**Investigating host plant selection of harlequin bug, *Murgantia histrionica* (Hahn), in order to improve a trap cropping system for its management.**

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ABSTRACT

Harlequin bug (HB), *Murgantia histrionica* (Hahn), is a pest of cole crops. Alternative control strategies were investigated for control of HB, including trap cropping and systemic neonicotinoid insecticide applications.

Potential trap crops, mustard (*Brassica juncea* 'Southern Giant Curled'), rapeseed (*B. napus* 'Athena'), rapini (*B. rapa*) and arugula (*Eruca sativa*) were preferred over collard (*B. oleracea* 'Champion'), and a non-brassica control, bean (*Phaseolus vulgaris* 'Bronco') in field-cage choice tests. Harlequin bug could not complete development on bean, developed poorly on arugula but was found to complete development on mustard, collard, rapeseed and rapini.

In the field, mustard was found to be an effective trap crop for reducing HB feeding injury on collard at three experimental sites in 2010 and 2011. Augmentation of the mustard trap crop with a systemic, neonicotinoid insecticide did not increase the level of control of harlequin bug for the duration of the ten week growing period.

In olfactometer choice tests, male HB responded to plant volatiles of bean, collard and mustard, but preferred Brassica volatiles over those from bean. Female response to plant volatiles alone was weak and inconsistent. Both males and females preferred volatiles from other males feeding on Brassica host plant over plant volatiles alone, and were deterred by volatiles from males feeding on bean versus the plant alone.

Laboratory toxicity assays revealed that the neonicotinoid insecticides imidacloprid, thiamethoxam, dinotefuran, and clothianidin were toxic to HB nymphs; $LC_{50}$ = 0.57, 0.52, 0.39, and 0.39 mg ai/liter, respectively. Field experiments were conducted to evaluate the efficacy of these insecticides over time when applied as a one-time drench, and all were found to provide significantly higher mortality of HB for at least 14 days after application.

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## Introduction

Harlequin bug (HB), *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae), is a conspicuous and important pest of cole crops (Brassicaceae), particularly in the southern United States. Although HB has been recorded in the majority of the continental U.S., it is not known to overwinter north of 40$^{o}$N latitude, and infestations in northern states are likely due to seasonal migration aided by wind currents (Hodson and Cook 1960, McPherson and McPherson 2000). Both adults and nymphs are piercing-sucking insects that feed on aboveground plant tissues, leaving white blotches, which make leafy cole crops such as collards and kale unmarketable. Under heavy feeding pressure plants can wilt and die, potentially destroying entire fields of untreated cabbage or collards (Paddock 1915, Wallingford et al. 2011).

There are several broad-spectrum insecticides that are effective at controlling HB (Rogers and Howell 1972, Wang 1978, McLeod 2005, Walgenbach and Schoof 2005, Kuhar and Doughty 2009); however, these classes of insecticides cannot be used in organic systems and are also detrimental to important natural enemies in the cole crop agroecosystem (Xu et al. 2001, 2004, Hill and Foster 2003, Cordero et al. 2007). Many growers are opting for newer insecticides that specifically target lepidopteran pests, which are safer for conservation of natural enemies, but do not control hemipteran pests, such as HB (Walgenbach and Schoof 2005, 2009).

Although there are several other commercially grown species within the Brassicaceae, traditional cole crops, such as cabbage, broccoli, cauliflower, and Brussels sprouts, are members of the same species, *Brassica oleracea* L. This plant species offers a remarkable variety of phenotypes and cultivars are grouped according to their growth patterns; “headless” varieties, such as collard greens and kale in the Acephala group, while cabbage is in the Capitata Group, or the “heading group” (Rubatzky andYamaguchi 1997). Broccoli, cauliflower, and Brussels sprouts are in the Italica, Botrytis, and Gemmifera Groups, respectively, and though not as commonly grown in the U.S., Chinese broccoli, and kohlrabi are also *B. oleracea* and are grouped as Alboglabra and Gongylodes, respectively (Peirce 1987).

The pest status of HB has risen over the past decade. This increase may be due to warming winter weather, which enables greater survival and reproduction (Walker and Anderson 1993). The increase in incidence of HB may also be due to the reduced use of broad-spectrum insecticides on brassicaceous crops, as most of the newer insecticides that specifically target either lepidopteran or aphid pest species but do not control HB (Walgenbach and Schoof 2005). There may be a need for an alternative management strategy for HB that does not rely on the use of broad-spectrum insecticides and can be integrated into current management strategies.

Chapter One is an overview of HB biology and discusses integrated pest management strategies for its control in cole crops (Wallingford et al. 2011). Chapter Two identifies plant species that are preferred over a typical cole crop, collard (*Brassica oleracea* L.), developmental performance of nymphs on those plant species, and a field-scales evaluation of border row trap crops augmented with a systemic neonicotinoid insecticide in their ability to control HB damage in collard. Chapter Three identifies a difference between genders in behavioral response to plant volatiles, and a proposed model is discussed to describe the role of olfactory cues in host plant searching and selection. Chapter Four presents lethal concentrations ($LC_{50}$) of the neonicotinoids, imidacloprid, thiamethoxam, dinotefuran, and clothianidin, and evaluates their residual efficacy over time when applied as a one-time soil drench.

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## Chapter One

### Harlequin bug biology and pest management in Brassicaceous crops.

(Wallingford, A.K., T.P. Kuhar, P.B. Schultz, and J.H. Freeman. 2011. Journal of Integrated Pest Management 2: H1-4.)

### Historical perspective

Harlequin bug, also known as harlequin cabbage bug or calico back, is likely native to Central America and was first described and identified as *Strachia histrionic*a by Hahn in 1834, then later moved to the genus *Murgantia* by Stal in 1872 (Paddock 1918). The pest was introduced to the U.S. most likely from Mexico in the 19$^{th}$ century, and was first reported in Texas in 1864 (Walsh 1866). Because of its timing with the U.S. Civil War and the high pest infestations that followed in the southern U.S., the bug was also referred to as the Sherman Bug named after the Union General William T. Sherman (Capinera 2001).

Considered a major pest of cabbage in the early 1900's, there were several reports of severe harlequin bug outbreaks following mild winters (Walker and Anderson 1933). Early management recommendations included destruction of overwintering habitat, handpicking, the use of soaps, and trap cropping with preferred host plants such as early radish, turnips, mustard, rape, and kale (Thomas 1915, Chittenden 1920, Fulton 1930).

With the introduction of broad-spectrum insecticides, harlequin bug was found to be easily controlled chemically with organophosphates and carbamates (Rogers and Howell 1972, Wang 1978), and more recently with pyrethroid and neonicotinoid insecticides (Edleson 2004a, McLeod 2005, Walgenbach and Schoof 2005, Kuhar and Doughty 2009).

However, there has been a shift toward the use of narrow-spectrum, reduced risk insecticides, such as *Bacillus thuringiensis* (*Bt*) for control of lepidopteran pests, which have typically been the primary pest concerns for cole crop management. The use of *Bt* and other narrow-spectrum insecticides serves the interests of both human and environmental safety as well as integrated pest management, but typically does not provide management of hemipteran pests such as harlequin bug. This trend away from broad

spectrum insecticides may result in a resurgence of harlequin bug as a major pest as has occurred with some other stink bug pests (McPherson and McPherson 2000).

## Life Cycle and Description

Harlequin bug typically completes two to three generations per year; a fourth generation may occur in warm climates (Paddock 1915). Adults survive the winter under shelter in field litter and debris; however, all stages of the bug can be found throughout the winter months if temperatures are warm and host plants are available (Paddock 1915, White and Brannon 1933). Moreover, mild winters have been implicated in larger than average densities of harlequin bug during the season (Walker and Anderson 1933). Thus, warm winter temperatures may favor higher harlequin bug infestations. In the spring, large aggregations of adults can often be found on desirable host plants such as mustard and wild radish (McClain 1981).

**Adult.** Adults are typical pentatomid shield-shaped bugs, 0.25-0.5 inches (7-11 mm) in length, and brightly colored light orange to red with black and white markings. Adults overwinter in crop debris and weeds, and become active in early spring when temperatures warm. Under laboratory conditions sex ratios are roughly equal to one and males and females live an average of 68.2 and 82 days, respectively (Streams and Pimentel 1963). Preoviposition periods have been estimated at a range of 7-30 days from eclosion (Canerday 1965, Zahn et al. 2008a) and variation of time to maturity can depend on temperature as well as host plant (Ludwig and Kok 2001). A female can lay 4-16 egg masses over her lifetime and a copulation event usually occurs between each oviposition, but is not necessary (Canerday 1965). Communication is aided by the use of vibratory signals through the host plant (Čokl et al. 2004, 2007) as well as a male-synthesized aggregation pheromone (Zahn et al. 2008b).

**Egg.** Egg masses are typically laid on the underside of host plant leaves in double rows of 6 (12 eggs total) (White and Brannon 1933). Eggs are barrel-shaped and striped black and white, roughly 0.04 inches (1 mm) in length. Optimal temperature for development is 84.2-89.6°F (29-32°C; Canerday

1965), and hatch occurs in 3-5 days in the summer, while springtime temperatures may necessitate 15-20 days for egg development (White and Brannon 1933).

**Nymphs.** Nymphs are black, orange, and white, but are smaller and do not have wings. There are five instars and the duration between molts increases with each instar, 2-5, 3-6, 7-11, 8-18, and 10-18 days, respectively, while total duration from egg to adult is 37-57 days at 75.2°F (24°C), 45% RH (Zahn et al. 2008a). First instars remain on or near the egg shell (White and Brannon 1933, Streams and Pimentel 1963) where they acquire symbiotic gut bacteria delivered from mother to offspring on the egg mass surface (Prado and Almeida 2009).

## Hosts

Harlequin bug has been reported to feed on over 50 species of plants, but shows a strong preference for members of the Brassicaceae family, feeding on alternate hosts only in their absence (McPherson and McPherson 2000). Economically important cole crop species include broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, kohlrabi (*Brassica oleracea* L.), rape, rutabaga (*B. napus* L.), Chinese cabbage, turnip, broccoli raab, mizuna (*B. rapa* L.), radish (*Raphanus sativa* L.), horseradish (*Armoracia rusticana* Gaertn, May and Scherb), and arugula (*Eruca sativa* Miller). Wild hosts include: wild mustard (*Brassica* spp.), shepherds purse (*Capsella bursa-pastoris* (L.)), peppergrass (*Lepidium* spp.), bittercress (*Cardamine hirsute* L.), watercress (*Nasturtium* spp.) and other brassicaceous weeds (McPherson and McPherson 2000). Some non-brassica weeds such as common pigweed (*Amaranthus* spp.) and lambsquarters (*Chenopodium album* L.) are also fed upon by harlequin bug (Capinera 2001).

Sullivan and Brett (1974) evaluated several varieties of cole crops for resistance to pests and concluded that the mechanism of plant resistance to harlequin bug was due to non-preference and determined that mustard, turnip, kale, rutabaga and Chinese cabbage to be preferred over cabbage, cauliflower, broccoli, collards, Brussels sprouts, radish, and kohlrabi.

## Injury

Both adults and nymphs are piercing-sucking insects that feed on aboveground plant tissues, leaving white blotches, which make leafy crucifer crops such as collards and kale unmarketable. Low pest densities often result in only cosmetic damage, but under heavy feeding pressure, plants can wilt and die (Ludwig and Kok 2001), and leaves can turn yellow and wilt. Entire fields of cabbage or collards can be completely destroyed by high densities of harlequin bug, if left untreated (Paddock 1915).

## Management options

There are several insecticides registered for use on cole crops that are effective in controlling harlequin bug, including systemic neonicotinoids, that can be drench applied at planting or upon first observation of the pest, providing several weeks of protection. However, there are other non-chemical strategies that show potential for harlequin bug management including destruction of crop residues, trap cropping and, to a lesser extent, biological control using parasitic wasps.

## Cultural management

Since harlequin bug overwinters in the previous crop residue, destruction or removal of the old crop can help manage the pest (Capinera 2001). Trap cropping, or the planting of one or more species of preferred plants near a protected crop in order to divert herbivore feeding (Hokkanen 1991) has great potential for the management of harlequin bug, and particularly because these insects use pheromones to aggregate in large numbers on preferred host plants (McClain 1981, Aldrich et al. 1996). Ludwig and Kok (1998) showed that early-planted broccoli can serve as a trap to draw bugs away from the later-planted main crop. Destruction of the bugs is necessary to prevent dispersal from the trap crop. Since harlequin bug prefers certain plant species such mustard, turnip, and Chinese cabbage over crops such as cabbage, cauliflower, broccoli, collards, and radish (Sullivan and Brett 1974), there is great potential for utilizing multiple cropping or intercropping approaches as a trap crop management strategy, particularly in the interest of reducing chemical sprays. Bender et al. (1999) found that intercropping cabbage (*B. oleracea*)

and Indian mustard (*B. juncea*) reduced the need for two insecticide sprays in a heavy infestation of harlequin bug. This management approach has potential for harlequin bug control but needs further investigation, such as addressing selection of the proper plant species or variety, selection of the proper deployment of a trap crop (e.g. perimeter, intercropping, multiple trap crops, push-pull strategies, semiochemically-assisted, etc.), as well as proper maintenance of that trap crop once the pest has established (Hokkanen 1991, Shelton and Badenes-Perez 2006).

