. DVR units stored video data as ".divx" format, thus these files were viewed with VLC media player v 2.2.4 Weatherwax (VideoLAN organization, Paris, France), an open source, multi-format multimedia player. Video playback was conducted at approximately 8x speed during assessment. When an organism contacted an egg mass in the footage, the time of day and duration of the interaction was recorded as well as the identity of the visiting organism. These "visitors" were identified to at least the ordinal level, with more specific identification made when possible. Organisms which could not be identified to the ordinal level were categorized as unknown. Egg mass visitors causing visible damage to egg masses were categorized separately for later analysis, although these predation events were often difficult to perceive.

Analysis. All statistical analysis was conducted using R Studio v3.2.2 "fire safety". Egg fate was modeled using generalized linear models with binomial error distribution and logit link. The following types of egg fate were each considered a binary for each egg and analyzed separately: chewing predation, sucking predation, parasitism (combining successful and unsuccessful parasitoid emergence), and total natural enemy mortality. For each analysis, significance of model terms was determined with likelihood ratio tests. First, I tested for significant differences in the frequency of each type of egg fate between 2013 and 2014 (*model:* $egg\ fate = year + farm + crop + eggs\ deployed + \varepsilon$). However, due to significant differences in methodology between years, further analysis was conducted separately for each year. In 2013, only pepper was grown at both sites and as a result egg fates were compared between farms and crops but the model terms for farm and crop could not be analyzed for potential interaction. In 2014, farm and crop were analyzed as potentially interacting factors. Post-hoc analysis and means separation was conducted using a modification of Tukey's method for general linear hypothesis

testing. Except where missing eggs were confirmed to be the result of predation, missing eggs masses were excluded from analysis and summary statistics.

Shannon diversity index and Pielou's evenness index were calculated for the community of visitors and were compared between crops and years using Kruskal-Wallis tests. To determine the relationship between visitation by potential predators and egg performance, I conducted a regression analyses of visit duration and egg mass hatch rate (*model: hatch rate = mean + duration of visit + crop + year + month + ε*). The diel periodicity of egg mass visitation was determined by converting the observed times of visits into hours before/after solar noon and binning visits into hour long increment.

Results

Sentinel Egg Masses. In total, 409 *H. halys* egg masses consisting of 12,644 individual eggs were deployed at three farms, in four crops. Of these, 11,420 were successfully recovered and assessed for symptoms of predation. The recovered eggs were affected by several types of feeding injury including sucking predation (3.70% ± 0.59% of eggs), chewing predation, (2.14% ± 0.63% of eggs) and parasitism (1.43% ± 0.36% of eggs) (Fig. 1). Between both years, 1224 eggs (9.68% ± 4.21%) were missing after the 48 h deployment (including egg masses which were 100% missing, but excluding predation from vertebrates confirmed by video recordings). Overall, 7.27% (± 0.90%) of deployed *H. halys* eggs were destroyed by natural enemies and 52.0% (± 1.98%) hatched normally. Total natural enemy mortality was significantly higher in 2013 than in 2014 ($df = 1, 407$; $P < 0.001$) (Fig. 1A). Chewing predation affected significantly more *H. halys* eggs in 2013 than 2014 ($df = 1, 407$; $P < 0.001$) (Fig. 1B). Similarly, rates of sucking predation were significantly higher in 2013 than in 2014 ($df = 1, 407$; $P < 0.001$) (Fig. 1C). Parasitism rates were significantly higher in 2014 than in 2013 ($df = 1, 407$; $P = 0.039$) (Fig. 1D).

2013. In 2013, 171 *H. halys* egg masses were deployed consisting of 4720 individual eggs. Of these, 11.1 % (± 1.54%) experienced mortality from predation or parasitism. Nymphs

successfully hatched from 60.0% (± 1.54%) of eggs. Eggs experienced significantly higher overall mortality from natural enemies at RAREC than at the Princeton farm (*df* = 1, 169; *P* < 0.0001) (Fig. 1A). Chewing predation was significantly more common at RAREC than at the Princeton farm (*df* = 1, 169; *P* < 0.0001) (Fig 1B), but sucking predation was significantly more common at the Princeton farm (*df* = 1, 169; *P* = 0.0025) (Fig. 1D). Parasitism rates of sentinel eggs were significantly higher at RAREC than at the Princeton Farm (*df* = 1, 169; *P* < 0.0001) (Fig. 1D).

Analysis revealed significant differences in chewing predation between crops (*df* = 2, 167; *P* < 0.0001), parasitism (*df* = 2, 167; *P* < 0.0001), and overall natural enemy mortality in 2013 (*df* = 2, 167; *P* < 0.0001) (Fig 2). Differences in sucking predation among crops were not statistically significant (*df* = 2, 167; *P* = 0.09) (Fig. 2C). Sentinel eggs in soybeans experienced significantly higher overall natural enemy mortality than those in apple or pepper (Fig. 2A). Chewing predation on *H. halys* eggs was more common in soybean than in peppers or apple, though only the former was statistically significant (Fig. 2B). In apples, chewing and sucking predation rates were not significantly different (Fig. 2C). Parasitism of *H. halys* sentinel egg masses was low overall, but significantly higher in soybean than in pepper or apple (Fig. 2D).

2014. In 2014, 238 *H. halys* egg masses were deployed consisting of 6188 individual eggs; 4.35% (± 1.02%) of these experienced predation. Total mortality was significantly different between sites (*df* = 1, 236; *P* < 0.0001) and between crops (*df* = 1, 235; *P* < 0.0001) (Fig. 3A). Chewing predation did not significantly differ between the two farms studied (*df* = 1, 236; *P* = 0.844) or between the two crops studied (*df* = 1, 235; *P* = 0.335) (Fig. 3B). Significantly more eggs were affected by sucking predation at RAREC than Monroe Twp. (*df* = 1, 236; *P* < 0.0001) and was significantly more common in raspberry than in peppers (*df* = 1, 235; *P* < 0.0001) (Fig. 3C). Parasitism of *H. halys* eggs was significantly lower at RAREC than Monroe Twp. (*df* = 1, 236; *P* = 0.027) but significantly higher in peppers than raspberries (*df* = 1, 235; *P* = 0.047) (Fig. 3D). The interaction between farm and crop explained significant model deviance for chewing

predation (*df* = 1, 233; *P* <0.0001), sucking predation (*df* = 1, 233; *P* <0.0001), parasitism (*df* = 1, 233; *P* <0.0001), and overall natural enemy mortality (*df* = 1, 233; *P* <0.0001).

Parasitoids. Between the two years of this study, 115 parasitoids successfully emerged from *H. halys* eggs (1.05% of total) affecting 18 egg masses (4.4% of total). The most abundant species of successful parasitoids was *Anastatus mirabilis* (Walsh and Riley) which constituted 56 parasitoids from 2 egg masses in 2013. *Telenomus podisi* (Ashmead) was the next most abundant parasitoid species, including 39 successfully developed parasitoids from 11 egg masses across both years. Additional species of parasitoids collected were: *Anustatus pearsalli* (Ashmead), eight parasitoids from one egg mass in 2013, *Trissolcus euschisti* (Ashmead), two parasitoids from one egg mass in 2013, and *Trissolcus brochymenae* (Ashmead), two parasitoids from two egg masses spanning both years.

Parasitoids of *H. halys* eggs did not always successfully complete development. Observations of post-deployment egg masses, and dissection of egg masses in 2014 revealed 75 unsuccessful parasitoids. In 2013, 11 parasitoids died after partially emerging from *H. halys* egg masses; seven of these died emerging from egg masses which also produced successfully emerged parasitoids. Six had parasitized an egg mass which produced a single *T. brochymenae* and one shared an egg mass with five successfully emerged *T. podisi*. Egg dissections in 2014 detected 64 partially developed but un-emerged parasitoids. Identification of these was often impossible due to incomplete development, but 21 were associated with egg masses which also produced successful and identifiable adult parasitoids. Five egg masses successfully parasitized by *T. podisi* also had 15 partially developed parasitoids. Similarly, an *A. pearsalli* parasitized egg mass yielded 2 partially developed parasitoids, and masses parasitized by *T. brochymenae* and *T. euschisti* were found to contain a single partially developed parasitoid each.

Vidoegraphy of Sentinel Egg Masses. Over the two years of this study, 193 *H. halys* sentinel egg masses (4,680 eggs) were deployed with video cameras. Footage collected by these cameras

identified 31 different arthropod taxa visiting *H. halys* egg masses constituting 688 individual visits. In 2013, cameras recorded 80 visits from 15 unique taxa; of these parasitic Hymenoptera visited most frequently. In 2014, 608 visits were observed from 27 taxa including 16 taxa which were not observed the previous year; Muscoid flies visited most often. Table 1 displays the identity of taxa observed visiting *H. halys* egg masses, and summarizes the frequency of these visits.

Taxa observed causing damage to eggs were Anthocoridae, Acrididae, Elateridae, Geocoridae, Formicidae, Gryllidae, Miridae, Pentatomidae, Rhyparochromidae and Tettigoniidae. Among these, the most frequent visitors were Formicidae (88 total visits, 1 with confirmed predation) and Pentatomidae (19 total visits, 2 with confirmed predation), although very few visits resulted in confirmable predation. In addition, three unidentifiable Hemiptera were also observed damaging eggs. Visits from Pentatomidae, Tettigoniidae, and Formicidae did not obligatorily result in damage to *H. halys* eggs. Of the 688 recorded visits to *H. halys* eggs, 14 of these (2.03% of all visits) resulted in confirmable egg predation or parasitism; during these visits 223 eggs were destroyed (15.9 eggs per damaging visitor). Of the taxa observed attacking *H. halys* egg masses, the most frequent visitors were parasitic Hymenoptera, Formicidae, and Pentatomidae (Table 1). Parasitoid wasps accounted for 130 total visitations and were observed on 25.0% of filmed egg masses in 2013 and 18.1% in 2014. However, successful parasitoids rarely emerged from these eggs visited by parasitoids (0.2% of eggs produced viable parasitoids). Formicidae visited egg masses 88 times, although no direct feeding was observed. Members of the family Pentatomidae visited *H. halys* egg masses 19 times, accounting for consumption of 52 eggs.

Orthoptera were observed visiting (n=20) and consuming egg masses in video recordings of sentinel eggs. In three of these visits (one Acrididae and two Tettigoniidae), the Orthoptera completely consumed the entire egg mass leaving no indication of predation and the eggs would have been assessed as missing. In total, 84 eggs were consumed by these Orthoptera, constituting

14.9% of all missing egg masses under video surveillance. The single video of an Acridid revealed that this organism consumed the leaf as well as the egg mass. Conversely, the two Tettigoniids observed consuming entire egg masses appeared to have a more directed search, consuming the *H. halys* egg mass without any leaf tissue. Other chewing predators, including some Gryllidae, consumed the eggs but left crushed pieces of *H. halys* egg chorion which was later used as a diagnostic criteria for assessing chewing predation. Each of these events was recorded during 2013 in soybeans at night and each event lasted from 1-3 h. Orthoptera constituted 2.9% of all visitors to *H. halys* egg masses but 28.6% of damaging visitors.

***Predator Diversity*.** The Shannon diversity index of all visitors identified from video data was 2.542 and evenness of this community was 0.747. When diversity indices were compared between years, 2014 was found to have significantly higher diversity and evenness compared to 2013 (*diversity*: $X^2 = 12.82$, $df = 1$, $P = 0.0003$; *evenness:* $X^2 = 19.26$, $df = 1$, $P < 0.0001$). Diversity indices were not significantly different between crops, farm, or months of deployment in 2013. In 2014, Shannon diversity index differed significantly between farms and months of deployment but not between crops (*farm*: $X^2 = 3.886$, $df = 1$, $P = 0.048$; *month*: $X^2 = 8.22$, $df = 3$, $P = 0.041$). Species evenness was not significantly affected by farm, crop, or month of deployment in 2014.

Significant correlation between sentinel egg mass hatch rate and visitors per egg mass was not detected in 2013 (*Slope* = -0.0042, $F_{1,83} = 0.0362$, $P = 0.8496$, $R^2 = 0.0004$)(Fig. 4a) or 2014 (*Slope* = -0.0079, $F_{1,83} = 0.029$, $P = 0.738$, $R^2 = -0.0155$) (Fig. 4b). Visitors to *H. halys* sentinel egg masses showed distinct diel dependency in their visitations. Of 688 total visits, 452 (65.7%) occurred during the daylight hours (600 to 2000 hours, adjusted for solar noon) while 236 (34.3%) occurred at night (Fig. 5). Visits occurred most frequently in the 2 hours preceding sunset (1700 to 1900 hours); 122 visits (17.7%) occurred during this period (Fig. 5).

Discussion

Identifying natural enemies of invasive pests is a key step in the development of ecologically-based pest control strategies for invading arthropods. After its discovery in the U.S., the natural enemy community affecting *H. halys* in the Eastern U.S. was unknown and the level of population suppression provided by these natural enemies was unquantified. My results show that natural control of *H. halys* egg masses in New Jersey agro-ecosystems is relatively low, affecting less than 10% of returned sentinel eggs (Fig. 1). Despite this, data from sentinel egg masses (and video recordings thereof) revealed frequent interactions between natural enemies and *H. halys* eggs: over one third of all egg masses contained at least one egg affected by natural enemies and hundreds of visits to sentinel egg masses were recorded by video (Table 2). Based on these findings, the observed low level of *H. halys* natural control is likely the result of poor host/prey acceptance, rather than poor host/prey finding. Effective models of *H. halys* enemy-prey dynamics must be developed to determine if the observed predation and parasitism rates have a meaningful impact on *H. halys* abundance and crop injury.

This study was conducted as part of a collaborative multistate effort (Ogburn et al., 2016). The findings of this work indicate similarity between the results from New Jersey and other states. Together, these results revealed low *H. halys* egg mass utilization by predators and endemic parasitoids across its invaded range. When data from all seven Eastern U.S. states was combined, chewing and sucking predation each affected < 5% of all *H. halys* sentinel eggs (Ogburn et al., 2016). As in New Jersey, chewing predation in West Virginia and Tennessee was numerically highest in soybean. Successful parasitism of *H. halys* egg masses was rare in other states, as it was in New Jersey; parasitoids emerged from less than 0.5% of all eggs across both years. Un-emerged parasitoids were dissected from *H. halys* sentinel eggs from all seven states including New Jersey. The rate of unsuccessful parasitism, although variable, was higher than the rate of successful parasitism at each site and in every crop. Differences in natural control were

often detected between states, possibly due to differences in natural enemy diversity or the duration of *H. halys* presence among states.

Natural enemy impacts on native Pentatomidae have also been determined with sentinel egg mass studies. Like *H. halys*, the highest rates of predation on *Euschistus servus* (Say) egg masses were observed in soybeans, with 0 – 2.4% of *Euschistus servus* (Say) eggs attacked by predators (Koppel et al., 2009). However, predation rates on sentinel egg masses of other species of native Pentatomids were higher: 13.2% of *Euschistus variolarius* (Palisot de Beauvois) eggs, 39.5% of *Podisus maculiventris* (Say) eggs and 27.2% of *Chinavia hilare* (Say) eggs were killed by predators (Yeargan, 1979). However, successful parasitism rate on sentinel *E. servus* eggs was 14.1-88.9% (Koppel et al., 2009), significantly higher than that of *H. halys* in any of the crops studied. Yeargan (1979) found similarly high parasitism of *E. servus, E. variolarious,* and *P. maculiventris* eggs, effecting 19.6-50.2% of recovered eggs. Low parasitism of *H. halys* may be related to its recent invasion, as laboratory studies have shown higher rates of acceptance and successful parasitoid oviposition on native Pentatomids than *H. halys* (Abram et al., 2013). Differences in natural enemy utilization of *H. halys* eggs were detected across its geographic range and may be related to this phenomenon (Ogburn et al., 2016).

Videography of *H. halys* sentinel egg masses deployed in Michigan identified a community of visitors similar to that detected in New Jersey (Poley et al., *unpublished*). Despite this, the taxa observed consuming eggs varied between the two states. For example, earwigs (F: Forficulidae) and spiders were common visitors to and consumers of sentinel eggs in Michigan apples but neither taxa was observed in New Jersey apples. Additionally, visits to sentinel egg masses in New Jersey were most frequent during the photophase, visits in Michigan did not show a clear pattern of diel periodicity. These differences in predator community and behavior might be explained by landscape or farm management differences. Alternatively, New Jersey is located very near the epicenter of *H. halys* introduction (Nielsen et al., 2013), while Michigan is on the leading edge of the *H. halys* invasion of the American Midwest (Leskey and Hamilton, 2014).

Time since invasion may have important implications for predator and parasitoid acceptance of novel prey (Jaworski et al., 2013), thus the changing utilization of invasive species by natural enemies during range expansion should be monitored.

Results presented in this study are the first documented evidence of consumption by Orthoptera of *H. halys* egg masses in the field. These findings were confirmed in laboratory no-choice predation bioassays: Tettigoniids accepted and consumed > 70% of *H. halys* egg masses presented to them, and consumed approximately 70% of the eggs in each egg mass (Morrison et al., 2016). In a multi-state survey, missing eggs accounted for 8-13% of all deployed eggs and 37% of sentinel egg predation in peach orchards was consistent with the feeding evidence associated with Orthoptera (Morrison et al., 2016; Ogburn et al., 2016). In Southeast Asia, Orthopterans (including F: Tettigoniidae G: *Conocephalus spp.*) are important predators of Lepidopterous rice pests (Chitra et al., 2002). Direct observations of *Nezara viridula* (L.) sentinel eggs revealed instances of Orthopteran feeding on deployed eggs, but feeding by Formicid and Hemipteran predators was significantly more common (Stam et al., 1987). Although the feeding behaviors and relative omnivory of Orthopterans is not well studied outside of rice systems, these insects are abundant in many cropping systems affected by *H. halys* (Pote, *unpublished*). Orthopteran omnivory may play an important role in suppressing *H. halys* populations, however, it is unclear if the observed omnivory behaviors are obligate (as in Asian *Conocephalus spp.*), facultative, or a coincidence resulting from herbivorous consumption of leaf tissue.

One notable departure from nation-wide sentinel egg mass results were the unusually high rates of sucking predation in 2013 at New Jersey sites. There are two potential explanations for this phenomenon. First, stylet sheaths were one of several indicators used to identify sucking predation, but may also occur after cannibalism within *H. halys* colonies (Iverson et al., 2016). Cannibalism and resulting stylet sheaths were first observed after the 2013 season and as a result, the pre-deployment egg mass assessment protocol was updated for 2014 to include a count of pre-existing stylet sheaths. Higher reported sucking predation in 2013 may also be the result of

unclear diagnostic criteria for assessing sucking predation. Morrison et al. (2016) developed a visual guide for diagnosing predation of *H. halys* eggs, and sentinel egg mass assessment criteria were updated for the 2014 field season. Data presented here have been revised to include the updated diagnostic methods but it is not possible to distinguish between stylet sheaths from predation and cannibalism *post-hoc* so the reported sucking predation in 2013 may still be unrealistically inflated.

Unsuccessful parasitism was observed more frequently in 2014 than the previous year, largely due to post-deployment egg dissections. These often revealed a black gelatinous substance within unhatched *H. halys* eggs. Eggs containing this "black goop" were frequently associated with indications of parasitoid activity. The death of a host egg as well as the developing parasitoid embryo within, known as egg abortion, has been observed in *H. halys* eggs parasitized by native parasitoids (Abram et al., 2016). This phenomenon may constitute an important but undervalued facet of parasitoid natural enemy services (Kaser in review). Despite this, *H. halys* may also be functioning as an "evolutionary trap" for native parasitoids by causing these organisms to expend scarce time and egg resources while parasitizing an unsuitable host (Abram et al., 2014a). In this way, *H. halys* may facilitate population growth in other Pentatomidae species which would have otherwise been suppressed by shared native parasitoids.

The results presented here must be augmented with additional research to understand the full impact of predation and parasitism on *H. halys* population dynamics. There are no known parasitoids of the *H. halys* nymphal stage, but predators may provide top-down control of the five immature instars of *H. halys.* In laboratory predation biosassays, high rates of predation were observed on 1st and 2nd instar *H. halys* nymphs by Nabids, Reduviids, and *P. maculiventris* nymphs which reduced nymphal survivorship by 40-50% compared to untreated controls (Pote and Nielsen 2017). Considering these results and the low utilization rates of *H. halys* eggs, it is possible that predators of nymphs may be the driving source of natural enemy mortality in this species. Furthermore, rates of *H. halys* predation and parasitism varied significantly between

crops. Natural control of *H. halys* may have a greater impact in other crops, or in systems augmented by conservation strips or “beetle banks” (Landis et al., 2000). Augmentation of natural enemy services with these methods may complement cultural pest control tactics such as trap cropping. Sunflower, attractive to parasitoids as well as *H. halys*, has been used as a part of a polyculture trap crop system for the management of *H. halys* in vegetable crops (Mathews et al., 2017; Nielsen et al., 2016; Soergel et al., 2015). Utilizing a trap crop which also attracts natural enemies may synergistically reduce pest pressure and increase biological control of *H. halys*.

References

Abram, P.K., Brodeur, J., Burte, V., Boivin, G., 2016. Parasitoid-induced host egg abortion: An underappreciated component of biological control services provided by egg parasitoids. Biol. Control 98, 52–60. doi:10.1016/j.biocontrol.2016.04.002

Abram, P.K., Doyon, J., Brodeur, J., Gariépy, T.D., Boivin, G., 2014a. Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Can. Entomol. 147, 1–5. doi:10.4039/tce.2014.41

Abram, P.K., Gariepy, T.D., Boivin, G., Brodeur, J., 2014b. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol. Invasions 16, 1387–1395. doi:10.1007/s10530-013-0576-y

Abram, P.K., Gariepy, T.D., Boivin, G., Brodeur, J., 2013. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol. Invasions 16, 1387–1395. doi:10.1007/s10530-013-0576-y

Baltz, D.M., Moyle, P.B., 2007. Invasion Resistance to Introduced Species by a Native Assemblage of California Stream Fishes. Ecol. Appl. 3, 246–255.

Basnet, S., Maxey, L.M., Laub, C.A., Kuhar, T.P., Pfeiffer, D.G., 2014. Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in Southwestern Virginia. J. Entomol. Sci. 49, 304–312.

Byers, J.E., 2002. Physical habitat attribute mediates biotic resistance to non-indigenous species invasion. Oecologia 130, 146–156. doi:10.1007/s004420100777

Caley, P., Groves, R.H., Barker, R., 2008. Estimating the invasion success of introduced plants. Divers. Distrib. 14, 196–203. doi:10.1111/j.1472-4642.2007.00440.x

Carlsson, N.O., Sarnelle, O., Strayer, D.L., 2009. Native predators and exotic prey - An acquired taste? Front. Ecol. Environ. 7, 525–532.

Case, T.J., 1991. Invasion resistence, species build-up and community collapse in metapopulation models with interespecies competition. Biol. J. Linn. Soc. 42, 239–266.

Case, T.J., 1990. Invasion resistance arises in strongly interacting species-rich model competition communities. Proc. Natl. Acad. Sci. 87, 5. doi:10.1073/pnas.87.24.9610

Chitra, N., Gunathilagaraj, K., Soundararajan, R.P., 2002. Prey preference of Orthopteran predators on rice insect pests. J. Biol. Control 16, 109–111. doi:10.18311/JBC/16/2/4117

Cornelius, M.L., Dieckhoff, C., Hoelmer, K.A., Olsen, R.T., Weber, D.C., Herlihy, M. V., Talamas, E.J., Vinyard, B.T., Greenstone, M.H., 2016. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control 103, 11–20. doi:10.1016/j.biocontrol.2016.07.011

Costamagna, A.C., Landis, D.A., 2007. Quantifying predation on soybean aphid through direct field observations. Biol. Control 42, 16–24. doi:10.1016/j.biocontrol.2007.04.001

DeRivera, C.E., Ruiz, G.M., Hines, A.H., Jivoff, P., 2005. Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab. Ecology 86, 3364–3376. doi:10.1890/05-0479

Fogain, R., Graff, S., 2011. First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. J. Entomol. Soc. Ontario 142, 45–48.

Gapon, D.A., 2016. First records of the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. Entomol. Rev. 96, 1086–1088. doi:10.1134/S001387381608011X

Grieshop, M.J., Werling, B., Buehrer, K., Perrone, J., Isaacs, R., Landis, D.A., 2012. Big brother is watching: studying insect predation in the age of digital surveillance. Am. Entomol. 172–182.

Gurevitch, J., Padilla, D.K., 2004. Are invasive species a major cause of species extinctions? Trends Ecol. Evol. 19, 470–474.

Hahn, N.G., Kaufman, A.J., Rodriguez-Saona, C., Nielsen, A.L., LaForest, J., Hamilton, G.C., 2016. Exploring the spread of brown marmorated stink bug in New Jersey through the use of crowdsourced reports. Am. Entomol. 36–45.

Hardie, J., Powell, G., 2002. Video analysis of aphid flight behaviour. Comput. Electron. Agric. 35, 229–242. doi:10.1016/S0168-1699(02)00020-0

Harris, A.C., 2010. *Halyomorpha halys* (Hemiptera: Pentatomidae) and *Protaetia brevitarsis* (Coleoptera: Scarabaeidae: Cetoniinae) intercepted in Dunedin . Weta 44, 42–44.

Herbold, B., Moyle, P.B., 1986. Introduced Species and Vacant Niches. Am. Nat. 128, 751–760. doi:10.2307/2678832

Herlihy, M. V., Talamas, E.J., Weber, D.C., 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11, e0150275. doi:10.1371/journal.pone.0150275

Hoebeke, E.R., Carter, M.E., 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105, 225–237.

Holtz, T., Kamminga, K., 2010. Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in the United States. USDA APHIS-PPQ.

Iverson, J.M., Cira, T.M., Burkness, E.C., Hutchison, W.D., 2016. Cannibalistic oophagy in Halyomorpha halys (Hemiptera: Pentatomidae) laboratory colonies. J. Entomol. Sci. 51, 122–128. doi:10.18474/JES15-29.1

Jaworski, C.C., Bompard, A., Genies, L., Amiens-Desneux, E., Desneux, N., 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. PLoS One 8, 1–10. doi:10.1371/journal.pone.0082231

Jones, A.L., Jennings, D.E., Hooks, C.R.R., Shrewsbury, P.M., 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control 78, 61–66. doi:10.1016/j.biocontrol.2014.07.011

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

Kindvall, O., Nordlander, G., Nordenhem, H., 2000. Movement behaviour of the pine weevil *Hylobius abietis* in relation to soil type: an arena experiment. Entomol. Exp. Appl. 95, 53–61. doi:10.1046/j.1570-7458.2000.00641.x

King, R. a, Read, D.S., Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17, 947–63. doi:10.1111/j.1365-294X.2007.03613.x

Koppel, A.L., Herbert, D.A., Kuhar, T.P., Kamminga, A.K., 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in Southeast Virginia. Environ. Entomol 38, 375–379. doi:10.1603/022.038.0209

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45, 175–201. doi:10.1146/annurev.ento.45.1.175

Lee, D.-H., Short, B.D., Joseph, S. V, Bergh, J.C., Leskey, T.C., 2013. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ. Entomol. 42, 627–41. doi:10.1603/EN13006

Leskey, T.C., 2010. 2010 Tree Fruit Damage Survey.

Leskey, T.C., Hamilton, G.C., 2014. H. halys invasion history, in: BMSB Working Group Meeting.

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp, M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K. a., Lee, D.-H., Wright, S.E., 2012. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Liu, L., Piper, B., 2016. Predicting the total economic impacts of invasive species: The case of *B. rubostriata* (red streaked leafhopper). Ecol. Econ. 128, 139–146. doi:10.1016/j.ecolecon.2016.04.014

Lonsdale, W.M., 1999. Global patterns of plant invasions and the concept of invasibility. Ecology 80, 1522–1536.

Maistrello, L., Dioli, P., Bariselli, M., Mazzoli, G.L., Giacalone-Forini, I., 2016. Citizen science and early detection of invasive species: phenology of first occurrences of *Halyomorpha halys* in Southern Europe. Biol. Invasions 18, 3109–3116. doi:10.1007/s10530-016-1217-z

Mathews, C.R., Blaauw, B., Dively, G., Kotcon, J., Moore, J., Ogburn, E., Pfeiffer, D.G., Trope,

T., Walgenbach, J.F., Welty, C., Zinati, G., Nielsen, A.L., 2017. Evaluating a polyculture trap crop for organic management of *Halyomorpha halys* and native stink bugs in peppers. J. Pest Sci. (2004). 1–11. doi:10.1007/s10340-017-0838-z

Morrison, W.R., Mathews, C.R., Leskey, T.C., 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97, 120–130. doi:10.1016/j.biocontrol.2016.03.008

Nielsen, A.L., Dively, G., Pote, J.M., Zinati, G., Mathews, C., 2016. Identifying a potential trap crop for a novel insect pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in organic farms. Environ. Entomol. 45, 472–478. doi:10.1093/ee/nvw006

Nielsen, A.L., Hamilton, G., 2009a. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 108, 608–616.

Nielsen, A.L., Hamilton, G.C., 2009b. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102, 1133–1140. doi:10.1603/029.102.0335

Nielsen, A.L., Holmstrom, K., Hamilton, G.C., Cambridge, J., Ingerson-Mahar, J., 2013. Use of black light traps to monitor the abundance, spread, and flight behavior of *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Econ. Entomol. 106, 1495–1502. doi:10.1603/EC12472

Ogburn, E.C., Bessin, R., Dieckhoff, C., Dobson, R., Grieshop, M., Hoelmer, K.A., Mathews, C., Moore, J., Nielsen, A.L., Poley, K., Pote, J.M., Rogers, M., Welty, C., Walgenbach, J.F., 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control 101, 39–51. doi:10.1016/j.biocontrol.2016.06.002

Pimentel, D., Zuniga, R., Monison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

Pimm, S., 1989. Theories of predicting success and impact of introduced species, in: Biological Invasions: A Global Perspective. pp. 167–351.

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by generalist predators. Biol. Control. 114: 1-7.

