HERBIVORE INDUCED PLANT VOLATILES OF ASPARAGUS (*ASPARAGUS OFFICINALIS* L.) AND THEIR ATTRACTION TO NATURAL ENEMIES OF KEY ASPARAGUS PESTS

By

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ABSTRACT

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Asparagus production in Michigan is limited in longevity, productivity, and market value by two key monophagous insect pests, the asparagus miner (*Ophiomyia simplex* Loew) (Diptera: Agromyzidae) and common asparagus beetle (*Crioceris asparagi* L.) (Coleoptera: Chrysomelidae). Asparagus producers have expressed interest in pursuing the development of biological control management tactics because the current chemical management tactics available are ineffective at controlling these pests. My research seeks to fill knowledge gaps that currently exist in our understanding in biological control tactics for these pests by: 1) establishing pest and natural enemy spatial distributions, 2) developing a molecular gut content analysis protocol for predators of the two pests, 3) determining key predators of these two pests, 4) investigating the influence border habitats have on predator abundance, 5) identifying herbivore induced volatiles of asparagus, 6) determining natural enemy and pest attraction to asparagus volatiles, 7) examining the attraction of volatile lures to pests and natural enemies in a field setting, and 8) investigating the use of volatile lures to increase biological control of the two key pests.

Pests and predator arthropods were collected from transects inside fields, on the field edges, or in margin habitats, weekly in 2014 and 2015, from commercial asparagus fields with different border habitat types. Key asparagus pests had significantly higher abundances on the field edge, while predator arthropods were found in higher abundance in the field margin. Key

pests had higher abundances in fields bordered by another asparagus field, while predators were found in higher abundance in fields bordered by forests. Molecular gut content analysis revealed predators testing positive for the DNA of either key pest were primarily collected from field margins with forested habitats or margins planted with other (non-asparagus) crops.

In 2014, headspace was collected from asparagus grown in field cages that were exposed to either no damage, mechanical damage, or feeding damage from the common asparagus beetle. I found that asparagus responds to specialist herbivory through upregulation in the production of (*E*)-β-ocimene, (*E,E*)-α-farnesene, and 1-tetradecanol. In 2015 and 2016, y-tube olfactometer tests revealed that adult asparagus beetles and predatory lady beetles had little attraction to asparagus volatiles. In 2016, field lures were developed from induced asparagus volatiles and tested in commercial fields; all attracted significantly more parasitoids than control lures but did not attract predators or pests. In 2017, the most attractive lure to parasitoids identified in the previous year's research was deployed in an effort to increase the biological control of key pests by parasitoids using two lure deployment densities. It was determined that lures deployed in a low-density arrangement led to increases in the number of asparagus miners attacked by individuals from the Pteromalidae parasitoid family. Overall, the results of this research offer the most comprehensive attempt, to date, to develop a biological pest control tactic in asparagus and represents a promising avenue for future pest management research in this specialty crop.

This dissertation is dedicated to my partner in life whose support and love has allowed me to achieve more than I ever thought possible, and my parents whose guidance and love has been unconditional.

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CHAPTER 1

Management challenges for key pests of asparagus

Introduction

Management of arthropod pests in agroecosystems of the United States (US) is characterized by the use of multifaceted management strategies that integrate compatible tactics to protect crops, the surrounding environment, and the profitability of agricultural operations. At its core, integrated pest management (IPM) recognizes the shortcomings of single strategy approaches to managing pests and the plasticity of pests to quickly adapt to narrow management strategies (Pedigo, 2002). Due to considerable efforts on the part of scientists, regulators, pest management professionals, and farmers to incorporate IPM into on-farm practices, massive reductions in the use of insecticides have been realized since the 1960's (Fernandez-Cornejo et al., 2014). However, IPM strategy and tactic development are dynamic processes and are often unique to each cropping system, location, and pest being managed, and require thorough research and testing before implementation in a commercial setting (Flint, 2012). The uniqueness of each system's pest problems and related management tactics can result in IPM knowledge gaps in understudied crops, like many specialty crops, that inadvertently promote the use of broad spectrum chemical controls due to the lack of viable and well established alternative management strategies and tactics, particularly in cases of key crop pests (Fennimore and Doohan, 2008; Trumble, 1998).

This has been the case with asparagus (*Asparagus officinalis* L.) production in the US where obligate arthropod pests, the asparagus miner (*Ophiomyia simplex* Loew) (Diptera: Agromyzidae) and the common asparagus beetle (*Crioceris asparagi* L.) (Coleoptera:

Chrysomelidae), are impacting crop longevity and value and are not managed effectively with currently available IPM strategies, resulting in the use of broad spectrum insecticides to control crop specific pests (Bird et al., 2014; Morrison et al., 2014b; Morrison and Szendrei, 2014). Recently, asparagus producers expressed interest in developing alternative management strategies to target crop specific pests using biological control by increasing natural enemy abundance in fields to aid in current pest control efforts. In agroecosystems where pest management relies mostly on pesticides, like asparagus, biological control strategies can be effectively incorporated to include the use of pesticides with selective modes of action, and consideration of time and space distributions of pests and natural enemies when applying pesticides (Gurr et al., 2000; Gurr and Kvedaras, 2010). However, to successfully develop any biological control strategy in asparagus we must first understand the biology of the agroecosystem to determine where knowledge gaps exist that are preventing producers from using biological pest control.

Target pests of interest

Asparagus miner was first reported in the US by Loew in 1869, and is an obligate specialist in asparagus (Chittendon, 1907; Spencer, 1973). Asparagus miners are bivoltine with the first generation occurring during the spring asparagus harvest period and the second generation occurring during the summer post-harvest period (Fig. 1.1) (Lampert et al., 1984; Morrison et al., 2014a; Tuell, 2003). Adult asparagus miners mate and then oviposit eggs directly beneath the epidermis of the asparagus stem, near the soil line, where the larval stage emerges and feeds creating mines that meander within the stem (Barnes, 1937; Ferro and Gilbertson,

1982; Lampert et al., 1984; Morrison et al., 2011; Tuell, 2003). The miner overwinters as a pupa within stems and emerges as an adult in the spring (Lampert et al., 1984; Morrison et al., 2014a).

The economic impact of asparagus miner larval feeding is not well understood, but it is compounded by the fact that the mines create entry points for secondary infection by pathogens and increases incidence of *Fusarium* crown rot (Tuell and Hausbeck, 2008). The primary causal strains responsible for asparagus crown rot in the US are *Fusarium oxysporum* Wollenw. f. sp. *asparagi* S.I. Cohen (Gordon and Martyn, 1997; Van Bakel and Kerstens, 1970) and *Fusarium proliferatum* (Matsushima) Nirenberg (teleomorph *Gibberella fujikuroi*) (Elmer, 1995). Overall, *Fusarium* infections result in roughly a 50% reduction in asparagus field longevity and has therefore made the asparagus miner a target pest of interest for producers (Elmer et al., 1996).

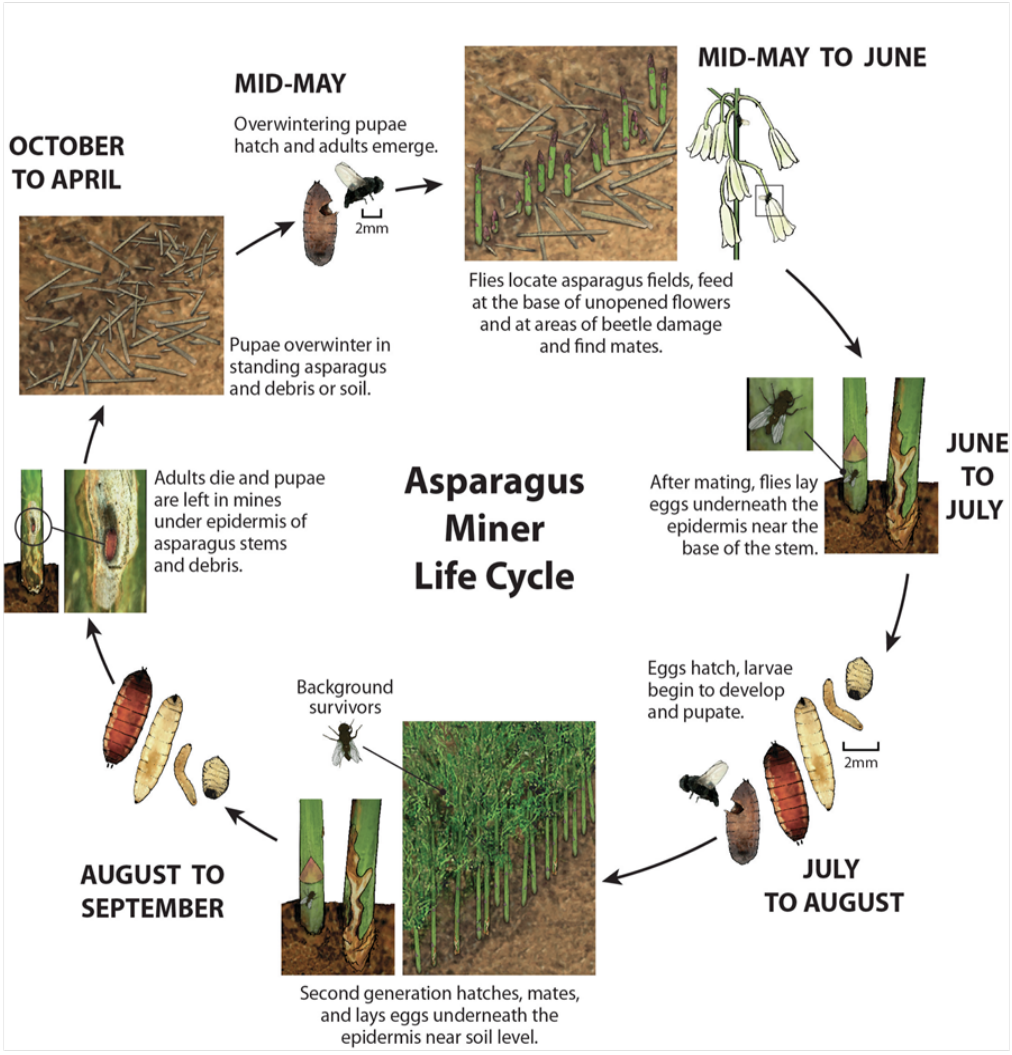

Figure 1.1. Asparagus miner life cycle drawn by Marlene Cameron, Michigan State University.

The common asparagus beetle is an obligate feeder on asparagus that was introduced into the US from Europe in 1860 (Chittenden, 1917), and usually completes three generations per year in temperate climates (Fig. 1.2) (Capinera and Lilly, 1975a; Taylor and Harcourt, 1975; Taylor and Harcourt, 1978). Damage by the common asparagus beetle can impact both the harvested and unharvested portions of the plant. During harvest, adults emerge from overwintering sites in old stems, debris from the previous season, and from underneath tree bark in surrounding woods, and feed on the emerging spears creating pock marks which result in

reduced market value (Chittenden, 1917; Gupta and Riley, 1967). Additionally, eggs oviposited on spears by the beetles can result in an unmarketable product because they aren't easily removed by washing (Voight and Gorb, 2010). Post-harvest, the beetles feed upon the cladophylls (needles) and axillary branches of the asparagus fern resulting in defoliation, reduced photosynthetic capacity and carbohydrate assimilation, and may ultimately cause fern death (Capinera, 2001; Grafius and Hutchinson, 1995). Collectively, control cost and loss estimates for damage and chemical control of the beetle have been reported between $1.4 – 1.6 million per year for Michigan, Washington, and Illinois combined (Hendrickson et al., 1991).

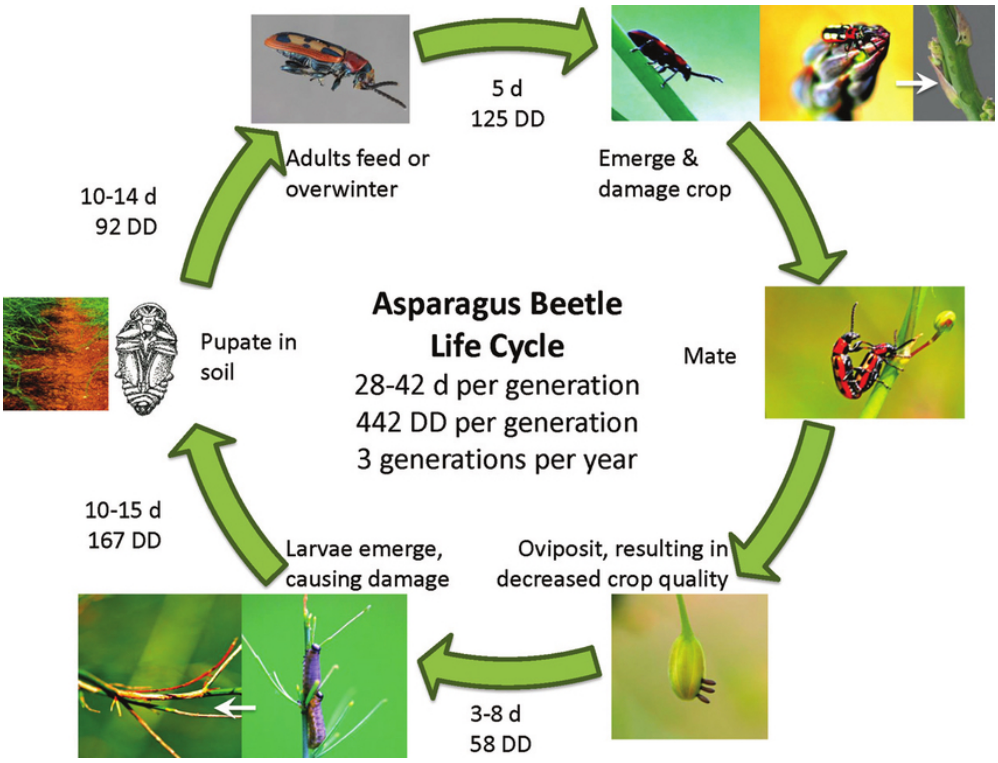

Figure 1.2. Common asparagus beetle life cycle by Morrison and Szendrei (2014).

Asparagus miner and the common asparagus beetle follow similar patterns of distribution during their post-harvest generations, congregating on the field edges (Ingrao et al., 2017; Morrison and Szendrei, 2013). Natural enemies also inhabit field borders and edges of production fields due to their requirement for additional resources available in diverse habitats outside agricultural fields (Landis et al., 2000). This creates the potential for multi-trophic interaction in field margins and edges in post-harvest asparagus and represents a potential opportunity to exploit this distribution to favor natural enemies, thereby enhancing biological control services for producers.

Chemical control

Broad-spectrum insecticides are the only chemicals registered to control asparagus miner in Michigan (Szendrei and Morrison, 2011). Compounds available are recommended to be applied as foliar treatments and must make contact with the pest to be effective. This is problematic in controlling asparagus miners because the adult is the only life stage that lives outside of the asparagus stem. A degree day model has been created for asparagus miner which may increase the effectiveness of chemical controls by allowing growers to target applications during peak adult flight; however, the second generation's peak flight is prolonged, covering several weeks during August making spray application timing problematic (Morrison et al., 2014a). Systemic pesticides have the potential to impact more life stages, but to date no systemic chemistries are labeled for use in asparagus. Additionally, no economic thresholds have been developed for asparagus miner, but due to its association as a passive vector for *Fusarium* any pest presence may warrant control measures. Due to the lack of effective chemical controls for

asparagus miner there is incentive for growers to implement biological control management strategies which may be able to target life stages that are protected from chemical controls.

While there are more insecticide chemistries available for controlling the common asparagus beetle, these pests still present challenges to growers. Broad-spectrum insecticides (*e.g.* carbamates, pyrethroids) are the most widely used control method for asparagus beetles among Michigan asparagus growers (Buchanan et al., 2015). Economic thresholds have been created for chemical control of asparagus beetles during harvest and post-harvest and require scouting 100 plants per field for beetle presence. During harvest the economic threshold is met if: > 2 % of the spears have eggs, > 50 % of the plants are infested with larvae, or > 5 % are infested with adults. The post-harvest threshold for chemical control is > 10 % defoliation (Bird et al., 2014; Delahout, 2005). When applied, chemistries labeled for beetle control require re-entry intervals of 12 – 72 h (Bird et al., 2014), making their use during harvest difficult because fields need to be harvested daily. Newer chemistries that have short re-entry times are being investigated for common asparagus beetle control. Spinosad and spinetoram both have re-entry times of 4 h; however, they are only labeled for use on asparagus ferns, thus they cannot be used to control beetles during the harvest period (Bird et al., 2014). The inability of growers to control common asparagus beetle outbreaks during the harvest period represents an opportunity for research into the development and application of biological control tactics that could enhance natural enemy populations and lead to better season-long control.

Biological control

Natural enemies, predator and parasitoid arthropods, can be important regulators of pest pressure in agroecosystems (Van Driesche et al., 2009). As a component of IPM, natural enemies

serving as biological pest control agents are reported to have the highest return on investment of any IPM tactic (Naranjo et al., 2015), and provide pest control services valued from $4.5 – $17 billion annually in the US alone (Losey and Vaughan, 2006; Pimentel et al., 1997). However, no proven biological control management tactics are currently available to US asparagus producers.

Morrison et al. (2014b) were the first to study natural enemies in Michigan asparagus and identified parasitoids of the asparagus miner by rearing parasitized miner pupae. In all, 12 parasitoid species were identified as feeding on the asparagus miner, 91 % of which were *Chorebus randanii* Giard (Hymenoptera: Braconidae) (24 %) and *Thinodytes cephalon* (Walker) (Hymenoptera: Pteromalidae) (67 %).

Several parasitoids of the common asparagus beetle have been identified in the US (Capinera and Lilly, 1975b; Poll et al., 1998; Watts, 1938). *Tetrastichus asparagi* Crawford (Hymenoptera: Eulophidae) is a host-specific koinobiont parasitoid of the beetle found throughout the US (Capinera and Lilly, 1975b; Poll et al., 1998). *Paralispe infernalis* Townsend (Diptera: Tachinidae) is a larval parasitoid of the beetle primarily found in the southern states of the US (Watts, 1938). Efforts to establish additional parasitoid species from Europe have been pursued; however, only *Lemophagus crioceritor* Aubert (Hymenoptera: Ichneumonidae) established in the northern regions of North America (Hendrickson et al., 1991).

Overall, the complex of predators attacking the asparagus miner in the US is undescribed; however, efforts to describe the complex of the common asparagus beetle have been made. Identified predators of the beetle include individuals from the Coccinellidae, Carabidae, Pentatomidae, Reduviidae, and Nabidae families (Capinera and Lilly, 1975a; Drake and Harris, 1932; Morrison and Szendrei, 2014; Watts, 1938). However, these studies have relied on

observational data and staged predation events in the lab that don't reliably identify relevant predators that could be targeted for biological control efforts in the field.