## Chemical control

Over the past thirty years, broad spectrum chemicals such as pyrethroids, organophosphates, and carbamates have been widely used for effective control of harlequin bug nymphs and adults (Rogers and Howell 1972; Edelson 2004b; Edelson and Mackey 2005b, 2006b; McLeod 2005; Walgenbach and Schoof 2005). More recently, neonicotinoid insecticides including imidacloprid, thiamethoxam, acetamiprid, chlothianidin, and dinotefuran have also been shown to be effective for harlequin bug control (Edelson 2004a; Edelson and Mackey 2005a, 2005c, 2006a; Kuhar and Doughty 2009). Surfactant type adjuvants are often recommended for cole crops to ensure good spray coverage when applying foliar insecticides. Soil applications (transplant water, soil drench, or drip chemigation) of the neonicotinoids imidacloprid, thiamethoxam, and dinotefuran have provided effective control of harlequin bug with residual efficacy lasting up to 30 days after application (Kuhar and Doughty 2009).

Chemical control options for organic producers are limited but there are several products which are registered for use in organic systems. Overall et al. (2007, 2008) reported significant control of harlequin bug nymphs on collards and turnips with spinosad, which demonstrated the highest toxicity (lowest $LC_{50}$) levels to harlequin bug nymphs in leaf dip bioassays over pyrethrins, and azadirachtin.

## Biological control

Harlequin bug has relatively few natural enemies. The bug produces several warning defense chemicals, some emitted from the metathoracic gland, and others expelled as a frothy liquid from the prothoracic

gland when disturbed (Aldrich et al. 1996). Harlequin bug also sequesters glucosinolates from its host plants, which likely makes it unpalatable to predators, such as wild starlings (Aliabadi et al. 2002).

At least three species of native hymenopteran wasps parasitize the eggs of harlequin bug including *Ooencyrtus johnsoni* Howard, *Trissolcus murgantiae* Ashmead, and *Telenomus podisi* Ashmead, (White and Brannon 1933, Huffaker 1941, Koppel et al. 2009). Because of its success in the southern U.S. (Huffaker 1941), *T. murgantiae* was introduced into California as a biological control agent and later recovered from harlequin bug eggs (DeBach 1942). Surveys evaluating egg parasitism rates range from 8-50% of field-collected eggs with higher rates of parasitism occurring in years of heavy harlequin bug infestation (Huffaker 1941, Ludwig and Kok 1998). In the 1990s, Ludwig and Kok (1998) primarily recovered *T. murgantiae* and *O. johnsoni* from harlequin bug collected in Virginia, but more recently Koppel et al. (2009) found only *T. podisi* in Virginia, with 63.5% of harlequin bug egg masses and 12.9% of the total eggs parasitized. As with a related species, *Trissolcus basalis* Wollaston, *T. murgantiae* and *T. podisi* may use chemical volatiles emitted by plants due to harlequin bug oviposition to find the host eggs (Salerno et al. 2006, 2009).

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## Chapter Two

## Part I

## Trap cropping literature review

A trap crop is a plant stand that attracts pest organisms away from a protected crop, and concentrates these pests in a certain part of the field where they can be economically managed (Hokkanen 1991). Trap cropping is not only a long time practice, but demonstrates a phenomenon that gardeners and farmers have discovered on their own when growing a diversity of plant species. Much work has been conducted by the scientific community to improve upon this practice, including proper selection of trap crop species, where and when trap crops should be planted, and methods to improve the efficacy of trap crops.

Trap cropping has the most potential to control pest insects that show a strong host plant preference, but there is also potential for control of pests demonstrating an "edge effect," a tendency to aggregate on field margins after migration into cultivated fields from overwintering habitats or from wild host plants. Behavior-mediated management strategies like trap cropping have been evaluated for control of Colorado potato beetle, *Leptinotarsa decemlineata* Say, but work has also shown potential for control by intercepting invading beetles with physical or chemical barriers without a dedicated trap crop (Weber et al. 1994, Hunt and Whitfield 1996, Hoy et al. 2000). Conventional trap crops are normally planted in a perimeter around the protected crop, or at least positioned to intercept movement between overwintering spots and cultivated fields.

Sequential trap crops are planted earlier than the main crop to allow invading pest species to settle where they can be controlled, and often a more mature plant is a more attractive plant (Shelton and Badenes-Perez 2006). An alternative to different planting times is the use of an earlier maturing variety. For example, there is potential for diverting stink bugs from cotton by using a soybean trap crop; stink bugs are more attracted to earlier maturing varieties of soybean and this is likely due to the earlier presence of the soybean pod on plants (Schumann and Todd 1982, Bundy and McPherson 2000).

A pest insect that shows a preference for one host plant and is deterred by another can be diverted by a "push-pull" system or a "stimulo-deterent" planting strategy (Miller and Cowels 1990, Cook et al. 2007). For example, *Desmodium* spp. grasses are inter-planted with maize to deter oviposition of the stem borer, *Chilo partellus* (Swinhoe) (push), and a perimeter of the Napier grass, *Pennisetum purpureum* Schumach, and Sudan grass, *Sorghum vulgare sudanense* Stapf., attracts ovipositing females away from maize (pull; Khan et al. 2000, 2001). These grasses also provide control of weeds, such as *Striga hermonthica* (Del.), and can be used as animal forage after the main crop is harvested (Hassanali et al. 2008). Push-pull systems may be unattractive to growers because of the complexity of the system, and the amount of land that cannot be used for cultivation of the main crop.

The loss of cultivated space is particularly unattractive when the trap crop is so target-specific that it controls only one pest. Often a mix of plant species is the most attractive trap crop (Hokkanen 1989), and has the potential to target more than one pest species (Shelton and Badenes-Perez 2006). A simpler system that diverts more than one pest would be ideal. Indian mustard, *B. juncea*, has been cited as a potential trap crop for HB (Ludwig and Kok 1998) as well as flea beetle, *Phyllotreta* spp. (J. Parker, personal communication), and several lepidopteran pests of cole crops, such as the cabbage head caterpillar, *Crocidolomia pavonana* (Fab.), cabbage webworm, *H. rogatalis*, cabbage looper, *Trichoplusia ni* (Hubner), green garden looper, *Chrysodeixis eriosoma* (Doubleday), and diamondback moth, *Plutella xyllostella* L. (Luther et al. 1996, Smyth et al. 2003).

Trap crops can be rendered more attractive by semiochemical augmentation. Kuhar et al. (2006) found more adult Colorado potato beetles were found aggregating on potato plants treated with a synthetic aggregation pheromone, and fewer egg masses and larvae were found in nearby plots, than an untreated control. Bark beetles can be more reliably attracted to a host plant with pheromone-baited trees (Borden 1995, Borden and Greenwood 2000). Injecting ethanol into host trees will attract more scolytid beetles, as the ethanol mimics plant response to stress caused by flood conditions (Kimmerer and Kozlowski 1982, Reding et al. 2011). Conversely, a semiochemically-aided control can be improved by the use of a

trap crop. For example, toxic baits are often used to attract and kill melon fly, *Bactrocera cucurbitae* Coquillett, and oriental fruit fly, *B. dorsalis* Hendel, in papaya, *Carica papaya* L., and control could be improved by applying these baits to the preferred roosting plant, cassava, *Manihot esculenta* Crantz (McQuate 2011).

Establishing a highly attractive trap crop on-farm does raise concern about creating a source of pest organisms. Once a pest population has congregated on a trap crop, management is crucial. Traditional methods were to burn, chop, or till under infested trap crops as a chemical-free option (Thomas 1915, Chittenden 1920, Fulton 1930). Alternatively, foliar applications of chemical pesticides can be applied to knock down populations. For example, traditional economic threshold numbers are used to time chemical sprays in trap crops of Blue Hubbard squash, *Cucurbita maxima* Duchesne, meant to control cucumber beetle, *Acalymma vittatum* Fab.; a practice that maintains quality and yield as well as pollinator visitation in butternut squash, *C. moschata* Duch., while reducing insecticide use by as much as 97% (Cavanagh et al. 2009, 2010).

A dead-end trap crop is highly attractive for feeding and oviposition, but does not allow for development of subsequent generations, through a toxic effect or because it does not meet the nutritional needs for development (Shelton and Nault 2004). Diamondback moth females prefer to oviposit on wild crucifer, *Barbarea vulgaris* R. Brown, over cabbage, but the resulting offspring do not develop past 1st instar (Lu et al. 2004, Shelton and Badenes-Perez 2006). Arugula, *Eruca sativa* L., has been shown to be an effective dead end trap crop for root-knot nematode, *Meloidogyne* spp. (Melakeberhan et al. 2010). Stinkweed, *Thlaspi arvense* L., shows promise as a dead end trap crop for cabbage looper (Cameron et al. 2007). Using a transgenically modified dead-end trap crop has been proposed for control of sugarcane stalk borer, *Eldana saccharina* Walker, using transgenic *Bt* maize (Keeping et al. 2007), and for control of diamondback moth using transgenic *Bt* collards (Shelton et al. 2008).

Application of a systemic pesticide, such as a neonicotinoid, to a trap crop can create a temporarily dead-end trap crop. Neonicotinoid insecticides are known for their low mammalian toxicity, reduced effects on non-target insects and low potential for environmental hazards and can be applied as a foliar spray or as a drench treatment (Thomson 2000). Several neonicotinoid products have been found to be effective in controlling HB when used as a foliar spray (Edelson 2004a,b, Edelson and Mackey 2005a,b, Edelson and Mackey 2006a, Walgenbach and Schoof 2011). Drench application allows for better reduction in non-target effects, and is a more attractive method for many growers as residual efficacy can be doubled, compared to a foliar application (Kuhar and Doughty 2009).

Trap cropping shows potential for resistance management by offering a refuge from exposure to insecticide, which conserves susceptible alleles; this could be of particular use in transgenic *Bt* maize, cotton, rice, and broccoli (Gould 1998, Tang et al. 2001).

## Chapter Two

## Part II

## Host plant preference and performance of harlequin bug, and evaluation of a trap cropping strategy for its control in collard.

In the early 1900s, prior to the widespread use of synthetic chemical controls, trap cropping was recommended for control of harlequin bug (HB), *Murgantia histrionica* (Hahn); suggested trap crops included radish (*Raphanus sativus* L.), turnips (*Brassica rapa* L.), mustard (*B. juncea* L.), rapeseed (*B. napus* L.), or kale (*B. oleracea* var. *acephala* L.) to draw pressure away from cabbage (*B. oleracea* var. *capitata* L.; Thomas 1915, Chittenden 1920, Fulton 1930). Although the efficacy of this control tactic was not well documented, Ludwig and Kok (1998) more recently demonstrated the potential of trap cropping to reduce HB injury to broccoli.

Harlequin bug displays many characteristics of a pest that can be successfully managed by trap cropping in that it displays host preference, is highly mobile, and aggregates on field margins. There is also potential for a complex of insects to be controlled by the same strategy (Shelton and Badenes-Perez 2006). It is important that a trap crop be deployed in a manner that fits into commercial cole crop practices and does not create an unexpected pest problem. Information on host plant preference will aid in the selection of a proper trap crop species. Augmenting a trap crop with a systemic insecticide could create a sink for pest populations on-farm rather than a potential source for subsequent plantings. Our objective is to (1) identify an attractive host plant for HB feeding, habitation and oviposition and (2) evaluate a method of trap cropping that is augmented by the use of a systemic insecticide.

## Materials and Methods

### Host preference and performance

**Host plants.** Potential trap crop species, mustard (*B. juncea* 'Southern Giant Curled'), rapeseed (*B. napus* 'Athena'), arugula (*E. sativa* 'Roquette'), and rapini (*B. rapa*), were compared to collard (*B.*

*oleracea* 'Champion') and bean (*Phaseolus vulgaris* 'Bronco'), a typical cash crop and a non-brassica control, respectively. Plants were grown from seed in the greenhouse in a mix of sphagnum peat moss, perlite and vermiculite (2:1:1), irrigated daily and fertilized weekly with Scott's Water Soluble Plant Food (18-18-21 NPK with micronutrients; Scotts-Sierra Horticultural Product Company, Marysville, OH). Plants used in all experiments were 8-10 weeks old, with no reproductive structures, and at least four true leaves on plants that remained in pots, while plants in field-cages had a minimum of eight true leaves.

