INVESTIGATION OF THE TRITROPHIC INTERACTIONS OF THE ASPARAGUS MINER (*OPHIOMYIA SIMPLEX*; DIPTERA: AGROMYZIDAE) AND THE INFLUENCE OF TEMPERATURE ON ITS POPULATION DYANMICS

By

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ABSTRACT

INVESTIGATION OF THE TRITROPHIC INTERACTIONS OF THE ASPARAGUS MINER (*OPHIOMYIA SIMPLEX*; DIPTERA: AGROMYZIDAE) AND THE INFLUENCE OF TEMPERATURE ON ITS POPULATION DYANMICS

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Asparagus is globally in decline due partially to increased pest prevalence. One of the main pests is the asparagus miner, a stem-mining fly, that may spread pathogenic fungi (*Fusarium* spp.). Because conventional pesticides are not sufficient in controlling the asparagus miner, an integrated management program needs to be developed to aid asparagus growers. This dissertation evaluates three different aspects of plant-insect interactions in asparagus fields in order to lay the basis for an IPM program for the miner. These include 1) developing a degree-day model and elucidating the spatial distribution of the asparagus miner within a field so growers can accurately time and place insecticides to achieve increased levels of control with decreased ecological and monetary costs; 2) investigating the natural enemy community of the asparagus miner to lay a foundation for a conservation biological control program; and 3) exploring the chemical ecology of asparagus and its interactions with its arthropod community in order to probe the potential for the use of plant volatiles in IPM. For the first objective, sticky traps were deployed from 2010-2012 at multiple distances into 3-5 commercial fields, and the abundance of miner adults was counted weekly. In addition, the lower developmental threshold for the miner pupae was assessed through environmental chambers set to 10 different temperatures to inform the DD model. Asparagus miner adults were uniformly distributed throughout the field during their first generation, whereas they were primarily clustered around the edges of the field during the second generation. Adults were greatest on field edges bordered

by neighboring asparagus, while the lowest abundance was found on edges bordered by forests. The lower developmental threshold for the miner was 12.1°C, and DDs reliably predicted important phenological events in the life cycle of the asparagus miner when using a biofix date of March 1. These results indicate that the conservation of the remaining forested habitats may be beneficial for pest mitigation and that growers may concentrate their insecticide sprays around the edges of asparagus fields during the second generation of the asparagus miner. For the second objective, we sampled for asparagus miner pupal parasitoids from 2010-2013. There were 12 parasitic wasps that used the asparagus miner as a host. Of these, *Chorebus rondanii* and *Thinodytes cephalon* were good candidates for biological control. From rearing on artificial diets, sugar-rich resources increased both the lifespan of miner adults and its parasitoids. Rearing on various flowering plants significantly impacted the lifespan of asparagus miner adults, with buckwheat and fava bean being unfavorable resources. This research lays the groundwork for a biocontrol program. For the third objective, asparagus headspace was significantly altered both quantitatively and qualitatively, depending on damage. The main component in all headspace blends, regardless of treatment was 3E-hexenyl acetate. Asparagus miner adults significantly preferred healthy asparagus stem blends over purified air, and preferred asparagus miner-damaged blends in the presence of healthy asparagus. Finally, in the field, the asparagus miner and parasitoids were most attracted to methyl salicylate-containing baits, while other pests were most attracted to cis-3-hexen-1-ol. Consequently, these compounds should not be used in asparagus fields. Future evaluation of the volatiles identified from asparagus headspace in this study should help illuminate those with biological activity for use in an IPM program for the miner. Overall, the results from this study are expected to significantly contribute to the IPM of the asparagus miner, and give growers alternative tactics for suppressing this important pest.

To all the courageous, intelligent, enlightening and wonderful women in my life, you are a continuing inspiration to me

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CHAPTER 1

Constraints on asparagus production:

The association of *Ophiomyia simplex* (Diptera: Agromyzidae) and *Fusarium* spp.

Introduction

Asparagus (*Asparagus officinalis*) production has become increasingly constrained globally during the past several decades, because of problems with replanting asparagus fields, and the early onset of asparagus stand decline (Grogan and Kimble, 1959; Elmer et al., 1996). Grogan and Kimble (1959) defined asparagus decline as "a slow decline in the productivity of old asparagus plantings…to the point where the plantings become unprofitable to maintain." The authors furthermore defined the replant problem as, "the inability to establish productive plantings…where plantings have declined." Early decline of asparagus can lead to a reduction in the lifespan of planted fields by 5 to 8 years; farmers are not able to recoup the investment associated with establishing asparagus fields (Elmer et al., 1996).

The fungi *Fusarium subglutinans*, *Fusarium proliferatum* (population D, *Gibberella fujikuroi*; Elmer, 1995), and *Fusarium oxysporum* f.sp. *asparagi* have been directly linked as partial casual agents in the premature decline (Keulder, 1999), and in the inability of new plantings to become established and be productive in locations where asparagus was previously grown (Grogan and Kimble, 1959; Elmer et al., 1996), even decades after asparagus was last grown (Poll and Huiskamp, 1992). The asparagus miner, *Ophiomyia simplex* Loew (Diptera: Agromyzidae), acts as a putative vector for infection of asparagus plants with *Fusarium*. The larvae cause damage to the plant (Tuell and Hausbeck, 2008), exacerbating the early decline of asparagus fields (Gilbertson et al., 1985).

The purpose of this review is to examine and synthesize the current information about this tripartite interaction among asparagus, the *Fusarium* spp. associated with *Fusarium* crown and root rot (FCRR), and the asparagus miner. We examine potential methods to limit the asparagus miner and thereby reduce FCRR, and discuss future research areas, including the need for an integrated pest management approach.

Methods

This review is a synthesis of information regarding *Fusarium* crown and root rot and the asparagus miner. The Michigan State University library collection and online databases including Web of Science, ScienceDirect, SpringerLink, GoogleScholar and JSTOR were searched for terms that included but were not limited to "asparagus miner," "early decline," "*Fusarium* crown and root rot," and "replant problem." For the purpose of the figures and tallying, the term "experiments" refers to individual experiments within studies, and it is possible to have more than one experiment dealing with a similar subject within a study. A full meta-analysis of the data in the reviewed articles was not possible, because of wide variability in dependent variables and treatments. However, when there was sufficient replication among studies within a subject, nonparametic statistics (Mann-Whitney U tests) were used to evaluate differences in variables, since the assumptions of normality were not fulfilled.

Results

Biology, biogeography and pathogenicity of Fusarium spp. Since FCRR was first described in 1908, *Fusarium* spp. have undergone extensive taxonomic revision, having originally been described as *F. moniliforme* (Snyder and Toussoun, 1965; Proctor et al., 2010).

In 1983, *F. moniliforme* was taxonomically split into *F. proliferatum* and *F. subglutinans* (Nelson et al., 1983). On the other hand, *F. oxysporum* was originally identified as one of the causal agents of FCRR by Cohen and Heald (1941) and later grouped into *formae specialis* based on subsets of isolates that can infect specific host crops (Snyder and Hansen, 1940; Grogan and Kimble, 1959). However, *F. oxysporum* f. sp. *Asparagi* may also be pathogenic to other crops, such as celery and onion (Armstrong and Armstrong, 1969; Blok and Bollen, 1997; Elmer, 2001). Elmer (2001) has called the monophyletic status of *F. oxysporum* into question, and recent studies have shown that *F. oxysporum* is in fact polyphyletic and may not be a good biological species (Wong and Jeffries, 2006; for review, see Lievens et al., 2008).

Fusarium spp. are anamorphic (Gordon and Martyn, 1997) and nearly ubiquitous in both agricultural soils and native soils around the world (Hartung et al., 1990; Vujanovic et al., 2006). Both pathogenic and nonpathogenic strains of *Fusarium* spp. can be found in soils, even those that have not been planted to asparagus (Hartung et al., 1990). *Fusarium oxysporum* may infect young feeder roots, gaining entry at the junction where the feeder roots emerge (Graham, 1955; Smith and Peterson, 1983) between epidermal cells. Subsequently, the fungus moves into the cells, radiating intercellularly into the cortex of the root. Lesions that are small, red and elliptical develop on the feeder root tips and along the root (Shoemaker, 1965 c.f. Elmer, 2001), and may also be evident on the underground portion of the plant stem. Asparagus that is damaged or stressed from cultural practices or other means is more susceptible to FCRR (Nigh, 1990). Different species of *Fusarium* are found in varying regions in the world: for example, in the Netherlands, *F. culmorum* is associated with FCRR, while *F. proliferatum* and *F. oxysporum* are absent (Blok and Bollen, 1996). It is likely that F. *subglutinans* plays a minor role in the North American FCRR (Elmer et al., 1996), as it is less often isolated from asparagus plants

(Vujanovic et al., 2006). In the United States, the primary pathogens associated with FCRR are considered to be *F. proliferatum* (teleomorph *Gibberella fujikuroi*; Elmer, 1995) and *F. oxysporum* f. sp. *Asparagi*, which primarily infects the crown/stem region and roots, respectively (Van Bakel and Kerstens, 1970; Gordon and Martyn, 1997). *Fusarium oxysporum* is implicated in infecting and causing FCRR in the root system of asparagus (Van Bakel and Kerstens, 1970) although it has also been isolated from other parts of the asparagus plant (Tuell and Hausbeck, 2008). *Fusarium oxysporum* is thought to play a significant role in newly-planted fields, often hindering establishment of asparagus (Cohen and Heald, 1941; Graham, 1955; Endo and Burkholder, 1971), while *F. proliferatum* is thought to affect older fields and thereby plays a key role in the early decline of asparagus.

Symptoms, severity and cost of Fusarium crown and root rot. Fusarium crown and root rot symptoms of asparagus includes: wilting, dwarfing, chlorosis, browning of vascular tissue, death to the growing point, and damping off in seedlings (Eskelsen and Schreiber, 1997; USDA, 1999). The pathogen spreads basipetally toward the crown, often causing premature plant death. Symptoms of infection are usually observed during midsummer, and infection with *Fusarium* spp. can also result in the complete destruction of the feeder roots, and withering of the storage roots (Elmer et al., 1996). Damage from FCRR can be exacerbated by exposure to viral agents including AV-1, AV-2 and tobacco streak virus (Evans and Stephens, 1989; Knaflewski et al., 2008), asparagus allelopathic residues (Hartung and Stephens, 1983), environmental stress (Nigh, 1990), and insect damage (i.e. asparagus miner damage, which serves as entry sites for *Fusarium* infection; Damicone et al., 1987).

The development of FCRR in asparagus plantings has negative economic impacts that include the loss of the initial costs associated with establishing an asparagus planting, which is

estimated to be over $8,600 per ha (pers. communication, J. Bakker, 2010). Yields may be decreased 2.22-3.03 kg/ha per year when a planting is affected by FCRR (Reid et al., 2001). Currently, an asparagus field planted in Michigan is productive for approximately one half as much time as one that was planted in the 1950s, as a result of FCRR and other pathogens, such as *Phytophthora* spp. as well as autotoxicity from asparagus residues (pers. communication, J. Bakker and N. Myers, 2010).

The biology and role of the asparagus miner as a vector. The asparagus miner is a bivoltine organism (Ferro and Gilbertson, 1982; Lampert et al., 1984; Tuell, 2003), and its only known host is asparagus (Spencer, 1973). In the United States, the asparagus miner occurs wherever asparagus is grown, including the major asparagus producing regions of Washington (Eichmann, 1943), Michigan (Tuell, 2003), and California (Essig, 1913). The asparagus miner has also been recorded from other asparagus growing regions in the world, including central Hungary, France and Germany (Dingler, 1934). The asparagus miner was likely introduced to the United States from Europe (Dingler, 1934; Spencer, 1973) when asparagus was brought to the New World by French Huguenots (Schofield, 1946), despite being first recorded from Pennsylvania in 1869 by H. Loew.