Pyšek, P., Richardson, D.M., 2010. Invasive species, environmental change and management, and health. Annu. Rev. Environ. Resour. 35, 25–55. doi:10.1146/annurev-environ-033009-095548

Reusch, T.B.H., 1998. Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in southern California, USA. Mar. Ecol. Prog. Ser. 170, 159–168. doi:10.3354/meps170159

Roy, H.E., Lawson Handley, L.-J., Schönrogge, K., Poland, R.L., Purse, B. V., 2011. Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? BioControl 56, 451–468. doi:10.1007/s10526-011-9349-7

Sheppard, S.K., Harwood, J.D., 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19, 751–762. doi:10.1111/j.1365-2435.2005.01041.x

Soergel, D.C., Ostiguy, N., Fleischer, S.J., Troyer, R.R., Rajotte, E.G., Krawczyk, G., 2015. Sunflower as a potential trap crop of *Halyomorpha halys* (Hemiptera: Pentatomidae) in pepper fields. Environ. Entomol. 44, 1581–1589. doi:10.1093/ee/nvv136

Stachowicz, J.J., Whitlatch, R.B., Osman, R.W., 1999. Species Diversity and Invasion Resistance in a Marine Ecosystem. Source Sci. New Ser. 286, 1577–1579. doi:10.1126/science.286.5444.1577

Stam, P.A., Newsom, L.D., Lambremont, E.N., 1987. Predation and food as factors affecting survival of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in a soybean ecosystem. Environ. Entomol. 16, 1211–1216. doi:10.1093/ee/16.6.1211

StopBMSB.org [WWW Document], 2017. . Northeast. IPM Cent. URL http://www.stopbmsb.org/where-is-bmsb/state-by-state/

Storer, J., Young, S., Hardie, J., 1999. Three-dimensional analysis of aphip landing behaviour in the laboratory and field. Physiol. Entomol. 24, 271–277.

Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11, 627–641. doi:10.1046/j.1365-294X.2002.01471.x

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561–94. doi:10.1146/annurev.ento.47.091201.145240

Talamas, E.J., Herlihy, M. V., Dieckhoff, C., Hoelmer, K.A., Buffington, M., Bon, M.-C., Weber, D.C., 2015. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43, 119–128. doi:10.3897/JHR.43.4661

Vétek, G., Papp, V., Haltrich, A., Rédei, D., 2014. First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780, 2010–2012.

Wyniger, D., History, N., Basel, M., 2014. First records of an invasive bug in Europe : *Halyomorpha halys* Stal (Heteroptera : Pentatomidae). Bull. la Soc. Entomol. Suisse 81, 1–8.

Yeargan, K. V., 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8, 715–719.

Tables and Figures

Table 1. Frequency of Visitors to *H. halys* Sentinel Egg Masses Identified with Video Recordings. Data were collected in New Jersey agroecosystems during the growing seasons of 2013 and 2014. Rows containing taxa which were confirmed as damaging predators of *H. halys* eggs from video recordings are shaded grey. Predators which could not be identified beyond Order are listed as "Unk. [Order]" while those which could not be identified to Order are listed as "Unknown." App. = Apple, Pepp. = Pepper, Rasp. = Raspberry, Unk. = Unkown.

	Crop					
Taxa	**App.**	**Pepp.**	**Rasp.**	**Soy**	**Total Visits**	**Damaging Visits**
Acari	0	13	16	0	**29**	**0**
Araneae	6	13	14	0	**33**	**0**
Carabidae	0	2	1	0	**3**	**0**
Coccinellidae	0	10	8	0	**18**	**0**
Curculionidae	0	0	2	0	**2**	**0**
Elateridae	0	0	4	0	**4**	**1**
Scarabaeidae	0	1	0	0	**1**	**0**
Unk. Coleoptera	0	3	5	0	**8**	**0**
Dermaptera	0	1	2	0	**3**	**0**
Culicidae	0	3	2	0	**5**	**0**
Muscomorpha	0	55	92	0	**148**	**0**
Syrphidae	0	0	1	0	**2**	**0**
Unk. Diptera	0	18	7	0	**25**	**0**
Anthocoridae	0	2	0	0	**2**	**1**
Berytidae	0	0	1	0	**1**	**0**
Geocoridae	0	5	0	0	**8**	**1**
Miridae	0	2	0	0	**2**	**1**
Nabidae	0	0	1	0	**1**	**0**
Pentatomidae	0	2	17	0	**19**	**2**
Rhyparochromidae	0	0	1	0	**1**	**1**
Unk. Hemiptera	0	1	26	0	**27**	**2**
Formicidae	2	82	3	0	**88**	**0**
Parasitic Hymenop.	4	68	55	2	**130**	**0**
Isopod	0	0	3	0	**3**	**0**
Lepidoptera	0	0	1	0	**1**	**0**
Neuroptera	1	0	0	0	**1**	**0**
Opiliones	0	5	13	0	**18**	**0**
Acrididae	0	0	0	4	**4**	**1**
Gryllidae	10	0	1	0	**11**	**1**
Tettigoniidae	0	0	5	0	**5**	**2**
Unknown	3	30	51	0	**84**	**0**
Total	**26**	**316**	**331**	**6**	**686**	**14**

Figure 1. Natural Enemy Mortality of *H. halys* Sentinel Egg Masses, 2013-2014. A. Total mortality from chewing predation, sucking predation, and parasitism combined, B. chewing predation mortality, C. sucking predation mortality, D. parasitism including unsuccessful or partially developed parasitoids. Lines and capital letters indicate significant differences between years ($P < 0.05$). Lowercase letters indicate significant differences between sites within each year ($P < 0.05$) while n.d. indicates no significant difference between sites.

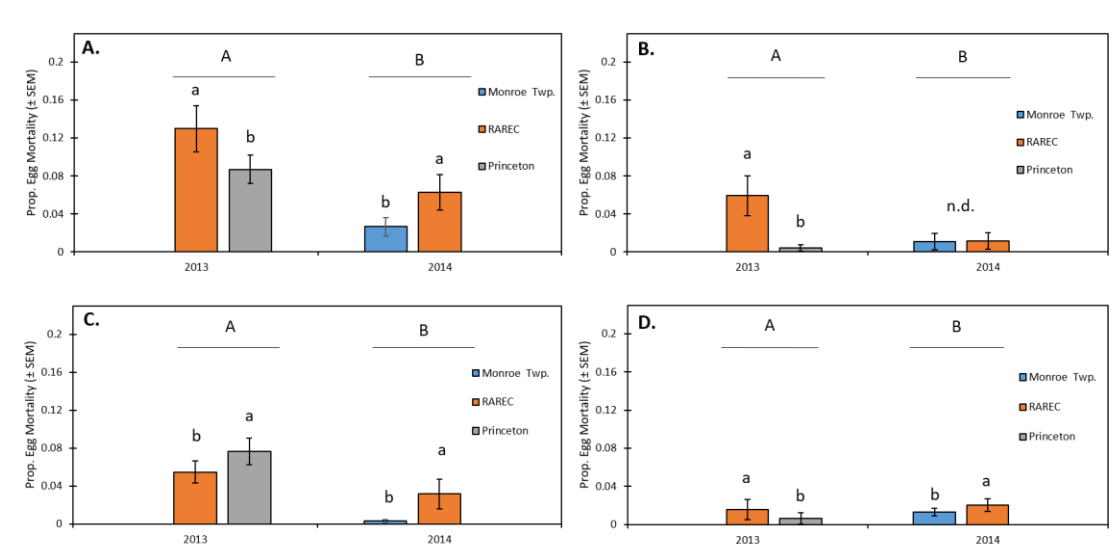


Figure 2. Natural Enemy Mortality of *H. halys* Egg Masses by Crop and Site, 2013. Note: the y-axis scale has been altered between Fig. 2a and Fig. 2b-d to highlight small values. A. Total mortality from chewing predation, sucking predation and parasitism combined, B. chewing predation mortality, C. sucking predation mortality, D. parasitism including unsuccessful or partially developed parasitoids. Lines and capital letters indicate significant differences between crops ($P < 0.05$) while n.d. indicates no significant difference.

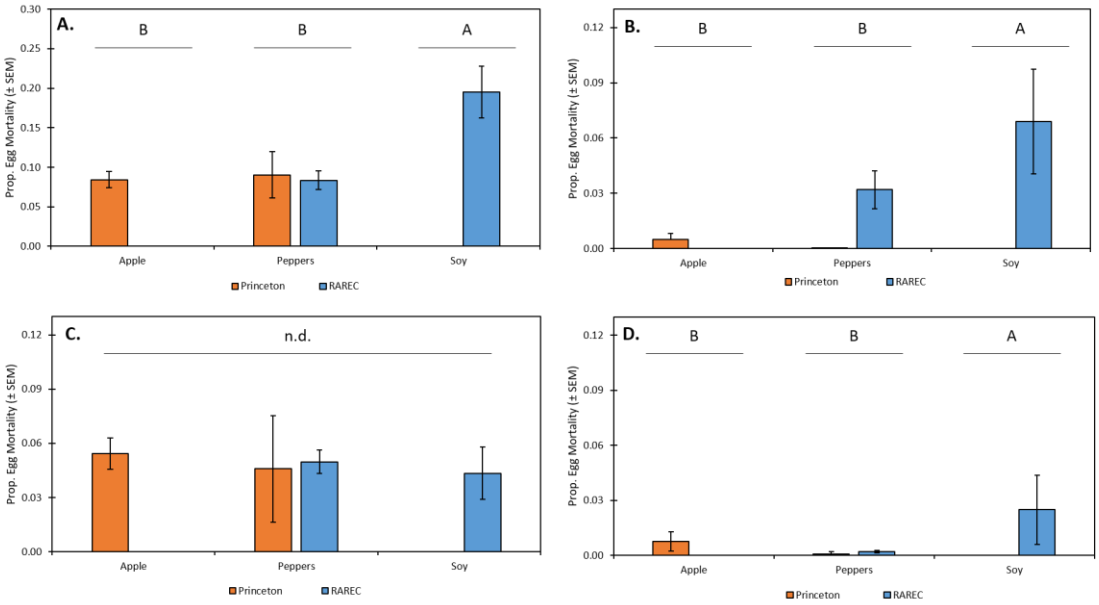


Figure 3. Natural Enemy Mortality of *H. halys* Egg Masses by Crop and Site, 2014. Note: the y-axis scale has been altered between Fig. 3a and Fig. 3b-d to highlight small values. A. Total mortality from chewing predation, sucking predation and parasitism combined, B. chewing predation mortality, C. sucking predation mortality, D. parasitism including unsuccessful or partially developed parasitoids. Lines and capital letters indicate significant differences between crops ($P < 0.05$) while n.d. indicates no significant difference.

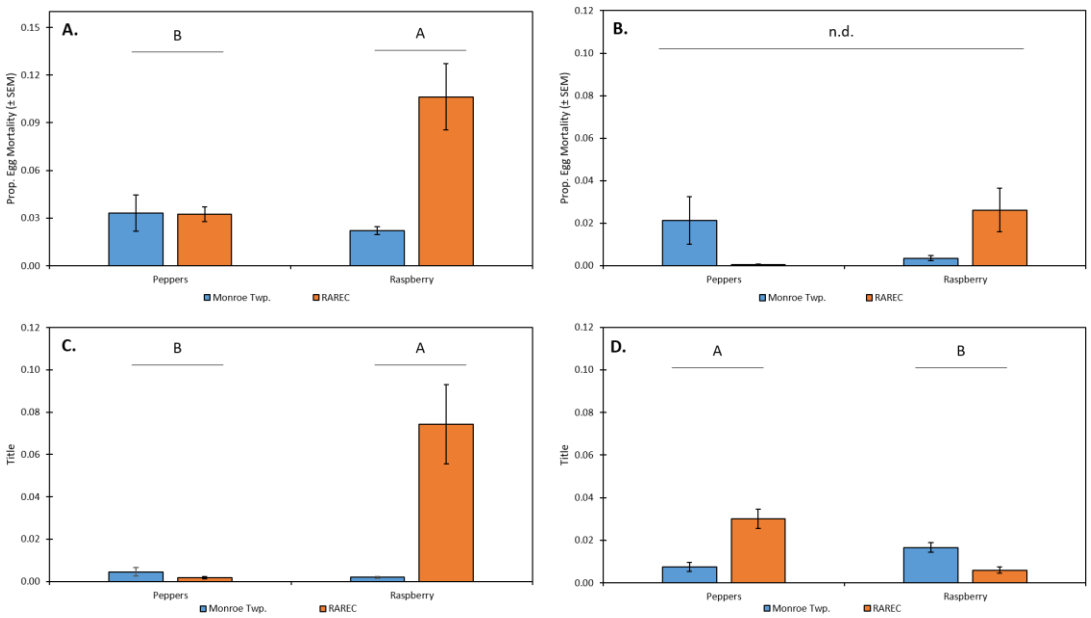


Figure 4. Analysis of Visit Duration on the Hatch Rate of *H. halys* Sentinel Egg Masses Under Video Surveillance.

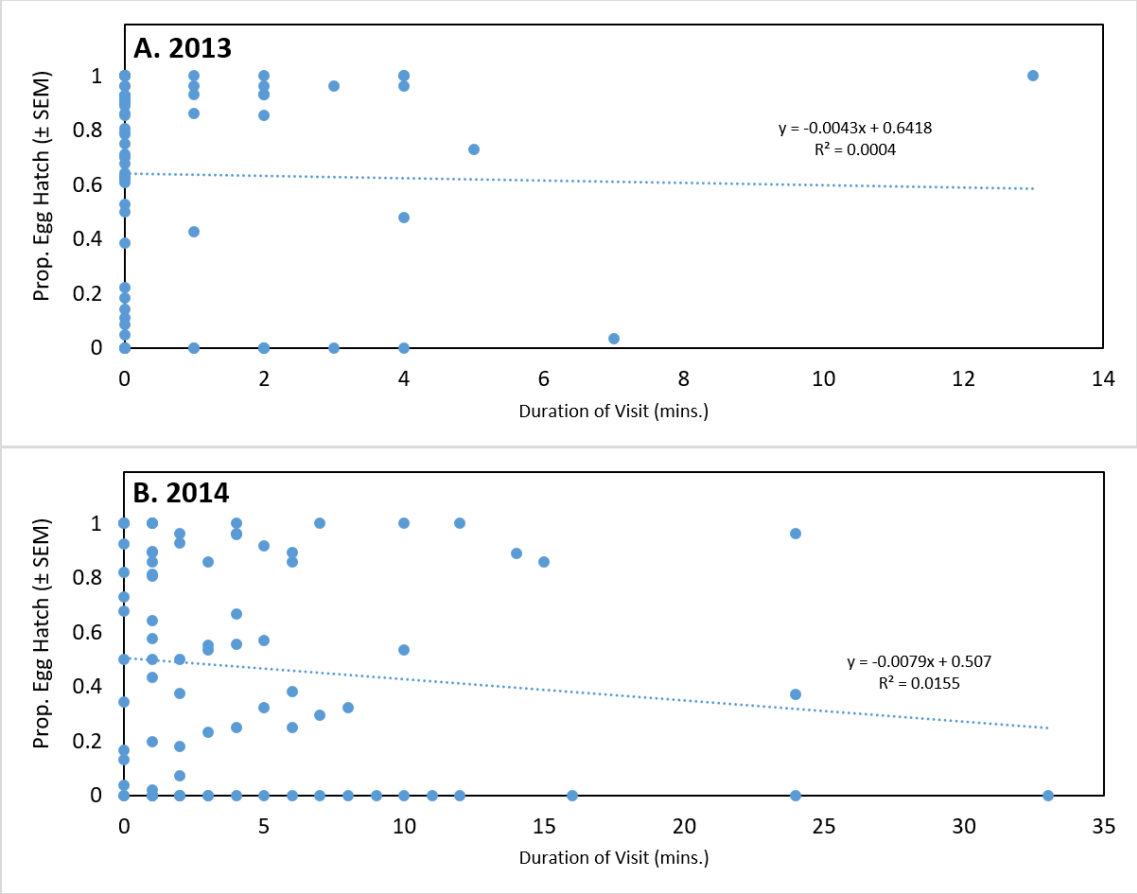
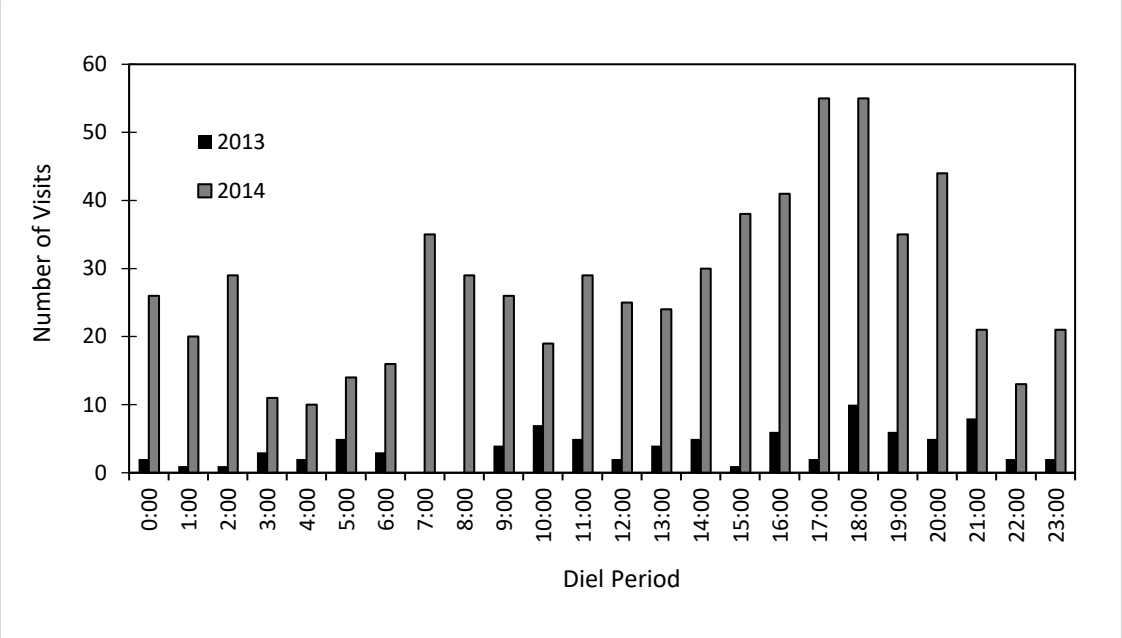


Figure 5. Diel Periodicity of Visitors to *H. halys* Sentinel Egg Masses. Periods along the x-axis indicate the start time of 1 hour time increments into which visitation data were binned. All visitation times were adjusted so 12:00 corresponded to the time of local solar noon in Bridgeton, NJ. Daylight hours during the growing season generally occurred between 6:00 and 20:00 hours.



Chapter 3a: The Effect of Amplification Method on Half-Life of Detectability of *Halyomorpha halys* (Stål) DNA in Laboratory-Fed Predators

Abstract:

Halyomorpha halys (Stål) is an invasive pest of agriculture in the U.S. As a recent invader, *H. halys* exists without many of its co-evolved natural enemies but molecular tools may help identify native predators affecting this important pest. We developed a novel primer set for detecting *Halyomorpha halys* DNA from the digestive tracts of predatory arthropods. These primers, HhalysCO1Spec, were designed from nucleotide sequences of *H. halys* and other North American Pentatomids published on GenBank. HhalysCO1Spec amplifies a 89-bp region of the CO1 mtDNA gene and was verified species-specific by BLASTn queries and empirical testing against non-target Pentatomidae and common predators. We compared the duration of prey DNA detectability of the HhalysCO1Spec primers to a TaqMan qPCR assay, BMITS1, for *H. halys* using laboratory-reared *Chrysoperla carnea* (Stephens) larvae as predators. Larvae were starved for 24 h, fed a single *H. halys* 1st instar nymph and allowed to digest for 0 h to 72 h. Samples were assayed with both *H. halys*-specific methods and the DNA detectability half-life (T_{50}) was modeled and calculated for each. Target DNA was detected by the HhalysCO1 assay after 24 h of digestion, but not after 32 h (T_{50} = 12.12 h). BMITS1 detected target DNA after 72 h, the longest digestion time tested (T_{50} = 48.87). Our findings indicate that TaqMan qPCR systems can detect dilute and degraded DNA, making them a valuable tool for molecular gut content analysis. We compare the detectability period of target sequences in the HhalysCO1 and BMITS1 systems to that of similar techniques and discuss causes of variation between them. Possible disadvantages of short target sequences and long DNA detection periods are also discussed.

Keywords: Molecular Gut Content Analysis, qPCR, DNA Detectability, Predation, brown marmorated stink bug

Introduction

Accurate and efficient detection of arthropod predation events is often difficult to accomplish, as their size and concealed/nocturnal feeding habits make it difficult to visually observe predation events in the wild difficult (King et al., 2008; Symondson, 2002). However, advances in molecular techniques allow researchers to identify and quantify patterns of arthropod predation without direct observation. Although a multitude of immunological and genetic methods exist, detection and amplification of the prey species' DNA through Polymerase Chain Reaction (PCR) is an accurate and cost-effective technique for tracking predation in laboratory and field experimental settings (Greenstone et al., 2014; King et al., 2008; Symondson, 2002). Molecular GCA is the application of PCR and other genetic techniques to study the flow of matter through ecosystems (Sheppard and Harwood, 2005). It relies on prey-specific oligonucleotide primers to screen potential predators for undigested copies of a target prey species' DNA sequence (King et al., 2008; Symondson, 2002).

Molecular GCA has been used to study community ecology in a number of ways. These include the quantification of specific predator-prey relationships (e.g. Harwood & Obrycki 2005; Zhang *et al.* 2007; Kobayashi *et al.* 2011), the study of intra-guild predation (e.g. Gagnon et al., 2011; Harwood et al., 2007; Sheppard et al., 2005; Yang et al., 2016), and the identification of effective generalist predators for the management of pests (e.g. Chen et al., 2000; Furlong et al., 2014; Greenstone et al., 2010). Unlike visual observations, molecular GCA allows for the quantification of predation events between small, concealed, and/or nocturnal organisms and, unlike sentinel prey studies, can be used to study predation across all prey life stages (Sheppard and Harwood, 2005). Molecular GCA is a robust and accurate technique for studying predation, but the degradation of DNA within predator digestive tracts limits the detection of target sequences by molecular methods (Greenstone et al., 2014).

Prey DNA begins to decay shortly after consumption as a result of digestive processes within the predator, and only persists at concentrations detectable by molecular techniques for a

limited time (Greenstone et al., 2014, 2007). For the purposes of comparison, the duration of DNA detectability is often quantified by measuring the time since feeding at which 50% of fed predators assay positive for target DNA (Greenstone et al., 2014; Greenstone and Hunt, 1993). This time point, known as the DNA detectability half-life (T_{50}), can vary widely due a number of factors and appears to be unique to each predator-prey-primer system (Greenstone et al., 2014, 2010, 2007). For example, T_{50} varies widely between predators of different taxa and by the nutritional status of field-collected predators (Greenstone et al., 2014).

Experimental methodology can also have a profound effect on T_{50}. Larger target sequences and higher temperatures during digestion are associated with shorter T_{50} values, while "chaser prey" (non-target prey offered after consumption of the target prey) may increase T_{50} (Agustí et al., 2003; Harper et al., 2005; Kobayashi et al., 2011; Naranjo and Hagler, 2001; Zaidi et al., 1999). Differing methods of sample preservation can also affect T_{50}. Among seven fixative protocols tested, predators stored in 70% ethanol prechilled to -20ºC contained the most intact DNA (Weber and Lundgren, 2009). The effect of differing extraction methods on T_{50} is currently unknown, but exploration of the relationship between amplification method and DNA detectability has begun.

Conventional PCR can provide data on the presence/absence of target prey within predator guts by amplifying target DNA, with target-specific primers, and visualizing these sequences as size-specific bands on an electrophoretic gel. More recent techniques (in the form of quantitative PCR, qPCR) improve the sensitivity of conventional PCR by using fluorescent dyes to detect small quantities of target DNA (Gomez-Polo et al., 2015). Molecular GCA first made use of this technology to detect traces of fish DNA from the feces of sea lions (Deagle et al., 2006) and it has since been used for exploring arthropod food webs (e.g. Lundgren and Ellsbury, 2009; Lundgren and Weber, 2010; Troedsson et al., 2009; Valentin et al., 2016; Weber and Lundgren, 2009; Zhang et al., 2007). Zhang *et al.* (2007) developed a novel qPCR assay to amplify DNA of *Bemesia tabaci* (Gennadius) in the digestive tracts of field-collected predators.

The qPCR assay was able to detect *B. tabaci* DNA in four samples which had scored negative when assayed by conventional PCR, indicating a higher degree of sensitivity in the quantitative method (Zhang et al., 2007). Predators of important lettuce pests were collected *in situ* and assayed for target sequences of two prey species using both conventional PCR and qPCR (Gomez-Polo et al., 2016, 2015). The quantitative approach detected higher frequencies of predation than conventional PCR indicating higher sensitivity in the former. Despite these results, the effect of qPCR techniques on the duration of T_{50} remains unquantified.

Molecular identification of predation may be particularly useful when studying invasive prey because new DNA amplification systems can be designed and employed rapidly (Harwood et al., 2009; Monzó et al., 2010; Valentin et al., 2016). *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an agriculturally and ecologically significant invasive pest. Native to East Asia, *H. halys* is highly polyphagous, capable of damaging a range of fruit, vegetable, berry, row, and ornamental crops (Hoebeke and Carter, 2003; Holtz and Kamminga, 2010; Leskey et al., 2012; Nielsen and Hamilton, 2009; Rice et al., 2014). According to the Enemy Release Hypothesis, the success of *H. halys* in its invaded range may be due to a lack of effective co-evolved natural enemies (Keane and Crawley, 2002). However, the impact of native predators and parasitoids on the population dynamics of *H. halys* was initially unclear.

Since its introduction, the identity and effectiveness of natural enemies affecting *H. halys* in its invaded range has been assessed through multiple observational studies. Sentinel egg mass studies revealed low utilization of *H. halys* eggs by natural enemies, accounting for < 10% of egg mortality in agricultural settings (Cornelius et al. 2016a, 2016b; Ogburn et al. 2016). Successful parasitism of sentinel egg masses was particularly infrequent in agricultural crops (< 0.5% of egg mortality), while predation affected 7.4% of sentinel eggs (Ogburn et al. 2016). This suggests that native predators may have a greater impact on *H. halys* abundance than native parasitoids in American agricultural landscapes (Ogburn et al., 2016). Laboratory-based no-choice studies have also been used to identify and study the behaviors of *H. halys* predators, but these have focused

primarily on the egg stage (Abram et al., 2014; Morrison et al., 2016). However, Pote and Nielsen (2017) found significant differences in predator complex between *H. halys* life stages. Molecular GCA could be used to identify predators of *H. halys* across all life stages, providing a clearer understanding of the ecology of this important pest.

Valentin *et al.* (2016) developed a novel species-specific qPCR assay for detecting *H. halys* DNA in the guano of insectivorous bats. This method, BMITS1, utilizes *H. halys*-specific primers and a TaqMan fluorescent probe to amplify a 96-bp section of the ITS region of ribosomal DNA. BMITS1 is highly sensitive, capable of detecting target DNA from degraded sources including vertebrate predator droppings (Valentin et al., 2016). The use of TaqMan probes theoretically increases assay specificity due to its requirement of a complete sequence match between target region and probe (Tyagi and Kramer, 1996). Despite this, a quantitative comparison of the sensitivity of TaqMan assays and those that rely on standard (non-quantitative) PCRs has yet to be completed. As such, the objectives of this study were to 1) develop a set of *H. halys*-specific standard PCR primers appropriate for use in gut content analysis, 2) test the specificity of this method against native Pentatomidae 3) determine and compare the detectability half-life (T_{50}) of *H. halys* DNA in lab-fed *Chrysoperla carnea* (Stephens) larvae using the BMITS1 and standard PCR methods of amplification.

Methods

Primer Design. To design the HhalysCO1spec primers, sequences from the mitochondrial CO1 gene of *H. halys* and other Nearctic Pentatomidae were obtained from GenBank. Sequences were aligned with Sequencher v 4.8 (Gene Codes, Ann Arbor, MI). Within the *H. halys* CO1 gene, we identified two single base pair polymorphisms unique to *H. halys.* We then designed a pair of primers (“HhalysCO1specFs” and “HhalysCO1specR”) which anneal at these polymorphisms and amplify an 89-bp region of CO1 between them. To ensure maximum

specificity, the HhalysCO1spec primers were designed such that the 3' terminus of each primer annealed at one of these unique polymorphisms.

Specificity Testing. The HhalysCO1Spec primers anneal to the loci of two single base pair mutations found only in the mitochondrial genome of *H. halys* and thus were designed to be specific to this species. Uniqueness of primer sequences was verified via BLASTn v2.6.1 on NCBI (Query_22237, Query_63695). Specificity of the HhalysCO1Spec primers was also tested against common predators and non-target Pentatomidae found in the *H. halys* invaded range. The species involved in this testing are provided in Table 1.

To confirm the fidelity of the HhalysCO1Spec assay to *H. halys* throughout its invaded range, we tested these primers against *H. halys* samples from California, Delaware, Indiana, Maryland, Massachusetts, Michigan, New Jersey, New York, Pennsylvania, and West Virginia. Samples from Indiana and Michigan were collected from residential areas in summer 2015, but all others were collected between 2006 and 2008 (Xu et al., 2014). At least three specimens from each state were assayed with the HhalysCO1Spec primers.

Optimization. Initial reactions using the HhalysCO1Spec primer system were conducted using a generalized arthropod PCR protocol (see Smith and Fonseca, 2004). The optimal thermal conditions for the HhalysCO1Spec system were determined with a multi-stage TouchDown PCR while optimal reagent concentrations were determined by factorial experiments. The results indicated strongest reactivity under the following conditions: 1× PCR buffer, 2.625 mM $MgCl_2$, 200µM of each dNTP, 300nM of each primer, 1 unit of Amplitaq Gold DNA polymerase, 0.1 mg/mL of bovine serum albumin and approximately 20ng DNA; the steps of the reaction included an initial denaturation phase for 10 min at 96°C, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72°C for 30s with a final extension period of 10 min at 72° C. PCR products from the HhalysCO1Spec primers were separated using gel electrophoresis at 90 V for 30 min on a 1% (w/v) agarose gel in 1× TAE buffer (40 mm Tris-

acetate, 1 mm EDTA) and visualized with gel electrophoresis. Each experiment included reactions with *H. halys* DNA and water serving as positive and negative controls, respectively.