With advances in molecular technologies, linking predators with prey through molecular gut content analysis can offer a clearer picture of predators of pests in asparagus; however, there has never been a study that has made these explicit predatory linkages. Without true confirmation of predation through molecular gut content analysis it is difficult to know all the key predators of these pests. To move forward with any biological pest control program, we must develop a better understanding of the community of natural enemies that are relevant to the pests of interest. Additionally, there has been no investigation into the role that border habitats play in the abundance and diversity of predator communities in asparagus. By understanding which arthropod predators are relevant for the two key pests, and where their populations reside within and near asparagus fields, measures can be taken to enhance their populations and interactions with pests.

Physical and cultural control

Physical and cultural control measures have not been researched specifically for the asparagus miner. This is likely because it was not considered a serious economic pest of asparagus until its association with asparagus decline syndrome and *Fusarium* was confirmed in the last 20 years (Eichmann, 1943; Morrison et al., 2011; Tuell, 2003; Tuell and Hausbeck, 2008). While grower's mowing practices at the end of the season may impact some pupae overwintering in stem debris, many pupae are protected from this cultural practice because they are beneath the soil line (Buchanan et al., 2015).

Physical and cultural control tactics for common asparagus beetle focus on field sanitation. Physical tactics include the mowing and burning of crop debris in the fall or spring, before crop emergence, to disrupt and destroy overwintering sites of the beetle (Buchanan et al., 2015). Cultural tactics include the removal of volunteer asparagus around production fields using herbicides to prevent recolonization of fields (Delahout, 2005).

Chemical ecology of asparagus

Volatile chemicals emitted by plants in response to herbivory play important roles in host location for natural enemies and act as indirect defenses for plants (Dicke and Van Loon, 2000; Turlings et al., 1990; Van Loon et al., 2000). As a mechanism to support biological control, herbivore induced plant volatiles (HIPVs) have been shown to attract and retain natural enemies in research settings and have resulted in the commercial production of volatile natural enemy lures, such as Benallure® (MSTRS Technologies, Ames, IA) and PredaLure® (AgBio Inc., Westminister, CO), that have been successfully used in research settings to increase predation of crop pests (Bottrell et al., 1998; Dicke et al., 1990; Pickett et al., 2006; Rodriguez-Saona et al., 2011; Sedlacek et al., 2009; Turlings and Ton, 2006).

The volatiles of asparagus have been investigated in three peer-reviewed studies so far, one of which investigated HIPVs (Morrison et al., 2016; Sun et al., 2001; Ulrich et al., 2001). Morrison et al. (2016) investigated the plant volatiles of healthy asparagus, mechanically damaged asparagus, and asparagus fed upon by the generalist black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). They found 28 different volatiles being produced by asparagus and found significant upregulation of pentadecane from herbivore damaged plants. Other compounds identified in the headspace of herbivore damaged asparagus were similar to

healthy plants, mechanically damaged plants, or both. However, there has been no investigation of HIPVs produced by asparagus in response to feeding by an obligate asparagus pest. Furthermore, there has been no published research exploring natural enemy attraction to asparagus HIPVs in a lab or field context. Identifying asparagus HIPVs that impact natural enemy chemotaxis could aid growers by allowing them to implement lures baited with HIPVs to attract natural enemies and support biological control of key pests.

Research objectives

This research seeks to aid in the development of biological pest control tactics for producers of asparagus in Michigan by:

Objective I: Developing predatory food webs for asparagus miner and common asparagus beetle and describing their spatial relationships relative to border habitat type.

Sub-objectives

- ***A.*** Evaluate asparagus miner, common asparagus beetle, and natural enemy spatial distributions in commercial asparagus fields.
- ***B.*** Develop molecular gut content analysis methods for predators of asparagus miner and common asparagus beetle.
- ***C.*** Determine the naturally-occurring predators of asparagus miner and common asparagus beetle in commercial asparagus fields.
- ***D.*** Investigate the impact of field margin habitat on incidents of predation for asparagus miner and common asparagus beetle.

Objective II: Determining the role asparagus HIPVs may play in enhancing biological control of key asparagus pests.

Sub-objectives

A. Identify HIPVs of asparagus fed upon by common asparagus beetle under field conditions.

B. Investigate the attraction of the common asparagus beetle and convergent lady beetle (*Hippodamia convergens* Guérin-Méneville) (Coleoptera: Coccinellidae) to asparagus HIPVs in olfactometer assays.

C. Examine natural enemy attraction to field deployed lures baited with asparagus HIPVs.

D. Determine if lures baited with asparagus HIPVs increase biological control of asparagus miner or common asparagus beetle.

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CHAPTER 2

Biocontrol on the edge: Field margin habitats in asparagus fields influence natural enemy-pest interactions

Introduction

Agricultural field margins are important sources of ecosystem services, but their beneficial contributions to pest management are not well understood (Bell et al., 2002; Dennis and Fry, 1992; O'Rourke and Jones, 2011; Vickery et al., 2009). Field margins represent crop field edges that interface areas of managed or unmanaged natural vegetation, crop fields, or anthropogenic structures, such as roads (Marshall and Moonen, 2002). Generally, higher arthropod abundance and diversity is observed in field edges than in the field interior (Botero-Garcés and Isaacs, 2004; Denys and Tscharntke, 2002). One proposed explanation for this is that intensively managed agroecosystems are frequently sprayed with insecticides, thus creating temporal arthropod deserts, and field margins can provide habitat for shelter and recolonization (Ramsden et al., 2015). Therefore, promoting the development of alternative non-cropped habitats outside fields could contribute to ecosystem friendly pest management if they provide biological control services (O'Rourke and Jones, 2011; Tschumi et al., 2016). However, there is concern about the effects of field margin habitat on pest control because they may harbor harmful arthropods (Duelli et al., 1990; O'Rourke and Jones, 2011).

Increasing plant diversity in field margins may lead to an improvement in resources for beneficial arthropods which in turn can enhance the magnitude and outcome of biocontrol (Dennis and Fry, 1992; Fiedler and Landis, 2007; Isaacs et al., 2009; Walton and Isaacs, 2011a; Walton and Isaacs, 2011b). Conversely, some plant species may be disproportionately attractive to pests, which would defeat the purpose of providing such habitat. For example, some arthropod

pests find and develop on alternate hosts, which would sustain pest populations in agricultural landscapes (Blitzer et al., 2012; Schellhorn et al., 2008). Encouragingly, studies show consensus that natural enemies are more commonly attracted to diverse high quality field margins and non-cropping areas in agricultural landscapes than pests and this leads to enhancing conservation biocontrol programs for key pests (Fielder and Landis, 2007; Isaacs et al., 2009; Letourneau et al., 2011; Thies and Tscharntke, 1999; Tscharntke et al., 2005).

Commonly, pest management is focused on a few key pests that are the top priorities for securing economically profitable yields (e.g., Reitz et al., 1999). The efficacy of habitat enhancement programs for key pest control hinges on whether pests and natural enemies spatially and temporally overlap (e.g., Woodcock et al., 2016). For instance, arthropod natural enemies may move into agricultural fields from field margins during periods of abundant prey, while others may only randomly disperse into the field looking for prey using margins as permanent homes. To advance our understanding of biocontrol in agricultural landscapes, we need to better understand the interactions that occur between pests and natural enemies across crop to field margin interfaces.

Characterizing interactions between arthropod herbivores and predators has been revolutionized by the use of molecular gut content analysis (Furlong, 2015; King et al., 2008; Sheppard and Harwood, 2005; Symondson and Harwood, 2014). This method provides a qualitative approach to unraveling food webs and determining which field-collected predators are providing biocontrol services. Studying trophic interactions with this approach has become increasingly used in agricultural systems; however, the primary focus previously has been on interactions taking place within managed fields (e.g., González-Chang et al., 2016; Szendrei et al., 2010). With a growing recognition of the importance of agricultural landscape structure on

pest management, research is needed on the effects of margin habitat and landscape elements on biocontrol services using molecular gut content analysis as a tool.

In this study, we focus on the interface between field margins and agricultural fields to aid in the development of a conservation biocontrol program for two key asparagus pests, the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) and common asparagus beetle (*Crioceris asparagi* L.; Coleoptera: Chrysomelidae) (Barnes, 1937; LeSage et al., 2008). Past studies in asparagus have determined asparagus miner to be spatially aggregated at field edges, providing the possibility for overlap with natural enemies preferring field margin habitat and the opportunity of designing habitat management programs to improve biological control (Morrison and Szendrei, 2013). Our specific goals were to: 1) evaluate pest and predator spatial distributions in relation to field margin types, 2) develop molecular gut content analysis methods for both key pests, 3) determine the predators of these key pests using molecular gut content analysis, and 4) investigate the impact of field margin type and spatial location (i.e., within field or near field margin) on the incidence of predation.

Materials and methods

Arthropod collections. We collected predators and pests weekly in 10 postharvest commercial asparagus fields in Oceana County, Michigan, USA, from July – August 2014 (five sampling dates), and June – August 2015 (nine sampling dates; Table S2.1). Two margin regions per field were designated as collection sites. For all fields, vegetation outside the field edge consisted of a ~5 m wide drive row that typically consisted of mowed weeds or grass, and is a common feature of agricultural fields in the US to allow the movement of farm equipment. Beyond the drive row, we classified the margins as one of four types: asparagus, crop (alfalfa, cherry, or corn), forest

(unmanaged areas with mixtures of deciduous hardwoods and coniferous evergreen softwoods, e.g., maple (Acer spp.), pine (Pinus spp.), beech (Fagus spp.), and hemlock (Tsuga spp.)) and non-crop (infrequently managed areas with mixtures of grasses, e.g., Poa spp., Lolium spp., Festuca spp., and Agrostis spp., and weeds, e.g., Plantago spp., Amaranthus spp., Anthemis spp., and Taraxacum spp., that were often adjacent to an anthropogenic structure, such as a building or road). Each sampled margin region was divided into three transects, each consisting of a 10 m x 1 m sampling area running parallel to the field margin. One sampling area was located 10 m away from the asparagus field in the margin habitat, another at the asparagus field edge, and the third was 20 m into the asparagus field (Fig. S2.1).

Collections of live pest and predatory arthropods were done using a sweep net for canopy-dwelling arthropods and a field vacuum (Toro® Power Vac, Bloomington, MN, USA) modified with a fitted mesh bag over an 11 cm diameter inlet for soil-dwelling arthropods. Five vacuum samples were taken at random within each transect's 10 m x 1 m sampling area for 10 s per sample and was consistent between all margin habitats. Sweep net sampling in asparagus fields was comprised of 40 sweeps in each sampling area from ~100 – 150 cm canopy height. In forested margins, sweep net samples were taken from low tree branches and understory flora ~100 – 150 cm from the soil surface. However, in crop (alfalfa and cherry) and non-crop habitats plant material below 100 cm in height were sampled because these plants are kept short with management by farmers. Arthropods were sorted in the field immediately after collection, predatory specimens were then placed individually into chilled vials containing 75 % ethanol, and stored on ice until they were frozen in the lab at -20 °C. Only those predatory arthropods were retained that were in a life-stage that was feeding on other arthropods; for example, only larval stages of Chrysopidae were collected for further processing since adults are not predatory.

Primer design for asparagus miner and common asparagus beetle DNA. Primers designed to amplify asparagus miner and common asparagus beetle DNA were developed to establish predatory linkages. Sequences for primer design were obtained using cytochrome c oxidase subunit I (COI) primers Nancy (5' – CCC GGT AAA ATT AAA ATA TAA ACT TC – 3') and Ron (5' – GGA TCA CCT GAT ATA GCA TTC CC – 3') (Simon et al., 1994). PCRs (50 µl) were comprised of 36.25 µl PCR certified H_2O (Teknova, Hollister, CA, USA), 5 µl 10x PCR buffer, 1.5 µl (50 mM $MgCl_2$), 1 µl (0.2 µM) dNTP, 1 µl (0.2 µM) of each general primer, 0.25 µl Taq (ThermoFisher Scientific Inc., Waltham, MA, USA), and 4 µl of asparagus miner or asparagus beetle DNA. PCR was conducted with an Eppendorf Mastercycler® Pro (Eppendorf, Hauppauge, NY, USA) thermal cycler using the PCR protocol of 94.5 °C for 3 min, followed by 40 cycles of 94.5 °C for 45 s, 41 °C for 1 min, 72 °C for 2 min, and a final extension period of 72 °C for 5 min. Gel electrophoresis (60 V for 3 h) confirmed amplification using 6 µl of PCR product in 3 % agarose gel (Invitrogen UltraPure® Agarose, ThermoFisher Scientific Inc.) stained with 7.5 µl GelRed nucleic acid stain (Phenix Research Products, Candler, NC, USA). Reactions with sufficient PCR product were purified and sequenced at the Michigan State University Genomics Core Facility (East Lansing, MI, USA).

Sequences for all available Agromyzidae and Chrysomelidae were downloaded from GenBank and aligned with asparagus miner and common asparagus beetle COI sequences using MUSCLE (Edgar, 2004). Primers for asparagus miner and common asparagus beetle were selected following testing in *Primer 3* (Rozen and Skaletsky, 2000). Primers selected for asparagus miner had sequences of 5' – CTT CAT TTA GCT GGA ATT TCT TCT ATT – 3' (AM_F, T_m = 59 °C) and 5' – ATA GGG TCT CCC CCT CCA G – 3' (AM_R, T_m = 60 °C) and

produced a 238 bp amplicon product. Primers selected for the common asparagus beetle had sequences of 5' – TCA CAG TTG GTG GTT TAA CAG GA – 3' (AB_F, T_m = 62 °C) and 5' – TGC AAA CAC TGC CCC TAT TG – 3' (AB_R, T_m = 62 °C) and produced a 122 bp amplicon product. Primer specificity was screened against a non-target library of 100 arthropods representing 44 families from 12 orders (Schmidt et al., 2016) and there was no amplification with any of the non-target species.

Predator gut content extraction. To establish trophic linkages to asparagus miner and common asparagus beetle, molecular gut content analysis was conducted on the field-collected predators. Predators were identified to family, genus or species prior to DNA extraction (Arnett, 2000; Arnett and Thomas, 2000; Arnette et al., 2002; Bradley, 2012; Stehr, 1987; Ubick et al., 2009). Specimens were then removed from their respective collection vials, rinsed with double-distilled H_2O and 95 % ethanol, dried, and placed in autoclaved 1.7 ml centrifuge vials. The whole predator was pulverized with a pestle and total DNA was extracted and purified using a QIAGEN DNeasy® Blood and Tissue kit using the protocol outline by the manufacturer for animal tissue extraction (QIAGEN Inc., Chatsworth, CA, USA).

Predator gut content screening. Predatory linkages were established by screening extracted predator DNA for the presence of asparagus miner and common asparagus beetle DNA using multiplex PCR and gel electrophoresis. The PCR mix contained 4.33 µl PCR certified H_2O (Teknova, Hollister, CA, USA), 6.25 µl 2x PCRBIO HS Taq Mix Red (PCR Biosystems Ltd., London, UK), 0.50 µl (10 mM) asparagus miner primer, and 0.42 µl (10 mM) common asparagus beetle primer were mixed with 1 µl of extracted whole predator DNA. Asparagus

miner and common asparagus beetle DNA were used as positive controls. PCR was conducted using an Eppendorf Mastercycler® Pro thermal cycler using the protocol of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 56.5 °C for 30 s, 72 °C for 45 s, and a final extension period of 72 °C for 5 min. Gel electrophoresis (2 %, Invitrogen UltraPure® Agarose; 7.5 µl GelRed nucleic acid stain) was conducted using 6 µl of PCR product at 90 V for 1.5 h. A reference (1.5 µl, GeneRuler LR, 25-700 bp, ThermoFisher Scientific Inc.) was used to verify correct product sizes.

Statistical analysis. Spatial autocorrelation between collection sites was checked using a Mantel's test (package = "ADE4") to ensure independence between collection sites for pests and predators prior to analysis (R Core Development Team, 2015). Asparagus miner and common asparagus beetle abundances were determined from sweep net samples only, as vacuum sampling resulted in few asparagus miners and no asparagus beetles, and predator abundances were the sum of vacuum and sweep net collections. All data were analyzed using a mixed effects model with a Poisson distribution GLMER (package = "LME4") with margin type and transect sampling location as fixed effects, and collection date and field as random effects. We compared reduced and full models using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) to select the model with the best fit for the data. Collection years were analyzed separately because 2014 represented a five-year low in degree days accumulated over the growing season, 18 % below the five-year average, and 2015 represented an above-average degree day accumulation at 2 % above the five-year average (MSU Enviro-weather, 2016). A post-hoc least squares means comparison with Bonferroni correction was made on fixed factors

detected as significant using generalized linear hypothesis test ($\alpha = 0.05$; package = "MULTCOMP").

We created food webs using the proportion of predators testing positive for pest DNA, corrected for overall predator abundance, which allowed visualization of predatory linkages and the relative strength of those links (package = "BIPARTITE"). To test for predation differences, we compared the total number of predators testing positive for asparagus miner and common asparagus beetle DNA by margin habitat type and collection transect sampling location using a Pearson's chi square test with post-hoc multiple pairwise comparisons ($\alpha = 0.05$; package = "STATS").

Predator community composition was analyzed by collection type (vacuum or sweep) and by transect sampling location. To meet acceptable stress levels for community analysis, predator totals from the field edge and 20 m sampling locations were summed (Clark,1993). Analysis was done at the family taxonomic level (except for Opiliones) with non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM; package = "VEGAN"; $\alpha = 0.05$). However, sweep net data had no convergent solutions; therefore, only vacuum samples were analyzed with NMDS. The exception was 2015 sweep net data from field margins, which produced convergent solutions with acceptable stress values for NMDS (Clark, 1993).

Results

Pest abundance. We confirmed for asparagus miners and beetles that collection sites were independent (Mantel's test: r = -0.12, $p = 0.92$). We collected 809 and 2102 asparagus miners in 2014 and 2015, respectively. In 2014, there was no significant margin effect on pest abundance; however, in 2015, a significant effect was detected (2014: $\chi^2 = 4.94$, df = 3, $p = 0.18$; 2015: $\chi^2 =$

12.34, df = 3, $p < 0.01$). For both years, significant transect sampling location (2014: $\chi^2 = 75.44$, df = 2, $p < 0.01$; 2015: $\chi^2 = 250.60$, df = 2, $p < 0.01$) and margin x transect sampling location interaction (2014: $\chi^2 = 172.34$, df = 6, $p < 0.01$; 2015: $\chi^2 = 170.53$, df = 6, $p < 0.01$) were found (Table S2.2a). In 2015, the abundance of asparagus miners was statistically higher in sites adjacent to asparagus borders than those bordered by crops and non-crop borders ($z > 3.15$, df = 3, $p < 0.01$; Fig. 2.1a). Asparagus miners were significantly more abundant in both years at the field edges when compared to the margins and inside the field (2014: $z > 8.92$, df = 2, $p < 0.001$; 2015: $z > 9.06$, df = 2, $p < 0.001$; Fig. 2.1b).