Adults of the asparagus miner oviposit on the stem of the asparagus near the soil surface, and once the eggs hatch, the larvae start producing mines and shafts in the cortex of the asparagus stems (Barnes, 1937). The damage from the miner is considered to have a negligible effect on plant vigor (Dingler, 1934; Barnes, 1937; Eichmann, 1943). The asparagus miner typically has two generations per season, and asparagus plantings may exhibit nearly 80-100% mining incidence in certain years (Tuell, 2003). In 2010, Michigan experienced an unusually warm and extended growing season, exacerbating miner activity and associated crop damage,

with a stem sometimes infested by more than 6 asparagus miner pupae (pers. obs., R. Morrison). By contrast, the total number of pupae collected in a normal year (e.g. 2002) ranged from 3 to 6 per stem (Tuell, 2003) in the same area. These sites of damage present openings for pathogenic *Fusarium* spp. to gain access to the plant and cause FCRR (Ferro and Gilbertson, 1982).

In Michigan, the asparagus miner overwinters as pupae in plant debris and the soil, with the first generation emerging in May. The first generation spans from May until mid-June, during which time the adults mate and the females oviposit on the asparagus stem near the soil surface (Figure 1.1.). The second generation begins to emerge at the beginning of August and spans until around September (Ferro and Gilbertson, 1982; Lampert et al., 1984; Tuell and Hausbeck, 2008). Adult populations can be sampled with yellow sticky traps (Ferro and Suchak, 1980). Tuell (2003) sampled for the asparagus miner with a regime that involved canopy (1.5m finished height) and ground (0.4m high) traps with sticky traps deployed at each height. Canopy-level traps caught higher numbers of adults than ground level traps (Tuell, 2003).

Within the last several decades, damage from asparagus miner has become recognized as a factor in exacerbating FCRR, despite long being considered unimportant after it was first described (Dingler, 1934; Eichmann, 1943). Newly planted fields are quickly colonized by the asparagus miner (Tuell, 2003; Tuell and Hausbeck, 2008), with pathogenic *Fusarium* spp. occurring on the pupae, mines, and adults of *O. simplex* (Gilbertson et al., 1985; Tuell and Hausbeck, 2008), as well as larvae and larval frass (Ferro and Gilbertson, 1982). In older fields, over 25% and 20% of the pupae from the asparagus miner had evidence of spores from *F. proliferatum* and *F. oxysporum*, respectively, on their exterior. A total of 44% and 4% of stem tissue pieces from above-ground mines were colonized by *F. proliferatum* and *F. oxysporum*, respectively (Tuell and Hausbeck, 2008). Larval mining predisposes the upper stems of

asparagus to *Fusarium* infection, and the overwintering pupae serve as a form of inoculum of *Fusarium* spp. for the next growing season (Ferro and Gilbertson, 1982).

Increased incidence of the asparagus miner has been linked to increased severity in FCRR infection in asparagus (Damicone et al., 1987) and decreased yields. High populations of asparagus miner may exacerbate FCRR, resulting in decline of the asparagus yield until it is no longer profitable to harvest.

Managing Fusarium disease. Most studies have focused on *F. oxysporum* (Figure 1.2.). There have been many attempts to limit FCRR through cultural, fungicidal and biological control approaches, including i) using nonpathogenic *Fusarium* spp. (Reid et al., 2002), ii) salting with NaCl (Reid et al., 2001; Elmer, 2004), iii) using arbuscular mycorrhizae (Counts and Hausbeck, 2008), iv) incorporating asparagus root residues (Blok and Bollen, 1996), v) performing biological soil disinfestation (Blok et al., 2008), vi) using fungicides, including benomyl, thiophanate-methyl and fludioxonil (Counts and Hausbeck, 2008), vii) employing antibiotics from *Streptomyces griseus* (Smith et al., 1990), and viii) developing genetic resistance against *Fusarium* by gametophyte selection (Pontaroli and Camadro, 2001). These measures have exhibited varying levels of efficacy (Table 1.1).

Of the examined experiments, the vast majority of them target *F. oxysporum* (Figure 1.2.). There are very few studies that have specifically looked at *F. proliferatum*, the main agent currently implicated in the early decline of asparagus. This is likely due to the high degree of morphological similarity between *F. proliferatum* and *F. oxysporum* (Proctor et al., 2010). Advances in PCR-based (Yergeau et al., 2005) and genomics methods make it possible to correctly identify *F. proliferatum* and *F. oxysporum* by using calmodulin gene sequences (Mule et al., 2004).

The most extensively examined management practices include salting, and the use of nonpathogenic *Fusarium* spp. to compete with pathogenic *Fusarium* spp. Salting with NaCl is significantly better than employing nonpathogenic *Fusarium* species (Mann-Whitney U Test: $U=0$, $P<0.0199$). Salting used to be standard practice among asparagus growers to control weeds in the 19th and beginning of the 20th centuries (Elmer et al., 1996), but fell into disuse with the advent of modern herbicides to counter weeds. The long-term consequences from salting fields may be a change in soil pH, salinity and other parameters important for the yield of asparagus plantings (Hodupp, 1983). Reid et al. (2001) found that two annual applications of NaCl to a commercial production field in Michigan did not significantly affect levels of pH, potassium, magnesium or calcium, nor did it increase the salinity in the soil from 15 cm. However, NaCl applications did increase salinity in the soil to 5.4 mS/m in a deeper (15-30 cm) layer. Most studies where NaCl successfully reduced FCRR severity were conducted in greenhouses, growth chambers or small field plots. The field plot research was typically conducted in severely declined asparagus plantings of a small, non-commercial scale and may not represent larger commercial production fields. When commercial field trials with salt were conducted in Michigan, there was no increase in the yield of asparagus (Reid et al., 2001). In addition, NaCl exacerbates *Phytophthora* crown and root rot, which is recognized as an important pathogen of asparagus in Michigan (Saude et al., 2008) and California (Falloon et al., 1991). As a result of these combined factors, salting is not a recommended strategy to control *Fusarium* spp.

Another cultural method that has been investigated is biological soil disinfestation (Table 1.1; Blok et al., 2008), which has been used to manage *F. redolens*, with positive results. This process requires growers to dig up to 80 cm in the ground to deposit grass clippings and subsequently to cover the entire field in airtight plastic. Because this method has not been

attempted for other species of *Fusarium* and is labor intensive, more research is needed before any conclusive recommendations can be made.

Biological control has also been investigated as a means to manage FCRR. This has been carried out using nonpathogenic *Fusarium* spp (Blok and Bollen, 1996; Elmer, 2004; Counts and Hausbeck, 2008).

Much prior investment has been directed to developing *Fusarium* resistant asparagus cultivars (e.g. Stephens et al., 1989; Dan, 1994; Dan and Stephens, 1995; He et al., 2002; He and Wolyn, 2005). However, this has not yielded a viable commercial cultivar. A promising long-term approach to combat FCRR involves gametophyte selection of asparagus plants resistant to *Fusarium* spp (Pontaroli et al., 2000; Pontaroli and Camadro, 2001).

Select fungicides, fumigants, and an antibiotic significantly reduced FCRR, particularly thiophanate-methyl, metam-potassium, Telone C-35, and faeriefungin (Table 1.1). Fungicides and fumigants have not been widely used to reduce FCRR, but field trials are ongoing (Hausbeck and Cortright, 2008). Further development and testing of fungicidal compounds targeting *Fusarium* spp. would benefit growers.

Stress is an important factor in promoting FCRR in asparagus (Nigh, 1990). Sandy soils, which are predominant in many asparagus growing regions, especially in Michigan, are very porous and do not retain water, which may lead to water stress conditions for asparagus fields. Research into irrigation methods and timing to reduce environmental stress for asparagus could reduce FCRR severity. Asparagus growers rely on herbicides for weed control, especially in young plantings. Growers have become concerned with specific herbicides and their observed negative effects on asparagus fern growth. Greenhouse trials were conducted to evaluate the effect of select herbicides on asparagus growth, and the application of mesotrione resulted in a

reduction in crown weight. Field trials revealed that mesotrione can be phytoxic to asparagus and could result in decreased yields (Rodriguez-Salamanca, 2010). Future research should investigate the effect that certain management regimes (including herbicides) have on asparagus plant vigor and the progression of FCRR. Overall, an effective strategy for combating FCRR should include a multi-pronged approach that includes fungicides, continuing selection for resistance against *Fusarium* spp., and certain cultural techniques such as avoiding herbicides that are phytotoxic to asparagus and irrigation to avoid environmental stress in asparagus.

Table 1.1. Overview of different techniques used to control Fusarium crown and root rot.

Management Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
Salting							
NaCl	Foa[a]	10g/L of H_2O	Greenhouse	100mL applied one week after planting	Sig. red. In FCRR	39% decr. In root lesions, 16% incr. in root weight	(Elmer, 2008)
NaCl	Foa	1% NaCl	Greenhouse	Added 100mL after infection with Foa	Sig. and strong red. In FCRR	35% decr. In root lesions, ns incr. in root weight	(Elmer, 2004)
NaCl	Foa & Fp[b]	560-1,120kg/ha	Commercial field	Applied 3 times during April	ns	ns	(Reid et al, 2001)
NaCl	Foa & Fp	1,120kg/ha	Research field	Applied 3 times during April	No direct measure of FCRR	13% incr. in the no. of stalks >0.79cm	(Reid et al, 2001)
NaCl	Foa & Fp	0.32g/pot	Greenhouse	Cl equivalent to other treatments	Sig. red. In FCRR	15.3% decr. In root rot, 0.55g more fresh weight per plant	(Reid et al, 2001)
NaCl	Foa & Fp	17.1-34.2mM	Growth chamber	Cl equivalent to other treatments	Sig. and strong red. In FCRR	27.4% decr. In root lesions from Fp, and 33.1% decr. In root lesions from Foa	(Reid et al, 2001)
$CaCl_2$	Foa & Fp	17.1mM	Growth chamber	Cl equivalent to other treatments	Sig. and strong red. In FCRR	14.8% decr. In root lesions from Fp, and ns decr. In root lesions from Foa	(Reid et al, 2001)
$CaCl_2$	Foa & Fp	0.40g/pot	Greenhouse	Cl equivalent to other treatments	ns	ns	(Reid et al, 2001)

Table 1.1. (cont'd)

Manage-ment Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
NH_4Cl	Foa & Fp	34.2mM	Growth chamber	Cl equivalent to other treatments	Sig. and strong incr. in FCRR	11.6% incr. in root lesions from Fp, and 16.1% incr. in root lesions from Foa	(Reid et al, 2001)
NH_4Cl	Foa & Fp	0.29g/ pot	Greenhouse	Cl equivalent to other treatments	ns	ns	(Reid et al, 2001)
$MnCl_2$	Foa & Fp	8.55mM	Growth chamber	Cl equivalent to other treatments	ns	ns	(Reid et al, 2001)
$MnCl_2$	Foa & Fp	0.53g/ pot	Greenhouse	Cl equivalent to other treatments	ns	ns	(Reid et al, 2001)
Cultural Practices							
Biological Soil Dis-infestation	Fr[c]	62-102t/ha	Abandoned field	Grass added at 80cm soil depth and covered w/airtight plastic	Sig. and strong red.	28.4-45.8% decr. In FCRR	(Blok et al, 2008)
AM fungi addition	Foa	1.2mL/plant or 1.57g/L	Commercial field	Addition by crown dip or irrigation	ns	ns	(Counts and Hausbeck, 2008)
Root residue	Foa	20gkg^{-1}	Greenhouse	Sterilized asparagus root residues added	ns	ns	(Blok and Bollen, 1996)
Tricho-derma harzianum	Foa	0.02kg/ L or 55.6g/row-m	Commercial field	Addition by crown dip or irrigation	Sig. better stand count	9.5% better stand count	(Counts and Hausbeck, 2008)

Table 1.1. (cont'd)