**Insects.** Adult HB were field-collected from collards grown at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (AREC) in Painter, VA. In 2009, participants were collected from the field in June and were likely a mix of overwintered and 1st generation adults. In 2010, participants were collected in September and were likely a mix of 2nd and 3rd generation adults. Lab-reared insects were originally collected from mustard and collards grown at the Virginia Tech Kentland Research Farm near Blacksburg, VA. Insects were reared in mesh cages (30 x 30 x 30 cm; Bioquip Products, Rancho Dominguez, CA) on a mix of collard leaves, cabbage leaves, and cauliflower florets, and maintained at 24+ 5$^{o}$C, ~10% RH, and a photoperiod of 16:8 (L:D) h.

**Field-cage choice tests.** Host plant preference for HB feeding, habitation, and oviposition was evaluated at the Eastern Shore AREC in Painter, VA, June 2009 and September 2010. A row of five plants of each species were randomly planted in each of four cages (2 x 2 x 1 m; Bioquip Products, Rancho Dominguez, CA) at 6-8 weeks and experiments were started at 8-10 weeks. Adults (30-50) were introduced in the center of each cage and plants were observed for insects and egg masses in the early afternoon (the time of highest HB feeding activity) at 24, 48, and 72 h after introduction. Weather conditions for the duration of both experiments were generally partly cloudy, and daytime temperatures ranged from 24-30$^{o}$C.

**Oviposition choice test.** Oviposition rates were low in both field-cage experiments, so an additional choice test was conducted in the greenhouse at Virginia Tech in Blacksburg, VA in May 2011.

One potted plant of each species was placed in each of six wooden framed cages with wire mesh sides (45 x 45 x 60 cm). Three mating pairs were taken from the colony and introduced to cages in the evening and plants were observed daily for egg masses over the following 72 h. This procedure was repeated twice for a total of 12 replications. Weather conditions were generally overcast and greenhouse temperatures ranged from 26-32$^{o}$C.

**Plants Part Choice Test.** Choice test experiments were conducted to determine if plant maturity plays a role in host preference, by evaluating what plant parts (stems, leaves, florets) HB adults were observed on most often. Assays were conducted using broccoli (*Brassica oleracea* 'Gypsy') grown at the Hampton Roads AREC in Virginia Beach, VA in 2009, and plants grown at Kentland Research farm in Blacksburg in 2011. Plants were used in assays when 1) crowns were formed, 2) after crowns had elongated and open flowers were present, and 3) post-flowering when seed pods (siliques) had formed.

Five HB adults (field-collected from broccoli in Blacksburg, VA) were given the choice between one piece of stem (7-10 cm length, 2-3 cm diameter), a floret (7-10 cm length), or a leaf (10-15 cm), cut from the same plant. Assays were conducted within vented plastic cages (30 x 15 x 15 cm, n = 10). The cut ends of each floret and leaf were inserted into wet floral foam to maintain moisture. HB were allowed to acclimate to their environments overnight (T = 25$\pm$1$^{o}$C, Day length 16D: 8N) and observations of their locations were made 24 hours after introduction. Each adult was counted as "choosing" the plant part when it was found on that part. Harlequin bugs on the walls of cage or underneath plant part were not counted.

**Developmental Performance.** Egg masses were removed from the colony and isolated until nymphs reached 2$^{nd}$ instar. Fifty 2$^{nd}$ instars were isolated to potted plants of each test species and observed every two days for the number of individuals in each stadia 2$^{nd}$ - 5$^{th}$, and adults. Plants were replaced twice weekly and maintained in a growth chamber at 24$\pm$5$^{o}$C, 14:10 (L:D) h. Observations ended when 50% of the insects observed on plants reached adulthood.

**Trap crop experiments**

Experiments were conducted in July 2010 at the Eastern Shore AREC in Painter, VA, and in May 2011 at Kentland Research Farm in Blacksburg, VA and the Hampton Roads AREC in Virginia Beach, VA. Collards (*B. oleracea* 'Champion') and mustard (*B. juncea* 'Southern Curled Giant') were direct seeded at 2-4 kg per hectare, and managed with minimal inputs other than weed management, which were applied according to conventional management practices (Wilson et al. 2010). Collard plots consisted of eight 5 m rows spaced 0.3 m apart, each plot being a minimum of 10 m from any other. Three treatments were evaluated and each treatment was replicated four times at each site: (1) no trap crop, collard plot as described, (2) mustard border rows, collard plot as described with the addition of a 5 m row of mustard seeded on both sides, and (3) insecticide-treated mustard border rows, collard plot as described with the addition of a 5 m row of mustard seeded on both sides to which a drench application of thiamethoxam + chlorantraniliprole (0.16 L a.i./hectare Durivo; Syngenta, Greensboro, NC) was applied at first observation of HB in plots.

Plots were scouted weekly for arrival of local populations and, when adults were first observed, insect densities were recorded twice weekly until collard greens reached harvest maturity (10 weeks). On each observation date, ten collard plants and ten mustard plants (when applicable) were observed in each plot for HB adults, egg masses, and nymphs. When collard greens reached harvest maturity, 20 leaves were randomly selected from each plot and observed for HB feeding scars (distinctive white blotches).

**Data Analysis**

All data analysis was conducted using JMP (SAS Inst. 2007, Cary, NC). Choice test data were non-normal and did not respond to transformation, so Kruskal-Wallis test was conducted to test for significant difference between the number of insects and egg masses observed on different plant species; mean separation was evaluated by nonparametric multiple comparisons based on rank sums ($\alpha = 0.05$; Zar 1984). ANOVA followed by Tukey-Kramer HSD comparison tests were conducted to evaluate

significant difference between percent damaged collards leaves observed in collard plots by treatment ($\alpha = 0.05$). A Student's t-test was conducted to evaluate significant difference between HB adults observed in collard plots versus their accompanying mustard border rows in the trap crop experiment ($\alpha = 0.05$).

## Results

### Host preference and performance

**Field-cage choice tests.** In 2009 and 2010, significantly more HB adults were observed on mustard than any other plant species with the exception of arugula at the 24 hour observation in 2010 (Table 2.1). More adults were observed on rapeseed and rapini than on collard, arugula or bean by the end of the experiment in 2009, while arugula was also preferred over collard and bean in 2010 (Table 2.1).

**Oviposition choice tests.** In 2009, more HB egg masses were observed on arugula, collard, mustard, and rapeseed than on rapini or bean, while in 2010, more egg masses were found on rapeseed and collard than any other species, and mustard was not different from bean (Table 2.1). There were more egg masses observed on rapeseed than any other species in the greenhouse cage tests, while mustard was not different from bean (Table 2.1).

**Plant parts choice tests.** More HB adults were observed on leaves than florets using broccoli plants with crowns (before flowering; Fig. 2.1); however florets attracted more HB adults in all other plant parts choice tests, using flowering broccoli plants and broccoli plants with seed pods (Figs. 2.2 and 2.3).

**Developmental performance.** Nymphs that were isolated to bean survived up to 10 days, but none molted to 3$^{rd}$ instar (Table 2.2). Only 6 (12%) of arugula-fed nymphs reached adulthood, compared to 25 and 21 (~50%) of collard and rapeseed-fed nymphs, and 44 (88%) of mustard and rapini-fed nymphs reached adulthood (Table 2.2).

### Trap crop experiments

Harlequin bugs appeared on plants well before harvest in all experiments and the insecticide applications to treatment three plots occurred at week 4, 5, and 3 for Painter, Virginia Beach, and Blacksburg, respectively. Peak numbers of adults did not occur until week 8 or 9, and the subsequent generation of nymphs reached a peak by week 10 or 11.

More damaged collard leaves were observed in plots with no trap crop (55-85%) than in plots with mustard border rows (5-25%) at Virginia Beach and Blacksburg ($F = 37.56$; $dF = 2, 9$; $p < 0.0001$, $F = 6.45$; $dF = 2,9$; $p = 0.0183$, respectively), while there was no difference between plots protected by either untreated or insecticide-treated trap crops (Fig. 2.4). The mustard plants in border rows at the Painter 2010 site failed due to hot and dry summer conditions, but one plot of each treatment remained intact and these data show a similar trend as the results seen at Virginia Beach and Blacksburg the following spring, although they were not replicated and could not be subjected to statistical analysis (Fig. 2.4).

More HB adults were observed in mustard border rows than in accompanying collard plots on several observation dates for trap crop treatments ($\alpha = 0.05$; Fig. 2.5). While this difference was seen immediately in untreated mustard plots, differences were not seen in insecticide-treated plots until 60-70 days after treatment, well after the residual efficacy of thiamethoxam (30-40 days; Chapter Four).

## Discussion

Harlequin bug demonstrated a strong preference for mustard over collard and other plants evaluated in our study, confirming historical recommendations and previous reports of host preference (Paddock 1915, Ludwig and Kok 1998). Harlequin bugs appear to be more attracted to florets of more mature host plants (Figs. 2.2, and 2.3). A trap crop of mustard decreased the feeding injury seen in collard for up to 10 weeks, a time frame comparable to that of a cabbage or broccoli crop started from transplants, although some cole crops started from seed would require a longer period of protection. Ludwig and Kok (1998) also achieved a reduction in HB damage to broccoli using a complete perimeter of the trap crop around

the cash crop; however, border rows would probably be a better fit in conventional cole crop production because it may not require an extra step at planting.

There was no difference in control between plots with untreated versus thiamethoxam-treated trap crops (Fig. 2.1) and this is likely due to a poorly timed application at 'first appearance of harlequin bug.' The rate of colonization was surprisingly slow, generally 3-4 weeks between the first observation of HB adults in plots and peak populations, while the expected efficacy of a drench application of thiamethoxam is slightly shorter (Chapter Four). Without a proper action threshold, the use of a systemic insecticide may be viewed as unnecessary for the 10 week time period evaluated, although control of the pest population is highly recommended. Control of congregated HB could also be accomplished with a foliar insecticide treatment or by burning or tilling under that trap crop, which will destroy eggs and nymphs.

Oviposition data differed between years, and this may be due to the variability in the time of year during which experiments were conducted (Table 2.1). Ovipositing females in the September 2010 experiment may have been more selective than those used in June 2009, because shorter days indicate that the season is ending. Variation in tactile cues from the plant surface may have played a role in oviposition choice. Although the effect on HB is unknown, presence of hairs on the leaf surface and increased waxy bloom have been reported to affect the behavior of phytophagous insects and their natural enemies (Lamb 1980, White and Eigenbrode 2000). Greenhouse choice test arenas offered more opportunity for participants to move back and forth between plant species, and oviposition choice may have been made based on texture of the leaf surface rather than suitability of the host plant.

Oviposition choice and the subsequent nymphal densities probably did not contribute to the overall damage observed on collard leaves during the time frame of the field experiments; however, earlier colonization may have led to higher nymphal densities with the potential to contribute to the overall injury level. Nymphs are highly mobile and capable of finding host plants, although nymphal host

preference is unknown. The movement of ovipositing females and their nymphs between trap crop and protected plants should be investigated for longer infestation periods.

Although mustard was found to be the most consistently preferred plant species in choice tests, rapeseed and rapini were also consistently preferred over collard (Table 2.1). There is potential for several plant species or varieties to be used as a trap crop and a mix of more than once plant species is potentially the most effective trap crop, as is the case when trap cropping flea beetles in cole crops (personal communication, J. Parker). There is potential for control of a complex of pests using one or more of these plant species. *Brassic juncea*, also known as Indian mustard, has been previously cited as an effective trap crop for HB (Ludwig and Kok 1998), as well as flea beetle and several lepidopteran pests of cole crops (Luther et al. 1996, Smyth et al. 2003).

Trap cropping is a viable option in several farming systems and is currently used regularly on organic farms, and particularly on farms that maintain a high diversity of plant varieties in production at any given time. Often a preferred host plant grown as a cash crop will also act as a trap crop, and slow movement of pest species through cultivated space, with no action taken by the farmer. Insects can be hand-picked or vacuumed off these plants, and there are several organically certified insecticides that provide some efficacy on HB, whose active ingredients include spinosad and azadirachtin (Edelson and Mackey 2006a,b, Overall et al. 2008). Trap cropping can also be augmented with natural enemies, such as entomopathogens, parasitoids and predators, and can improve overall arthropod diversity which can maintain pest populations below economic thresholds (Correa-Ferreira and Moscardi 1996, Aguilar-Fenollosa et al. 2011).