We collected 40 and 95 common asparagus beetles in 2014 and 2015, respectively. The effect of margin type on the number of asparagus beetles in either year was not significant (2014: $\chi^2 = 2.25$, df = 3, $p = 0.81$; 2015: $\chi^2 = 2.70$, df = 3, $p = 0.75$; Fig. 2.1c). In 2014, transect sampling location was not a significant predictor of asparagus beetle abundance ($\chi^2 = 7.32$, df = 2, $p = 0.12$); however, in 2015, significantly more asparagus beetles were found at the field edge when compared to the other sampling locations ($\chi^2 = 11.92$, df = 2, $p = 0.02$; Fig. 2.1d). No interaction between margin and transect sampling locations were detected in either year (2014: $\chi^2 = 1.61$, df = 6, $p = 0.95$; 2015: $\chi^2 = 8.91$, df = 6, $p = 0.18$; Table S2.2b).

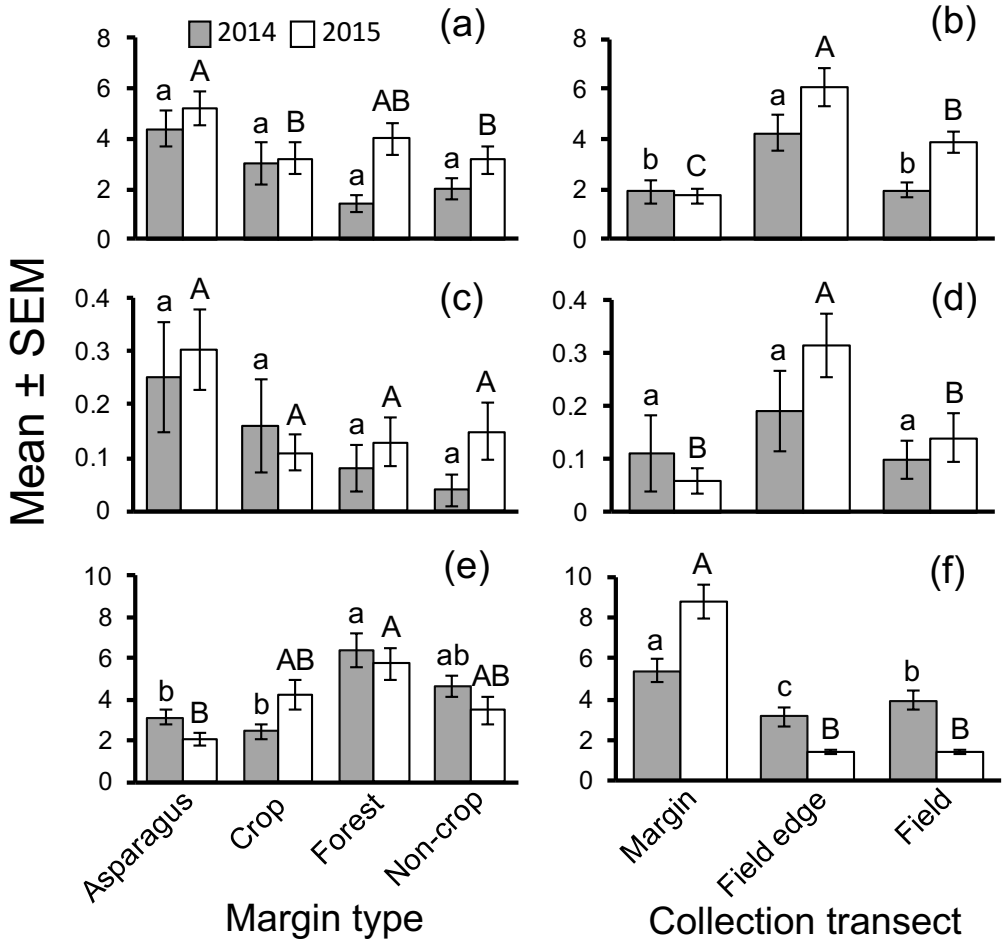

Figure 2.1. Mean ± SEM number of asparagus miner, common asparagus beetle, and predators collected in asparagus fields in 2014 (grey bars) and 2015 (white bars). Asparagus miner abundance by margin type (a) and transect (b) and asparagus beetle abundance by margin type (c) and transect (d). Both pests were collected by sweep nets. Predator abundance by margin (e) and transect (f). Predators were collected with vacuum and sweep nets.

Predators of asparagus miner and common asparagus beetle. Spatial autocorrelation was not found among our sites for predators (Mantel's Test: r = -0.01, $p = 0.47$). In 2014 and 2015, there were significant differences in arthropod predator abundance across margin types (2014: $\chi^2 = 17.88$, df = 3, $p < 0.01$; 2015: $\chi^2 = 9.43$, df = 2, $p = 0.02$) and transect sampling locations (2014: $\chi^2 = 60.54$, df = 2, $p < 0.01$; 2015: $\chi^2 = 1167.31$, df = 2, $p < 0.01$). Significant interactions

between margin and transect sampling locations were also detected in both years (2014: χ^2 = 36.60, df = 6, $p < 0.01$; 2015: $\chi^2 = 71.47$, df = 6, $p < 0.01$; Table S2.2c).

Predator abundance was significantly higher in fields with forested margins than fields with asparagus or crop margins in 2014 ($z > 2.61$, df = 3, $p < 0.04$). In 2015, forested margins also had the highest predator abundance of all margin types and was significantly higher than fields with asparagus margins ($z = 3.61$, df = 3, $p < 0.01$; Fig. 2.1e). Significant differences in predator abundance relative to transect sampling location was found in both years with significantly more predators collected from the field margins than at the field edge or within the field (2014: $z > 2.85$, df = 2, $p < 0.01$; 2015: $z > 25.00$, df = 2, $p < 0.01$; Fig. 2.1f).

Predator communities. Predator communities collected from inside the asparagus fields by vacuum relative to margin vegetation type in both years were similar to each other (2014: ANOSIM R = -0.05, $p = 0.77$, NMDS stress = 0.11; 2015: R = -0.10, $p = 0.94$, NMDS stress = 0.17; Fig. S2.2). In 2014, predators in forested margins had a distinct community compared to the other margin types (ANOSIM R = 0.21, $p < 0.05$, NMDS stress = 0.15; Fig. S2.3a). However, this pattern did not continue in 2015, when all margin predator communities were similar to each other (R = -0.02, $p = 0.56$, NMDS stress = 0.13; Fig. S2.3b). Sweep net-collected samples from inside asparagus fields gave no convergent solutions in either year, and therefore could not be analyzed with NMDS. However, in 2015, sweep net collections from forested margins had a significantly different predator community composition than all other margin vegetation types (R = 0.27, $p < 0.01$, NMDS stress = 0.14; Fig. S2.4).

Molecular gut content analysis summary: Food webs of key asparagus pests. Of the 1456 predators we screened in 2014, 80 (6 %) tested positive for asparagus miner DNA and 16 (1 %) tested positive for asparagus beetle DNA. The arthropods that tested positive for asparagus miner represented 22 groups (13 spider groups and 9 insect families; Fig. 2.2a). In total, we collected 1244 individuals that belonged to these taxonomic groups (Table S2.3a). We found 400 individuals that came from six taxonomic groups (two spider groups and four insect families; Fig. 2.2a), which tested positive for asparagus beetle DNA (Table S2.4a). In 2014, two individuals tested positive for DNA of both pests; a *Nabis americoferus* Carayon (Hemiptera: Nabidae) and a rove beetle from the subfamily Aleocharinae (Coleoptera: Staphylinidae).

In 2015, we screened 2190 predators and had 307 individuals (14 %) test positive for asparagus miner DNA, and 64 individuals (3 %) positive for asparagus beetle DNA in gut contents. These predators represented 24 predatory groups for asparagus miner (12 spider groups and 12 insect families; Fig. 2.2b; Table S2.3b), and 12 predatory groups (6 spider groups and 6 insect families) that tested positive for asparagus beetle DNA (Fig. 2.2b; Table S2.4b). We had 2091 and 1419 individuals that came from families that tested positive for asparagus miner and asparagus beetle, respectively. Similar to 2014, we only had a few individual predators that tested positive for both prey. Staphylinids were positive for both pests in 2015, with one individual from the subfamily Aleocharinae and seven individuals from the genus *Tachyporus.*

Asparagus miner predators. Overall, spiders from the Linyphiidae family had the most individuals testing positive for asparagus miner in 2014, and Thomisidae had the most positive individuals in 2015 (Fig. 2.2; Table S2.3). Among the Insecta predators testing positive for

asparagus miner DNA, we found that in both years staphylinids and ground beetles (Carabidae) were prominent predatory groups for asparagus miner (Fig. 2.2; Table S2.3).

Margin type significantly influenced the number of individuals positive for asparagus miner DNA in both years (2014: $\chi^2 = 38.70$, df = 3, $p < 0.01$; 2015: $\chi^2 = 80.69$, df = 3, $p < 0.01$; Fig. 2.3a). In 2014, in the presence of forested margins, the number of positive samples increased by 3-fold, compared to all other margin types ($\chi^2 > 15.52$, df = 1, $p < 0.01$). In 2015, fields with crop and forested margins had significantly more predators testing positive for asparagus miner compared to the other margin types ($\chi^2 > 20.86$, df = 1, $p < 0.01$; Fig. 2.3a). When comparing sampling locations (transects in relation to margin habitat), we observed significant differences in the total number of predators positive for asparagus miner DNA in 2014 and 2015 (2014: $\chi^2 = 10.00$, df = 2, $p < 0.01$; 2015: $\chi^2 = 252.71$, df = 2, $p < 0.01$; Fig. 2.3b). Field margins in 2014 had double the number of predators testing positive for asparagus miner DNA compared to the other sampling locations ($\chi^2 > 6.67$, df = 1, $p < 0.01$). In 2015, we found more than a 5-fold greater abundance of predators outside asparagus fields than inside that tested positive for asparagus miner DNA ($\chi^2 > 131.70$, df = 1, $p < 0.01$; Fig. 2.3b).

Asparagus beetle predators. Comparatively fewer predators tested positive for asparagus beetle in 2014 (Fig. 2.2.; Table S2.4), likely related to low abundance of this pest (Fig. 2.1c; Fig. 2.1d). In both years, a diversity of Insecta and Arachnida predators tested positive for asparagus beetle DNA (Fig. 2.2; Table S2.4). Insects from Coccinellidae and Staphylinidae were prominent predatory families for asparagus beetle, with coccinellids making up 50 % of the predators testing positive in 2014 and staphylinids accounting for 59 % of the predators testing positive in 2015 (Fig. 2.2; Table S2.4).

Margin habitat type had no significant effect on the total number of predators positive for asparagus beetles in 2014 ($\chi^2 = 1.50$, df = 3, $p = 0.68$), but significantly affected the number of predators testing positive in 2015 ($\chi^2 = 77.00$, df = 3, $p < 0.01$; Fig. 2.3c). In 2015, 87 % of predators testing positive for asparagus beetles came from crop margins ($\chi^2 > 25.14$, df = 1, $p < 0.01$). Forested margins accounted for 16 % of the total number of predators testing positive, which was significantly more than in non-crop margins ($\chi^2 = 5.33$, df = 1, $p = 0.02$; Fig. 2.3c). The effect of transect sampling location on predators testing positive for beetle DNA was significant in both years (2014: $\chi^2 = 6.13$, df = 2, $p < 0.05$; 2015: $\chi^2 = 70.72$, df = 2, $p < 0.01$). In 2014, 56 % predators testing positive for beetle DNA came from the margin, which was significantly more than from the field ($\chi^2 = 6.4$, df = 1, $p = 0.01$). Margin transects had 83 % of the predators positive in 2015, which was significantly more than the other transect sampling locations ($\chi^2 > 35.27$, df = 1, $p < 0.01$; Fig. 2.3d).

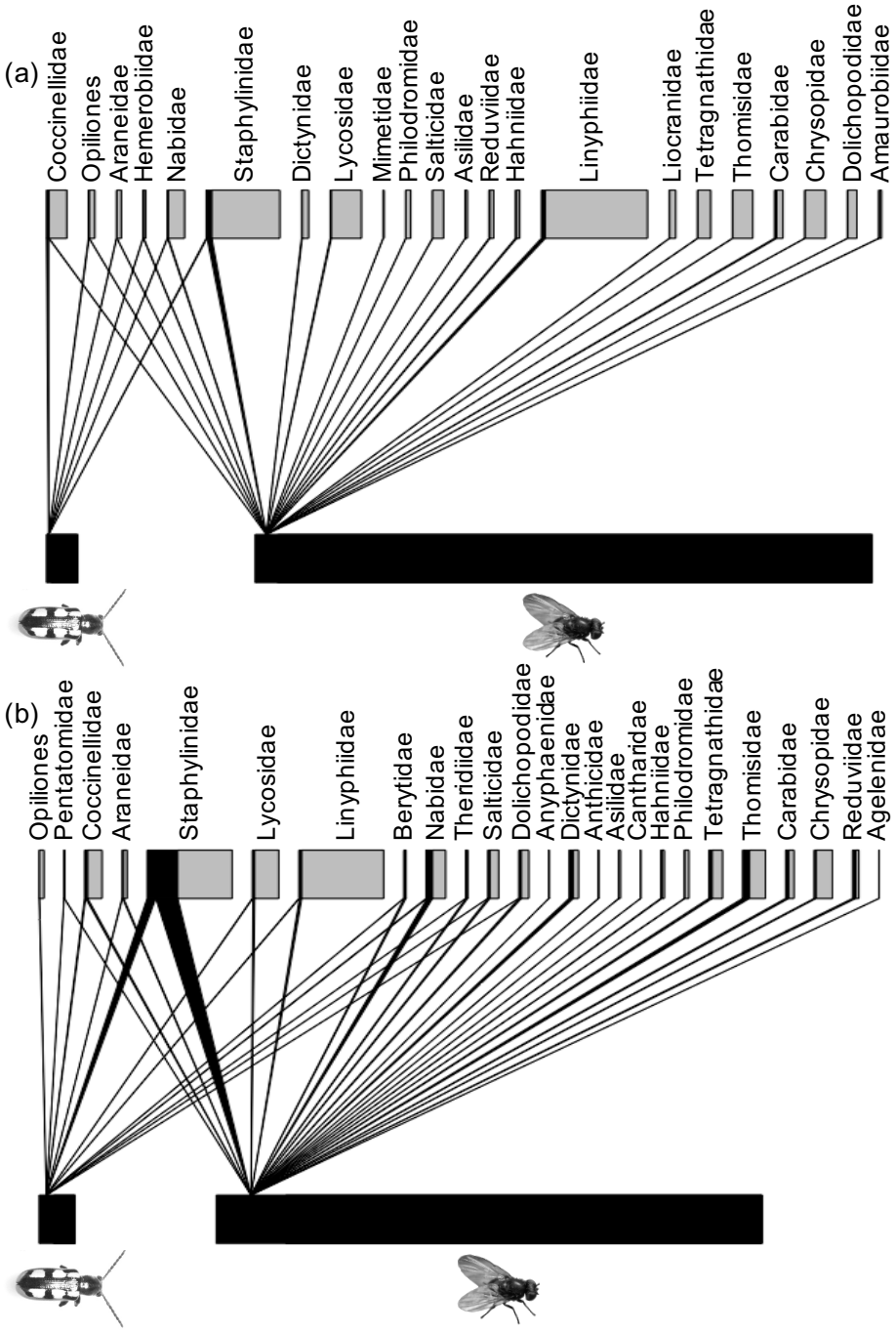

Figure 2.2. Predatory linkages visualized using food webs for common asparagus beetle and asparagus miner for 2014 (a) and 2015 (b). In each year, the width of upper and lower horizontal bars represents total abundance of the arthropod groups. Lower horizontal bars represent the relative abundance of asparagus beetle and asparagus miner. Upper horizontal bars represent relative abundance of predators. Lines connecting the upper and lower axes, and the corresponding black area of upper horizontal bars indicate the proportion of each predatory group that were positive for asparagus miner and/or asparagus beetle DNA determined by molecular gut content analysis.

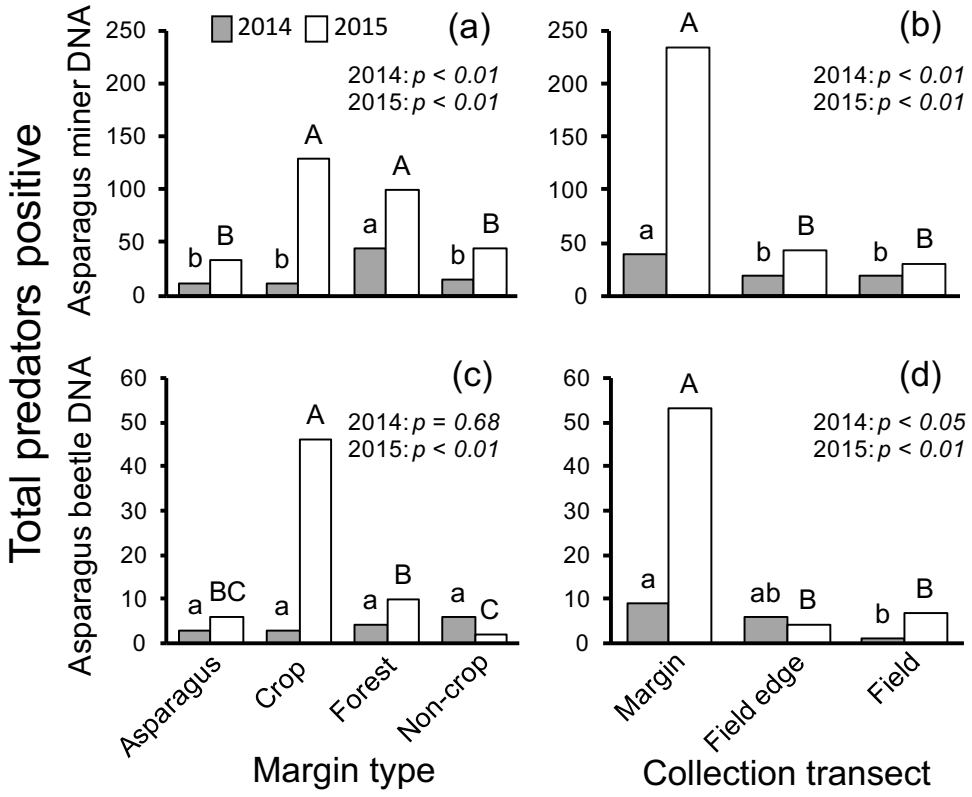

Figure 2.3. Total number of predators collected from commercial asparagus fields that tested positive for asparagus miner DNA (a, b) and common asparagus beetle (c, d) with molecular gut content analysis in 2014 and 2015. Significant differences among bars of the same color, within years, were determined with a Pearson's chi square test with post-hoc multiple pairwise comparisons ($\alpha = 0.05$).