Extraction. Thawed samples were removed from ethanol, placed in empty microcentrifuge vials and allowed to dry at room temperature. DNA was extracted using the HotSHOT method (Truett et al., 2000): First, 50 µL of lysis reagent (consisting of 25 mM NaOH, and 0.2 mM disodium EDTA) was added to each tube. Samples were incubated at 95º C for 60 minutes before 50 µL of neutralizing agent (40 mM Tris-HCl) was added to complete the extraction. Each plate of extractions included two empty wells containing only the extraction reagents used for that plate. Extraction control samples were treated identically to all other wells during the extraction and amplification processes, including manipulation with sterilized forceps.

Amplification. To compare the effect of amplification method on the duration of DNA detectability, extracted samples were assayed for *H. halys* DNA using the HhalysCO1Spec primer system (standard PCR treatment) as well as the BMITS1 method (qPCR treatment). Samples were amplified with HhalysCO1Spec primers according to the protocol discussed above. Amplification with the BMITS1 method was performed according to the protocols of Valentin et al. (2016): samples were amplified in 20 µL reactions containing 500 nM of each primer, 250 nM of the *H. halys* specific probe, 1× TaqMan® Universal Master Mix II with no Uracil-N glycoslyase (UNG) and 1–2 µL of extracted DNA. Reactions included an initial denaturing step at 96º C for 10 min, 45 cycles of denaturing for 15 s and annealing and extension at 60º C for 1 min (Valentin et al., 2016).

Predators and Target Prey. *Chrysoperla carnea* were acquired from Natural Insect Control (Stevensville, Ontario, Canada) as early-instar larvae. These were reared individually in closed 1 oz plastic deli cups at 25º C with a relative humidity of approximately 40% and a photoperiod of 16:8 (L:D). Larvae were provided a diet of *Ephestia kuehniella* eggs and moist dental wicks as needed until they reached the 3rd instar. Target prey (*Halyomorpha halys* 1st instar nymphs) was reared from egg masses obtained from the New Jersey Department of Agriculture

Beneficial Insects Laboratory. *Halyomorpha halys* egg masses were kept at 25º C with a relative humidity of approximately 40% until hatching.

Feeding Protocol. All containers, forceps and surfaces were sterilized with 10% bleach solution and triple rinsed with deionized water prior the beginning of the experiment, and forceps were re-sterilized after each use. Preliminary experiments indicated that early instar *C. carnea* larvae rarely consumed *H. halys* nymphs; therefore only larvae in the 3rd and final instar were used in this study. Predators were starved for 48 h prior the experiment and provided only a moist dental wick during this time. After starvation, a subset of the unfed predators were stored in 100% ethanol and frozen immediately, constituting the unfed control group.

Starved predators were transferred to individual 1 oz. plastic deli cups containing one 1st instar *H. halys* nymph. Moist dental wicking was not provided during feeding. For each predator, the time (rounded to the nearest minute) was recorded when the prey was introduced, when feeding began and when feeding ceased. Predators were allowed to consume the prey to satiation, and feeding was considered complete when the predator moved away from the prey remains. After feeding, predators were transferred to new deli cups and randomly assigned to one of the following digestion time treatments: 0 h (positive control), 1.5 h, 3 h, 6 h, 12 h, 18 h, 24 h, 32 h, 48 h, and 72 h. The desired number of samples per treatment was 20, however predators frequently died during this starvation period (especially those in the 48 h and 72 h treatments). As a result, the pre-starvation number of samples varied between treatments: the 0 h treatment started with 21 samples, the 1.5 h – 32 h treatments started with an initial count of 25 samples, and the 48 h – 72 h treatments started with 30 samples (post-mortality sample size is reported in Table 3). During the digestion period, predators were kept at room temperature and were not provided with alternate ("chaser") prey. After the allotted digestion time had elapsed, predators were stored in 1.5 mL microcentrifuge tubes containing 100% reagent grade ethanol, and kept in a -20°C freezer until extraction.

Environmental Contamination. To guard against contamination as a result of predators physically contacting *H. halys* nymphs or their droppings during the feeding period, a subset of unfed predators were exposed to deli cups that had recently housed a 1st instar *H. halys*. Twelve *H. halys* 1st instars were individually introduced to sealed 29.5 mL plastic deli cups and kept for 60 min. After this period, the nymphs were removed and replaced with unfed *C. carnea* larvae. After 95 min (the average time required for predators to complete feeding), all samples were immediately deposited into 1.5 mL centrifuge tubes containing 100% ethanol and frozen to -20ºC. These samples, constituting the "environmental control", were otherwise handled and assayed identically to those in treatment groups.

Analysis. The proportion of samples testing positive for *H. halys* DNA was calculated for each digestion time treatment for both methods of amplification. For each method, the rate at which the proportion of positive samples decreased over time was modeled using non-linear least squares to estimate the coefficients of a logistic decay curve. A logistic regression analysis was performed in R studio (v. 3.2.2 "Fire Safety") using the commands "nls" and "SSlogis", both of which are included in the R package "stats" (R Development Core Team, 2011). A Wald test, performed using "linearHypothesis" in the R package "car", was used to compare the regression coefficient "xmid" between amplification methods to check for significant differences in duration of DNA detectability. This coefficient corresponds to the x-value at the inflection point of the decay curve, and represents a statistical proxy for T_{50}.

Results

We designed the HhalysCO1Spec primer system, consisting of HhalysCO1SpecFs (5' - CCC TGA ACG AAT CCC ATT G - 3') and HhalysCO1SpecR (5' - TGC TAA CAC AGG TAA GGA TAA TAA C - 3'), which amplifies an 89-bp segment of the *H. halys* CO1 mitochondrial gene. The results of a BLAST search of the HhalysCO1Spec primer sequences indicated a high degree of species specificity among published sequences. The only entries with a perfect match

for both primer sequences were submissions from the *H. halys* mitochondrial genome from which these primers were designed, however, each primer perfectly matched with one sequence from a non-target organism. HhalysCO1SpecFs matched a sequence of mt-DNA from a freshwater crayfish species and HhalysCO1SpecR matched chromosomal DNA from bighorn sheep. However, neither organism was a perfect match for both primers, therefore DNA from these non-targets would not have elicited amplification. The HhalysCO1Spec primers did not amplify DNA in any of the non-target predators or Pentatomid species tested (Table 1).

Halyomorpha halys DNA was successfully detected in laboratory-fed *C. carnea* when assayed with the HhalysCO1Spec standard PCR primers as well as the BMITS1 qPCR system. Both methods successfully detected *H. halys* DNA in all positive control samples (0 h digestion time) but no target DNA was detected in any of the unfed control, extraction control, or environmental control samples with either method (Figs. 1-2, Table 2). When assayed with the HhalysCO1Spec method, target DNA was detected for a maximum of 24 h (amp. rate at 24 h: 9.5% ± 6%; Fig. 1, Table 2). In contrast, target DNA was detected by the BMITS1 method in all of the tested treatment times including 72 h, the longest digestion time tested in this experiment (amp. rate at 72 h: 23.8± 9%; Fig. 2, Table 2). Target DNA detection rate decreased rapidly with the HhalysCO1Spec primer system (Fig. 1). After 1.5 h of digestion, target DNA was detected only in 75% of samples. This effect was not observed with the BMITS1 amplification method, which detected target DNA in at least 75% of samples for 32 h (Figs. 1-2).

The decreasing rate of DNA detectability over time was successfully modeled for both methods of amplification using a logistic decay curve (Figs. 1-2). The best-fit curves for both primer systems fit the observed data well, as indicated by high R^2 values (HhalysCO1Spec: $R^2 = 0.96$; BMITS1: $R^2 = 0.94$). Regression analysis revealed significant differences in proportion of amplification between amplification methods ($P < 0.05$). A Wald test of the model coefficient "xmid" revealed significant differences in the value of this coefficient between amplification

methods (χ^2 = 212.69, P < 0.0001). Fitted model coefficients were used to estimate T_{50} for each method of amplification (HhalysCO1Spec: T_{50} = 12.12 h; BMITS1: T_{50} = 48.87 h).

Discussion

Identifying and implementing highly sensitive assay methodologies for the detection of dilute or degraded DNA is central to the field of molecular gut content analysis. In this study, we demonstrated that qPCR (using the BMITS1 primer) was able to detect *H. halys* DNA four times longer than standard PCR (using the HhalysCO1Spec primer), although different regions of the genome were assayed. This indicates that the BMITS1 system is significantly more sensitive to low levels of degraded DNA like that found in the digestive tract of recently fed predators. These results agree with similar studies comparing the relative sensitivities of conventional PCR and qPCR methods (Gomez-Polo et al., 2016, 2015; Zhang et al., 2007). However, feeding trials with controlled digestion times and the calculation of T_{50} are needed to best quantify differences in sensitivity (Greenstone et al., 2014).

The sensitivity of the BMITS1 system, T_{50} = 48.8 h, is similar to other reported qPCR assays used to detect target prey in predatory arthropods and fish. TaqMan probes were used to detect a 72-bp segment of DNA from fish eggs (T_{50} = 31 h) and larvae (T_{50} = 26 h) consumed by a whiting predator (Albaina et al., 2010; Hunter et al., 2012). Similarly, a multiplex TaqMan assay was used to screen predators of rice planthoppers for target sequences of 3 prey species simultaneously (Wang et al., 2013). Amplicon sizes in this assay ranged from 104 to 136-bp and could be detected for up to 42 h. In the San Francisco Bay estuarial system, a short segment (<150-bp) of salmon DNA can be detected in bass predators using a species specific TaqMan qPCR for up to 120 h (T_{50} = 66.2 h) (Brandl et al., 2015, 2016).

The methods described in this study (HhalysCO1Spec and BMITS1) were intentionally designed to amplify target segments shorter than 100-bp. Amplicon length is negatively correlated with the duration of target DNA detectability, therefore GCA methods which screen

for long sequences are less likely to detect predation events (Greenstone et al., 2014; Hoogendoorn and Heimpel, 2001). Hoogendoorn and Heimpel (2001) developed four sets of primers which amplify *Ostrinia nubilalis* (Hübner) ribosomal DNA of differing lengths (ranging from 492-bp to 150-bp) and determined that T_{50} was inversely proportional to the length of the fragment being amplified. In *Chrysoperla plorabunda*, the T_{50} of 198-bp and 339-bp sequences of aphid mtDNA are 3.95 h and 2.56 h, respectively. Increasing the likelihood of detecting predation on this somewhat uncommon prey was considered during the design of each of these amplification method. A small amplicon size allows a more sensitive test for detecting rare, or partially digested prey to be identified. However, short amplicons are also associated with increased likelihood of false positive results from contamination (Greenstone et al., 2014). No contamination was detected in the negative control samples of the experiment presented here, but a preliminary feeding trial using *Nabis spp.* as the focal predator has been omitted from this chapter as target DNA was detected in un-fed negative control samples. Methods employing short amplicons may enable the detection of some predation when this would not otherwise be possible, but care should always be taken to prevent and monitor for contamination during GCA.

The duration of DNA detectability in the HhalysCO1Spec system (T_{50} = 12.1 h) is slightly longer than found in earlier research studying DNA detectability rates in *Chrysoperla spp.* The T_{50} of a 197-bp fragment of *Homalodisca vitripennis* (Germar) mtDNA in *C. carnea* was 11 h (Fournier et al., 2008) while that of a 198-bp segment of cereal aphid mtDNA in *C. plorabunda* (Fitch) was only 3.95 h (Chen et al., 2000). Differences between the T_{50} of HhalysCO1Spec and that of earlier work may be due to variable rates of DNA digestion within the *Chrysoperla* genus, differences in primer and/or amplicon nucleotide composition, differences in PCR protocols, or differing amplicon sizes. Regardless, HhalysCO1Spec can detect target DNA for longer periods than several other comparable PCR systems and may be effectively used in studies that do not rely on long DNA detection periods, or when the higher cost of qPCR methods would be prohibitive.

The molecular methods compared here increase the duration of target DNA detectability compared to similar methods by amplifying small target sequences and, in the case of BMITS1, using fluorescent probes to detect low levels of amplification. However, short amplicons and lengthy detection periods may interfere with the objectives of some experiments. Small target sequences are associated with an increased risk of non-target reactivity and contamination, while longer detection periods increase the likelihood of false positives identified as a result of secondary predation and/or scavenging (Greenstone et al., 2014; King et al., 2008; Sheppard and Harwood, 2005). The appropriate molecular technique for use in GCA depends on the nature of the research being conducted and should be considered carefully during experimental design.

Although the HhalysCO1Spec primers had a significantly shorter T_{50} than the BMITS1 qPCR primer system, both of these systems may have utility in GCA studies of *H. halys* ecology. Given the longer detection period of the BMITS1 primers, this system may be more appropriate for identifying predation in field settings where *H. halys* is rare, or on in predator taxa which are known to digest quickly (e.g. Coccinellidae) (Greenstone et al., 2014). Novel prey are unlikely to be consumed by any predator, so using a method which can detect predation for longer periods increases the likelihood of collecting predators which consumed the target prey. However, due to the higher up-front costs associated with qPCR (Garland et al., 2011) the HhalysCO1Spec standard PCR primers may be useful in some research applications. Standard PCR amplification would be adequate for determining if predation occurred in controlled settings where predators were exposed to prey for a short time like predation bioassays or field cage experiments. Conventional PCR methods have also been used to study predation in field settings when the target prey are abundant, its predators well known, but the relative effect of these predators is unknown (e.g. Greenstone 2010). Accuracy is an important factor in GCA, but accurate results can be attained in two ways: a large number of samples assayed with less expensive and less sensitive conventional primers or fewer samples assayed with more sensitive but more costly qPCR methods. Given the inconsistent and “patchy” distribution of *H. halys* in many field

settings (Hahn et al., 2017), the latter approach may be more appropriate for identifying predators of *H. halys* in the field, but this will not be the case for all pests. There is no universally appropriate amplification method for GCA and the selection of molecular methods used will depend heavily on the specific predators, prey and research objectives of a given experiment.

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References

Abram, P.K., Doyon, J., Brodeur, J., Gariépy, T.D., Boivin, G., 2014. Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Can. Entomol. 147, 1–5. doi:10.4039/tce.2014.41

Agustí, N., Shayler, S.P., Harwood, J.D., Vaughan, I.P., Sunderland, K.D., Symondson, W.O.C., 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: Prey detection within predators using molecular markers. Mol. Ecol. 12, 3467–3475. doi:10.1046/j.1365-294X.2003.02014.x

Albaina, A., Fox, C.J., Taylor, N., Hunter, E., Maillard, M., Taylor, M.I., 2010. A TaqMan real-time PCR based assay targeting plaice (*Pleuronectes platessa* L.) DNA to detect predation by the brown shrimp (*Crangon crangon* L.) and the shore crab (*Carcinus maenas* L.)—Assay development and validation. J. Exp. Mar. Bio. Ecol. 391, 178–189. doi:10.1016/j.jembe.2010.06.029

Brandl, S., Schumer, G., Schreier, B.M., Conrad, J.L., May, B., Baerwald, M.R., 2015. Ten real-time PCR assays for detection of fish predation at the community level in the San Francisco Estuary-Delta. Mol. Ecol. Resour. 15, 278–284. doi:10.1111/1755-0998.12305

Brandl, S.C., Schreier, B.M., Conrad, J.L., May, B., Baerwald, M.R., 2016. Generation of quantitative polymerase chain reaction Detectability Half-Lives and comparison of sampling protocols for genetic diet studies of San Francisco estuary fishes. Trans. Am. Fish. Soc. 145, 441–449. doi:10.1080/00028487.2015.1131745

Chen, Y., Giles, K.L., Payton, M.E., Greenstone, M.H., 2000. Identifying key cereal aphid predators by molecular gut analysis. Mol. Ecol. 9, 1887–1898. doi:10.1046/j.1365-294x.2000.01100.x

Cornelius, M.L., Dieckhoff, C., Hoelmer, K.A., Olsen, R.T., Weber, D.C., Herlihy, M. V., Talamas, E.J., Vinyard, B.T., Greenstone, M.H., 2016a. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control 103, 11–20. doi:10.1016/j.biocontrol.2016.07.011

Cornelius, M. L., C. Dieckhoff, B. T. Vinyard, and K. A. Hoelmer. 2016b. Parasitism and Predation on Sentinel Egg Masses of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in Three Vegetable Crops: Importance of Dissections for Evaluating the Impact of Native Parasitoids on an Exotic Pest. Environ. Entomol. 45: 1536–1542.

Deagle, B.E., Eveson, J.P., Jarman, S.N., 2006. Quantification of damage in DNA recovered from highly degraded samples--a case study on DNA in faeces. Front. Zool. 3, 1–10. doi:10.1186/1742-9994-3-11

Fournier, V., Hagler, J., Daane, K., De León, J., Groves, R., 2008. Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): A comparative study of the efficacy of an ELISA and PCR gut content assay. Oecologia 157, 629–640. doi:10.1007/s00442-008-1095-x

Furlong, M.J., Rowley, D.L., Murtiningsih, R., Greenstone, M.H., 2014. Combining ecological methods and molecular gut-content analysis to investigate predation of a Lepidopteran pest complex of Brassica crops. Entomol. Exp. Appl. 153, 128–141. doi:10.1111/eea.12231

Gagnon, A.È., Heimpel, G.E., Brodeur, J., 2011. The ubiquity of intraguild predation among predatory arthropods. PLoS One 6, 1–7. doi:10.1371/journal.pone.0028061

Garland, S., Wood, J., Skerratt, L., 2011. Comparison of sensitivity between real-time detection

of a TaqMan assay for Batrachochytrium dendrobatidis and conventional detection. Dis. Aquat. Organ.

Gomez-Polo, P., Alomar, O., Castañé, C., Aznar-Fernández, T., Lundgren, J.G., Piñol, J., Agustí, N., 2016. Understanding trophic interactions of *Orius spp.* (Hemiptera: Anthocoridae) in lettuce crops by molecular methods. Pest Manag. Sci. 72, 272–279. doi:10.1002/ps.3989

Gomez-Polo, P., Alomar, O., Castañé, C., Lundgren, J.G., Piñol, J., Agustí, N., 2015. Molecular assessment of predation by hoverflies (Diptera: Syrphidae) in Mediterranean lettuce crops. Pest Manag. Sci. 71, 1219–1227. doi:10.1002/ps.3910

Greenstone, M.H., Hunt, J.H., 1993. Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. Entomol. Exp. Appl. 68, 1–7. doi:10.1111/j.1570-7458.1993.tb01682.x

Greenstone, M.H., Payton, M.E., Weber, D.C., Simmons, A.M., 2014. The detectability half-life in arthropod predator-prey research: what it is, why we need it, how to measure it, and how to use it. Mol. Ecol. 23, 3799–813. doi:10.1111/mec.12552

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E., Hawthorne, D.J., 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. Bull. Entomol. Res. 97, 201–209.

Greenstone, M.H., Szendrei, Z., Payton, M.E., Rowley, D.L., Coudron, T.C., Weber, D.C., 2010. Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. Entomol. Exp. Appl. 136, 97–107. doi:10.1111/j.1570-7458.2010.01006.x

Hahn, N., Rodriguez-Saona, C., Hamilton, G., 2017. Characterizing the spatial distribution of brown marmorated stink bug, Halyomorpha halys Stål (Hemiptera: Pentatomidae), populations in peach orchards. PLoS One.

Harper, G.L., King, R.A., Dodd, C.S., Harwood, J.D., Glen, D.M., Bruford, M.W., Symondson, W.O.C., 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Mol. Ecol. 14, 819–827. doi:10.1111/j.1365-294x.2005.02442.x

Harwood, J.D., Desneux, N., Yoo, H.J.S., Rowley, D.L., Greenstone, M.H., Obrycki, J.J., O'Neil, R.J., 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: A molecular approach. Mol. Ecol. 16, 4390–4400. doi:10.1111/j.1365-294X.2007.03482.x

Harwood, J.D., Obrycki, J.J., 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102, 335–350. doi:10.14411/eje.2005.051

Harwood, J.D., Yoo, H.J.S., Greenstone, M.H., Rowley, D.L., O'Neil, R.J., 2009. Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis. Biol. Invasions 11, 895–903. doi:10.1007/s10530-008-9302-6

Hoebeke, E.R., Carter, M.E., 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105, 225–237.

Holtz, T., Kamminga, K., 2010. Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in the United States. USDA APHIS-PPQ.

Hoogendoorn, M., Heimpel, G., 2001. PCR-based gut content analysis of insect predators: using

ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol. Ecol. 10, 2059–2067.

Hunter, E., Taylor, N., Fox, C.J., Maillard, M., Taylor, M.I., 2012. Effectiveness of TaqMan probes for detection of fish eggs and larvae in the stomach contents of a teleost predator. J. Fish Biol. 81, 320–328. doi:10.1111/j.1095-8649.2012.03298.x

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

King, R. a, Read, D.S., Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17, 947–63. doi:10.1111/j.1365-294X.2007.03613.x

Kobayashi, T., Takada, M., Takagi, S., Yoshioka, A., Washitani, I., 2011. Spider predation on a mirid pest in Japanese rice fields. Basic Appl. Ecol. 12, 532–539. doi:10.1016/j.baae.2011.07.007

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp, M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K. a., Lee, D.-H., Wright, S.E., 2012. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Lundgren, J.G., Ellsbury, M.E., Prischmann, D.A., 2009. Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. Ecol. Appl. 19, 2157–2166. doi:10.1890/08-1882.1

Lundgren, J.G., Weber, D.C., 2010. Changes in digestive rate of a predatory beetle over its larval stage: Implications for dietary breadth. J. Insect Physiol. 56, 431–437. doi:10.1016/j.jinsphys.2009.11.020

Monzó, C., Sabater-Muñoz, B., Urbaneja, A., Castañera, P., 2010. Tracking medfly predation by the wolf spider, *Pardosa cribata* Simon, in citrus orchards using PCR-based gut-content analysis. Bull. Entomol. Res. 100, 145–152. doi:10.1017/S0007485309006920

Morrison, W.R., Mathews, C.R., Leskey, T.C., 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97, 120–130. doi:10.1016/j.biocontrol.2016.03.008

Naranjo, S.E., Hagler, J.R., 2001. Toward the quantification of predation with predator gut immunoassays: a new approach integrating functional response behavior. Biol. Control 20, 175–189. doi:10.1006/bcon.2000.0892

Nielsen, A.L., Hamilton, G.C., 2009. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102, 1133–1140. doi:10.1603/029.102.0335

Ogburn, E.C., Bessin, R., Dieckhoff, C., Dobson, R., Grieshop, M., Hoelmer, K.A., Mathews, C., Moore, J., Nielsen, A.L., Poley, K., Pote, J.M., Rogers, M., Welty, C., Walgenbach, J.F., 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control 101, 39–51. doi:10.1016/j.biocontrol.2016.06.002

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by

generalist predators. Biol. Control. 114: 1-7.

R Development Core Team, R., 2011. R: A language and environment for statistical computing. R Found. Stat. Comput., R Foundation for Statistical Computing. doi:10.1007/978-3-540-74686-7

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K., Hooks, C.R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A.L., Pfeiffer, D.G., Raupp, M.J., Rodriguez-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P.D., Whalen, J., Wiman, N.G., Leskey, T.C., Tooker, J.F., 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5, 1–13. doi:10.1603/IPM14002

Sheppard, S.K., Bell, J., Sunderland, K.D., Fenlon, J., Skervin, D., Symondson, W.O.C., 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol. Ecol. 14, 4461–4468. doi:10.1111/j.1365-294X.2005.02742.x

Sheppard, S.K., Harwood, J.D., 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19, 751–762. doi:10.1111/j.1365-2435.2005.01041.x

Smith, J.L., Fonseca, D.M., 2004. Rapid assays for identification of members of the Culex (Culex) pipiens complex, their hybrids, and other sibling species (Diptera: Culicidae). Am. J. Trop. Med. Hyg. 70, 339–345. doi:10.4269/AJTMH.2004.70.339

Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11, 627–641. doi:10.1046/j.1365-294X.2002.01471.x

Troedsson, C., Simonelli, P., Nägele, V., Nejstgaard, J.C., Frischer, M.E., 2009. Quantification of copepod gut content by differential length amplification quantitative PCR (dla-qPCR). Mar. Biol. 156, 253–259. doi:10.1007/s00227-008-1079-8

Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., Warman, M.L., 2000. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques 29, 52–54.

Tyagi, S., Kramer, F.R., 1996. Molecular beacons: Probes that fluoresce upon hybridization. Nat. Biotechnol. 14, 303–308. doi:10.1038/nbt0696-765

Valentin, R.E., Maslo, B., Lockwood, J.L., Pote, J., Fonseca, D.M., 2016. Real-time PCR assay to detect brown marmorated stink bug, *Halyomorpha halys* (Stal), in environmental DNA. Pest Manag. Sci. 72, 1854–1861. doi:10.1002/ps.4217

Wang, G.-H., Wang, X.-Q., Qiao, F., Zhu, Z.-R., Cheng, J.-A., 2013. Development and preliminary application of a triplex real-time polymerase chain reaction assay for evaluating predation on three planthoppers in a rice ecosystem. Mol. Ecol. Resour. 13, 811–819. doi:10.1111/1755-0998.12127

Weber, D.C., Lundgren, J.G., 2009. Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. J. Insect Sci. 9, 41. doi:10.1673/031.009.4101

Xu, J., Fonseca, D.M., Hamilton, G.C., Hoelmer, K. a., Nielsen, A.L., 2014. Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*. Biol. Invasions 16, 153–166. doi:10.1007/s10530-013-0510-3

Yang, F., Wang, Q., Wang, D., Xu, B., Xu, J., Lu, Y., Harwood, J.D., 2016. Intraguild predation

among three common Coccinellids (Coleoptera: Coccinellidae) in China: detection using DNA-based gut-content analysis. Environ. Entomol. 42, 1–10. doi:10.1093/ee/nvw154

Zaidi, R.H., Jaal, Z., Hawkes, N.J., Hemingway, J., Symondson, W.O.C., 1999. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Mol. Ecol. 8, 2081–2087. doi:10.1046/j.1365-294x.1999.00823.x

Zhang, G.F., Lü, Z.C., Wan, F.H., Lövei, G.L., 2007. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Mol. Ecol. Notes 7, 947–954. doi:10.1111/j.1471-8286.2007.01819.x

Tables and Figures

Table 1. List of Species Tested for Reactivity with the HhalysCO1Spec Primer System. Specimens tested for cross-reactivity with HhalysCO1Spec primers were collected from New Jersey agricultural ecosystems between 2001-2015.

Order	Family	Latin Name
Coleoptera	Carabidae	*Harpalus pennsylvanicus*
"	Coccinellidae	*Coleomegilla maculata*
"	"	*Harmonia axyridis*
Hemiptera	Nabidae	*Nabis spp.*
"	Pentatomidae	*Acrosternum hilare*
"	"	*Banasa calva*
"	"	*Banasa dimidiata*
"	"	*Banasa euchlora*
"	"	*Cosmopela bimaculata*
"	"	*Dendrocoris humeralis*
"	"	*Euschistus conspersus*
"	"	*Euschistus servus*
"	"	*Euschistus tristigmus*
"	"	*Euschistus variolarius*
"	"	*Murgantia histrionica*
"	"	*Peribalus limbolarius*
"	"	*Podisus maculiventris*
"	"	*Thyanta cursator*
"	Reduviidae	*Arilus cristatus*
"	"	*Pselliopus spp.*
Neuroptera	Chrysopidae	*Chrysoperla carnea*
Orthoptera	Tettigoniidae	*Conocephalus spp.*

Table 2. The Effect of Digestion Time and Amplification Method on the Detection of *Halyomorpha halys* DNA in Laboratory-Fed *Chrysoperla carnea* Larvae. Prop. Detection refers to the proportion of samples assayed which resulted in positive detection of *H. halys* DNA. Only a subset of all samples were assayed with the rt-PCR method thus the number of samples varies between the two methods.

Method:	**HhalysCO1Spec (Std PCR)**			**BMITS1 (qPCR)**		
Trt (h)	**Prop. Detection**	**SEM**	**No. Samples**	**Prop. Detection**	**SEM**	**No. Samples**
0	1.000	0.00	21	1.000	0.00	21
1.5	0.750	0.10	20	1.000	0.00	18
3	0.778	0.10	18	1.000	0.00	9
6	0.762	0.09	21	0.846	0.10	13
12	0.526	0.11	19	0.895	0.07	19
18	0.65	0.11	20	1.000	0.00	20
24	0.095	0.06	21	0.900	0.07	20
32	0.000	0.00	20	0.750	0.10	20
48	0.000	0.00	24	0.444	0.12	18
72	0.000	0.00	27	0.238	0.09	21
Unfed Ctrl	0.000	0.00	20	0.000	0.00	10
Environ. Ctrl	0.000	0.00	12	0.000	0.00	12
Extract. Ctrl.	0.000	0.00	6	0.000	0.00	6

Figure 1. Detection of *Halyomorpha halys* DNA in Laboratory-Fed *Chrysopa carnea* Larvae Amplified with the HhalysCO1Spec Primer System. Average proportion of predators positive for *H. halys* DNA is indicated by black diamonds, and logistic regression curve for these data are indicated with a solid black line. Finely dotted lines represent upper and lower 95% confidence interval for the decay curve, and the thick dashed line corresponds to 50% amplification. The R-squared value for the logistic regression model is also displayed.