Even when incorporating insecticides into a trap crop strategy, these materials are used in discrete areas. In theory, applications of insecticide concentrated to one small area would leave the rest of the protected crop as a 'refuge' for natural enemies, such as *Diadegma insulare*, a larval parasitoid which significantly reduces diamondback moth in cole crops (Mitchell et al. 1997). Higher rates of parasitism, and less

damage from cabbage maggot has also been observed in broccoli protected by a trap crop of turnip (*B. rapa*; Rousse et al. 2003). Although there is potential to improve parasitism rates, the direct effects of landscape management practices on conservation biological control are somewhat intractable, but should be considered as part of a larger integrated pest management strategy.

In summary, our results show a predictable behavior in HB for the advantage of its management in cole crops. A border row of mustard reduced HB injury in collard by roughly 50%, without the use of broad-spectrum insecticides. Although there was no difference in control between untreated and insecticide treated plots, management of HB aggregations on trap crops is highly recommended for reducing on-farm pest populations, which may include the use of insecticides.

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Table 2.1: Harlequin bug adults and egg masses (EM) observed on plants in choice tests in June 2009, September 2010, and May 2011; mean number of adults observed on each plant type 24, 48, and 72 hours after introduction to field cages, and mean number egg masses observed on plants over 72 hours in field cages (n = 4), and in greenhouse experiment (n = 12).

| | 2009 | | | | 2010 | | | | 2011 |
|---|---|---|---|---|---|---|---|---|---|
| | Adults | | | EM | Adults | | | EM | Greenhouse |
| **Plant** | **24 hr** | **48 hr** | **72 hr** | **Total** | **24 hr** | **48 hr** | **72 hr** | **Total** | **Total EM** |
| **arugula** | 1.0 b | 0.3 b | 0.0 c | 1.8 ab | 5.8 ab | 4.8 b | 4.0 b | 0.0 b | 0.1 c |
| **bean** | 0.0 c | 0.3 b | 0.0 c | 0.3 c | 0.0 c | 0.0 c | 0.0 c | 0.0 b | 0.0 c |
| **collard** | 1.5 b | 1.0 b | 0.0 c | 3.3 ab | 2.3 b | 3.0 b | 1.0 c | 2.3 ab | 0.7 b |
| **mustard** | 10.0 a | 11.3 a | 7.3 a | 3.8 a | 10.0 a | 9.0 a | 11.8 a | 0.3 b | 0.0 c |
| **rapeseed** | 1.0 b | 0.5 b | 0.5 b | 3.5 a | 3.3 b | 3.8 b | 3.3 b | 2.5 a | 2.2 a |
| **rapini** | 1.5 b | 0.3 b | 1.3 b | 0.8 bc | 4.8 b | 5.3 b | 3.0 b | 0.8 b | 0.3 bc |

Values in the same column, followed by the same letter are not significantly different according to a Kruskal-Wallis test and means separated by nonparametric multiple comparisons based on rank sums ($p < 0.05$ for all experiments).

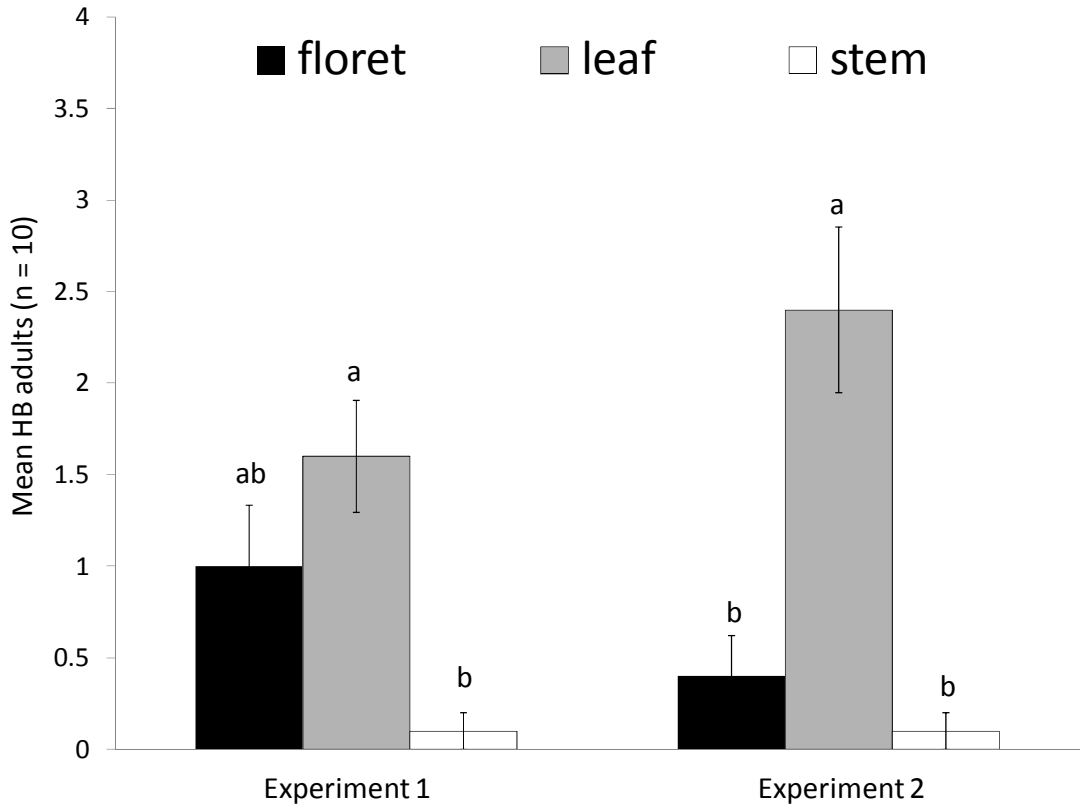


Figure 2.1: HB adults observed on plant parts from broccoli plants with crowns (before flowering). Experiments 1 and 2 occurred in August and September, respectively, using plants grown in Blacksburg, VA, 2011. Bars with the same letter were not significantly different according to ANOVA, Tukey's HSD ($p<0.001$).

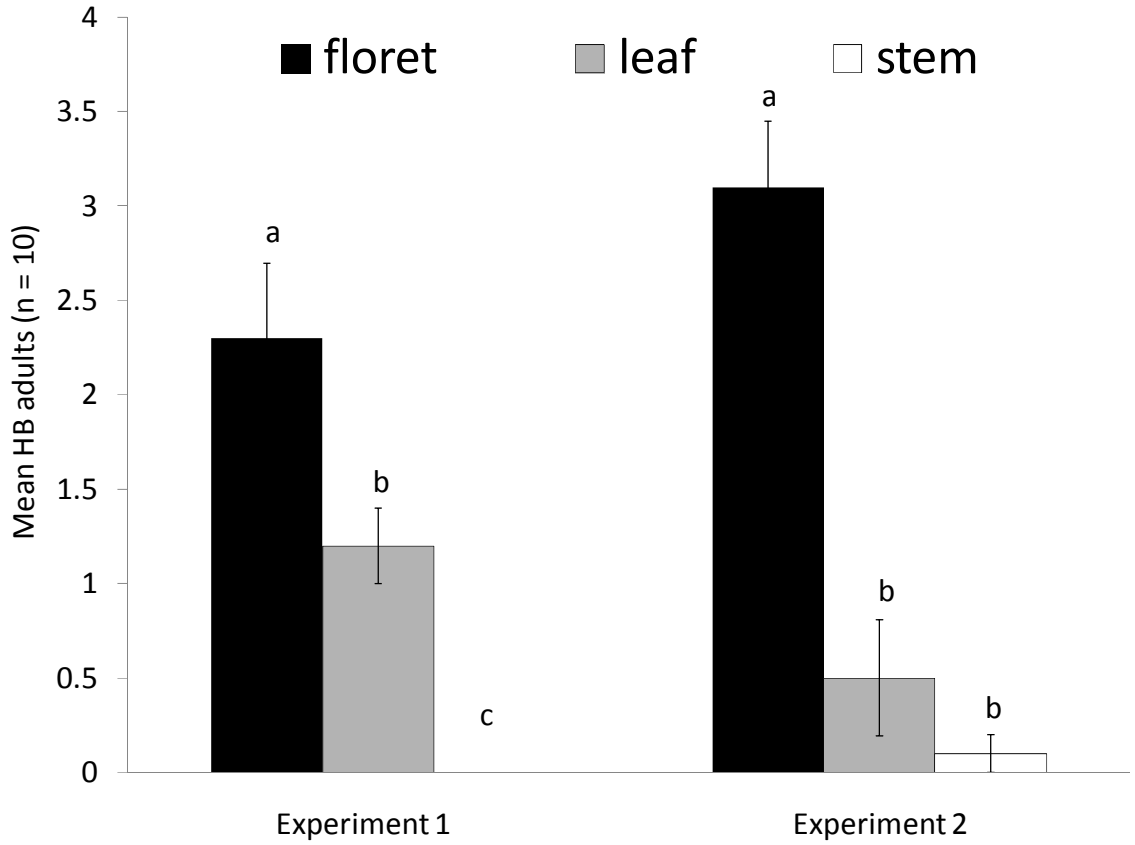


Figure 2.2: HB adults observed on plant parts from flowering broccoli plants. Experiments 1 and 2 occurred in August and September, respectively, using plants grown in Blacksburg, VA, 2011. Bars with the same letter were not significantly different according to ANOVA, Tukey's HSD ($p<0.001$).

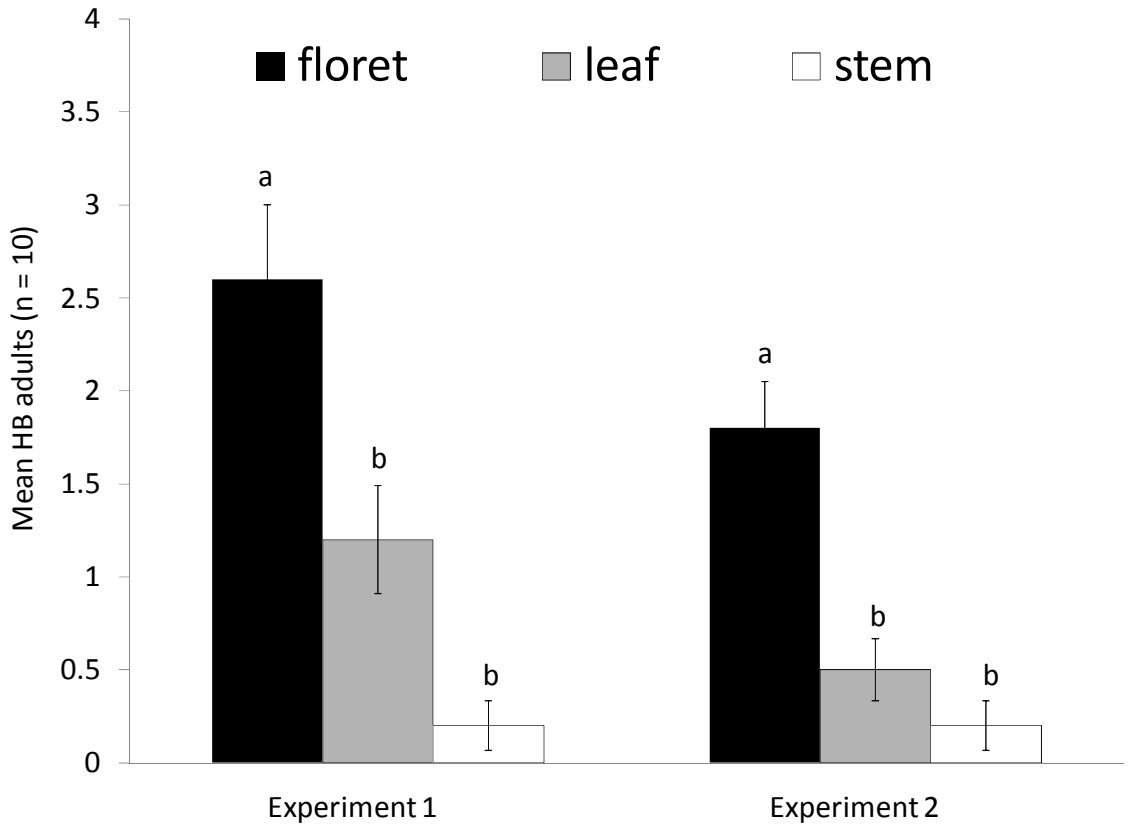


Figure 2.3: HB adults observed on plant parts from broccoli plants with seed pods (after flowering). Experiment 1 was conducted July 2009, using plants grown in Virginia Beach, VA. Experiment 2 was conducted August 2011, using plants grown in Blacksburg, VA. Bars with the same letter were not significantly different according to ANOVA, Tukey's HSD ($p<0.001$).