Management Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
Formononetin	Foa	20mg/L H_2O	Greenhouse	Isoflavone that promotes AM fungi colonization, applied as 100mL drench once after planting	Sig. red. In FCRR	7% red. In root lesions	(Elmer, 2008)
Formononetin	Foa	20mg/L H_2O	Research field	Soaked for 20min in field	FCRR not directly measured	12% incr. in the 3yr total marketable spear weights	(Elmer, 2008)
Gametophyte selection	Foa	50-200 seeds/trt	Greenhouse	Controlled crosses exposed to 6% TCF	Sig. red. In FCRR	3.78-10.34% decr. In affected root area for certain crosses	(Pontaroli and Camadro, 2001)
Lime	Foa & Fp	6,719kg/ha	Commercial field	Applied in the spring	No direct measure of FCRR	ns	(Reid et al, 2001)
Nonpathogenic Fusarium							
Non-pathogenic *Fusarium* spp.	Foa	427kg/ha, or 397g/12 m	Commercial field	Addition by crown dip or irrigation	ns	ns	(Counts and Hausbeck 2008)
CWB 312	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	ns	ns	(Elmer, 2004)
CWB 314	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	ns	ns	(Elmer, 2004)

Table 1.1. (cont'd)

Management Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
CWB 314	Foa	$5x10^5$ spores/ml	Research field	Crowns dipped in 10L for 20min	ns	ns decr. In root lesions, ns incr. in stand counts, ns decr. In disease rating	(Elmer, 2004)
CWB 318	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	ns	ns	(Elmer, 2004)
CWB 318	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	Sig. red. In FCRR	10% red. In root lesions	(Elmer, 2004)
CWB 318	Foa	10^4 spores/ml	Research field	Soaked for 20min in field	FCRR not directly measured	36% incr. in the 3yr total marketable spear weights	(Elmer, 2004)
CWB 318	Foa	$5x10^5$ spores/ml	Research field	Crowns dipped in 10L for 20min	Sig. decrease in FCRR	ns decr. In root lesions, ns incr. in stand counts, 13.8% decr. In disease rating	(Elmer, 2004)
CS-20	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	Sig. increase in root weight	27.3% greater root weight w/ NaCl, 16.7% greater root weight w/o NaCl	(Elmer, 2004)
CS-20	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	Sig. red. In FCRR	8% red. In root lesions	(Elmer, 2008)
CS-20	Foa	10^4 spores/ml	Research field	Soaked for 20min in field	FCRR not directly measured	12% incr. in the 3yr total marketable spear weights	(Elmer, 2008)

Table 1.1. (cont'd)

Manage-ment Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
CS-20	Foa	$5x10^5$ spores/ml	Research field	Crowns dipped in 10L for 20min	Sig. decrease in FCRR	ns decr. In root lesions, ns incr. in stand counts, 15.4% decr. In disease rating	(Elmer, 2004)
FO47	Foa	10^4 spores/ml	Greenhouse	Roots of plants soaked in 500mL	ns	ns	(Elmer, 2004)
Cultivars							
Mary Washington	Fma & Foa	N=288 or N=144	Research field	Non-resistant cultivar	ns	ns	(Damicone et al, 1987)
Fungicides							
benomyl	Foa	1.20g/L or 5.67g/30.5 row-m	Commercial field	Fungicide addition by crown drip or irrigation trial	ns	ns	(Counts and Hausbeck, 2008)
thiophanate-methyl	Foa	1.20g/L	Commercial field	Fungicide addition by crown drip	Sig. better stand condition	25.6% better stand count	(Counts and Hausbeck, 2008)
Fludioxonil	Foa	0.6g/L, or 76.5kg/ha	Commercial field	Fungicide addition by crown drip or irrigation trial	ns	ns	(Counts and Hausbeck, 2008)
Metam-Potassium	Foa & Fp	227.1L/A	Research field	Shank applied at 25-30cm depth once	Sig. red. In FCRR	Reduced Foa and Fp by 1520 CFU/gram of soil	(Hausbeck et al, 2006)

Table 1.1. (cont'd)

Manage-ment Strategy	Target	Amount	Type	Description	Efficacy against FCRR	Change in Condition	Citations
Telone C-35	Foa & Fp	132.5L/A	Research field	Shank applied at 25-30cm depth once	Sig. red. In FCRR	Reduced Foa and Fp by 1620 CFU/gram of soil	(Hausbeck et al, 2006)
Cannonball 50WP	Foa & Fp	14.2g/37 8.5L	Commerical Field	Crown soak	Sig. incr. in vigor	18.5 more ferns per 20ft. & 3.3cm grtr. Height	(Hausbeck and Cortright, 2006)
Antibiotics							
Faeriefungin	Foa	12.5-50ppm	Growth chamber	from *Streptomyces griseus*	Sig. and strong red. In FCRR	36mm incr. in asparagus shoot length,133% incr. in dry fibrous root weight	(Smith et al, 1990)
Insecticides							
Vorlex	Fma & Foa	363L/ha	Research field	Fumigated in fall	ns	ns	(Damicone et al, 1987)
Diazinon	Fma & Foa	0.63kg/ha	Research field	Sprayed when warranted by insect activity	Sig. and strong red. In FCRR and mines	decr. In External & Internal stem rot index and mines from O. simplex, & incr. yield	(Damicone et al, 1987)
Herbicides							
Simazine	Fma & Foa	6.20kg/ha	Research field	Sprayed beginning of season	ns	ns	(Damicone et al, 1987)

[a] *Fusarium oxysporum f.sp. asparagi*

[b] *Fusarium proliferatum*

Table 1.1. (cont'd)

[c] *Fusarium redolens*

Figure 1.1. Life cycle of the asparagus miner based on previously published research. Drawn by Marlene Cameron, MSU.

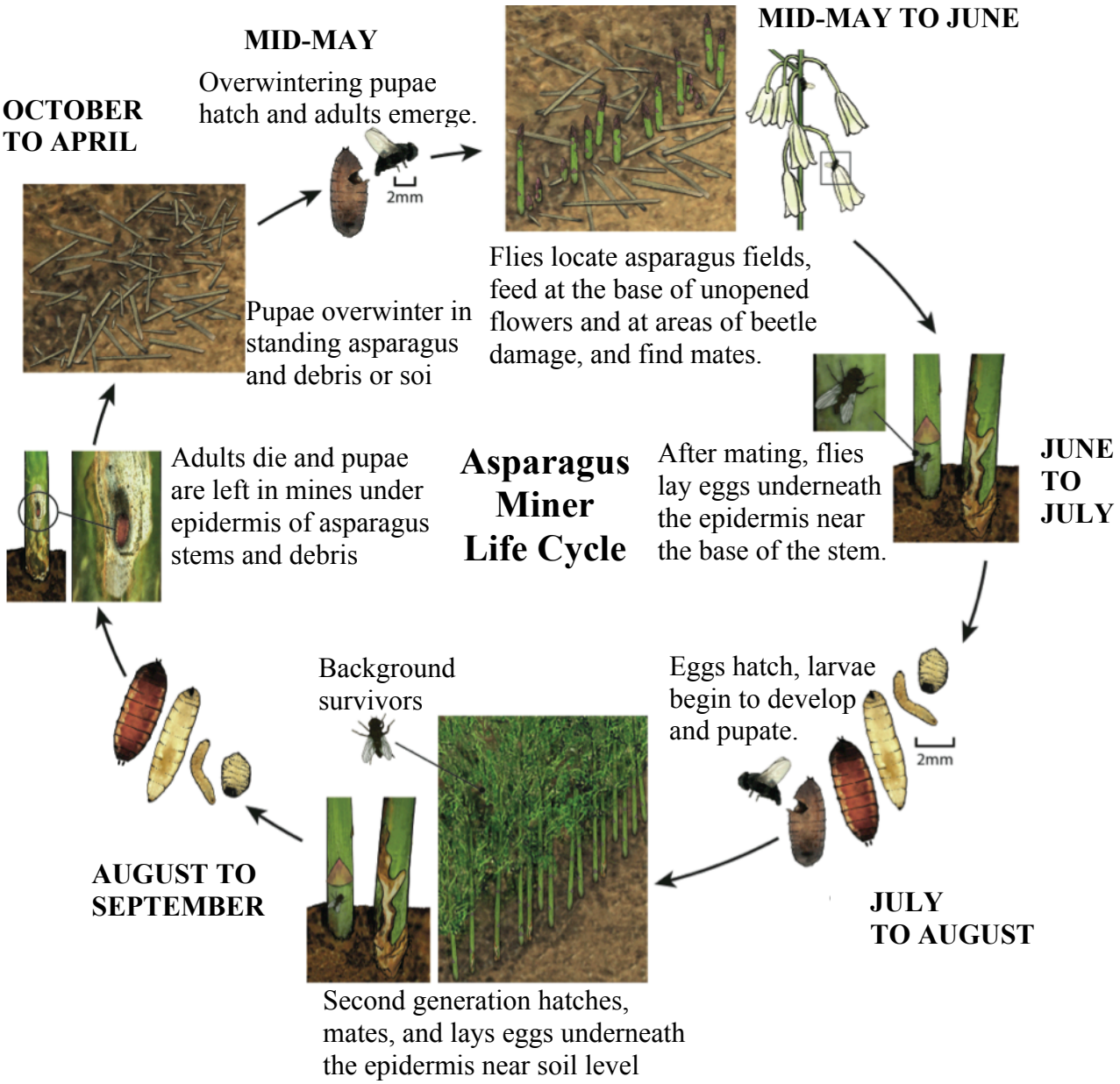
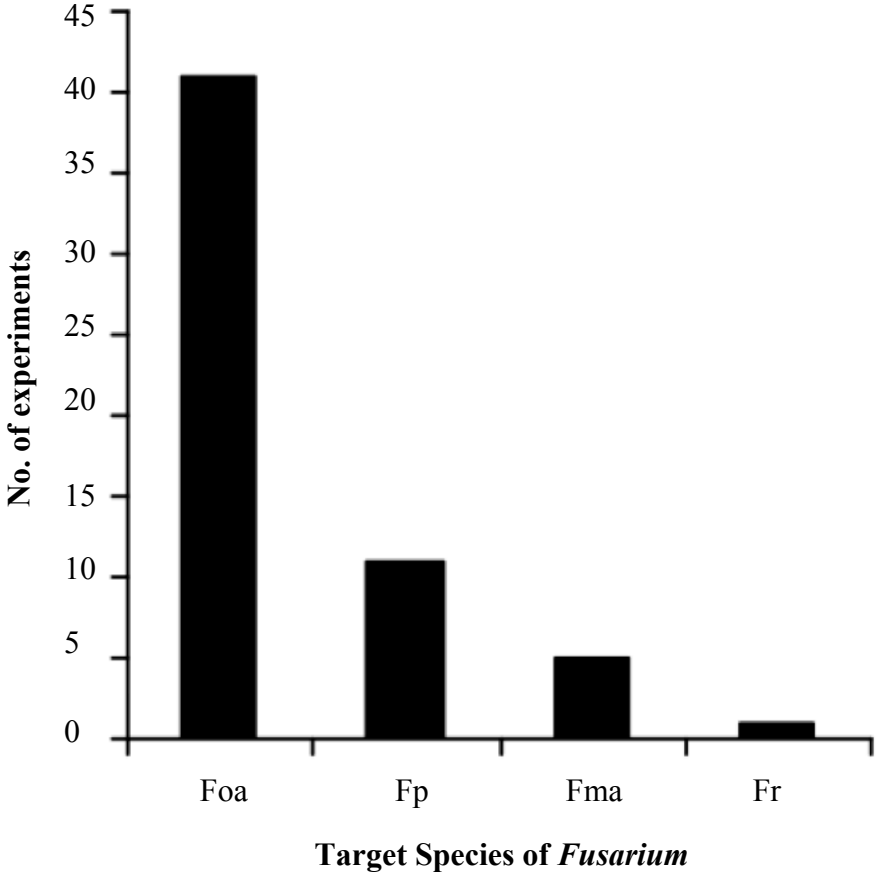


Figure 1.2. Number of experiments in managing *Fusarium* species reviewed in this dissertation. Abbreviations: Foa – *F. oxysporum* f.sp. *asparagi*, Fp – *F. proliferatum*, Fma – *F. monoliforme*, Fr – *F. redolens*.



Controlling asparagus miner populations. The asparagus miner remains an understudied species, especially considering its role as a putative vector in the spread of FCRR in asparagus fields. Repeated applications of the insecticide diazinon after the harvesting season reduced asparagus miner incidence, severity of FCRR and increased yield (Damicone et al., 1987). An action threshold has not been established for timing insecticide applications that target the asparagus miner, presenting added difficulty. Although a sampling regime for the asparagus miner that uses canopy-level sticky traps has been shown to help (Tuell, 2003), sticky traps are not species-specific, and can therefore be time-consuming to process. A sampling regime that uses fewer traps, or a trap with a species-specific lure may increase the efficiency of monitoring asparagus miner populations.

