BIOLOGICAL CONTROL OF *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) USING NATIVE NATURAL ENEMIES IN MICHIGAN

By

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ABSTRACT

BIOLOGICAL CONTROL OF *HALYOMORPHA HALYS* (HEMIPTERA: PENTATOMIDAE) USING NATIVE NATURAL ENEMIES IN MICHIGAN

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The goal of this thesis was to investigate top-down pressures that exist at the beginning of the biological invasion process and to translate this into potential sources of biological control for a novel pest in Michigan's agroecosystems. *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive agricultural pest and the potential for a biological control program that utilizes existing natural enemies for *H. halys* is currently unknown. I deployed sentinel egg masses under video surveillance to determine a baseline frequency of natural enemy activity. Six predators were observed attacking eggs. Forficulidae attacked the highest number of eggs, followed by Gryllidae, then Miridae. Parasitoids were less prevalent than predators, occurring only in the first year. We found the baseline frequency of natural enemy activity to be a minimum of 2.2% and a maximum of 3.1% when considering eggs that were likely missing due to natural enemy activity. I then selected predators we observed and determined their functional response to *H. halys* eggs in a laboratory environment. Four generalist predators- *Acheta domesticus* L. (Gryllidae), *Melanoplus femurrubrum* DeGeer (Acrididae), *Orius insidiosus* Say (Anthocoridae), and *Conocephalus fasciatus* DeGeer (Tettigoniidae)- were tested against four densities of *H. halys* eggs. *A. domesticus* was the only predator that exhibited a Type II response with viable estimates of handling time and attack rate produced, suggesting the potential to be a density dependent mortality factor when *H. halys* egg densities are low. The remaining predators exhibited a Type I response and are unlikely to be a stabilizing force in *H. halys* population dynamics.

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CHAPTER 1

Introduction

When invasive species are introduced into a new ecosystem, extensive damage to natural and managed environments can lead to biodiversity losses, local and regional extinctions of indigenous species, and altered ecosystem structure and function (Pimentel et al. 2000; Crowl et al. 2008; Pyšek and Richardson 2010). In addition, invasive species can negatively affect resource availability and can result in economic losses (Pimentel et al. 2000; Didham et al. 2005; Pimentel 2005). Each of these factors must be assessed to effectively manage invasive pest species (Pyšek and Richardson 2010).

The invasion potential of invasive species is increasing due to global connectivity through transportation and importation (Crowl et al. 2008). Often these species will thrive in new environments due to lack of natural enemies and the ability to adapt to disturbances and switch hosts (Pimentel et al. 2000). Invasive species can threaten the structure and function of forests at the ecosystem level through decreases in productivity, shifts throughout food webs (Lovett et al. 2006), and can also establish long-term economic consequences (Holmes et al. 2009). Invasive species also serve as disease vectors, negatively affecting ecosystem function and human health (Lounibos 2002; Crowl et al. 2008).

In agriculture, invasive arthropods impose threats directly through yield loss and indirectly through the loss of biodiversity and ecosystem services (Pimentel et al. 2000). Managing for pests that are quick to adapt to change can be challenging without efficient management options. In organic agriculture, which relies heavily on biological control as part of a set of ecological practices for pest management (Zehnder et al. 2007; Gomiero et al. 2011), the problem posed by invasive species is compounded by a reduced set of pest management options (Zehnder et al. 2007). Managing for newly introduced invasive species in agriculture requires an understanding of ecologically sound practices that incorporate various approaches, such as biological control, before pest damage exceeds the economic threshold (Wyss et al. 2005; Zehnder et al. 2007).

1.1. Pest management tactics

Chemical tactics can provide immediate and effective control of target pests; however, extensive use of chemical pesticides can be harmful to the environment and can lead to resistance in the target species (van Driesche and Bellows 1996; Hajek 2004). Sub-lethal and lethal effects of chemicals on non-target species can disrupt natural control of pests, which can then lead to pest resurgences and secondary pest outbreaks (Hajek 2004; Desneux et al. 2007). The application of insecticides to crops is a reactive approach that is viewed as the final step in the hierarchy of organic pest management (USDA National Organic Program 2000). Implementing biologically based tactics of pest suppression can reduce dependency on chemical pesticides, and unlike chemical tactics, are sustainable.

Mechanical and physical pest control tactics are designed to make the environment unsuitable for survival and reproduction of pest species. Methods include creating physical barriers to prevent establishment or bagging fruit to prevent feeding damage. These methods of pest control are often labor-intensive and only effective for small-scale, short-term management (Banks 1976; Hajek 2004). Cultural pest management tactics aim to make the environment less favorable to the pest species through modifications in existing management practices. These modifications could include crop rotation, sanitation (Hajek 2004), and trap crops (Hokkanen 1991). Mechanical, physical, and cultural tactics will not prevent all damage from agricultural pests. Using these methods in concert with a biological control program can provide an effective alternative to chemical insecticides (Pimentel 2005).

Biological control is the use of live natural organisms to suppress another organism at the population level making it less abundant and damaging (Eilenberg et al. 2001). Natural enemies include predators, parasitoids, and pathogens with varying degrees of host-specificity (Hajek 2004). Biological control programs traditionally employ specialist natural enemies that have coevolved with the target pest (Doutt 1964; Beddington et al. 1978; Hassell 1978) however, generalist predators are thought to be effective as biological control agents due to early-season build-up of populations that allows for attack on pests during colonization (Curry 1993; Wiedenmann and Smith 1997). Furthermore, native generalist predators may be advantageous because they can persist in an environment in the absence of target pests by feeding on alternate prey (Curry 1993; Symondson et al. 2002; Messelink et al. 2012).

The three types of biological control that can be implemented in agroecosystems are classical, augmentative, and conservation. In classical biological control, host-specific natural enemies from the same region as the pest are introduced into the invaded environment for intended long-term control the invasive pest species. By contrast, in augmentative biological control, natural enemies are mass-reared and released without the goal of permanent establishment. In conservation biological control, natural enemies are released to enhance native natural enemy populations, or habitat supporting natural enemies is created. Although biological control can be effective, sustainable and affordable, it is not without its challenges, including: a potentially long period of time before establishment of released natural enemies and difficulty in successfully predicting non-target effects (Hajek 2004).

Before any management decisions can be made regarding suppression of invasive pests, biological and ecological information on the entire ecosystem is necessary; such as the biology of the pest, the potential for economic and ecological damage by the pest, and the potential for suppression by natural enemies (Pyšek and Richardson 2010). Preliminary studies examining natural enemies, including identification of primary predators and parasitoids and fundamental ecological concepts such as functional response of predators to the novel prey, must be conducted. These factors will help guide management decisions and will define how the generalist predators may affect interactions with novel prey.

1.2. *Halyomorpha halys*

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive pest that is native to eastern China, Korea, Japan and Taiwan (Hoebeke and Carter 2003). *H. halys* was first discovered in Allentown, Pennsylvania in 1996 (Hoebeke and Carter 2003) and has since spread to 43 states, including Michigan, and two Canadian Provinces (stopBMSB.org 2016). Adult *H. halys* are 12-17 mm long with dark brown, marbled dorsal coloration and legs that are pale, reddish-yellow. The antennae are black, except for the fourth and fifth antennal segments, which are pale white in part (Hoebeke and Carter 2003).

In the Northeastern United States, adult *H. halys* emerge from overwintering sites between mid-April and late June with peak emergence mid-May through early June (Bergh et al. 2017). Mating begins when diapause is terminated, which is photoperiod-dependent and usually occurs in mid-April to mid-May for the mid-Atlantic region (Nielsen et al. 2016b). A sexually mature female will mate as many as five times per day (Kawada and Kitamura 1983). Egg masses are deposited on the underside of leaves from May to late August. Eggs are approximately 1.6 mm in length, 1.3 mm in diameter, and are greenish-white in color. Egg masses will consist of 20-30 eggs. The egg breaker is black and T-shaped (Hoffmann 1931; Kobayashi 1956, 1967; Hoebeke and Carter 2003). The mean number of eggs produced by a female who mates several times is 244, or approximately nine egg masses. The first egg mass produced is the least fertile; fertility increases and remains constant for the remaining egg masses produced (Nielsen et al. 2008a).

After deposition, eggs hatch in 4-5 d (Hoebeke and Carter 2003; Nielsen and Hamilton 2008a). *H. halys* has five nymphal stages, similar to the pentatomids native to the United States. Upon emergence from the egg, first instar nymphs feed on the egg chorion and obtain beneficial gut symbionts that can enhance survival (Hirose et al. 2006, Taylor et al. 2014). The required number of degree days to complete development from the egg to a pre-oviposition adult is 538 (DD - 14°C). An additional 68-148 degree days are required for oviposition to begin (Yanagi and Hagihara 1980; Nielsen et al. 2008a).

One generation is typical throughout most of its native range, but 1-2 generations have been observed in Japan, and 4-6 generations have been observed in south China (Hoffmann 1931; Zhang et al. 1993; Hoebeke and Carter 2003). Throughout most of the United States *H. halys* has the capacity to be bivoltine (Nielsen et al. 2016b).

1.3. Impact

H. halys is a particularly challenging pest to manage because it is a highly mobile species with a wide host range (Hoebeke and Carter 2003; Rice et al. 2014). When introduced to an agroecosystem, *H. halys* has become the predominant pentatomid pest species (Nielsen and Hamilton 2009a; Leskey et al. 2012a). Nielsen and Hamilton (2009a, b) suggest that the seasonality of *H. halys* in the United States will coincide with critical damage stages in tree fruit. Starting in 2006, *H. halys* feeding injury has been observed on commercially grown apples, pears, and peaches in New Jersey and Pennsylvania (Nielsen et al. 2008a). In 2010, *H. halys* caused $37 million in damage to the apple crop in New Jersey and the surrounding region (U.S. Apple Association 2011). In Michigan, the agricultural impact of *H. halys* is just beginning, but citizen science reports to the Midwest Invasive Species Information Network (MISIN; http://www.misin.msu.edu) have totaled 6,500 records in Michigan (Wilson 2016). Michigan's agroecosystems could face comparable economic losses to the mid-Atlantic region when *H. halys* colonizes agricultural crops.

The combined developmental rate, fecundity, and degree-day requirements for *H. halys* will not limit its distribution to the mid-Atlantic region. Similarities in host plant ranges of native stink bug species suggest that *H. halys* will spread into other areas with economically important cropping systems throughout the United States (Nielsen et al. 2008a; Holtz and Kamminga 2010). Further, climate niche modeling indicates the potential distribution to include latitudes between 30° and 50° (Zhu et al. 2012).

H. halys is a highly polyphagous pest and is reported to feed on more than 150 host plants, with the families Fabaceae and Rosaceae most commonly cited (Hoffmann 1931; Hoebeke and Carter 2003; Bernon 2004; Nielsen and Hamilton 2009b). Among crops of economic importance in the United States, damage has been reported on tree fruit (apples, pears and peaches), small fruit (grapes, raspberry, blueberry), row crops (soybean, wheat and field corn), and vegetables (Leskey et al. 2012c; Rice et al. 2014). Damage to crops along the edges of the cropping system and those bordering forests are at the highest risk of attack by *H. halys* (Kuhar et al. 2012b; Venugopal et al. 2014; Blaauw et al. 2016; Rice et al. 2016).

H. halys will also feed on ornamental trees and shrubs including those in the genus *Paulownia*, which is native to China (Bergmann et al. 2016). At least two species of this genus are naturalized in the United States (Hoebeke and Carter 2003). *Paulownia* spp. are popular in agroforestry and urban areas due to their rapid growth and high canopy (Hiruki 1999). Nymphs feed on leaves, stems, and fruit while adults generally feed primarily on fruit (Hoebeke and Carter 2003). Stink bugs have piercing-sucking mouthparts that penetrate a food source to feed through a combination of mechanical pressure and enzymes in the saliva that dissolve components of the cell wall (Miles 1959; Brown 2003). Puncture marks by stink bugs are circular in shape.

Damage caused to apples by stink bugs is often confused with two disorders caused by calcium deficiency: cork spot and bitter pit (Brown 2003). Damage due to cork spot occur separately from the skin and may have healthy flesh in-between. On the fruit surface, cork spot will appear irregular in outline and can be found randomly distributed at multiple locations on the fruit. In contrast, corking due to stink bug damage appears immediately below the skin; it is generally uniform and clustered in distribution (Brown 2003). Bitter pit in apples appears as small black depressions with a small amount of corky flesh under the skin of the apple. Bitter pit often occurs during apple storage, but damage caused by stink bugs does not progress post-harvest (Brown 2003).

Damage caused to tree fruit including apples and peaches by *H. halys* occurs throughout the growing season (Brown 2003; Nielsen and Hamilton 2009a; Acebes-Doria et al. 2016). Early-season damage to peaches, occurring immediately after bloom or when fruit is small, results in gummosis or fruit that is cat-faced. Damage that occurs during mid or late-season fruit development results in fruit that has a depressed surface at the feeding site with internal necrosis (Nielsen and Hamilton 2009a; Acebes-Doria et al. 2016). Damage to any of these critical development stages results in unmarketable fruit (Rings 1957). In apple, higher levels of economic injury occur during the swell period of fruit development, late-season, compared to early- and mid-season development (Nielsen and Hamilton 2009a; Acebes-Doria et al. 2016).

Damage at different tree fruit developmental periods is caused by different developmental stages of *H. halys,* based on the seasonal phenology of the crop. Mid-season damage is likely caused by nymphal stages. Early- and late-season damage is the result of adults recently emerged from overwintering sites and late-instar nymphs or adults, respectively (Nielsen and Hamilton 2009a; Acebes-Doria et al. 2016).

H. halys nymphs and adults prefer vegetable crops with reproductive structures for feeding and will readily switch hosts when suitable feeding sites are unavailable (Zobel et al. 2016). Feeding injury to vegetable crops is variable. Sweet corn kernels become discolored, collapsed, or aborted. Pods of green beans, soybeans, and okra can become scarred and deformed. Fruit rot, spongy tissue, and abortion can occur in peppers, tomatoes, and eggplant. Damage to okra could indicate potential damage to a related crop of economic importance, cotton, as *H. halys* expands its range throughout the United States. Little damage has been reported on cucurbit or cruciferous vegetables to date (Kuhar et al. 2012b; Zobel et al. 2016).

In its native and invaded range, *H. halys* overwinters in natural landscapes in tree holes and litter (Qin 1990), under the bark of trees (Ueno and Shoji 1978; Lee et al. 2014), and in mountain terrain at high elevations (Wang and Wang 1988). *H. halys* can also be a nuisance to homeowners during its overwintering stage as it moves into human-made structures (Hamilton 2009; Inkley 2012). *H. halys* begins to aggregate on surfaces of buildings at the end of September through October before moving indoors (Hoebeke and Carter 2003; Hamilton et al. 2008). Once indoors, *H. halys* congregates in cool, dry areas within homes and businesses including inside walls, attics, and other suitable areas. *H. halys* emits an unpleasant odor when disturbed or crushed (Hamilton et al. 2008; Leskey et al. 2012c). Additionally, the frass of *H. halys* can stain walls. Adults move to exterior surfaces of walls and other surfaces as temperatures warm (Leskey et al. 2012c).

1.4. Insecticidal control of *Halyomorpha halys*

Pentatomid species native to the United States are generally considered to be secondary pests of tree fruit and have previously been successfully managed with applications of broad-spectrum, contact insecticides that were directed at other pests (Leskey et al. 2012c). Organophosphates have provided consistent control of the brown stink bug, *Euschistus servus* (Say), but reductions in broad-spectrum use because of the Food Quality Protection Act (1996) have led stink bugs to become an increasingly serious pest in orchard agroecosystems (Willrich et al. 2003; Leskey et al. 2012b). Recommendations for insecticidal control of native stink bugs are dependent on the species; the predominant species in many areas are the *E. servus*, the green stink bug, *Chinavia hilaris* (Say), and the Southern green stink bug, *Nezara viridula* (Linnaeus). Organophosphates such as acephate, dicrotophos, and methyl parathion are often recommended for *Euschistus* spp., while pyrethroids are often recommended for control of *C. hilaris* and *N. viridula* (Willrich et al. 2003). Glass-vial bioassays have indicated that *E. servus* is more tolerant to pyrethroids than *C. hilaris* or *N. viridula*. The pyrethroid bifenthrin was equally effective against the adults and nymphs of *E. servus* and *N. viridula* (Snodgrass et al. 2005).

When *H. halys* is added to the stink bug complex, it becomes the key pest driving management practices. Because *H. halys* is a newly established pest, there is little information from which to develop an effective management strategy, which has led to insecticide-based management (Leskey et al. 2012b). Insecticide programs in some regions like the mid-Atlantic, have drastically changed growers' spray regimes. For example, the number of insecticide applications by some growers increased four-fold and the time between applications decreased in apple and peach (Leskey et al. 2012b). This method of control may provide short-term relief, but is not sustainable long-term, as *H. halys* will reinvade from the surrounding landscape.

Insecticidal approaches may increase the likelihood that *H. halys* will develop resistance to insecticides used by selecting for resistant individuals and killing susceptible individuals (Leskey et al. 2012c). A second consequence of insecticidal control is the occurrence of secondary pest outbreaks due to a breakdown in biological control of pests (Hajek 2004). Insecticide applications may reduce populations of non-target arthropods, including natural enemies that prey on various pests, as well as the target pest. This disruption in biological control results in outbreaks of secondary pests that were previously kept in check by non-target natural enemies (Harper and Zilberman 1989). As a result, insecticides, at times increasing in toxicity, must be applied more frequently to control target and secondary pest populations (Pimentel et al. 1992; Hajek 2004).

Insecticide classes including pyrethroids, neonicotinoids, carbamates, and organophosphates have proven very effective in some laboratory studies (Leskey et al. 2012a). Leskey et al. (2012a) found high initial efficacy of pyrethroids and neonicotinoids to *H. halys*, but recovery after knockdown was > 33% of individuals. Results from glass-vial bioassays using pyrethroids showed knockdown and recovery of *H. halys* to -cyfluthrin, cyfluthrin, -cyhalothrin, fenpropathrin, and bifenthrin (Nielsen et al. 2008b). Horizontal and vertical movement of *H. halys* became erratic or ceased when exposed to pyrethroids, organophosphates, neonicotinoids, and carbamates. However, upon recovery from insecticidal knockdown, *H. halys* was able to regain full vertical mobility (Lee et al. 2013a). Similar results were obtained by Morrison et al. (2016b) when considering sub-lethal effects of insecticide applications, suggesting brief exposure to insecticides may result in dispersion to other areas. Knockdown and recovery was not observed in laboratory bioassays of *H. halys* on organophosphates or carbamates (Leskey et al. 2012a).

Field testing of these insecticide classes indicate that mortality in the field can be substantially lower with high knockdown and recovery, possibly due to the nature of laboratory testing (forced contact no-choice testing on a flat smooth surface) or the potential ability of *H. halys* to detect and avoid insecticide application (Kuhar et al. 2012a; Leskey et al. 2012a; Leskey et al. 2013).

The timing of insecticide applications can affect their efficacy. Significantly higher mortality rates were observed early in the season when compared to applications made later in the growing season. The reason for this could be that adult *H. halys* emerging from overwintering sites are older than generations that appear later in the year and have depleted their stored resources making them more susceptible (Leskey et al. 2013). Additionally, Leskey et al. (2013) found that mortality was lower if the residue of the insecticide had aged for 3 or 7 d, as compared to fresh insecticide residue. For up to 7 d after application, certain insecticides such as fenpropathrin and dinotefuran can cause an antifeedant effect which prevents live insects from injuring fruit, but does not cause mortality to the pest (Leskey et al. 2013; Lee et al. 2013a).

1.5. Organic management tactics

Organic control methods that are National Organic Program-compliant are limited for this pest. Mechanical control of *H. halys* by removing egg masses from crops has been suggested by Hoffman (1931) and Qin (1990); however, this is labor-intensive and not applicable for large-scale operations. Brown (2003) demonstrated that putting exclusion cages over apple trees as a form of physical control prevented stink bug damage. Other suggested physical control tactics include placing sticky barriers around tree trunks and bagging fruit (Lee et al. 2013b; Quarles 2014). However, Li et al. (2007) found that bagging fruit did not prevent feeding injury during one study. There have been a number of publications outlining the success of using trap crops as a cultural control tactic (Panizzi 1980; McPherson and Newson 1984; Corrêa-Ferreira and Moscardi 1996; Osakabe and Honda 2002).