Table 2.2: Development time, or days until 50% of harlequin bugs isolated to each test plant species reached 3rd, 4th, 5th instar and adult, and the percent (out of 50) that survived the duration of the experiment.

| | Days to 50% | | | | |
|---|---|---|---|---|---|
| **Plant** | **3rd instar** | **4th instar** | **5th instar** | **Adult** | **% survival** |
| **arugula** | 6 | 18 | 29 | 45 | 12% |
| **bean** | - | - | - | - | 0% |
| **collard** | 6 | 12 | 24 | 42 | 50% |
| **mustard** | 4 | 12 | 24 | 38 | 88% |
| **rapeseed** | 10 | 16 | 22 | 40 | 42% |
| **rapini** | 4 | 10 | 20 | 34 | 88% |

Table 2.3: Mean number of harlequin bugs observed on collard greens grown at Virginia Beach and Blacksburg, VA in 2011 (n = 4). Ten collard plants were observed twice weekly for adults, egg masses and nymphs after first appearance.

| | | Virginia Beach (days after treatment) | | | | | Blacksburg (days after treatment) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Treatment** | **62** | **69** | **71** | **76** | **78** | **54** | **56** | **65** | **70** |
| **Adults** | No trap crop | 57.3 a | 34.0 a | 39.0 a | 20.0 | 10.5 | 6.5 a | 5.5 | 3.8 a | 2.3 |
| | Mustard borders | 0.0 b | 2.3 b | 2.5 b | 2.3 | 2.0 | 0.3 b | 0.0 | 0.5 b | 0.0 |
| | Treated mustard | 0.3 b | 2.3 b | 0.3 b | 11.0 | 1.8 | 0.3 b | 0.5 | 0.3 b | 0.5 |
| **Egg Masses** | No trap crop | 0.0 | 16.5 a | 9.5 | 25.5 a | 15.5 a | 6.5 a | 6.0 | 7.8 | 9.5 |
| | Mustard borders | 0.0 | 0.0 b | 1.0 | 0.3 b | 3.8 b | 0.3 b | 1.5 | 2.8 | 2.8 |
| | Treated mustard | 0.0 | 0.0 b | 0.5 | 0.5 b | 0.8 c | 0.3 b | 0.5 | 0.3 | 1.3 |
| **Nymphs** | No trap crop | 7.0 | 6.0 | 17.0 | 56.8 a | 70.3 a | 2.0 | 0.5 | 3.0 a | 12.3 |
| | Mustard borders | 1.5 | 1.8 | 2.0 | 3.8 b | 6.0 b | 0.0 | 0.3 | 0.0 b | 0.5 |
| | Treated mustard | 2.0 | 2.3 | 2.5 | 0.8 b | 1.3 c | 0.8 | 0.0 | 0.0 b | 0.8 |

Values in the same column, followed by the same letter are not significantly different according to a Kruskal-Wallis test and means separated by nonparametric multiple comparisons based on rank sums ($p < 0.05$). Those columns without letters were not significantly different.

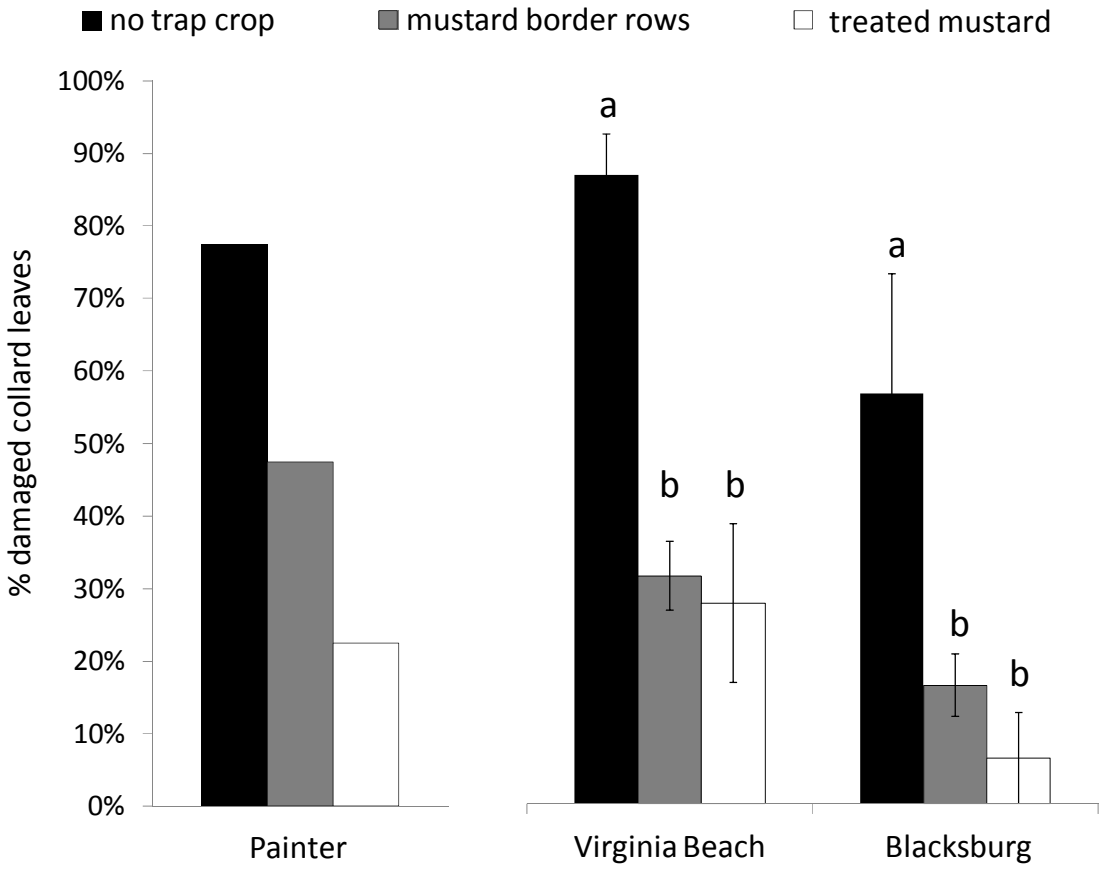


Figure 2.4: Percent of collard leaves damaged by harlequin bug observed at harvest (10 wks) at three Virginia locations. All but one plot was destroyed by drought at Painter in July 2010, so only one replication is reported. Values from Virginia Beach and Blacksburg sites are the mean (±SE) of four replications planted in May 2011. Bars with the same letter are not significantly different according to ANOVA, Tukey's HSD ($\alpha = 0.05$).

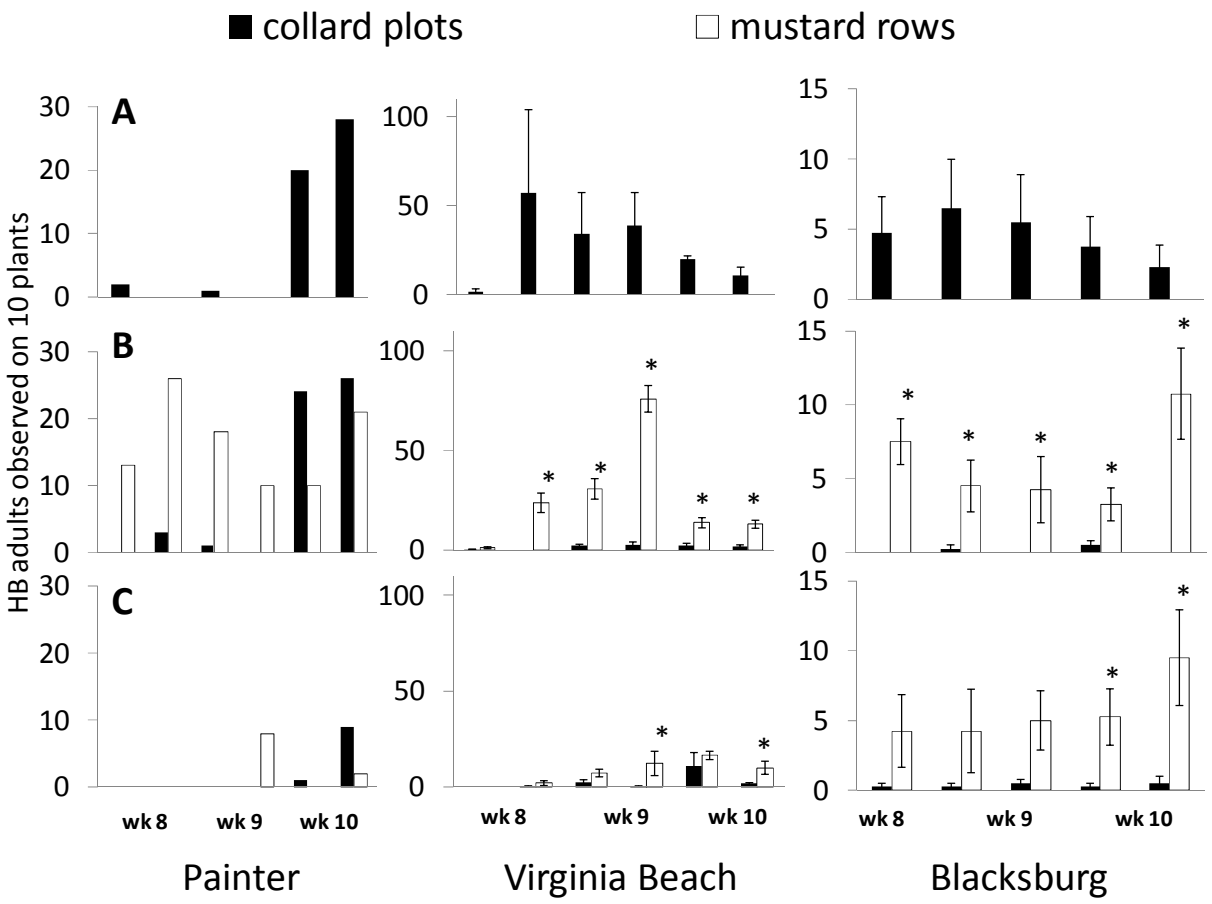


Figure 2.5: Harlequin bug adults observed in collard plots and their accompanying mustard border rows, the number observed on 10 plants, (A) collard plot alone, (B) collards with untreated mustard border rows, and (C) collard plots with insecticide treated mustard border rows. All but one plot was destroyed by drought at Painter in July 2010, so only one replication is reported. Values from Virginia Beach and Blacksburg sites are the mean (±SE) of four replications planted in May 2011. Bars with asterisks indicate observation dates where there were significantly more adults observed on mustard border rows than their accompanying collard plots, according to a Student's t-test ($\alpha = 0.05$).

## Chapter Three

## The role of olfactory cues in host plant selection of harlequin bug.

The chemical ecology of stink bugs is intriguing, particularly harlequin bugs (HB), *Murgantia histrionica* (Hahn), which feeds on the chemically-noxious Brassicaceae. Brassica plants produce glucosinolates, a family of chemical compounds that provide plant defense against herbivory (Fahey et al. 2001). Often referred to as mustard oil glycosides, these compounds give cole crops a hot or sharp taste, and act as an antifeedant or toxin to many herbivores. When plant cell walls are ruptured, the glucosinolate is degraded by the plant enzyme myrosinase; glucosinolates and myrosinases are stored separately to avoid autotoxicity (Louda and Mole 1991). Harlequin bug not only tolerates glucosinolates, but sequesters them for its own chemical defense from predators (Aliabadi et al. 2002). Like other pentatomids, HB secretes a defense chemical from the metathoracic gland when disturbed, but also expels a frothy liquid from the margins of the prothorax. This liquid has been shown to contain glucosinolate breakdown products (Aldrich et al. 1996).

Male HB produce an aggregation pheromone, Mugantiol (one of the stereoisomers of the sesquiterpene epoxyalcohol 4-[3-(3,3- dimethyloxiran-2-yl)-1-methylpropyl]-1-methylcyclohex-2-en-1-ol; Zahn et al. 2008a, 2012), that attracts both females and males in olfactometer choice tests. Precopulatory and copulatory behavior has been reported for this species by Lanigan and Barrows (1977) and, although the aggregation pheromone has a likely role in drawing female HB to their mates, courtship and mate recognition relies on short range substrate vibration (Čokl et al. 2004). The presence of this aggregation pheromone in males may imply that they play an important role in host plant finding/selection in this species.

The role of host plant volatiles in HB host searching and selection is not well understood, and understanding factors that affect host plant selection by HB is essential to trap cropping. This study seeks

to determine HB response to plant and insect-produced volatile organic compounds as well as evaluate plant preference based on olfactory cues alone.

## Materials and Methods

**Plants.** Mustard (*Brassica juncea* 'Southern Giant Curled'), collard (*B. oleracea* 'Champion'), and bean (*Phaseolus vulgaris* 'Bronco') were grown from seed in the greenhouse, in a mix of sphagnum peat moss, perlite and vermiculite (2:1:1), irrigated daily, and fertilized weekly with Scott's Water Soluble Plant Food (18-18-21 NPK with micronutrients; Scotts-Sierra Horticultural Product Company, Marysville, OH). Mustard and collard plants used in all experiments were 8-10 weeks old, with a minimum of four true leaves, and no reproductive structures. Bean plants matured faster than the brassicas, and were used at a minimum of six weeks old, with a minimum of three trifoliate leaves, and no flowers.