Discussion

Our analysis of asparagus food webs is among the first studies to characterize predatory communities in a landscape context using molecular gut content analysis (Hagler et al., 2004; Sheppard et al., 2004). In general, our results indicated that the abundance of natural enemies is higher outside asparagus fields than inside, and this coincided with higher predation levels on two key pest species. Furthermore, we found that margin habitat type shapes predator communities; asparagus fields bordered by forests contained more abundant predator communities as compared to other types of field margins. However, overall incidents of

predation were relatively low in both years which makes establishing key predators as potential targets for biological control programs difficult. A diversity of predators was found to have fed on the two key pests, indicating that predator community diversity may be important for biological control in this system. This supports the growing consensus in the literature about the importance of biodiversity for ecosystem functions, such as biological control (Cardinale et al., 2006), and emphasizes that agroecosystem function depends on sustaining biodiversity in field margins to help maintain biocontrol agents in agricultural landscapes (Wratten, 1988; Wratten et al., 1998).

Although asparagus is a commonly grown crop around the world, few studies have documented the predatory communities of these systems (Angalet and Stevens, 1977; Capinera and Lilly, 1975; Drake and Harris, 1932; Starý, 1990; Watts, 1938). Only three studies have documented predators of asparagus beetle (Capinera and Lilly, 1975; Drake and Harris, 1932; Watts, 1938), while none have described predators of asparagus miner. The predators we collected, especially arachnids, had higher incidences of predation in asparagus fields with forested borders as compared to other margin types, suggesting that increasing vegetation structural complexity, especially vegetation cover, may be an important factor for these groups of arthropods (Bell et al., 2002; Dennis and Fry, 1992; White and Hassall, 1994;). Many of the predators in our study were flightless and soil-dwelling with a diffuse distribution relative to the field margin, indicating that these species are habitat generalists, moving between field margins and agricultural fields in search of prey ("soft-edge" species, Duelli et al., 1990). Forested field margins seem to be an important source of refugia, likely increasing the number of predator immigrants into asparagus fields.

Field margins may be sources of pests, and in our system, the abundances of the two

herbivorous pests were generally lower outside asparagus fields than inside. This was expected since both pests are obligate asparagus feeders (Barnes, 1937; Drake and Harris, 1932; LeSage et al., 2008) and are most likely visiting volunteer asparagus plants in field margins, although asparagus miner adults (the life stage we collected) feed on nectar and can be seen visiting many species of flowers (*Z.S. pers. obs.*). Furthermore, the forested margins had a favorable effect on predators and predation, with a correspondingly low abundance of asparagus miners and beetles. This result suggests that the interaction between predators and these pests is particularly high on the forested margins of fields, and the efficacy of biological control in this system may be related to the amount of forested area in the landscape.

Arachnids testing positive for asparagus miner DNA were a mixture of soil-dwelling, arboreal, web-building, and wandering spiders. In 2014, linyphiids were the most abundant predator inside asparagus fields, with 67 % of all linyphiids testing positive for asparagus miner coming from inside the fields. Linyphiids are a particularly interesting arachnid family as a potential target for conservation biocontrol as they seem to tolerate disturbance and can make up 93 – 99 % of the total spiders in many different field and vegetable crops (reviewed in Nyffeler and Sunderland, 2003). Interactions between the miners and web-building linyphiids is most likely to occur when adults are captured as they move on and between plants. In 2015, Thomisidae spiders had relatively high abundance in forested borders and frequently tested positive for asparagus miners. These predators sit-and-wait for their prey, often at flowers. Therefore, it is possible that they could capture miner adults visiting flowers outside the asparagus fields. In both years, arachnids made up less than 25 % of the total predators testing positive for asparagus beetle DNA with no clearly dominate predatory taxa. However, those that did test positive represented taxa that utilize the same hunting modes and occupy the same

spatial niches within the landscape as those described for the asparagus miner.

Among the insect predators, staphylinids represented one of the numerically dominant groups and frequently tested positive for the two key pests. Many staphylinids are known facultative predators (Frank and Thomas, 1999) and, as omnivores, they can establish early in crop fields before pest populations are high and can feed on plants when prey are unavailable, mitigating mortality (Capinera, 2008). Staphylinids are also known scavengers and can test positive for prey DNA after feeding on carrion (Von Berg et al., 2012). Therefore, the roles of staphylinids and other generalist predators in these food webs are complex, and we may have overestimated predation on live pests due to secondary predation (Mansfield and Hagler, 2016; Sheppard and Harwood, 2005). False positives for predation can also occur when secondary predators (hyperpredators) feed on primary predators, creating food chain errors in molecular predation studies (Hagler, 2016; Harwood et al., 2001; Sheppard and Harwood, 2005). We hypothesized, that if staphylinids feed on live prey, they are most likely to feed on the immobile pupal stages of the two pests due to spatial separation and differences in mobility during the other prey life-stages. It is also difficult to discern if the positive occurrences we found for the two pests were not simply the result of staphylinids scavenging on dead or dying prey on the ground. Considering the propensity of staphylinids to feed on carrion and the potential of secondary predation it is difficult to verify our results without direct observations. Further studies on the roles of staphylinids in terrestrial food webs are clearly needed to better understand these issues.

We hesitate to make comparisons among predator groups for effectiveness as biocontrol agents because there is known variability in prey DNA detectability caused by differences in biotic conditions, the size, type and frequency of meals consumed, and the life stage of the

predator (Greenstone et al., 2014). This is a challenge and out of the scope for the current study given the diversity of predator taxa observed. While our current analysis of the system provides the first food web characterizing the communities of predators feeding on key asparagus pests, and their relationship to landscape characteristics, future work will clarify the importance of individual predatory taxa (e.g., Szendrei et al., 2010).

Conclusions

In summary, our study contributes to filling the knowledge gap in linking predators and prey through direct trophic linkages. We also highlight the importance of unmanaged field margins, particularly forested ones, in providing biocontrol services in agricultural fields. Many of the predator taxa that we confirmed to feed on key pests are not pollen and nectar feeders; therefore, in this system, predation and margin management with flowers may not be positively correlated. In the absence of forested borders, floral resources in margins may provide habitat for predators and attract parasitoids which could synergize with predators for more efficient biocontrol. While forested field margins tend to be only a small part of agricultural landscapes, their conservation should be promoted for increasing ecosystem services and biodiversity, and their benefits should be integrated into pest management programs.

Acknowledgement of prior publication

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APPENDIX

Supplementary Tables

Table S2.1. Field collection sites used to collect predators and pests of asparagus in 2014 and 2015.

Field	Coll. Site	Location (Dec. Degrees)	Cultivar	Field Area (ha)	Margin Type
1	C1	43.712561, -86.422514	Guelph Millennium	3.88	Forest
1	C2	43.013536, -86.422514	Guelph Millennium		Crop
2	C3	43.712258, -86.438431	Guelph Millennium	16.19	Non-crop
2	C4	43.709161, -86.436450	Guelph Millennium		Asparagus
3	C5	43.710436, -86.441680	Guelph Millennium	7.28	Asparagus [A]
3	C6	43.710883, -86.443439	Guelph Millennium		Crop
4	C7	43.707197, -86.445067	Guelph Millennium	4.65	Crop [B]
4	C8	43.706753, -86.448111	Guelph Millennium		Non-crop
5	C9	43.714506, -86.434872	Guelph Millennium	7.89	Forest
5	C10	43.712750, -86.435733	Guelph Millennium		Asparagus
6	C11	43.716017, -86.424547	Guelph Millennium	5.22	Forest
6	C12	43.718061, -86.424375	Guelph Millennium		Non-crop
7	C13	43.709052, -86.440906	Guelph Millennium	7.28	Crop
7	C14	43.707391, -86.439744	Guelph Millennium		Forest
8	C15	43.741341, -86.235439	Tyson & Millennium	10.26	Forest
8	C16	43.744708, -86.235106	Tyson & Millennium		Non-crop
9	C17	43.741836, -86.233261	Jersey Giant	5.09	Crop
9	C18	43.741181, -86.232119 [C]	Jersey Giant		Asparagus
10	C19	43.744033, -86.242864	Jersey G. & G. Millennium	15.33	Non-crop
10	C20	43.742867, -86.238042 [D]	Jersey G. & G. Millennium		Asparagus

[A] Site margin habitat changed in 2015 to crop.
[B] Site margin habitat changed in 2015 to asparagus.
[C] Collection transect was moved in 2015 to another side of the field (43.741414, -86.234583) due to field margin habitat change.
[D] Collection transect was moved in 2015 to another side of the field (43.742969, -86.240344) due to field margin habitat change.

Table S2.2. Results of mixed model evaluating fixed effects of margin type "Margin" (asparagus, crop, forest, non-crop) and collection transect "Transect" (10 m outside of the field, on the field edge, or 20 m into the field, on abundance of asparagus miners (a), asparagus beetles (b), and predators (c) collected in 2014 and 2015.

(a)

2014

Source of variation	df	χ^2	*P*
Margin	3	4.94	0.18
Transect	2	75.44	< 0.001***
Margin * Transect	6	172.34	< 0.001***

2015

Source of variation	df	χ^2	*P*
Margin	3	12.34	< 0.01**
Transect	2	250.60	< 0.001***
Margin * Transect	6	170.53	< 0.001***

(b)

2014

Source of variation	df	χ^2	*P*
Margin	3	2.25	0.81
Transect	2	7.32	0.12
Margin * Transect	6	1.61	0.95

2015

Source of variation	df	χ^2	*P*
Margin	3	2.70	0.75
Transect	2	11.92	0.02*
Margin * Transect	6	8.91	0.18

(c)

2014

Source of variation	df	χ^2	*P*
Margin	3	17.88	< 0.001***
Transect	2	60.54	< 0.001***
Margin * Transect	6	36.60	< 0.001***

Table S2.2 (cont'd)

2015

Source of variation	df	χ^2	*P*
Margin	3	9.43	0.02*
Transect	2	1167.31	< 0.001***
Margin * Transect	6	71.47	< 0.001***

Table S2.3. Total number of predators collected from commercial asparagus fields testing positive for asparagus miner DNA in their gut contents by margin habitat type and the field transect that were collected using a vacuum for soil-dwelling predators and sweep net for arboreal predators in 2014 (a) and 2015 (b). Total predator abundance was the seasonal total of all predators collected from each predator group. Stars indicate significant differences in the numbers of predators testing positive for asparagus miner within margin habitat type or field transect, respectively.

(a)

		Total number positive with molecular gut content analysis						
	Total	Margin habitat type				Field transect		
Predator group	abundance	Asparagus	Crop	Forest	Non-crop	Margin	Field edge	Field
Arachnida								
Amaurobiidae	7	0	0	3	0	3	0	0
Araneidae	15	0	0	0	1	1	0	0
Dictynidae	22	0	0	0	3	3	0	0
Hahniidae	10	**0**	**0**	**5**	**0****	**5**	**0**	**0****
Linyphiidae	361	**1**	**0**	**8**	**5****	**2**	**2**	**10****
Liocranidae	21	0	0	2	0	2	0	0
Lycosidae	106	0	2	2	0	2	1	1
Mimetidae	2	0	0	0	1	1	0	0
Opiliones	19	0	0	1	0	1	0	0
Philodromidae	17	0	0	1	0	1	0	0
Salticidae	38	0	0	1	0	0	1	0
Tetragnathidae	45	1	0	1	0	1	1	0
Thomisidae	70	0	0	2	0	2	0	0
Arachnida Total	*733*	***2***	***2***	***26***	***10****	***24***	***5***	***11****
Insecta								
Asilidae	6	0	0	1	0	0	1	0
Carabidae	22	3	2	3	0	3	3	2
Chrysopidae	72	1	0	1	0	1	1	0
Coccinellidae	63	1	0	0	0	0	1	0
Dolichopodidae	32	0	0	2	0	2	0	0

Table S2.3 (cont'd)

Hemerobiidae	6	0	1	0	1	1	1	0
Nabidae	57	0	2	0	1	2	0	1
Reduviidae	13	0	0	4	0	3	1	0
Staphylinidae	240	4	4	7	2	4	7	6
Insecta Total	*511*	***9***	***9***	***18***	***4****	*16*	*15*	*9*
Predator Total	*1244*	***11***	***11***	***44***	***14****	***40***	***20***	***20****

Pearson's chi-square test for each taxonomic group ($\alpha = 0.05$): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

(b)

		Total number positive with molecular gut content analysis						
	Total	Margin habitat type				Field transect		
Predator group	abundance	Asparagus	Crop	Forest	Non-crop	Margin	Field edge	Field
Arachnida								
Agelenidae	3	0	0	1	0	1	0	0
Anyphaenidae	8	0	0	1	0	1	0	0
Araneidae	69	1	0	3	1	4	1	0
Dictynidae	143	**4**	**2**	**3**	**10***	**17**	**1**	**1*****
Hahniidae	14	**0**	**0**	**5**	**0****	**5**	**0**	**0****
Linyphiidae	203	1	2	5	2	**8**	**1**	**1****
Lycosidae	146	1	1	1	5	**6**	**0**	**2***
Philodromidae	48	1	0	2	0	2	1	0
Salticidae	65	0	4	3	2	5	2	2
Tetragnathidae	43	**1**	**11**	**1**	**2*****	**5**	**10**	**0****
Theridiidae	27	0	2	3	3	5	1	2
Thomisidae	175	**1**	**4**	**23**	**3*****	**29**	**2**	**0*****
Arachnida Total	*944*	***10***	***26***	***51***	***28*****	***88***	***19***	***8*****

Table S2.3 (cont'd)

		Total number positive with molecular gut content analysis						
	Total	Margin habitat type				Field transect		
Predator group	abundance	Asparagus	Crop	Forest	Non-crop	Margin	Field edge	Field
Insecta								
Anthicidae	31	0	1	0	0	0	1	0
Asilidae	3	0	0	1	0	1	0	0
Berytidae	74	**0**	**1**	**7**	**0*****	**7**	**1**	**0****
Cantharidae	21	1	0	0	0	1	0	0
Carabidae	51	2	7	4	2	**12**	**1**	**2*****
Chrysopidae	80	1	4	3	1	4	2	3
Coccinellidae	113	2	1	1	5	5	2	2
Dolichopodidae	154	**0**	**2**	**8**	**1****	**11**	**0**	**0*****
Nabidae	216	**10**	**13**	**1**	**0*****	**13**	**10**	**1****
Pentatomidae	19	0	0	1	0	1	0	0
Reduviidae	59	**0**	**0**	**14**	**1*****	**13**	**2**	**0*****
Staphylinidae	324	**7**	**74**	**9**	**7*****	**77**	**5**	**15*****
Insecta Total	*1145*	***23***	***103***	***49***	***17******	***145***	***24***	***23******
Predator Total	2089	**33**	**129**	**100**	**46*****	**234**	**43**	**31*****

Pearson's chi-square test for each taxonomic group ($\alpha = 0.05$): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table S2.4. Predators collected from commercial asparagus fields testing positive for asparagus beetle DNA in their gut contents by margin habitat type and the field transect that were collected using a vacuum for soil-dwelling predators and sweep net for arboreal predators in 2014 (a) and 2015 (b). Total predator abundance was the seasonal total of all predators collected from each predator group. Stars indicate significant differences in the numbers of predators testing positive for asparagus miner within margin habitat type or field transect, respectively.

(a)

		Total number positive with molecular gut content analysis						
	Total	Margin habitat type				Field transect		
Predator group	abundance	Asparagus	Crop	Forest	Non-crop	Margin	Field edge	Field
Arachnida								
Araneidae	15	0	0	0	1	1	0	0
Opiliones	19	0	0	2	0	1	1	0
Arachnida Total	*34*	*0*	*0*	*2*	*1*	*2*	*1*	*0*
Insecta								
Coccinellidae	63	2	2	0	4	3	4	1
Hemerobiidae	6	0	0	1	0	0	1	0
Nabidae	57	0	0	0	1	1	0	0
Staphylinidae	240	1	1	1	0	3	0	0
Insecta Total	*366*	*3*	*3*	*2*	*5*	*7*	*5*	*1*
Predator Total	400	3	3	4	6	**9**	**6**	**1***

Pearson's chi-square test for each taxonomic group ($\alpha = 0.05$): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table S2.4 (cont'd)

(b)

		Total number positive with molecular gut content analysis						
	Total	Margin habitat type				Field transect		
Predator group	abundance	Asparagus	Crop	Forest	Non-crop	Margin	Field edge	Field
Arachnida								
Araneidae	69	1	0	1	0	1	0	1
Linyphiidae	203	2	0	1	0	3	0	0
Lycosidae	146	0	1	1	1	3	0	0
Opiliones	9	0	0	2	0	2	0	0
Salticidae	65	0	0	1	0	1	0	0
Theridiidae	27	0	0	1	0	1	0	0
Arachnida Total	*519*	***3***	***1***	***7***	***1****	***11***	***0***	***1*****
Insecta								
Berytidae	74	0	0	2	0	2	0	0
Coccinellidae	113	2	3	0	0	1	2	2
Dolichopodidae	154	0	0	1	0	1	0	0
Nabidae	216	**1**	**4**	**0**	**0***	4	1	0
Pentatomidae	19	0	1	0	0	1	0	0
Staphylinidae	324	**0**	**37**	**0**	**1*****	**33**	**1**	**4*****
Insecta Total	*900*	***3***	***45***	***3***	***1*****	***42***	***4***	***6*****
Predator Total	1419	**6**	**46**	**10**	**2*****	**53**	**4**	**7*****

Pearson's chi-square test for each taxonomic group ($\alpha = 0.05$): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Supplementary Figures

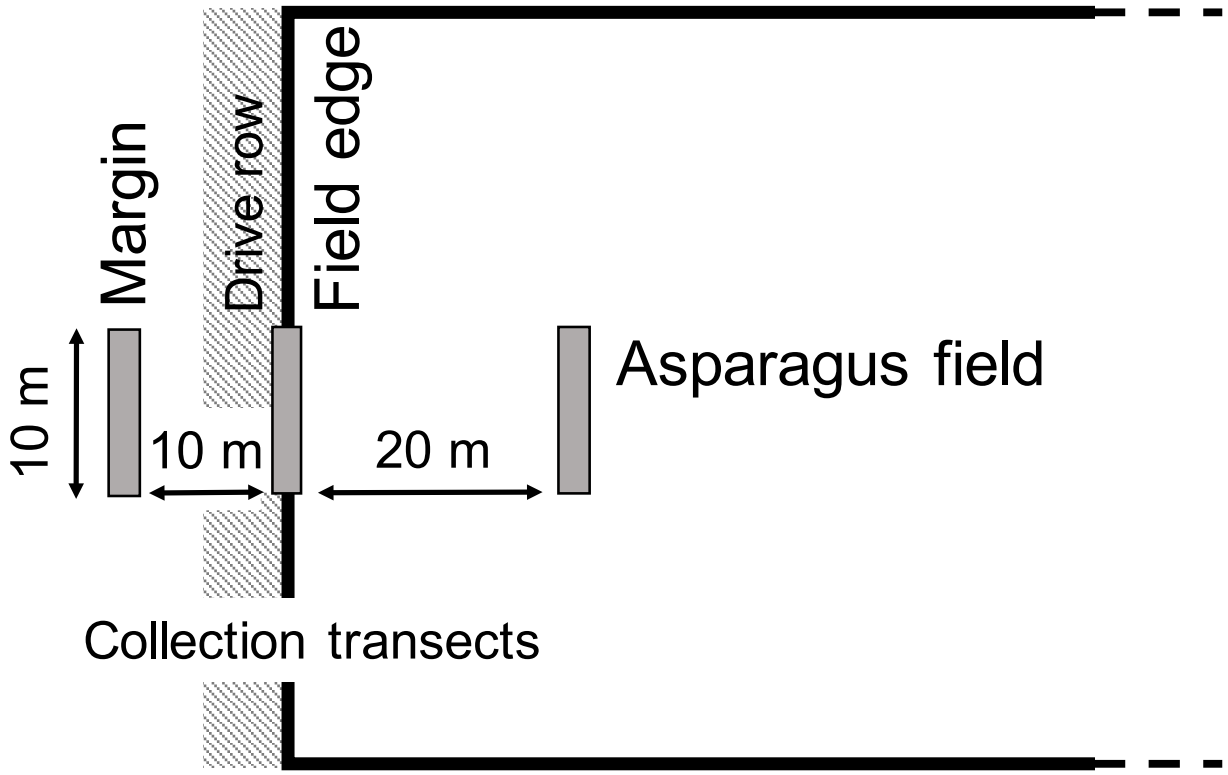


Figure S2.1. Top-down view of an asparagus field showing the layout for predator and pest collection in one location out of 20, in 2014 and 2015. Grey bars represent 10 m x 1 m transects from which arthropods were collected using sweep nets and an insect vacuum. Drive rows occupied the first ~5 m outside of the field edge and are represented by the shaded grey area. Distances between collections sites ranged from 108 – 17,972 m and were located in Oceana County, MI, USA.