H. halys is a perimeter-driven pest that causes substantially greater damage to the outer edge of agricultural fields, particularly fields bordering woodlots (Kuhar et al. 2012b; Venugopal et al. 2014; Blaauw et al. 2016). Planting early-maturing soybeans or runner beans along field edges to protect varieties that mature later in the season has proven effective at attracting and retaining large portions of native stink bug populations, up to 80% in one study (McPherson and Newson 1984). The use of sorghum as a trap crop attracted *H. halys* at high densities during the time when pest populations are peaking (Nielsen et al. 2016a) and sunflower has also been suggested, although it may not be effective on its own (Soergel et al. 2015).

The sunflower and sorghum trap crops increase the retention time of *H. halys* (Nielsen et al. 2016a; Blaauw et al. 2017) but injury was only reduced to minor damage (Mathews et al. 2017). If retention of *H. halys* can be maintained in a trap crop, various management tactics can then be implemented to keep populations below economic thresholds, while at the same time promoting natural enemy populations by providing shelter and floral resources (Corrêa-Ferreira and Moscardi 1996; Hajek 2004; Nielsen et al. 2016a). In addition, releasing parasitoids into trap crops could adequately suppress stink bug populations without the use of chemical insecticides. Inoculative release of the egg parasitoid *Trissolcus basalis* Wollaston (Hymenoptera: Platygastridae) reduced population densities of a native stink bug by 54% in a trap crop (Corrêa-Ferreira and Moscardi 1996).

1.6. Biological control of Pentatomidae

Biological control is a sustainable management tactic for native stink bugs across the United States. The target of most pentatomid biological control programs is the sessile egg stage although some predators consume nymphs and adults. There are several examples of successful predation on native stink bug eggs and nymphs in a variety of cropping systems including corn, soybean, rice, tomato, bean, wheat, alfalfa, and peanut (Ingram 1927; Buschman and Whitcomb 1980; Ehler 2002; Koppel et al. 2009; Tillman 2008, 2010). The complex of predators within these cropping systems will change depending on the time of year and the phenology of the crop (Tillman 2010). The native stink bugs that are commonly used for natural enemy assessments are *C. hilaris*, *N. viridula*, *E. servus*, the rice stink bug, *Oebalus pugnax* F., and the predatory spined soldier bug, *Podisus Maculiventris* (Say).

Generalist predators are often considered to be important factors in keeping stink bug pest populations below economic thresholds (Symondson et al. 2002). Predators will attack stink bug eggs, nymphs, or adults. Results from laboratory feeding trials suggest that little predation on nymphs will occur beyond the third instar (Ragsdale et al. 1981). Predators are classified as either stylet sucking, punctured sucking, or chewing depending on their mouthparts. A stink bug egg that is attacked by a stylet sucking predator will have a small hole or a stylet sheath on the chorion. The chorion will generally be intact but the contents will be dry. Punctured sucking predators, such as spiders, leave characteristic punctures in eggs and suck out the contents. Chewing predators consume the entire egg (complete chewing) or leave behind bits of the egg shell (incomplete chewing) (Tillman 2010; Morrison et al. 2016a).

In a study using sentinel and naturally laid egg masses of native stink bugs in Georgia corn fields, chewing predators consumed more eggs than sucking predators (Tillman 2010).

Similar results were obtained by Yeargan (1979) in a study examining predation in soybean and alfalfa. Chewing predators from at least four Orders have been described attacking native stink bug eggs or nymphs. In the Order Coleoptera, coccinellid larvae (*Hippodamia convergens* Guérin-Méneville, *Coccinella septempunctata* L., *Coleomegilla maculata* De Geer, and *Harmonia axyridis* Pallas) have been observed chewing eggs of *N. viridula* and *E. servus* on corn, peanut, and soybean (Ragsdale et al. 1981; Stam et al. 1987; Tillman 2008, 2010). Stam et al. (1987) observed coccinellid larvae feeding on second instar *N. viridula* and at least one adult antlike flower beetle species, *Anthicus cervinus* Laf., (Coleoptera: Anthicidae) consuming a first instar *N. viridula* in soybean. Adult Coccinellidae (*Cycloneda sanguinea* L. and *C. maculata*) have been observed feeding on *N. viridula* eggs and nymphs in soybean. Ragsdale et al. (1981) identified a ground beetle, *Lebia analis* Dejean (Coleoptera: Carabidae), feeding on eggs in soybean.

In the Order Neuroptera, green lacewing larvae, *Chrysoperla carnea* Stephens (Chrysopidae), consumed eggs and nymphs of *E. servus* and *N. viridula* in the lab and in the field, in corn (Tillman 2010). Similarly, Stam et al. (1987) detected this predator in soybean, but consuming only *N. viridula* first instars. In the Order Orthoptera, members of the family Gryllidae and at least three species of grasshopper, adults and nymphs, in the family Acrididae have been observed attacking native stink bug eggs in corn and soybean. Grasshopper species include *Schistocerca obscura* (F.), *Orchelimum nigripes* (Scudder), and *Conocephalus fasciatus* (De Geer).

In the Order Hymenoptera, at least three species of ants, including red imported fire ants, *Solenopsis invicta* Buren (Formicidae), have been identified as egg predators of *N. viridula* and *E. servus*. Ants may become primary predators of stink bug eggs in soybean during vegetative stages (Ragsdale et al. 1981; Stam et al. 1987; Tillman 2010). Spiders are also important predators of nymphal stink bugs in soybean. Stam et al. (1987) identified four species of spiders attacking second instars of *N. viridula*. Similarly, Ragsdale et al. (1981) identified three species of spiders consuming nymphs of *N. viridula*.

Sucking predators of native stink bugs have been identified from the Order Hemiptera. *Orius insidiosus* Say (Anthocoridae) is commonly stated as the primary and most abundant predator of native stink bug eggs (Yeargan 1979; Tillman 2010). Other important sucking predators that attack native stink bug eggs and nymphs are *Geocoris punctipes* Say (Lygaeidae) nymphs and adults; *Reduviolus roseipennis* Reuter (Nabidae); *Largus succinctus* L. nymphs (Largidae); and one *Sinea* sp. nymph (Reduviidae). One important factor of egg predation by sucking predators is cannibalism- stink bugs of other pentatomid species, or as the same species as the prey, consuming the eggs. Stam et al. (1987) identified *N. viridula* adults and nymphs, *E. servus* adults, and *Euschistus tristigmus* (Say) adults feeding of the eggs of *N. viridula*. This cannibalism is common in many agroecosystems and could signal of a lack of suitable food sources during various times of crop development (Tillman 2010).

An important predator of native stink bug eggs and nymphs throughout the United States is *P. maculiventris*. This species is sold commercially in the United States and Europe for augmentative biological control in greenhouses and field systems (Aldrich et al. 1984; Valicente and O’Neil 1995). *P. maculiventris* will generally attack eggs of phytophagous insects, as well as first and second instars of native stink bug pests (Aldrich et al. 1984). In a laboratory feeding study, Ragsdale et al. (1981) found that *P. maculiventris* will accept stink bug nymphs above the third instar. However, the authors note that laboratory conditions may have altered typical feeding behavior.

Although predators have been observed in the field attacking various life stages of stink bug pests, most of these are generalists. They will likely not prevent stink bug populations from increasing in density and causing damage above the economic threshold unless an effort is made to conserve these predators and to avoid excess chemical applications (Ragsdale et al. 1981; Stam et al. 1987). Thus, specialist parasitoids are often released as a primary source of control for native stink bug pests.

Egg parasitoids are important natural enemies of native stink bugs in the United States and generally have higher prey mortality associated with them than do predators (Laumann et al. 2008; Tillman 2010). Parasitoids of pentatomid eggs belong to the families Encyrtidae, Eupelmidae, and Platygastridae, with the genera *Trissolcus* and *Telenomus* in the Platygastridae the most abundant in North America (Yeargan 1979; Tillman 2010). Specifically, *T. basalis* and *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) have been reared from *N. viridula*, *Euschistus* spp. and *Podisus* spp. In Georgia, parasitism rates of native stink bugs ranged from 25.8% to 90.9% during the duration of one study in corn (Tillman 2010). In Kentucky, Yeargan (1979) reports parasitism rates of 72.5% in soybean. These differences in parasitism rates of the same parasitoid species, attacking the same host, could be attributed to the host plant composition, structure, stage of development, the relative abundance of hosts in different cropping systems, regional or seasonal differences, or the occurrence of a disturbance to the parasitoid by pesticides. Parasitism rates could be related to the size of the egg mass. Tillman (2010) found that the percent eggs parasitized by native parasitoids increased as the number of eggs in the egg mass decreased.

There are many attributes of parasitoids that make them suitable for use as biological control agents, such as multiple generations per season; high parasitism rate; and monophagous or oligophagous host ranges (Hajek 2004; Laumann et al. 2008). Another important attribute is the type of functional response. Parasitoids with a Type III, or sigmoidal response, can be a density-dependent mortality factor, which will reduce pest populations in patches where growth is highest. Population regulation at the local-scale population regulation is then possible (Laumann et al. 2008). Egg parasitoids in the family Platygastridae are regularly considered for biological control programs because they possess these attributes. Specifically, *T. basalis* and *T. podisi* have been considered and released in many countries for classical and augmentative biological control of stink bugs with varying results (Clarke 1990; Laumann et al. 2008).

Yeargan (1979) reports that substantial mortality of native stink bug eggs and nymphs is possible through the use of natural enemies. The use of multiple species of natural enemies can lead to greater pest control than a single-species approach (Laumann et al. 2008). Releasing multiple species of Platygastridae has been suggested to increase pest management efficiency, however releasing multiple agents that fill the same niche could lead to interference competition (Ehler 2000; Laumann et al. 2008). For example, chewing predators will attack both parasitized and unparasitized eggs (Yeargan 1979), which will lower the overall natural enemy impact.

1.7. Biological control of *Halyomorpha halys*

Natural enemies of *H. halys* in its native range are thought to be important factors in the mortality of multiple life stages. *Flavipes* spp. of the genus *Trissolcus* are considered to be the most specialized parasitoids of *H. halys*. In Japan, *Trissolcus mitsukurii* Ashmead (Hymenoptera: Platygastridae) was identified to be the most efficient parasitoid (Arakawa and Namura 2002). In China, *Trissolcus halyomorphae* (syn. *Trissolcus japonicus*) Ashmead (Hymenoptera: Platygastridae) was identified to be the most efficient parasitoid (Yang et al. 2009). Parasitism rates of *H. halys* in Asia of at least 50% are common with a maximum rate of 70% for the first generation (Yang et al. 2009). *T. japonicus* is currently being evaluated in the United States as a biological control agent and has recently been detected in the eastern and western United States (Talamas et al. 2015). Flies in the family Tachinidae (Diptera) parasitize adult *H. halys* in Asia (Leskey et al. 2012c) and in the United States, although parasitism rates were < 2% and only found in one county of Pennsylvania. Floral resources providing nectar could increase longevity and fecundity of tachinid flies (Biddinger et al. 2012).

A regional assessment of natural enemies found variation in mortality by natural enemies across the invaded range of *H. halys* (Ogburn et al. 2016). Mortality was influenced by state and crop but was low overall. Predators and parasitoids were responsible for 10.5±2.2% and 7.8±2.1% of egg mortality in 2013 and 2014, respectively. Predators accounted for 80% of natural enemy activity. Chewing predators were more prevalent than sucking predators for sentinel egg masses for both years of the study. Through this study, soldier beetle larvae (Cantharidae), arachnids, and slugs (Gastropoda) were observed attacking eggs. Native parasitoid activity was low in sentinel eggs with 3% of eggs parasitized; the majority of those parasitoids failed to emerge. The most common native parasitoid encountered was *T. podisi* (Ogburn et al. 2016).

Leskey et al. (2012c) reported predation by spiders (Araneae) and big-eyed bugs (*Geocoris* spp.) to be at approximately 47% of egg masses in Maryland. In laboratory predation studies *C. carnea* attacked *H. halys* eggs as a 4th instar but not as a 2nd instar, perhaps because the chorion of the egg is too tough for smaller instars to puncture. Eggs that were exposed to, but not punctured by, *C. carnea* had a lower nymphal developmental success, suggesting some sort of external damage to the eggs. Fourth instar twelve-spotted lady beetles, *C. maculata*, will consume *H. halys* eggs, but 2nd instar and adults will regularly attack but are unable to pierce the egg chorion. *P. maculiventris* has also been observed feeding on *H. halys* eggs in the laboratory (Abram et al. 2015).

Web-building spiders commonly found in and around homes could be an important source of predation for overwintering adults (Morrison et al. 2017). In Pennsylvania, predatory sand wasps (Hymenoptera: Sphecidae) such as *Bicyrtes quadrifasciatus* (Say) and *Astata unicolor* (Say) have been observed predating on *H. halys* nymphs. These wasps will sting and paralyze stink bug nymphs, typically later instars, and transport the nymph to the wasp burrow where larval wasps will consume the nymph (Biddinger et al. 2012).

Without oligophagous natural enemies for use in classical biological control, including primary parasitoids like *T. japonicus*, the population density of *H. halys* has the potential to increase, as predicted by the natural enemy release hypothesis (Keane and Crawley 2002). With this unchecked population growth, it is likely that *H. halys* will become an economically important pest in a variety of cropping systems throughout the United States (Nielsen et al. 2008a) with a distribution that mirrors that of *C. hilaris* (Bernon 2004).

1.8. Monitoring

Monitoring for fluctuations in population density will be crucial to designing management strategies. However, *H. halys* can be difficult to detect due to its nocturnal lifestyle and hiding behaviors. Sampling techniques such as sweep nets and beat sampling are likely not suitable because they fail to dislodge adults and therefore will not give an adequate representation of the population size. Visual samples in orchards may also not be suitable because of the hiding behaviors and a tendency to hang out in high areas of trees (Leskey et al. 2012b); however visual inspection in soybeans has proven effective (Aigner et al. 2016).

Using trap-based thresholds to monitor populations and inform management decisions can reduce crop injury and insecticide use. A treatment threshold of 10 adult *H. halys* per trap in apples reduced insecticide application by 40% (Short et al. 2016). Traps baited with aggregation pheromone can be an important tool for monitoring pest populations. The two primary components of the *H. halys* aggregation pheromone have been identified as (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (Khrimian et al. 2013, 2014). Methyl (2E,4E,6Z)-decatrienoate (MDT) is the aggregation pheromone produced by the brown-winged green bug, *Plautia stali* Scott (Hemiptera: Pentatomidae), a sympatric species to *H. halys*. MDT is attractive to *H. halys*, particularly in the late summer, and acts as a synergist with the pheromone (Khrimian et al. 2008; Leskey et al. 2012b). Black pyramid traps baited with the *H. halys* aggregation pheromone and MDT are more attractive to *H. halys* nymphs and adults season-long than traps baited with the aggregation pheromone or MDT alone (Weber et al. 2014). However, aggregation pheromone-baited traps can increase crop injury to surrounding crops within a 2.5 m radius (Sargent et al. 2014; Morrison et al. 2015b; Short et al. 2016).

Morrison et al. (2015a) evaluated trap designs and found that black coroplast pyramid traps deployed on the ground captured more adults and nymphs than wooden traps of the same design and are also cheaper to purchase. At the landscape level, blacklight traps are also an effective way to monitor early-season or low-density populations and can help detect seasonal flight activity (Tada et al. 2001; Nielsen et al. 2013).

1.9. Research objectives

The goal of my thesis was to explore the top-down pressures that exist at the beginning of the biological invasion period (Fagan et al. 2002) and to translate this into potential sources of biological control for *H. halys* in Michigan's agroecosystems. Specific objects to meet this goal were 1a) determine baseline frequency (Franklin 1989) of *H. halys* egg utilization by native natural enemy community (Chapter 2), 1b) to identify existing sources of biological control of *H. halys* using video surveillance (Grieshop et al. 2012) in Michigan (Chapter 2), and 2) determine the functional response (Hassell et al. 1997; Murdoch and Oaten 1975) of generalist predators of *H. halys* egg masses in a laboratory environment (Chapter 3).

CHAPTER 2

Monitoring for natural enemies of *Halyomorpha halys* using video surveillance in Michigan

1. Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive agricultural pest and homeowner nuisance that is native to China, Korea, Japan and Taiwan (Hoebeke and Carter 2003). *H. halys* was first discovered in Allentown, Pennsylvania in 1996 (Hoebeke and Carter 2003) and has since spread to 43 states and two Canadian provinces (stopBMSB.org 2016). Michigan is on the leading edge of the invasion from the Eastern United States with *H. halys* first detected in 2010 and currently reported in 55 counties (Wilson 2016; http://www.ipm.msu.edu/invasive_species/brown_marmorated_stink_bug). *H. halys* is highly polyphagous, attacking a wide range of ornamental plants and agricultural crops (Hoffmann 1931; Hoebeke and Carter 2003, Bernon 2004). Among crops of economic importance, damage has been reported on tree fruit (apples, pears and peaches), small fruit (grapes, caneberry, blueberry), row crops (soybean, wheat and field corn), and vegetables (Leskey et al. 2012c; Rice et al. 2014).

When introduced to an agroecosystem, *H. halys* quickly becomes the predominant hemipteran pest (Nielsen and Hamilton 2009a, Leskey et al. 2012a). High pest pressures in New Jersey and the surrounding region resulted in agricultural damage of more than $37 million in 2010 (U.S. Apple Association 2011). In Michigan, the agricultural impact of *H. halys* has yet to be wholly realized, but citizen science reports to the Midwest Invasive Species Information Network (MISIN; http://www.misin.msu.edu) have totaled 6,500 records in Michigan (Wilson

2016). Michigan’s agroecosystems could face comparable economic losses to the mid-Atlantic region when *H. halys* colonizes agricultural crops.

The ability of invasive species to thrive in a new environment can be affected, positively or negatively, by species that already inhabit that environment (Pyšek and Richardson 2010). Utilizing pre-existing natural enemies as a source of top-down pressure could provide an effective source of control for invasive species (Sheldon and Creed 1995) under certain conditions, such as early in the season during pest colonization (Curry 1993; Wiedenmann and Smith 1997). In response to an invasion, native predators may adapt to using the novel prey as a viable food source, provided the new prey is within the feeding capabilities of the predator (Carlsson et al. 2009).

Biological control programs traditionally employ specialist natural enemies that have coevolved with the target pest (Doutt 1964; Beddington et al. 1978; Hassell 1978); however, the use of indigenous generalist predators as biological control agents may be feasible because generalists can persist on alternative prey in the absence of the target pest (Symondson et al. 2002; Messelink et al. 2012). Predators with this advantage can theoretically slow the spread of local pest populations (Fagan et al. 2002) and have demonstrated the ability to reduce pest populations in agroecosystems (Riechert and Lockley 1984; Rosenheim et al. 1993; Snyder and Wise 1999).

Natural enemies, primarily parasitoids, of *H. halys* in its native range are thought to be an important mortality factor (Yang et al. 2009). Due to uncertainty in host-specificity of *Trissolcus japonicus* Ashmead (Hymenoptera: Platygastridae), currently being evaluated for release as a classical biological control agent (Talamas et al. 2015), the identity and efficacy of native natural enemies should be investigated.

Few studies have been published quantifying the effect of predators on *H. halys* (Abram et al. 2015; Morrison et al. 2016a; Ogburn et al. 2016). Identification of predators in the field using sentinel prey is difficult. Video surveillance is a direct approach that has been used to identify predators for biological control in some studies (Schenk and Bacher 2002; Merfield et al. 2004; Grieshop et al. 2012). Video surveillance allows the researcher to conduct simultaneous observations in multiple locations for long periods of time.