**Insects.** Participants in olfactometer choice tests were either "field-collected" or "lab-reared." Field-collected insects were obtained from collard plots grown at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter, VA, June-August 2010. After these insects were collected from plants in the afternoon, they were returned to the lab and isolated to individual Petri-dishes (9 cm diameter), deprived of light and starved for a minimum 24 hours before entering choice test (24$\pm$ 5$^{o}$C, ~40% RH). Lab-reared insects were the offspring of HB collected from mustard or collard greens grown at Virginia Tech's Kentland Research Farm near Blacksburg, VA, June-September 2011. Insects were reared on cauliflower florets in clear plastic containers with mesh lids (10 cm diameter, 6 cm height), and maintained at 24$\pm$ 5$^{o}$C, ~40% RH, photoperiod of 16:8 (L:D) h. Food sources were changed twice weekly. Late instars were observed every other day for their final molt into adulthood, and males and females were then isolated from each other to maintain virgin status. These adults were reared in a similar manner until 14 days past eclosion, the time necessary for HB to reach sexual maturity (Zahn et al.

2008b), and then each insect was isolated to an individual Petri dish (9 cm diameter), deprived of light and starved for a minimum of 24 hours before choice test (24± 5°C, ~40% RH).

Males that were used as a source of stimulus in choice tests were lab-reared, 14 day-old virgin adults, reared in a similar manner as described previously. Five stimulus-males were used in each olfactometer choice test, and were allowed to feed for a minimum of 6 h on the host plant (bean, collard, or mustard) evaluated that day before they were moved to olfactometer chambers. Stimulus-males were then allowed to acclimate for 20 minutes in olfactometer chambers before the start of the choice test.

**Olfactometer choice tests.** The response of HB adults to leaf surface volatiles was evaluated by using the following olfactometer choice tests: bean v. clean air, collard v. air, mustard v. air, bean v. collard, bean v. mustard, and collard v. mustard volatiles. The response of HB to insect and plant volatiles was evaluated by offering participants a choice of bean v. bean with male HB, collard v. collard with male HB, and mustard v. mustard with male HB.

Choice tests were conducted in a "Flying T" olfactometer, also referred to as an open Y-track olfactometer, described in detail by Dickens (1999). Hydrocarbon-free air supplied at the rate of 1 liter/min was humidified by bubbling through distilled water before delivery to the apparatus. Assays took place in a darkened room (24°C), in which the only light source was placed at the top of the "T" so that an insect released at the bottom would walk up the "T," due to its tendency to be positively phototactic and negatively geotactic. In follow-up experiments with lab-reared insects, the apparatus was kept under a frame that allowed the assay arena to be in dark conditions, while Ehrlenmeyer flasks containing plant materials could be exposed to ambient light augmented by 75 W full spectrum incandescent bulbs (Osram Sylvania, Danvers, MA). After performing behaviors such as turning or antennating within the volatile plumes, HB were considered to have made a choice after traveling 1 cm up either arm of the "T."

Plants used as stimulus in olfactometer choice tests were taken directly from the greenhouse before assay, 2-3 leaves were removed and cut ends were wrapped with a piece of wet paper towel. Foliage was used for no longer than 30 minutes during each assay.

Three assays were conducted for each experimental pairing. Each assay consisted of 20 field-collected males and 20 females, and was conducted after participant insects were isolated and starved for 24 h. Significant difference was determined by testing the hypothesis that the binomial proportion was different from 50:50 chance using the standard normal approximation (Ott and Longnecker 2001).

## Results

Harlequin bug males were attracted to volatile organic compounds from all three test plant species, choosing plant volatiles over clean air in olfactometer choice tests (Fig. 3.1). Field-collected female participants were attracted to collard volatiles only, but lab-reared virgin females did not respond to any plant volatiles (Fig. 3.1). When given a choice between a host and a non-host plant, males chose volatiles from collard and mustard over bean plants, but made no distinction between collard and mustard volatiles (Fig 3.2). Given the same set of choices, females made no distinction between any of the volatiles offered in olfactory choice tests (Fig. 3.2). Both males and females chose volatiles from males isolated on mustard and collard over the volatiles from those plants alone, while they appeared deterred from volatiles of males isolated on bean (Fig. 3.3).

## Discussion

Male HB were attracted to volatile organic compounds from all plant species offered, while females showed little response to plant volatiles alone. Males preferred volatiles from Brassicas over those from bean, but made no distinction between the two Brassicas, collard and mustard (Fig. 3.1 and 3.2); however, both genders have been reported to prefer mustard over broccoli (*B. oleracea*; Ludwig and Kok 1998, Chapter 2). These data support a hypothesis that males are the primary "colonizers" of new food sources and long distance olfactory cues help them to orient to cole crops. The ultimate choice of whether or not

to feed on a host plant, how long to stay on a host plant, and whether or not to emit a chemical signal to others, is likely made based on gustatory or tactile cues, rather than olfactory cues, given by the plant.

All plants produce green leaf volatiles, six carbon alcohols and aldehydes that are products of oxidative degradation of plant lipids, which are known to elicit electrophysiological responses from the antennae of several insects (Visser et al. 1979, Dickens 1989). While all plants produce volatile organic compounds, *Brassica* spp. produce a wider variety of volatiles than *Phaseolus* spp., particularly alcohols and terpenes (Mumm et al. 2008, Velikova et al. 2010). Generic green leaf plant volatiles may aid in orienting male HB to a field of young actively growing plants, and this mix of brassica-specific alchohols and terpenes may also play a role in the ability of these insects to orient specifically to cole crops.

Females showed little response to plant volatiles, and likely rely on male aggregation pheromones to find their host plants in the long range. In only one experimental pairing did female participants make a choice based on olfactory cues (Fig 3.1 and 3.2); field-collected females preferred collard volatiles over air, while virgin females made no choice in the same experimental pairing. Research has shown that past experience can influence feeding and oviposition preference in insects (Hopkins 1917, Barron 2001). Learning is not well reported in Pentatomidae; however, other hemipterans are reported to associate novel olfactory stimulus with a host plant (Patt and Setamou 2010). Nonetheless, we cannot rule out the possibility that the response of field-collected females was learned, as they may have connected the volatiles from the collard plants to previously-experienced gustatory cues.

Mating status was unknown for field-collected participants, but it can be assumed that many female participants were mated and carrying eggs, as experiments were conducted during a time of high reproductive activity (June-August). Females have been found to oviposit on collard more often than mustard in choice tests, even though they prefer to feed on mustard (Chapter Two). Collard plants used in these experiments were a glaucous variety while the mustard variety was not; glaucous plants are characterized by the presence of a waxy bloom, or extra-cuticular wax, produced by the plant that imparts

a bluish or whitish appearance to the leaves and reduces water loss by preventing cuticular transpiration (Denna 1979). A wax layer such as this has been shown to interfere with host searching of predators and parasitoids, which may make collard a more attractive oviposition site than mustard (Eigenbrode 2004). While HB may prefer to feed on mustard, both mustard and collard are suitable hosts for development (Chapter Two). It is possible that the positive response of female HB to collard volatiles may have to do with a response to olfactory cues that were associated with the waxy bloom of the plant.

Both males and female HB preferred the volatiles from males feeding on mustard and collard over the plant alone, while they appeared deterred from males feeding on bean (Fig. 3.3). Male HB may produce their aggregation pheromone only when they are feeding on a proper host plant, in this case the Brassicas, collard and mustard. On the other hand participant insects may be responding to plant volatiles that were produced by the plants in response to attack. Brassicas respond to HB feeding and oviposition with an increase in emission of certain mono- and sesquiterpenes, such as β-phellandrene, sabinene, eucalypton and β-caryophyllene, while feeding does not induce terpene emission in bean (Velikova et al. 2010).

Several species of insects that feed on Brassicales have been found to use volatile glucosinolate breakdown products as chemical cues for host finding, oviposition and/or feeding stimulants (Aliabadi and Whitman 2001). Orientation to crucifer compounds (i.e. isothiocynates) has been demonstrated in the lepidopteran pests *Plutella maculipennis* (L.) (Bartlett 1996), *Mamestra brassica* (L. ) (Rojas 1999), and several species of beetles (Pivnick et al. 1992, Smart et al. 1997, Blight and Smart 1999). These chemicals would not be released from an intact plant, rather they would be released as a volatile gas when the plant was damaged physically or by herbivory.

Like other pentatomids, HB secretes a defense chemical from the metathoracic gland when disturbed, but also expels a frothy liquid from the margins of its prothorax. This liquid has been shown to contain glucosinolate breakdown products, sequestered from the insect's host plant (Aldrich et al. 1996). Male HB produce an aggregation pheromone, mugantiol (Zahn et al. 2008, 2012), that attracts both females and

males to a food source from long range. The presence of a male produced aggregation pheromone may imply that males play a critical role in host plant finding and selection.

In addition, there may be a synergistic effect of insect and plant-produced volatiles. Green leaf volatiles have been found to be synergists in insect semiochemistry (Dickens 1989). For example the flea beetle, *Phyllotreta striata* (Fab.), does not respond to a synthetic version of their male-produced aggregation pheromone without the presence of plant volatiles, such as allyl isothiocyanate (Beran et al. 2011).

In summary, male HB are attracted to plant volatiles and are able to distinguish Brassica volatiles from a non-Brassica, but do not distinguish between collard, *B. oleracea,* and mustard, *B. juncea*, even though mustard is preferred in the field. The lack of distinction between long distance olfactory cues indicates that the location of a trap crop should be positioned to intercept movement from wild hosts or overwintering areas, in a perimeter or a border row. Though both males and females respond to volatiles from males feeding on host plants, the organic compounds responsible for this behavior should be investigated as this knowledge would impact the potential of using synthetic aggregation pheromones for semiochemically augmenting trap crops or baits.

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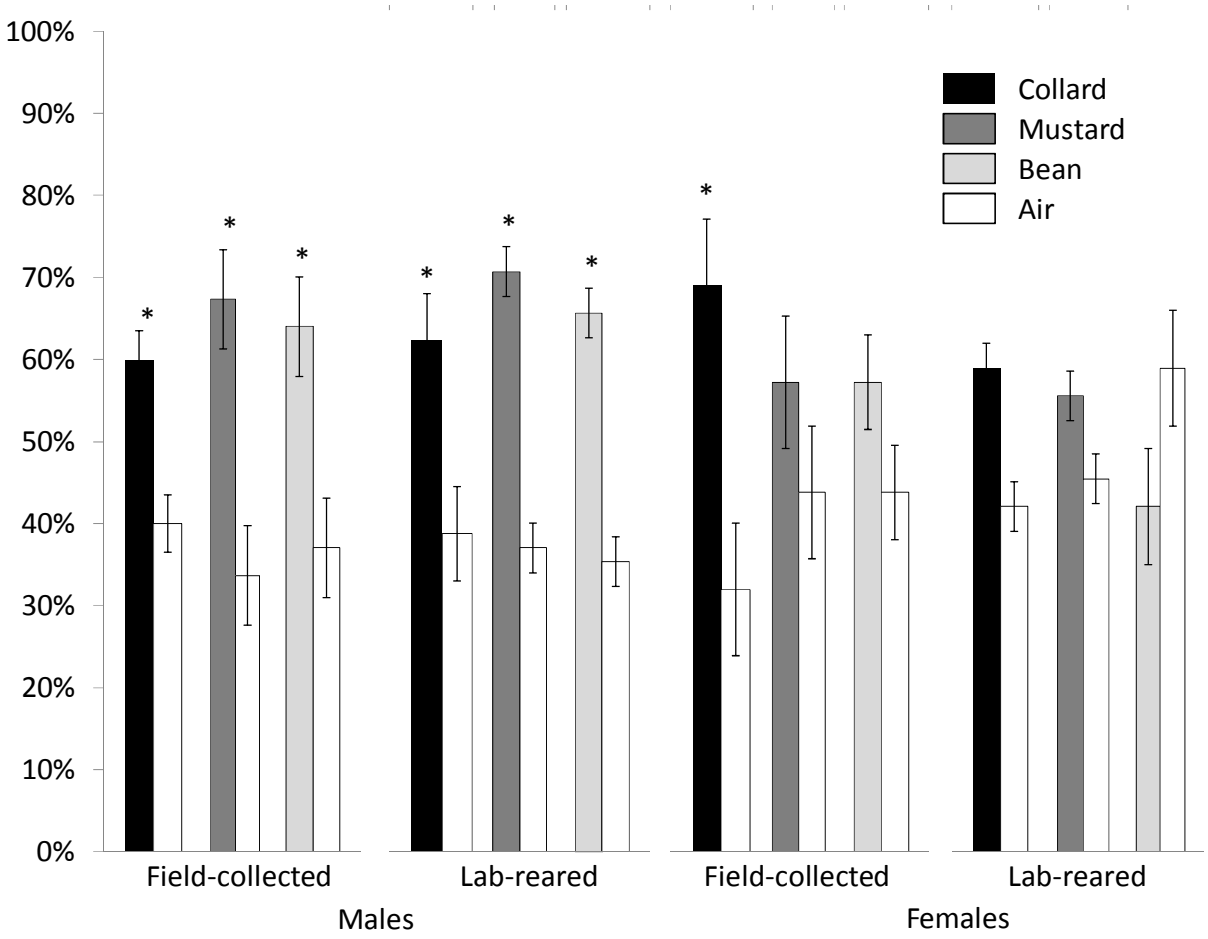


Figure 3.1: Choice of plant volatiles versus clean air in olfactometer choice tests using field-collected and lab-reared, virgin participants; percent mean (± SE) of choices in three bouts. Bars with asterisks indicate volatile sources that were selected significantly more often than 50% of the time according to a test of binomial proportions ($n = 60$, $p < 0.05$).