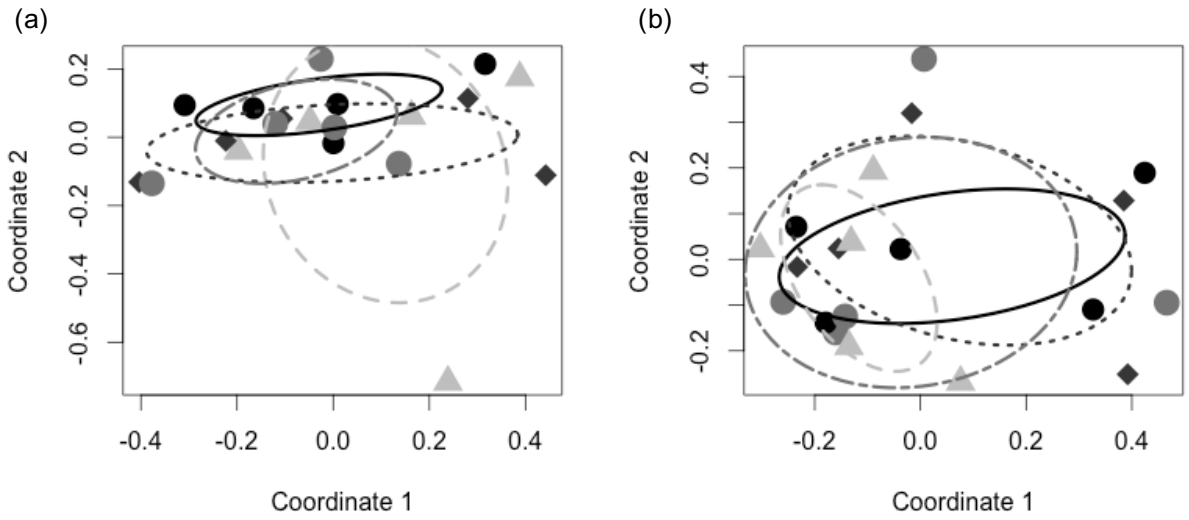


Figure S2.2. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) of soil-dwelling arthropod predator communities collected in 2014 (a) and 2015 (b) by a vacuum from within asparagus fields bordered by four habitat types commonly found around asparagus fields in Michigan, USA (2014: ANOSIM R = -0.05, $p = 0.77$, NMDS stress = 0.11; 2015: ANOSIM R = -0.10, $p = 0.94$, NMDS stress = 0.17). Margin types are represented by: black circle and black line = asparagus, light grey triangle and light grey dash = crop, grey circle and grey dash = non-crop, dark grey diamond and dark grey dash = forest.

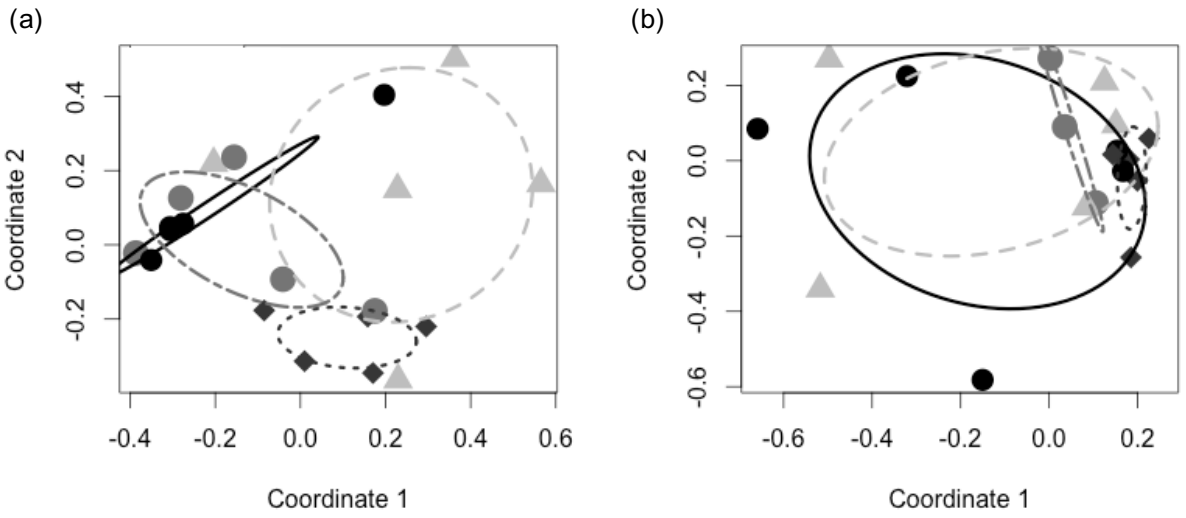


Figure S2.3. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) of soil-dwelling arthropod predator communities collected by a vacuum from four margin types commonly found around asparagus fields in Michigan, USA in 2014 (a; ANOSIM R = 0.21, $p < 0.05$, NMDS stress = 0.15) and, 2015 (b; ANOSIM R = -0.02, $p = 0.56$, NMDS stress = 0.13). Margin types are represented by: black circle and black line = asparagus, light grey triangle and light grey dash = crop, grey circle and grey dash = non-crop, dark grey diamond and dark grey dash = forest.

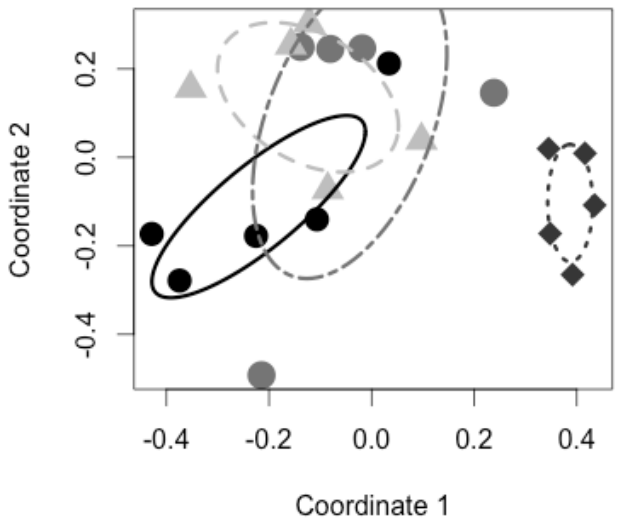
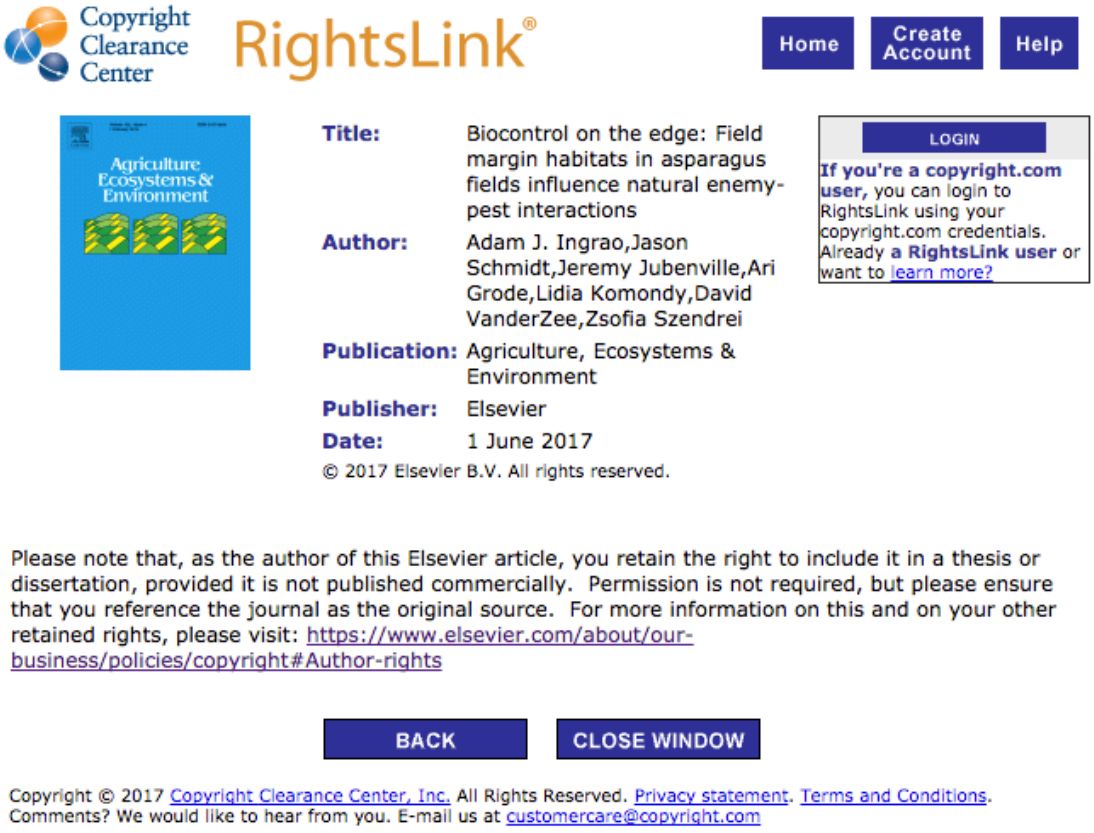


Figure S2.4. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) of arboreal arthropod predator communities collected with a sweep net from four margin types commonly found around asparagus fields in Michigan, USA, in 2015 (ANOSIM R = 0.27, $p < 0.01$, NMDS stress = 0.14). The 2014 data did not meet the stress requirements for NMDS due to low overall predator abundance. Margin types are represented by: black circle and black line = asparagus, light grey triangle and light grey dash = crop, grey circle and grey dash = non-crop, dark grey diamond and dark grey dash = forest.



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CHAPTER 3

Natural enemy attraction to herbivore induced asparagus volatiles

Introduction

Biological control is one of the foundations of sustainable pest management and can effectively complement other pest management strategies such as cultural and chemical control (Gurr and Kvedaras, 2010; Van Driesche and Bellows, 1996). Herbivore induced plant volatiles (HIPVs) are indirect plant defenses that can attract biological control agents, such as arthropod natural enemies, to plants when damaged by pests (Dicke and van Loon, 2000; Turlings et al., 1990; Van Loon et al., 2000). Although plants produce low levels of volatile chemicals constitutively, herbivore feeding can result in the upregulation of constitutive compounds or the *de novo* production of volatiles (Paré and Tumlinson, 1997; Vet and Dicke, 1992). The information provided by HIPVs to natural enemies can be reliable signals serving as infochemical webs that influence natural enemy foraging behavior and chemotaxis (Baldwin, 2010; Vet and Dicke, 1992). HIPVs can illicit innate responses in specialist natural enemies and can be learned by generalist natural enemies through associative learning (Allison and Hare, 2009; De Boer and Dicke, 2005; Dukas and Duan, 2000; Giunti, et al. 2015). Although our understanding of these interactions is improving, the applications in agriculture for pest management are still largely lacking and research focused on the development of lures baited with HIPVs to enhance biological control programs and support pest management should be the next step to engage crop producers with these technologies (Kaplan, 2012; Turlings and Ton, 2006).

Much of the published research on HIPVs to attract natural enemies has been conducted in the laboratory; however, some field experiments deployed lures effectively in agroecosystems (Hunter, 2002; Kaplan, 2012). Natural enemy attraction to lures baited with HIPVs has been successful in perennial agroecosystems, such as apples (Jones et al., 2016), cotton (Yu et al., 2008), cranberries (Rodriguez-Saona et al., 2011), grapes (James and Grasswitz, 2005; James and Price, 2004), hops (James, 2003a; James, 2003b; James, 2005), pears and walnuts (Jones et al., 2016). Promising results from these types of studies led to the development of commercially available arthropod predator lures containing the plant volatiles methyl salicylate (PredaLure®, AgBio Inc., Westminister, CO, USA) and 2-phenylethanol (Benallure®, MSTRS Technologies, Ames, IA, USA), which were effectively used in some crops (Rodriguez-Saona et al., 2011; Sedlacek et al., 2009). However, many challenges still face successful development of lures baited with HIPVs in agroecosystems for attracting natural enemies.

HIPVs produced by plants are often complex and can include hundreds of compounds making selection of HIPVs for experimentation challenging (Kaplan, 2012; Mumm and Dicke, 2010). In addition, lack of knowledge of food webs, determination of effective HIPVs concentrations, chemical release rates and non-target effects, logistics of field scale testing of lures, and identification of natural enemy responses to lures that are predictable and reliable are important to understand when developing these technologies (Kaplan, 2012). Complicating matters, volatile signals can also serve as attractants for pests resulting in negative outcomes for pest management (Bolter et al., 1997; Halitschke et al., 2008). Therefore, to narrow the scope of inquiry and address many of these issues it is important for researchers to focus on specific agroecosystems with targeted management goals.

Perennial specialty crops, such as asparagus (*Asparagus officinalis* L.), are a particularly interesting target for using HIPVs in lures as part of a pest management program because of the unique challenges facing producers and the high crop value. In the United States, specialty crops make up 40 % of the total value of the agricultural market, but account for only 1.5 % of the total hectares farmed (USDA, 2015; USDA ERS, 2017). Due to the small total area of these crops, compared to field/row crops, agro-chemical companies often have little financial incentive to register pesticides that target obligate pests of specialty crops and growers are left looking for alternative pest management options, such as biological control (Miller and Leschewski, 2012).

Our research aimed to understand the use of HIPV lures to attract natural enemies in asparagus, a crop grown in 62 countries (Benson, 2009), in an effort to control two key specialist asparagus pests, the asparagus miner (*Ophiomyia simplex* Loew, Diptera: Agromyzidae) and the common asparagus beetle (*Crioceris asparagi* L., Coleoptera: Chrysomelidae). We explored this topic by: 1) identifying HIPVs of asparagus under field conditions, 2) investigating responses of common asparagus beetle and a known predator, the convergent lady beetle (*Hippodamia convergens* Guérin-Méneville, Coleoptera: Coccinellidae) (Ingrao et al., 2017), to asparagus HIPVs in an olfactometer bioassay, 3) examining natural enemy responses to field deployed lures baited with asparagus HIPVs, and 4) determining if HIPV lures increase biological control of asparagus miner or common asparagus beetle.

Methods and materials

HIPV collection and analysis. Investigation of asparagus HIPVs were conducted using common asparagus beetle larvae in field trials at the Entomology Research Farm, Michigan State University (East Lansing, MI, USA), from July – August 2014. Beetle larva were chosen as a

target subject because they are voracious feeders, easy to collect and handle, and co-occur with asparagus miner on field edges of post-harvest commercial asparagus fields (Ingrao et al., 2017).

Sixteen insect exclusion cages (183 × 183 × 183 cm, 32 × 32 mesh Lumite® screen, BioQuip, Rancho Dominguez, CA, USA), were set up in a 0.2 ha fallow field. Field cages were spaced 5 m apart in all cardinal directions to create a 4 × 4 randomized block design. Six, one-year-old asparagus crowns (cv. 'Guelph Millennium', Oomen Farms Ltd., Hart, MI, USA) were planted at 25 cm depth into each cage in two rows running north to south in a 2 × 3 design with 90 cm row spacing and 60 cm crown spacing within rows. Plants grew under natural conditions, without supplemental fertilizer or irrigation for the duration of the experiment and were monitored twice weekly for pests using visual scouting and yellow sticky traps (13 × 8 cm, Great Lakes IPM, Inc., Vestaburg, MI, USA) and any insects found were removed from the cages. Plants were used in experiments when at least one stem reached the fern stage with all cladophylls fully expanded, approximately six weeks after planting.

Herbivore treatments to induce the plants were assigned to field cages and administered to one randomly selected asparagus plant within each cage, other plants in cages were used in later replications. Treatments consisted of (1) empty collection bag (used to identify background contamination), (2) control (undamaged healthy asparagus plant), (3) mechanically damaged plant, and (4) common asparagus beetle larvae damaged plant. Mechanical damage was inflicted on ferns by removing 8 cm of plant tissue from the terminal end of five randomly selected branches using a scalpel, 48 and 24 h prior to volatile collection. Preliminary tests determined that 20 asparagus beetle larvae (2^{nd} – 4^{th} instar) removed approximately the same amount of plant tissue in 48 h of feeding as our mechanical damage treatment. Common asparagus beetle larvae damage treatments were inflicted upon plants with 20 larvae (2^{nd} – 4^{th} instars). Larvae were hand

collected from a five-year-old, 0.2 ha asparagus field (cv. 'Guelph Millennium') located at Michigan State University and were used within 3 h of collection for experiments. Asparagus beetle larvae were randomly placed on axillary branches of a caged asparagus ferns with a fine tipped paintbrush and were allowed to feed *ad libitum* over a 48 h period prior to volatile collection. All beetle larvae were removed from plants one hour prior to volatile collection.