As a recent invading species, *H. halys* is a new threat to Michigan's agroecosystems. The full impact of its presence hasn't yet been realized, and the potential for a biological control program that utilizes indigenous natural enemies is currently unknown. Targeting augmentative or conservation biological control efforts in recently colonized areas, such as Michigan, in advance of the main body of the invasion could have implications for slowing the overall spread of the invasion (Fagan et al. 2002). The first step in this process is to determine the suite of indigenous natural enemies in Michigan that might attack *H. halys* eggs. Therefore, the objectives of this study were (1) to determine baseline frequency of *H. halys* egg utilization by the native natural enemy community and (2) to identify potential *H. halys* egg predators.

2. Materials and Methods

We conducted an observational study using colony reared *H. halys* sentinel egg masses under video surveillance over two growing seasons to explore the potential of existing sources of biological control. Two organically managed fruit and vegetable farms in Ingham County Michigan, USA were used as research sites. The Michigan State University Student Organic Farm (SOF) is a 6 ha university-sponsored, multi-crop CSA located in East Lansing, Michigan, USA (42.675441, -84.486916). Three Brothers Farm is a 1.6 ha multi-crop operation located in

Lansing, Michigan, USA (42.704853, -84.517606). Data were collected weekly throughout the summer months of 2013 and 2014.

2.1. Laboratory colony

H. halys were reared using a method modified from Medal et al. (2012). Insects were kept in an environmental growth chamber set to a 16L:8D photoperiod, 24°C and 60-70% RH. Approximately 100 adults were kept in each 30 cm cube vinyl cage (BioQuip, Rancho Dominguez, CA) containing two live green bean plants grown from seed (Bush Green Bean, Fedco Seeds, Waterville, ME) as an egg laying substrate. Insects were fed a mixture of organic green beans, broccoli, sunflower seeds, and carrots twice per week. Egg masses were collected from the green bean plants daily to ensure an age of 24 h or less for placement in the field.

2.2. Sentinel egg deployment

At both field locations, colony-reared *H. halys* egg masses were placed in tomato and apple crops in 2013, and in pepper and apple crops in 2014. At each location, and in each crop, *H. halys* egg masses containing 20-30 eggs were pinned using insect pins on the underside of leaves to mimic natural egg deposition (Takahashi 1930). Each week, six egg masses less than 24 h of age were placed in each crop at each research site for 11 consecutive weeks in 2013. In 2014, each week six egg masses were placed in both crops at one location, and on alternate weeks six egg masses were deployed in the same manner at the other field site. Field sites alternated weekly in this way for 14 consecutive weeks. For each crop type, three egg masses were placed under video surveillance (described below) and three were placed without a video camera. Egg masses were deployed 10 m apart and left pinned to the crop for 48 h. Egg masses deployed without video surveillance in 2013 were stored in a -80°C freezer until deployment due to rearing limitations. All other egg masses used in both years were viable.

Utilization of egg masses, defined as any damage to the egg mass caused by a predator or a parasitoid, was recorded and categorized after each 48 h deployment period. Damage categories included predation by chewing, predation by sucking, parasitism, or missing eggs (Figure 2.1A-C). Also recorded were the number of eggs per mass before and after the deployment session and the number of *H. halys* that hatched out of each egg mass. Egg masses were retained and placed in an environmental growth chamber (described above) to allow for *H. halys* nymph and parasitoid emergence. Six weeks after deployment, eggs were dissected following protocol described by Tatman et al. (2013) and the number of partially developed parasitoids (Figure 2.1D) was recorded.

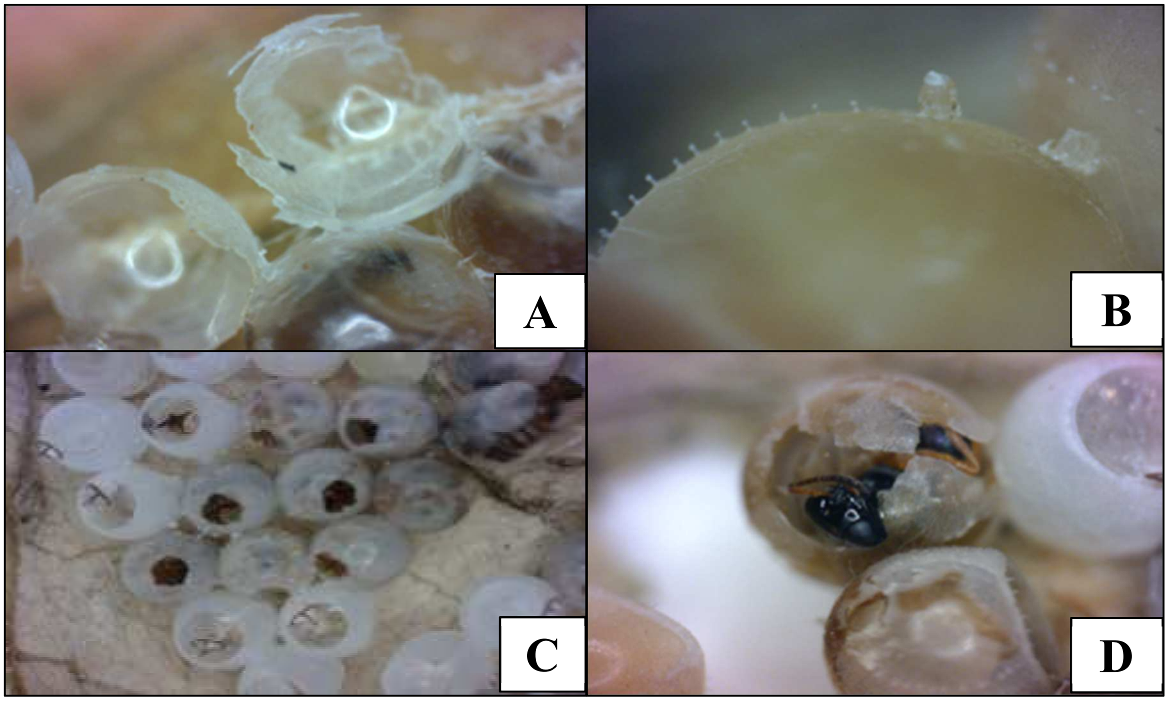


Figure 2.1. Examples of sentinel egg fates. A) Evidence of chewing predator. B) Evidence of sucking predator. C) Egg mass with *H. halys* hatched eggs and parasitized eggs. Parasitized eggs have a characteristic emergence chew-hole. D) A partially developed parasitoid found upon dissection of egg.

2.3. Video recording

Video equipment was modified from Grieshop et al. (2012). Modifications included the use of 12-volt, 7 ah batteries (eComElectronics, Brooklyn, NY) and a single channel, high definition mini-DVR (model MDVR25HR, Supercircuits, Austin, TX). We used an indoor/outdoor surveillance camera equipped with 12 infrared Light Emitting Diodes (LEDs) for night viewing (model CFC6044IR, Supercircuits, Austin, TX). The camera was mounted to a polyvinyl chloride (PVC) pipe that was staked into the ground in the vegetable crop rows or bungee strapped to apple tree limbs (Figure 2.2).

All other parts of the recording system were stored inside a waterproof toolbox (48.26 x 22.86 x 25.4 cm) to protect from the weather. A white board approximately the same length and width as the toolbox was attached to the top of the toolbox to reflect sunlight and prevent overheating of equipment inside. A square piece of plastic board, approximately 15 x 15 cm, and marked with a 2.5 cm grid was attached to the PVC pipe and oriented facing the camera approximately 20 cm away (Figure 2.2). The leaf with the egg mass attached remained connected to the plant but was also secured to this board using rubber bands to hold the leaf in place for optimal camera viewing. The batteries were replaced with fresh-charged batteries at the 24 h mark of the 48 h deployment period.

Video recordings were continuous through the 48 h deployment session. We determined the backdrop boards and camera equipment were attracting birds at Three Brothers Farm. To prevent this, egg masses deployed in apple trees were placed inside 1.27 cm square plastic mesh cages to protect from non-arthropod damage after week 2 when deployed masses were immediately consumed by birds. After installment of mesh cages, all bird predation was prevented. Non-arthropod damage was omitted from analysis. Birds were not an issue at the Student Organic Farm.

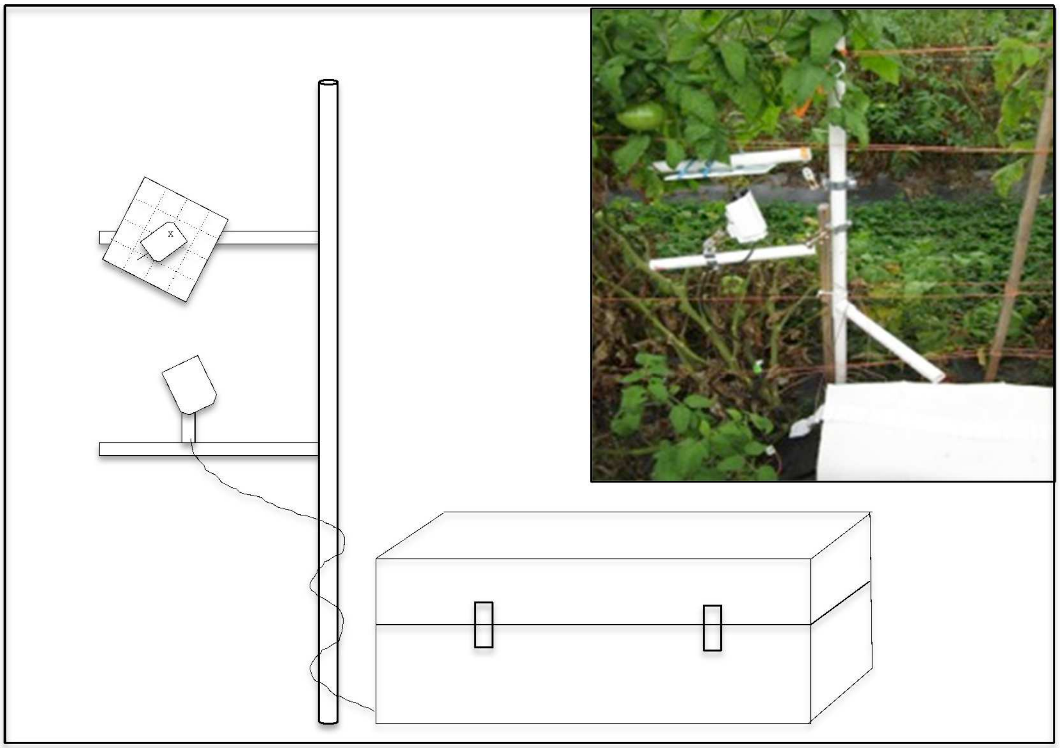


Figure 2.2. Schematic of camera set-up. PVC pipe stand staked into the crop row with camera attached in vegetable systems. Stand was adapted for use in tree fruit systems by strapping PVC stand to the tree limb of choice. A white board marked with a 2.5 cm grid for reference is attached to the PVC pipe facing the camera in both crops.

2.4. Data analysis

Data were analyzed using appropriate parametric and non-parametric methods in the R statistical language (R core development team 2014). When using parametric tests, data was not transformed. The level of significance for all tests was set to *P* = 0.05.

2.4.1. Natural enemy impact

Data on egg damage were examined as proportions of both individual eggs and the number of whole egg masses damaged. Damage to each egg was quantified in the damage categories previously listed. Each year of the study was analyzed separately. A proportions t-test was conducted to compare any two samples represented as proportions.

2.4.2. Natural enemy community

After each deployment session, the DVRs were brought back to the laboratory and the recorded footage was copied to a hard drive on a desktop computer. Video was watched using VLC media player (VideoLAN organization, Paris, France) at 8x real speed. An event was defined to be any interaction by an organism with the egg mass. Organisms observed interacting with the egg mass were identified to at least the ordinal level, but more specific identifications were made when possible. Organisms were labeled as "unknown" if identifications could not be made. The start time, end time, and total duration of the interaction with the egg mass was recorded.

Observed organisms were split into two categories: causing damage while visiting the egg mass, and interacting with the egg mass but causing no damage. A diversity index was quantified using the Shannon-Wiener equation (Wiener 1948; Shannon and Weaver 1949). Species abundance and Pielou's Evenness of Predators (Pielou 1969) were also quantified. Indices were compared using the nonparametric Kruskal-Wallis test (Kruskal and Wallis 1952). A regression analysis was conducted to examine the effect of arthropod visitation and duration on *H. halys* hatch rate.

2.4.3. Natural enemy behavior

A diel rhythm chart was created based on video observations using solar noon as a reference point to examine diel rhythm of non-damaging events and predators. Seasonal occurrence of egg predators were plotted against cumulative degree-days above 14°C starting from Jan 1 each year. Cumulated degree-day records were accessed from the Michigan State University Enviro-Weather database using the East Lansing weather station.

3. Results

3.1. Natural enemy impact

A total of 6593 eggs were deployed in 2013 and 3633 in 2014 with an average of 26.9 and 26.3 eggs per mass, respectively (Table 2.1). Of these, 3057 and 1815 were deployed under video surveillance in 2013 and 2014, respectively. Total egg damage by an arthropod predator or parasitoid did not exceed 2% in either of the deployment years. When missing eggs are included as a source of egg damage, total egg damage increases to a maximum of 6.5% (Figure 2.3). Consumption by a chewing predator was consistently low across both years and occurred in 0.5 and 0.4% of eggs deployed in 2013 and 2014, respectively. Eggs consumed by a chewing predator were more frequent in apples than in tomatoes in 2013 with 1.0 and 0.06% of eggs consumed, respectively (Figure 2.4). Incidence of chewing predation decreased in the apple crop in 2014 with 0.4% eggs consumed. At Three Brothers Farm in 2014, chewing predation in the vegetable crop was higher than in the apples at either location, but overall vegetable crop chewing predation was consistent with the apple crop at 0.4%. The percentage of eggs consumed by chewing predators was higher in both years than eggs consumed by sucking predators with the exception of the vegetable crop at the MSU Student Organic Farm in 2013.

The overall percent of eggs consumed by a sucking predator increased from 0.06% in 2013 to 0.3% in 2014 (Table 2.1). There was no consumption by a sucking predator at Three Brothers Farm in either crop in 2013. Eggs consumed by a sucking predator increased in the vegetable crop from 0.03% and 0.4% of eggs damaged and in the apple crop 0.13% to 0.23% in 2013 and 2014, respectively. One egg mass, located in the vegetable crop in 2014, had chewing and sucking damage.

Table. 2.1. Fate of *H. halys* sentinel eggs deployed at two organic field sites in 2013 and 2014.

Year	Total eggs deployed	Eggs with video surveillance	Missing eggs	Chewing predation	Sucking predation	Eggs parasitized	Emerged parasitoids	Partially developed parasitoids[1]	Available to hatch[2]	Hatched eggs
2013	6593	3057	252	33	4	21	42	3	2743	1713
2014	3633	1815	172	15	11	13	0	13	3422	1661

[1] Partially developed parasitoids = parasitoids discovered upon dissection of eggs

[2] Available to hatch = eggs deployed as viable and not missing or lost to predation or parasitism

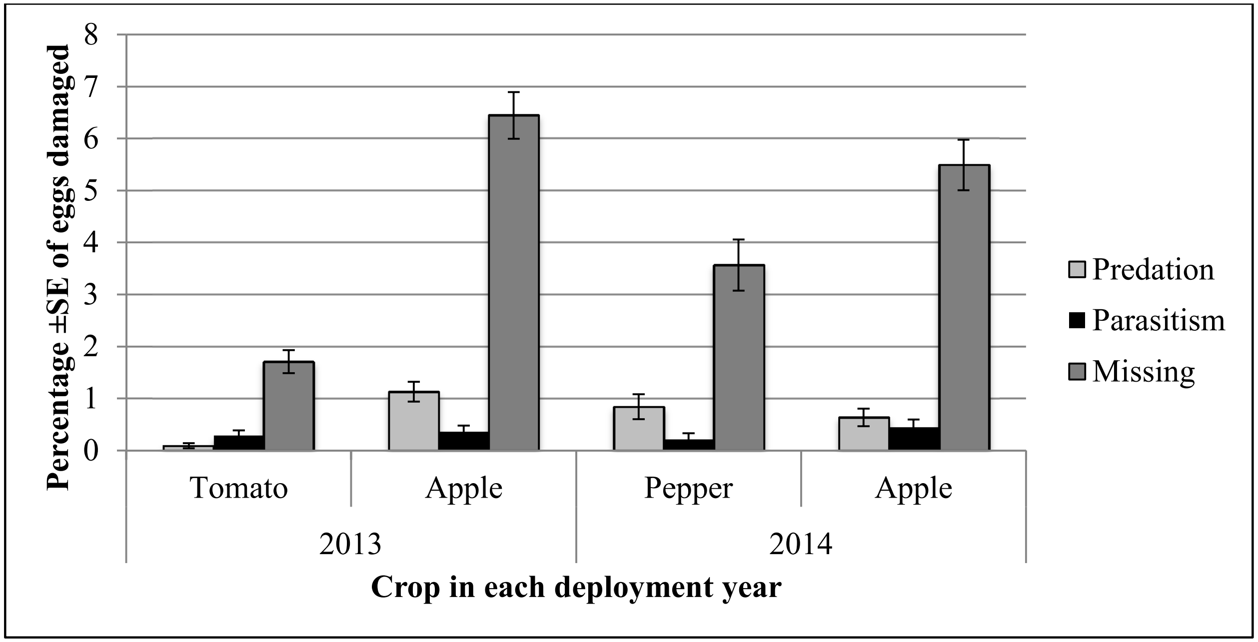


Figure 2.3. Percentage ± SE of *H. halys* eggs damaged in 2013 and 2014 in each crop. Total egg damage was less than 2% when missing eggs are excluded as a source of damage and increases to a maximum of 8% when including missing eggs.