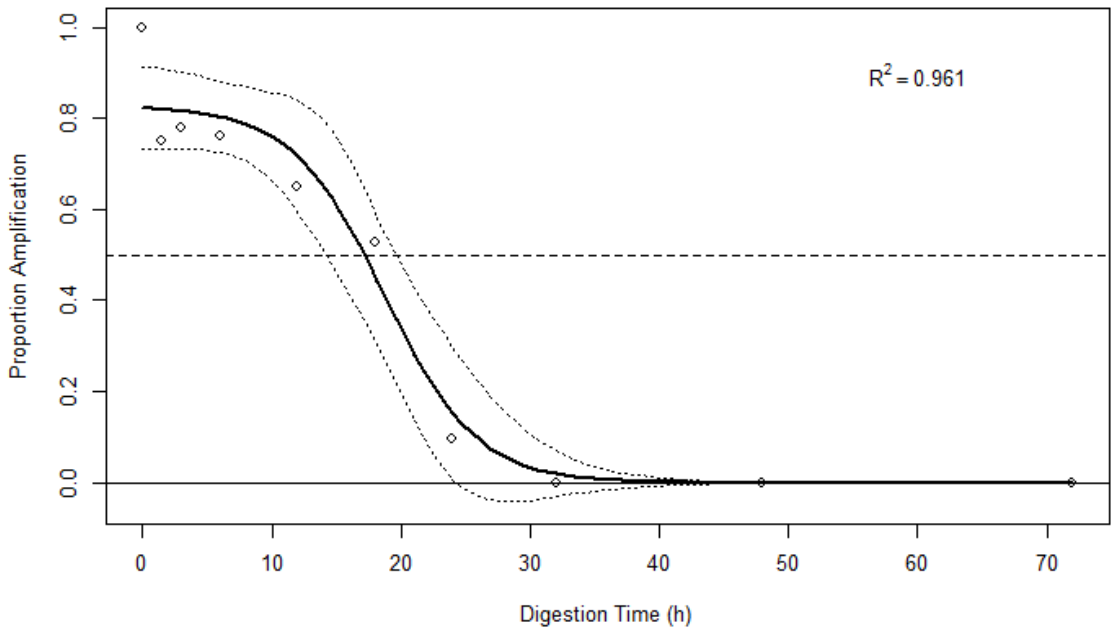
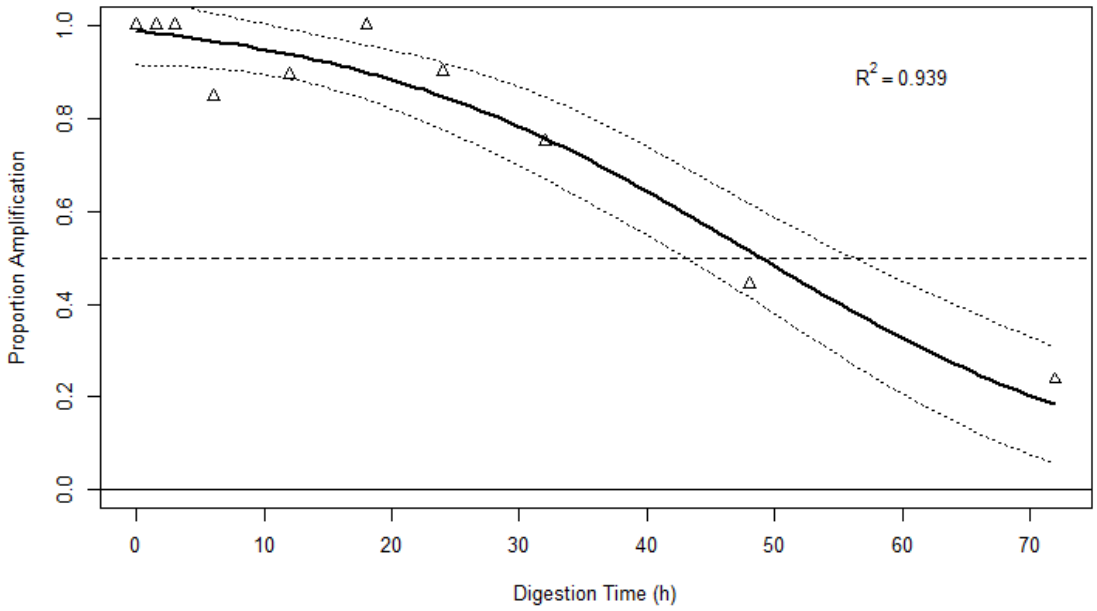


Figure 2. Detection of *Halyomorpha halys* DNA in Laboratory-Fed *Chrysopa carnea* Larvae Amplified by "TaqMan" Real-Time Polymerase Chain Reaction. Average proportion of predators positive for *H. halys* DNA is indicated by black diamonds, and logistic regression curve for these data are indicated with a solid black line. Finely dotted lines represent upper and lower 95% confidence interval for the decay curve, and the thick dashed line corresponds to 50% amplification. The R-squared value for the logistic regression model is also displayed.



Chapter 3b: Identifying the Predators of *Halyomorpha halys* (Stål) with Molecular Gut Content Analysis

Abstract:

Halyomorpha halys (Stål) is an invasive pest of agriculture in the U.S. As a relatively novel exotic species, little is known about the identity of predators affecting *H. halys* in its invaded range. Molecular gut content analysis is a powerful tool capable of elucidating trophic linkages by detecting prey DNA within predator guts. To identify the predators affecting *H. halys* in New Jersey agroecosystems, field collected predators were assayed with the BMITS1 qPCR system for presence of *H. halys* ITS1 rDNA. In total, 850 predators were collected from five crops at two farms over three years with sweep net or beat sheet sampling. Of these, 13.6% samples assayed were positive for target DNA. The highest target DNA incidence rate was observed in Nabidae (29.4% ± 6.4%), followed by Tettigoniidae (26.3% ± 7.2%), Acrididae (14.7% ± 6.2%), Dermaptera (12.8% ± 4.0%), and Coccinellidae (11.7% ± 1.6%). Incidence rate was significantly higher in sunflowers, peppers, and raspberry than soybean while samples collected in peaches had the lowest observed incidence rate. Incidence rate was not significantly different between beat-sampled and sweep-collected samples. *Harmonia axyridis* (Pallas) was the most heavily sampled predator species in the present study, but *H. halys* DNA incidence rate did not vary significantly between *H. axyridis* and other species of Coccinellidae. Predator and *H. halys* abundance were also studied. Predator abundance varied by taxa and crop, but predators were generally most abundant during the second half of the summer, when *H. halys* populations were highest. Peak *H. halys* abundance coincided with an increase in overall detection of *H. halys* DNA in field collected predators.

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive species native to East Asia which has become a major agricultural and homeowner pest since its establishment in the United States (Hoebeke and Carter 2003, Leskey, Hamilton, et al. 2012, Lee et al. 2013). First detected in Allentown, Pennsylvania in 1996, *H. halys* has subsequently spread to 43 states, and has been detected in Canada, and Europe (Fogain and Graff 2011, Vétek et al. 2014, Cesari et al. 2015, "StopBMSB.org" 2017). *Halyomorpha halys* is a mobile and highly polyphagous pest capable of feeding on over 200 species of plant, many of which are economically significant in American agriculture (Nielsen and Hamilton 2009, Leskey, Hamilton, et al. 2012, Rice et al. 2014). First recorded in 2006, damage from *H. halys* is the result of feeding by the nymph or adult stages on sensitive fruiting or reproductive plant tissue (Nielsen and Hamilton 2009, Acebes-Doria et al. 2016). *Halyomorpha halys* populations and resultant economic loses have increased since 2006 with damage to the Mid-Atlantic apple industry estimated at $37 million in 2010 alone (Leskey 2010). As a result, Integrated Pest Management (IPM) practices have been disrupted throughout the region with insecticide usage quadrupling in some affected areas (Leskey, Short, et al. 2012). Increases in insecticide use are often associated with declines in natural enemy abundance and pest suppression (Ruberson et al. 1998), however little is known about the effect of local natural enemies on *H. halys* population dynamics.

Invasive species, like *H. halys*, may proliferate outside of their historical range due to the absence of closely evolved natural enemies (Elton 1958, Keane and Crawley 2002, Pyšek and Richardson 2010). Attempts to control invasive pests often focus on the importation of specialist natural enemies from the pest's native range "classical biological control", but this is not always possible (see Howarth 1991, Barratt et al. 2010). However, generalist natural enemies in the invaded range may help guard against the establishment and spread of would-be invaders by decreasing initial propagule size and limiting population growth (Ehler 1998, Reusch 1998, Fagan et al. 2002, Liebhold and Bascompte 2003, Pyšek and Richardson 2010). Generalists may also

help suppress established populations of invasive species through prey switching: predator preference changes to favor the most abundant prey (Jaworski et al. 2013). Many invasive species, including *H. halys*, have extremely high intrinsic rates of increase in their invaded range, which may disrupt species of native competitors and dominate the local ecosystem (Keane and Crawley 2002, Nielsen et al. 2008, Pyšek and Richardson 2010, Basnet et al. 2014). As a result, native generalists may undergo a prey switch and begin favoring the abundant invasive pest and, in doing so, protect scarce native species and stabilize local prey abundance and diversity (Symondson et al. 2002, Venzon et al. 2002, Jaworski et al. 2013). Native generalists may also initially reject invasive prey due to novel anti-predator adaptations or predator/parasitoid naiveté. For these natural enemies, prey switching can only occur after a period of learning and/or adaptation (King et al. 2006, Carlsson et al. 2009). The relationship between exotic prey and endemic natural enemies is complex and often difficult to predict, thus studying enemy-prey interactions is a key component of understanding the life-history and population dynamics of novel exotic species.

Interactions between *H. halys* and natural enemies in the U.S. were initially poorly understood, but recent studies have attempted to identify and quantify the effect of parasitoids and predators on this invasive pest. Studies aimed at measuring predation of *H. halys* egg masses deployed in agricultural and ornamental crops revealed low utilization by endemic natural enemies, affecting less than 10% of eggs (Cornelius et al. 2016, Ogburn et al. 2016). Parasitoids may play an important and underestimated role in suppressing *H. halys* populations in ornamental crops (Jones et al. 2014), but successful parasitism in agricultural crops occurred in less than 0.5% of all eggs (Ogburn et al. 2016). *Trissolcus japonicus* (Asmead), a parasitoid of *H. halys* eggs native to East Asia, was discovered in the U.S. in 2014 (Talamas et al. 2015). Although *T. japonicus* (Hymenoptera: Scelionidae) may eventually impact *H. halys* population dynamics, this parasitoid is not yet widely distributed and its effectiveness against *H. halys* in agricultural crops is uncertain (Herlihy et al. 2016). Given the uncertain distribution and establishment of *T.*

japonicus and the low success rate of endemic species, egg parasitoids are not currently effective at reducing populations of *H. halys.*

The impact of generalist predators on *H. halys* has been explored in the U.S.. Ogburn et al. (2016) found that predation accounted for approximately 10% of *H. halys* sentinel egg mass mortality, however missing eggs, which accounted for 10.7% of deployed eggs, may have been consumed by chewing predators which ingest eggs entirely, leaving no diagnostic indication of predation (Morrison et al. 2016). A subset of these egg masses were filmed using battery operated closed-circuit cameras to aid in the identification of natural enemies visiting and attacking the eggs (Poley et al. *unpublished*). The most common taxa filmed visiting the *H. halys* sentinel eggs were Acrididae, Anthocoridae, Araneae, Coccinellidae, Forficulidae, Gryllidae, Miridae, Parasitica, Pentatomidae, and Tettigoniidae (Poley et al. *unpublished*).

In laboratory bioassays, arthropod predators of the families Carabidae, Tettigoniidae, and Salticidae frequently attacked *H. halys* egg masses and consumed the most eggs per mass (Morrison et al. 2016). In a separate laboratory no-choice study, immature and adult stages of common natural enemies were exposed to *H. halys* eggs (Abram et al. 2014). Of these, late-instar *Chrysopa carnea* (Stephens) larvae attacked *H. halys* eggs most frequently and consumed more eggs per attack, while early-instar *C. carnea* and *Coleomegilla maculata* (De Geers) (Coleoptera: Coccinellidae) larvae and adults attacked eggs at the lowest rate and ate the fewest eggs per attack (Abram et al. 2014). In larger arena-based laboratory bioassays, Orthopteran predators most frequently consumed eggs while Hemipteran predators most frequently attacked nymphs (Pote and Nielsen 2017). These experiments have helped identify the *H. halys* predator community in its invaded range, but the findings therein have only been partially corroborated with field studies.

The larger community of predators can be studied *in situ* using molecular genetics tools and has become an important tool for studying predator-prey interactions in field settings. Polymerase chain reaction (PCR) analysis has been used for almost 20 years to detect prey-specific DNA sequences in the digestive tract of predators (Symondson 2002). Molecular gut

content analysis (GCA) can be used to identify the taxa of predators feeding on target prey and can provide estimates of the relative frequency of predator-prey associations in the field (Hoogendoorn and Heimpel 2001, Symondson 2002). These results can be used (with estimates of predator digestion speed) to develop an index of biological control efficiency which may help inform Integrated Pest Management decision making (Greenstone et al. 2010, 2014).

Using more advanced methods for DNA amplification (i.e. quantitative PCR, qPCR), target prey DNA can be detected within predator guts for longer durations, increasing the accuracy of predation rate estimations made from these data (Zhang et al. 2007, Weber and Lundgren 2009). Valentin et al. (2016) created and tested a qPCR-based species-specific method for identifying and amplifying *H. halys* DNA. This method, BMITS1, has been used to successfully amplify *H. halys* DNA from bat guano and from the guts of laboratory-fed insect predators (Valentin et al. 2016). The BMITS1 method is capable of detecting *H. halys* DNA for significantly longer than a similar non-quantitative method but has not been used to assay field collected arthropod predators of this invasive species.

The objectives of this experiment were to 1) determine the abundance and seasonality of generalist predators in *H. halys* host plants, 2) identify the community of predators affecting *H. halys* in New Jersey agroecosystems and 3) determine the rates of *H. halys* DNA detection in predators commonly found in New Jersey agroecosystems.

Methods

Insects were collected from organic and conventionally managed plots at Rutgers Agriculture Research and Extension Center (RAREC) in Bridgeton, NJ (39.518719, -75.205849) and, in 2014 only, from a multi-crop organic farm in Monroe Township, NJ (39.703274, -75059854). In 2014, predators were sampled once per week for six weeks from early July through mid-August. Crops sampled in 2014 included peaches, peppers, raspberry, soybean, and sunflower. In 2015, weekly predator sampling was conducted from May through September in

peaches, raspberries, and soybean at RAREC. Sampling was reduced in 2016, with predators collected in peaches from May through September.

Field Sampling. Predators were collected via beat sampling (1 m^2 square white canvas beat sheet, Bioquip Inc., Rancho Dominguez, CA) from conventionally managed peaches (RAREC only), organic raspberries (Monroe Twp. and RAREC) and organic sunflower (Monroe Twp. and RAREC). Any potential predators on the beat sheet were transferred to sterile 1.5 ml microcentrifuge tubes containing chilled (~40ºC) 100% ethanol with sterilized forceps. In peppers (Monroe Co. and RAREC), sweep netting was used to sample predators from grass and weeds adjacent to pepper rows. Similarly, sweep netting was used to sample predators in organic soybean (RAREC only). To minimize the risk of regurgitation and external contamination, a maximum of five sweeps per sample were used when sampling in soybean and peppers (Greenstone et al. 2011). Later, a subset of field collected predator samples were tested for external contamination by assaying legs from these samples for *H. halys* DNA. A more detailed description of these external contamination controls is provided below. All samples were kept chilled in a cooler until collecting was completed, and then transferred to a -20ºC freezer until extraction.

In 2014, sampling included the collection of a wide variety of predatory and omnivorous arthropods, and specimens were only rejected from sampling if they were considered too small/too large to feed on any stage of *H. halys*. Sampling effort during 2015-2016 was limited to those taxa that were identified as likely *H. halys* predators by laboratory studies (Morrison et al. 2016, Pote and Nielsen 2017). Sampling was conducted between the hours of 10:00 am and 2:00 pm on days with little wind and no precipitation. Sampling effort varied between predator taxa and sampling dates. Local predator abundance was monitored twice weekly and these data informed decisions about the desired number of samples per taxa (see *Predator Seasonality Sampling* below). A predator taxa was considered “common” if 10 samples of that taxa could be collected from a single crop in under two person-hours. On each collection date, sampling

continued until 10 individuals from each common taxa had been collected. Samples of "uncommon" taxa were collected as frequently as possible but the total number per collection date was often less than ten. Predators were identified to the family level in the field, when possible, and commonness was primarily assigned at the taxonomic level for collection purposes. Notable exceptions include: Aranaea (considered one taxa for collections but later identified to family), Dermaptera (considered one taxa for collections and not identified further), and Coccinellidae (identified to species in the field and considered separate groups for collections).

Gut Content Analysis. DNA from field collected predators was extracted using a DNeasy blood and tissue kit (Quiagen Sciences, Germantown, MD). Predators which could fit into a 20 µL well were extracted whole, but for larger predators, non-digestive tissues (wings, legs etc.) were removed by dissection with a flame-sterilized razor blade prior to extraction. Extracted samples were amplified with *H. halys*-specific qPCR primers and Taqman fluorescent probe as in Valentin et al. (2016). From a subset of specimens, a leg was removed prior to extraction. If a specimen tested positive for target DNA, the leg was then also assayed to test for false positive results from external contamination. These external contamination controls (legs) were handled, extracted, and amplified according to the same protocol as non-control samples.

Predator Seasonality Sampling. To identify abundant taxa for field sampling and subsequent GCA assay, predator seasonality was studied by sampling predators at RAREC throughout the growing seasons of 2015 and 2016. Predator abundance and seasonality were sampled from the same plots at RAREC where predators were collected for molecular assay. Predator sampling started in mid-June in raspberry, mid-May in peaches and early July in soybean (2015 only) and continued through late August in all crops. During the summer of 2015, predators were beat sampled in peaches and raspberries and sweep sampled in soybeans 1-2 times per week. In 2016, predator sampling was limited to weekly beat samples in peaches and raspberry. In both years, predator sampling in peaches was conducted in mixed variety peach plantings from early to mid-May through late August. Preliminary sampling indicated high

variability in predator abundance between peach plots at RAREC. As a result, predator seasonality was sampled in multiple plantings (three plots in 2015, four plots in 2016) of peaches at RAREC on each sample date. Additional sampling locations of raspberry and soybeans were not available at RAREC either year.

In peach, one beat sample consisted of firmly tapping three peach limbs (>3 cm in diameter) at different heights within the peach tree canopy; ten beat samples were collected per plot per sampling date. In raspberry, each beat sample consisted of tapping canes from three plants within a single 4 m panel; this was repeated ten times per sample date. When beat sampling in peach or raspberry, predators which landed on a 1 m^2 mesh sheet held beneath the limbs/canes were identified and counted as was the abundance and life stage (egg mass, nymph, adult) of *H. halys*. Similarly, sweep sampling in soybeans consisted of ten sweeps per sample replicated ten times per sampling date. Sweep net contents were then deposited into 4 L zip top plastic bags and any predators or *H. halys* within were identified and recorded. In all crops, predators were identified to at least the ordinal level, but family identifications were made when possible. Coccinellidae were further identified as either *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an extremely abundant predator species in these samples, or "other Coccinellids" which included all other species of this family. Similarly, Arachnids were identified as Opiliones, Salticidae or "Other Aranaea" which included all other groups of spiders. The abundance of non-predatory life-stages (e.g. lacewing adults) and taxa with specialized predatory behaviors (e.g. Lampyridae) were not recorded.

Data Analysis. To determine the seasonality of predators, the average abundance of predator taxa and functional feeding groups (e.g. sum of all chewing predators) for each Julian week was calculated. I then calculated average predator abundance by crop and by year. Statistical analyses were not completed for this data due to unbalanced experimental design, but mean abundances are compared numerically between crops and years and to the average weekly incidence rate of *H. halys* DNA in assayed predators. Data are presented as organisms per ten

samples because the average number of organisms collected per sweep was often less than one. For data summary purposes, lacewings (Neuroptera: Chrysopidae and Hemerobiidae) and all Hemipterans were considered sucking predators.

All statistical analysis was conducted in R Studio v3.2.2 “fire safety”. Factors affecting target DNA incidence rate (proportion of samples testing positive *H. halys* DNA) were studied with logistic regression analysis. Assay results were treated a binary response variable for target DNA and modeled using generalized linear models with binomial error distribution and logit link. Predator taxa with fewer than ten total samples were excluded from analysis but are included in summary statistics. Preliminary testing analyzed the relationship between incidence rate and sampling method to check for differences in assay results between beat- and sweep-collected samples (*collection method model:* result = year + site + collection method + ε). Collection method was not included as a model term in subsequent analysis. Primary hypotheses tested for differences in assay results between years, sites, crops and predator taxa. These hypotheses were tested by performing likelihood ratio tests on model terms (*full model:* result = year + site + crop + predator taxa + ε). Wilcoxon Rank Sum test was used to determine if the target DNA incidence rate in *Harmonia axyridis* varied significantly from other Coccinellidae due to its high representation in sampling. Tukey’s HSD was used to separate means for factors with three or more levels (year, crop and predator taxa); differences between factor levels were considered significant when $P < 0.05$.

Results

Gut Content Analysis. Of 850 predators tested with the BMITS1 amplification system, 116 assayed positive for the presence of *H. halys* DNA (13.6% overall incidence rate). Incidence rates ranged from 0.0% (± 0.0%) in several taxa to 27.3% (± 6.8%) in Nabidae (Fig. 1). *Halyomorpha halys* DNA was detected in 8 of 11 families and in 6 of 7 orders assayed. External contamination controls (legs) were collected from twelve predators which tested positive for

target DNA: all twelve legs assayed negative for target DNA, and thus we do not suspect positive samples were due to contamination. Target prey detection rate (proportion of detection) did not differ significantly between collection methods (df = 1, 684; F = 0.034; P = 0.27).

Crop had a significant effect on incidence rate (df = 3, 682; F = 3.7; P = 0.011) (Table 1a). Proportion of detection was significantly higher in sunflowers (33.3% ± 9.8%), peppers (22.9% ± 6.1%), and raspberry (17.6% ± 4.7%) than soybean (10.7% ± 3.4%) (Table 1a). The rate of target prey detection was lowest in peaches (9.1% ± 1.2%) although this was not significantly different from any other crop (Table 1a). Incidence rate was significantly affected by year (df = 2, 686; F = 3.93; P = 0.019) (Table 1b). Proportion of detection did not differ significantly between sites (df = 1, 685; F = 0.064; P = 0.80).

There were significant differences in incidence rate between predator taxa (df = 12, 672; F = 2.47; P = 0.006) but pairwise comparisons did not differentiate between crops or predator taxa (Table 1; Figure 1). The highest incidence rate of *H. halys* was detected in Nabidae (27.3% ± 6.8%), followed by Tettigoniidae (26.3 ± 7.2), and Acrididae (14.7% ± 6.2%) (Fig. 1). Coccinellidae was the most frequently collected predator taxa (n= 377, 44.3% of all samples). Five species of Coccinellidae were collected for molecular assay: *Coccinella septempunctata* (L.), *Coleomegilla maculata*, *H. axyridis*, *Hippodamia convergens* (Guérin-Méneville), and *Propylea quatuordecimpunctata* (L.). *Harmonia axyridis* was the most commonly collected Coccinellid and the most commonly collected predator overall, comprising 276 samples (73.2 % of Coccinellidae, 32.5% of all samples). Fourty-four Coccinellidae samples tested positive for *H. halys* DNA (11.7 % ± 1.7%). Although the incidence rate was lower in *H. axyridis* (10.1% ± 1.8%) compared to other species of Coccinellidae (15.8% ± 3.6) this effect was not statistically significant (W = 13144, P = 0.127).

Predator Seasonality and Abundance. Predator abundance changed considerably throughout the growing season, as well as between crops and years. When data from all years and crops was combined, overall predator abundance in late May and early June was relatively low,

with fewer than ten predators per ten samples for four consecutive weeks (Fig. 2). By the 3rd week of June, predator abundance increased nearly 50% (≥ 15 predators per ten samples) and remained at or above this level until after the end of August (Fig. 2). The abundance of *H. halys* was considerably lower than that of predators. The average number of *H. halys* (adults + nymphs) collected in ten samples was 0.5 compared to 16.5 predators. The highest densities of *H. halys* were observed during the first three weeks of August (Julian weeks 31-33), when the average count per ten samples was over 1.1 for three consecutive weeks (Fig. 2). Incidence rate of *H. halys* DNA detection was similarly binned by Julian week across all years and crops, revealing slightly declining incidence rates from early June through the end of July. Two of the highest incidence rates occurred during consecutive weeks at the beginning of August (Julian weeks 31 and 32), which correspond to peak *H. halys* abundance (Fig. 2).

Predator seasonality also varied between functional feeding groups, crops, and years. Chewing predators reached their highest abundance (> 11 predators / ten samples) from late June through early July, concurrent with the lowest abundances of spiders (Fig. 3). By late July however, spiders were more abundant than chewing predators which declined in mid-July and remained less abundant for the remainder of the year. Coccinellidae and Orthoptera were the most common chewing predators, averaging 3.8 (± 1.1) and 1.3 (± 0.3) individuals per ten sweeps, respectively (Table 2). Orthopterans in soybean were the most abundant predator-crop combination, with 12.1 (± 1.38) individuals per ten samples (Table 2). Outside of Coccinellidae and Orthoptera, chewing predators were uncommon, especially in raspberry and soybeans. Overall, Arachnids were the most abundant feeding group (6.7 ± 0.3 per 10 samples) and among these, unidentified spiders were the most common (3.8 ± 0.2), followed by Salticids (2.8 ± 0.2) (Table 2). Many of the unidentified spiders were very small and it was unclear if these could have consumed any stage of *H. halys*. Sucking predators were less common than spiders and chewing predators but were most abundant in early July (Julian week 27) (Fig. 2). The most common taxa

of sucking predators were Anthocoridae and Nabidae, both of which were most abundant in soybean (Table 2). In 2015, predator abundance was higher in soybean than peaches or raspberry. Predator abundance was higher in raspberry than peaches both years and higher in 2015 than in 2016 (Table 2).

Discussion

The seasonal abundance of generalist predators and assayed field-collected predators for the presence of *H. halys* DNA was studied in southern NJ approximately 20 years post-introduction. The overall incidence of *H. halys* predation was identified as 13.6% with a range of 0.0-27.3%. Several predatory taxa with high rates of *H. halys* DNA incidence have been identified as *H. halys* predators in earlier work. Nabidae, which had the highest incidence rate of any taxa tested, has been shown to attack *H. halys* nymphs but not eggs in laboratory bio-assays (Morrison et al. 2016, Pote and Nielsen 2017). Tettigoniidae, Acrididae, and Dermaptera (each with incidence rates >10%) were all observed feeding on *H. halys* eggs in laboratory feeding trials and video recordings of sentinel egg masses in the field (Morrison et al. 2016; Poley et al. *in prep*). Predatory Pentatomidae (consisting mostly of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) in this study) and Gryllidae species feed on *H. halys* nymphs and eggs, respectively, in laboratory settings (Morrison et al. 2016, Pote and Nielsen 2017), and although not robustly sampled, all samples of these taxa assayed negative for *H. halys* DNA. Conversely, we found relatively high incidence rate in Coccinellidae which previous research has indicated are ineffective predators of *H. halys* eggs and early nymphs (Morrison et al. 2016, Pote and Nielsen 2017). The cause of these discrepancies is currently unknown, but they highlight the importance of studying predation with multiple complementary approaches.

Molecular GCA allows researchers to quantify predation while avoiding pitfalls associated with other techniques used to study predation. Sentinel egg mass and laboratory studies have provided valuable insights about the identity of the community of *H. halys* predators,

but these techniques rely on experimental manipulation of prey and/or environmental conditions which may artificially influence results (Sheppard and Harwood 2005, King et al. 2008). Molecular techniques are also capable of identifying prey regardless of developmental stage while sentinel prey and laboratory bioassay studies require increased replication to study multiple prey life-stages. In general, molecular assays of field collected predators have much larger sample sizes than other predation studies thanks to reduced labor, time, and space requirements. However, molecular techniques are unable to differentiate between DNA consumed during primary predation, secondary predation (consumption of a predator which had already consumed target prey), or scavenging (King et al. 2008). Every method of predator identification exists with limitations and thus utilizing multiple supplementary methods of predation detection is imperative for accurate results.

Halyomorpha halys is an important pest of New Jersey peaches (Nielsen and Hamilton 2009), and the majority of the sampling in this study was conducted in peaches. Some peach varieties produce extrafloral nectaries which emit carbohydrate resources to attract certain natural enemies (Gregory 1915, Mathews et al. 2007). Despite this, peaches had the lowest predator abundance and the lowest incidence of *H. halys* DNA of any crop studied. This may be due to the most abundant predator taxa collected from peaches were Coccinellidae, which do not readily attack *H. halys* eggs or early instar nymphs in laboratory settings (Pote and Nielsen 2017). However, the rate of *H. halys* DNA incidence was higher in Coccinellidae than Chrysopidae, the larvae of which will feed on *H. halys* under laboratory conditions (Abrams 2016, Pote *unpublished*). The acceptability of *H. halys* as prey for Coccinellidae is still unclear, but it is clear that predators are not effectively controlling *H. halys* in peaches. Although initial studies found low rates of parasitism on *H. halys* eggs in peaches (Pote and Nielsen, *unpublished*), extrafloral nectaries are known to attract parasitoids which attack Lepidopteran pests of the peach fruit (Mathews et al. 2007). Although *T. japonicus* is not currently widely distributed in the U.S., extrafloral nectaries may play an important role in attracting and arresting *T. japonicus* which has

only been detected in non-agricultural landscapes (Talamas et al. 2015). Given the lack of effective natural enemies in peaches, chemical management will likely continue to be the primary source of *H. halys* population regulation in this crop, thus it is imperative that reduced-input tactics be employed (Blaauw et al. 2015).