Plant volatiles were collected (1 l min^{-1}) on an inline volatile trap (30 mg HayeSep Q®, Sigma Aldrich, St. Louis, MO, USA) during headspace collection of a treated plant for 24 h. Headspace was sampled by enclosing the entire damaged plant in a collection bag (polyvinyl fluoride film collection bag 56 × 40 cm, Tedlar®, DuPont Inc., Wilmington, DE, USA). The inline volatile trap was inserted into the bag while being attached to a push pull vacuum pump (Model 8R1110-101-1049, Gast Manufacturing, Benton Harbor, MI, USA), powered by a 12V battery (Model UB1280, Universal Power Group Inc., Coppell, TX, USA), and housed in a water proof case (Seahorse SE-300F, The Waterproof Case Company LLC., La Mesa, CA, USA).

Volatiles were eluted from each inline volatile trap using 150 µl dichloromethane and tetradecane (500 µM/ sample) was added as an internal standard to each sample. Volatile extractions were analyzed using an Agilent 7890A gas chromatograph (GC) paired with an Agilent 5975C mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA, USA). The GC-MS was equipped with an Agilent HP-5 column (30 m length, 0.320 mm ID, film thickness 0.25 µm). Helium was used as the carrier gas at 30 cm s^{-1} flow velocity. Aliquots (1 µl) of each sample were injected into the GC-MS and separated with a program of 1 min at 40 °C followed by increasing temperature at a rate of 10 °C min^{-1} to 260 °C. The reagent gas used for chemical ionization was isobutane. Ion source temperature was 250 °C in chemical ionization mode and was 220 °C in electron impact mode. GC-MS results were analyzed using MSD ChemStation

v.2.00 (Agilent Technologies, Santa Clara, CA, USA). Detected compounds were identified by comparing the mass spectrum of each compound to those in reference libraries: Adams 2 terpenoid/natural product library (Adams, 1995) and NIST 11 (National Institute of Standards and Technology, Springfield, VA, USA). Compound identifications were confirmed by comparing calculated Kovats Indexes (KI) to reference KI (Adams, 1995).

Prior to statistical analysis, background contamination identified in the empty collection bag treatment and rare compounds, only appearing in a less than three samples, were removed from sample profiles. The amount of individual volatile compounds released from each treatment were calculated relative to the hours of collection and the biomass of the plant (volatile (ng) / plant tissue (g) / collection (h)) and were analyzed to determine their relative contributions to the overall headspace profile of asparagus. Differences between treatments among individual compounds were determined using a Kruskal-Wallis test (package = "STATS"). When significant differences were found between treatments, a post-hoc Dunn's multiple comparisons test with Bonferroni correction was conducted ($\alpha = 0.05$; package = "DUNN.TEST"). All statistical analyses were conducted using R software (R Core Development Team, 2015).

Y-tube olfactometer assays. Y-tube choice tests were conducted with common asparagus beetle and convergent lady beetle adults (a known predator of common asparagus beetle (Ingrao et al., 2017)) to identify their chemotactic responses to synthetic asparagus odors (ocimene, farnesene, and tetradecanol – HIPVs identified in *Experiment 1*) and biological odors (healthy plants, asparagus beetle larvae damaged plants, and asparagus beetle larvae). Individual y-tube assays lasted for a maximum of 10 min. Individuals were recorded as making a choice if the beetle passed the half way point of one of the 6 cm arms of the y-tube. A no choice was recorded if an

individual did not pass the halfway point of either arm after 10 min. All assays were conducted in a climate controlled room (25 ± 0.5°C, 70 ± 5 % RH, 16: 8 L: D). After each assay, all glassware was rinsed with methanol and hexane and then dried in an oven at 60 °C for 10 min.

Convergent lady beetle adults (Rincon-Vitova Insectaries, Ventura, CA, USA) were maintained according to the protocol outlined in Bryant et al. (2014) until use in y-tube assays. Adult asparagus beetles were hand collected for y-tube assays from the field described in *Experiment 1* and were kept in 60 × 15 mm petri dishes (Falcon®, Durham, NC, USA) with a damp cotton ball and placed in the environmental chamber where y-tube assays were conducted for a 24 h acclimatization period prior to experimentation. Individuals were isolated and acclimatized in the same manner for both beetle species.

In the y-tube olfactometer (2 cm diameter, 12 cm length bottom arm and two 6 cm length top arms with ground glass joints, Michigan State University Glass Blowing Facility, East Lansing, MI, USA), air was passed through an activated charcoal filtered and then was split into two 0.5 l min^{-1} flows. Synthetic odor dilutions were offered on a 1 × 1 cm piece of filter paper (Grade 1, Whatman®, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) with 10 µl of treatment solution, dried for 5 min prior to use. Once dry, filter papers were placed in the y-tube odor source chamber. Whole plant treatments consisted of an entire potted asparagus plant (1.5 l nursery pot) in an inline sealed 7.6 l glass chamber (Michigan State University Glass Blowing Facility, East Lansing, MI, USA) which allowed clean air to move over the plant prior to entering the y-tube. Plants used in assays were grown within an environmental chamber (25 ± 0.5°C, 70 ± 5 % RH, 16: 8 L: D) in a media blend of 50 % play sand (Quikrete® Play Sand, The Quikrete Companies, Atlanta, GA, USA) and 50 % potting soil (SureMix Perlite, Michigan

Grower Products Inc., Galesburg, MI, USA). Plants were fertilized once at planting with a water soluble 20–20–20 N–P–K fertilizer (Scotts Miracle-Gro Products, Marysville, OH, USA), and watered twice weekly. Asparagus beetle damaged plants used in assays were induced using the same method outlined in *Experiment 1*. When using asparagus beetle larvae as an odor source, 20 larvae (2^{nd} – 4^{th} instar) collected from the field described in *Experiment 1* were placed in a 0.1 l inline sealed glass container (The Glass Group, Park Hills, MO, USA) to allow air to flow over the larvae before entering the y-tube.

Assays were first conducted to test lady beetle responses to volatiles induced by asparagus beetle larval feeding. Synthetic odor treatments identified as asparagus HIPVs were purchased from commercial suppliers in their available forms most closely related to those produced by asparagus plants (farnesene mixture of isomers and ocimene mixture of isomers – Sigma Aldrich, St. Louis, MO, USA; tetradecanol – Matrix Scientific, Columbia, SC, USA) and were diluted with pentane to three concentrations that represented volatile release rates above, near, and below the release rate identified from asparagus beetle damaged ferns in *Experiment 1*. This resulted in the following volatile treatments tested in all dual combinations with the convergent lady beetle: clean air (control), pentane (control), farnesene high (40.87 mM), farnesene mid (27.25 mM), farnesene low (20.44 mM), ocimene high (1362.30 mM), ocimene mid (136.23 mM), ocimene low (13.62 mM), tetradecanol high (214.39 mM), tetradecanol mid (53.60 mM), tetradecanol low (35.73 mM), and a 1:1 mixture of farnesene mid (27.25 mM) and ocimene mid (136.23 mM) solutions. Each synthetic treatment combination was replicated in y-tube choice tests 12-39 times. We measured convergent lady beetles' responses to biological odors in the y-tube using the following treatments: clean air (control), undamaged asparagus

fern, 20 asparagus beetle larvae (2^{nd} – 4^{th} instar), and an asparagus beetle induced fern. All biological treatment combinations were replicated 36-39 times.

All compounds that showed any indication of attraction to lady beetles in y-tube assays were tested on common asparagus beetles to ensure pests did not demonstrate attraction to odors that may attract natural enemies. Therefore, treatments used in common asparagus beetle y-tube assays included: clean air (control), pentane (control), farnesene mid, ocimene high, undamaged asparagus fern, and an asparagus beetle induced fern (20, 2^{nd} – 4^{th} instar larvae feeding for 48 h). All combinations of treatments were replicated 30-39 times. The number of beetles making a choice for treatments in y-tube assays were analyzed using a G-test with a William's correction (Sokal and Rohlf ,1995).

HIPV lures. Lures were developed for experiments to test the attraction of asparagus HIPVs to arthropods in the field using ocimene and farnesene (ocimene mixture of isomers and farnesene mixture of isomers – Sigma Aldrich, St. Louis, MO, USA) because of their presence in herbivore induced asparagus plants, availability, and low cost. Lures were comprised of a cotton ball (~0.28 g, Covidien LLC, Mansfield, MA, USA) placed in a 2 ml microcentrifuge vial (Denville Scientific Inc., Holliston, MA, USA) and wrapped with black tape (Scotch Duct Tape, The 3M Company, St. Paul, MN, USA) to prevent photolysis of compounds. Ocimene and farnesene lures were tested at different concentrations either as isolates or as mixtures of the two compounds. The following lures were evaluated: no lure (negative control), blank lure (positive control), farnesene high (1000 µl farnesene), farnesene low (750 µl farnesene), ocimene high (500 µl ocimene), ocimene low (300 µl ocimene), mixture high (1000 µl farnesene + 500 µl ocimene) and mixture low (750 µl farnesene + 350 µl ocimene). Vials were opened and attached

horizontally to the top of a 1 m tall metal pole with garden wire, directly below a yellow sticky trap (13 × 8 cm, Great Lakes IPM, Inc., Vestaburg, MI, USA). Lure field-release rates were established by collecting volatiles from each lure type over a seven-day period on day one, four, and seven, for 2 – 7.5 h (Table S3.1). Average release rates per h were calculated from the weekly mean of three replications with the same headspace collection equipment described in *Experiment 1*.

Field testing of lures was initially conducted from July – August 2016, in six commercial asparagus fields in Oceana County (MI, USA). Field sites were all within 8 km of Lake Michigan and had a consistent eastwardly prevailing wind from the lake. All fields used in the experiment had eastern field margins that were along unmanaged forests (mixtures of conifers and deciduous hardwoods) and lures were placed on the eastern crop edges 10 m apart so that the prevailing wind carried volatile signals into the wooded field border to attract natural enemies into the asparagus field from these natural habitats. Sticky traps and lures were replaced weekly for five weeks and pests, predators, and parasitoids collected on the traps were identified to lowest possible taxonomic level and quantified (Arnett, 2000; Arnett and Thomas, 2000; Arnette et al., 2002; Bradley, 2012; Goulet and Huber, 1993; Stehr, 1987; Ubick et al., 2009).

To test the effect of field position on the efficacy of lures, we continued sampling for an additional three weeks from August – September 2016, adding six research sites with lures on the southern field edge of asparagus fields with forested southern margins. Following the same protocol outlined above, we collected sticky traps and determined abundance of pests, predators, and parasitoids weekly.

The effect of field position on the number of arthropods trapped was determined; however, since it had no effect position was dropped as a fixed factor and total abundance for pests, predators, and parasitoids were analyzed with a mixed effects model GLMER (package = "LME4") with Poisson distribution. This analysis can account for an unbalanced experimental design since lures and traps were sometimes run over by farm equipment and destroyed. Lure treatments were fixed effects, and field and date were random effects. Full and reduced models were considered and models were selected for best fit based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Least squares means multiple comparisons, with Bonferroni correction, were conducted post-hoc ($\alpha = 0.05$; package = "MULTCOMP").

Effects of lures on biological control of key pests. In 2017, performance of the lure that attracted the most parasitoids in *Experiment 3* (ocimene high; 500 µl) was investigated in the field to understand the impact of the ocimene lure on the biological control of common asparagus beetle and asparagus miner (a co-occurring pest in Michigan asparagus that has been shown to be attacked by some of the same parasitoid taxa observed on sticky cards in *Experiment 3* (Morrison et al., 2014)). Lures were constructed and deployed as described in *Experiment 3*; however, yellow sticky cards were not used. Lures were deployed near forested margins on the eastern field edge of four commercial asparagus fields in Oceana County. Lures were distributed on the asparagus field edge at two densities: control (no lures), low density (three lures on the field edge), and high density (three lures on the field edge and three lures 5 m into the field) (Fig. S3.1). Treatments were separated by 20 m and lures within treatments were spaced at 10 m intervals. Lures were replaced weekly for six weeks.

Arthropods were collected from a 1 × 20 m transect on the field edge of each treatment area weekly from July 24 – August 30, starting one week after lure deployment (Fig. S3.1). Within each collection transect we hand collected 3rd – 4th instar asparagus beetle larvae and asparagus miner pupae. Collected larvae were placed in a plastic bag and transported to the lab where they were reared in a climate controlled room (25 ± 0.5°C, 70 ± 5 % RH, 16: 8 L: D) to determine larval parasitization. Larval rearing cages were comprised of an asparagus axillary branch cut from a mature fern with the cut end inserted through a small hole in the bottom of a plastic cup (59 ml, Solo®, Dart Container Corp., Mason, MI, USA) that was filled with potting soil (SureMix Perlite, Michigan Grower Products Inc., Galesburg, MI, USA) to allow larvae to fall to the soil to pupate. The bottom of the asparagus stem was inserted into 4 × 4 × 3 cm piece of saturated wet foam (FloraCraft®, Ludington, MI, USA) and placed in a cup (0.35 L, Letica® Corp., Rochester, MI, USA). One to 10 larvae were placed on the asparagus stems with a fine tipped paint brush and were covered with a 30 × 10 cm cylindrical chamber constructed of plastic transparency film (ACCO Brands, Inc., Lincolnshire, IL, USA), covered with a 160 μm screen mesh at the top to allow for ventilation (Fig. S3.2). Once larvae dropped from the plant and began pupating in the soil, the asparagus stem was removed and the soil filled cups were capped with a perforated lid. Cups were then monitored daily and emerged asparagus beetles and parasitoids were quantified and identified to species using reference vouchers from the A.J. Cook Arthropod Research Collection (Michigan State University).

Asparagus miner pupae were collected by randomly cutting 20 stems/collection transect, ~6 cm below the soil surface and at the highest mine on the stem. Samples were placed in plastic bags and returned to the lab. All asparagus miner pupae were excised from each of the mined stems and placed individually into ventilated plastic cups (59 ml, Solo®, Dart Container Corp.,

Mason, MI, USA) with 72 h. Rearing cups were then held in a climate controlled growth chamber (26.0 ± 1.0 °C, 80 ± 5.0 % RH, 16: 8 L: D) until an asparagus miner or parasitoid hatched. Samples were discarded if nothing hatched after five weeks. Asparagus miners and parasitoids that emerged from pupae were quantified and identified to genus or species using voucher specimens from the A.J. Cook Arthropod Research Collection.

Due to the absence of asparagus beetles on all collection dates except August 14th (41 larvae collected and reared) and August 21st (3 larvae collected and reared), statistical analysis on the number of asparagus beetles and the proportion of asparagus beetles parasitized are not presented here. For asparagus miners and its associated parasitoids, the hatch rates were analyzed with a generalized linear mixed model with binomial distribution where treatment was a fixed factor and date and field were random factors (package = "LME4"). When significant main effects were detected, a post-hoc least squares means comparison with Bonferroni correction was used to determine differences between treatments (package = "MULTCOMP"). The total number of parasitoids that hatched from asparagus miner pupae were summed over the season and were analyzed with a Pearson's chi-squared test with post-hoc multiple pairwise comparisons (α = 0.05; package = "STATS").

Results

HIPV collection and analysis. We detected 21 volatile compounds that were produced by asparagus ferns in response to herbivory by asparagus beetle larvae (Table 3.1). Healthy asparagus ferns produced 20 volatile compounds in the headspace ((*E*)-β-ocimene not present), while mechanical damaged plants produced 18 compounds (undecane, dodecane, and 1-tetradecanol not present). Herbivory by asparagus beetle larvae significantly upregulated the

production of (*E*)-β-ocimene (χ^2 = 9.30, df = 2, p = 0.01) and 1-tetradecanol (χ^2 = 12.83, df = 2, $p < 0.01$) in beetle damaged plants when compared to mechanically damaged or healthy plants. Asparagus beetle damaged plants also had significantly higher concentrations of (*E,E*)-α-farnesene compared to undamaged plants, but had similar concentrations to that of mechanically damaged plants (χ^2 = 16.43, df = 2, $p < 0.01$; Table 3.1, Fig. 3.1).

Table 3.1. Mean ± SEM ng / g fresh plant tissue / h plant volatiles released from healthy asparagus (undamaged), mechanically damaged asparagus, and asparagus beetle larvae damaged plants. Samples were collected in the field over a 24 h sampling period.