It is important to utilize integrated pest management strategies to reduce reliance on insecticides and to manage populations of asparagus miner. One method is to incorporate biological control into a management regime for the asparagus miner, but there has only been limited research on the parasitoids of the species. In the United Kingdom, Giard (1904 c.f. Barnes, 1937)) described a parasitoid, *Dacnusa rondanii* (Hymenoptera: Braconidae) on asparagus miner. About three decades later, also in the United Kingdom, Barnes (1937) described three additional hymenopteran parasitoids of the asparagus miner: *Pediobius epigonus* (Eulophidae; formerly *Pleurotropis epigonus*; Spencer, 1973), *Sphegigaster* sp (Pteromalidae) and misidentified *Chorebus rondanii* (Braconidae) as *Dacnusa bathyzona* (Griffiths, 1967). However, none of these biological control agents were evaluated for their efficacy, nor have any parasitoids been described in the United States. This aspect of research provides potential for the future management of both the asparagus miner and the associated FCRR.

Semiochemicals are chemicals emitted from plants or insects that may be used as a tool in an IPM program to manage the asparagus miner. To our knowledge, there have been no studies on the response of the asparagus miner to the volatiles of asparagus. If compounds that are attractive to the asparagus miner are identified, these could potentially be used for baits in traps, making population sampling more precise and effective.

Research is also needed on the identification of source populations of asparagus miner in new plantings of asparagus. It is not known where asparagus miner populations originate, and if they use alternative hosts; it is assumed that they come from volunteer asparagus plants (Personal communication, Myers, 2010). The types of habitats or vegetation outside production fields that harbor the asparagus miner should be identified and methods pursued to suppress immigrating populations.

Conclusions and future research

There are over 250,000 ha of asparagus globally (Benson, 2009), representing a major investment of economic resources. Fusarium crown and root rot is a significant barrier to increased productivity. Moreover, FCRR is a difficult disease to manage and therefore efforts to date have focused on exclusion of the pathogen via soil fumigation of seedling nurseries, crown fungicidal soaks, and cultural strategies, including a neutral pH of the soil, no tillage and other horticultural techniques (e.g. no over-picking) to enhance plant vigor. Due to the link between FCRR and the asparagus miner, it is necessary to address each. An integrated pest management strategy is needed to address the following: 1) the role of semiochemicals in attracting asparagus miner to asparagus, and the pheromones driving mating behavior, 2) identifying habitats, which act as reservoirs of asparagus miner for newly planted fields, 3) identifying and increasing the

efficacy of natural enemies of the asparagus miner, 4) developing an economic threshold for pesticide application to guide management of the asparagus miner, 5) alleviating human-induced plant damage (e.g. the impact of specific herbicides weakening the asparagus crown and making it more vulnerable), 6) using cultural techniques to reduce natural stresses to asparagus, and 7) delivering effective pesticides and fungicides via drip irrigation to the root zone. A program that results from research advances will manage asparagus miner and FCRR in a cost-effective manner with minimal impact on ecosystems.

Acknowledgement of Prior Publication

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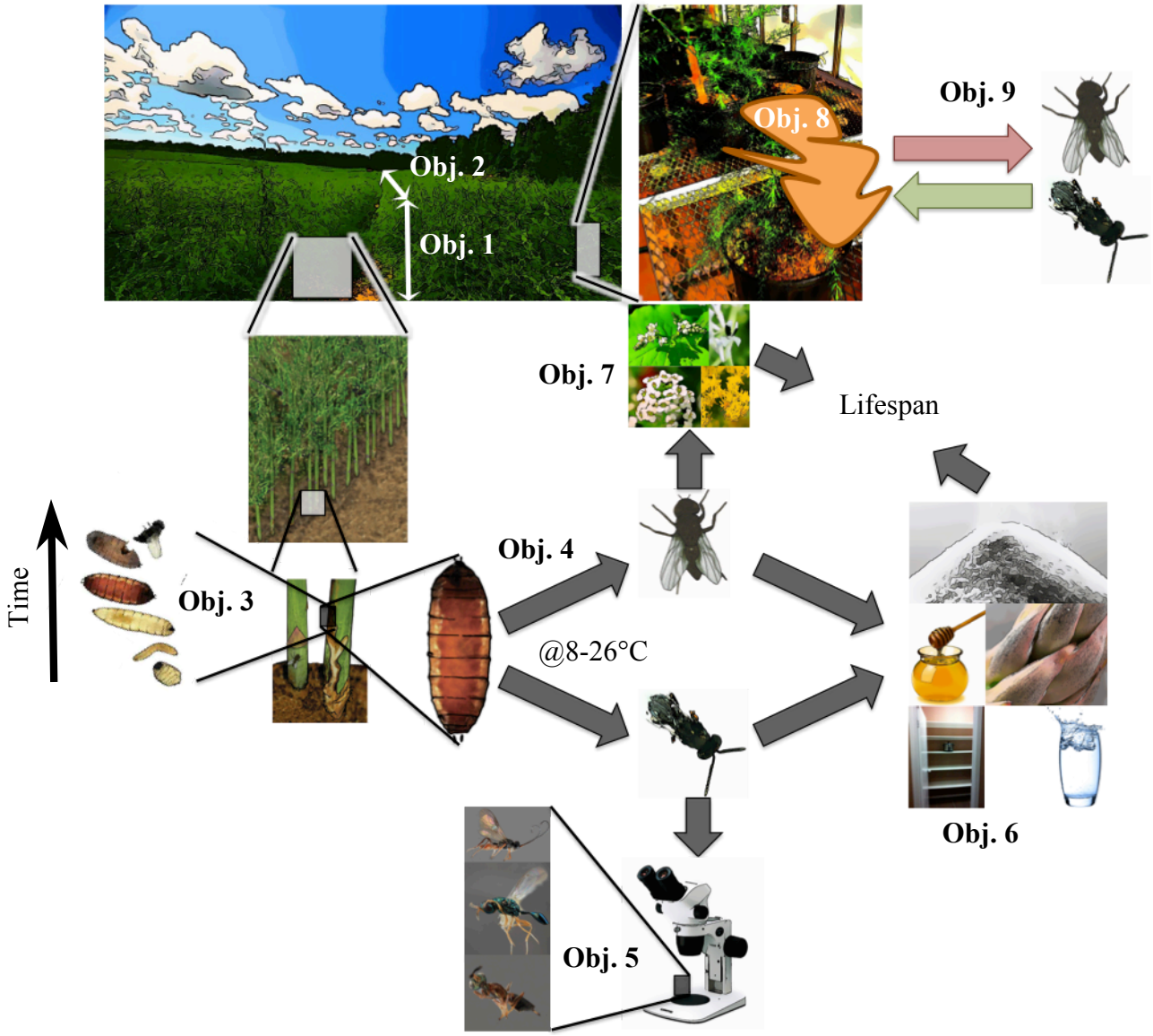
Table 1.2. Enumeration of research objectives in dissertation.

Objective No.	Objective Description
1	Vertical distribution of the asparagus miner
2	Horizontal distribution of the asparagus miner
3	Degree-day model for the phenological events in the life cycle of the miner
4	Lower developmental threshold for the asparagus miner
5	Identity and abundance of pupal parasitoids for the miner
6	Life span of the asparagus miner and its parasitoids on artificial diets
7	Life span of the asparagus miner on various floral resources
8	Damage's effects on the volatile emissions of asparagus
9	Effect of volatiles on the behavior of the asparagus miner and its natural enemies in the field and lab.

Figure 1.3. Overview of research objectives. Objective definitions can be found in table 1.2.



Thesis objectives

Ultimately, the goals of this dissertation involved investigating applied and basic questions regarding asparagus and its arthropods to contribute to an integrated pest management program for the asparagus miner. The first project of this dissertation elucidated the spatial distribution of the asparagus miner, both vertically and horizontally in time, to fine-tune management strategies for asparagus miner (Objectives 1 & 2; Figure 1.3.; Table 1.2.). Part of this objective involved developing a degree-day model to develop accurate predictions for important phenological events in the life cycle of the asparagus miner, both for adults and immatures (Objective 3). The second project's aim was to lay the foundation for a conservation biological control program for the asparagus miner, which involved describing the identity and abundance of its pupal parasitoids (Objective 5), evaluating other potential predators in the system, and understanding how artificial and flower diets impact the life span of both the asparagus miner and its parasitoids (Objective 6 & 7). Finally, the third project involved examining the volatiles emitted by asparagus plants exposed to various kinds of damage (Objective 8), how these affect the asparagus miner in the lab, and how the use of specific volatiles in the headspace may affect the attraction of the asparagus miner, its natural enemies, and other pests in the field (Objective 9).

CHAPTER 2

Patterns of spatial and temporal distribution of the

asparagus miner (Diptera: Agromyzidae): Implications for management

Introduction

The asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) is an obligatory asparagus (*Asparagus officinalis* L.) feeder and was likely introduced when asparagus was first brought to the USA from Europe (Barnes 1937). It is a cosmopolitan species, commonly found in asparagus growing regions of the world (Giard 1904, Fink 1913, Dingler 1934, Barnes 1937). It overwinters as a pupa and adults appear in the field in Michigan during May (Lampert et al. 1984). During spring, asparagus spears are picked regularly, resulting in no canopy, but after harvest, asparagus plants branch out to form a canopy, often referred to as fern. Throughout the growing season eggs are deposited on the asparagus stems near the soil-air interface (Ferro and Gilbertson 1982). Once larvae hatch, they burrow into the stem underneath the epidermis to feed, and create tunnels. In asparagus, a portion of the photosynthesis occurs in the green stems (Downton and Törökfalvy 1975) and the mines destroy tissue that is essential for the development and survival of the plant. Mines and oviposition scars from the asparagus miner create infection sites for *Fusarium* spp., and stem rot from the fungus linearly increases with the number of mines (Damicone et al. 1987). Furthermore, *Fusarium* has been associated with all the life stages of the asparagus miner (Tuell and Hausbeck 2008), and has been linked to increasing inoculum and disease incidence in the field (Gilbertson et al. 1985). The disease has been implicated in the early decline of asparagus (Grogan and Kimble 1959) and in reducing asparagus yields over the past 30 years (Elmer et al. 1996). Although there has been extensive

research into controlling the fungus (e.g. Stephens et al. 1989, Pontaroli and Camadro 2001, Reid et al. 2001, Reid et al. 2002, Counts and Hausbeck 2008), research on the biology and management of the insect vector has been lacking (Morrison et al. 2011).

Distribution of asparagus miner adults and the patterns of larval damage in asparagus fields are poorly understood. Previous studies have demonstrated that adult asparagus miners are more abundant in the canopy than near the ground, and prefer recently planted fields to older ones (Tuell 2003). Early in the season, most miner pupae occur about 3 cm below the soil surface, but as the miners prepare to overwinter, most pupae can be found between 5-7 cm belowground in the stem (Lampert et al. 1984). However, knowledge of asparagus miner distribution at the field and between-field scale is lacking, which our study aimed to address.

Spatial dynamics have implications for effective pest management in the field. For example, if pests primarily occur in a particular area of the field, management methods such as insecticides can be targeted to that location to reduce pesticide use and harmful environmental effects (Ferguson et al. 2003). Some pest flies, such as the papaya fruit fly, have a greater abundance near the edge of orchards than further into the field (Aluja et al. 1997). However, the distribution of other insects, such as the apple maggot fly, changes over the course of the season, ranging from being aggregated, to randomly and finally to evenly distributed in hawthorn trees (Averill and Prokopy 1989). The asparagus miner is a strong flier (Ferro and Gilbertson 1982), which may allow for directed dispersal, aggregated distribution in the field and active host-finding, which is dissimilar from weakly flying insects that are often wind-dispersed such as aphids (Pasek 1988). Information on the spatial distribution of the asparagus miner in the field, both horizontally and vertically, may provide pest managers much needed precision for their management actions, reducing the overall usage of potentially harmful chemicals.