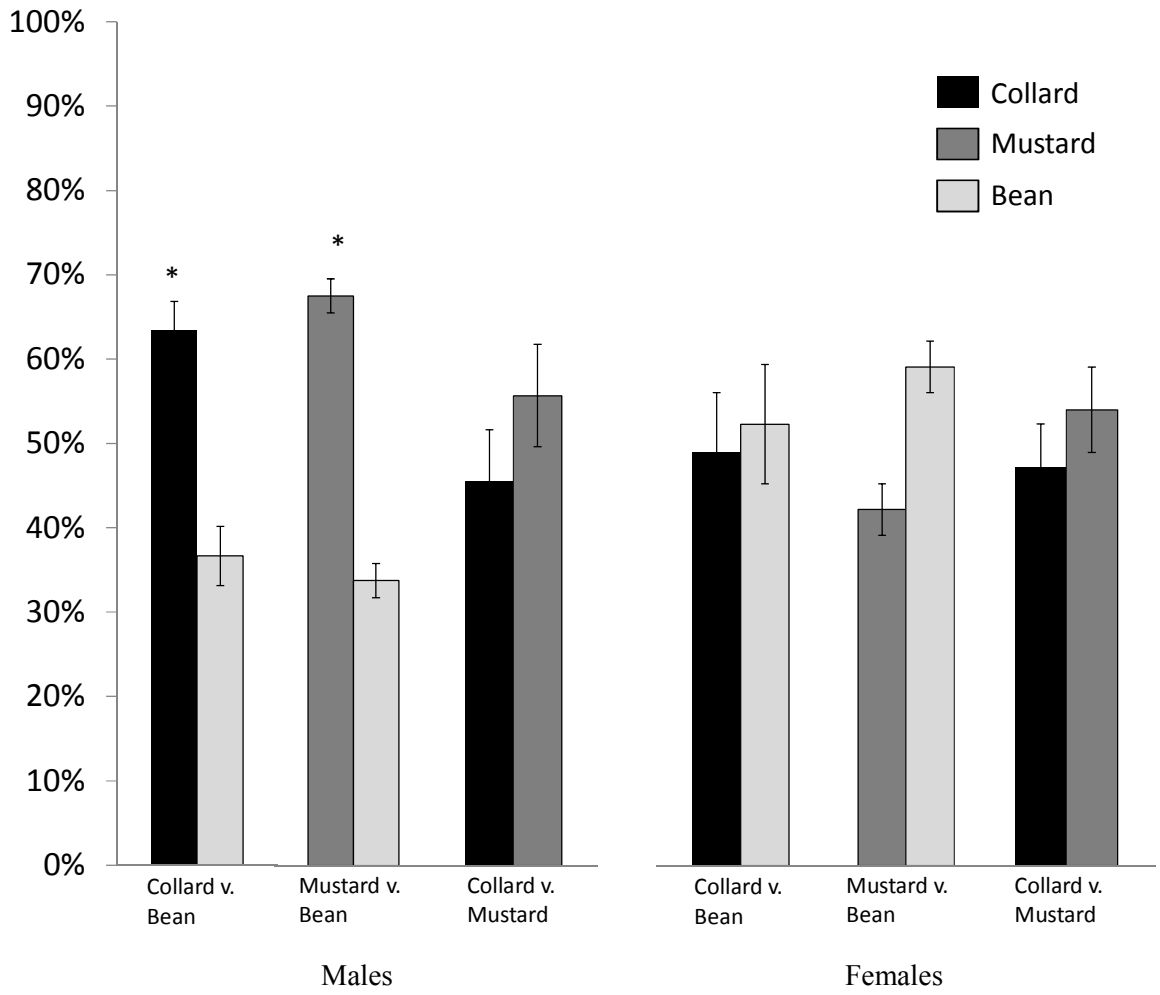


Figure 3.2: Choice between plant volatiles in olfactometer choice tests of field-collected male and female participants; percent mean (± SE) of choices in three bouts. Bars with asterisks indicate volatile sources that were selected significantly more often than 50% of the time according to a test of binomial proportions ($n = 60$, $p < 0.05$).

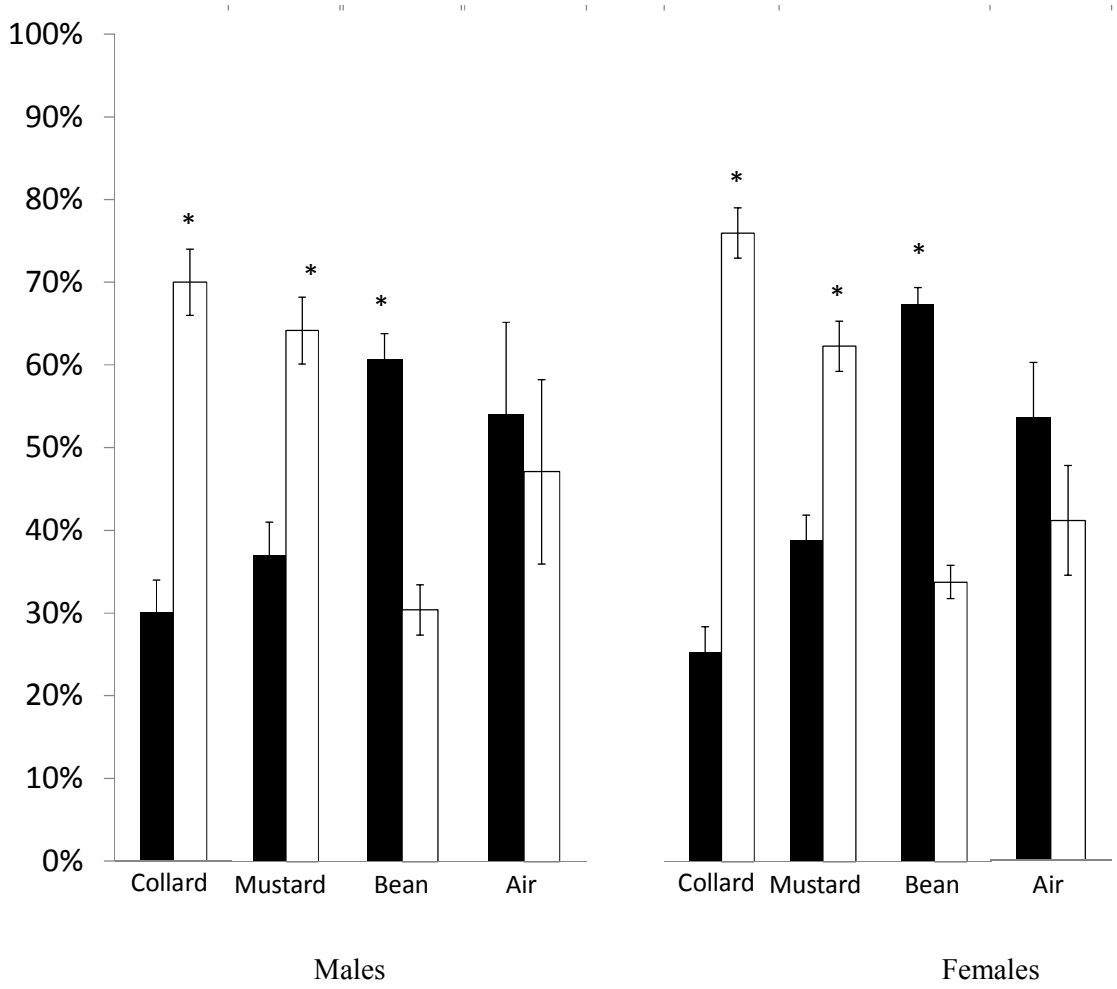


Figure 3.3: Choice between volatiles from virgin, male harlequin bugs and plants versus plant volatiles alone in olfactometer choice tests of male and female participants; percent mean (± SE) of choices in three bouts. Dark bars indicate plants only or clean air, while white bars indicate stimulus with insects. Bars with asterisks indicate volatile sources that were selected significantly more often than 50:50 according to a test of binomial proportions ($n = 60$, $p < 0.05$).

# Chapter Four

## Lethal concentration and field efficacy of four neonicotinoids on harlequin bug.

Neonicotinoid insecticides offer control of hemipteran insects, such as harlequin bug (HB), *Murgantia histrionica* (Hahn), that is less disruptive to natural enemies than broad-spectrum insecticides; they are water soluble and can be taken up by plants through the roots and translocated through the xylem vessels to plant tissues, exposing herbivores to the toxin only when they feed (Sur and Stork 2003, Tomizawa and Casida 2005). Neonicotinoids target the nicotinic acetylcholine receptors in insects, and cause an over-stimulation of neurons, which leads to the paralysis and the ultimate failure of the central nervous system (Thomson 2000). There is potential for both positive and negative interactions between neonicotinoids in the soil and biological control agents used to control soil pests, such as entomopathic fungi, nematodes, and bacteria (Koppenhöfer et al. 2002, Morales-Rodriguez and Peck 2009). In addition to toxic effects, neonicotinoids cause paralysis which could make insects more susceptible to attack from biological control agents.

Several neonicotinoid insecticides have been found to be effective in controlling HB using foliar applications, including acetameprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam (Edelson 2004, Edelson and Mackey 2005a,b,c,d, 2006, Walgenbach and Schoof 2011). However, soil application of neonicotinoids could allow for greater residual efficacy against the target pest while reducing non-target effects by eliminating the foliar application. The objectives of this study were to compare and contrast the relative toxicity of four neonicotinoid insecticides on HB and to assess the residual efficacy of these compounds when applied as a soil drench.

## Materials and Methods

### Dipped cabbage leaf disk toxicity bioassays to determine $LC_{50}$

Dose-mortality was estimated for four insecticides (Table 4.1). Leaf disks (8.5 cm diameter) were cut from the wrapper leaves of cabbage heads and dipped for 10 seconds in each insecticide solutions (concentration of 0, 0.001, 0.01, 0.1 and 1mg a.i./L) and allowed to dry for 2 h. Dry leaf disks were placed into individual Petri dishes (9 cm diameter) along with five HB $3^{rd}$-$4^{th}$ instars (n = 4). Mortality was determined after 48 h of exposure for imidacloprid, thiamethoxam, and dinotefuran assays, and after 72 h for clothianidin. Nymphs were considered dead when no movement was observed when prodded. Moribund nymphs exposed to clothianidin took up to 72 h before they were reliably determined dead, while 48 h was sufficient for the other three products. The experiment was repeated three times for each insecticide.

### Excised leaf toxicity bioassays to determine residual efficacy in the field

Collards (*Brassica oleracea* 'Vates') were seeded in May and again in July, 2010 at the Virginia Tech Eastern Shore Agricultural Research and Extension Center in Painter, VA. Insecticides were applied to 6 m, single-row plots in a randomized block design at the highest labeled rate once plants reached at least one true leaf (n = 4; Table 4.1). Drenches were applied with the equivalent of 378.5 liters of water per 300 m rows and 94.6 liters of water per 300 m rows, in May and July experiments, respectively. Leaves were removed from plots 7, 14, 21 and 28 days after treatment and five HB $3^{rd}$-$4^{th}$ instars were isolated to these leaves in Petri dishes (9 cm diameter).