			Plant Volatile Release ng/g/h					
			Undamaged		Mechanical Damage		Beetle Damage	
Compound	K.I. (c)[a]	K.I. (r)[b]	Mean ± SEM	% Total	Mean ± SEM	% Total	Mean ± SEM	% Total
1. α-Pinene	941	939	25.15 ± 8.30 a	2.76	9.34 ± 6.40 a	1.00	26.99 ± 7.02 a	2.61
2. Octanal	1000	998	27.05 ± 7.06 a	2.96	30.03 ± 12.00 a	3.22	22.39 ± 6.54 a	2.17
3. *(Z)*-3-Hexenyl acetate	1008	1005	38.78 ± 15.47 a	4.25	10.03 ± 6.05 a	1.08	34.15 ± 20.04 a	3.30
4. 1-Hexanol, 2-ethyl	1020	1012[d]	167.17 ± 37.09 a	18.32	150.86 ± 38.71 a	16.16	132.79 ± 23.29 a	12.85
5. (E)-ß-Ocimene	**1046**	**1037**	**0.00 ± 0.00 b**	**0.00**	**12.58 ± 8.93 ab**	**1.35**	**29.53 ± 10.40 a[c]**	**2.86**
6. Undecane	1107	1100	10.00 ± 5.38 a	1.10	0.00 ± 0.00 a	0.00	7.81 ± 5.42 a	0.76
7. Nonanal	1108	1100	144.44 ± 32.24 a	15.83	201.70 ± 63.34 a	21.62	126.25 ± 27.12 a	12.22
8. Ethyl hexyl acetate	1156	1153	165.64 ± 37.86 a	18.16	133.92 ± 36.77 a	14.35	128.34 ± 29.21 a	12.42
9. Dodecane	1207	1200	6.05 ± 3.36 a	0.66	0.00 ± 0.00 a	0.00	4.47 ± 3.08 a	0.43
10. Unknown 1	-	-	1.59 ± 1.59 a	0.17	13.31 ± 10.82 a	1.43	4.49 ± 2.70 a	0.43
11. Decanal	1208	1201	24.40 ± 11.28 a	2.67	36.83 ± 14.35 a	3.95	21.39 ± 8.06 a	2.07
12. Ethyl acetophenone	1271	1281	10.70 ± 4.69 a	1.17	7.68 ± 4.43 a	0.82	8.68 ± 4.67 a	0.84
13. Tridecane	1308	1300	15.11 ± 5.39 a	1.66	7.06 ± 3.88 a	0.76	19.48 ± 6.59 a	1.89
14. Unknown 2	-	-	6.55 ± 3.06 a	0.72	5.22 ± 4.36 a	0.56	2.90 ± 1.56 a	0.28
15. Pentadecane	1509	1500	67.82 ± 10.10 a	7.43	79.20 ± 25.56 a	8.49	75.05 ± 11.89 a	7.26
16. (E,E)-α-Farnesene	**1512**	**1505**	**9.08 ± 4.94 b**	**1.00**	**21.21 ± 10.92 b**	**2.27**	**82.69 ± 24.36 a[c]**	**8.00**
17. Hexadecane	1609	1600	37.38 ± 8.68 a	4.10	41.98 ± 13.76 a	4.50	35.92 ± 4.40 a	3.48
18. Heptadecane	1709	1700	21.22 ± 6.45 a	2.33	22.23 ± 9.89 a	2.38	15.90 ± 3.80 a	1.54
19. Methyl tetradecanoate	1727	1723	127.92 ± 33.08 a	14.02	137.97 ± 51.02 a	14.79	100.50 ± 21.34 a	9.73
20. Unknown 3	-	-	3.16 ± 2.19 a	0.35	11.85 ± 7.72 a	1.27	9.87 ± 4.19 a	0.96
21. 1-Tetradecanol	**1813**	**1811[e]**	**3.13 ± 3.13 b**	**0.34**	**0.00 ± 0.00 b**	**0.00**	**143.57 ± 59.43 a[c]**	**13.90**

[a] K.I. = Kovats indices calculated

[b] K.I. = Kovats indices referenced from Adams 1995

[c] Significant Kruskal-Wallis test with Dunn's post hoc multiple comparisons and Bonferroni correction (n = 16, α = 0.05)

[d] From da Silva Junkes et al. 2003

[e] From De Marques et al. 2000

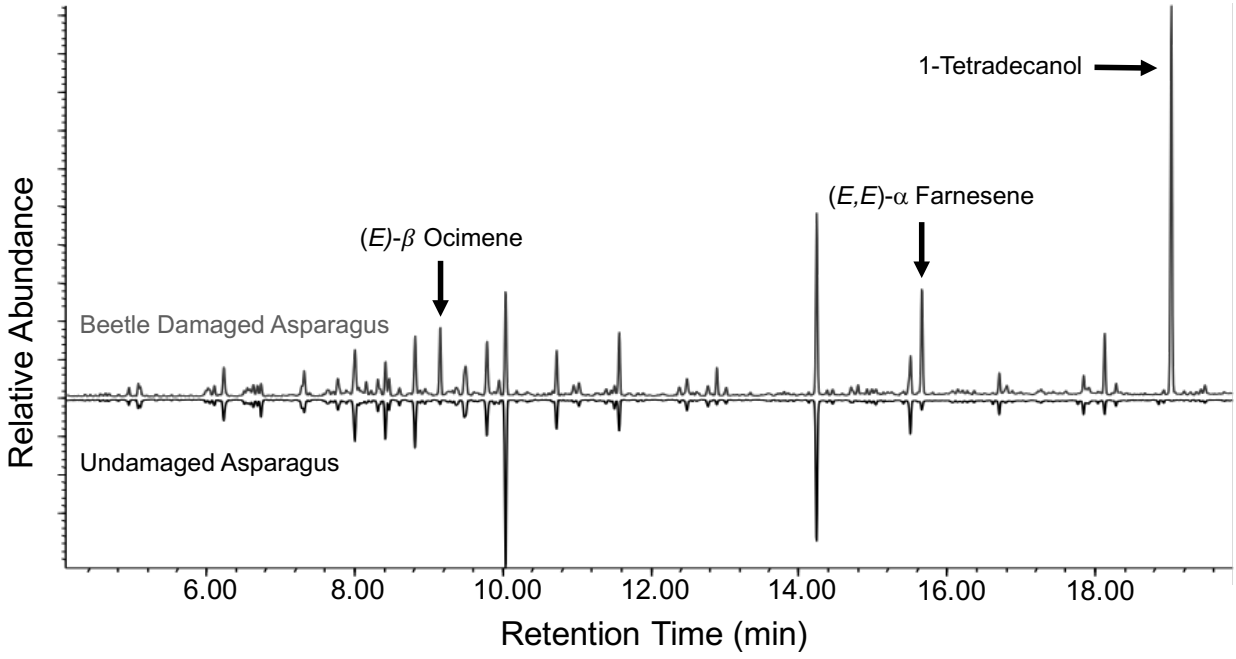

Figure 3.1. Representative GC/MS headspace profiles collected in the field from one-year-old asparagus ferns treated with either 20 asparagus beetle larvae, fed *ad libitum* for 48 h, or an undamaged asparagus plant. Arrows indicate compounds that were upregulated in response to beetle feeding. Mechanically damaged ferns had profiles similar to undamaged asparagus (data not shown).

Ocimene was not present in any of the control plants' headspace, but it made up 1 % and 3 % of the mechanical and asparagus beetle damaged plants' profiles, respectively. Farnesene was found in all treatments, but asparagus beetle damaged plants had an eight-fold increase in its production over healthy plants and a four-fold increase over mechanically damaged plants. Tetradecanol was not found in the headspace of mechanically damaged treatments and comprised < 1 % of the headspace of healthy plants; however, it made up 14 % of the headspace of asparagus beetle damaged plants. Overall, the three compounds upregulated by asparagus beetle feeding comprised 25 % of the overall headspace profile collected from asparagus beetle damaged plants, but only 4 % of the mechanically damaged plants and 1 % of the control plants' headspace.

Y-tube olfactometer assays. Convergent lady beetles demonstrated no clear attraction to synthetic odors in the treatment combinations tested. Lady beetles were only found to be significantly attracted to high concentrations of ocimene over high concentrations of farnesene ($\chi^2 = 7.63$, $N = 27$, $p = 0.01$), mid concentrations of farnesene over pentane controls ($\chi^2 = 12.06$, $N = 18$, $p < 0.01$), and mid concentrations of tetradecanol over high concentrations of tetradecanol ($\chi^2 = 4.86$, $N = 32$, $p < 0.05$; Table S3.2). In olfactometer assays with biological odor stimuli, lady beetles only showed significant chemotaxis towards asparagus beetle larvae when compared to clean air controls ($\chi^2 = 4.61$, $N = 36$, $p < 0.05$) and when compared to asparagus beetle induced plants ($\chi^2 = 4.33$, $N = 36$, $p < 0.05$; Table S3.3). Common asparagus beetle olfactometer assays resulted in no clear preference for any of the synthetic or biological volatile treatments tested in this study ($\chi^2 \leq 2.50$, $N \geq 30$, $p \geq 0.12$; Table S3.4).

HIPV lures. All lures developed from volatile compounds found in the headspace of asparagus beetle damaged plants attracted more parasitoid wasps to yellow sticky traps over the eight-week sampling period than controls ($\chi^2 = 316.14$, df = 7, $p < 0.01$; Fig. 3.2). High concentration ocimene lures attracted significantly more parasitoids than all other treatments ($z \leq -4.55$, $p < 0.01$), except low farnesene concentration lures ($z = -1.99$, $p = 0.48$). Low farnesene concentration lures attracted 19 % more parasitoids than high concentration lure mixtures of ocimene + farnesene ($z = 3.84$, $p < 0.01$), and at least 37 % more than controls ($z < -10.17$, $p < 0.01$); however, they performed similar to high farnesene ($z = -2.58$, $p = 0.16$), low ocimene ($z = 1.82$, $p = 0.61$), and mixtures of low ocimene + farnesene lures ($z = 2.58$, $p = 0.17$). High farnesene, low ocimene and both mixture lures all performed similarly and all attracted significantly more parasitoids than the control treatments ($z \leq -6.24$, $p < 0.01$). Predatory

arthropods did not respond differently to our lures compared to the control treatments ($\chi^2 = 5.71$, df = 7, $p = 0.57$). Likewise, key obligate asparagus pests, common asparagus beetle and asparagus miner, showed no significant attraction to any of the lures tested compared to the controls ($\chi^2 = 4.88$, df = 7, $P = 0.68$).

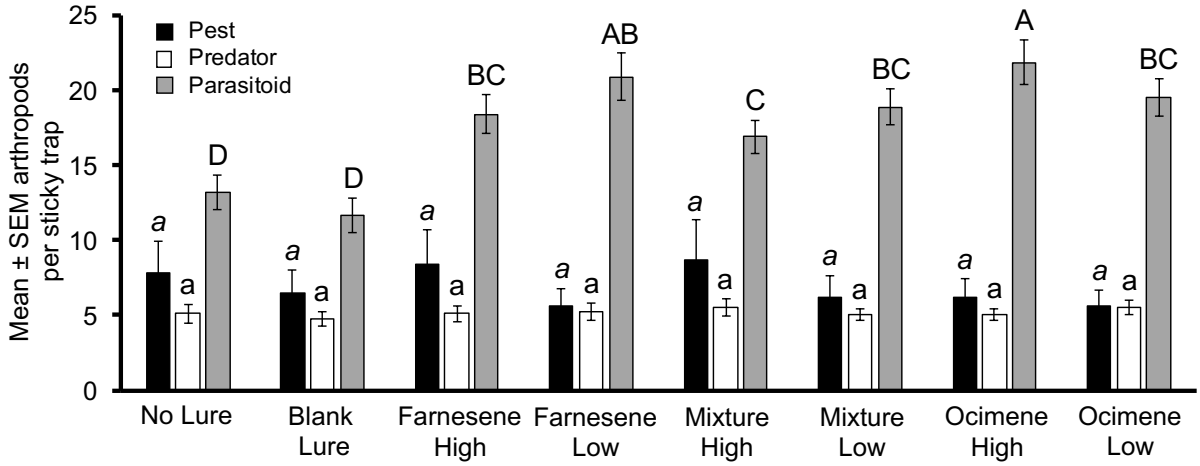

Figure 3.2. Volatile lures were deployed in commercial asparagus fields to determine attraction of pests, parasitoids and predator arthropods to baited yellow sticky traps. Lure treatments consisted of: no lure (negative control), blank lure (positive control), farnesene high (1000 µl farnesene), farnesene low (750 µl farnesene), mixture high (1000 µl farnesene + 500 µl ocimene), mixture low (750 µl farnesene + 350 µl ocimene), ocimene high (500µl ocimene), and ocimene low (300 µl ocimene).

Effects of lures on biological control of key pests. Although common asparagus beetle abundance was low throughout the season (44 individuals collected), 32 % (14 individuals) of those we collected were parasitized. Of those, 86 % were parasitized by *Tetrastichus asparagi* Crawford (Hymenoptera: Eulophidae) and 14 % were parasitized by *Paralispe infernalis* Townsend (Diptera: Tachinidae). All parasitoids reared from asparagus beetles were collected from high density ocimene treatments, except one *P. infernalis* which was collected from the low density treatment.

Of the 251 viable asparagus miner pupae excised from asparagus stems collected in 2017, 54 % (136 individuals) were parasitized. Asparagus miner hatch rates were significantly higher in high density ocimene treatments than in low density treatments, but neither were significantly different from the controls ($\chi^2 = 7.95$, df = 2, $p < 0.05$) (Fig. 3.3a). Hatch rates of parasitoids were similar across treatments ($\chi^2 = 4.27$, df = 2, $p = 0.12$) (Fig. 3.3a). Asparagus miner was parasitized by Braconidae, Pteromalidae, Eulophidae and Eupelmidae. *Chorebus rondanii* Giard (Hymenoptera: Braconidae) accounted for 49 % of all parasitoids hatched from asparagus miner pupae and was the most common parasitoid found in this study; however, the seasonal total of braconids was not affected by lure treatments ($\chi^2 = 1.91$, df = 2, $p = 0.39$) (Fig. 3.3b). Pteromalids were the second most common family found parasitizing the miner, accounting for 46 % of all parasitoids hatched. Three pteromalid species were found parasitizing the miner: *Thinodytes cephalon* Walker (92 % of all pteromalids), *Cyrtogaster vulgaris* Walker (5 %), and *Sphegigaster cracentis* Heydon and LaBerge (3 %). The seasonal total of pteromalids parasitizing miners was significantly higher in low density ocimene treatments when compared to all other treatments ($\chi^2 = 7.97$, df = 2, $p < 0.05$) (Fig. 3.3b); however, pteromalids were only found in two of the four fields we sampled over the entire season. Other parasitoids attacking miners in low numbers were: *Neochrysocharis* sp. (Hymenoptera: Eulophidae) (4 %) and *Eupelmus vesicularis* Retzius (Hymenoptera: Eupelmidae) (1%).

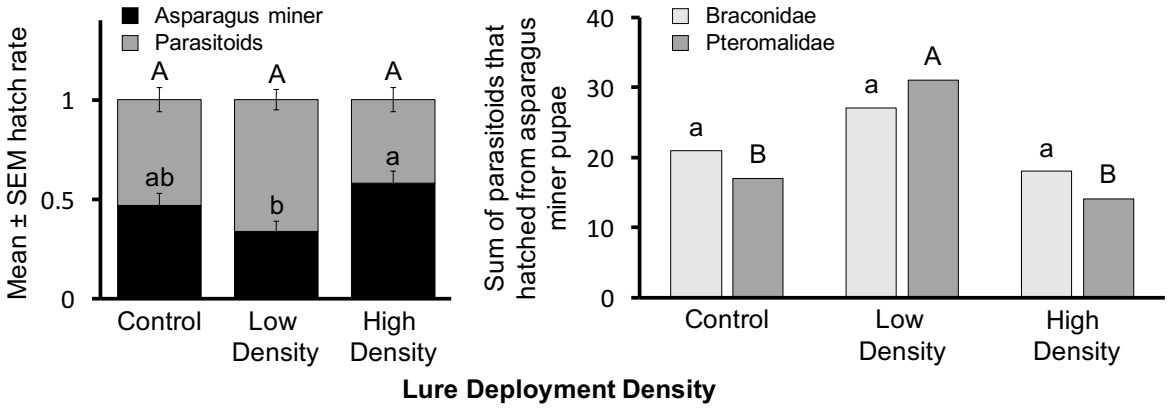

Figure 3.3. Ocimene lures (500µl ocimene) deployed in high and low densities in asparagus fields were used to determine biological control of asparagus miner by parasitoids with the mean hatch rate of asparagus miner and all parasitoids reared from asparagus miner pupae (a) and the seasonal total of parasitoids from Braconidae and Pteromalidae (b).

Discussion

Successful use of HIPVs for improving biological control in agroecosystems partly depends on identifying plant volatiles that are attractive to natural enemies of key pests but are not attractive to pests. Here, we identified three plant volatiles from asparagus that had elevated emissions in response to chewing herbivore damage, allowing us to focus on these as potential targets for use in pest management (Fig. 3.1). In y-tube olfactometer assays, we determined that a predatory lady beetle and common asparagus beetle were not attracted to asparagus HIPVs. In field trials we confirmed that pests and predators were not attracted to asparagus HIPV lures, but parasitoids demonstrated strong attraction that may increase biological control of the asparagus miner, a specialist pest that co-occurs with the common asparagus beetle.

Previous studies have indicated that parasitoids often use volatile cues for host location which makes them ideal targets for biological control programs (De Moraes and Lewis, 1999; De Moraes et al., 1998; Du et al., 1998). Our results from the field experiments support this, with parasitoids significantly more attracted to farnesene and ocimene lures, but other natural enemies

and pests not recognizing these as attractive cues (Fig. 3.2). Interestingly, in our research, HIPVs resulting from a specialist chewing pest, attracted parasitoids of a specialist stem mining insect. While we were not able to compare asparagus volatile profiles induced by both herbivores, it is possible that there are similarities in the HIPV profiles induced by the two types of pests and that natural enemies use these as generic host recognition cues. On the other hand, insect stem mining causes minimal emissions of HIPVs compared to chewing (Turlings et al., 1998), thus parasitoids of mining pests might rely on cues emitted by other co-occurring specialist herbivores that cause prominent but reliable cues (Vet and Dicke, 1992). In our system, it is not uncommon to find asparagus beetles and asparagus miners feeding on the same plants simultaneously, thus asparagus beetle feeding might lead to associational susceptibility of asparagus miners, which should be tested in future studies.

Two families of pupal parasitoids dominated the parasitoid community of the asparagus miner in our study and these groups have been previously reported in the literature in asparagus fields from our region (Morrison et al., 2014). One of these two groups of parasitoids, the pteromalids, had significantly higher parasitism of asparagus miners in response to the low density ocimene lure treatment in the field, thus our results provide the first evidence of ocimene as a potential pteromalid attractant leading to improved pest control. While braconids are known to be attracted to some HIPVs, we did not observe this with ocimene lures (Giunti et al., 2016; Ngumbi et al., 2005; Takemoto and Takabayashi, 2015; Zimba et al., 2015). It is interesting to note that the pteromalid species present in our system generally have broad host ranges while the one braconid species is a specialist on asparagus miner, which might explain the lack of the braconid's response to the ocimene lure (Morrison et al., 2014). While the generalist pteromalids are able to use the volatile induced by a chewing herbivore as a host recognition cue, the

specialist braconid might not be able to use it in host finding. Our work highlights the importance of resolving certain insect traits, such as host breadth, that may explain behavioral responses of parasitoids to plant volatiles.

From a pest management perspective, it is fortunate that pteromalids are typically three times more abundant in Michigan commercial asparagus fields than braconids (Morrison et al., 2014). Therefore, our ocimene lures were increasing the abundance of the most prominent group of parasitoids in our system. However, despite their abundance in our study we only collected pteromalids from two of the four fields we sampled. Interestingly, these two fields had similar border habitat compositions with one field border habitat that was forested, two that were in asparagus and one that was in a non-asparagus crop. Conversely, the two fields with no pteromalids had three field border habitats in forest and one in a non-asparagus crop. Habitat simplification is typically associated with decreases in natural rates of biological control in agricultural systems (Rusch et al., 2016); however, our data seems to support the hypothesis that pteromalids rely more on resources provided by crops than natural habitat (Tscharntke et al., 2016). Future studies should focus on teasing out the connection between pteromalid abundance and habitat complexity of agroecosystems.

Temporal and spatial relationships between pests and natural enemies are important to consider when developing volatile lures to support biological control programs (Braasch and Kaplan, 2012). In our system, the two key pests co-occur and congregate on asparagus field edges, post-harvest, while natural enemies are primarily found in the field margins, ~10 m outside of the field (Ingrao et al., 2017; Morrison and Szendrei, 2013). This spatial arrangement provides a unique opportunity to strengthen the relationship between these two groups in space and time using volatile lures. Lures can be deployed on asparagus field edges to attract natural

enemies from field margins, but they should only be deployed when the pest is in a vulnerable life stage, and reaches a management threshold, otherwise lures should be removed to release natural enemies from a habitat devoid of their hosts (Kaplan, 2012). Pest phenology is particularly important to consider with HIPV based biological control because pests are often only vulnerable to particular natural enemies during certain life stages. As HIPV driven pest management tactics are explored in specialty crop systems, the use of pest degree day models to inform deployment timing will provide important information in developing 'attract and release' strategies that consider pest phenology and target life stage.