Currently, asparagus growers do not have a targeted management plan for the asparagus miner (Morrison et al. 2011). The organophosphate, diazinon, has been shown to reduce the abundance of asparagus miner adults in the field, as well as stem rot from *Fusarium* (Damicone et al. 1987). However, the EPA is reevaluating and phasing out many organophosphates (USEPA 2012). The asparagus insect control program for growers consists of multiple broad-spectrum insecticides applied at various times in the season. In total, there were 24.9 metric tons of broad-spectrum insecticides used on asparagus in the United States in 2010 (USDA-NASS 2011), and their use has been associated with harmful effects on beneficial insects in other perennial systems (Epstein et al. 2000). Therefore, there is a need to develop a sustainable asparagus miner management strategy that decreases the reliance on broad-spectrum insecticides and increases the use of appropriately timed, environmentally sound, economically feasible, and socially acceptable management alternatives. Because the immature life stages of the asparagus miner remain protected within the stem of asparagus and are impervious to registered pesticides, we focused our study on asparagus miner adults since this life stage is susceptible to currently available insecticides. Reducing adults will ultimately decrease immature abundance in the field and the damage associated with that life stage. The aim of our study was to evaluate the field-level spatial and temporal distribution of adult asparagus miner as well as its damage in commercial fields.

Materials and Methods

Study Site. The five commercial asparagus fields used in this study in 2011 and three fields in 2012 were located 1.21-16.14 km apart in Oceana County, MI (Table 2.1.). The asparagus fields were bordered with some combination of the following types of habitats: mixed

coniferous-deciduous forest patches, grassland, other asparagus fields or some type of anthropogenic modification, such as a road or building. This fragmented habitat is typical of this part of Michigan, and is representative of the asparagus-producing region of the state.

Adult asparagus miner sampling. Asparagus miner adult abundance was measured with yellow sticky traps (7.5 × 12.3 cm, Great Lakes IPM, Vestaburg, MI). Thirty-two sticky traps were set up per field (N=160 for all fields combined), and these were checked and changed every 6-10 days. Asparagus miners were counted on traps and their numbers recorded. Sampling occurred from the beginning of asparagus emergence on 11 May to end of adult flight on 3 October 2011 and 22 March to 7 October 2012. The traps were arranged in transects, with sampling points at 0, 10, 20 and 30 m into the asparagus field from the field edge. Three sets of transects were placed 10 m apart in each field. Additionally, there were two traps placed 10 m from the field edge outside the field in border habitats. At each sampling point, there was a canopy trap, placed on a 1 m long steel conduit, paired with a ground-level trap (10 cm above ground), except during asparagus harvest when only ground traps were used for practical reasons. This experimental design was intended to assess vertical and the horizontal distribution of the asparagus miner.

In addition, in order to approximate how neighboring habitat affects asparagus miner abundance, the type of habitat on each border of the field was assigned to one of four types, including: forest, grass, agricultural land (agricultural), or interspace between asparagus fields (asparagus). Forest included either deciduous forest or coniferous forest, while agricultural land consisted of other crop types (rye or cherry orchard) or landscapes that have been modified by humans (e.g. barns, buildings, roads, etc.). These categories were noted for each yellow sticky

trap on the field edge and were later used in the analysis of asparagus miner distribution as described in the statistical analysis section.

In 2012, sampling for adults was performed in a cherry orchard located next to an asparagus field to evaluate abundance of the asparagus miner in areas where they may be using alternative resources. Ground-level yellow sticky traps were placed in three transects spaced 16 m apart, with traps located in the cherry orchard at 9.3 m, 21.8 m, and 34.3 m away from the adjacent asparagus field. Similarly to above, traps were changed weekly from 26 April to 7 October 2012 and the abundance of asparagus miner adults were recorded.

Damage data collection. In 2012, two measures were taken to quantify the damage in asparagus fields. The percent damage (intensity of damage) within 5 cm of the base of an asparagus stem was recorded for 10 stems at 0, 10, 20, and 30 m points into the field, offset from the sticky traps by 5 m to avoid confounding effects near the traps. Percent damage was estimated visually by the area of the epidermis that exhibited mining damage around the circumference of asparagus stem. This was done for the three transects set up for sampling the abundance, each separated by 10 m. The first measurement for damage was taken two weeks after the asparagus harvest ended in a field and the plants went to fern (Range: 159-172 Julian days). Thereafter, sampling for damage happened every 2-3 weeks in the growing season at three time points. Secondly, the proportion of stems damaged (out of the 10 sampled at each point above) was noted to quantify the extent of damage in the field.

Statistical analysis. A repeated measures analysis of variance (ANOVA) with first order autoregressive correlation among the time points was used to evaluate the fit of the abundance of adult asparagus miners' horizontal and vertical distribution within and outside the asparagus field. This was done with the Julian date as the time parameter and sticky trap at a specific height in a

given field and at a certain distance into the field as the repeated subject. Asparagus miner adult abundance was used as the dependent variable and trap height, distance of trap into field, and year of data collection were used as independent variables in the linear mixed model with a generalized least squares function (R Development Core Team, 2012). The field was used as a random variable. Two additional random variables were the error terms, which was an interaction among field, distance and transect, and an interaction among field, distance, transect number, height of trap, and year. The autoregressive model was the best fit to the data when comparing AIC and BIC criteria among similar models with different correlation structures. A Kenward-Rogers correction was used to calculate the degrees of freedom to avoid its artificial inflation due to the repeated measures. The residuals were analyzed to evaluate assumptions of normality and the data were log-transformed to meet assumptions of homogeneity of variances and normal distribution. Results were considered to be significant at $\alpha=0.05$ for this analysis and all subsequent ones.

An additional repeated measures ANOVA with the same structure as above was used to assess how the abundance of asparagus miners was affected by habitat type on the edges of the fields. Abundance of asparagus miner adults was the dependent variable, while height of trap, habitat type and year were independent variables. A separate ANOVA was required because of different sampling points not included in the above analysis outside of the field and unequal replication between treatments. The two error terms for this model (both random variables) were an interaction among direction (of field edge), field and habitat, and an interaction among height (of traps), direction, field, habitat, and year. Post-hoc Tukey's HSD test was used to assess pairwise comparisons between asparagus miner abundance at different distances into the field, pairwise differences between habitat types on the field edge in adult abundance, and was also

used to evaluate pairwise comparisons between different distances into the cherry orchard for asparagus miner abundance.

A final repeated measures ANOVA was performed to assess overall differences in percent mining damage (intensity of mining per stem) at different distances into the field, since this was a separate dataset from the abundance data and did not contain the same sampling points. The percent mining damage was used as a dependent variable and the distance into the field as the independent variable, with a first-order autoregressive correlation structure. There were two random variables: field and an interaction among field, distance and transect number (error term). Residuals were inspected for normality assumptions, which were not fulfilled. The data were inverse transformed to meet normality assumptions, and pairwise comparisons between mining damage at different distances into the field was assessed with Tukey's HSD. A different measure of damage, the proportion of stems damaged (extent of mining damage) out of 30 stems sampled per point at the different distances into the field was analyzed using a Pearson's Chi-square test and Monte Carlo simulations with 10,000 replicates to obtain a P-value. The data were pooled between the three fields for overall frequencies of damage and comparisons were made to the null hypothesis that damage was equally distributed at the edge, 10, 20 and 30 m into the field.

Results

Temporal and spatial within-field distribution of adults. Adult miner abundance was significantly different in the two sampling years (repeated measures ANOVA, year: $F = 38.96$; df = 1, 152; $P < 0.01$). Specifically, there was an almost 3-fold greater abundance of adult asparagus miners caught in 2011 compared with 2012. As a result, all further analyses were done separately for 2011 and 2012 for the adults.

Overall, there were two generations in a year for adults in 2011 and 2012 (Figure 2.1.F, J). Despite increased broad-spectrum insecticide sprays in certain fields (Figure 2.1. A, C, E, G, I), there was a greater abundance of asparagus miner adults in these fields compared with ones that received fewer sprays (Figure 2.1. B, D, H). Regardless of whether asparagus harvesting stopped during (Figure 2.1. A, C, G, I) or after the first adult generation's peak (Figure 2.1. B, D, E, H), this did not seem to influence the overall abundance of adults.

Asparagus miners were more evenly distributed in fields in the beginning of the season (Figure 2.2.). On the other hand, in the latter half of the season, adults were found primarily on the edges and outside the field. This pattern was true in 2011 for the first generation where there were no significant differences across different distances (repeated measures ANOVA, distance into field: $F = 0.22$; $df = 4, 16.4$; $P = 0.65$) and the second generation which showed a greater abundance near the edge of the field ($F = 5.73$; $df = 4, 16.4$; $P<0.01$). The case was also similar in 2012 for both generations (repeated measures ANOVA, first generation, distance into field: $F = 3.73$; $df = 4, 9.95$; $P = 0.05$; second generation: $F = 5.83$; $df = 4, 19.8$; $P<0.01$). Overall for both years, adult asparagus miners occurred in greater frequency on the edges and outside the field rather than within the asparagus fields (distances in 2011: $F = 2.69$; $df = 4, 36$; $P<0.05$; 2012: $F = 3.41$; $df = 1, 20.5$; $P<0.03$; Figure 2.3.). On average, there were 8.1 ± 0.67 flies per yellow sticky trap at the edge or outside the field in 2011 compared with 3.2 ± 0.23 flies inside the field. In 2012, on average there was an almost two-fold greater number of adults on the edges (2.70 ± 0.28 flies per yellow sticky trap) compared with adults within the field (1.45 ± 0.10). In addition, 2-fold greater abundance of asparagus miners were caught in canopy compared with ground-level traps in both years (2011: $F = 9.73$; $df = 1, 36.6$; $P<0.01$; 2012: $F = 45.9$; $df = 1, 22.3$; $P<0.01$; Figure 2.3.).

Asparagus miner damage. Asparagus miner damage was greater near the edge than further inside the field in 2012 (Figure 2.3.). Distance into the field significantly affected the percent mining damage found on stems ($F = 19.63$; $df = 3, 6$; $P<0.01$), as well as the proportion of stems damaged (χ^2-test: $df = 3$; $\chi^2 = 59.82$; $P=0.02$). About 40% of the total damaged stems were found along the edge of the field, compared with about 16% at locations 30 m into the field. Around 15% of the total damage occurred during the first generation of adults, while 85% occurred during the second generation.

Asparagus miner adults in field-border habitats. There was a significant effect of habitat type on asparagus miner abundance for 2011 (repeated measures ANOVA, habitat type: $F = 50.58$; $df = 3, 30.3$; $P<0.01$) and for 2012 (habitat type: $F =$; $df = 3, 12.6$; $P<0.01$; Figure 2.4.). In each year, the traps located between two asparagus fields had the greatest abundance of asparagus miner adults, followed by agricultural areas. Traps in forests around asparagus fields had the fewest asparagus miners both years, with about 15 times and 5 times fewer adults than the traps in between two asparagus fields in 2011 and 2012, respectively.

Asparagus miners were significantly more abundant on traps located near the edge of the cherry orchard closest to the asparagus field compared with traps further away from the asparagus field (Tukey's HSD; Figure 2.5.). On average, there were about 3 times as many asparagus miners on traps located 9.3 m as 34.3 m away from the asparagus field. The total number of asparagus miners over the season in the cherry orchard was 338 adults, 61 of which were found 35 m away from the asparagus field. Overall, adult miners averaged 0.91 ± 0.16 flies per sticky trap (Figure 2.5.).

Table 2.1. Summary information of asparagus field sites located in Oceana Co., MI.