Orthopteran predators had high incidence rates of *H. halys* DNA, and were more abundant in soybean than any other predator-crop combination. These organisms, including Tettigoniidae and Acrididae, were also among the largest predators sampled during this study, and are known to consume *H. halys* egg masses whole (Morrison et al. 2016). Predation analysis using ELISA is sensitive to meal size and predator size (Sunderland 1996, Hagler and Naranjo 1997) and this may true for the qPCR assay used in this study. The high incidence rate observed in Tettigoniidae and Acrididae may be somewhat inflated by these factors, but given the high abundance of Orthoptera in soybeans, the pest control potential of this taxa is still promising. Orthopteran predators are important consumers of the eggs of Lepidopterous rice pests in southeast Asia (Chitra et al. 2002, Ito et al. 2008) and feed on the eggs of *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Given their unique feeding mode, high rates of *H. halys* DNA detection and high abundance, Orthoptera may play an important role in *H. halys* population suppression in soybeans.

Predator preferences for *H. halys* vary between life stages, with chewing predators and spiders consuming mostly eggs, and sucking predators attacking mostly nymphs (Pote and Nielsen 2017). However, rate of *H. halys* predation did not differ significantly by predator feeding mode in this study. This is particularly unexpected, given the major differences in digestive anatomy and physiology between arachnids, manipulate insect predators (chewing) and stylet-feeding insect predators (sucking). Sucking insect predators and spiders digest their food extraorally by injecting digestive enzymes into their prey, but Hemipteran and Chrysopid predators differ from spiders in the origin and utilization of these enzymes (Cohen 1998). Spider digestive enzymes originate in the gut and may be cycled into and out of the prey multiple times

during the process of prey consumption (Cohen 1995), which should, in theory, increase the rate of DNA digestion. In fact, the detectability half-life of prey DNA is significantly different between some chewing and sucking predators (Greenstone et al. 2007). In order to use our GCA results to compare the biological control potential of *H. halys* predators, we must compare the rates of target DNA incidence among a diverse set of predatory taxa with a variety of feeding behaviors, any of which could affect the detectability of target DNA (Greenstone et al. 2010). Before comparisons can be made across taxa, we must quantify the differences in digestion speed that inherently exist between all groups of predators.

An important outcome of many agricultural predation studies is to determine the relative importance of various predators in the population dynamics of pests. However, raw incidence rate data should not be compared between predator taxa without first determining the digestion speed of each taxa (Greenstone et al. 2010). Slow digesting predators retain prey DNA for longer than those with rapid digestion and, as a result, slow digesting species have naturally higher incidence rates of target DNA (Greenstone et al. 2010, 2014). For example: although Tettigoniidae resulted in the 2nd highest observed *H. halys* DNA incidence rate of any taxa in the present study, they were also the largest organisms sampled (often > 2 cm in length). Predator digestion speed is correlated with size (Sunderland 1996, Hagler and Naranjo 1997), thus it would be inappropriate to directly compare the incidence rate in Tettigoniidae to smaller, quicker digesting taxa. Between-taxa comparisons can be made following the completion of time-series digestion studies for the specific predator-prey-amplification system of interest (see Greenstone et al. 2014).

Harmonia axyridis, the most abundant predator in the present study, is an invasive species of Coccinellidae. Like *H. halys*, *H. axyridis* is native to East Asia and is relatively abundant in *H. halys* host crops across their invaded and native ranges (Tedders and Schaefer 1994, Koch 2003). The Enemy Release Hypothesis predicts that invasive pests may succeed in the absence of co-evolved natural enemies (Jeffries and Lawton 1984, Keane and Crawley 2002). *Harmonia axyridis* and *H. halys* existed in similar geographic ranges and on similar host plants

throughout evolutionary time, yet we found no statistical difference in *H. halys* DNA incidence rate between *H. axyridis* and endemic species of Coccinellidae. *Halyomorpha halys* may exhibit defensive behaviors or chemical defenses that affect all members of Coccinellidae with equal intensity or, the size of *H. halys* life stages may be inappropriate for consumption by Coccinellid mouth parts. Regardless, these findings indicate that shared evolutionary history alone may not be sufficient to predict the intensity of a predator-prey interaction. Due to the economic and ecological costs of successful invasive species, future research should seek to determine those prey characteristics, which may override evolutionary history, inhibit predation, and thus facilitate future invasions.

References

(StopBMSB.org) Northeastern IPM Center. 2017. StopBMSB.org. (http://www.stopbmsb.org/where-is-bmsb/state-by-state/).

Abram, P. K., J. Doyon, J. Brodeur, T. D. Gariépy, and G. Boivin. 2014. Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Can. Entomol. 147: 1–5.

Acebes-Doria, A., T. C. Leskey, and J. Bergh. 2016. Injury to apples and peaches at harvest from feeding by *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs early and late in the season. Crop Prot. 89: 58–65.

Barratt, B., F. Howarth, T. Withers, J. Kean, and G. S. Ridley. 2010. Progress in risk assessment for classical biological control. Biol. Control. 52: 245–254.

Basnet, S., L. M. Maxey, C. A. Laub, T. P. Kuhar, and D. G. Pfeiffer. 2014. Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in Southwestern Virginia. J. Entomol. Sci. 49: 304–312.

Blaauw, B. R., D. Polk, and A. L. Nielsen. 2015. IPM-CPR for peaches: Incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach. Pest Manag. Sci. 71: 1513–22.

Cesari, M., L. Maistrello, F. Ganzerli, P. Dioli, L. Rebecchi, and R. Guidetti. 2015. A pest alien invasion in progress: potential pathways of origin of the brown marmorated stink bug *Halyomorpha halys* populations in Italy. J. Pest Sci. (2004). 88.

Chitra, N., K. Gunathilagaraj, and R. P. Soundararajan. 2002. Prey preference of Orthopteran predators on rice insect pests. J. Biol. Control. 16: 109–111.

Cohen, A. 1998. Solid-to-liquid feeding: the inside (s) story of extra-oral digestion in predaceous Arthropoda. Am. Entomol. 93: 395–401.

Cohen, A. C. 1995. Extra-oral digestion in predaceous terrestrial arthropoda. Annu. Rev. Entomol. 40: 85–103.

Cornelius, M. L., C. Dieckhoff, K. A. Hoelmer, R. T. Olsen, D. C. Weber, M. V. Herlihy, E. J. Talamas, B. T. Vinyard, and M. H. Greenstone. 2016. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control. 103: 11–20.

Ehler, L. E. 1998. Invasion biology and biological control. Biol. Control. 13: 127–133.

Elton, C. S. 1958. The ecology of invasions by animals and plants. J. Chem. Inf. Model. 53: 1689–1699.

Fagan, W. F., M. A. Lewis, M. G. Neubert, and P. Van Den Driessche. 2002. Invasion theory and biological control. Ecol. Lett.

Fogain, R., and S. Graff. 2011. First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. J. Entomol. Soc. Ontario. 142: 45–48.

Greenstone, M. H., M. E. Payton, D. C. Weber, and A. M. Simmons. 2014. The detectability half-life in arthropod predator-prey research: what it is, why we need it, how to measure it, and how to use it. Mol. Ecol. 23: 3799–813.

Greenstone, M. H., D. L. Rowley, D. C. Weber, M. E. Payton, and D. J. Hawthorne. 2007. Feeding mode and prey detectability half-lives in molecular gut-content analysis: an

example with two predators of the Colorado potato beetle. Bull. Entomol. Res. 97: 201–209.

Greenstone, M. H., Z. Szendrei, M. E. Payton, D. L. Rowley, T. C. Coudron, and D. C. Weber. 2010. Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle. Entomol. Exp. Appl. 136: 97–107.

Greenstone, M. H., D. C. Weber, T. C. Coudron, and M. E. Payton. 2011. Unnecessary roughness? Testing the hypothesis that predators destined for molecular gut-content analysis must be hand-collected to avoid cross-contamination. Mol. Ecol. Resour. 11: 286–93.

Gregory, C. T. 1915. The taxonomic value and structure of the peach leaf glands. Bull. Cornell Univ. Agric. Exp. Stn.

Hagler, J. R., and S. E. Naranjo. 1997. Measuring the Sensitivity of an Indirect Predator Gut Content ELISA: Detectability of Prey Remains in Relation to Predator Species, Temperature, Time, and Meal Size. Biol. Control. 9: 112–119.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One. 11: e0150275.

Hoebeke, E. R., and M. E. Carter. 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105: 225–237.

Hoogendoorn, M., and G. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol. Ecol. 10: 2059–2067.

Howarth, F. G. 1991. Environmental Impacts of Classical Biological Control. Annu. Rev. Entomol. 36: 485–509.

Ito, K., N. H. Kin, and C. P. Min. 2008. *Conocephalus longipennis* (De Haan) (Orthoptera: Tettigonidae): A suspected egg-predator of the rice bug in the Muda Area, west Malaysia. Appl. Entomol. Zool. 30: 599–601.

Jaworski, C. C., A. Bompard, L. Genies, E. Amiens-Desneux, and N. Desneux. 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. PLoS One. 8: 1–10.

Jeffries, M. J., and J. H. Lawton. 1984. Enemy-free space and the structure of ecological communities. Biol. J. Linn. Soc. 23: 269–286.

Jones, A. L., D. E. Jennings, C. R. R. Hooks, and P. M. Shrewsbury. 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control. 78: 61–66.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17: 164–170.

King, R. a, D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17: 947–63.

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3: 32.

Lee, D.-H., B. D. Short, S. V Joseph, J. C. Bergh, and T. C. Leskey. 2013. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China,

Japan, and the Republic of Korea. Environ. Entomol. 42: 627–41.

Leskey, T. C. 2010. 2010 Tree Fruit Damage Survey.

Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. C. Bergh, D. A. Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. a. Hoelmer, D.-H. Lee, and S. E. Wright. 2012. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23: 218–226.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche (Stuttg). 2012: 1–14.

Liebhold, A., and J. Bascompte. 2003. The Allee effect, stochastic dynamics and the eradication of alien species. Ecol. Lett. 6: 133–140.

Mathews, C. R., M. W. Brown, and D. G. Bottrell. 2007. Leaf extrafloral nectaries enhance biological control of a key economic pest, *Grapholita molesta* (Lepidoptera: Tortricidae), in peach (Rosales: Rosaceae). Environ. Entomol. 36: 383–389.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control. 97: 120–130.

Nielsen, A. L., and G. C. Hamilton. 2009. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102: 1133–1140.

Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environ. Entomol. 37: 348–355.

Ogburn, E. C., R. Bessin, C. Dieckhoff, R. Dobson, M. Grieshop, K. A. Hoelmer, C. Mathews, J. Moore, A. L. Nielsen, K. Poley, J. M. Pote, M. Rogers, C. Welty, and J. F. Walgenbach. 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control. 101: 39–51.

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by generalist predators. Biol. Control. 114: 1-7.

Pyšek, P., and D. M. Richardson. 2010. Invasive species, environmental change and management, and health. Annu. Rev. Environ. Resour. 35: 25–55.

Reusch, T. B. H. 1998. Native predators contribute to invasion resistance to the non-indigenous bivalve *Musculista senhousia* in southern California, USA. Mar. Ecol. Prog. Ser. 170: 159–168.

Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Gariepy, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5: 1–13.

Ruberson, J., H. Nemoto, and Y. Hirose. 1998. Pesticides and conservation of natural enemies in pest management, pp. 207–220. *In* Barbosa, P. (ed.), Conserv. Biol. Control. Academic Press.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19: 751–762.

Sunderland, K. D. 1996. Progress in quantifying predation using antibody techniques., pp. 419–455. *In* Symondson, W.O.C. (ed.), Ecol. Agric. Pests Bio-Chemical Approaches. Chapman & Hall, London.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11: 627–641.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47: 561–94.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. Buffington, M.-C. Bon, and D. C. Weber. 2015. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Tedders, W. L., and P. W. Schaefer. 1994. Release and establishment of *Harmonia axyridis* (Coleoptera, Coccinellidae) in the Southeastern United States. Entomol. News. 105: 228–243.

Valentin, R. E., B. Maslo, J. L. Lockwood, J. Pote, and D. M. Fonseca. 2016. Real-time PCR assay to detect brown marmorated stink bug, *Halyomorpha halys* (Stal), in environmental DNA. Pest Manag. Sci. 72: 1854–1861.

Venzon, M., A. Janssen, and M. W. Sabelis. 2002. Prey preference and reproductive success of the generalist predator *Orius laevigatus*. Oikos. 97: 116–124.

Vétek, G., V. Papp, A. Haltrich, and D. Rédei. 2014. First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa. 3780: 2010–2012.

Weber, D. C., and J. G. Lundgren. 2009. Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. J. Insect Sci. 9: 41.

Zhang, G. F., Z. C. Lü, F. H. Wan, and G. L. Lövei. 2007. Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. Mol. Ecol. Notes. 7: 947–954.

Tables and Figures

Table 1. Molecular gut content analysis of field collected predators, by crop and year. Total indicates the number of individual predators sampled in each crop/year. Positives refers to the number of individuals positive for *H. halys* DNA assayed by the BMITS1 qPCR method. Means followed by shared letters are not significantly different (Tukey's HSD, $\alpha = 0.05$).

A. *Crop*	Total	Number Positives	Proportion Positive Samples	
Sunflower	24	8	33.3% (± 9.8%)	A
Pepper	49	11	22.4% (± 6.0%)	A
Raspberry	70	12	17.1% (± 4.5%)	A
Soybean	84	9	10.7% (± 3.4%)	B
Peach	623	50	8.0% (± 1.1%)	AB

B. *Year*	Total	Number Positives	Proportion Positive Samples	
2014	156	28	17.9% (± 3.1%)	a
2015	524	54	10.3% (± 1.3%)	a
2016	170	8	4.7% (± 1.6%)	a

Table 2. Abundance of Predators in *H. halys* Host Plants in New Jersey, 2015-2016. Data presented here are the average (± SEM) number of predators collected per 10 samples. Note: Sampling effort in peaches was considerably higher than raspberry or soybean, the latter of which was only sampled in 2015. The entries for "*Harmonia axyridis*" include only adult *H. axyridis*, as Coccinellid larvae were not identified and are reported here as "Other Coccinellidae."

		Crop			
	Taxa	**Peach**	**Raspberry**	**Soybean**	**Total**
Arachnida	Thomisdiae	0.63 (± 0.09)	1.04 (± 0.24)	1.08 (± 0.31)	0.73 (± 0.08)
	Opiliones	0.03 (± 0.02)	1.00 (± 0.23)	0.08 (± 0.08)	0.19 (± 0.04)
	Salticidae	2.40 (± 0.19)	4.91 (± 0.66)	2.25 (± 0.47)	2.80 (± 0.18)
	Other Araneae	3.68 (± 0.23)	3.78 (± 0.59)	4.25 (± 0.80)	3.75 (± 0.21)
Chewing	Cantheridae	0.86 (± 0.11)	0.35 (± 0.12)	0.08 (± 0.08)	0.71 (± 0.09)
	Carabidae	0.05 (± 0.02)	0	0	0.04 (± 0.01)
	Dermaptera	0.69 (± 0.11)	0.04 (± 0.04)	0	0.53 (± 0.09)
	Harmonia axyridis	3.76 (± 0.26)	2.22 (± 0.35)	0.42 (± 0.18)	3.23 (± 0.21)
	Other Coccinellidae	0.47 (± 0.07)	0.87 (± 0.24)	0.75 (± 0.26)	0.56 (± 0.07)
	Orthoptera	0.10 (± 0.03)	0.91 (± 0.20)	12.08 (± 1.38)	1.25 (± 0.15)
Sucking	Anthocoridae	0.77 (± 0.09)	0.26 (± 0.11)	8.42 (± 1.05)	1.33 (± 0.13)
	Geocoridae	0.01 (± 0.01)	0	1.08 (± 0.35)	0.10 (± 0.03)
	Nabidae	0.02 (± 0.01)	0.02 (± 0.09)	2.60 (± 0.55)	0.26 (± 0.05)
	Neuroptera	1.77 (± 0.17)	0.04 (± 0.04)	0	1.34 (± 0.13)
	Pentatomidae	0.18 (± 0.04)	0.91 (± 0.24)	0.17 (± 0.11)	0.30 (± 0.05)
	Reduviidae	0.24 (± 0.05)	0.04 (± 0.04)	0.17 (± 0.12)	0.20 (± 0.04)
	Total	15.70 (± 0.51)	15.71 (± 1.14)	32.58 (± 1.97)	17.1 (± 4.7)
	# Samples (*N*)	1070	230	120	1420

Figure 1. Detection of *H. halys* DNA in field collected predators. Incidence rate is the proportion of samples which test positive for target DNA within a given predator taxa. Significant differences in incidence rate between predators was not detected (Tukey's HSD, $\alpha = 0.05$).

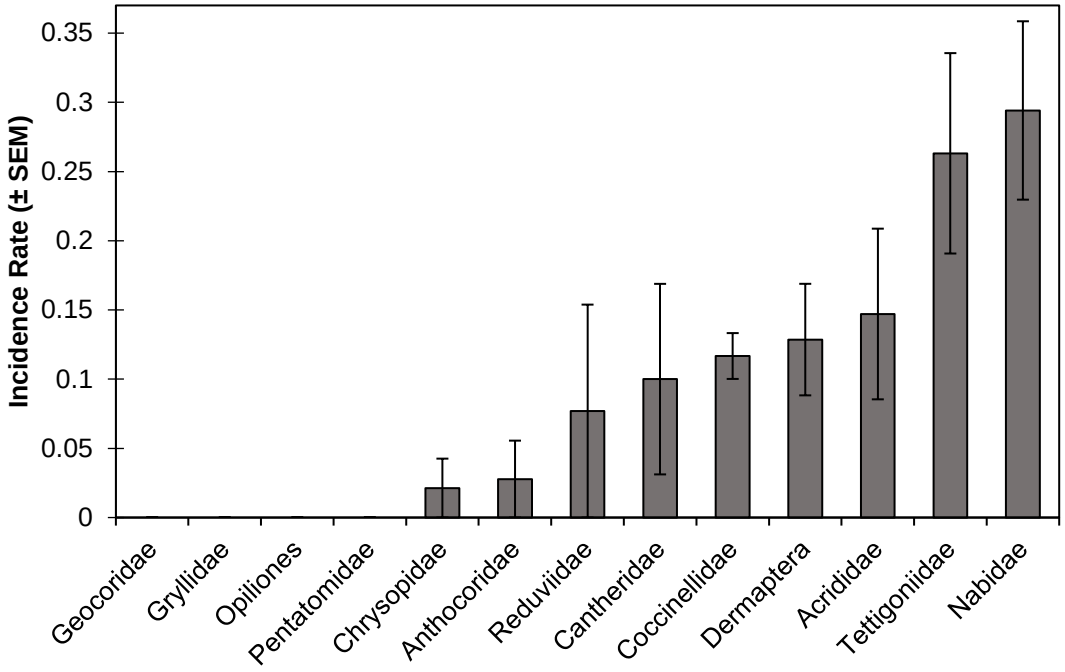


Figure 2. Comparing the Abundance of H. halys and Generalist Predators to the Incidence Rate of H. halys DNA in Field Collected Predators, 2015-2016. Data presented here are weekly averages calculated across all crops and years. Incidence rate is the proportion of field collected predators which assayed positive for *H. halys* DNA with the BMITS1 TaqMan qPCR primer system. *Note:* To ensure similarity in scale, the abundance of *H. halys* is expressed as the relative proportion of the highest average weekly abundance (1.23 *H. halys* per ten samples, recorded during week 33).

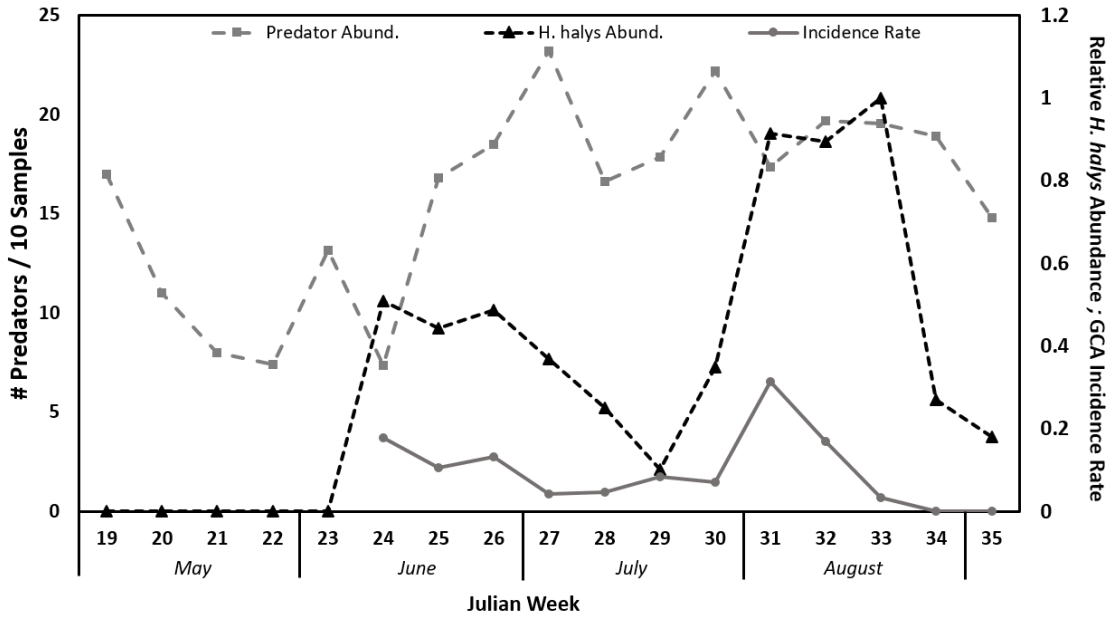
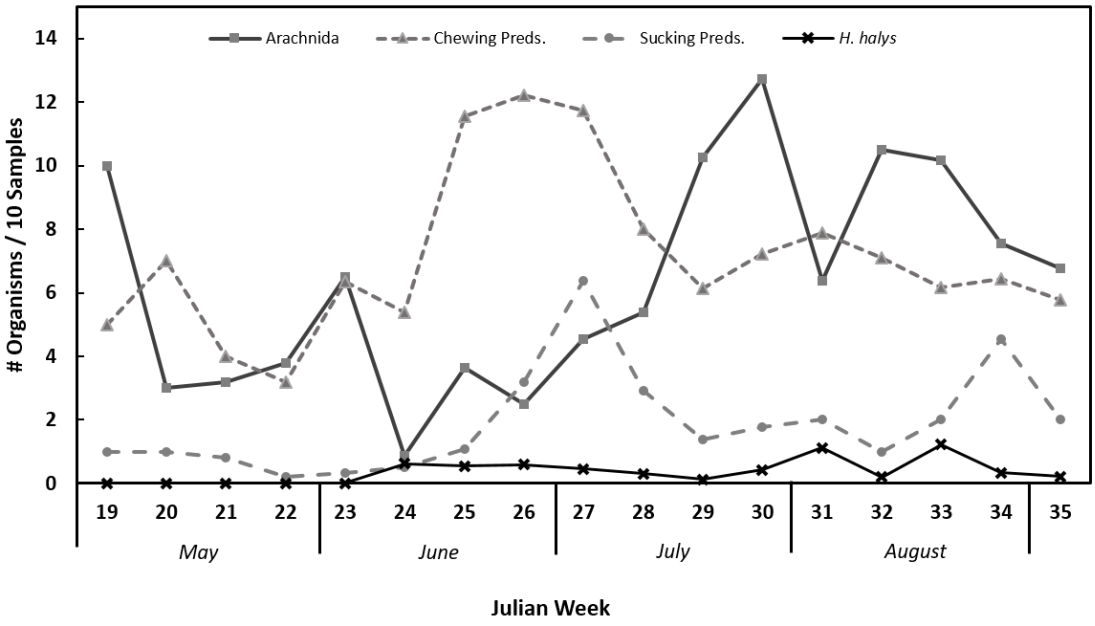


Figure 3. The Seasonality of *H. halys* and Generalist Predators in New Jersey Agro-Ecosystems, 2015-2016. Data presented here are weekly averages calculated across all crops and years. *Note:* Abundance of *H. halys* is expressed as mean insects per ten sweeps, not as a proportion of the maximum.



Chapter 4: Life Stage Specific Predation of *Halyomorpha halys* (Stål) by Generalist Predators

Abstract:

Halyomorpha halys (Stål) is an invasive pest of agriculture in the U.S. Feeding damage from *H. halys* affects dozens of crops yet little is known about the community of predators which prey on *H. halys* in its invaded range. Ten taxa of predatory or omnivorous insects were evaluated for their capacity to consume eggs and nymphs of *Halyomorpha halys* in laboratory mesocosm experiments. Predators were collected from agricultural ecosystems in New Jersey, starved for 24-48 hours, and then exposed to *H. halys* eggs, first instar, or second instar nymphs. Survivorship of control prey in predator-excluding containers within the arenas was compared to that of predator treatment groups to determine the effect of predator presence. Stage-specific differences in *H. halys* survivorship among life stages were observed for several predator taxa indicating stage-specific predation. Acrididae, *Coccinella septempunctata* (L.), *Podisus maculiventris* (Say) (nymphs and adults), and Tettigoniidae reduced the hatch rate of *H. halys* eggs. Hemipteran predators, including *Nabis* spp. and Reduviidae, reduced the survivorship of first instar nymphs. Similarly, *Nabis* spp. and *P. maculiventris* nymphs reduced the survivorship of second instar nymphs. Acrididae, *Nabis* spp., *P. maculiventris* nymphs, Reduviidae, and Tettigoniidae showed stage-specific tendencies in their consumption of *H. halys.* Morphological similarities between the immature stages of *H. halys* may facilitate predator suppression of these mobile stages. These results indicate that predation estimates that rely solely on sentinel egg masses may underestimate the impact of generalist predators on other *H. halys* life stages.

Key words: Brown marmorated stink bug; predator bioassay; invasive prey; native predators

Introduction

Natural enemies play an important role in the regulation of insect pest populations (Hassell and May, 1986; Symondson et al., 2002). A meta-analysis of biological control literature over the last 10 years showed that, overall, biological control agents significantly reduce pest abundance compared to predator-protected control groups (Stiling and Cornelissen, 2005). Although predators may generally suppress insect herbivore populations, the effect of individual predator taxa on prey abundance can vary widely among prey life stages. Lycosid spiders can cause 91% mortality of small, third instar grassland acridids, but only 63.5% and 30.4% mortality of larger fifth instars and adults, respectively (Oedekoven and Joern, 1998). Predation on *Frankliniella occidentalis* (Pergande) flower thrips by *Orius insidiosus* (Say) varies among prey life stages and is mediated by shifts in prey behavior between nymph and adult stages (Baez et al., 2004). Predatory mites that consume eggs and larvae of *Tetranychus urticae* (Koch), also show life-stage preferences that vary with their diet breadth; oligophagous species preferg eggs, whereas generalist species show no preference for prey life stage (Blackwood et al., 2001).

Variation in behavior and other predatory cues among prey life stages may drive the demographic prey preferences of generalist predators. Although olfactory kairomones emitted by prey are often used by specialists for prey location, many generalists rely heavily on visual cues (Vet and Dicke, 1992). To reduce the risk of predation, arthropods often limit their visual detectability by reducing movement and foraging behaviors in the presence of predators (Lind and Cresswell, 2005; Nelson, 2007; Persons et al., 2001; Sih, 1986). Sessile prey such as eggs or female scale insects may provide weaker visual cues than mobile life stages, which may affect rates of predator attack. Alternatively, mobile prey may be more susceptible to attack by more visually-oriented predation strategies such as 'sit-and-wait' predation (Nelson, 2007; Olberg et al., 2000).

Differential impacts of predation on various pest life stages can have important implications for monitoring pest abundance, especially when the severity of pest damage varies

among life stages. *Lygus hesperus* (Knight) is a pest of cotton at all life stages, but feeding by late instar nymphs and adults causes the majority of economic damage (Zink and Rosenheim, 2005). The primary predators of *L. hesperus* in cotton are *Geocoris* spp. which feed preferentially on the early, less damaging, nymphal instars. However, nymphs are more easily sampled than the winged adult *L. hesperus* and as a result, high nymphal predation complicates attempts to monitor the economically damaging adult populations. The disconnect between predator preferences and developmental changes in prey damage has important consequences for patterns of pest damage and for efforts to monitor pest populations (Zink and Rosenheim, 2008).

Brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), is an invasive pest of agriculture across the U.S. and parts of Europe (Hoebeke and Carter, 2003; Leskey et al., 2012a; Maistrello et al., 2016; Nielsen and Hamilton, 2009b). As a recently invaded species, *H. halys* can be a useful model organism for studying the role of generalist natural enemies on suppressing invasive prey populations. *Halyomorpha halys* feeds on multiple agriculturally important crops including apple, peach, tomato, pepper, corn, and soybean, making it a landscape-level pest (Leskey et al., 2012b; Rice et al., 2016, 2014). Late instar *H. halys* nymphs cause significantly more injury to peaches and more discolored depressions in apples than do younger nymphs (Acebes-Doria et al., 2016). As a result, predator impacts on early instars may result in less damage reduction than similar predation rates on older instars. The high density of *H. halys* in many agricultural settings may accelerate predator adaptation due to the high frequency of predator-prey encounters (Basnet et al., 2014; Nielsen et al., 2011; Nielsen and Hamilton, 2009b). Sentinel egg masses have been used across habitats in the invaded region to study the effect of natural enemies on *H. halys*. Sentinel *H. halys* egg masses placed within agricultural sites in seven states across the lower Midwest and Mid-Atlantic regions during 2013-2014 were parasitized at very low rates (< 1 % of eggs produced an adult parasitoid) (Ogburn et al., 2016). In Mid-Atlantic ornamental nurseries, parasitism of *H. halys* egg masses varies greatly, possibly due to differences in parasitoid community (Cornelius et al., 2016; Jones et al., 2014). Predation

on these egg masses, at least in agricultural settings, was markedly higher (up to 20% in some crops), suggesting that predators may have a greater effect on *H. halys* populations than do native parasitoids (Ogburn et al., 2016).