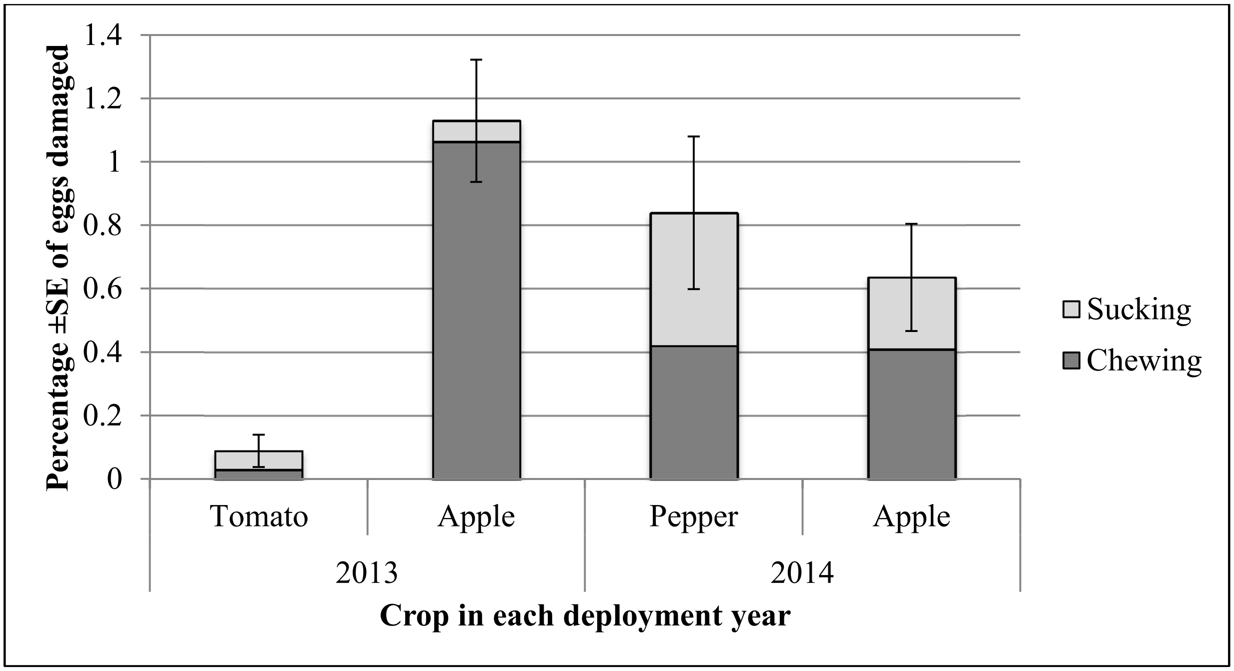
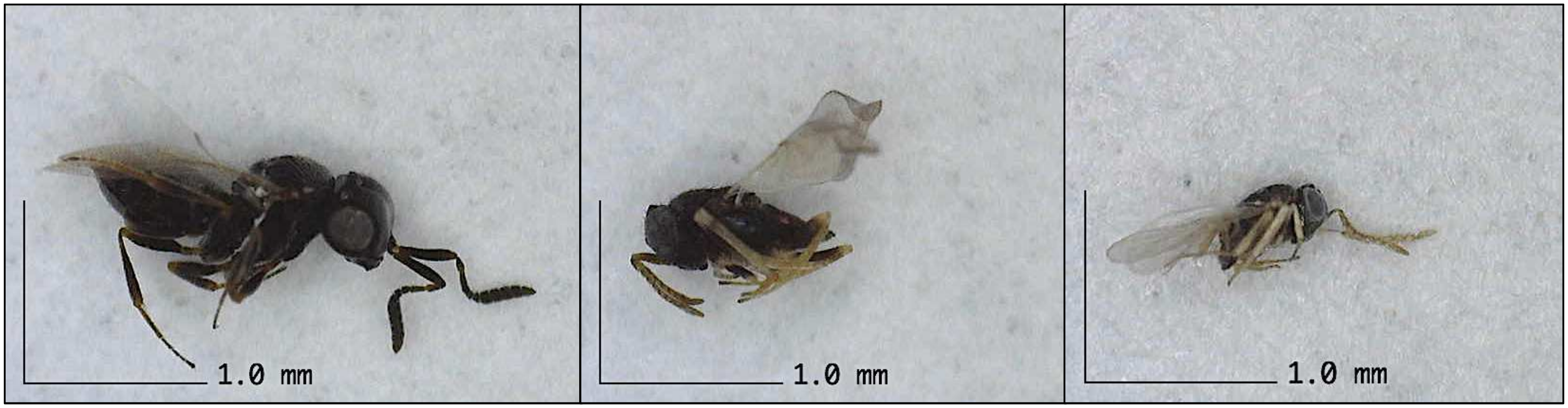


Figure 2.4. Percentage ± SE of *H. halys* eggs damaged by chewing and sucking arthropod predators in 2013 and 2014. Chewing predators attacked more eggs than sucking predators in all crops for each deployment year with the exception of tomato in 2013 in which a sucking predator attacked 1 additional egg.

Parasitism occurred only at Three Brothers Farm in 2013 with 0.5% of eggs deployed in tomatoes parasitized and 0.6% of eggs in apples. Emerged parasitoids were keyed out to *Trissolcus* spp. (Hymenoptera: Platygastridae), *Ooencyrtus* spp. (Hymenoptera: Encyrtidae), and two unidentified, but suspected hyperparasitoids (Figure 2.5). A total of 42 parasitoids emerged from 21 eggs. In each instance of more than one emergence from a single egg, the parasitoids appear to be the same species indicating successful superparasitism. Of the total number of emerged parasitoids, 88% emerged from viable, not frozen, eggs. Upon dissection of eggs, 3 partially developed parasitoids were found in 3 viable *H. halys* eggs (Figure 2.1D).

In 2014, there were no emerged parasitoids. Thirteen partially developed parasitoids were found upon dissection from 13 *H. halys* eggs. All observed parasitism occurred only at the MSU Student Organic Farm, opposite of parasitism from the previous year, with 0.03% of eggs in peppers parasitized and 0.09% of eggs in apples.



Trissolcus spp.

Ooencyrtus spp.

Unknown

Figure 2.5. Identifications of parasitoids that emerged from *H. halys* egg masses deployed at Three Brothers Farm in 2013. One *Trissolcus* spp. emerged from one egg. 39 *Ooencyrtus spp.* and two suspected hyperparasitoids emerged singly and in pairs from 20 single eggs. Pictures take with Dino-Lite Edge Digital Microscope and DinoCapture 2.0 software (BigC, Torrance, CA).

Partially developed parasitoids were not identified.

Missing eggs accounted for the majority of egg damage and was consistent at an average of 3.8 and 4.7% in 2013 and 2014, respectively. Each year, the percentage of missing eggs was highly variable between crops and ranges from 1.0 to 10.9% in 2013 and 0.1 to 6.2% in 2014.

The proportion of whole-egg masses that had ≥1 egg damaged was significantly higher than the proportion of total individual eggs damaged ($X^2 = 112.32$, $df = 1$, $P < 0.0001$). There was no whole-egg mass utilization by a parasitoid. 2.5 and 2.8% of whole egg masses were completely missing in 2013 and 2014, respectively, which could be attributed to predation. The proportion of whole egg masses with damage in 2013 was significantly greater in apples (11.5%) over tomatoes (2.4%) in 2013 ($X^2 = 6.46$, $df = 1$, $P = 0.011$). In 2014, the proportion of whole egg masses with damage was slightly higher in peppers (14.8%) over apples (14.2%); this difference was not significant ($X^2 = 0$, $df = 1$, $P = 1.0$) (Figure 2.6).

When expressed as whole egg masses, in 2013, 6.7% of masses were consumed by a predator, 2.1% had an emerged parasitoid, and 16.3% had missing eggs for a total of 25.2% of masses with some form of damage. In 2014, 14.5% of masses were attacked by a predator, 1.4% contained a developing parasitoid, and 19.6% had missing eggs for a total of 35.5% of masses with damage, an increase from the previous year. 96.5 and 81.8% of viable egg masses had at least one *H. halys* hatch out in 2013 and 2014, respectively.

In 2013, egg masses deployed without video surveillance were frozen due to rearing limitations. The proportion of frozen whole egg masses damaged (19.5%) was significantly lower than fresh whole egg masses (31.3%) ($X^2 = 3.78$, $df = 1$, $P = 0.05$). The total proportion of frozen individual eggs damaged, 5.3%, was significantly higher than fresh eggs, 3.9% ($X^2 = 6.14$, $df = 1$, $P = 0.011$); this includes missing eggs.

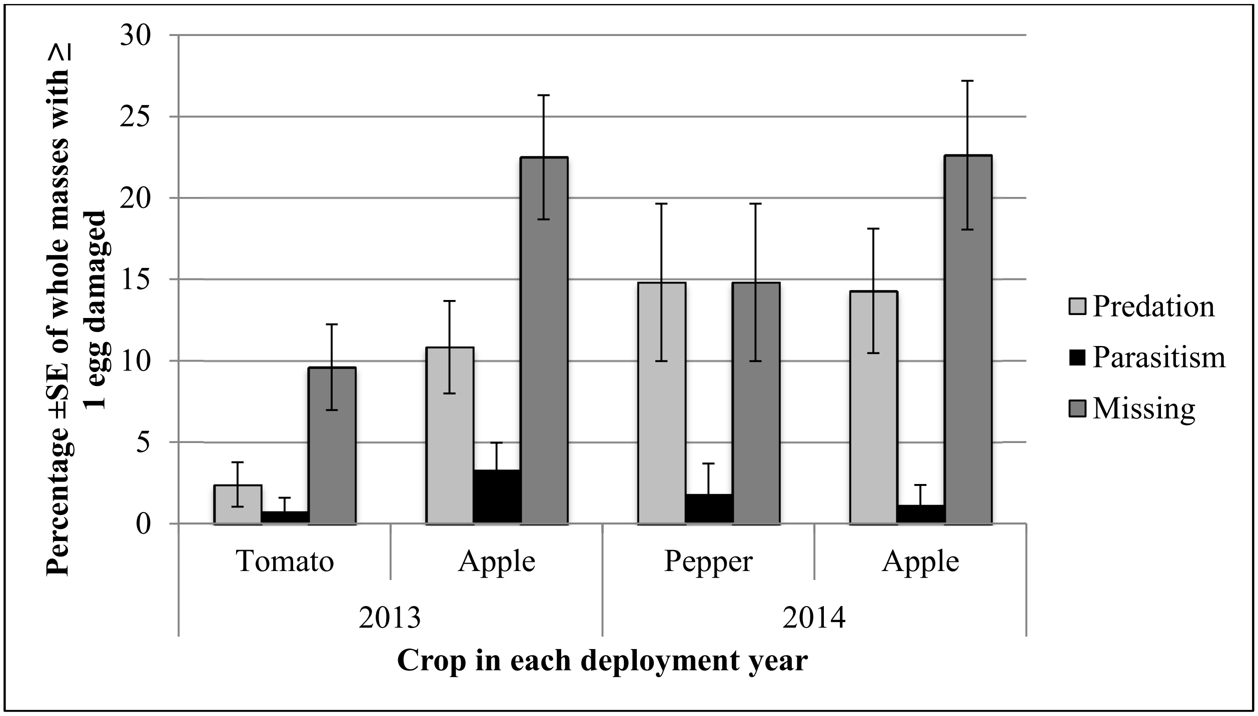


Figure 2.6. Percentage ± SE of whole *H. halys* egg masses with ≥ 1 egg damaged in 2013 and 2014 in each crop. A greater proportion of whole egg masses had ≥ 1 damaged in the vegetable crop in 2013 than in apples, this trend is reversed in 2014.

The proportion of chewing predation (X^2 = 0.0048, *df* = 1, *P* = 0.944) and sucking predation (X^2 = 2.723, *df* = 1, *P* = 0.0981) were not significantly different for frozen and fresh eggs. There was no predation on frozen eggs deployed in tomatoes at either location. The proportion of parasitism was significantly higher is fresh eggs (X^2 = 11.572, *df* = 1, *P* = 0.0006) over frozen. The proportion of missing eggs was significantly higher in frozen eggs over fresh (X^2 = 17.358, *df* = 1, *P* <0.0001) (Figure 2.7).

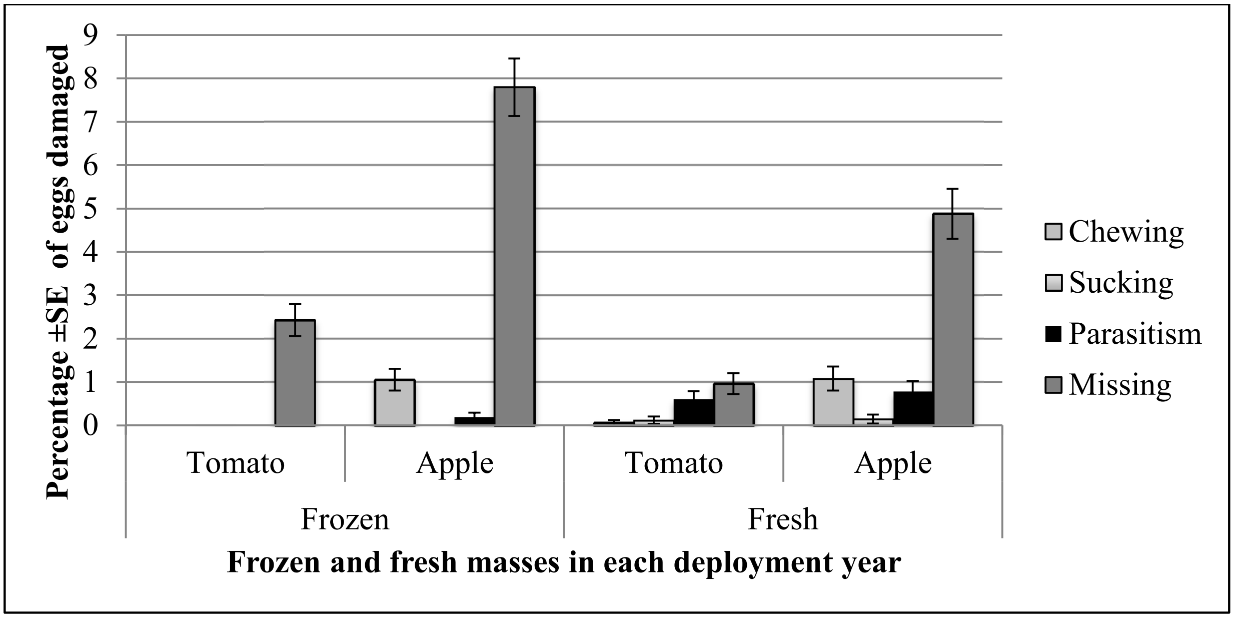


Figure 2.7. Percentage ± SE of *H. halys* eggs attacked in frozen and fresh egg masses in 2013. The proportion of missing eggs was greater in frozen masses but the number of eggs damaged due to predation or parasitism was greater in fresh eggs.

3.2. Natural enemy community

A total of 21 different arthropods spanning 11 orders were identified interacting with *H. halys* egg masses (Table 2.2). Taxa observed damaging *H. halys* egg masses in 2013 include Acrididae, Anthocoridae, Araneae, Forficulidae, Gryllidae, Miridae, and Parasitica (Figure 2.8). Each taxon observed damaging eggs were also observed at least one additional time visiting eggs with no damage, with the exception of Acrididae, Anthocoridae, and Miridae. Each visit by an anthocorid or acridid in 2013 led to egg damage. Miridae was observed only once.

The Order with the highest number of recorded events on egg masses in 2013 was Dermaptera with 28 total visits, including four damaging visits in which nine eggs were consumed (Table 2.3). Each of these visits occurred in apples at Three Brothers Farm. The second most frequent taxon in 2013 was Hymenoptera: Parasitica. Parasitoids were observed 21 times visiting 14 egg different egg masses, or 12.2% of the masses deployed under video surveillance.

Table 2.2. The number of organisms observed visiting *H. halys* sentinel egg masses in 2013 and 2014. Identifications were taken to the furthest possible level. Shaded taxa indicate organisms that caused damage to eggs. A Shannon-Wiener diversity index and Pielou's evenness index were calculated for predators in each crop.

	2013		2014	
Order	Apple	Tomato	Apple	Pepper
Araneae	10	5	29	11
Coleoptera				
Coccinellidae A.[1]				3
Coccinellidae L.[2]			3	2
Chrysomelidae		1		1
Elateridae			1	
Scarabaeidae		1		
Dermaptera				
Forficulidae	28		36	
Diptera				
Muscomorpha			18	5
Other	1	2	1	
Gastropoda		1		4
Hemiptera				
Anthocoridae		2	1	
Miridae		1	1	4
Other				1
Hymenoptera				
Formicidae	4	2	58	
Parasitica	1	20	12	55
Other			1	
Lepidoptera			1	
Neuroptera				
Planipennia L.[3]		1	1	2
Opiliones		3		1
Orthoptera				
Acrididae	2		1	
Gryllidae	2	1	4	4
Unknown	3	23	13	26
Predator Diversity	0.90	1.15	0.96	1.19
Predator Evenness	0.82	0.82	0.59	0.86

[1] Adults in the family Coccinellidae of the order Coleoptera
[2] Larvae in the family Coccinellidae of the order Coleoptera
[3] Larvae in the suborder Planipennia of the order Neuroptera

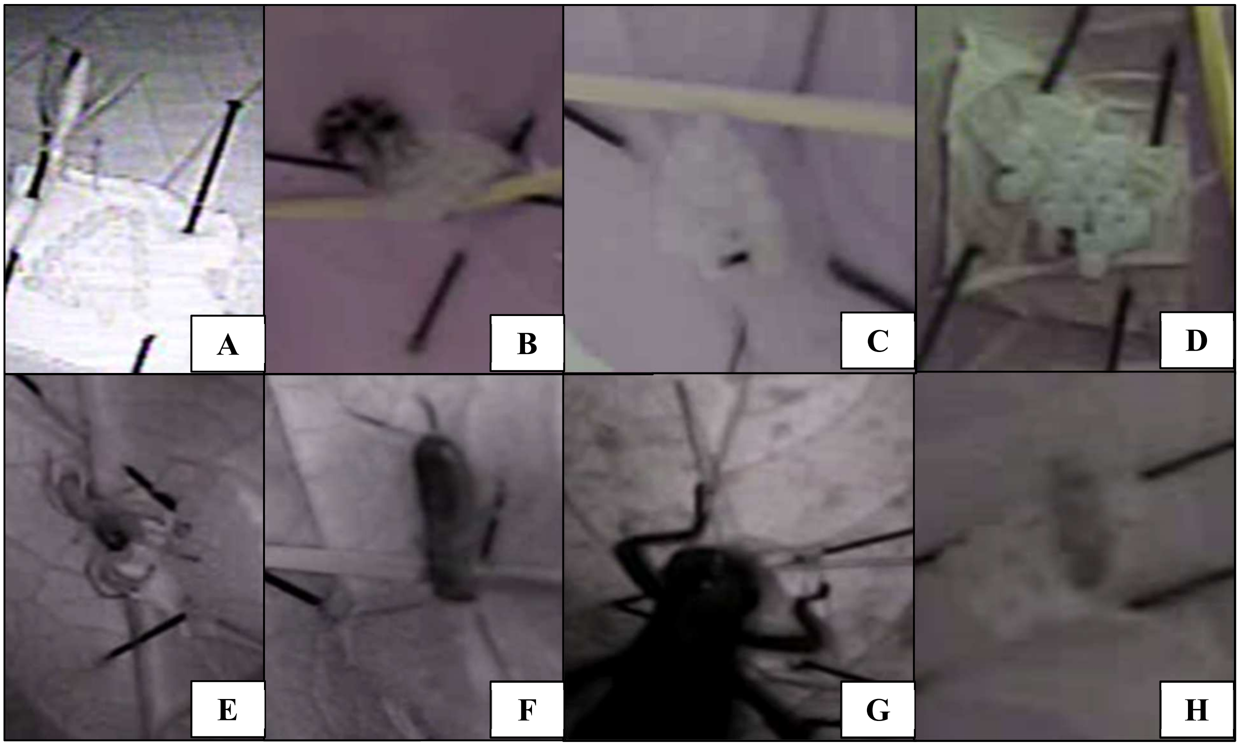


Figure 2.8. Still shots from video surveillance of predators that caused damage to *H. halys* egg masses taken from surveillance video. A) Tree cricket (Orthoptera: Gryllidae). B) Spotted lady beetle (Coleoptera: Coccinellidae). C) Parasitoid (Hymenoptera: Parasitica). D) Minute pirate bug (Hemiptera: Anthocoridae). E) Spider (Araneae). F) European earwig (Dermaptera: Forficulidae). G) Grasshopper (Orthoptera: Acrididae). H) Plant bug (Hemiptera: Miridae).

Table 2.3. The number of *H. halys* eggs each predator attacked in 2013 and 2014. The maximum number of eggs attacked was 9 by Forficulidae and the mode attacked was 2.

Predator	2013	2014
Acrididae	2	2
Anthocoridae	2	0
Araneae	2	2
Coccinellidae	0	1
Forficulidae	9	2
Gryllidae	8	0
Miridae	2	3

Of the remaining predators observed, Acrididae were observed twice in apples, Anthocoridae twice in tomatoes, Araneae 15 times in both apples and tomatoes, Miridae were seen once in tomatoes, and Gryllidae were observed in three instances in both crops.

Taxa observed damaging *H. halys* egg masses in 2014 include Acrididae, Araneae, Coccinellidae (adult), Forficulidae, Miridae, and Parasitica (Figure 2.8). Again, each taxon observed damaging the eggs was also observed at least one other time visiting the eggs without causing damage with the exception of Acrididae and Coccinellidae (adult). In 2014 the Order with the most recorded events was Hymenoptera. Within Hymenoptera, parasitoids were observed more frequently in 2014 than 2013. Parasitoids were observed on 67 occasions visiting 13 different egg masses, or 18.8% of masses under video surveillance. All observed parasitism in 2014 occurred at one location.

The Order with the second most frequent visitation in 2014 was Araneae with 40 total observations, including three damaging events. Dermapterans were again important predators in 2014 with high visitation of 36 total visits, including three damaging visits in which three eggs were consumed (Table 2.3). Visitation of dermapterans was observed only in apples. Of the remaining predators observed damaging eggs in 2014, Coccinellidae (adult), and Acrididae were each observed just once.