ID	Area (ha)	Year Planted	Variety	Sampled	Edge Habitat
Field 1	6.06	2009	Millennium	2011, 2012	As[a], As, Ag, Ag
Field 2	3.77	2009	Millennium	2011, 2012	Ag, As, F, F
Field 3	3.54	2009	Millennium	2011, 2012	F, F, F, G
Field 4	1.82	2008	Millennium	2011	F, F, G, G
Field 5	3.66	2010	Millennium	2011	Ag, Ag, Ag, Ag

[a]Abbreviations: F – forest, G – grassland, Ag – agricultural, As – interspaced between asparagus fields

Figure 2.1. Variation in mean ± SEM asparagus miner abundance by field per yellow sticky trap for 2011 (A-F) and 2012 (G-J) for field 1 (A, G), Field 2 (B, H), Field 3 (C, I), Field 4 (D), Field 5 (E), and overall (F, J). Dashed lines indicate the date at which asparagus harvesting stopped, and arrows indicate insecticide applications. Some graphs are not present because certain fields were only sampled in 2011. The overall graph shows average abundance combined over the fields for a year. The horizontal bars underneath each graph show the first generation (black solid line) and whether the second generation was full (solid grey line) or reduced (dotted grey line) compared to the first generation.

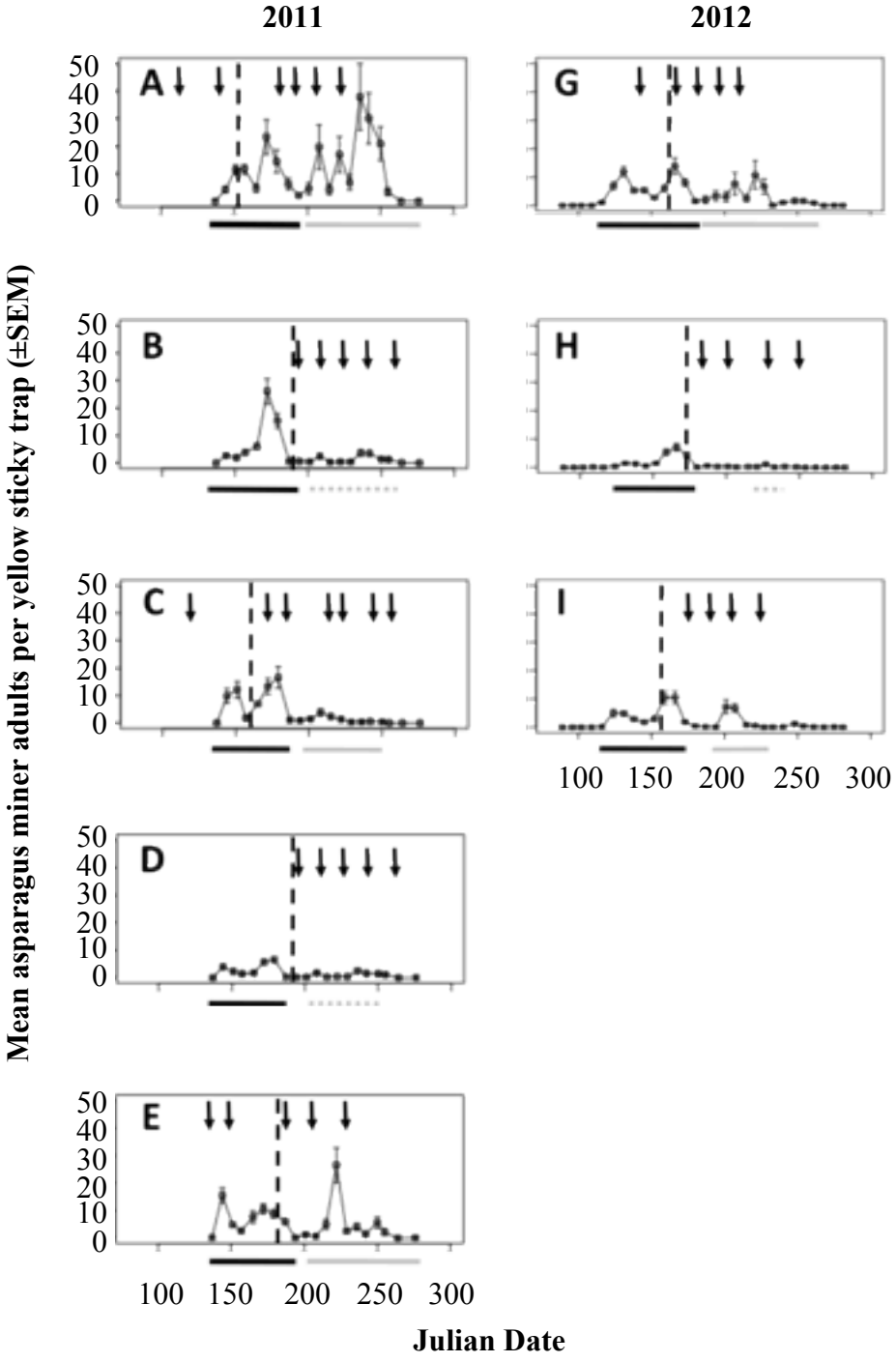


Figure 2.1. (cont'd)

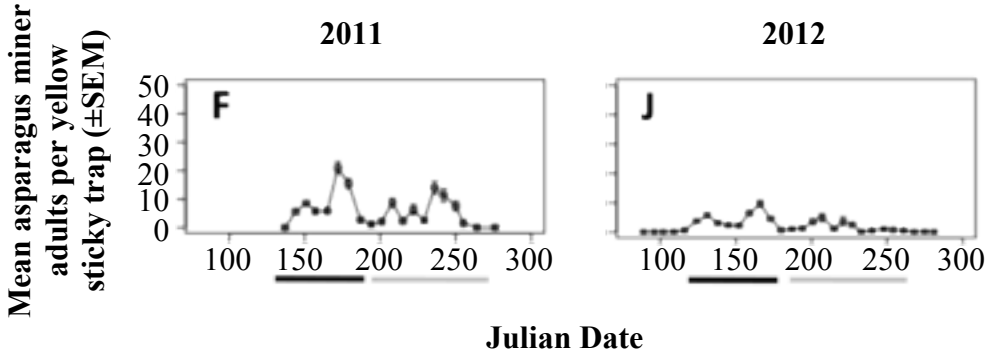


Figure 2.2. Mean distribution of adult asparagus miners over all sampled fields by Julian date at different distances into commercial asparagus fields in Oceana Co., MI for A) 2011 and B) 2012. Darker shades indicate a greater abundance of asparagus miners.

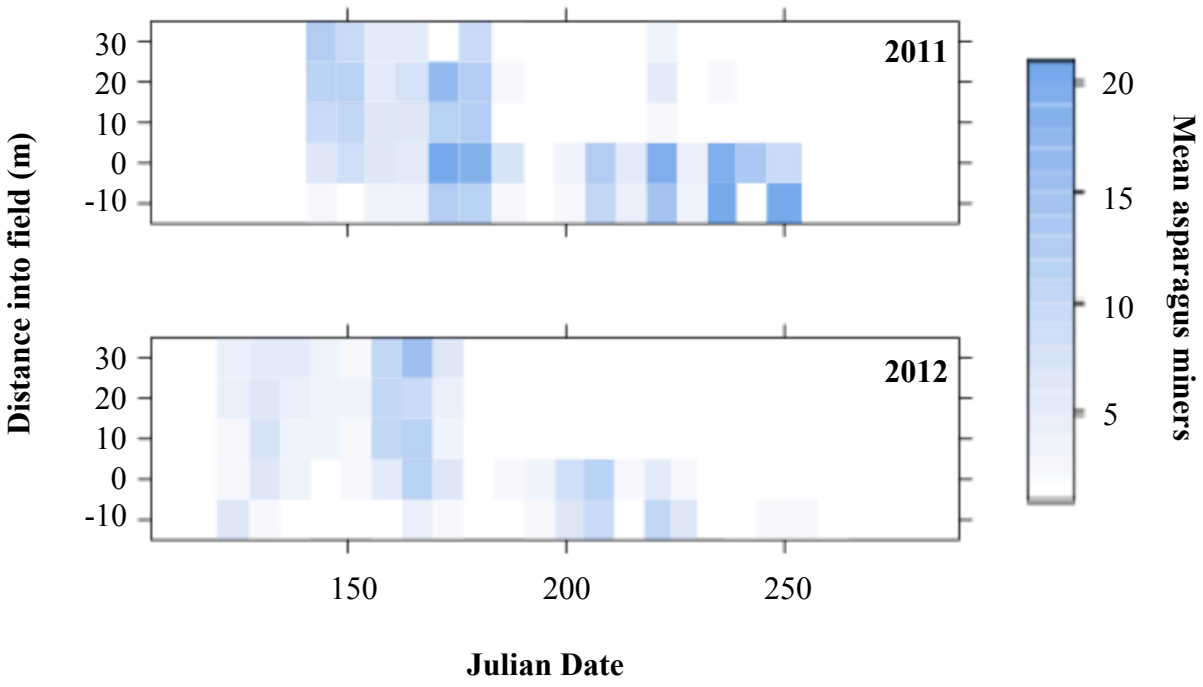


Figure 2.3. Mean ± SEM abundance of asparagus miner adults at different distances (m) into commercial asparagus fields in Oceana Co., MI for A) 2011 and B) 2012, with damage data (average % stem mined) at different distances into the field for 2012. Black bars are canopy-level yellow sticky traps while white bars are ground-level traps. Upper case letters are for pairwise comparison (Tukey's HSD) within canopy-level traps for, while lower case letters are for comparisons within ground-level traps for a specific year; shared letters indicate no significant differences. All damage measurements are significantly different at the various distances into the field (Tukey's HSD). Damage was not evaluated in 2011.

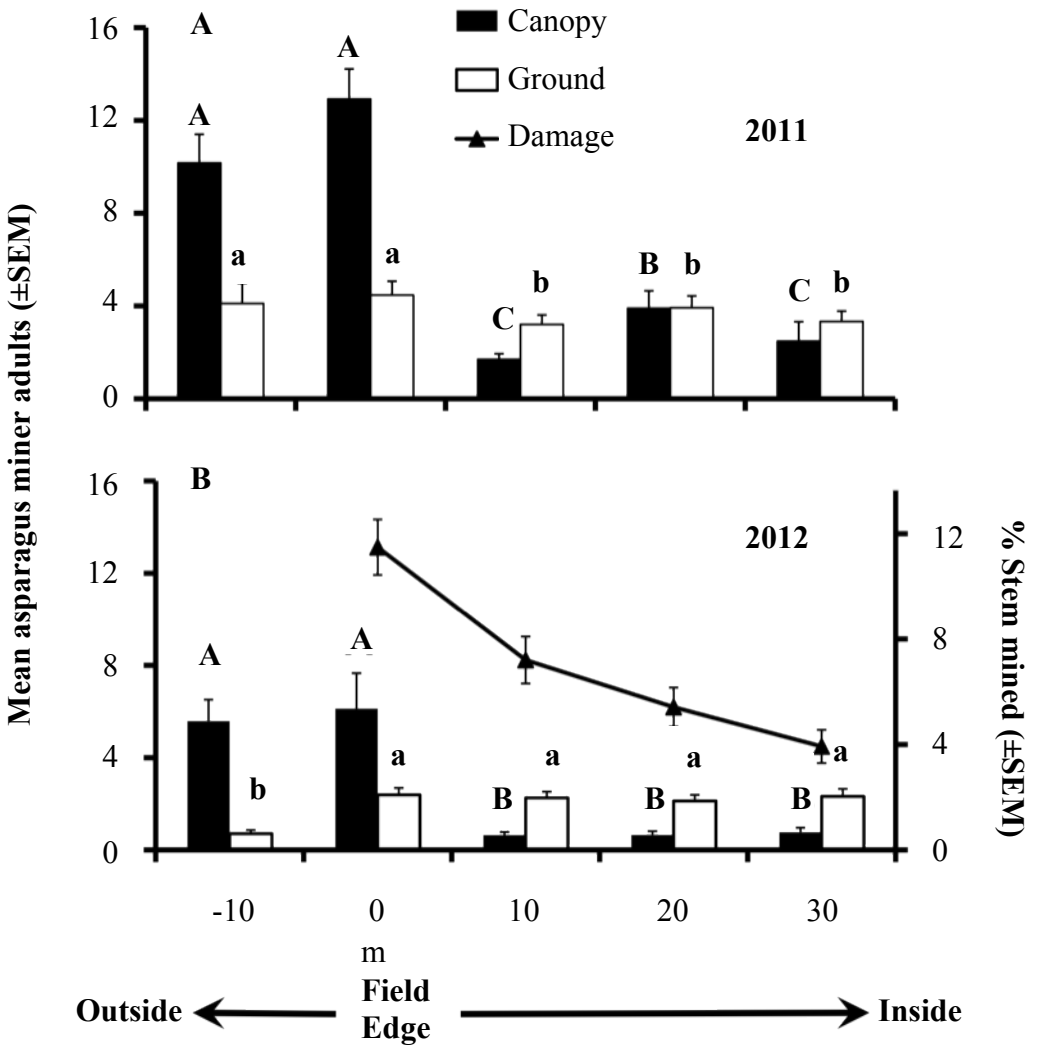


Figure 2.4. Mean ± SEM adult asparagus miner abundance in different edge habitats. Uppercase letters are for pairwise comparisons within habitat type for 2011, while lowercase letters are for 2012. Shared letters above bars indicate values that are not significantly different from one another (Tukey's HSD, α=0.05). The number of field edges in each habitat type is displayed above the columns. The N above each group of bars represents the number of independent field edges classified as a given type of habitat for the analysis.