Due to the high abundance and impact of *H. halys* within diverse agroecosystems, it is important to understand how the generalist predator community is responding to this novel resource. Our understanding of predator impacts on *H. halys* to date has focused on one sessile life stage (eggs) and thus does not present a complete picture of predation. Evidence from *Nezara viridula* (L.) suggests that the community of predators concuming eggs and nymphs may be largely non-overlapping (Ragsdale et al., 1981). Behavioral differences exist between sessile *H. halys* eggs, aggregated first instar nymphs, and highly mobile second instar nymphs (Nielsen and Hamilton, 2009a), which may affect predation of these stages. Thus, the objectives of this research were to 1) determine the community of generalist predators that attack *H. halys* eggs, first and second instar nymphs in semi-natural arenas and 2) determine if predators differentially attack the various early life stages of *H. halys.*

Methods

Predators and Prey

To test the acceptability of *H. halys* to natural enemies in laboratory, field collected predators were exposed to *H. halys* eggs, first instars, and second instar nymphs in no-choice predation assays. Predators were collected at the Rutgers Agriculture Research and Extension Center (RAREC) in Bridgeton, NJ from organic soybean and rye by sweep-netting and beat sampling in conventionally managed peaches. From May through October of 2015-2016, preliminary sampling determined which taxa were sufficiently abundant to include in the study. Individuals of each abundant predator taxa were first tested in experimental arenas to determine if their behavior was significantly altered by this confinement and, as a result, ants and spiders were excluded from this study. Predator taxa tested in this experiment included Acrididae (represented

by one unidentified morpho-species), *Coccinella septempunctata* (L.), *Coleomegilla maculata* (DeGeer), *Geocoris* spp., *Harmonia axyridis* (Pallas), *Hippodamia convergens* (Guérin-Méneville), *Nabis* spp., adults and 3rd-4th instar nymphs of *Podisus maculiventris* (Say), Reduviidae (consisting of *Arilus cristatus* (L.) and *Sinea spinipes* (Herrich-Schaeffer)), and *Conocephalus spp.* (see Table 1). *Halyomorpha halys* egg masses were acquired from a laboratory colony at the New Jersey Department of Agriculture Philip J. Alampi Beneficial Insect Laboratory in Trenton, NJ and nymphs were reared at RAREC using organic carrot and green bean.

Predation Arenas

Arenas used for predator assays were rectangular boxes constructed of 6 mm acrylic sheeting measuring 15 cm wide, 30 cm deep, and 30 cm tall (Figure 1). A five cm hole was drilled into the bottom of each arena to allow for the insertion of a small sunflower plant. Sunflowers, *Helianthus annuus* L., are a known *H. halys* host plant and were selected because they grow easily in small pots (Soergel et al., 2015). Plants were grown from organic seed (var: grey striped sunflower, Johnny's Selected Seeds, Fairfield, ME) in individual 500 mL pots (seedling pads, grow light, room temperature and humidity) until the R1-R2 stage. Plants were used for multiple experiments, but were rinsed with water and searched for prey remains between runs. Sterile sand was spread across the bottom of each arena to simulate a natural substrate and to cover the soil. Two smaller 3 cm holes were drilled into one of the narrow vertical surfaces of the arenas and covered with window screening for ventilation. The top of each arena was covered by a hinged lid of acrylic sheeting sealed with masking tape to prevent insects from escaping. To deter insects from climbing the walls of the arenas, the base of each wall was painted with a five cm strip of fluon (Teflon PTFE 30, Dupont, Wilmington, DE). Matte grey spray paint was applied to three of the four walls to prevent activity outside the arenas from altering predator or

prey behavior. The lid of each arena and one large vertical surface were left unpainted to allow light to enter the arenas (Figure 1).

Assays

Each predator-life stage treatment was evaluated in two or three temporal blocks each consisting of four replicates. Although a maximum of eight arena experiments could be conducted simultaneously, half-runs were occasionally conducted if predator species or prey stages were limiting. Predator treatments were selected each week based on availability and field abundance of predatory taxa in various crops at RAREC (listed above). After a predator treatment had been selected, ten individuals of this taxa were collected and starved for 48 h by providing only deionized water and a 10% sugar water solution. If fewer than eight individuals survived 48 h of starvation, this taxa was recollected and starved for only 24 h. Prey treatments consisted of 1) one egg mass consisting of ca. 28 *H. halys* eggs (< 48 h old), 2) approximately 28 *H. halys* first instar nymphs, or 3) five second instar nymphs. Egg masses and first instar nymphs aggregated on the hatched egg mass were affixed to the underside of a leaf of the sunflower plant with non-toxic craft glue (School Glue Gel, Elmer's, High Point, NC). Glue was applied only to the ovipositional substrate, not to eggs themselves. Second instar nymphs were transferred directly onto the leaves of the sunflower plant. In addition to the *H. halys* used as prey in the arenas, bugs of the same stage were stored in a small deli cup (30 mL, Solo Cup Company, Lake Forest, IL) within the arena containing moistened sand substrate and sealed with a paper lid to constitute negative controls. Prey were allowed to settle for 30 minutes prior to the introduction of a single predator in each arena. Throughout each trial, the sand substrate in the arenas and the cups containing the controls were misted with deionized water at least once a day and the sunflower plants were watered as needed. Aside from semi-daily misting, arenas were kept at ambient temperature, humidity and lighting (mixed fluorescent and natural light during daylight hours, half-intensity fluorescent at night). After 48 h, predators were removed and prey were assessed

for mortality. Individual predators were used only once. Egg masses were assessed for signs of predation based on the symptoms identified by Morrison et al. (2016). Each predator-prey stage combination was repeated at least eight times, within two blocks of four simultaneously conducted assays. Replicates where the predator did not survive the 48 h assay were discarded (Tables 2-4).

Data Analysis

A preliminary Welch two-sample t-test was performed to test for differences in prey survivorship between *A. cristatus* and *S. spinipes*, the two reduviid species in the experiment, to determine if these species could be considered and analyzed collectively as Reduviidae. To determine if predators caused a significant decrease in the survivorship of *H. halys* nymphs (or the hatch rate of *H. halys* eggs), Welch two-sample t-tests were performed. Arcsine-square root transformed proportion of survivorship (or proportion of hatch for egg masses) in treatment prey were compared to that of control prey for each predator and prey-stage combination. For each predator causing significantly lower survivorship than the control in at least one prey life stage, an analysis of variance (ANOVA) was used to test for significant differences in survivorship among prey life stages. First, prey survivorship in treatment groups was adjusted by the survivorship of the control group using Abbott's Formula (Abbott, 1925). Next, a one-way ANOVA was performed for each predator to test for significant differences in corrected survivorship between prey life stages. Tukey's HSD method was used for means separation in a post-hoc analysis. Differences in survivorship means were considered significant at $\alpha = 0.05$. All data analyses were performed in R Studio v3.2.2 ("Fire Safety").

Results

Predation on Eggs and Nymphs

We detected no statistically significant difference in *H. halys* survivorship between *A. cristatus* and *S. spinipes* so these species were combined for subsequent analyses and are referred to simply as Reduviidae (t = 0.39, P = 0.627). Independent Welch t-tests revealed significantly lower hatch rate when egg masses were exposed to Acrididae (t = 2.379, P = 0.0483), *C. septempunctata* (t = 2.658, P = 0.0287), *P. maculiventris* adults (t = 2.313, P = 0.0436), *P. maculiventris* nymphs (t = 2.789, P = 0.0215), and Tettigoniidae (t = 2.788, P = 0.0145) (Table 2). When exposed to acridids and tettigoniids, 40.8% and 46.8% fewer *H. halys* eggs hatched, respectively, compared to unexposed controls (Table 2).

Survival of *H. halys* first instar nymphs was significantly lower than that of the controls when exposed to *Nabis* spp. (t = 3.609, P = 0.0015) and Reduviidae (t = 2.568, P = 0.0311) (Table 3). Reduviidae caused a 34.3% reduction in survivorship, whereas *Nabis* spp. reduced survivorship by 9.7%.

Only *Nabis* spp. (t = 4.94, P = 0.0001) and *P. maculiventris* nymphs (t = 4.413, P = 0.0014) reduced the survivorship of second instar nymphs compared to controls (Table 4), lowering it by 48.3% and 40%, respectively.

Survivorship of Prey Life Stages

Life stage-specific differences in *H. halys* survivorship were significant for *Nabis* spp. (F = 13.72; df = 2,33; P < 0.001), Acrididae (F = 4.382; df = 2,21; P = 0.0261), *P. maculiventris* nymphs (F = 3.967, df = 2,21; P = 0.0352), Reduviidae (F = 3.698; df = 2,21; P = 0.0425) and Tettigoniidae (F = 11.54; df = 2, 20; P < 0.001). *Nabis* spp. and *P. maculiventris* nymphs significantly reduced survivorship of second instar nymphs more than that of first instar nymphs, whereas Acrididae and Tettigoniidae reduced egg hatch more than the survivorship of either first

or second instar nymphs (Figure 2). Reduviidae reduced first instar nymph survivorship more than the hatch rate of eggs, but preyed on first and second instar nymphs equally (Figure 2).

Discussion

The differences we observed among taxa in predation on various *H. halys* life stages is consistent with the findings of previous research on predators affecting other North American Pentatomidae. The predator guild consuming eggs of *N. viridula*, *Euschistus servus* (Say), *E. variolarius* (P. De B.), *Chinavia hilare* (Say), and *P. maculiventris* were primarily those with chewing mouthparts (Koppel et al., 2009; Ragsdale et al., 1981; Yeargan, 1979). In addition to chewing predators, *P. maculiventris* was identified as a predator of *N. viridula* eggs, consistent with the present study (Ragsdale et al., 1981). Similarly, *N. viridula* and *H. halys* nymphs were most commonly preyed upon by sucking predators, including Reduviidae and *P. maculiventris* and markedly different predator communities were shown to feed on the eggs and nymphs of *N. viridula* (Ragsdale et al., 1981).

We observed significant differences in survivorship between first and second instar *H. halys* nymphs exposed to *Nabis spp.* and *P. maculiventris* nymphs. Variation in predation between nymphal instars may be explained by stage-specific differences in defensive compounds. Pentatomidae are known for the odorous compounds they release when disturbed (Aldrich, 1988) and these compounds can vary with life stage (Borges and Aldrich, 1992). Members of the subfamily Pentatominae excrete (E)-4-oxo-2-decenal when agitated, but only during the first instar (Borges and Aldrich, 1992). In other species this compound is emitted by all nymphal instars but is notably absent in the secretions of adults (Pareja et al., 2007). The chemical composition of *H. halys* nymphal secretions has not been thoroughly studied, but variation in these compounds could lead to the observed variation in predation on different nymphal instars.

Our work is one of several recent studies attempting to identify the native natural enemies affecting *H. halys* eggs in agricultural settings. Visitors to *H. halys* sentinel egg masses in

agricultural settings in Michigan and New Jersey were identified using closed circuit video cameras (Poley et al. *unpublished*) and included members of Acrididae, Anthocoridae, Araneae, Coccinellidae, Forficulidae, Gryllidae, Miridae, Parasitica, Pentatomidae, and Tettigoniidae. Many of these also fed on *H. halys* eggs in the present study. Our findings confirm the findings of earlier Petri dish assays in which Tettigoniidae were among the predator taxa feeding most frequently on *H. halys* eggs (Morrison et al., 2016). Abram et al. (2014) also tested the acceptance of *H. halys* eggs by several endemic generalist predators and, as in the current study, *C. maculata* proved to be a poor predator of *H. halys* eggs, with adults accepting them in less than seven percent of trials and consuming few of them (< 0.1 egg per predator in 24 h).

Many studies attempting to identify or quantify predation on phytophagous Pentatomidae have focused on the egg stage (e.g. Ogburn et al., 2016; Yeargan, 1979) but the present results broaden our understanding of *H. halys* natural enemies by identifying predation on nymphal stages. In the present study, three taxa were confirmed as predators of nymphs (*P. maculiventris*, *Nabis* spp., and Reduviidae). Due to the observed preference of hemipteran predators for the nymph stage of *H. halys*, it is possible these 'sucking' predators may attack the nymphs of other members of Pentatomidae. Our findings suggest that sentinel egg mass studies alone underestimate the role of predation in the population dynamics of *H. halys*, as well as other pentatomid pests.

Orthopteran omnivores readily attacked the eggs of *H. halys* but, unlike other chewing predators, consumed the entire egg mass leaving no trace of their activity (Morrison et al., 2016). Studies using sentinel egg masses to quantify predation may underestimate predation by tallying eggs consumed by orthopterans as lost or missing. Missing egg masses accounted for 9.7-12.8% of sentinel eggs in the survey by Ogburn et al. (2016) and 37% of egg predation observed in peach orchards was consistent with feeding by Orthoptera (Morrison et al. 2016). In Southeast Asia, Orthoptera regularly consume the eggs of many rice pests and are considered effective natural enemies in these cropping systems (Chitra et al., 2002). Orthopteran omnivores are

abundant in many cropping systems affected by *H. halys*, but it is unclear whether the consumption of *H. halys* eggs is supplemental, coincidental or actually preferred over plant matter.

Variation between the predator complex attacking *H. halys* and other native or less recently invasive Pentatomidae may be partially explained by the 'enemy release hypothesis' (ERH). According to the predictions of the ERH, *H. halys* arrived in the U.S. without its coevolved natural enemies and is outcompeting native species as a result. Although the mechanism for the invasive success of *H. halys* is unclear, native predators may be maladapted to specific behavioral or chemical defenses of *H. halys*, allowing it to thrive in its invaded range (Keane and Crawley, 2002). Although Pentatomidae employ defensive secretions to avoid predation, the chemical composition of these may vary widely among local and invasive species. Invasive plant species often produce novel defensive compounds not found in native species and, as a result, experience less herbivory than non-invasive species (Cappuccino and Arnason, 2006; Cappuccino and Carpenter, 2005). Similarly, invasive *Harmonia axyridis* eggs are chemically defended from predation while eggs of native species of coccinellids are more prone to predation and cannibalism (Cottrell, 2004). However, the primary defensive compound emitted by *H. halys*, (E)-2-decenal, is a component of the secretions of at least five species of New World Pentatomidae including *N. viridula* (Borges and Aldrich, 1992; Harris et al., 2015). Although this compound is common throughout Pentatomidae, further study might determine if the secretions of *H. halys* and indigenous pentatomids vary in minor components. Direct comparisons of defensive secretions and other anti-predator behaviors between exotic and native Pentatomidae are needed to test the predictions of the ERH and to clarify the role of predators in the success of *H. halys* in its invaded range.

Although the ecological root of this phenomenon remains unclear, *H. halys* is outcompeting and displacing native pentatomids in agroecosystems across the Eastern U.S.. Evidence in ornamental crops and soybean suggests that *H. halys* has become the most abundant

pentatomid species in these systems, comprising over 50% of all Pentatomidae in many locations (Bakken et al., 2015; Nielsen and Hamilton, 2009a). Although the current utilization of *H. halys* by natural enemies is relatively low, the high relative abundance of this pest may change local predator preferences and adaptations over time (Carlsson et al., 2009; Carlsson and Strayer, 2009). High *H. halys* abundance in the absence of competitors could create strong selection pressure for predators able to consume this abundant, but underutilized, food resource. The enhanced fitness of individuals that successfully utilize *H. halys* as prey may drive dietary shifts toward more *H. halys* consumption (Carlsson et al., 2009; Elliott, 2004; Jaworski et al., 2013). Continuing surveys of natural enemy impacts on *H. halys* could reveal dietary shifts and how these affect overall pest mortality and abundance.

The absence of a single predator capable of competently attacking all life stages of *H. halys* implies that biological control of this pest will only be provided by a community of predators, not by an individual species. However, the lack of any highly effective single natural enemy will affect the potential roles of biological control in pest management. For example, augmentative releases or chemical attractants for a single predator species are unlikely to aid in the suppression *H. halys* populations in agricultural settings. However, the predator community may be enhanced through cultural tactics such as conservation biological control, use of trap crops, or intercropping with predator-attracting crops (Blaauw and Isaacs, 2012; Nielsen et al., 2016; Soergel et al., 2015). Additionally, the responsible usage of chemical control methods, such as border-only spray programs, may further protect natural enemies in the crop interior while simultaneously suppressing *H. halys* at the crop edge where they are most abundant (Blaauw et al., 2015).

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References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Acebes-Doria, A., Leskey, T.C., Bergh, J., 2016. Injury to apples and peaches at harvest from feeding by *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs early and late in the season. Crop Prot. 89, 58–65.

Aldrich, J.R., 1988. Chemical ecology of the Heteroptera. Annu. Rev. Entomol. 33, 211–238. doi:10.1146/annurev.ento.33.1.211

Baez, I., Reitz, S.R., Funderburk, J.E., 2004. Predation by *Orius insidiosus* (Heteroptera: Anthocoridae) on life stages and species of *Frankliniella* flower thrips (Thysanoptera: Thripidae) in pepper flowers. Environ. Entomol. 33, 662–670.

Bakken, A.J., Schoof, S.C., Bickerton, M., Kamminga, K.L., Jenrette, J.C., Malone, S., Abney, M.A., Herbert, D.A., Reisig, D., Kuhar, T.P., Walgenbach, J.F., 2015. Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. Environ. Entomol. 44, 1011–1021. doi:10.1093/ee/nvv092

Basnet, S., Maxey, L.M., Laub, C.A., Kuhar, T.P., Pfeiffer, D.G., 2014. Stink bugs (Hemiptera: Pentatomidae) in primocane-bearing raspberries in Southwestern Virginia. J. Entomol. Sci. 49, 304–312.

Blaauw, B.R., Isaacs, R., 2012. Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density. Ecol. Entomol. 37, 386–394. doi:10.1111/j.1365-2311.2012.01376.x

Blaauw, B.R., Polk, D., Nielsen, A.L., 2015. IPM-CPR for peaches: Incorporating behaviorally-based methods to manage *Halyomorpha halys* and key pests in peach. Pest Manag. Sci. 71, 1513–22. doi:10.1002/ps.3955

Blackwood, J.S., Schausberger, P., Croft, B.A., 2001. Prey-Stage preference in generalist and specialist Phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. Environ. Entomol. 30, 1103–1111. doi:10.1603/0046-225X-30.6.1103

Borges, M., Aldrich, J.R., 1992. Instar-specific defensive secretions of stink bugs (Heteroptera: Pentatomidae). Experientia 48, 893–896. doi:10.1007/BF02118429

Cappuccino, N., Arnason, J.T., 2006. Novel chemistry of invasive exotic plants. Biol. Lett. 2, 189–193. doi:10.1098/rsbl.2005.0433

Cappuccino, N., Carpenter, D., 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. Biol. Lett. 1, 435–438. doi:10.1098/rsbl.2005.0341

Carlsson, N.O., Sarnelle, O., Strayer, D.L., 2009. Native predators and exotic prey - An acquired taste? Front. Ecol. Environ. 7, 525–532.

Carlsson, N.O.L., Strayer, D.L., 2009. Intraspecific variation in the consumption of exotic prey - A mechanism that increases biotic resistance against invasive species? Freshw. Biol. 54, 2315–2319. doi:10.1111/j.1365-2427.2009.02263.x

Chitra, N., Gunathilagaraj, K., Soundararajan, R.P., 2002. Prey preference of Orthopteran predators on rice insect pests. J. Biol. Control 16, 109–111. doi:10.18311/JBC/16/2/4117

Cornelius, M.L., Dieckhoff, C., Hoelmer, K.A., Olsen, R.T., Weber, D.C., Herlihy, M. V., Talamas, E.J., Vinyard, B.T., Greenstone, M.H., 2016. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control 103, 11–20. doi:10.1016/j.biocontrol.2016.07.011

Cottrell, T.E., 2004. Suitability of exotic and native lady beetle eggs (Coleoptera: Coccinellidae) for development of lady beetle larvae. Biol. Control 31, 362–371. doi:10.1016/j.biocontrol.2004.06.004

Elliott, J.M., 2004. Prey switching in four species of carnivorous stoneflies. Freshw. Biol. 49, 709–720. doi:10.1111/j.1365-2427.2004.01222.x

Harris, C., Abubeker, S., Yu, M., Leskey, T., Zhang, A., 2015. Semiochemical production and laboratory behavior response of the brown marmorated stink bug, *Halyomorpha halys*. PLoS One 10. doi:10.1371/journal.pone.0140876

Hassell, M.P., May, R.M., 1986. Generalist and specialist natural enemies in insect predator-prey interactions. J. Anim. Ecol. 55, 923–940. doi:10.2307/4425

Hoebeke, E.R., Carter, M.E., 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105, 225–237.

Jaworski, C.C., Bompard, A., Genies, L., Amiens-Desneux, E., Desneux, N., 2013. Preference and prey switching in a generalist predator attacking local and invasive alien pests. PLoS One 8, 1–10. doi:10.1371/journal.pone.0082231

Jones, A.L., Jennings, D.E., Hooks, C.R.R., Shrewsbury, P.M., 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control 78, 61–66. doi:10.1016/j.biocontrol.2014.07.011

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

Koppel, A.L., Herbert, D.A., Kuhar, T.P., Kamminga, A.K., 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in Southeast Virginia. Environ. Entomol 38, 375–379. doi:10.1603/022.038.0209

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp, M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K. a., Lee, D.-H., Wright, S.E., 2012a. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Leskey, T.C., Short, B.D., Butler, B.R., Wright, S.E., 2012b. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche (Stuttg). 2012, 1–14. doi:10.1155/2012/535062

Lind, J., Cresswell, W., 2005. Determining the fitness consequences of antipredation behavior. Behav. Ecol. 16, 945–956.

Maistrello, L., Dioli, P., Bariselli, M., Mazzoli, G.L., Giacalone-Forini, I., 2016. Citizen science and early detection of invasive species: phenology of first occurrences of *Halyomorpha halys* in Southern Europe. Biol. Invasions 18, 3109–3116. doi:10.1007/s10530-016-1217-z

Morrison, W.R., Mathews, C.R., Leskey, T.C., 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97, 120–130. doi:10.1016/j.biocontrol.2016.03.008

Nelson, E.H., 2007. Predator avoidance behavior in the pea aphid: Costs, frequency, and population consequences. Oecologia 151, 22–32. doi:10.1007/s00442-006-0573-2

Nielsen, A.L., Dively, G., Pote, J.M., Zinati, G., Mathews, C., 2016. Identifying a potential trap crop for a novel insect pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in organic farms. Environ. Entomol. 45, 472–478. doi:10.1093/ee/nvw006

Nielsen, A.L., Hamilton, G., 2009a. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 108, 608–616.

Nielsen, A.L., Hamilton, G.C., 2009b. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102, 1133–1140. doi:10.1603/029.102.0335

Nielsen, A.L., Hamilton, G.C., Shearer, P.W., 2011. Seasonal phenology and monitoring of the non-native *Halyomorpha halys* (Hemiptera: Pentatomidae) in soybean. Environ. Entomol. 40, 231–238. doi:10.1603/EN10187

Oedekoven, M.A., Joern, A., 1998. Stage-based mortality of grassland grasshoppers (Acrididae) from wandering spider (Lycosidae) predation. Acta Oecologica 19, 507–515. doi:10.1016/S1146-609X(99)80005-8

Ogburn, E.C., Bessin, R., Dieckhoff, C., Dobson, R., Grieshop, M., Hoelmer, K.A., Mathews, C., Moore, J., Nielsen, A.L., Poley, K., Pote, J.M., Rogers, M., Welty, C., Walgenbach, J.F., 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control 101, 39–51. doi:10.1016/j.biocontrol.2016.06.002

Olberg, R.M., Worthington, A.H., Venator, K.R., 2000. Prey pursuit and interception in dragonflies. J. Comp. Physiol. 186, 155–162. doi:10.1007/s003590050015

Pareja, M., Borges, M., Laumann, R.A., Moraes, M.C.B., 2007. Inter- and intraspecific variation in defensive compounds produced by five neotropical stink bug species (Hemiptera:

Pentatomidae). J. Insect Physiol. 53, 639–648. doi:10.1016/j.jinsphys.2007.04.004

Persons, M.H., Walker, S.E., Rypstra, A.L., Marshall, S.D., 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: Lycosidae). Anim. Behav. 61, 43–51. doi:10.1006/anbe.2000.1594

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by generalist predators. Biol. Control. 114, 1-7.

Ragsdale, D.W., Larson, A.D., Newsom, L.D., 1981. Quantitative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELISA. Environ. Entomol. 10, 402–405. doi:10.1093/ee/10.3.402

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K., Hooks, C.R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A.L., Pfeiffer, D.G., Raupp, M.J., Rodriguez-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P.D., Whalen, J., Wiman, N.G., Leskey, T.C., Tooker, J.F., 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5, 1–13. doi:10.1603/IPM14002

Rice, K.B., Troyer, R.R., Watrous, K.M., Tooker, J.F., Fleischer, S.J., 2016. Landscape factors influencing stink bug injury in mid-Atlantic tomato fields. J. Econ. Entomol. 110, 94–100. doi:https://doi.org/10.1093/jee/tow252

Sih, A., 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67, 434–441. doi:10.2307/1938587

Soergel, D.C., Ostiguy, N., Fleischer, S.J., Troyer, R.R., Rajotte, E.G., Krawczyk, G., 2015. Sunflower as a potential trap crop of *Halyomorpha halys* (Hemiptera: Pentatomidae) in pepper fields. Environ. Entomol. 44, 1581–1589. doi:10.1093/ee/nvv136

Stiling, P., Cornelissen, T., 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biol. Control 34, 236–246. doi:10.1016/j.biocontrol.2005.02.017

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561–94. doi:10.1146/annurev.ento.47.091201.145240

Vet, L.E.M., Dicke, M., 1992. Ecology of info chemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37, 141–72. doi:10.1146/annurev.ento.37.1.141

Yeargan, K. V., 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8, 715–719.

Zink, A.G., Rosenheim, J.A., 2007. Stage-specific predation on *Lygus hesperus* affects its population stage structure. Entomol. Exp. Appl. 24, 896–900. doi:10.1111/j.1570-7458.2007.00634.x

Zink, A.G., Rosenheim, J.A., 2005. Stage-dependent feeding behavior by western tarnished plant

bugs influences flower bud abscission in cotton. Entomol. Exp. Appl. 117, 235–242. doi:10.1111/j.1570-7458.2005.00356.x

Tables and Figures

Table 1. Identity and life stages of taxa assessed for predation on *H. halys*.

Predator Taxa	**Life Stage Tested**
Acrididae	Adults
Coccinella septempunctata	Adults
Coleomegilla maculata	Adults
Geocoris spp.	Adults
Harmonia axyridis	Adults
Hippodamia convergens	Adults
Nabis spp.	Adults
Podisus maculiventris	Adults and Late Instar Nymphs
Reduviidae	Mixed [1]
Tettigoniidae	Adults

[1]Reduviidae consisted of a combination of *Sinea sinipes* adults and *Arillus cristatus* nymphs.

Table 2. Mean (± SEM) effects of predators on *H. halys* egg hatch. *P* values were obtained from Welch two-sample t-tests; *P*-values in bold face were significant ($P < 0.05$).

Predator (*n*)	**Treatment Hatch**	**Control Hatch**	***P***
Acrididae (8)	0.549 (±0.17)	0.957 (±0.02)	**0.048**
Coccinella septempunctata (8)	0.886 (±0.03)	0.978 (±0.01)	**0.029**
Coleomegilla maculata (8)	0.867 (±0.07)	0.772 (±0.11)	0.480
Geocoris spp. (8)	0.572 (±0.14)	0.472 (±0.15)	0.633
Harmonia axyridis (8)	0.858 (±0.12)	0.764 (±0.10)	0.566
Hippodamia convergens (8)	0.655 (±0.04)	0.797 (±0.08)	0.152
Nabis spp. (8)	0.750 (±0.10)	0.749 (±0.09)	0.994
Podisus maculiventris adult (8)	0.581 (±0.12)	0.879 (±0.05)	**0.044**
Podisus maculiventris nymph (8)	0.699 (±0.07)	0.917 (±0.03)	**0.021**
Reduviidae (8)	0.901 (±0.02)	0.875 (±0.06)	0.677
Tettigoniidae (8)	0.241 (±0.12)	0.709 (±0.12)	**0.015**

Table 3. Mean (± SEM) survivorship of treatment and control *H. halys* first instar nymphs in various predator treatments. *P* values were obtained from Welch Two-Sample t-Tests; *P*-values in bold face were significant ($P < 0.05$).

Predator (n)	**Treatment Survivorship**	**Control Survivorship**	***P***
Acrididae (8)	0.934 (±0.02)	0.970 (±0.02)	0.167
Coccinella septempunctata (8)	0.861 (±0.07)	0.875 (±0.02)	0.850
Coleomegilla maculata (8)	0.559 (±0.14)	0.812 (±0.05)	0.116
Geocoris spp (8)	0.894 (±0.03)	0.956 (±0.03)	0.138
Harmonia axyridis (8)	0.932 (±0.03)	0.949 (±0.03)	0.701
Hippodamia convergens (8)	0.823 (±0.02)	0.855 (±0.03)	0.423
Nabis spp (16)	0.869 (±0.02)	0.967 (±0.01)	**0.002**
Podisus maculiventris adult (8)	0.759 (±0.10)	0.828 (±0.07)	0.579
Podisus maculiventris nymph (8)	0.816 (±0.09)	0.886 (±0.03)	0.490
Reduviidae (8)	0.389 (±0.13)	0.733 (±0.04)	**0.031**
Tettigoniidae (7)	0.980 (±0.01)	0.983 (±0.01)	0.885

Table 4. Mean (± SEM**)** survivorship of treatment and control *H. halys* second instar nymphs in various predator treatments. *P* values were obtained from Welch two-sample t-tests; *P*-values in bold face were significant ($P < 0.05$).