While the bioactive range of plant volatile lures is variable (Braasch and Kaplan, 2012; Mallinger et al., 2011; Rodriguez-Saona et al., 2011), our findings indicate that the concentration of volatiles emitted by lures against the natural background of plant volatiles can have an impact on the outcome of biological control (Dicke et al., 2003; Schröder and Hilker, 2008). In our study, the low density deployment of an ocimene lure was more attractive for parasitoids than when we doubled the number of lures on the field edge, suggesting that otherwise attractive plant volatiles can become repellent for insects at high concentrations (Hilker and McNeil, 2008; Kaplan, 2012; Whitman and Eller, 1992). In addition, the spatial arrangement of lures may also have a profound effect on attraction, for example, we may need to consider increasing the space among lures to adjust the concentration of ocimene in the air. Although the bioactive range, deployment density, and spatial arrangement of lures needs further study, our research provides strong evidence that ocimene lures may increase parasitism of asparagus miner by pteromalids.

Conclusions

Specialty crops, such as asparagus, have high economic value per hectare but often are limited in pest management tools. This requires that alternative pest management tactics, such as utilizing HIPV lures for improving biological control, are given much more research attention. One of the greatest challenges for specialty crops is that alternative pest management strategies must be developed and tested for each specialty crop and pest combination due to the variability across systems. Coordinated efforts between specialty crop producers, pest managers, and chemical ecologists could facilitate meaningful pest management solutions and further our understandings of the role semiochemicals play in pest management.

APPENDIX

Supplementary Tables

Table S3.1. Average release rates of field deployed lures.

	Mean ± SE Lure Release Rate (mg/day)					
Compound	Farnesene high	Farnesene low	Ocimene high	Ocimene low	Mixture high	Mixture low
(Z) – *β* – Farnesene	2.47 ± 1.17	1.53 ± 0.74			2.71 ± 1.34	1.10 ± 0.51
(E) – *β* – Farnesene	2.17 ± 1.05	1.00 ± 0.49			2.19 ± 1.06	0.80 ± 0.29
(Z) – *β* – Ocimene			8.66 ± 3.10	4.09 ± 1.51	16.67 ± 5.63	9.80 ± 3.22
(E) – *β* – Ocimene			13.69 ± 5.04	7.51 ± 3.12	28.84 ± 9.90	16.06 ± 4.85

Table S3.2. Y-tube choice test responses of convergent lady beetle to synthetic volatile compounds.

Choice 1	Choice 2	Choice 1[a]	Choice 2[a]	No choice	N^b	χ^2	p
Clean air	Oci. mid + farn. mid	10	17	6	33	1.80	0.18
Clean air	Tetradecanol high	10	7	4	21	0.52	0.47
Clean air	Tetradecanol low	7	7	1	15	0.00	1.00
Clean air	Tetradecanol mid	6	8	4	18	0.28	0.60
Farnesene low	Clean air	17	15	4	36	0.12	0.73
Farnesene low	Farnesene high	14	19	3	36	0.75	0.39
Farnesene low	Ocimene high	8	8	2	18	0.00	1.00
Farnesene low	Oci. mid + farn. mid	18	14	7	39	0.49	0.48
Farnesene mid	Clean air	15	13	5	33	0.14	0.71
Farnesene mid	Farnesene high	10	10	1	21	0.00	1.00
Farnesene mid	Farnesene low	9	9	6	24	0.00	1.00
Farnesene mid	Ocimene high	8	9	1	18	0.06	0.81
Farnesene mid	Oci. mid + farn. mid	13	11	0	24	0.16	0.69
Farnesene mid	Tetradecanol high	10	9	2	21	0.05	0.82
Farnesene mid	Tetradecanol low	5	8	2	15	0.67	0.41
Farnesene mid	Tetradecanol mid	8	9	4	21	0.06	0.81
Farnesene high	Clean air	17	11	8	36	1.27	0.26
Farnesene high	Oci. mid + farn. mid	10	11	3	24	0.05	0.83
Ocimene high	Clean air	14	16	3	33	0.13	0.72
Ocimene high	**Farnesene high**	**18**	**5**	**4**	**27**	**7.63**	**0.01 ***
Ocimene high	Oci. mid + farn. mid	9	14	7	30	1.07	0.30
Ocimene high	Tetradecanol high	4	6	5	15	0.38	0.54
Ocimene high	Tetradecanol low	5	5	2	12	0.00	1.00
Ocimene high	Tetradecanol mid	7	12	5	24	1.30	0.25
Ocimene low	Clean air	15	9	6	30	1.49	0.22
Ocimene low	Farnesene high	12	17	7	36	0.85	0.36
Ocimene low	Farnesene low	7	9	2	18	0.24	0.62
Ocimene low	Farnesene mid	8	7	3	18	0.06	0.80
Ocimene low	Ocimene high	11	6	4	21	1.45	0.23
Ocimene low	Ocimene mid	9	7	2	18	0.24	0.62
Ocimene low	Oci. mid + farn. mid	20	13	6	39	1.47	0.22
Ocimene low	Pentane	17	13	3	33	0.53	0.47
Ocimene mid	Clean air	17	11	8	36	1.27	0.26
Ocimene mid	Farnesene high	19	12	2	33	1.57	0.21
Ocimene mid	Farnesene low	9	11	1	21	0.20	0.66
Ocimene mid	Farnesene mid	13	17	6	36	0.53	0.47
Ocimene mid	Ocimene high	12	17	1	30	0.85	0.36
Ocimene mid	Oci. mid + farn. mid	16	10	10	36	1.37	0.24
Pentane	Clean air	10	20	9	39	3.34	0.07

Table S3.2. (cont'd)

Pentane	Farnesene high	11	17	2	30	1.27	0.26
Pentane	Farnesene low	9	16	2	27	1.95	0.16
Pentane	**Farnesene mid**	**2**	**16**	**0**	**18**	**12.06**	**< 0.01 ***
Pentane	Ocimene high	14	9	7	30	1.07	0.30
Pentane	Ocimene mid	19	13	4	36	1.11	0.29
Pentane	Oci. mid + farn. mid	11	19	3	33	2.12	0.15
Pentane	Tetradecanol high	7	10	1	18	0.52	0.47
Pentane	Tetradecanol low	8	9	4	21	0.06	0.81
Pentane	Tetradecanol mid	10	6	11	27	0.98	0.32
Tetradecanol high	Tetradecanol low	8	8	5	21	0.00	1.00
Tetradecanol high	**Tetradecanol mid**	**9**	**21**	**2**	**32**	**4.86**	**0.03 ***
Tetradecanol mid	Tetradecanol low	5	6	1	12	0.09	0.77

[a] Total number of insects choosing either side of the y-tube arm
[b] Total number of replications of the assay with a particular treatment combination
* Statistically significant G-test with William's correction, $\alpha = 0.05$

Table S3.3. Responses of convergent lady beetles to biological volatile signals in y-tube choice tests.

Choice 1	Choice 2	Choice 1[a]	Choice 2[a]	No choice	N^b	χ^2	p
AB damaged	**AB larvae**	**11**	**23**	**2**	**36**	**4.33**	**0.04 ***
AB damaged	Undamaged	23	14	2	39	2.21	0.14
Clean Air	AB damaged	20	13	3	36	1.50	0.22
Clean Air	**AB larvae**	**10**	**22**	**4**	**36**	**4.61**	**0.03 ***
Clean Air	Undamaged	20	14	2	36	1.06	0.31
Undamaged	AB larvae	12	22	2	36	2.99	0.09

[a] Total number of insects choosing either side of the y-tube arm

[b] Total number of replications of the assay with a particular treatment combination

* Statistically significant G-test with William's correction, $\alpha = 0.05$

Table S3.4. Common asparagus beetle responses to synthetic and biological volatile signals in y-tube choice tests.

Choice 1	Choice 2	Choice 1[a]	Choice 2[a]	No choice	N^b	χ^2	p
AB damaged	AB larvae	17	10	9	36	1.84	0.18
AB damaged	Undamaged	17	9	10	36	2.50	0.12
AB larvae	Undamaged	18	14	4	36	0.50	0.48
Clean Air	AB damaged	10	15	8	33	1.01	0.32
Clean Air	AB larvae	17	12	7	36	0.87	0.36
Clean Air	Farnesene mid	16	16	7	39	0.00	1.00
Clean Air	Ocimene high	11	9	10	30	0.20	0.66
Clean Air	Pentane	19	11	6	36	2.16	0.15
Clean Air	Undamaged	14	15	7	36	0.03	0.85
Farnesene mid	Ocimene high	15	14	7	36	0.03	0.85
Pentane	Farnesene mid	15	15	6	36	0.00	1.00
Pentane	Ocimene high	15	9	12	36	1.52	0.22

[a] Total number of insects choosing either side of the y-tube arm

[b] Total number of replications of the assay with a particular treatment combination

Supplementary Figures

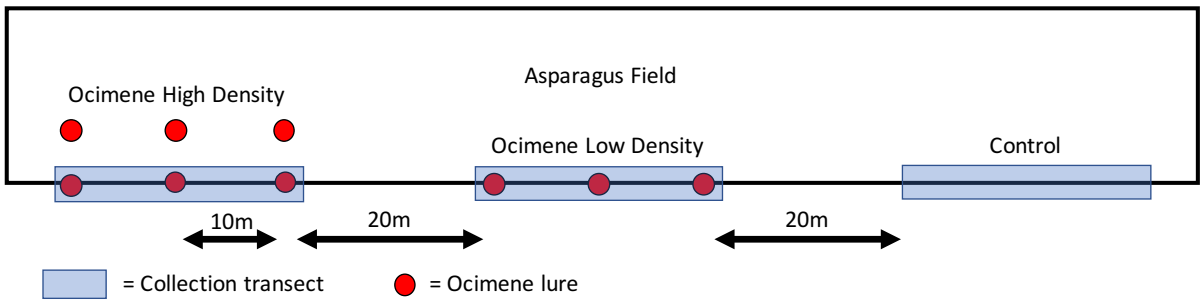

Figure S3.1. Field layout of 2017 lure experiment investigating parasitism rates of asparagus beetle and asparagus miner relative to lure deployment density with lures placed on the eastern edge of fields with forested borders in high and low density arrangements. Asparagus beetles and miners were monitored within collection transects, and beetle larvae and miner pupae were collected weekly or when present.

Figure S3.2. Asparagus beetle larvae (3rd – 4th instar) were field collected and brought to the lab where they were placed in a rearing apparatus in a climate controlled chamber (25 ± 0.5°C, 70 ± 5 % RH, 16: 8 L: D). The rearing apparatus was comprised of a 0.35 l plastic cup with a 4 × 4 × 3 cm piece of saturated wet foam in the bottom of the cup. Inside of the plastic cup, and on top of the foam, was a small 59 ml plastic cup filled with potting soil with a 1 cm hole in the bottom of the cup. A branch from a mature asparagus plant was then placed in the apparatus, through the hole in the small hole, into the wet foam. Field collected larvae were then placed on the asparagus branch with a fine tipped paint brush. The asparagus branch with larvae was then covered with a cylindrical chamber made of plastic transparency film and covered with screen mesh. Larvae were fed *ad libitum* until they dropped from the plants into the soil where they pupated. Once larvae were in the soil filled cup, the cup was removed from the growing apparatus, capped with a ventilated lid and were monitored daily for adult beetle or parasitoid emergence.

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LITERATURE CITED

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CHAPTER 4

Conclusions and future directions

Overall, this dissertation represents the most comprehensive attempt, to date, to establish an applied biological control tactic in Michigan asparagus for the control of asparagus miner and common asparagus beetle. For the scientific community, this research has identified the predatory complex of asparagus miner and the common asparagus beetle and the role that border habitats play in their abundance and distribution, as well as the volatile chemicals of asparagus induced by common asparagus beetle feeding and the role of HIPV lures may play in biological control of the asparagus miner. For the grower community, this research has the potential to address the inadequacy of current control measures for crop specific pests using a HIPV driven management tactic.

In Chapter 2, I sought to describe the asparagus miner's and common asparagus beetle's natural enemy communities in Michigan commercial asparagus fields to identify which taxa should be considered as potential biological control agents. My research was the first to investigate the predatory community feeding on the asparagus miner. While some of the North American predators of asparagus beetle have been described in previous studies (Capinera and Lilly, 1975b; Poll et al., 1998; Watts, 1938); the only evidence of predatory linkages for the beetle came from observational data and lab based feeding trials (Capinera and Lilly, 1975a; Drake and Harris, 1932; Morrison and Szendrei, 2014; Watts, 1938). Therefore, I used molecular gut content analysis to produce predatory food webs for each of the two pests that has resulted in the most complete understanding of these food webs to date. In the future, this information should be further improved by the inclusion of prey detectability half-life for the key predators in

the asparagus system.

One of the biggest challenges in determining the importance of specific predator taxa testing positive for pest DNA is scavenging (King et al., 2008). In my data, Staphylinids (Aleocharinae, Tachyporinae, and Staphylininae) had strong predatory linkages with both asparagus pests in 2014 and 2015. The three staphylinid subfamilies I found are primarily facultative predators (Frank and Thomas, 1999) and interactions of these epigeal predators with miners and beetles is unlikely due to the differences in spatial distributions. However, staphylinids may feed on miner pupae that are exposed due to desication and cracking of the epidermal layer of the asparagus stem near the soil line, while beetle pupa in the soil may become exposed to predation with soil disturbance. Predation on pupae by staphylinids may be offering some biological control of the miner and beetle; however, these incidents may be rare and feeding could be occurring on cadavers that have fallen from the canopy. Therefore, it is difficult to support targeting staphylinids as potential biological control agents with my findings. Future research should invesitgate the feeding behaviors of staphylinids occuring in asparagus fields to determine if the predation identified in this research is in fact predation and not scavaging. If predation is occurring, then efforts to support populations of staphylinids through cover crops, mulching, and other floor habitat management approaches may support control of both target pests.

Spatial relationships between pests and predators were also investigated in Chapter 2 to determine the field distribution of both of these groups and whether their distributions could be manipulated to support increased biological pest control. Although it is broadly understood that natural habitats around agricultural fields are vital for providing refuges for invertebrates, potentially ameliorating the negative effects of pest management methods on biological control

agents (Wratten 1988; Wratten et al. 1998), it is less clear from system to system how pest distributions may be affected by these habitats. I determined that predators of the miner and beetle primarily resided in diversified field margins, such as forests, while both pests primarily occupied the field edge of asparagus fields. Therefore, my remaining research focused on better understanding how this spatial relationship could be exploited to favor increased natural enemy-prey interactions on the field edge.

In Chapter 3, my research focused on bridging the natural enemy-pest spatial gap on field edges to increase biological control of asparagus miner and asparagus beetle using volatile signals. This study was the first to investigate the herbivore induced volatiles of asparagus under herbivory by a monophagous pest and has resulted in the identification of three novel compounds being upregulated in response to herbivore feeding: (*E*)-β-ocimene, (*E,E*)-α-farnesene, and 1-tetradecanol. Although I tested all compounds in olfactometer assays with a predator of asparagus beetle no clear attraction was found to asparagus HIPV's. Future researchers should investigate these compounds with parasitoids identified in this research, braconid and pteromalids, in olfactometers and wind tunnels to get a broader sense of the role these compounds may be playing in attracting parasitoids. The abundance of both parasitoid families identified in Chapter 3 and in Morrison et al. (2014) is a clear indication of the importance of these taxa in controlling asparagus miners; thus, they should be primary targets for future biological control research.

In Chapter 3, pteromalids attacked the most asparagus miners on field edges with ocimene lures in low density arrangements. Pteromalids have been identified in this research and that of Morrison et al. (2014) as abundant natural enemies in Michigan asparagus. The attraction of pteromalids to lures resulting in increased biological control of a target pest is a key finding of

my research and presents a clear target for future research into asparagus miner management. However, the lures developed and tested as part of Chapter 3 were rudimentary and improvements in lure performance and attractiveness could be realized with some refinement of both the lure and volatile attractants. The lure tested had a measurable release rate for one week, but the release rate declined non-linearly over that period. Additionally, the ocimene lure may be improved by blending ocimene with other volatiles, such as green leaf volatiles (Maeda et al., 2015). Future, researchers should coordinate with a lure manufacturer to produce a lure that has a constant release rate to stabilize the attractiveness to natural enemies and explore the attractiveness of formulations with ocimene blends.

Although I attempted to test the effects of ocimene lures on asparagus miner and asparagus beetle management, I was unable to collect enough data to make any conclusions regarding the effectiveness of the lure on biological control of the beetle. Throughout my research, asparagus beetle populations fluctuated and in 2017 beetle abundance was at the lowest I witnessed in four years of working in Oceana County, Michigan. However, despite not being able to make conclusions regarding parasitism I was able to identify two parasitoid species attacking beetle larvae that had been described in previous studies (Capinera and Lilly, 1975b; Poll et al., 1998; Watts, 1938). Therefore, future research should investigate the use of natural enemy lures on parasitism of asparagus beetle during high population density years in Michigan.

This dissertation provides new opportunities for asparagus producers to control crop pests through the recruitment of natural enemies using HIPV lures. HIPVs represent complex communications in agroecosystems among trophic levels and research into their ecological roles offers us opportunities to take part in these dynamic “conversations” to support favorable pest management outcomes. However, our knowledge regarding the role of HIPVs in agroecosystems

is still in its infancy and as our ability to detect and synthesize these compounds increases with technological advances, so too should our efforts to understand their ecological roles. The uniqueness of specialty crop pest management makes alternative management tactics for pests, such as HIPV driven biological control, an area that should be given much more research attention, and perhaps represents the best opportunity for researchers to demonstrate the value of such a new management tool compared to field crops, which often have a suite of management tactics to choose from. However, the use of HIPV lures in agricultural pest management should be regarded as one of the many IPM tools available and should be incorporated as part of a multifaceted strategy that includes scouting, management thresholds, and degree day models to ensure timing of lure deployment is synchronous with pest life stages that are targets for control. It is my sincere belief that HIPVs represent the next great revolution in agricultural pest management which will allow researchers and pest managers to become active participants in the "conversations" taking place between plants and arthropods residing in managed systems.

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: 2017-15

Author: Adam J. Ingrao

Title of thesis: Herbivore induced plant volatiles of asparagus (*Asparagus officinalis* L.) and their attraction to natural enemies of asparagus miner (*Ophiomyia simplex* Loew) and common asparagus beetle (*Crioceris asparagi* L.)

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Agromyzidae	*Ophiomyia simplex*	adult	10	pinned
Braconidae	*Chorebus rondanii*	adult	9	pinned
Chrysomelidae	*Crioceris asparagi*	adult	10	pinned
Coccinellidae	*Hippodamia convergens*	adult	10	pinned
Eulophidae	*Neochrysocharis* sp.	adult	10	pinned
Eupelmidae	*Eupelmus vesicularis*	adult	3	pinned
Pteromalidae	*Thinodytes cephalon*	adult	10	pinned
Pteromalidae	*Cyrtogaster vulgaris*	adult	2	pinned

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