Video data revealed that Araneae were the only predators observed at all crops and locations, consuming a total of five and three eggs in 2013 and 2014, respectively. There was one instance where >1 predator damaged at ≥ 1 egg on an egg mass in 2013 (Araneae and Acrididae) and one in 2014 (Coccinellidae, Araneae, and Miridae). There was one instance in 2013 where a parasitoid and predator damaged the same egg mass. A parasitoid visited and parasitized ≥ 8 eggs. After this event, a gryllid visited the mass and consumed eight eggs. Parasitoids emerged out of eight eggs, and *H. halys* nymphs hatched from the remaining eggs.

Diversity, abundance, and evenness indices were calculated for each year. Species diversity, abundance, and evenness of all arthropods identified interacting with the egg masses were significantly higher in 2014 than in 2013 (*diversity*: $X^2 = 14.25$, $df = 1$, $P = 0.0001$ *abundance*: $X^2 = 27.8166$, $df = 1$, $P < 0.0001$; *evenness*: $X^2 = 19.028$, $df = 1$, $P = 0.0001$). When comparing species abundance and diversity of predators observed, all indices were also greater in 2014 (*diversity*: $X^2 = 3.7952$, $df = 1$, $P = 0.0514$; *abundance*: $X^2 = 17.3221$, $df = 1$, $P < 0.0001$; *evenness*: $X^2 = 6.157$, $df = 1$, $P = 0.013$). In 2013, there were no significant differences in abundance or diversity among factors when looking at observed predators. The evenness score for observed predators was 0.61 (on a scale from 0-1). There were no significant differences in the evenness index of all arthropods observed, but the evenness index is significant for predators observed between the two crops ($X^2 = 3.6594$, $df = 1$, $P = 0.055$) with the tomatoes scoring slightly higher than apples.

In 2014, diversity of all observed arthropods was significantly higher at the MSU Student Organic Farm ($X^2 = 7.6935$, $df = 1$, $P = 0.005$). Similarly, the MSU Student Organic Farm had a significantly higher abundance ($X^2 = 7.2737$, $df = 1$, $P = 0.006$) and diversity of predators ($X^2 = 7.6935$, $df = 1$, $P = 0.005$) than the Three Brothers Farm. There were no additional significant differences found for either index in 2014. The evenness score for observed predators was 0.65. There were no significant differences in the evenness index for all arthropods or predators among factors in 2014.

The length of time each predator spent with the egg mass during a consumption event varied (Table 2.4). Araneae feeding events were the longest on average in 2013 but the shortest in 2014. Of the total time spent by predators consuming eggs, sucking predators spent significantly higher proportion of time with the egg mass over chewing predators in 2013. This was reversed in 2014 with chewing predators spending a significantly higher proportion of time at the egg mass (2013: $X^2 = 2079.729$, $df = 1$, $P < 0.0001$; 2014: $X^2 = 59854.73$, $df = 1$, P

Table 2.4. Average length of time in seconds each predator spent interacting with the egg mass during consumption events in 2013 and 2014. Dotted lines indicate no attack event recorded.

Predator	2013	2014
Acrididae	38.5	196
Anthocoridae	555.5	…
Araneae	3666	108
Coccinellidae	…	297
Forficulidae	3302	1791
Gryllidae	1810	…
Miridae	269	320

<0.0001). When comparing consumption visits to non-damaging visits by the same predator, the average time spent by a predator consuming eggs was always longer on average than the amount of time spent interacting with the egg masses and not causing damage for 2013. This does not apply in 2014 where Araneae and Miridae both had longer average interactions with no damage than damaging events (Figure 2.9).

It has been suggested that missing eggs are the result of chewing predation that leaves no trace of the eggs behind (Ogburn et al. 2016). Using video observations, we calculated the 95% confidence interval to find the percent of missing eggs that were likely consumed by a predator. There were a total of 84 and 67 eggs under video surveillance missing in 2013 and 2014, respectively. In 2013, 48.8% of missing eggs (95% CI: 38.2 – 59.4%) could be attributed to visitation by natural enemies including one Dermaptera, one Araneae, and one Gryllidae. Forty-three missing eggs (51.2%) were not visited by an arthropod. In 2014, 29.8% of missing eggs (95% CI: 18.9 – 40.7%) could be attributed to interactions with 15 dermapterans, eight Araneae, two Coccinellidae (larvae) and one Formicidae.

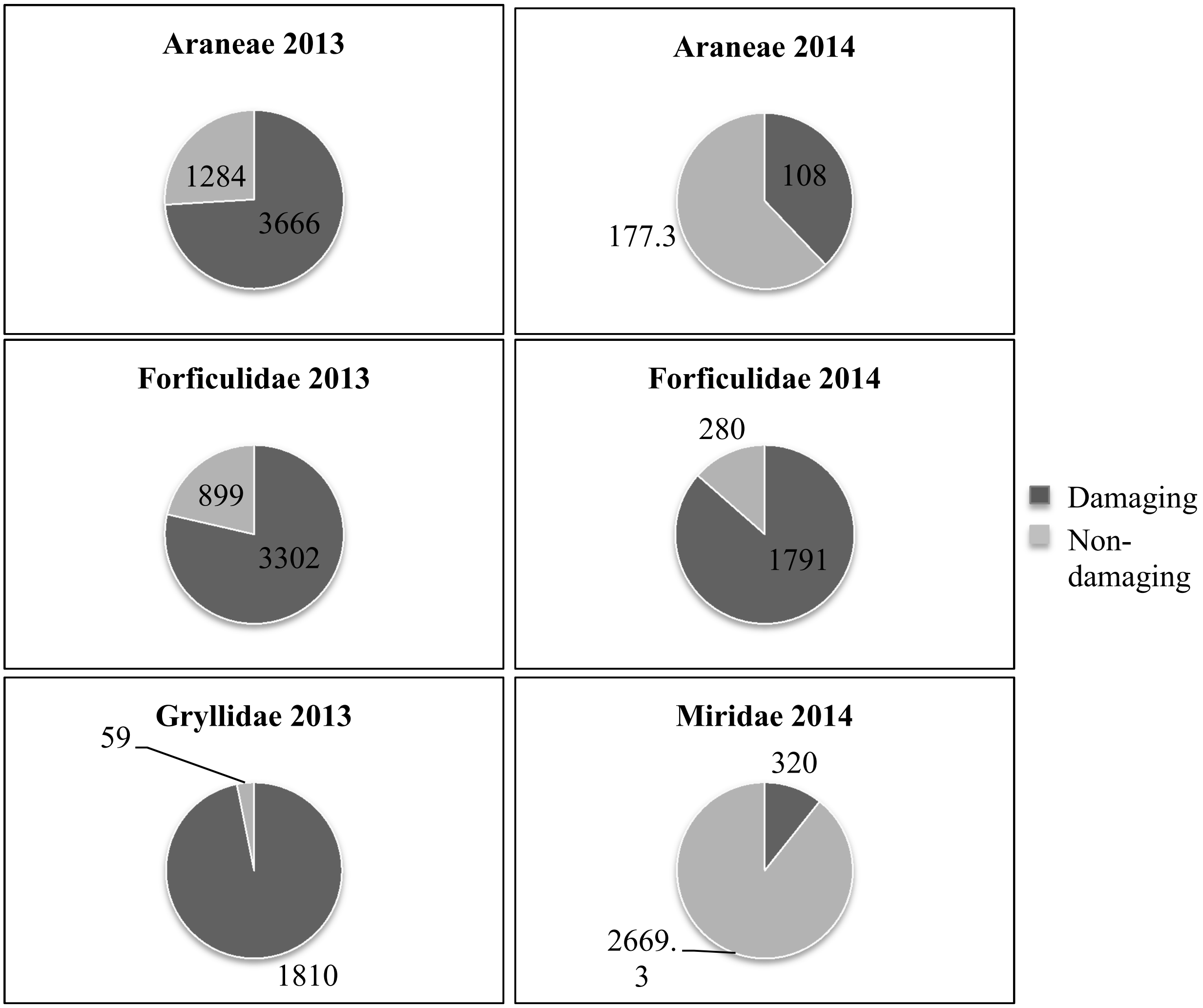


Figure 2.9. The average length of time in seconds for predators observed both attacking *H. halys* eggs and interacting with eggs without causing damage. Two predators in 2014, Araneae and Miridae, spent more time on average on non-damaging interactions than damaging interactions.

If interactions by all arthropods observed are taken into account, then 88.1% of missing eggs (95% CI: 80.4 – 95.8%) could be attributed to arthropod interactions, however this is less likely given the identity of some of these arthropods as the suborder Planipennia (larvae), the Infraorder Muscomorpha, Families Cicadellidae and Formicidae. Eight missing eggs (11.9%) had no visitation in 2014. The number of times an egg mass was visited during deployment affected the *H. halys* hatch rate. There was a significant negative correlation between the number of non-damaging visits by arthropods to egg masses and the *H. halys* hatch rate from those visited masses in 2013 (*Slope* = -0.051, $F_{1,50}$ = 4.318, *P* = 0.043, R^2 = 0.061). In contrast, there was no correlation between arthropod visitation and *H. halys* hatch rate in 2014 (*Slope* = 0.002, $F_{1,47}$ = 0.1353, *P* = 0.715, R^2 = -0.018). The total amount of interaction time by an arthropod with an egg mass did not have a significant effect on the hatch rate of *H. halys* in either year (2013: *Slope* = -7.42e-06, $F_{1,50}$ = 3.566, *P* = 0.06, R^2 = 0.04; 2014: *Slope* = 5.88e-07, $F_{1,47}$ = 0.04, *P* = 0.842, R^2 = -0.02).

3.3. Natural enemy behavior

The time of day in which arthropods began their interactions, both damaging and non-damaging, with the egg mass was similar among crops in each year. In 2013, 53% of observed events began at night between the hours of 2000 and 0400. The time frame in which the most events occurred in tomato was 1400 to 2100 h. There was an increase in activity in tomatoes just before sunset that was not prevalent in apples and the peak of activity occurred at 2000 h. Parasitoids and two unidentifiable insects are responsible for this peak. In apple, the peak in activity occurred at 2100 h and the organism responsible for those events belonged to Forficulidae (Figure 2.10).

The seven egg predators that consumed *H. halys* eggs in 2013 were observed across a range of h covering both the scoto- and photophases (Table 2.5). Acrididae were observed in the late afternoon during the hours of 1600 and 1800. Anthocoridae were observed in the scotophase during the 2000, 2100, and 2200 h. Araneae and Forficulidae were observed across a wide range of the scotophase from 2000 to 0300 h. Gryllidae were observed at two distinct times; the first during the scotophase and the second crepuscular, occurring around sunrise. Miridae and the unknown predator were the only two observed during the photophase at 1300 and 1800 h, respectively. Parasitoids were observed interacting with egg masses nearly every hour of the day, but we did not record parasitism.

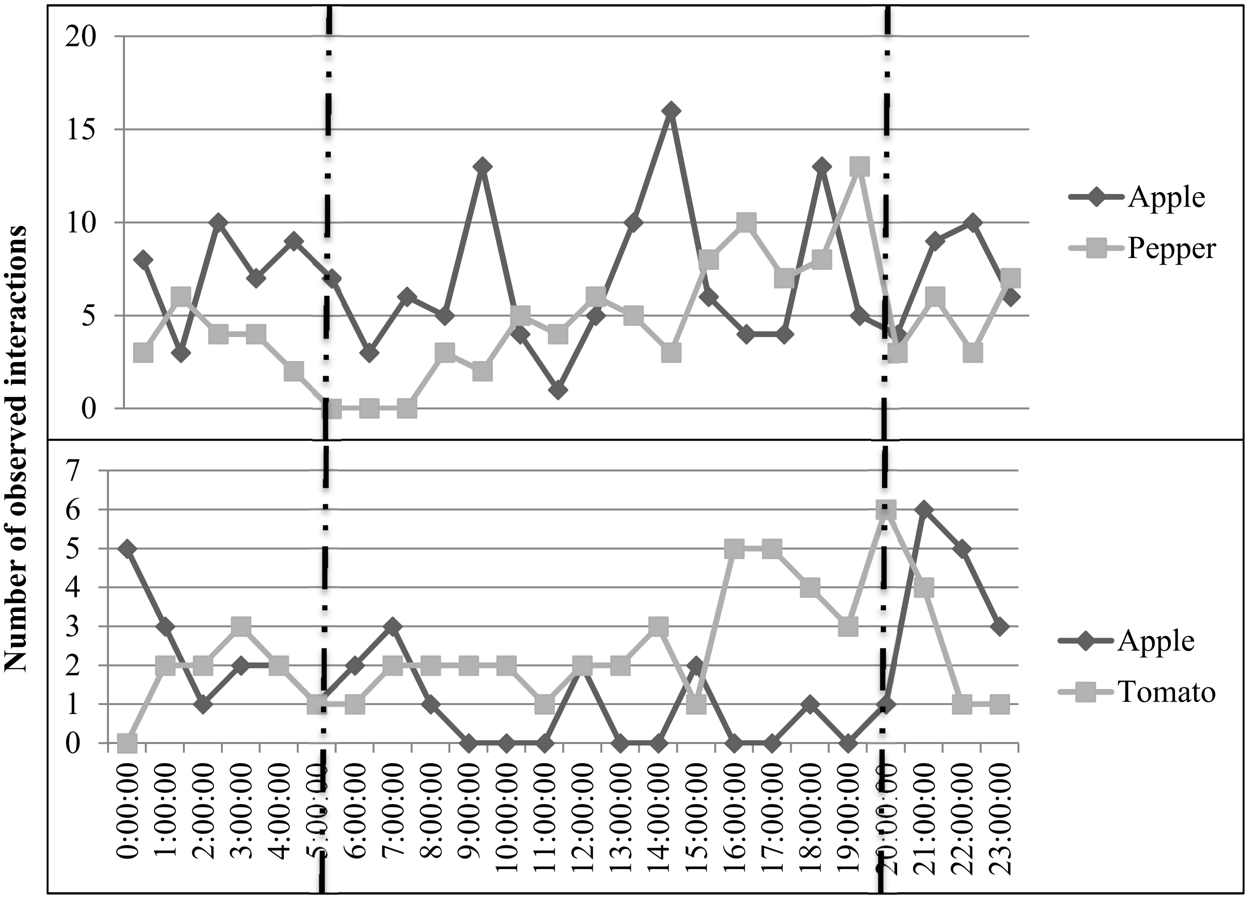


Figure 2.10. The time of day each observed arthropod began its interaction with the egg mass, adjusted for solar noon. Includes damaging and non-damaging events. Dashed lines denote average sunrise/sunset. In 2013, the majority of events started at night while the majority of events started during the day in 2014.

In contrast with 2013, the majority of events, 54%, were observed during the day in 2014. There is a noticeable increase in the number of observed visits in the peppers starting at sunrise and continuing until sunset when the number of visits drops (Figure 2.10). In pepper, there is one peak in activity at sunset, 1900 h. The arthropods responsible for this peak are primarily parasitoids followed by Coccinellidae larvae. The trend of the apple crop in 2014 was more oscillating with multiple peaks. The largest peak in the apples at 1400 h was the overall largest peak. The arthropods responsible for this belong primarily to Formicidae.

Table 2.5. The time of day each predator attacked *H. halys* eggs, adjusted for solar noon. Both years of deployment combined. Time of attack covers both the scoto- and photophase.

Time	Acrididae	Anthocoridae	Araneae	Coccinellidae	Forficulidae	Gryllidae	Miridae
0:00:00			X		X		
1:00:00			X		X	X	
2:00:00					X		
3:00:00					X		
4:00:00	X		X		X	X	
5:00:00	X		X		X	X	
6:00:00	X						
7:00:00	X						
8:00:00							
9:00:00							
10:00:00							
11:00:00							
12:00:00				X			
13:00:00				X			X
14:00:00							X
15:00:00							
16:00:00							
17:00:00							
18:00:00							
19:00:00							
20:00:00		X	X		X		
21:00:00		X	X		X		
22:00:00		X	X		X		
23:00:00			X		X		

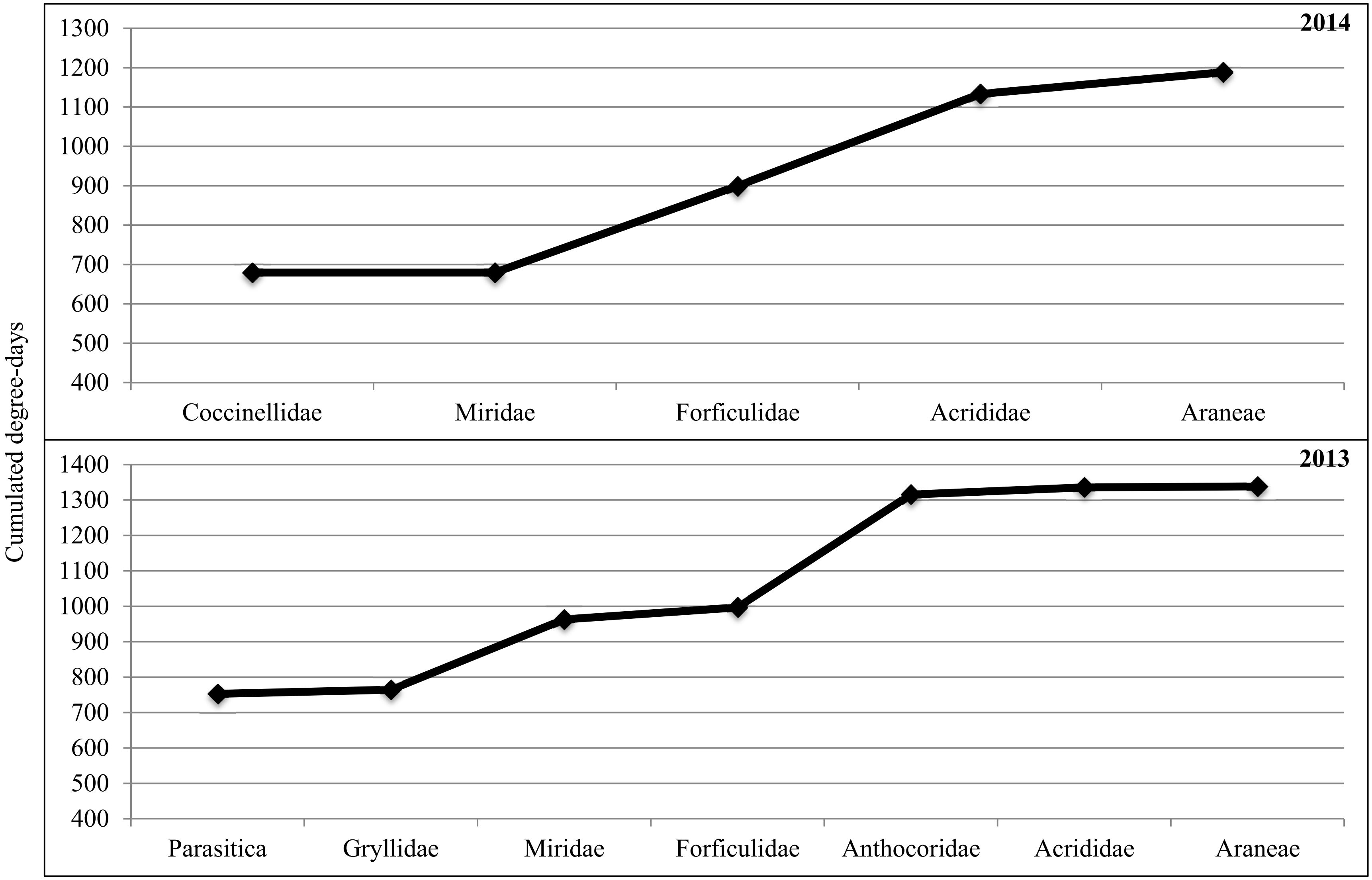


Figure 2.11. Cumulative degree-days of each predator attack on *H. halys* eggs in 2013 and 2014. Degree-day base temperature is 14°C starting from Jan 1 each year.

The five egg predators that consumed *H. halys* eggs in 2014 were observed across a wide range of the day (Table 2.5). Acrididae were observed during the 1600 h, this was not different from 2013. Araneae and Forficulidae were observed during the scotophase from 2000 to just before sunrise at 0500. Coccinellidae and Miridae were each observed exclusively during the photophase at 1200 h.