Predator	**Treatment Survivorship**	**Control Survivorship**	***P***
Acrididae (8)	0.819 (±0.06)	0.825 (±0.07)	0.947
Coccinella septempunctata (8)	0.725 (±0.09)	0.850 (±0.06)	0.283
Coleomegilla maculata (7)	0.800 (±0.08)	0.914 (±0.06)	0.259
Geocoris spp (8)	0.588 (±0.14)	0.875 (±0.05)	0.094
Harmonia axyridis (8)	0.875 (±0.08)	0.825 (±0.07)	0.634
Hippodamia convergens (8)	0.708 (±0.06)	0.775 (±0.08)	0.526
Nabis spp (8)	0.383 (±0.09)	0.866 (±0.05)	**<0.001**
Podisus maculiventris adult (8)	0.900 (±0.04)	0.900 (±0.04)	1
Podisus maculiventris nymph (8)	0.500 (±0.08)	0.900 (±0.04)	**0.001**
Reduviidae (8)	0.700 (±0.13)	0.875 (±0.05)	0.229
Tettigoniidae (8)	0.775 (±0.05)	0.825 (±0.06)	0.513

Figure 1. Diagram of Arenas Used to Study Predation of *H. halys* Eggs and Nymphs. A. Hinged unpainted lid, shown in an open position, was closed and secured with masking tape during experiments, B. Vertical face, unpainted to allow observation of interactions inside the arena, C. exterior of all other vertical faces painted with opaque matte grey spray paint (illustrated as translucent for clarity), D. bottom surface with 8 cm opening for sunflower plants, grown from a small pot below each arena, E. two 3 cm ventilation holes covered with fine mesh. Note: although the sunflower is illustrated in full bloom, actual plants used in the present study were at the R2 stage of reproductive development.

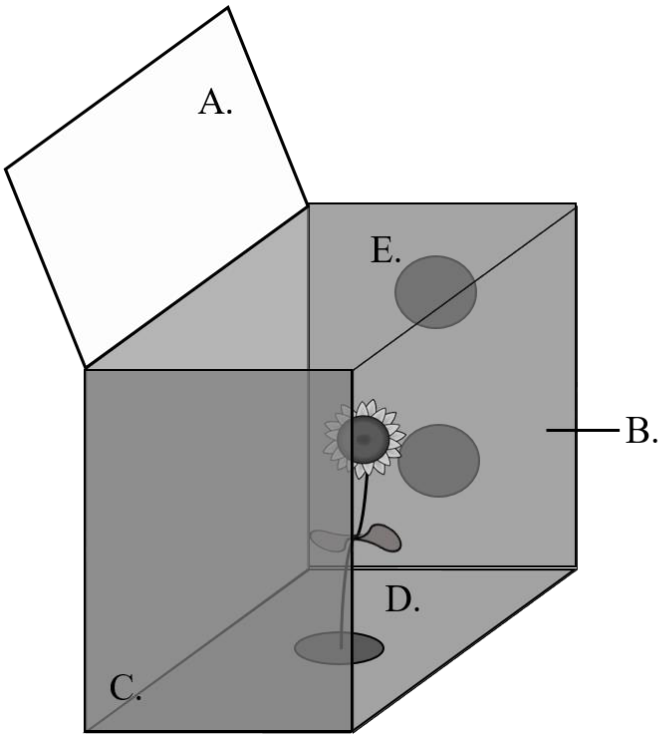
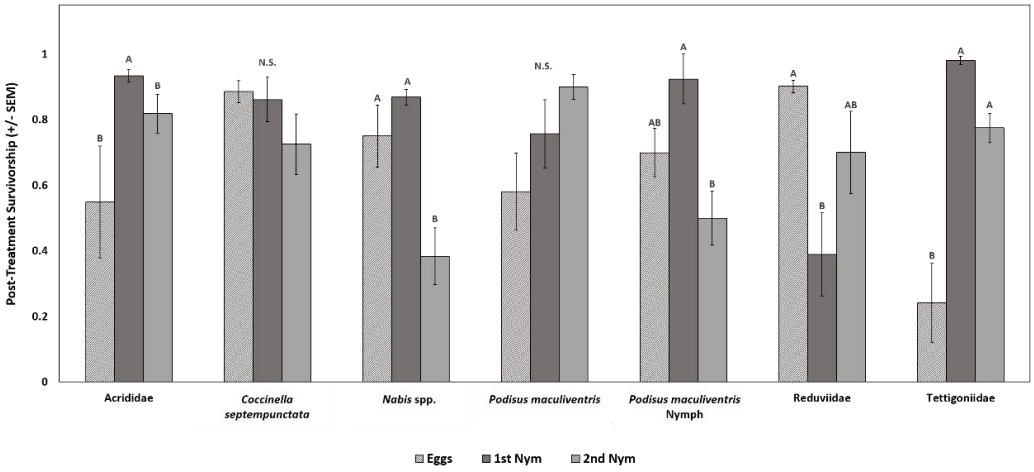


Figure 2. Survivorship of *H. halys* Life Stages after Exposure to Predator Treatments. Differential prey survivorship was analyzed for each predator separately (ANOVA). Differing letters indicate significant differences between prey treatments. "N.S." indicates no significant differences in survivorship between prey treatments. Means separation determined with Tukey's HSD post-hoc analysis ($P < 0.05$ were considered significant).

Note: Values displayed above are uncorrected values, however analysis was completed on control-corrected survivorship.



Chapter 5: The Effect of Alternate Prey on Predation of *Halyomoprha halys* (Stål) in Greenhouse Mesocosms

Abstract:

Halyomorpha halys (Stål) is a serious invasive pest of agriculture in the Northeast. *Halyomorpha halys* feeding damage is economically injurious in a multitude of crops in this region including tree fruit, vegetable crops, and row crops. Many growers in the Mid-Atlantic have abandoned long-standing IPM programs in response to *H. halys* pressure in favor of insecticide-based programs. Sustainable management options are being explored to combat this new pest including the potential impact of natural enemies. Current research indicates that native predators and parasitoids are ineffective at providing natural control of this pest in the egg stage however, little is known about the predator complex affecting the nymphal stage. The goal of this study was to quantify predation rates on *H. halys* nymphs alone and in the presence of an alternate prey, soybean aphid (*Aphis glycines*, Matsumura). Prey treatments included *H. halys* alone, *H. halys* plus *A. glycines* and *A. glycines* alone. These prey were exposed to potential predation from either *Hippodamia convergens* (Guerin-Meneville) or *Podisus maculiventris* (Say) nymphs on immature soybean (*Glycine max* L.) plants for three weeks. After the each experiment, metrics of plant growth and the abundance of prey species were assessed. Prey treatments significantly affected plant vertical growth, lateral bud development and final dry mass; but in most cases, predator treatments did not significantly reduce the negative effects of herbivory. Plant health metrics did not differ between the *A. glycines* alone and *A. glycines* plus *H. halys* prey treatments. Unexpectedly, *H. halys* survivorship was significantly higher on plants with *A. glycines* than on those without aphids.

Keywords: *Halyomorpha halys, Aphis glycines,* predation, soybean, alternate prey

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an economically significant invasive agricultural pest in the mid-Atlantic region. Native to East Asia, *H. halys* was first confirmed in the U.S. in 2001 (Hoebeke and Carter, 2003). Since its initial detection in Allentown, PA, *H. halys* has spread to over 40 states and has been detected in Canada, and Europe (Fogain and Graff, 2011; Harris, 2010; Vétek et al., 2014; Wyniger et al., 2014). *Halyomorpha halys* is highly mobile and capable of feeding on over 170 species of plants, leading to its classification as a high risk pest by the Animal and Plant Health Inspection Service, Plant Protection and Quarantine program (Holtz and Kamminga, 2010; "StopBMSB.org," 2017). Economic damage from BMSB was first recorded in 2006 and subsequent economic losses have increased drastically (Leskey et al., 2012b; Nielsen and Hamilton, 2009b). During the 2010 growing season, *H. halys* caused significant economic injury to peach, Asian pear, cherry, tomato, corn, and soybeans (Holtz and Kamminga, 2010; Leskey, 2010) and an estimated $37 million in damage to Mid-Atlantic apple production (Leskey et al., 2012a). In New Jersey, Maryland, and West Virginia, some stone fruit growers have reported up to 90% yield loss from BMSB damage (Leskey et al., 2012b). In response to high levels of *H. halys* damage, insecticide applications in some Mid-Atlantic tree fruit orchards have increased by ca. fourfold (Leskey et al., 2012b). Such an approach is disruptive to the agroecosystem and resurgences of secondary pests have occurred in multiple crops due to *H. halys* management programs (Rice et al., 2014).

According to the Enemy Release Hypothesis, *H. halys* may succeed in invaded ranges due to a lack of closely evolved natural enemies (Keane and Crawley, 2002). However, understanding the effect of predators on *H. halys* could lead to a decrease in the intensity of insecticide-based management programs and allow for the implementation of conservation biological control as a preventative management tool for this invasive pest. As a polyphagous pest species, *H. halys* provides the opportunity to study the impact of naïve natural enemies across multiple agricultural systems. The effect of predators and parasitoids on *H. halys* eggs in

U.S. agricultural systems was initially evaluated in a multi-state study using sentinel egg masses in the field and in laboratory studies. Ogburn et al. (2016) found low utilization of sentinel *H. halys* eggs by endemic Hymenopteran parasitoids but predators had significantly higher impact. In New Jersey, predators were responsible for 4-9% of egg mortality compared to < 1% mortality from parasitoids (Ogburn et al., 2016). In New Jersey, more sentinel egg masses were preyed upon in soybean than any other crop tested. Based on the results of laboratory no-choice predation bioassays, generalist predators in the families Acrididae, Carabidae, Forficulidae, Gryllidae, Pentatomidae, Salticidae, and Tettigoniidae will consume *H. halys* eggs (Abram et al., 2014; Morrison et al., 2016, Pote and Nielsen, 2017). Generalist predators in the families Nabidae, Reduviidae, and Pentatomidae caused significant reduction in survivorship of *H. halys* 1st and 2nd instar nymphs in laboratory predation assays (Pote and Nielsen 2017).

Field crops like soybean represent an abundant food resource for *H. halys* prior to overwintering (Venugopal et al., 2015). In the U.S., *H. halys* was first described in soybean in 2006 and has subsequently become the dominant species of Pentatomidae found in soybean across several Mid-Atlantic states (Nielsen et al., 2011; Nielsen and Hamilton, 2009a; Owens et al., 2013). Stink bug feeding damage on soybean is caused by the insertion of the piercing sucking mouthparts into pod or seed tissues and by the release of digestive enzymes which destroys cells and dissolves proteins (McPherson and McPherson, 2000). Owens et al. (2013) found that intense *H. halys* feeding damage can delay soybean maturation, decrease final seed weight, and can destroy developing pods which is consistent with feeding by endemic stink bugs (McPherson and McPherson 2000). *Halyomorpha halys* feeding can also lead to a “stay green” effect wherein soybean plant senescence is delayed in patches which have experienced high feeding damage by *H. halys*, particularly along the edge (Leskey et al., 2012a; Venugopal et al., 2014). Inconsistent maturation can lead to delayed harvest times and lost yield (Venugopal et al., 2014), but little is known about the effect of predation and parasitism on *H. halys* populations and subsequent plant damage in this cropping system.

Generalist natural enemies are abundant in soybean (Costamagna and Landis, 2006; Rutledge et al., 2004). Multiple studies have shown how these organisms, specifically at the landscape scale, can provide natural enemy services in the form of herbivory suppression (Gardiner et al., 2009; Landis et al., 2000; Rutledge et al., 2004; Tonhasca, 1993). *Aphis glycines* (L.) (Hemiptera: Aphididae), an invasive aphid pest of soybeans, is widely consumed by generalist predators in soybean systems (Rutledge et al., 2004). These predators include aphidophagous Coccinellids like *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae) which have substantially increased in abundance since the invasion of *A. glycines* and are now considered effective natural enemies of this pest (Ragsdale et al., 2011). Generalist natural enemies have been shown to decrease aphid populations by 95% in some cases and can cause a detectable trophic cascade, doubling plant biomass and yield compared to those grown in predator exclusion cages (Costamagna et al., 2008, 2007; Fox et al., 2004; Ragsdale et al., 2011; Rutledge et al., 2004).

Endemic predators are less successful at controlling stink bug pests in soybean (Yeargan, 1979). One study found that predation accounted for <20% of the egg mortality of *Euschistus servus* (Say) (Hemiptera: Pentatomidae) (Stam et al., 1987). *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), a predator of *H. halys* eggs and nymphs (Morrison et al. 2016, Pote and Nielsen 2017), is relatively common in soybeans (O'Neil, 1988) although the impact of *P. maculiventris* and other native predators on *H. halys* populations and resultant crop damage in soy has not been assessed. For many native stink bugs in soybean, parasitoids are the primary agent of top-down suppression, such as *Telenomus podisi* (Ashmead) (Hymenoptera: Platygastridae), a parasitoid used as an augmentative biological control agent for control of *Euschistus servus* (Say) (Moraes et al., 2005, 2009). In soybeans, *H. halys* sentinel egg masses are successfully parasitized very infrequently, but experience relatively high predation, indicating that the latter may be a more important source of *H. halys* suppression (Ogburn et al., 2016). Given the shared native range of *H. halys* and *A. glycines* (Blackman and Eastop, 2014; Xu et al.,

2014), their interactions within soybean ecosystems could have important implications for the ecology and management of these pests.

Aphis glycines, like *H. halys,* is an invasive pest of soybean native to eastern Asia (Blackman and Eastop, 2014). *A. glycines* was first recorded in the U.S. in 2000 (Alleman et al., 2002), but by the end of that year *A. glycines* had been detected in 10 states in the Upper Midwest (Venette and Ragsdale, 2004). Although *A. glycines* is believed to have existed within the U.S. prior to 2000, within three years of its first official detection, it had spread to 30 U.S. states and three Canadian provinces (Venette and Ragsdale, 2004). Winged morphs produced in the Spring and Fall allow *A. glycines* populations to cycle between buckthorn (*Rhamnus spp.*), their preferred overwintering and early spring host, and soybean, their summer or secondary host, (Ragsdale et al., 2004). Winged morphs may also be produced during the summer months in response to high population densities. Winged morphs allow *A. glycines* to invade soybean fields in geographic regions distant from their overwintering range, threatening soybean production across central North America (Ragsdale et al., 2004). Although they share a common host plant and an evolutionarily ancestral range, potential natural enemy- or host plant-mediated interactions between *H. halys* and *A. glycines* have not been well studied.

Our research has shown that multiple generalist predator species will attack *H. halys* nymphs in laboratory microcosm experiments (Pote and Nielsen 2017). However, this research has not been replicated in more natural settings or in the presence of alternate prey items. Many of the predators known to affect *H. halys* in laboratory settings are relatively abundant in soybean agroecosystems (Pote, *unpublished*, O'Neil, 1988) but it is unclear if impact of this predation is sufficient to protect plant health and development. Given the shared ecological traits of *H. halys* and *A. glycines*, the trophic interactions between these pests and potential predators should be more clearly understood. Thus, the objectives of this research were to 1) quantify realized predation rates on *H. halys* in greenhouse soybean mesocosms, 2) quantify realized predation

rates on *H. halys* in the presence of alternate prey (*A. glycines*), and 3) determine the effect of predator and prey presence on the growth of soybean plants within greenhouse mesocosms.

Methods

General. This research was conducted from November 2016 to May 2017 in greenhouses at the Rutgers Agriculture Research and Extension Center (RAREC) in Bridgeton, NJ. Mesh cages (Bugdorm-2120, MegaView Science, Taiwan) served as mesocosms in this experiment, each containing six soybean plants. This experiment was conducted as a 3 x 3 randomized complete block bioassay (three predator treatments, three herbivore treatments). Treatments were randomly assigned within spatial blocks, with three fully replicated spatial blocks per experiment (temporal blocks), over three experiments for a total of nine replicates per treatment. Experiments started on 5 December, 2016, 7 February, 2017, and 11 April, 2017 and each lasted 21 days. Herbivore treatments included the following: 1) eight 2nd instar *H. halys* nymphs, 2) eight 2nd instar *H. halys* nymphs plus 75 *A. glycines* or 3) no herbivores (negative control). These treatments were selected to simulate herbivore abundance at high density, such as that found in untreated organic soybean. The predator treatments consisted of 1) two 3rd-4th instar *P. maculiventris* 2) two adult *Hippodamia convergens* (Guerin-Meneville) (Coleoptera: Coccinellidae) or 3) no predator control. During the third temporal block, an additional aphid only treatment was added but, due to space constraints, this treatment was only studied in the absence of predators.

Plants. Soybeans (brand: 34A7, variety: 76347; Blue River Hybrids, Ames, IA, USA) were grown from seeds in 50 well trays in the greenhouses at RAREC until the V2 stage of soybean development (Pedersen and Lauer, 2004). Soybean seedlings were transplanted, three plants per pot, into larger 500 mL pots. Plants were fertilized with Miracle-Gro All Purpose Plant Food (NPK: 24-8-16; Miracle-Gro, Marysville, OH, USA) according to manufacturer specifications immediately after being transplanted and again 7 days thereafter. Pots were

randomly assigned to cages, two per cage, and treatments were randomly assigned to cages within each block. During experiments, fertilizer treatment was suspended and only soybeans were grown in the room of the greenhouse where experiments were conducted. Plants were lightly watered every 1-2 days. After watering, the contents of each cage were lightly misted via hose-end sprayer through mesh side panels to provide additional moisture for the insects within.

Insects. Aphids were kept in colony in the greenhouse. The colonized individuals were sustained on trays of young soybean seedlings. As plant health within the colony declined and more winged morphs developed, stems of plants with aphids were cut at soil-level and transferred to new trays of seedlings (Kaser, J. *personal comm.*). Predators used in this study were acquired from commercial biological control suppliers (*H. convergens:* Arbico Organics, Oro Valley, AZ, USA; *P. maculiventris:* Natural Insect Control, Stevensville, ON, Canada). Predators were kept in colony for at least one generation prior to use in experiments to ensure consistent nutritional status. *Podisus maculiventris* was reared on *Galleria mellonella* (L.) (O: Lepidoptera, F: Noctuidae) and 10% honey-water solution while *H. convergens* was sustained on *A. glycines* from colony. *Halyomorpha halys* were acquired from a colony kept at RAREC founded originally with individuals from the New Jersey Department of Agriculture's Philip J. Alampi Beneficial Insects Laboratory.

Sampling Procedure. During each temporal block, metrics of plant growth were assessed prior to prey introduction and twice per week after that. These metrics included a count of the number of trifoliate vertical nodes (omitting the cotyledon and auxiliary bud), and a count of the number of lateral trifoliate buds (those not arising from vertical growth) (Pedersen and Lauer, 2004). Buds at the vertical growing point were considered a single node unless the leaflets of the older node had begun to unfold and were no longer touching the leading bud. Lateral buds were only counted when the leaflets of the developing bud had unfolded enough that they no longer touched one another.

Experiments. Once all transplanted soybean plants had reached at least the V3 stage, an initial sample of plant growth metrics was assessed (mean vertical nodes during initial samples: 3.4 ± 0.16). Next, *A. glycines* were collected from the colony by removing whole soybean leaves, and aphid abundance was counted under magnification with a compound dissecting scope (Stemi 2000, Zeiss, Oberkochen, Germany). Aphids were gently removed from leaves with a soft tipped paint brush until the total abundance was 75 aphids distributed between two leaves. Preliminary experiments indicated inoculating one plant in each pot with aphids led to a more even distribution of aphid density. Total aphid load (75) was therefore distributed between two leaves so plants in each pot could be inoculated simultaneously. Due to high transfer mortality, *A. glycines* were given three days to colonize the soybean plants prior to the introduction of other organisms.

Three days after the introduction of aphids, plant growth metrics were assessed again. Eight 2nd instar *H. halys* were then introduced onto the plants with forceps for herbivore treatments 1 and 2. After 30 minutes, predators were introduced into the appropriate cages. During the course of 21 d experiments, predator and prey abundance was visually monitored and plant health metrics were assessed twice per week. Abundances of *H. halys* and the predators were counted directly, but aphid abundance was estimated by counting the approximate number of 25-aphid groups per cage. Dead *H. halys* and predators were removed from the cages at this time. Those in predator treatments may have been killed by predation, and those in no predator control treatments served as checks of environmental mortality. To ensure constant predation pressure, predator abundance was kept constant by replacing dead predators found during the biweekly assessments. *Podisus maculiventris* nymphs that had molted to adulthood were replaced with appropriate instar nymphs from the colony.

Vertical and lateral buds were often heavily damaged by herbivory. Vertical plant nodes were counted even if both buds at a node had withered or broken off entirely, but side buds were not counted if they appeared dead. Lateral buds were considered dead if their leaves were no

longer green and were dry and brittle to the touch. After three weeks, a final assessment of insect abundance and plant growth was conducted. Live predators were not reused between experiments. Any plants that died during the course of the 21 d experiments were cut at the base of the stem, removed from cages and discarded.

Plant Biomass. After final assessments were completed, full plant dry biomass (g) was measured. Soil was removed from roots and plants were transferred to paper bags and stored at -20º C. After at least 24 h in the freezer, plants were desiccated in a 55º C drying oven for 24 h (Jensen and Newsom, 1972) and weighed to the nearest 0.01g (OHAUS Scout Pro, OHAUS Corporation, Parsippany, NJ).

Statistical Analysis. All statistical analysis was conducted in R Studio v3.2.2 "fire safety" (R Development Core Team, 2011). Final plant health metrics and prey abundance counts were checked for assumptions of normality and analyzed for differences between treatments. Due to the possibility of plant death, plant growth metrics were calculated and analyzed per plant. In addition to the number of vertical nodes per plant (NPP), the change in vertical nodes per plant (ΔNPP) was calculated and analyzed for differences between treatments (final NPP – initial NPP). Plants did not have lateral buds at the onset of the experiments, so these data were analyzed only as lateral buds per plant (LBPP). Vertical growth metrics (NPP and ΔNPP) and plant biomass data conformed to assumptions of normality and were analyzed as a 3x3 two-way ANOVA (model: variable = predator × prey treatment + spatial block + temporal block + ε). Prey treatments which did not include stink bugs were omitted from the analysis of stink bug abundance, as was the case for aphids.

Data from the aphid only-no predator treatment, which was only included in the final temporal block, were analyzed separately. To do this, we excluded treatments with predators (since the aphid alone treatment was not tested in the presence of predators) and then analyzed plant metrics from the final temporal block for differences between the four prey treatments (*H. halys* alone, *A glycines* alone, *H. halys* + *A. glycines*, and no prey control). Analyses were

conducted as above but without model terms for predator treatment, which were not included in this analysis (model: variable = prey treatment + spatial block + ε). We then compared aphid abundance alone and in the presence of *H. halys*. Data that did not meet assumptions of normality were analyzed using generalized linear models with Poisson error distribution and log link function. Where appropriate, analysis of deviance tests were used to determine the statistical significance of model terms, and Tukey's HSD was used to separate significantly different means (P-values < 0.05 were considered significant).

Results

Plant Growth Characteristics. Development of vertical nodes was significantly impacted by the presence of herbivorous insects. Nodes per plant (NPP) was significantly affected by prey treatments (df = 2, 68, F = 54.17, P < 0.0001) but not predator treatments (df= 2, 68, F = 0.796, P = 0.315) (Fig. 1a). Plants in the no prey-no predator treatment had the most vertical nodes (NPP = 7.7 ± 0.17), while those in both prey-no predator treatment ended with the fewest (5.2 ± 0.11) (Fig. 1a). Plants in the no prey-control cages had the highest NPP (7.6 ± 0.2), while those exposed to both *H. halys* + *A. glycines* had the lowest NPP (5.6 ± 0.1) (Fig. 1a). The NPP of plants in the *H. halys* alone treatment was 6.8 ± 0.1. All pair-wise comparisons between prey treatments were statistically significant. The interaction between predator and prey treatments did not significantly affect NPP (df = 4, 68, F = 2.0, P = 0.10). We detected marginally significant evidence for an interaction between predator and prey treatment effects on NPP (df = 2, 68, F = 2.393, P = 0.053). Values of NPP were significantly different between temporal blocks (df = 2, 68, F = 26.50, P < 0.0001).

Change in Nodes per Plant. The growth of new vertical nodes (described by ΔNPP) was significantly affected by prey treatments (df= 2, 68, F =64.96, P < 0.0001) (Fig. 1b). Plants in the no prey control cages had higher ΔNPP (4.2 ± 0.2) than those exposed to *H. halys* alone (3.5 ± 0.2) and those exposed to both *H. halys* and *A. glycines* (2.2 ± 0.2) (Fig. 1b). All pair-wise

comparisons of ΔNPP between prey treatments were statistically significant. Differences in ΔNPP between predator treatments were not statistically significant (*df*= 2, 68, *F* = 2.17, *P* = 0.118), however, there was some evidence for an interaction between predator and prey treatments (*df* = 4, 68, *F* = 2.393, *P* = 0.0531). Values of ΔNPP varied significantly between temporal blocks (*df* = 2, 68, *F* = 127.1, *P* < 0.0001) and spatial blocks (*df* = 2, 68, *F* = 4.70, *P* = 0.0318).

Lateral Buds per Plant. Development of lateral buds (LBPP) was also significantly affected by prey treatments (*df* = 2, 76, *F* = 28.39, *P* < 0.0001) (Fig. 1c). Plants exposed to *H. halys* and *A. glycines* together produced significantly fewer SBPP (1.4 ± 0.2) than those exposed to *H. halys* alone (3.0 ± 0.5) or those in the no prey control (3.7 ± 0.5). Differences in LBPP between the *H. halys* alone treatment and the no prey control were not statistically significant. Predator treatments did not have a significant effect on LBPP (*df* = 2, 78, *F* =1.17 *P* = 0.31) (Fig. 1c). Interactions between predator and prey treatments showed a marginally significant effect on LBPP (*df* = 4, 68, *F* = 2.08, *P* = 0.079). Similarly, LBPP was affected by spatial blocking but this was only marginally significant (*df* = 2, 72, *F* = 3.57, *P* = 0.058).

Plant Biomass. Average plant biomass was significantly affected by prey treatment (*df* = 2, 68, *F* = 24.51, *P* < 0.0001) and predator treatment (*df* = 2, 68, *F* = 5.94, *P* = 0.004) (Fig. 1d). Mass of plants exposed to *H. halys* alone (1.906g ± 0.140g) was significantly lower than that in no prey control cages (2.276g ± 0.169g) (Fig. 1d). However, the mass of plants exposed to *H. halys* + *A. glycines* (1.424g ± 0.132g) was significantly lower than each of these. Averaged across all prey treatment, plants grown without predators had the lowest average mass (1.628g ± 0.164g); significantly lower than that of *H. convergens* (2.013g ± 0.162g) or *P. maculiventris* (1.967g ± 0.152g) treated plants. Predator-prey treatment interaction did not significantly affect plant mass (*df* = 4, 68, *F* = 1.62, *P* = 0.177), nor did spatial blocking (*df* = 2, 68, *F* = 1.62, *P* = 0.151). However, differences in plant mass between temporal blocks were statistically significant (*df* = 2, 68, *F* = 139.55, *P* < 0.0001).

Prey Abundance. The abundance of *H. halys* was significantly higher in the presence of *A. glycines* (df = 1, 50, F = 33.01, P < 0.0001). On average, 3.0 ± 0.4 *H. halys* nymphs survived the 21 d experiments in the presence of aphids, while only 0.5 ± 0.1 survived in treatments without *A. glycines*. More *H. halys* nymphs survived in the no predator control (2.3 ± 0.5) than those caged with *P. maculiventris* (1.0 ± 0.3) or *H. convergens* (1.7 ± 0.4), although the latter contrast was not statistically significant (Fig. 2). *Podisus maculiventris* was observed feeding on *H. halys* during the course of the experiments, but *H. convergens* was not. Effects of predator-prey treatment interaction (df = 2, 44, F = 0.388, P = 0.678) and spatial block (df = 1, 46, F = 0.973, P = 0.378) were not statistically significant.

Final aphid abundance was significantly affected by predator treatments (df = 2, 27, F = 1242.7, P < 0.0001) (Fig. 3). Significantly higher *A. glycines* abundance was observed in the no predator control (386.1 ± 65.8) than either predator treatment. Aphid abundance in cages with *H. convergens* (63.9 ± 36.1) was significantly lower than those with *P. maculiventris* (313.9 ± 46.8). Both predator species were observed feeding on *A. glycines* during the experiments. Unlike *H. halys* presence, which declined throughout experiments, *A. glycines* continued reproduction and final populations were often considerably higher than initial.

Plant Health Impacts of A. glycines. The effect of *A. glycines* without additional prey or predator was only evaluated during the final temporal block. When data from this block were separately analyzed, prey treatments significantly affected NPP (df = 3, 19, F = 32.89, P < 0.0001), ΔNPP (df = 3, 19, F = 31.7, P < 0.0001), SBPP (df = 3, 23, F = 2.83, P = 0.037) and plant mass (df = 3, 19, F = 446.5, P < 0.0001). Plants grown in the no prey control treatment had significantly higher NPP (8.8 ± 0.1) than those with *H. halys* alone (7.4 ± 0.2), or *A. glycines* alone (6.6 ± 0.2) (Fig. 4). The lowest NPP was observed on plants grown with both prey species together (5.3 ± 0.4); significantly lower than all other prey treatments. The NPP of plants grown with *H. halys* alone or *A. glycines* alone were not significantly different. Similarly, plants grown without prey had significantly higher ΔNPP (5.8 ± 0.1) than those with *H. halys* alone (4.6 ± 0.2),

A. glycines alone (3.9 ± 0.2) (Fig. 4). Plants exposed to both prey species had significantly lower ΔNPP (2.3 ± 0.4) than all other prey treatments. The ΔNPP of plants grown with *H. halys* alone or *A. glycines* alone were not significantly different. There were no significant differences in LBPP between prey treatments during pair-wise comparison (Fig. 4). Plants in the no prey control had significantly higher mass (2.852g ± 0.169 g) than those exposed to *H. halys* alone (2.487 g ± 0.140 g) (Fig. 4). Plant mass did not vary significantly between the *A. glycines* alone treatment (1.108 g ± 0.117 g) and the *H. halys* + *A. glycines* treatment (0.988 g ± 0.132 g), but each of these was significantly lower than that of the no prey control and the *H. halys* alone treatment (Fig. 4).