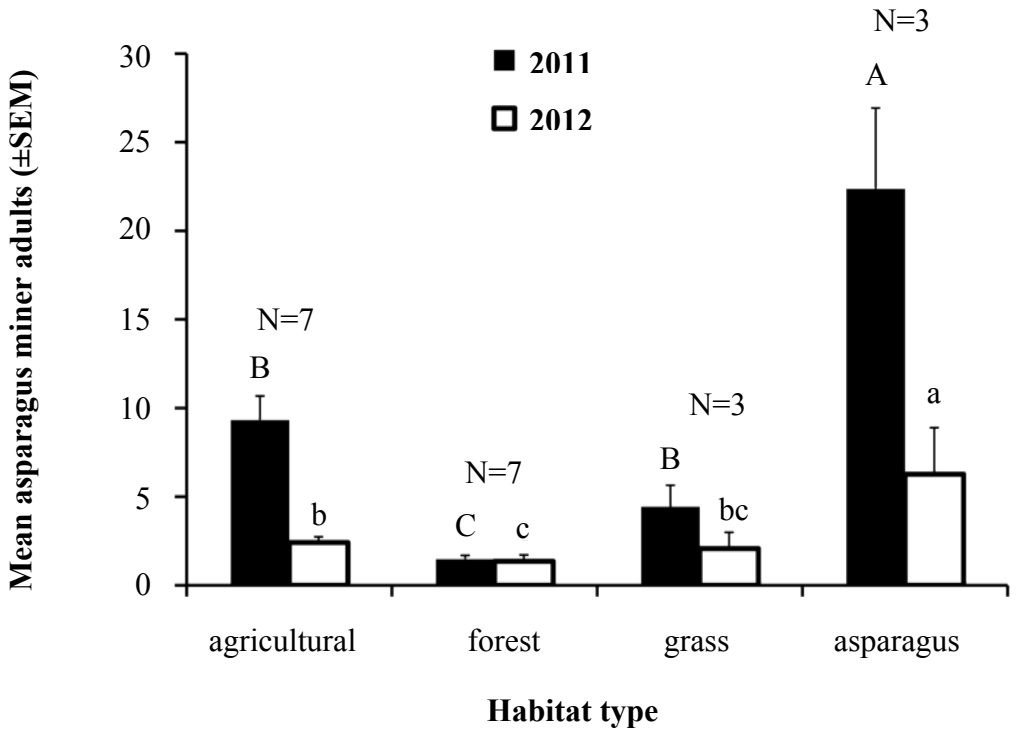
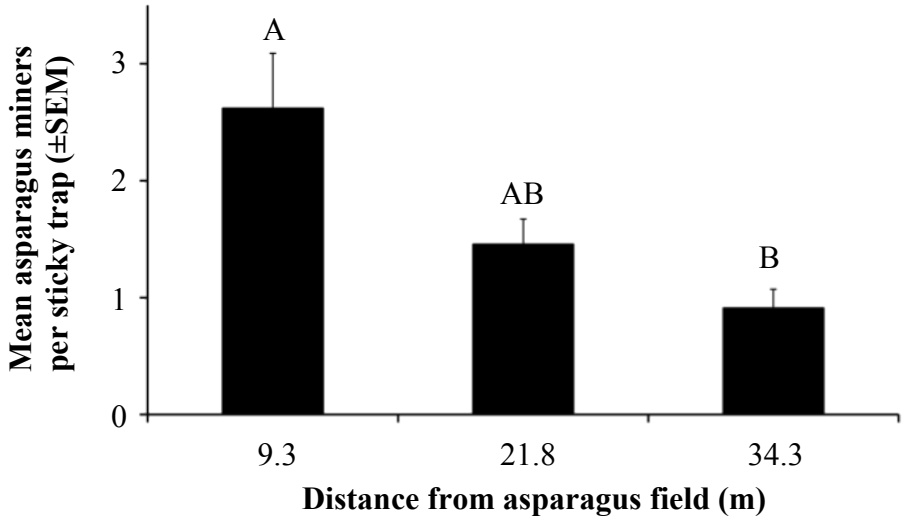


Figure 2.5. Mean ± SEM overall abundance of adult asparagus miners in a cherry orchard adjacent to an asparagus field in Oceana Co., MI at different distances into the orchard in 2012 Traps were placed at the edge of the orchard (9.5m away from the asparagus field), and at 21.8 m and 34.3 m from the edge, in three transects with ground-level yellow sticky traps. Traps were changed weekly. Different letters above bars represent statistically significant differences (Tukey's test, α=0.05).



Discussion

This study investigated the horizontal and vertical distribution of asparagus miner adults in commercial asparagus fields. In addition, we have examined the spatial variation of asparagus miner damage. Asparagus miner adults are evenly distributed in the field during the first generation, and located primarily on the edges or outside the field during the second generation. There are several possible explanations for this pattern. During asparagus harvest, plants are broken off at the soil surface, and the stubs of picked stalks have phloem fluid at the top, which adults frequently feed from (Morrison, personal observation). These small point sources of valuable resource are evenly distributed throughout the field, and miner adults may easily access them. As a result, there is a more even distribution of adults in the beginning of the season. In contrast, the second generation might emigrate from the field in search of mates or new oviposition sites, especially as population density increases in the first generation postharvest and fewer optimal oviposition sites remain. According to our findings, it may be beneficial for growers to concentrate their asparagus miner management programs during the latter half of the season to the edges of fields and along the field margins. This would result in lower amounts of pesticide loading in agricultural landscapes, ameliorating the deleterious effects of synthetic insecticides on non-target organisms (Epstein et al. 2000). In addition, treating only the field edges would give the natural enemies of the asparagus miner a refuge, while still eliminating most of the miner adults in the area. Refuges for natural enemies have been shown to be important in agricultural systems for increasing biological control (Schellhorn et al. 2008), thus such an edge-based tactic would have the added benefit of increasing the suppression of the remaining asparagus miners by its natural enemies. Reducing the area treated for asparagus miners can have significant economic benefits, since in 2011 there were 28 broad-spectrum

insecticide applications in five fields and in 2012 there were 13 in three fields. In other insect species, certain generations have corresponded to the dispersing stage, for example in *Anticarsia gemmatalis* (Lepidoptera: Noctuidae), the second generation disperses when population densities exceed a certain threshold (Fescemyer and Hammond 1988). Another explanation of the differential distribution between first and second generation adults is that this could be due to a sampling artifact created by an interaction between the asparagus fern and the visually attractive traps used for sampling. Asparagus plants during the first adult miner generation are short stalks, thus traps are clearly visible during this time, but asparagus miner adults might not see the traps as readily in the second generation as a result of occlusion by the fern when asparagus plants can reach up to 2 m. However this explanation is not likely, given our findings with the damage in the field, which was greater near the edge of the field.

We found that the date at which asparagus harvest stopped had no relationship with the severity of the first or second generation of asparagus miners in the field. This may indicate that most asparagus miners can deposit eggs far enough down on the asparagus stalk that they are not disturbed when harvesters pick spears. Indeed, previous studies have found pupae up to 3-7.6 cm below the soil surface (Lampert et al. 1984), which would be enough refuge from pickers. In addition, picking asparagus spears also did not affect emergence of adults, indicating that asparagus miner adults remain protected within the base of the plant as pupae. However, it is notable that intensive use of broad-spectrum insecticides resulted in reducing the abundance of the second generation's adults in two of our fields in 2011 and one in 2012. Nonetheless, we suggest that it is more economical, less time-intensive, and ecologically safer to have fewer well-timed applications during the growing season.

Similarly to Tuell (2003), adults were more abundant in the canopy than near the ground. The fact that adults were more abundant in canopy-level traps, despite the fern, suggests that the observed preference of asparagus miners for the edge and outside the field might not be a sampling artifact. Adult miners have been previously observed mating on asparagus fern (Ferro and Suchak 1980), and the adult flies feed on sugar, nectar and plant sap, which are resources found in the asparagus canopy (Ferro and Gilbertson 1982). When sampling for asparagus miners, it may be useful to use both canopy- and ground-level traps, because ground-level traps capture abundance early in the season, during asparagus harvest when the canopy is absent. Ground-level traps likely are not needed after the asparagus has grown fern, since only low numbers of adults are found in these traps later in the season.

Ours is the first study to characterize how landscape features affect asparagus miner distribution, and we found that asparagus miner adults cross the field margins and move into habitats near commercial asparagus fields. Here we show that asparagus miner adults can be found up to 34 m away from an asparagus field. Because adult asparagus miners feed on sugar and nectar, it is thus plausible that these adults move away from asparagus fields to find flowers as alternative food sources, especially when asparagus plants drop their flowers during the latter half of the season.

Forested borders in our study had fewer asparagus miner adults than agricultural, grassy and asparagus borders. This indicates that there is value in conserving the remaining forested segments of the landscape, which are characteristic of the Michigan asparagus-growing region. Creating borders around asparagus fields that are less suitable for the asparagus miners may lead to a reduction in their abundance inside the field. The impact of the type of habitat near the field border on the abundance of a pest has been documented with other insect species. For example,

the grape berry moth was less abundant where grasses bordered vineyards compared to coniferous woods (Botero-Garcés and Isaacs 2004). In this case, the presence of an alternative host plant in the woods was causing increased moth pressure at the field borders. We did not observe volunteer asparagus plants in forests (Szendrei, personal observation), but in grassy areas sometimes these plants can be present, which can explain the slight increase in miner abundance in these border areas relative to the forest borders. Another potential benefit to conserving forest habitat around asparagus fields is that these may have food and refuges for natural enemies that increase their abundance and efficacy as biocontrol agents (Tscharntke et al. 2007) of the asparagus miner. Because the asparagus miner is a specialist on asparagus, we expected it to be more abundant in between two asparagus fields and it is likely that these insects are moving between asparagus fields. Thus, it is likely common for an asparagus miner to leave the field in which it developed and seek out resources in the nearby habitat. Future studies with asparagus miners will address potential dispersal capacity and resource use in the landscape.

Overall, our results suggest that growers may be able to spare economic and ecological costs by targeting asparagus miner management along field edges in the latter part of the season. In addition, conserving existing forested borders around fields may help with decreasing asparagus miner movement and abundance. These strategies can be used as part of an integrated pest management program for controlling the asparagus miner and mitigating against infection by *Fusarium* spp.