Seasonality among predators differed for each year of deployment with predation events in 2014 occurring after a smaller number of cumulated degree-days than in 2013 (Figure 2.11). The first predation events in each year did not occur until after 600 cumulated degree-days in 2013 and 700 in 2014 (2013: 679.4 DD; 2014: 752.7 DD). The predators responsible for these events were Coccinellidae, Gryllidae, Miridae, and Parasitica. A higher number of degree-days accumulated in 2013 but the predators that attacked eggs at the higher end of the accumulated degree-day range were the same in both years; they were Acrididae and Araneae.

4. Discussion

Understanding the impact of natural enemies on invasive species early in the invasion process will give insights into the population dynamics of the species and the ability of the environment to implement top-down pressure on the invader. These insights can help determine whether the use of native natural enemies as a source of biological control can slow the invasion process or provide control of the pest once established.

We found the baseline level of biological control exerted by Michigan's natural enemy community to be < 1% when not including eggs that went missing. Using video observations, we are able to examine the fate of missing eggs and reject the hypothesis that all missing eggs are the result of chewing predators leaving no trace of eggs behind. We can infer the number of missing eggs due to arthropod interaction for eggs that were not under video surveillance using

95% confidence intervals. The baseline level of biological control will increase to a minimum of 2.2% and a maximum of 3.1% when including those missing eggs that were likely to have been consumed without leaving a trace behind based on video observations.

Egg damage was consistent across locations, which could indicate little to no differences in the potential for *H. halys* suppression across agroecosystems at the local level. Differences in predator diversity and abundance between locations and egg damage among crop types could be due to landscape factors including complexity and crop diversity. These factors were not quantified in this study but have shown to be important for natural enemy communities (Bianchi et al. 2006; Werling and Gratton 2008; Gardiner et al. 2009) and should be further explored in this system.

Predators were more prevalent than parasitoids in this study, accounting for 64.9% of all biological control. We observed a greater number of chewing predators than sucking predators attacking *H. halys* eggs. This result is consistent with other studies reporting predation on native stink bug eggs (Yeargan 1979; Tillman 2011) and *H. halys* eggs (Morrison et al. 2016a; Ogburn et al. 2016).

Video analysis revealed a complex of generalist predators that will interact with and attack *H. halys* egg masses. Generalist predators have been recognized as an important regulator of insect populations in various ecological systems (Jones 1995; Symondson et al. 2002; Messelink et al. 2010). We were able to corroborate the identity of predators previously observed attacking *H. halys* eggs in the field such as: Anthocoridae, Araneae, Coccinellidae, and Forficulidae (Rice et al. 2014; Ogburn et al. 2016). Gryllidae will attack *H. halys* eggs in laboratory testing, consuming 10% of masses given (Morrison et al. 2016a), and we can provide evidence that gryllids will also attack eggs in the field. Acrididae have been observed attacking native stink bug eggs and we can show they will attack *H. halys* eggs as well (Tillman 2011; Olson and Ruberson 2012). Tettigoniidae is another Orthopteran Family that has been described attacking *H. halys* eggs in the laboratory and the field (Morrison et al. 2016a; Poley et al. unpublished), although we did not observed tettigoniids in Michigan. We are the first to describe the Family Miridae as an *H. halys* egg predator.

The predators we observed have proved successful as biological control agents in other systems. Dermaptera were observed most frequently in our study but consumed only 11 eggs. Earwigs are a significant predator on the eggs and active stages of a variety of insect pests and have been considered as biological control agents in apple orchards in New Zealand. Earwigs have the attributes of a successful predator including wide distribution and high abundance and predation rates (Buxton 1974; Suckling et al. 2006). In addition, researchers in the United Kingdom found densities of earwigs in apple canopies were highest from July- September (Alford et al. 1980), which coincides with the highest *H. halys* populations (Hoebeke and Carter 2003). Understanding and predicting the predation efficiency of earwigs on *H. halys* eggs and nymphs is critical before consideration as a significant biological control agent in Michigan's agroecosystems.

Araneae were the only predators observed in each crop type at both locations. The impact of spiders on various prey species is well known (Riechert and Lockley 1984; Symondson et al. 2002) but spiders rarely show specificity toward one particular prey. Although functional response curves of spiders have high plateaus indicating effective density dependent response, the sit-and-wait prey capture tendencies of spiders (Riechert and Luczak 1982; Riechert and Lockley 1984) may make them unlikely candidates for significant *H. halys* suppression at the sessile egg stage. We also observed insect predators that are readily available for release as biological control agents in Michigan including Anthocoridae and Coccinellidae. Members of both families are frequently used in the control of numerous insect pests at the egg stage (Barber 1936; Obrycki and Kring 1998; Quarles 2015).

Video surveillance revealed predators could be attributed to the fate of 51% of missing eggs at most, which leaves roughly half of missing eggs unaccounted for. Alternative explanations for missing eggs include the actions of insects visiting or preying on the egg mass cause the egg to come loose and drop from the leaf or abiotic factors including rain or wind causing eggs to fall away; these explanations should be explored further.

Parasitoids were observed on video more frequently in 2014 with a total of 149 events recorded and an average visitation time of 49 min. However, less parasitism of eggs was recorded that year than in 2013. The parasitoids that emerged out of sentinel egg masses in 2013 are native species with generalist foraging behaviors. These species have previously been recovered from *H. halys* egg masses (Cornelius et al. 2016; Ogburn et al. 2016). The small number of parasitoids in 2013 and the lack of emerged parasitoids in 2014 highlights potential maladaptation to this novel host or a potential lack of available host finding cues for female parasitoids.

One explanation for low parasitism rates is the use of colony-reared egg masses instead of naturally laid masses. Jones et al. (2014) found higher rates of parasitism in naturally laid *H. halys* masses (30 to 35%) over sentinel masses (<5%), possibly due to lack of associated semiochemicals. Herlihy et al. (2016) found higher parasitism rates in frozen *H. halys* egg masses (89.3% of frozen masses) over fresh masses, the opposite of what we observed. This supports the hypothesis that *H. halys* is not a suitable host for native parasitoids and will not be effectively controlled by them at this time. Landscape factors such as habitat connectivity and complexity may be affecting the success of parasitoids at our study sites. Parasitism in 2013 occurred only at Three Brothers Farm which is surrounded on three sides by the wooded areas of a nature center. Conversely, the MSU Student Organic Farm, which saw no parasitoid emergence in either year, is surrounded by urban areas and agricultural fields. Several studies have demonstrated that Pentatomoidea-associated parasitoids native to the United States prefer forested habitats (Jones et al. 2014; Talamas et al. 2015; Cornelius et al. 2016; Ogburn et al. 2016). The Asian parasitoid responsible for significant *H. halys* population control in Asia (Yang et al. 2009), *Trissolcus japonicus* (Ashmead) (Hymenoptera: Platygastridae), was detected in the United States only in woody habitats in a sentinel egg study; however population levels of this species may be too low to declare habitat preference at this time (Talamas et al. 2015). Despite success of native parasitoids emerging from eggs in 2013, there was no parasitism recorded in 2014 at Three Brothers Farm, perhaps indicating there are other factors working against parasitism in our system.

The accidental introduction and spread of the Asian parasitoid, *T. japonicus*, could have implications on *H. halys* population control. *T. japonicus* is a significant source of *H. halys* control in its native range (Yang et al. 2009) and has been tested in quarantine facilities in the US for release as a biological control agent since 2007. It was first detected in sentinel eggs in Maryland (Talamas et al. 2015) and has since been found in eight states - Washington DC, DE, MD, NY, NJ, PA, VA, WA. If *T. japonicus* continues its spread across the country, it could become a significant source of *H. halys* population control.

Sixty-two percent of eggs deployed were undamaged by natural enemies and the *H. halys* hatch rate over both years of deployment from viable eggs was 50.4%. The high number of non-damaging interactions by arthropods suggests that *H. halys* eggs are not a preferred prey source in Michigan at this time. Native natural enemies have not been enough to keep *H. halys* populations below economic thresholds in the Eastern United States where *H. halys* has been an established pest for 15 yrs (Leskey et al. 2012b). *H. halys* is a novel food source for natural enemies in Michigan's agro-ecosystems. The impact of natural enemies on this pest in Michigan was previously unknown and our results suggest that there is little potential for native natural enemies to provide effective control at this point in the invasion.

In response to an invasion, native natural enemies that once interacted without damaging egg masses may change their diets and adapt to using the novel prey as a viable food source, however there may be a considerable lag time before these natural enemies become an effective source of population control (Carlsson et al. 2009) The natural enemies we observed may exert more pressure on this novel pest in time (Carroll et al. 2005; Grabenweger et al. 2010). However, host switching by natural enemies could lessen or potentially eliminate top-down pressures on indigenous pests.

As *H. halys* populations become damaging in Michigan's agroecosystems, sustainable management options need to be developed. Habitat conservation and manipulation will be crucial to the long-term success of natural enemies. Alterations in pesticide applications and the addition of weed strips or other habitat manipulations can conserve and enhance natural enemy populations (Hajek 2004).

This study represents the first attempt at identifying native natural enemies of *H. halys* in a state where pest populations are low. Few studies have been done using predators of *H. halys* nymph or adults and this is something that should be explored as management programs are developed **(**Ragsdale et al. 1981; Stam et al. 1987; Tillman 2008, 2010; Abram et al. 2015). Our current baseline data will be used in further natural enemy trials as we begin to develop recommendations for the control of *H. halys*. Further predator feeding investigations are necessary to determine the efficiency at which identified predators will suppress *H. halys*.

CHAPTER 3

Functional response of generalist predators to *Halyomorpha halys* eggs under laboratory conditions

1. Introduction

Arthropod predators are an important mortality factor for insect pest populations (Price 1997; Luck 1984; DeBach and Rosen 1991; Symondson et al. 2002) that have been demonstrated to be important sources of biological control for insect pests (Price 1997; Chang and Kareiva 1999). Generalist predators are important sources of biological control due to their ability to persist on alternative prey in the absence of the target pest (Curry 1993; Symondson et al. 2002; Messelink et al. 2012) and for early-season build-up of populations that allows for attack on pests during colonization (Curry 1993; Wiedenmann and Smith 1997).

Understanding the effects of generalist predators on prey populations is key to developing sound pest management practices. The functional response of a predator defines the relationship between rate of prey consumption and prey density (Solomon 1949), and allows the estimation of the daily maximum number of prey that can be attacked per predator (Holling 1959a). Functional response experiments aim to understand the efficiency of potential predators and are a common method of assessing if a predator is a suitable biological control agent (Isikber 2005) by determining whether or not the predator contributes a stabilizing factor in predator-prey population dynamics (Murdoch and Oaten 1975; Juliano 2001).

There are three common functional response types, defined mathematically by Holling (1959b). Each type reflects differences in the proportion of prey killed during a fixed timeframe. Type I responses are the simplest mathematically. Predators with a Type I response show an increasing linear response where the number of prey killed per predator is directly proportional to prey density until satiation is reached (Hassell 1978). The slope reflects the predator's searching efficiency. This type of response is density-independent and is not typically associated with arthropod predators. In a Type II functional response, the number of prey consumed approaches an asymptote hyperbolically as prey density increases. The asymptote is the maximum attack rate. Type II includes a measure of handling time, which refers to the time it takes a predator to pursue, subdue, consume, and recover (Holling 1959b).

The third form of functional response, Type III, is sigmoidal in shape with a slowly increasing attack rate at low densities (Hassell et al. 1977). As the natural enemy gains experience the attack rate quickly increases until a maximum point of satiation is reached. This type of response is density-dependent; predators respond to higher prey densities by consuming an increasing proportion of available prey. Type III responses are often associated with prey switching by the predator (Schenk and Bacher 2002) and natural enemies of this Type may be considered effective biological control agents because pest populations are regulated as densities increase (Murdoch and Oaten 1975; Hassell et al. 1977). Beneficial arthropod predators are typically classified as Type II or Type III (Murdoch and Oaten 1975; Luck 1984; Fernández-Arhex and Corley 2003).

Halyomorpha halys (Hemiptera: Pentatomidae) is expanding its range in North America and Michigan is on the leading edge of the invasion. Targeting biological control efforts at recently colonized areas in advance of the main body of the invasion could have implications for slowing the spread of the invasion (Hajek et al. 1996; Fagan et al. 2002). Generalist predators have been shown to delay or eliminate the spread of invading species when applied to the nascent foci (Cook et al. 1995; Ehler 1998). Potential generalist predators of *H. halys* eggs have been identified. Predators in the families Acrididae, Anthocoridae, Coccinellidae, Forficulidae, Gryllidae, Miridae, Tettigoniidae, and the Order Araneae have been observed attacking *H. halys* eggs in previous field and laboratory studies (Morrison et al. 2016a; Poley 2017a). *H. halys* egg susceptibility to predatory pentatomids and various instars of Chrysopidae have also been tested (Abram et al. 2015). Our objective was to determine the relative efficiency of generalist predators as biological control agents to *H. halys* egg masses by determining their functional response in a laboratory environment.

2. Materials and Methods

We conducted replicated laboratory experiments using colony-reared *H. halys* egg masses and generalist predators to explore the efficiency of these predators as density-dependent mortality factors. These experiments took place in a laboratory at Michigan State University (42.721750, -84.474531). We used a randomized complete block design with six blocks and four treatments. A positive control of specific predator diet and a negative control void of any food source were included in each replication. When available, 60 individuals of each predator were tested. If tested individually by sex, 60 individuals of each sex were tested.

2.1. Arthropod culture

H. halys egg masses were collected in the method described in Ch. 2. In addition, egg masses were acquired from the Delfosse Biological Control Laboratory at Michigan State University. Egg masses were frozen at < 24 h of age and stored in a -80°C freezer until use in experiments. Predators were kept in an insectary with controlled environmental conditions: 16L:8D photoperiod, 24°C, and 60-70% RH.

Predators were chosen for preliminary testing based previous *H. halys* egg predator results (Poley 2017a). *Phidippus audax* Hentz (Araneae: Salticidae) and *Forficula auricularia* Linnaeus (Dermaptera: Forficulidae) were hand-collected from Three Brothers Farm in Lansing, MI (42.704853, -84.517606). *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) larvae and adults and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) adults were hand-collected from the Michigan State University campus (42.721254, -84.474087). *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae), *Melanoplus femurrubrum* DeGeer (Orthoptera: Acrididae), and *Conocephalus fasciatus* DeGeer (Orthoptera: Tettigoniidae) were collected using sweep nets from the Michigan State University Entomology Farm (42.690943, -84.498095). *Orius insidiosus* Say (Hemiptera: Anthocoridae) were purchased from Koppert Biological Systems (Howell, MI). *Acheta domesticus* Linnaeus (Orthoptera: Gryllidae) were purchased from Petco Animal Supplies in Lansing, MI. *Podisus maculiventris* Say (Hemiptera: Pentatomidae) were colony-reared under the conditions described above and using *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) as a food source.

2.2. Preliminary predator testing

Predators were screened for their initial efficiency before use in functional response experiments. After a 24 h starvation period, predators were placed in a 473.18 ml (16 oz) deli cup (Deli Serve, WNA, Chattanooga, TN) arena with 110 mm filter paper (Cat No 1004 110, Whatman®, Pittsburgh, PA) and an egg mass affixed to the bottom using permanent double-sided tape (Scotch™, 3M, St. Paul, MN). The number of eggs per mass were not standardized and ranged from 20 to 28 eggs. Arenas were left under insectary conditions for 24 h. If no eggs were attacked in the first 24 h, predators were left for a second 24 h period, for a total of 48 h in the arena. *F. auricularia* and *P. audax* were kept in arenas until feeding or death occurred. Preliminary predator testing was not under video surveillance.

2.3. Functional response

Four predators were chosen for functional response testing based on results of preliminary testing: *A. domesticus*, *O. insidiosus*, *M. femurrubrum,* and *C. fasciatus*. Individual predators were randomly assigned to one of four prey densities. The range of densities tested was 26 eggs (one mass), 52 eggs (two masses), 78 eggs (three masses), and 104 eggs (four masses). Frozen *H. halys* egg masses were standardized at 26 eggs per mass. This number of eggs was chosen because it was the average number of eggs per mass produced by our colony. Egg masses with > 26 eggs were pared down to reach the target density. The egg mass was soaked in deionized water for 30 s to loosen the individual eggs from surrounding eggs and the bottom substrate. Eggs were then removed using narrow-tipped featherweight forceps (BioQuip, Rancho Dominguez, CA) without damaging the remaining eggs on the mass. Each egg mass was inspected under a dissecting microscope before use in experiments to ensure eggs were undamaged.

The average length of time an egg mass was stored in the freezer varied for each predator: 8.34 wk for *A. domesticus*, 17.3 wk for *O. insidiosus*, 17.9 wk for *M. femurrubrum*, and 18 wk for *C. fasciatus*. Egg masses were placed on 3.96 x 22.22 mm fender washers using double-sided tape. Sterilized sand was applied to the excess tape areas. Fender washers were used to weigh down the egg masses so the predator could not manipulate the masses and carry them out of the video frame. Washers were not used during *O. insidiosus* experiments because they created an obstacle for the predator. Egg masses for *O. insidiosus* were affixed directly to filter paper on the bottom of the arena using non-toxic glue (Elmer’s Products Inc., Columbus, OH).

Egg masses were arranged 5.08 cm apart in the center of the arena (Figure 3.1). For predators where sex was included as a factor (*A. domesticus* and *M. femurrubrum*), 10 individuals of each sex were exposed to each prey density. Due to difficulty in determining sex of individuals, 10 individuals were tested at each prey density for *O. insidiosus.* Due to prey availability and difficulty rearing, six females of *C. fasciatus* were tested at each density.

Predators were starved and without water for 24 h prior to experiments. Arenas differed among predators due to camera visibility and size of the arena. *A. domesticus*, *M. femurrubrum,* and *C. fasciatus* were placed in polyethylene terephthalate (PET) plastic arenas measuring 19.05 x 17.78 x 10.16 cm (Paper Mart, Orange, CA). Sterilized play sand evenly lined the bottom of the arena at approximately 1 cm in depth to create a natural substrate. Sand was

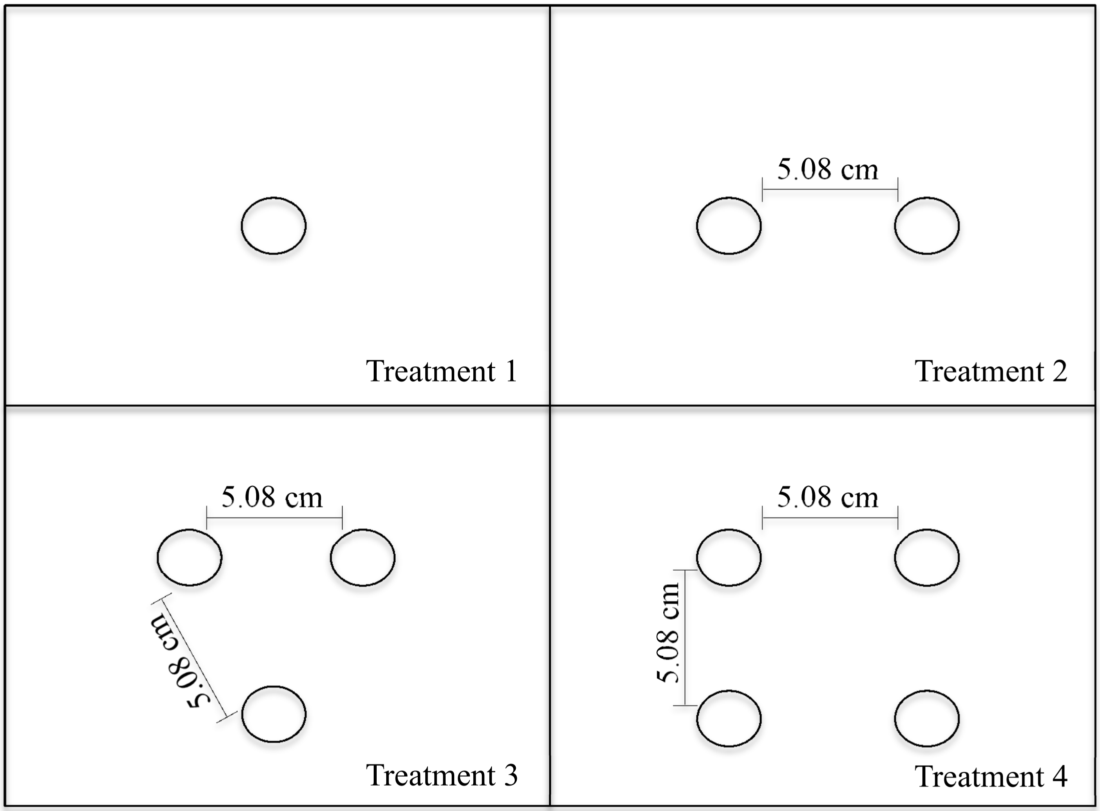


Figure 3.1. Arrangement of egg masses inside arenas for treatments 1-4. Egg masses were placed 5.08 cm apart in all directions. The positive control was placed in the manner of Treatment 1.

cleaned sterilized by rinsing three times with deionized water and then baked in a drying oven at 93°C for 4 h.