### On-farm test of residual efficacy of thiamethoxam in the transplant water

Another test of residual efficacy was conducted in collaboration with a commercial grower in Hillsville, VA. Broccoli and cabbage were transplanted in September 2011 and treated with high and low rates of thiamethoxam (Durivo, Syngenta, Greensboro, NC) in the transplant water. Leaves were removed from

plots 14, 21, and 30 days after drench treatment and ten HB 3rd-4th instar nymphs were isolated to these leaves in Petri dishes and observed for mortality after 48 hours of feeding (n = 4). Bioassays for all three experiments were observed for mortality or signs of intoxication after 48 hours of feeding; a nymph was considered dead when no movement was observed when prodded, and considered moribund when unable to right itself and displayed overactive movement of legs.

**Data analysis**

Analysis of variance was conducted using JMP (SAS Institute Inc. 2007, Cary, NC) to test significant difference between percent mortality of treatments in 1 mg a.i./L concentration leaf dip bioassays, and all residual bioassays; means separation was determined using Tukey's HSD. Dose-mortality was estimated for each insecticide using probit analysis, correcting control mortality using Abbott's formula (EPA Probit Software 2007).

## Results

**Dipped cabbage leaf disk toxicity bioassays to determine $LC_{50}$**

There was no difference in toxicity amoung insecticides in leaf dip bioassays ($\alpha = 0.05$), and the 1 mg a.i./liter concentrations resulted in 60-70% mortality of HB nymphs (Table 4.2). The $LC_{50}$ for all active ingredients was well below the equivalent of the recommended field rate for all four products (Table 4.3).

**Excised leaf toxicity bioassays to determine residual efficacy in the field**

All four neonicotinoids provided significantly higher mortality than the control in bioassays conducted 7 and 14 days after treatment (Table 4.4) in the May experiment ($F = 20.27$; $df = 6, 21$; $p < 0.0001$, $F = 17.68$; $df = 6, 21$; $p < 0.0001$, respectively) and in the June experiment ($F = 4.89$; $df = 6, 21$; $p = 0.0028$, $F = 12.18$; $df = 6, 21$; $p < 0.0001$, respectively). There were no differences between insecticides with the exception of imidacloprid, which provided only 7 days of residual efficacy in the May experiment and

was not different from the control 7 days after treatment in the July experiment, although mortality was higher than the control 14 days after treatment (Table 4.4).

**On-farm test of residual efficacy of thiamethoxam in the transplant water**

Excised leaves from thiamethoxam treated broccoli and cabbage leaves provided significantly higher toxicity to HB nymphs than the control for 21 and 30 days, respectively (Table 4.5).

## Discussion

All four insecticides were toxic to HB in leaf dip assays and residual efficacy trials. However, based on toxicity alone, these data indicate that these insecticides may be effective for a shorter period of time than findings from previous field studies (Table 4.4). Mortality was no different from the control 14 days after treatment in the May experiment. Kuhar and Doughty (2009) reported 29 days of control in the field.

Sub-lethal effects, can contribute to control of pests in the field, such as feeding cessation, avoidance, and reduction in oviposition or reduction in the health of offspring. When in low concentrations, imidacloprid had been described as an anti-feedant for several species of insects, including green peach aphid, *Myzus persicae* (Sulzer), whitefly, *Bemisia tabaci* Gennadius, and Japanese beetle, *Popillia japonica* Newman (Nauen 1995, Nauen et al. 1998, George et al. 2007, Wise et al. 2007). On the other hand, there are several examples where there is no effect on host choice when neonicotinoids are used as a systemic insecticide. Held and Parker (2011) found no avoidance behavior of azalea lace bug, *Stephanitis pyrioides* (Scott), when given the choice between treated and untreated cuttings for systemic applications of imidacloprid, thiamethoxam, dinotefuran or clothianidin. It is possible that avoidance behaviors are only seen when neonicotinoids are applied to leaf surfaces. It is also possible that avoidance is induced by plant defense mechanisms, as neonicotinoids have also been reported to induce a salicylate-associated plant defense response that increases vigor as well as the plants natural defenses against attack from insects and pathogens (Ford et al. 2010). The behavioral effects of sub-lethal doses of neonicotinoids are unknown in HB and may need further investigation.

Critical to the use of neonicotinoids as a systemic insecticide is delivery to the root zone, accomplished through either seed treatment, drench application to the soil surface, through a drip irrigation system, or in transplant water. Residual efficacy over time will be influenced by how quickly the insecticide can be taken up into the plant and the life of the insecticide in the soil, whether it will be leached from the rootzone or bind to the soil, and how quickly it degrades in the environment. Imidacloprid, thiamethoxam, dinotefuran, and clothianidin are all water soluble, but dinotefuran is more water soluble than the rest, faster to be taken up by plants and the least likely to bind to soil, while imidacloprid is most likely to be bound by soil (Byrne et al. 2007, 2010, Ali and Caldwell 2010).

The half-lives of these chemicals in the soil are variable as well, but thiamethoxam and dinotefuran have shorter half-lives relative to imidacloprid and clothianidin (EPA 2003, 2004). The soil half-life for imidacloprid has been reported to be as short as 60 days (Liu et al. 2011) or as long as 280 days (Saran and Kamble 2008). The half-life for thiamethoxam may range from 9 days to 75 days (Karmakar and Kulshrestha 2009, Maienfisch et al. 2001). Soil type, pH, groundcover, cultivation (i.e. exposure to sunlight), moisture, temperature, and microbial communities present in the soil all play a role in the residual life of an insecticide in the soil. Stable in neutral and acidic water, neonicotinoids will slowly degrade in higher pH solutions and are very susceptible to photodegredation in solution (Liu et al. 2006). Bare-ground soils will see longer half-lives than soils with groundcover, and higher levels of organic matter in the soil make for longer half-life as sorption of neonicotinoids increases as organic carbon content increases (Scholz and Spiteller 1992, Cox et al. 1997, 1998). When neonicotinoids are bound to the soil, this decreases the bioavailability to microorganisms that degrade the compound. Soil-dwelling microorganisms have been described that degrade neonicotinoids, such as imidacloprid and thiamethoxam (Anhalt et al. 2007, Pandey et al. 2009).

Imidacloprid had a shorter period of efficacy than the other insecticides in the May experiment and was also slower to provide control in the July experiment (Table 4.4). A slow uptake of imidacloprid can be expected due to its higher affinity to soil. All insecticides demonstrated shorter than anticipated residual

efficacy, and it is possible that the volume of water used in these drench treatments was not adequate to deliver the full rate of insecticide to the root-zone, or insecticide percolated to areas beyond the root structure.

The longer residual efficacy in the fall experiment in Hillsville, VA is likely due to the more efficient method of application, delivering the insecticide in the transplant water directly to the root-zone (Table 4.5). In addition, colder, wetter temperatures made for a slower plant growth rate during this experiment compared to May and July experiments, and a slower uptake of insecticide from the soil.

In summary, neonicotinoids provide control of HB and the residual efficacy of a drench application can vary. Other factors may influence insect control once these insecticides are deployed in the field. A method of application that puts the active ingredient directly in the root zone may be preferred over an application to the soil surface.

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Table 4.1: Neonicotinoid nsecticides evaluated and application rates for use on cole crops, with the exception of Belay for which the rates for potato are listed.

| Manufacturer | Product | Active Ingredient | Foliar | Soil |
|---|---|---|---|---|
| Bayer (Research Triangle Park, NC) | Admire PRO | imidacloprid | 1.3 fl oz/A | 4.44-10.5 fl oz/A |
| Syngenta (Greensboro, NC) | Platinum 75SG | thiamethoxam | no foliar rate | 1.66-3.67 oz/A |
| | Durivo | thiamethoxam (+ chlorantraniliprole) | no foliar rate | 10-13 fl oz/A |
| Valent (Libertyville, IL) | Venom 70SG | dinotefuran | 1-4 oz/A | 5-6 oz/A |
| | Belay | clothianidin | 2-3 fl oz/A | 9-12 fl oz/A |

Table 4.2: Mortality observed after 48 or 72 hours in dipped cabbage leaf bioassays. Each experiment assayed 20 nymphs for each rate and each experiment was repeated three times. Values reported are the mean of the percent mortality adjusted using Abbott's formula.

| Rate (mg a.i./L) | imidacloprid (48 h) | dinotefuran (48 h) | thiamethoxam (48 h) | clothianidin (72 h) |
|---|---|---|---|---|
| 0.001 | 5.20% | 2.10% | 5.30% | 5.30% |
| 0.01 | 4.00% | 2.10% | 8.00% | 7.40% |
| 0.1 | 14.60% | 16.00% | 31.10% | 32.90% |
| 1 | 67.70% | 65.80% | 73.40% | 61.90% |

Table 4.3: Lethal concentration ($LC_{50}$) and the lower and upper 95% confidence intervals (mg a.i./L) of listed neonicotinoid insecticides. Values are the mean of probit analysis results from three experiments.

| **Insecticide** | $LC_{50}$ | **lower C.I.** | **upper C.I.** |
|---|---|---|---|
| imidacloprid | 0.573 | 0.317 | 1.256 |
| clothianidin | 0.394 | 0.164 | 1.495 |
| thiamethoxam | 0.518 | 0.280 | 1.064 |
| dinotefuran | 0.385 | 0.204 | 0.917 |

Table 4.4: Percent mortality (dead + moribund) harlequin bug nymphs exposed to excised collard leaves 7, 12, 21 and 28 days after treatment (DAT) with soil drench applications of neonicotinoid insecticides at their highest registered rates.

| | Experiment 1 (%) | | | | Experiment 2 (%) | | | |
|---|---|---|---|---|---|---|---|---|
| **Insecticides** | **7 DAT** | **14** | **21** | **28** | **7 DAT** | **14** | **21** | **28** |
| untreated | 5 b | 2.5 c | 18 | 8 | 3 c | 10 b | 0 | 5 |
| imidacloprid | 80 a | 45 b | 20 | 15 | 25 bc | 95 a | 25 | 0 |
| thiamethoxam | 100 a | 100 a | 48 | 40 | 63 ab | 95 a | 25 | 10 |
| dinotefuran | 98 a | 100 a | 75 | 63 | 83 a | 90 a | 10 | 5 |
| clothianidin | 93 a | 88 a | 28 | 18 | 68 ab | 90 a | 20 | 25 |

Data within a column followed by the same letter are not significantly different ($n = 4$, $\alpha = 0.05$); there was no significant treatment effect on mortality at 21 and 28 days after treatment.

Table 4.5: Percent mortality (dead + moribund) of harlequin bug nymphs exposed to excised broccoli and cabbage leaves 14, 21 and 30 days after treatment (DAT) with thiamethoxam in transplant water at highest and lowest registered rates, Hillsville, VA.

| | | % mortality | | |
|---|---|---|---|---|
| **Crop** | | **14 (DAT)** | **21** | **30** |
| **Broccoli** | **Untreated** | 25 b | 3 b | 30 |
| | **Low** | 48 b | 60 a | 45 |
| | **High** | 90 a | 88 a | 45 |
| | F | 14.13 | 38.7 | |
| | p | 0.0017 | <0.0001 | |
| **Cabbage** | **Untreated** | 40 b | 8 b | 33 b |
| | **Low** | 85 a | 75a | 95 a |
| | **High** | 85 a | 70 a | 75 a |
| | F | 20.25 | 17.12 | 17.67 |
| | p | 0.0005 | 0.0009 | 0.0008 |

Data within the same column, followed by the same letter are not significantly different ($n = 4$, $\alpha = 0.05$).

## Summary

Our overall goal was to gain information about the behavior of harlequin bug (HB), *Murgantia histrionica* (Hahn), in order to reduce HB feeding injury using trap cropping and without the use of broad-spectrum insecticides. Our results show a predictable behavior in HB for the advantage of its management in cole crops.

Mustard was selected as a highly attractive trap crop from a group of plant species that were preferred by HB adults over collard in a field-cage choice test. A border row of mustard reduced HB injury in collard by roughly 50%, and there was no added benefit of using an insecticide-treated trap crop. Although there was no difference in control between untreated and insecticide treated plots, management of HB aggregations on trap crops is highly recommended for reducing on-farm pest populations, which may include the use of insecticides.

Neonicotinoids provide control of HB and the residual efficacy of a drench application is longer than that of a foliar application. Other factors may influence insect control once these insecticides are deployed in the field, and neonicotinoids may act as anti-feedants for HB. A method of application that puts the active ingredient directly in the root zone may be preferred over an application to the soil surface.

Male HB are attracted to plant volatiles and are able to distinguish Brassica volatiles from a non-Brassica, but do not distinguish between collard, *B. oleracea,* and mustard, *B. juncea*, even though mustard is preferred in the field. The lack of distinction between long distance olfactory cues indicates that the location of a trap crop should be positioned to intercept movement from wild hosts or overwintering areas, in a perimeter or a border row. Females did not respond to plant volatiles alone. Both males and females respond to volatiles from males feeding on host plants; however, the organic compounds present responsible for this behavior should be identified as this knowledge would impact the potential of using synthetic aggregation pheromones for semiochemically augmenting trap crops or baits.