Discussion

To determine the realized predation of *H. halys* nymphs on soybean, we performed greenhouse mesocosm experiments using *A. glycines* as an alternate prey. The findings of this study indicate that although predators were able to significantly decrease prey populations, plant growth and development were still strongly impacted by prey treatments. Predator treatments significantly affected average plant mass, but not metrics of vertical or lateral growth. Similarly, the presence of *H. halys* significantly affected NPP, ΔNPP, and plant biomass, but our results have demonstrated that aphid presence was a more significant factor in determining final plant health. Among the treatments that included only *H. halys* as the prey item, none of the plant growth metrics evaluated indicated a top-down effect by the predator. Although plant growth metrics in treatments with *H. halys* alone were significantly lower than those in the no prey control, these metrics were consistently and significantly lower in treatments that included *A. glycines.*

The cause of low *H. halys* survivorship in no predator control treatments is currently unknown but may be due to the effect of the experimental environment. Other stink bug species are known to develop poorly on pre-pod set soybeans, possibly due to the lack of nitrogen rich reproductive structures (Stam et al., 1987) and under natural settings, *H. halys* does not colonize

soybean until the R3-R4 stage (Nielsen et al. 2011) so poor the survivorship observed could be due to a nutritional mismatch. *Halyomorpha halys* survivorship also differed between temporal blocks, which may indicate significant differences in greenhouse conditions over time. Temperature data collected within the greenhouse showed that average temperature increased slightly between each successive block, but during the final replicate temperatures exceed 33º C on three non-consecutive days. At this temperature *H. halys* generational mortality is 95% (Nielsen et al., 2008). The final abundance of *H. halys* in this final temporal block were nearly half that observed in the first temporal block.

The results of this study indicated weak interactions between the selected predator and prey treatments. Weak interactions between predator and prey treatments are likely the result of unpredicted predator preferences. *Hippodamia convergens* did not significantly affect the survivorship of *H. halys* 2nd instars in bench-top predation bioassays, but this species is a predator of *A. glycines* (Rutledge et al., 2004, Pote and Nielsen 2017). Conversely, *P. maculiventris* is a predator of *H. halys* nymphs (Pote and Nielsen 2017) but was not expected to consume *A. glycines.* These predator species were intentionally selected to help clarify how predators respond to *H. halys* in the presence of a preferred secondary prey species, and how secondary prey species are affected by the presence of predators which prefer *H. halys.* However, the lack of a strong interaction effect on plant growth metrics may be the result of unexpected predation by *P. maculiventris* on *A. glycines*. Final aphid abundance was significantly lower in the presence of *P. maculiventris* compared to no predator controls. However, the magnitude of this population reduction may have been insufficient to protect soybean plant development.

The inclusion of an aphid only-no predator treatment during the final temporal block allowed for the direct comparison of *H. halys* feeding damage to that of *A. glycines.* Metrics of vertical and lateral growth were not statistically different between the aphid only and stink bug only treatments, but these treatments did differ significantly in their impact on plant mass. The average mass of plants grown with only *H. halys* nymphs was more than double that of plants

grown with only *A. glycines*. Furthermore, plant mass was not significantly different between the aphid only prey treatment, and that which included both prey items. These findings further reinforce the conclusion that *A. glycines* feeding was significantly more damaging to plant health and development than that of *H. halys*.

The relative impacts of *A. glycines* and *H. halys* on soybean seedlings may vary widely from their impacts on mature plants with developing reproductive tissues. The rapid proliferation of *A. glycines* observed in this study may be diminished on mature plants with greater energy reserves to dedicate to defense, while relatively little stink bug feeding can dramatically affect the quantity and quality of developing soybean seedpods (Jensen and Newsom, 1972; Owens et al., 2013). Plant age may have also affected the survival of *H. halys* nymphs on soybean plants. Despite the fact that soybean is a known *H. halys* host plant (Owens et al., 2013; Venugopal et al., 2014), relatively few *H. halys* nymphs survived the course of the 21 d experiments. However, *H. halys* only infests soybean fields once bean pods or other reproductive tissues are present on plants (Nielsen et al., 2011; Venugopal et al., 2015, 2014) thus pre-reproductive *G. max*, like those used in this study, may be a sub-optimal *H. halys* host plant. Additional experimentation is recommended to clarify the effect of plant age on the predator-prey interactions discussed here.

Unexpectedly, the presence of *A. glycines* significantly increased the survivorship of *H. halys* regardless of predator treatment. Fewer nymphs survived in the *H. halys* alone-no predator treatment combination (0.77 ± 0.5) than those deployed with *A. glycines* and *P. maculiventris* (1.77 ± 0.5), a known *H. halys* predator which was repeatedly observed feeding on *H. halys* nymphs in this study. The cause of this phenomenon is currently unclear. Aphid feeding is known to alter plant defensive chemistry and photosynthetic physiology (Bell et al., 1995; Macedo et al., 2003), either of which could affect the survivorship of *H. halys* nymphs. Plants exposed to treatments including *A. glycines* were often coated in honeydew which may have been consumed by *H. halys* nymphs, extending their lifespans. Interestingly, the observed increased survivorship of *H. halys* in the presence of *A. glycines* was mitigated somewhat by the presence of *H.*

convergens, a predator of *A. glycines* (Rutledge et al., 2004). The average final abundance of *H. halys* in both prey-no predator treatments was significantly lower than that of treatments with both prey and *H. convergens*. *Hippodamia convergens* may reduce *H. halys* survivorship by reducing aphid abundance thereby reducing the magnitude of the beneficial presence of *A. glycines*, or *H. convergens* may impact stink bug survival through direct predation or sub-lethal effects. Regardless of the cause, facilitative interactions between *H. halys* and *A. glycines* could be important for the ecology and management of these pests. As the *H. halys* invasion continues into the major soybean growing regions of the U.S., understanding the relationship between *H. halys*, *A. glycines* and their shared natural enemies may become a key concern for growers and researchers.

References

Abram, P.K., Doyon, J., Brodeur, J., Gariépy, T.D., Boivin, G., 2014. Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Can. Entomol. 147, 1–5. doi:10.4039/tce.2014.41

Alleman, R., Grau, C., Hogg, D., 2002. Soybean aphid host range and virus transmission efficiency, in: Proceedings: Wisconsin Fertilizer Agline Pest Management Conference. pp. 1–3.

Bell, E., Creelman, R. a, Mullet, J.E., 1995. A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. Proc. Natl. Acad. Sci. U. S. A. 92, 8675–8679. doi:10.1073/pnas.92.19.8675

Blackman, R., Eastop, V., 2014. Aphids on the world's plants. John Wiley & Sons Ltd.

Costamagna, A., Landis, D., 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecol. Appl. 16, 1619–1628.

Costamagna, A.C., Landis, D.A., Brewer, M.J., 2008. The role of natural enemy guilds in *Aphis glycines* suppression. Biol. Control 45, 368–379. doi:10.1016/j.biocontrol.2008.01.018

Costamagna, A.C., Landis, D.A., Difonzo, C.D., 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. Ecol. Appl. 17, 441–451. doi:10.1890/06-0284

Fogain, R., Graff, S., 2011. First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. J. Entomol. Soc. Ontario 142, 45–48.

Fox, T.B., Landis, D.A., Cardoso, F.F., Difonzo, C.D., 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environ. Entomol. 33, 608–618. doi:10.1603/0046-225X-33.3.608

Gardiner, M.M., Landis, D.A., Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., Heimpel, G.E., 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecol. Appl. 19, 143–154. doi:10.1890/07-1265.1

Harris, A.C., 2010. *Halyomorpha halys* (Hemiptera: Pentatomidae) and *Protaetia brevitarsis* (Coleoptera: Scarabaeidae: Cetoniinae) intercepted in Dunedin. Weta 44, 42–44.

Hoebeke, E.R., Carter, M.E., 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105, 225–237.

Holtz, T., Kamminga, K., 2010. Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål), in the United States. USDA APHIS-PPQ.

Jensen, R.L., Newsom, L.D., 1972. Effect of stink bug-damaged soybean seeds on germination, emergence, and yield. J. Econ. Entomol. 65, 261–264. doi:10.1093/jee/65.1.261

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45, 175–201. doi:10.1146/annurev.ento.45.1.175

Leskey, T.C., 2010. 2010 Tree Fruit Damage Survey.

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P., Hooks, C.R.R., Raupp, M.J., Shrewsbury, P.M., Krawczyk, G., Shearer, P.W., Whalen, J., Koplinka-Loehr, C., Myers, E., Inkley, D., Hoelmer, K. a., Lee, D.-H., Wright, S.E., 2012a. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23, 218–226. doi:10.1564/23oct07

Leskey, T.C., Short, B.D., Butler, B.R., Wright, S.E., 2012b. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche (Stuttg). 2012, 1–14. doi:10.1155/2012/535062

Macedo, T.B., Bastos, C.S., Higley, L.G., Ostlie, K.R., Madhaven, S., 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. J. Econ. Entomol. 96, 188–193 doi: 10.1603/0022–0493(2003)096[0188:PROST. doi:10.1093/jee/96.1.188

McPherson, J., McPherson, R., 2000. Stink bugs of economic importance in America north of Mexico. CRC Press, Boca Raton.

Moraes, M., Laumann, R., Sujii, E.R., Pires, C., Borges, M., 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. Entomol. Exp. Appl. 115, 227–237. doi:10.1111/j.1570-7458.2005.00290.x

Moraes, M.C.B., Laumann, R.A., Pareja, M., Sereno, F.T.P.S., Michereff, M.F.F., Birkett, M.A., Pickett, J.A., Borges, M., 2009. Attraction of the stink bug egg parasitoid *Telenomus podisi* to defence signals from soybean activated by treatment with cis -jasmone. Entomol. Exp. Appl. 131, 178–188. doi:10.1111/j.1570-7458.2009.00836.x

Morrison, W.R., Mathews, C.R., Leskey, T.C., 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97, 120–130. doi:10.1016/j.biocontrol.2016.03.008

Nielsen, A.L., Hamilton, G., 2009a. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 108, 608–616.

Nielsen, A.L., Hamilton, G.C., 2009b. Seasonal occurrence and impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in tree fruit. J. Econ. Entomol. 102, 1133–1140. doi:10.1603/029.102.0335

Nielsen, A.L., Hamilton, G.C., Matadha, D., 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environ. Entomol. 37, 348–355. doi:10.1603/0046-225X(2008)37[348:DREALT]2.0.CO;2

Nielsen, A.L., Hamilton, G.C., Shearer, P.W., 2011. Seasonal phenology and monitoring of the non-native *Halyomorpha halys* (Hemiptera: Pentatomidae) in soybean. Environ. Entomol. 40, 231–238. doi:10.1603/EN10187

O'Neil, R.J., 1988. Predation by *Podisus maculiventris* (Say) on Mexican bean beetle, *Epilachna varivestis* Mulsant, in Indiana soybeans. Can. Entomol. 120, 161–166. doi:doi:10.4039/Ent120161-2

Ogburn, E.C., Bessin, R., Dieckhoff, C., Dobson, R., Grieshop, M., Hoelmer, K.A., Mathews, C., Moore, J., Nielsen, A.L., Poley, K., Pote, J.M., Rogers, M., Welty, C., Walgenbach, J.F., 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: A regional assessment. Biol. Control 101, 39–51. doi:10.1016/j.biocontrol.2016.06.002

Owens, D.R., Herbert, D.A., Dively, G.P., Reisig, D.D., Kuhar, T.P., 2013. Does feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) reduce soybean seed quality and yield? J. Econ. Entomol. 106, 1317–1323. doi:10.1603/EC12488

Pedersen, P., Lauer, J.G., 2004. Soybean Growth and Development in Various Management Systems and Planting Dates. Crop Sci. 44, 508. doi:10.2135/cropsci2004.5080

Pote, J. M., Nielsen, A. L. 2017. Life stage specific predation of *Halyomorpha halys* (Stål) by generalist predators. Biol. Control. 114, 1-7.

R Development Core Team, R., 2011. R: A language and environment for statistical computing. R Found. Stat. Comput., R Foundation for Statistical Computing. doi:10.1007/978-3-540-74686-7

Ragsdale, D., Landis, D., Brodeur, J., 2011. Ecology and management of the soybean aphid in North America. Annu. Rev. Entomol. 56, 375–399.

Ragsdale, D.W., Voegtlin, D.J., Neil, R.J.O., Ragsdale, D.W., Voegtlin, D.J., 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. 97, 204–208.

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K., Hooks, C.R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A.L., Pfeiffer, D.G., Raupp, M.J., Rodriguez-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P.D., Whalen, J., Wiman, N.G., Leskey, T.C., Tooker, J.F., 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5, 1–13. doi:10.1603/IPM14002

Rutledge, C.E., O'Neil, R.J., Fox, T.B., Landis, D.A., 2004. Soybean aphid predators and their use in integrated pest management. Ann. Entomol. Soc. Am. 97, 240–248.

Stam, P.A., Newsom, L.D., Lambremont, E.N., 1987. Predation and food as factors affecting survival of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in a soybean ecosystem.

Environ. Entomol. 16, 1211–1216. doi:10.1093/ee/16.6.1211

StopBMSB.org [WWW Document], 2017. . Northeast. IPM Cent. URL http://www.stopbmsb.org/where-is-bmsb/state-by-state/

Tonhasca, A., 1993. Effects of agroecosystem diversification on natural enemies of soybean herbivores. Entomol. Exp. Appl. 69, 83–90. doi:10.1111/j.1570-7458.1993.tb01731.x

Venette, R.C., Ragsdale, D.W., 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97, 219–226. doi:10.1603/0013-8746(2004)097[0219:ATIBSA]2.0.CO;2

Venugopal, P.D., Coffey, P.L., Dively, G.P., Lamp, W.O., 2014. Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. PLoS One 9, 1–10. doi:10.1371/journal.pone.0109917

Venugopal, P.D., Dively, G.P., Lamp, W.O., 2015. Spatiotemporal dynamics of the invasive Halyomorpha halys (Hemiptera: Pentatomidae) in and between adjacent corn and soybean fields. J. Econ. Entomol. 108, 2231–41. doi:10.1093/jee/tov188

Vétek, G., Papp, V., Haltrich, A., Rédei, D., 2014. First record of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes. Zootaxa 3780, 2010–2012.

Wyniger, D., History, N., Basel, M., 2014. First records of an invasive bug in Europe : *Halyomorpha halys* Stal (Heteroptera : Pentatomidae). Bull. la Soc. Entomol. Suisse 81, 1–8.

Xu, J., Fonseca, D.M., Hamilton, G.C., Hoelmer, K.A., Nielsen, A.L., 2014. Tracing the origin of US brown marmorated stink bugs, *Halyomorpha halys*. Biol. Invasions 16, 153–166. doi:10.1007/s10530-013-0510-3

Yeargan, K. V., 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields. Environ. Entomol. 8, 715–719.

Figures

Figure 1. The Effect of Predator and Prey Treatments on Plant Growth Characteristics. Treatment effects on A) final mean soybean vertical nodes (NPP), B) mean growth of new soybean vertical nodes (ΔNPP), C) final mean Soybean Lateral Buds per Plant (LBPP) Development, D) mean soybean dry biomass. Data presented includes only that from the final sample of each temporal block. Differing letters above columns indicate significant differences in treatment means, non-significant treatment effects are indicated with "n.s." (Tukey's HSD, $P > 0.05$). H.c. = *Hippodamia convergens*, P.m. = *Podisus maculiventris*, H.h. = *Halyomorpha halys*, A.g. = *Aphis glycines.*

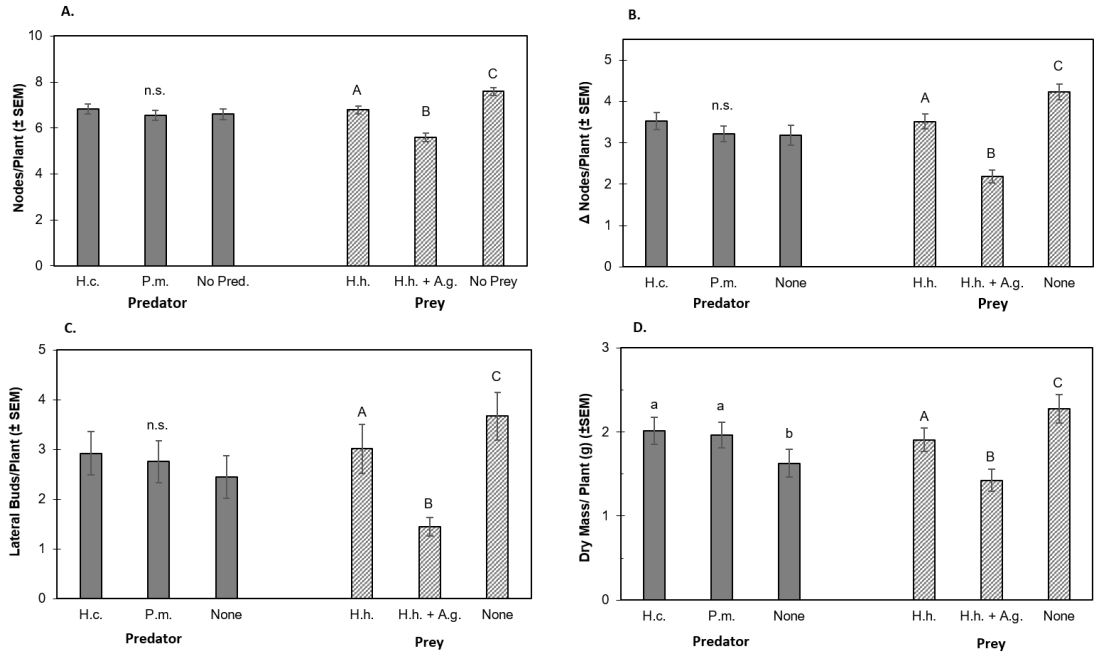


Figure 2. The Effect of Predator and Prey Treatments on Final Mean (±SEM) *H. halys* Abundance. Differing letters above columns indicate significant differences in *H. halys* abundance between predator (lower case letters) and prey (capital letters) treatment means. Tukey's HSD ($P < 0.05$) H.c. = *Hippodamia convergens*, P.m. = *Podisus maculiventris*, H.h. = *Halyomorpha halys*, A.g. = *Aphis glycines.*

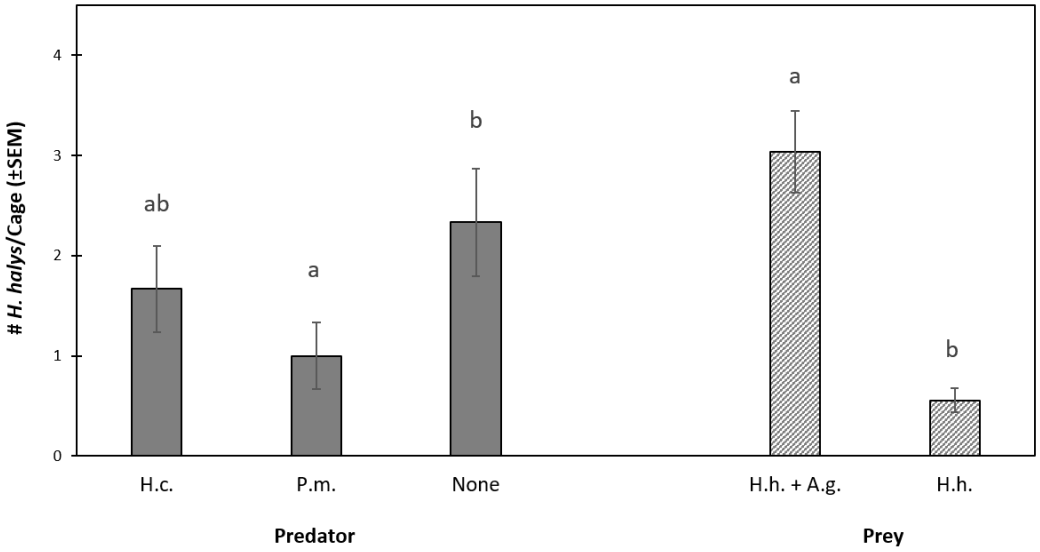


Figure 3. The Effect of Predator and Prey Treatments on Final Mean (± SEM) *A. glycines* Abundance. H.c. = *Hippodamia convergens*, P.m. = *Podisus maculiventris* Differing letters above columns indicate significant differences in *A. glycines* abundance between predator treatment means, Tukey's HSD ($P < 0.05$).

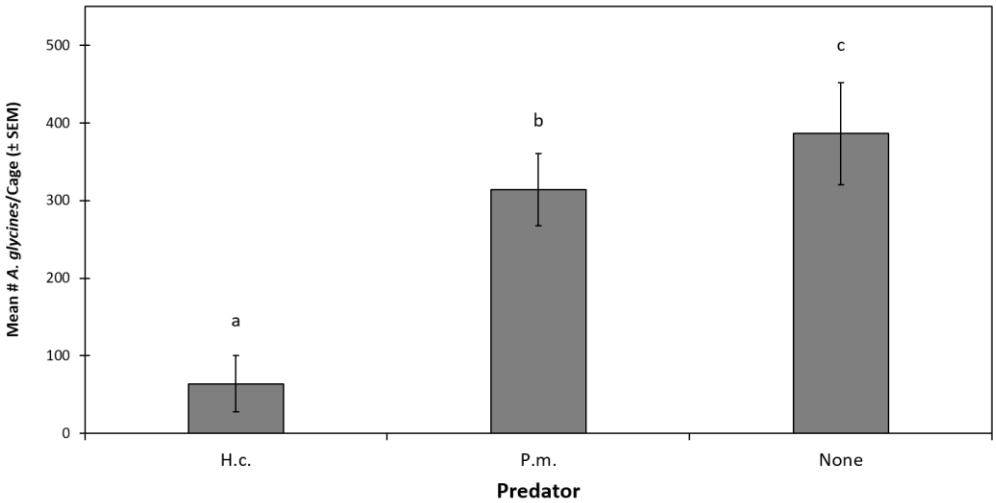
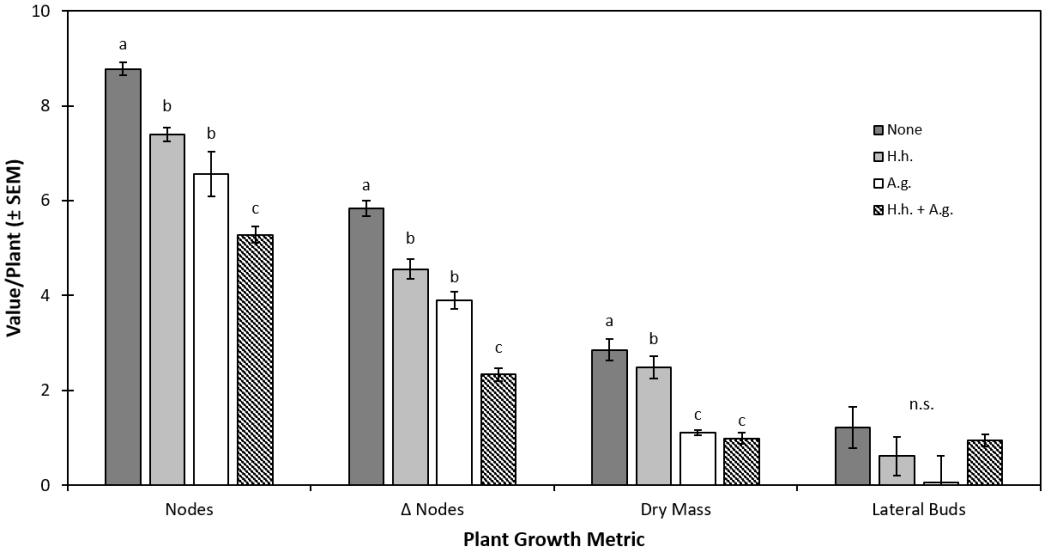


Figure 4. The Effect of Exposure to *A. glycines* on Metrics of Soybean Health. Results presented here include only that collected during third temporal block, the only one which include the *A. glycines* only treatment (white bars). H.c. = *Hippodamia convergens*, P.m. = *Podisus maculiventris*, H.h. = *Halyomorpha halys,* A.g. = *Aphis glycines.* Differing letters above columns indicate significant differences in mean between prey treatments by ANOVA, Tukey's HSD ($P < 0.05$) *Note*: Values for Dry Mass are reported in grams (g).



Chapter 6: Conclusions

The overall goal of my dissertation was to identify endemic predators which accept the invasive pest, *Halyomorpha halys* as prey and to quantify the magnitude of these predator-prey interactions. *Halyomorpha halys* is a severe pest in many fruit growing regions in the U.S. and has caused a disruption of standard IPM practices in many states. Biological control programs may help decrease the intensity of insecticide programs required to adequately manage *H. halys*, thus quantifying the effect of natural enemies on this pest is an important step in the development of manipulative or augmentative natural enemy tactics. Additionally, *H. halys* represents a valuable case study for understanding how invasive pests are affected by native predators and parasitoids which may provide valuable insights applicable to the control of future invaders.

The identification of *H. halys* predators in New Jersey was accomplished by a multi-disciplinary approach including the utilization of sentinel egg masses, molecular genetics, and controlled predator bioassays. Sentinel egg masses revealed a relatively low overall rate of natural enemy utilization on *H. halys* eggs. Successful parasitoid emergence was particularly low, but egg dissections in the second year of the study revealed a number of partially developed parasitoids which failed to successfully emerge from the sentinel eggs. These instances do represent a source of *H. halys* mortality but also of parasitoid egg mortality. The parasitoids which affect *H. halys* are considered generalists within Pentatomidae (Thompson 1946, Herting and Simmonds 1971), so these failed parasitization events may have important consequences for the population dynamics of other stink bug pests (Abram et al. 2013). This study was conducted prior to the discovery of *Trissolcus japonicus*, an effective parasitoid of *H. halys* in Asian agro-ecosystems, within the U.S. (Talamas et al. 2015). If *T. japonicus* successfully establishes throughout the American range of *H. halys*, parasitoids may become a larger source of *H. halys* mortality. Currently however, parasitoids play only a minor role in suppressing *H. halys* populations thus subsequent experiments focused primarily on predators and their potential as mortality agents.

Due to low rates of predation on *H. halys* egg masses, the central goal of Chapters 3-5 of this dissertation were to identify predators of *H. halys* immature stages and quantity their effects. The multidisciplinary approach used in these chapters identified several taxa as predators of *H. halys.* Most notably laboratory bioassays identified Nabidae and Reduviidae as predators of *H. halys* nymphs, and Tettigoniidae and Acrididae as predators of *H. halys* eggs. Molecular analysis confirmed the presence of *H. halys* DNA in a high proportion of field-collected Nabidae, Acrididae and Tettigoniidae. Common generalist natural enemies like Coccinellids and lacewings were conspicuously not among the predators which commonly attacked the immature stages of *H. halys.* This phenomenon may be due to the brief history of exposure between these predators and *H. halys,* which has only been present in the U.S. for ca. 30 years (Hoebeke and Carter 2003). However, *Harmonia axyridis,* a Coccinellid which shares a native range with *H. halys* (Koch 2003), was not identified as a key predator of *H. halys* in these studies. Thus evolutionary history alone may not be an accurate predictor of strong predator-prey associations. Alternate mechanisms may prevent common predators from consuming *H. halys* such as defensive adaptations (Aldrich 1988).

In addition to the identification and quantification of *H. halys* natural enemy mortality, this dissertation yielded several noteworthy findings. The experiment described in Chapter 2 was the first to observe Orthopteran predation on *H. halys* egg masses. Although the significance of Orthoptera as a source of *H. halys* predation remains unclear, this finding was relatively unexpected given the misconception that Orthopterans are exclusively herbivorous. The laboratory bioassays conducted in Chapter 4 also revealed a unique observation: predatory taxa which attacked *H. halys* eggs were distinct from those which attacked nymphs, and vice versa. This result has important implications for management. Attempts to implement conservation biological control programs for *H. halys* must focus on a community of predators each exerting a small pressure on one lifestage, rather than one “cure-all” predatory taxa as is the case for aphids and their Coccinellid predators (Rutledge et al. 2004). Finally, the greenhouse bioassays

conducted in Chapter 5 demonstrated that the presence of *Aphis glycines* on soybean plants increased the survivorship of *H. halys* on the same plants, regardless of the presence of predators. The mechanism for this facilitation remains unclear, but the management implications of this finding are not. The invasion of *H. halys* is currently spreading into the Upper Midwest, including those states most severely affected by *A. glycines* populations (Ragsdale et al. 2004). Positive interactions between these pests could increase the severity of pest damage throughout the primary soybean growing region of the U.S.

The results of this dissertation indicate that generalist natural enemies provide %-% mortality for *H. halys* eggs and early nymphs. Although several taxa were identified as predators of *H. halys*, these predators exist at very low densities in peach orchards and other *H. halys*-affected cropping systems. Furthermore, predator abundances may be augmented through habitat modification tactics such as conservation biological control leading to increased rates of natural enemy-mediated mortality (Landis et al. 2000). However, until such tactics are thoroughly researched, natural enemies will only represent an important source of *H. halys* mortality in special circumstances such as organic crop production.

References

Abram, P. K., T. D. Gariepy, G. Boivin, and J. Brodeur. 2013. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol. Invasions. 16: 1387–1395.

Aldrich, J. R. 1988. Chemical ecology of the Heteroptera. Annu. Rev. Entomol. 33: 211–238.

Herting, B., and F. Simmonds. 1971. A catalogue of parasites and predators of terrestrial arthropods.

Hoebeke, E. R., and M. E. Carter. 2003. *Halyomorpha halys* Stål (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Washingt. 105: 225–237.

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3: 32.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45: 175–201.

Ragsdale, D. W., D. J. Voegtlin, R. J. O. Neil, D. W. Ragsdale, and D. J. Voegtlin. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. 97: 204–208.

Rutledge, C. E., R. J. O'Neil, T. B. Fox, and D. A. Landis. 2004. Soybean aphid predators and their use in integrated pest management. Ann. Entomol. Soc. Am. 97: 240–248.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. Buffington, M.-C. Bon, and D. C. Weber. 2015. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Thompson, W. 1946. A catalogue of the parasites and predators of insect pests.