The arena for *O. insidiosus* was a 100 wide x 15 mm high Petri dish with moist filter paper as a substrate. Arenas were sealed shut using Parafilm® stretched around the inside rim of the lid and modeling clay around the outside seam to keep humidity levels high and prevent predators from escaping. A mesh-covered vent was cut into the lid of the dish to prevent condensation build-up from obscuring the video image.

Predators were removed from arenas after 24 h. Prey was not replaced throughout the experiment. Experiments were conducted under the same environmental conditions as predator storage. Each experiment contained a negative control void of any food source and a positive control with a food source specific to the predator tested. Positive control food sources were as follows: 1 g Fluker's® Cricket Diet (Petco Animal Supplies, Lansing, MI) for *A. domesticus*; 1 larval *G. mellonella* (Grand River Bait & Tackle, Lansing, MI) for *O. insidiosus*; 1 g TetraFin® Goldfish Flakes (Tetra Holding Inc., Blacksburg, VA) for *M. femurrubrum* and *C. fasciatus*.

Experiments were under video surveillance for the entire duration in order to calculate handling time of the predator. The video surveillance system outlined in Ch. 2 was modified for use in the laboratory (Figure 3.2). Modifications included the use of a 12 v AC to DC power supply as the power source. Cameras were placed directly above the arenas with the entire arena in the field of view. To reduce the amount of glare on the video, the lights were removed from the recording camera and a second camera was angled away from the arena to provide infrared light during the nighttime hours.

Due to the small size of *O. insidiosus*, handling time was collected through direct observation. Experiments were directly observed for 4 h after placement in the arena. The time

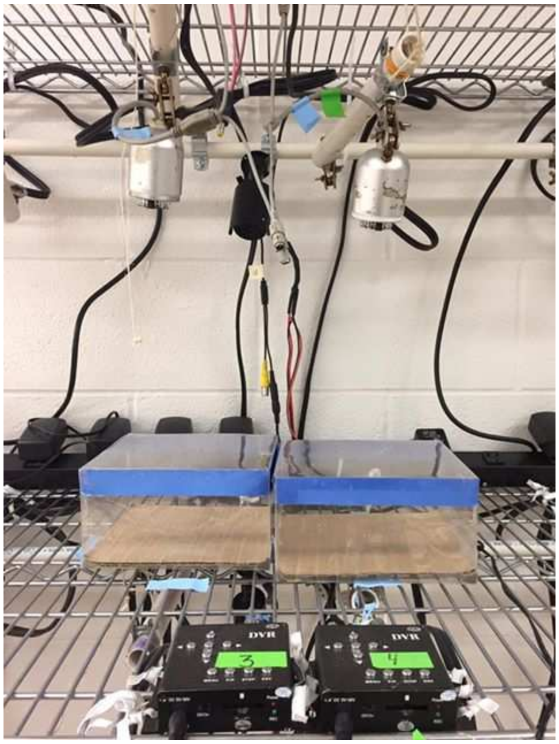


Figure 3.2. Functional response experiment video set-up. Cameras were placed directly above arenas with an additional camera placed behind and angled away to provide infrared light during nighttime hours.

from placement to discovery of the first prey item was recorded as well as the length of attack for that first prey item. After that, the time between discovery of the first and second prey item and the length of attack of the second prey item were recorded. This process was repeated until four prey items were attacked or until 4 h had passed. In addition, arenas were directly observed 12 h into the experiment for 1 h.

The number of eggs remaining after 24 h were counted and pictures were taken of each egg mass using a digital camera. Pictures of eggs attacked by *O. insidiosus* were taken using a microscope camera (Dino-Lite Digital Microscope, Torrance, CA) to see the stylet sheath. Damage was defined as puncturing the egg chorion.

2.4. Data analysis

A one-way analysis of variance (ANOVA) was conducted to compare differences in the mean predation rate in 24 h at each prey density. The predation rate is defined as the number of prey attacked per predator per 24 h. When the overall ANOVA was significant, post hoc multiple comparisons among treatments were made using Tukey's Honest Significant Difference (Tukey 1953). A t-test was performed to compare average predation rates between sexes of the same predator when applicable. The level of significance for all tests was set to $P = 0.05$.

2.4.1. Attack rate and handling time

Video observations allowed us to estimate the attack rate (a) and handling time (T_h) of each predator tested, with the exception of *O. insidiosus,* which was directly observed. The attack rate coefficient (a) is a measure of searching efficiency of the predator and is found by solving eqn (3) for a (Hassell et al. 1977) or as follows:

$$a = \frac{-\ln(1-\frac{N_e}{N_0})}{T-T_h N_e} \quad \textbf{(1)}$$

The attack rate was calculated for each recording of an attack event. The value of T was set to 24 h and handling time estimates were obtained from direct or video observations. Spearman's rank correlation (Spearman 1904) was used to analyze for density dependence of a and T_h. The slope of the attack rate relative to prey density can be used to discriminate among functional response types. The observed handling times of each predator were compared using 95% confidence intervals. Overlapping 95% confidence intervals are considered not significantly different. Observed a and T_h coefficients were compared to model outputs using a t-test.

2.4.2. Model fitting

Discriminating between functional responses of Type II and III can be difficult (Trexler et al. 1988), therefore model fitting was completed in two steps. A polynomial function was first fit to the data to determine the shape of the curve (Juliano 2001). A logistic regression of the proportion of eggs eaten (N_e/N_0) as a function of initial prey density (N_0) can provide a reliable determination (Trexler et al. 1988).

$$\frac{N_e}{N_0} = \frac{exp(P_0+P_1N_0+P_2N_0^2+P_3N_0^3)}{1+exp(P_0+P_1N_0+P_2N_0^2+P_3N_0^3)} \quad \textbf{(2)}$$

Where P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively. Coefficients were estimated using the method of maximum likelihood. A significant negative or positive linear coefficient (P_1) will determine whether the data fits a Type II or Type III curve, respectively (Juliano 2001). After the shape of the curve was determined, the functional response parameters handling time and attack rate were estimated using the appropriate functional response equation. Experiments were conducted with prey depletion, therefore the “random predator equation” is the appropriate model because it accounts for changing prey densities (Rogers 1972).

$$N_e = N_0[1 - \exp(-a(T - T_hN_e))] \quad \textbf{(3)}$$

Where N_e is the number of prey consumed by the predator, N_0 is the initial prey density, a is the attack rate, T is the amount of time the predator is exposed to the prey, and T_h is the handling time associated with each prey consumed. Eqn (3) models a Type II response with a constant attach rate (a).

Holling’s (1959b) disc equation (4) was also fitted to the data if the polynomial model (2) suggested a Type II relationship. The fit of eqns (3) and (4) were compared using AIC values. Holling’s disc equation assumes unchanging prey density (Royama 1971; Rogers 1972) and therefore is expected to have a lower fit to our dataset.

$$N_a = \frac{aTN_t}{1+aT_hN_t} \quad \textbf{(4)}$$

Here, N_a is the number of prey encountered, a is the attack rate, T is the amount of time the predator is exposed to the prey, T_h is the handling time, and N_t is the total prey available.

3. Results

3.1. Preliminary predator testing

Of the 10 predator species initially tested, six species consumed ≥1 egg (Table 3.1). The predators that consistently attacked the greatest number of eggs per mass, from highest to lowest, were *A. domesticus*, *M. femurrubrum*, *C. fasciatus* and *O. insidiosus*. Each *P. audax* individual attacked > 50% of the egg mass, however no individuals attacked eggs after 48 h in the arena, instead attacking only after left in the arena for 7 d or longer. One *F. auricularia* attacked the egg mass, this individual was kept in the arena for 5 d and attacked two eggs.

Table 3.1. Results of preliminary predator testing before inclusion in function response experiments. *Indicates egg attack did not occur within the 48 h period given to all predators.

Predator	n tested	n attacked	eggs given	eggs attacked
Acheta domesticus	6	6	158	149
Coleomegilla maculata	6	0	143	0
Conocephalus fasciatus	5	3	125	47
Forficula auricularia	10	1	259	2*
Harmonia axyridis Adult	6	0	153	0
Harmonia axyridis Larvae	3	0	80	0
Lygus lineolaris	5	0	136	0
Melanoplus femurrubrum	6	5	156	86
Orius insidiosus	6	5	150	9
Phidippus audax	3	3	82	69*
Podisus maculiventris	5	0	129	0

3.2. Attack rate and handling time

There were significant differences in the predation rate among treatments for *A. domesticus* males and females (*male*: $F = 12.87$, $df = 3$, $P < 0.001$; *female*: $F = 14.99$, $df = 3$, $P < 0.001$, ANOVA), *O. insidiosus* ($F = 2.90$, $df = 3$, $P = 0.04$, ANOVA), and *M. femurrubrum* females ($F = 3.28$, $df = 3$, $P = 0.03$, ANOVA). There were no significant differences in predation rate for *M. femurrubrum* males ($F = 1.23$, $df = 3$, $P = 0.31$, ANOVA) or *C. fasciatus* ($F = 2.18$, $df = 3$, $P = 0.12$, ANOVA) (Table 3.2). The predation rate of *M. femurrubrum* females was significantly higher than males ($t = -1.94$, $df = 69.05$, $P = 0.055$ t-test). There was no significant difference in predation rate between sexes of *A. domesticus* ($t = -0.62$, $df = 77.66$, $P = 0.53$ t-test).

Handling time estimates from video observations and calculated attack rates are given for each predator in Table 3.2, with the exception of *O. insidiosus*, which was never observed attacking eggs during the 5 total h of direct observation. Spearman's rank correlation showed observed handling time significantly decreased with increasing prey density for *A. domesticus* males ($r_s = -0.51$, $P = 0.0002$) and females ($r_s = -0.54$, $P = 0.0007$), and showed a weak positive correlation for *C. fasciatus* ($r_s = 0.45$, $P = 0.0282$). Handling time was not different between sexes of *A. domesticus* ($t = -.077$, $df = 53.17$, $P = 0.44$, t-test) or *M. femurrubrum* ($t = -.053$, $df = 67.28$, $P = 0.59$, t-test). The handling time of *M. femurrubrum* males and females were significantly lower than any other predators. *A. domesticus* males and females demonstrate the longest handling time on average (Table 3.2).

Spearman's rank correlation also showed calculated attack rates significantly decreased with increasing prey density for *A. domesticus* males ($r_s = -0.59$, $P = 0.0002$) and females ($r_s = -0.72$, $P < 0.0001$). Density-dependence in attack rate suggests a Type II response for *A. domesticus*. There was no correlation between attack rate and prey density for any other predator. Attack rates were not different between males and females (*A. domesticus*: $t = 0.23$. $df = 68.9$, $P = 0.81$; *M. femurrubrum*: $t = 1.55$, $df = 38.7$, $P = 0.12$, t-test). Observed attack rates were greatest for *A. domesticus* and shortest least for *M. femurrubrum* females (Table 3.2).

Table 3.2. Mean number of *H. halys* eggs attacked at each density level for each predator tested, by sex when applicable (M for male, F for female). Letters in the same column represent statistically different means (ANOVA *a* = 0.05). Calculated attack rate and handling time also for each predator are also given. Letters in the same row represent non-overlapping 95% confidence intervals.

	Mean number of prey attacked after 24 h (+SE)					
Prey Density	*A. domesticus* F	*A domesticus* M	*O. insidiosus*	*M. femurrubrum* F	*M. femurrubrum* M	*C. fasciatus*
26	25 (1.0)a	25.2 (0.69)a	0.5 (0.52)a	8.2 (3.45)a	4.7 (2.48)a	5.2 (1.0)a
52	43.8 (2.47)b	44.2 (3.46)b	0.9 (1.28)ab	12.1 (5.32)ab	7.9 (3.33)a	0 (0)a
78	51.8 (6.49)bc	46.1 (5.75)bc	1.6 (2.06)ab	15.7 (5.68)ab	13.7 (3.99)a	5.7 (6.49)a
104	71.4 (7.02)d	63.9 (5.67)d	2.3 (1.56)b	29.6 (5.77)b	9 (3.44)a	9.2 (7.02)a
Attack rate (*a*) (95% CI)	4.10E-05a (2.9E-05-5.2E-05)	3.78E-05a (2.8E-05-4.8E-05)	NA	1.11E-05b (-4.3E-07-2.2E-05)	2.28E-06b (1.0E-06-3.4E-06)	1.41E-06b (6.5E-08-2.7E-06)
Handling time (*h*) (95% CI)	0.321a (0.25-0.38)	0.3797a (0.25-0.50)	NA	0.0581b (0.034-0.081)	0.0709b (0.034-0.107)	0.1577c (0.055-0.25)

3.3. Functional response

The functional responses of each predator species to *H. halys* eggs are given in Figure 3.3. The proportion of prey consumed decreased with increasing prey density, then increased at the highest prey density for *A. domesticus*, *O. insidiosus*, and *M. femurrubrum* females. This relationship suggests a Type III functional response. The proportion of prey consumed decreased with increasing prey density for *M. femurrubrum* males and *C. fasciatus*, indicating a Type II response.

Logistic regression of eqn (2), revealed significant linear parameters for *A. domesticus* females ($P < 0.1$) and males ($P < 0.05$), suggesting a Type II functional response (Table 3.3). The linear parameter for *O. insidiosus, M. femurrubrum* females, and *C. fasciatus* was negative but not significant, indicating weak evidence for a Type II response. The linear parameter for *M. femurrubrum* males was positive, suggesting a Type III functional response. However this parameter was not significant so a Type I response was fit to the data (Table 3.3).

All *A. domesticus* individuals tested consumed ≥1 egg from an egg mass, with the exception of one male. Considering all eggs given across replicates, 26.1% were left uneaten by females and 31% by males. When presented with one egg mass, females consistently consumed all eggs with the exception of one female that consumed 16 eggs. Males also consumed all eggs when given one egg mass except for two individuals that left one and seven eggs uneaten. The random predator eqn (3) provided a better fit but Holling's disc eqn (4) provided estimates of handling time and attack rate (Table 3.4). The theoretical maximum predation rates (T/T_h) for females was 189.36 eggs (±95.3), or roughly seven egg masses assuming 26 eggs per mass. For males, the theoretical maximum was 116.23 (±35.07), or 4.5 egg masses.

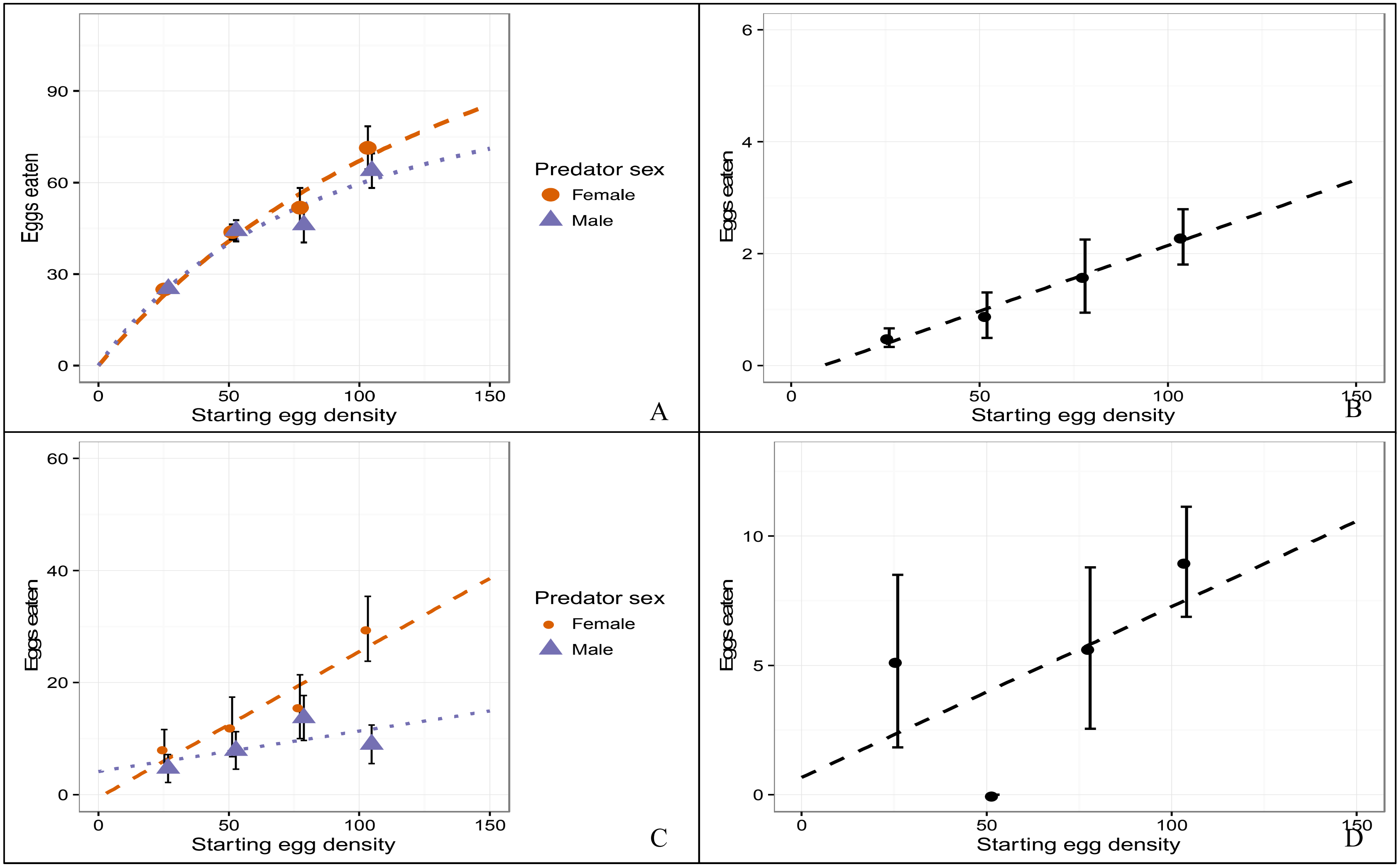


Figure 3.3. Functional response curves for A) *A. domesticus*, B) *O. insidiosus*, C) *M. femurrubrum*, and D) *C. fasciatus*

Table. 3.3. Results of logistic regression for each predator tested, by sex when applicable (M for male, F for female). A significant negative or positive linear coefficient will determines a Type II or Type III curve, respectively. *Indicates significant P value produced (*a* = 0.05).

Predator	Parameter	Estimate		SE		*t*		*P*	
		M	F	M	F	M	F	M	F
A. domesticus	Intercept	8.52	6.5	3.18	2.92	2.67	2.22	0.01*	0.03*
	Linear	-0.18	-0.13	0.08	0.07	-2.29	-1.7	0.02*	0.09
	Quadratic	0	0	0	0	2.1	1.56	0.04*	0.12
	Cubic	1.05E-05	5.10E-06	2.40E-05	2.20E-05	0.44	0.23	0.6	0.8
M. femurrubrum	Intercept	-1.82	0.16	1.34	1.31	-1.36	0.12	0.18	0.9
	Linear	0.01	-0.04	0.05	0.04	0.29	-0.8	0.7	0.3
	Quadratic	0	0	0	0	-0.44	0.85	0.6	0.3
	Cubic	-1.30E-05	3.80E-06	2.10E-05	2.30E-05	-0.61	0.16	0.5	0.8
O. insidiosus	Intercept	-3.83		1.05		-3.64		<0.001*	
	Linear	-4.27E-03		3.64E-02		-0.11		0.9	
	Quadratic	4.96E-05		2.72E-04		0.18		0.8	
	Cubic	-3.73E-06		1.63E-05		-0.22		0.8	
C. fasciatus	Intercept	2.1		3.37		0.62		0.5	
	Linear	-0.16		0.15		-1.05		0.3	
	Quadratic	0		0		0.97		0.3	
	Cubic	NA		NA		NA		NA	

Of the 40 *O. insidiosus* tested, 25 consumed ≥1 egg and 11 consumed > 1 egg. The maximum number of eggs consumed by one predator was seven and the mean was two. Logistic regression indicated *O. insidiosus* exhibit a weak Type II response. The random predator equation provided the best fit but no viable estimates for T_h could be produced. A Type I response produced a significant slope (Table 3.5).

Table 3.4. Parameter estimates from Holling's Disc equation for a Type II functional response.

		A. domesticus	
Sex	a±SE	T_h±SE	T_t/T_h
Male	1.22±0.29	0.206±0.048	116.22
Female	1.03±0.23	0.126±0.048	189.36

Table 3.5. Parameter estimates from linear model for a Type I functional response (M for male, F for female). *Indicates a significant parameter ($a = 0.05$).

Predator	Intercept ±SE	Slope ±SE	*P*	r^2
M. Femurrubrum M	4.15 ± 4.12	0.071 ± 0.05	0.22	0.013
M. femurrubrum F	-0.55	0.26	0.005*	0.16
O. insidiosus	-0.2	0.023	0.004*	0.17
C. fasciatus	0.66	0.06	0.16	0.04

Of the 40 *M. femurrubrum* females tested, 22 attacked eggs during the experiment. Females consumed all eggs in a mass on 12 occasions, and had a mean predation rate of 59.7% per mass. As with *O. insidiosus*, *M. femurrubrum* females exhibit weak evidence for a Type II response but no predictive parameters were produced. A Type I linear response produced a significant slope (Table 3.5). The same proportion of *M. femurrubrum* males attacked eggs as females, but linear regression provided no evidence for a Type II response so a Type I response was fit; this did not produce significant slope. Males consumed an entire egg mass on four occasions and the mean predation rate was 40.9% per egg mass.

Ten of the 24 *C. fasciatus* tested attacked ≥1 egg. There were no egg masses completely consumed. Maximum consumption across all replicates was 69% of one mass, with a mean of

32.6% eggs per mass. Linear regression showed weak indication for a Type II response but this this did not produce viable estimates. A Type I response was fit to the data and the result was not significant.

Viable (non-negative) estimates for handling time and attack rate were produced only for *A. domesticus* (Table 3.3). Handling time estimates produced by the model were significantly lower than estimates determined through video observations for both sexes of *A. domesticus* (*male*: $t = 2.81$, $df = 37$, $P = 0.007$; *female*: $t = 5.81$, $df = 39$, $P < 0.0001$ one-tailed t-test). The same is true for attack rate (*male*: $t = -239757.4$, $df = 34$, $P < 0.0001$; *female*: $t = -204409.7$, $df = 35$, $P < 0.001$ one-tailed t-test). The handling time and attack rate given by the model is higher for *A. domesticus* males but the observed estimates are higher for females.

4. Discussion

Determining the functional response of natural enemies will improve our understanding of their efficiency at potential population regulation. *H. halys* is a new threat to Michigan's agroecosystems and the potential for a biological control program utilizing native natural enemies is uncertain. Understanding the ability of generalist predators native to Michigan to be density dependent mortality factors will help guide pest management decisions for this novel pest.

Invertebrate predators are typically assigned a Type II functional response (Hassell et al. 1976), but our results suggest that, of the four predators tested, a Type II response curve describes the data of just *A. domesticus* well. We found weak evidence for Type II responses in *O. insidiosus* and *M. femurrubrum* females. When curve-fitting a Type II response, the Random Predator eqn (2) consistently provided a better fit but predictive estimates were produced by Holling's Disc eqn (3). We were not able to extract viable (non-negative) estimates of handling time or attack rate for *O. insidiosus* or *M. femurrubrum* females, indicating a Type I response better explained the data. *M. femurrubrum* males and *C. fasciatus* were not well-described by any response Type, suggesting the data are too variable or the range of prey densities was insufficient to obtain a significant result.

Population models using the Random Predator eqn (2) have shown that predators with a Type II functional response cannot contribute to the stability of pest populations (Murdoch and Oaten 1975), however Fernández-Arhex and Corley (2003) concluded that Types II and III may exert some degree of stabilizing force. Many studies have related functional response of natural enemies to their potential as biological control agents (van Lenteren and Bakker 1976; Hughes et al. 1992; Berryman 1999). In a review of literature on functional responses of biological control agents, Fernández-Arhex and Corley (2003) found there is no general relationship between success in biological control and the Type of functional response. This suggests that functional response is just one factor to consider when selecting for efficient biological control agents. Additional considerations of prey-predator dynamics such as prey preference, predator competition, and host patchiness can affect the efficiency of a biological control agent (Murdoch et al. 1985; Obrycki and Kring 1998; Pervez and Omkar 2005; Farhadi et al. 2010).

The attack rate and handling time estimated by the models and video observations were substantially different between and among predators, which indicates differences in each predator's ability to respond to changes in prey density. Differences in these values could be attributed to predator size, satiation time, and speed of digestion (Pervez and Omkar 2005). These factors should be included in further studies before considering these predators as part of a management program. The attack rate was highest at low prey densities for *A. domesticus* and *M.*

femurrubrum females. This relationship suggests these predators may be most efficient at suppressing pests at lower population densities.

Handling time estimated by functional response models includes time for resting, pruning, and digestion (Holling 1965; Hassel 1978), which we were unable to account for in our video observations. Despite this, our handling time estimates were longer than those produced by the model for *A. domesticus*, the only predator for which we were able to retrieve viable estimates. Montserrat et al. (2000) suggest that predators with a longer handling time may have a higher rate nutrient intake and a longer life span.

There were no significant differences between sexes of *A. domesticus* or *M. femurrubrum* for attack rate or handling time, however females of both species attacked a greater number of eggs than males. The ability of females to consume more eggs in a 24 h period could be attributed to differences in body size, delayed satiation (Mills 1982), faster digestion (Pervez and Omkar 2005), or reproductive processes like egg production (DeBach and Smith 1941). Future studies involving *A. domesticus* as predators for biological control should consider testing different life stages to test for differences in these factors.

We chose predators for preliminary testing based on results of sentinel egg studies and previous laboratory studies (Abram et al. 2015; Ogburn et al. 2016; Poley 2017a) that demonstrated egg predation. Lack of consumption in our preliminary testing does not necessarily indicate poor potential as predators of *H. halys* eggs. As *H. halys* pest densities increase in our agroecosystems and these predators gain more experience with eggs as prey, we may see more efficient predation, however there may be a considerable lag time before we can consider them as an effective source of population control (Carlsson et al. 2009).

Of the 10 predator species tested during preliminary experiments, *C. maculata*, *H. axyridis* adults and larvae, *L. lineolaris*, and *P. maculiventris* did not attack a single egg. Morrison et al. (2016a) also tested these predators against *H. halys* egg masses, with the exception of *L. lineolaris*, and saw minimal consumption in a laboratory environment. In that study, one *C. maculata* and one *H. axyridis* individual attacked an egg mass out of 11 and 21 individuals tested, respectfully. However, seven of eight *H. axyridis* larvae tested attacked eggs. *P. maculiventris* were tested as nymphs and five of 44 attacked the egg mass. Abram et al. (2015) also tested *C. maculata* and *P. maculiventris* in the laboratory and found that both would consume *H. halys* eggs, however they noted that *C. maculata* consistently attacked eggs but had difficulty breaking through the chorion.

We had moderate success with *P. audax* as an egg predator. Jumping spiders (Salticidae) have been found to attack sentinel egg masses in the field and are abundant in tree fruit where *H. halys* eggs are readily found (Morrison et al. 2016a). *P. audax* attacked eggs after 7 d or longer in the arena during our preliminary testing, suggesting either the need for a longer starvation period or that the tendency of jumping spiders to be sit-and-wait predators (Riechert and Luczak 1982) is not conducive to capturing stationary prey. *P. audax* and other species should be tested for their efficiency predating on other *H. halys* life stages.

Forficulidae species were the prominent predator observed in sentinel egg studies and attacked more eggs than any other observed predator (Poley 2017a). We were unsuccessful at demonstrating *F. auricularia* attack on eggs in the laboratory environment, where just one individual attacked two eggs in preliminary experiments. To try and understand the conditions necessary to induce *F. auricularia* attack, we manipulated the experimental environment. Manipulations included changes in starvation period, affixing the egg masses to synthetic plants to mimic the natural environment, day length and temperature changes, and competition where 2+ individuals were placed in the arena together. Recent studies have been successful at demonstrating Forficulidae spp. attack on *H. halys* eggs, where 56% of individuals tested attacked eggs (Morrison et al. 2016a). Forficulidae spp. have been cited as important predators of insect pests in tree fruit (Buxton 1974; Suckling et al. 2006) and should be further studied to examine predation efficiency against first and second *H. halys* instars.

Of the predators tested to determine functional response, *O. insidiosus* attacked the lowest proportion of eggs. *O. insidiosus* exhibited a weak Type II response but the data were better explained with a linear model. We can interpret this to mean that there is potential for this predator to have a stabilizing effect on *H. halys* eggs but it is unable to be clearly defined by this data. Coll and Ridgway (1995) suggest *O. insidiosus* predation efficiency is influenced by the structure of the host plant they are foraging on. *O. insidiosus* exhibited a Type II response on peppers and beans and a Type I response on tomatoes, suggesting a lower searching efficiency when foraging on this plant. In sentinel egg studies (Poley 2017a), *O. insidiosus* were observed interacting with egg masses only on tomatoes in Michigan.

We investigated the functional response of three Orthopteran families and the results were variable. The gryllid species tested, *A. domesticus*, was the only predators for which theoretical maximum consumption could be estimated, approximately seven and four egg masses per 24 h for females and males, respectively. Our results suggest that gryllids could be an effective source of *H. halys* pest suppression, particularly at low pest densities, which coincides with the findings of a recent sentinel egg and laboratory study (Morrison et al. 2016a). However, *A. domesticus* is a field cricket and may not encounter *H. halys* eggs in nature. We observed tree crickets (Oecanthinae) attacking eggs on video surveillance (Poley 2017a), therefore effort should be made to understand the predation efficiency of additional gryllid species on *H. halys* eggs.

We also tested species of Acrididae and Tettigoniidae. *M. femurrubrum* females exhibited a weak Type II response while males initially indicated a Type III, density-dependent response, although the data were better described by a Type I response. The data were likely too variable to see this relationship clearly. If males do indeed exhibit a Type III response under field conditions, there is high potential for *M. femurrubrum* to have a stabilizing effect on *H. halys* eggs but further testing should be conducted to clarify this relationship and to determine whether this predator is likely encounter *H. halys* egg masses in nature. *M. femurrubrum* is typically found in grasses and forbs, and is also considered a crop pest in alfalfa, soybean, and various vegetables (Pfadt 1994) which should be taken into account when considering this predator as a source of pest suppression.

Tettigoniids have the ability to consume a large number of *H. halys* eggs and are abundant in agroecosystems where *H. halys* are also found (Morrison et al. 2016a). In this study, *C. fasciatus* were not well described by any response Type and showed high variability in predation rates. We were unable to complete the intended ten replicates of our functional response testing due to predator availability, which could explain the apparent lack of trend in the data. In addition, only females were used due to prey availability. Further testing should include both sexes and other life stages of this predator to obtain a clearer understanding of the species' efficiency against *H. halys*. *C. fasciatus* are primarily found in grassland habitats, which is not where high pest densities of *H. halys* eggs are likely to be encountered. Further, widespread omnivory in tettigoniids may make *C. fasciatus* an unpredictable predator (Gwynne 2008; Morrison et al. 2016a) Tettigoniids are typically classified as a "chewing" predator

(Morrison et al. 2016a; Poley 2017a), however I found predation by *C. fasciatus* to appear more like the "punctured sucking" of predators such as spiders indicating potential underestimation of tettigoniids as predators.

A number of studies in the functional response literature discuss the shortcomings of simplified, laboratory experiments to investigate the functional response of arthropod predators (O'Neil 1989; Wiedenmann and O'Neil 1991; Schenk and Bacher 2002; Hassanpour et al. 2011). Laboratory studies such as ours do not take into account alternative prey as could be encountered in nature. Further, under laboratory conditions, the attack rate of a predator is limited by its handling time but under field conditions predators are limited more by size of the searching area and time (O'Neil 1989; Wiedenmann and O'Neil 1991), which could overinflate the likelihood of obtaining a Type II response. We tested predator efficiency with a single, unfamiliar prey choice, which might be of little value for predicting predator behavior in nature where multiple prey are available (Schenk and Bacher 2002). A multiple-choice test using a preferred prey source could aid in determining whether or not a predator will be a density dependent mortality factor for *H. halys* (Murdoch and Oaten 1975). Future studies should examine each predators' relative preference for other prey species; evaluate the nutritional value of *H. halys* eggs to each predator; investigate the predation efficiency of generalist predators under field conditions; and incorporate information on where the predators live vis-à-vis where *H. halys* lays its eggs.

This study improved our understanding of generalist predator interactions to this novel pest. We can conclude that the utilization of these predators as biological control agents, with the possible exception of *A. domesticus*, will not provide pest suppression at high densities.

CHAPTER 4

Conclusions and Future Research

Invasion ecology is a predictive science in which the population dynamics of natural enemy-prey interactions is a key concept. Predicting these interactions between native and invasive species is essential for implementing successful management practices (Fagan et al. 2002; Pyšek and Richardson 2010). Biological control is the foundation for sustainable management of invasive species (Pyšek and Richardson 2010) but requires an understanding of the invasion process of an exotic arthropod before decisions on the introduction or release of biological control agents can be made (Ehler 1998; Fagan et al. 2002).

Native natural enemies increase the probability of suppressing founding populations of invasive species as a form of environmental resistance (Ehler and Andres 1983) and, with particular emphasis on generalist predators, have demonstrated the ability to eliminate the spread of invading species when applied to the leading edge of an invasion (Cook et al. 1995; Ehler 1998). Targeting biological control efforts at the beginning of the invasion period, when recently colonized areas are present in advance of the main body of the invasion, could have implications for slowing the spread of exotic species (Hajek et al. 1996; Fagan et al. 2002). In agriculture, slowing the spread of exotic insect pests can lessen economic impacts and biodiversity losses.

Predicting the impact native natural enemies will have when pest pressures of an invasive species are high requires a baseline understanding of natural enemy-prey interactions. The goal of this thesis was to explore the top-down pressures that exist at the beginning of the biological invasion period and to translate this into potential sources of biological control for a novel pest in Michigan's agroecosystems. The results presented within the thesis were consistent with this goal and have brought to light additional questions to be investigated regarding biological control of *H. halys*.

My thesis provided a baseline level of biological control exerted by natural enemies native to Michigan. The results presented indicate Michigan's natural enemy community will not be effective for suppression of *H. halys* populations at this point in the invasion process. The absence of highly effective natural enemies is consistent with the natural enemy release hypothesis (Keane and Crawley 2002), in which exotic insect populations will increase in the absence of coevolved natural enemies. This is evident in number of whole egg masses that were retrieved from the field undamaged.

Egg parasitoids are an important source of population regulation for *H. halys* in its native range, but do not seem to be an effective source of suppression in Michigan's agroecosystems at this point in the biological invasion. Parasitoids did not consistently emerge from eggs deployed at any crop or location, and the presence of partially developed parasitoids in viable egg masses indicates that *H. halys* eggs are not ideal hosts for native parasitoid species, although the causal mechanisms were not investigated in the present study.

Video surveillance results revealed a complex of generalist predators that will attack *H. halys* egg masses, however the mode of eggs attacked over a 48 h period was two. Further, two predators spent more time on non-damaging interactions than damaging interactions. The small number of eggs attacked, and the amount of time spent by arthropods investigating but not damaging an egg mass suggests that generalist predators may not have an affinity for *H. halys,* as it is a novel pest (Fagan et al. 2002). Future studies should determine whether this relationship between predator preference toward *H. halys* as prey will change over time.

The results presented in Chapter 3 suggest that although generalist predators were observed attacking *H. halys* eggs in the field, these predators are not likely to provide a stabilizing force in *H. halys* population suppression. Functional response modeling demonstrated that, of the generalist predators tested, only *Acheta domesticus* has the potential to be a density-dependent mortality factor for *H. halys* eggs at low pest densities.

With the adventive introduction of *T. japonicus* and its ensuing range expansion in North America, and assuming it will expand its range into Michigan, there is the possibility of this *H. halys*-specific natural enemy inducing parasitoid-prey population dynamics similar to that of its native range. This new alternative to pest control raises questions of the possibility of native predators working synergistically with *T. japonicus* to control *H. halys*. However, generalist predators may not discriminate between parasitized and unparasitized eggs, leading to intraguild predation and the opportunity to negative long-term pest control (Snyder and Ives 2001). Indeed, sentinel egg results presented in Chapter 2 demonstrate this where on one egg mass we found at least 8 eggs parasitized followed by eight eggs attacked by a predator (Gryllidae) after parasitization.

The results presented in this thesis have opened up many avenues of future work. We demonstrated the feeding efficiency of generalist predators to a novel prey source. Future studies should investigate generalist predator feeding efficiency with choice-tests using preferred prey and *H. halys* eggs to gain a better understanding of each predator's potential as a density-dependent mortality factor. In addition, the effects of generalist predators on the environment and non-target organisms commonly found in Michigan's agroecosystems should be evaluated before a biological control program utilizing such predators is implemented.

H. halys egg masses are abundant throughout the growing season and are a good target for pest management because they are sessile with little defense against predators. However, the egg stage is short and placement can be cryptic, especially in complex environments. Effort should be made to sample predators in the field, with associated efficiency testing, for each *H. halys* life stage.

Finally, *H. halys* sentinel egg deployments should continue in Michigan's agroecosystems to monitor the baseline frequency of utilization by native natural enemies over time. Natural enemy activity can then be compared to regions like the mid-Atlantic where pest pressures are high, which will give researchers insights into the invasion ecology of *H. halys* and the long-term potential for native natural enemies to regulate pest populations. In addition, sentinel egg studies could detect the presence of *T. japonicus* and monitor its effectiveness as a natural enemy in its invaded range.

As *H. halys* continues its range expansion, Michigan is likely to face similar environmental and economic challenges as have been described in the mid-Atlantic and surrounding regions. At this time, biological control by native natural enemies is not likely to provide density-dependent population regulation of *H. halys* in Michigan. Management programs utilizing a combination of biological control with additional Integrated Pest Management strategies are recommended to maintain adequate pest suppression above economic thresholds.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2017-01

Author and Title of thesis:
Kristin Rae Poley, Biological control of *Halyomorpha halys* (Hemiptera: Pentatomidae) using native natural enemies in Michigan.

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Acrididae	*Melanoplus femurrubrum*	adult	5 male, 5 female	pinned
Anthocoridae	*Orius insidiosus*	adult	10	pinned
Encyrtidae	*Ooencyrtus* spp	adult	10	alcohol
Gryllidae	*Acheta domesticus*	adult	5 male, 5 female	pinned
Pentatomidae	*Halyomorpha halys*	adult	5 male, 5 female	pinned
Platygastridae	*Trissolcus flavipes*	adult	1	alcohol
Tettigoniidae	*Conocephalus fasciatus*	adult	10 female	pinned
Unknown Identification of Parasitoids		adult	2	alcohol

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