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CHAPTER 3

The development of the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) in temperate zones: A degree-day model

Introduction

A key to successful insect pest management is to be able to adequately time control measures to suppress populations before crop damage occurs. While there are many methods of deducing when to make management actions, one widespread and accurate tool is to employ a degree-day model (Herms, 1998; Petitt et al., 1991; Snyder et al., 1999; Wilson and Barnett, 1983; Zalom et al., 1983). Degree-day models help time management actions such as pesticide applications in integrated pest management programs (Dent, 2000), and their use can successfully reduce the number of applications necessary while improving pest control (Broatch et al., 2006; Davis and Pedigo, 1990; Elliott et al., 2009; Lindblad and Sigvald, 1996; Zalom et al., 1983). Degree-day modeling takes advantage of the fact that the development of insects is dependent on ambient environmental temperatures, and is typically limited by upper and lower temperature boundaries. In the calculation of a degree-day model, the upper and lower developmental thresholds - the temperature below or above which development occurs, respectively(McMaster and Wilhelm, 1997)- for a species must be determined. Insect species living in temperate climates seldom encounter the upper limit (Morrison WR, unpublished data, 2010, 2011, 2012), which is generally about 20°C above their lower developmental threshold (Dixon et al., 2009); therefore, determining the lower developmental threshold may be more important in these cases. The accumulation of daily average temperatures above the lower

developmental threshold from the biofix date (a calendar day that marks the beginning of the recording of temperature accumulation) can then be correlated to when particular biological events take place over the course of an insect's lifecycle. Predictions for the accumulated growing degree-days at which life stages occur must be formulated independently from the dataset used for model validation (Nowatzki et al., 2002; Welch et al., 1981). Growers can then use the degree-day model to tailor their management actions to the pest in the geographic area where the model was developed.

Asparagus is produced globally in 62 countries, representing about 196,000 ha of production (Benson, 2009), and accounting for $83.4 million in market value in the United States alone (USDA-NASS, 2012). However, asparagus has been in global decline due to a variety of factors, including the prevalence of insect (Gilbertson et al., 1985; Tuell, 2003; Tuell and Hausbeck, 2008) and fungal pests (Grogan and Kimble, 1959). The asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) is one such pest, and is a putative vector for pathogenic *Fusarium* spp fungus (Bishop et al., 2004). It is a specialist on asparagus, where it deposits its eggs under the epidermis of the stem near soil level (Ferro and Gilbertson, 1982; Fink, 1913). Larvae bore into the stem after hatching and, feed on cortical tissue (Eichmann, 1943). The asparagus miner is usually bivoltine in temperate climates, and overwinters as a pupa (Lampert et al., 1984). Adults emerge in Michigan in mid- to late-May, with the first generation flight peaking around mid-June (Tuell, 2003). Adults begin mating upon emergence, and soon thereafter start laying eggs into asparagus stems where the larvae feed (Barnes, 1937). The second generation adult peak occurs in late July to mid-August (for an illustrated life cycle see Morrison III et al., 2011), which produce the overwintering pupae.

In addition to vectoring pathogens, the asparagus miner can impair the ability of the plant to fix carbon by damaging photosynthetic tissue, which may in part result in harvest losses in subsequent years. While there has been ample research into controlling the pathogenic fungi *Fusarium* spp. (e.g., Blok et al., 2008; Counts and Hausbeck, 2008; Hausbeck and Cortright, 2006; Reid et al., 2001; Reid et al., 2002; Smith et al., 1990; Stephens et al., 1989), little work has been devoted to controlling the asparagus miner (Morrison III et al., 2011; Damicone et al., 1987; Elmer at al., 1996). There are currently no management recommendations or programs for the asparagus miner; however, control is imperative, since it preferentially attacks younger fields (Tuell, 2003) especially susceptible to *Fusarium* spp. infection. As a result of infection with *Fusarium* spp., the life span of a commercial asparagus field may be reduced by 5-8 years (Elmer at al., 1996).

Degree-day models have been successfully used in the management of related agromyzid species. For example, in the case of the vegetable leafminer (*Liriomyza sativae* Blanchard), a degree-day model was able to adequately predict the species' population dynamics in greenhouses (Petitt et al., 1991). Schuster and Patel (1985) developed a degree-day model for another agromyzid, a serious pest of tomatoes (*L. trifolii* Burgess), in order to accurately predict larval development. More specifically for the asparagus miner, Tuell (2003) provided charts with degree-day accumulations and adult abundance data, but did not actually relate the degree-days to phenological events or develop predictions for specific life stages. In addition, the degree-day model in that study was not validated, nor was the lower developmental threshold investigated.

The aims of our study were three-fold: 1) determine the lower developmental threshold of the asparagus miner, 2) develop and validate a degree-day model, and, 3) create a developmental time budget for the asparagus miner so that asparagus growers can appropriately plan

management actions as part of an integrated pest management approach to control the asparagus miner.

Materials and Methods

Study site. This study was conducted in five commercial asparagus fields in 2010 and three each in 2011 and 2012, which were located 1.21-16.14 km apart in Oceana County, MI (Table 3.1.). Sampling in 2010 started later and was shorter than usual, beginning on June 21st and continuing until September 17th according to the sampling protocol described below for 2011 and 2012. As a result, the 2010 data was used only for verification of the overall 2011 predictions for specific phenological events. All asparagus fields were of var. Millennium, an all-male hybrid of asparagus.

Phenological events. Accumulated degree-days for predicted asparagus miner phenological events were calculated from weather and insect data collected in 2011 and 2012. The degree-days for critical adult asparagus miner life cycle events were determined for the beginning of flight, first generation peak, beginning of the second generation, second generation peak, and the end of flight. For the immature stages, critical events included first appearance of larvae and pupae in the field, first population peak, and second population peak.

The phenological events for adults were evaluated by monitoring asparagus miner abundance with yellow sticky traps (7.5 × 12.3 cm, Great Lakes IPM, Vestaburg, MI). Thirty-two sticky traps were set up per field (N=160 for all fields combined), and these were checked and changed every 6-10 days. Sampling occurred from the beginning of asparagus emergence on 11 May to end of adult flight on 3 October 2011 and from 22 March to 7 October 2012. The traps were arranged in transects, with sampling points at 0, 10, 20 and 30 m into the asparagus

field from the field edge. Three sets of transects were placed 10 m apart in each field. At each sampling point, there was a canopy trap (placed at 1 m from the ground on a steel conduit) paired with a ground-level trap (10 cm above ground), except during the asparagus harvest when only ground traps were used due to the low height of spear-picking machines. The traps were located in the same place for the duration of the season, and were placed in roughly the same spot in subsequent sampling seasons. Asparagus miners were counted on traps and their numbers recorded weekly.

Immature asparagus miner stages were sampled by collecting 8-53 stems from each field (mean ± SEM: 33.8 ± 11 stems) every 6-10 days throughout the growing season from 1-5 fields per sampling date. The dates of collection ranged from 31 May to 3 October in 2011 and from 14 June to 7 October in 2012. Stems were collected 30 m into the field from a randomly selected point along the field margin. These were cut 5 cm below the surface of the soil, and cut again at the height of the longest mine. Stems were sealed in plastic bags and placed in a cooler with ice packs until they were transported to the laboratory. Samples were immediately stored at 5°C in the laboratory, and the stems were processed within 1-14 days. A razor blade was used to carefully peel the epidermis from the asparagus stem to reveal the immature stages. For each stem, the number of larvae and pupae were recorded.

Weather data collection and degree-day model development. Daily temperature data were obtained from Michigan State University's Enviro-weather station (Andresen et al., 2012) located in Hart, MI at the Michigan Asparagus Research Farm (43°44'11.33"N, 86°21'31.48"W) for 2010-2012. The weather station was centrally located among the sampled fields with all the fields located within 10 km, and temperature information was automatically recorded in an electronic database at 5-minute intervals.

Summarized monthly and seasonal climatic statistics (National Climatic Center Data, 2013) for Hart, MI are given in Supplemental Table S1. All three of the individual March-October growing seasons were on average warmer and wetter than the 30-year normals, which is consistent with regional climatic trends during the past few decades (Andresen et al., 2012). Mean seasonal temperatures during the three seasons ranged from 1.4°C above normal in 2011 to 2.9°C above normal in 2012, while total seasonal precipitation ranged from 52.5mm above normal in 2011 to 72.2mm above normal in 2012. Early season GDD accumulation was exceptionally high during the 2012 season due to a much warmer than normal March (the monthly mean temperature was 8.8°C above normal). A biofix date of 1 March was used, because this has historically resulted in the best fit for degree-day models in the Great Lakes region (Andresen J, unpublished data). Accumulated GDD were calculated using the Baskerville-Emin method, which approximates the temperature diurnal course using a sine wave and the maximum and minimum temperature for a 24-hour period (Baskerville and Emin, 1969). The minimum and maximum daily temperatures from the Enviro-weather station were used in the calculation of accumulated degree-days, and the lower developmental threshold reported in this study was used as the base temperature in the model. The Enviro-weather-based accumulated GDD corresponding to important phenological events in the life cycle of the asparagus miner in 2011 were used as the "predicted GDD" for each event and this was also used in comparisons with other years' asparagus miner abundance data from Michigan and other regions.

Lower developmental threshold. The lower developmental threshold of the asparagus miner was investigated using environmental growth chambers. Pupae were the focus of the lower developmental threshold experiment. Asparagus miner pupae were collected from the field on 18

September 2010 as well as 3 October 2011 from fields 1 and 4. The exact age of the asparagus miner pupae were not known. In the laboratory, pupae were dissected from the asparagus stems, placed individually into plastic cups (84.8 cm^3, 6 x 3 cm D:H, Solo Co., Chicago, IL, USA), and stored at 3°C until used in experiments (range 3-196 d; duration of storage did not effect viability of the pupae, Morrison, unpublished data). Pupae were placed into 10 environmental chambers (I-41VL or I-36VL, Percival Scientific, Inc., Perry, IA, USA) with a 16:8 L:D photoperiod and 75% humidity, by assigning batches of 20-60 pupae randomly to one of 10 constant temperatures with 3-7 replications per temperature: 8°C, 9°C, 10°C, 10.5°C, 11°C, 11.5°C, 12°C, 14°C, 16°C, and 26°C. For determining the lower developmental threshold, we focused on temperatures around 10°C, because the asparagus miner emerges in spring so we expected the threshold to be relatively low. About halfway through the experiment, temperatures were randomly reassigned to different environmental chambers to account for any effects due to the chambers. Digital thermometers were placed into each environmental chamber and checked daily to ensure proper temperature conditions, which never varied more than the given temp±0.06°C (SEM). The pupae were also checked daily for emergence of adults, and pupal duration was noted at time of emergence.

Comparison to previous years' asparagus miner abundance. In order to evaluate the accuracy of the degree-day model and its universality, the phenological events' predicted GDD from 2011 were regressed against the observed accumulated GDD at which phenological events occurred in previous years from this and previously published studies (see regression for validation (RV), Supplemental Table S2). This was done for data ranging from 1912 to the present collected from Michigan and the United Kingdom (Barnes, 1937; Gilbertson et al., 1985; Bishop et al., 2004; Ferro and Gilbertson, 1982; Eichmann, 1943). This included validation of

the model against observed phenological events for 2012, and the deviation from expectations was calculated for each event between 2011 and 2012. The predicted accumulated GDD for phenological events used in comparisons for this section are derived from the 2011 Enviro-weather station temperature data from Hart, MI. The calculated degree-days for previously observed phenological events in the literature were based on weather data collected in the vicinity of insect sampling, in the same year (Supplemental Table S2), using the lower developmental threshold elucidated in this study and the Baskerville-Emin method with a 1 March biofix date.

Developmental time budget. In order to characterize the life cycle of the asparagus miner, a time budget in days and equivalent degree-days was created that included estimates for the egg, larval, pupal and adult stages. Egg duration was estimated from previously published literature, specifically from Fink (1913) and Barnes (1937) (see developmental time budget (DTB), Supplemental Table S2). Larval duration was estimated in three ways. First, it was approximated by determining how many degree-days had elapsed since the first appearance of larvae compared with pupae in the field. Second, the period between the peak larval abundance and the peak pupal abundance was compared. Finally, estimates were made by consulting previously published literature (Barnes, 1937; Ferro and Gilbertson, 1982; Lampert et al., 1984). In developing degree-day estimates from previous literature for larvae and other stages, we used data from the nearest weather station to where the research was conducted (Supplemental Table S2). We applied the Baskerville-Emin method of calculation for the duration of a specific life stage and, combined it with the base temperature from our current work. The pupal duration was estimated by comparing the length of time that it took pupae to develop into adults at the beginning of the season in the field. Additional estimates were obtained by noting the pupal duration from pupae

raised in environmental chambers (as described earlier), and by consulting previously published information in a similar manner as to that described above (Tuell, 2003; Ferro and Gilbertson, 1982; Fink, 1913). Lastly, adult lifespan was calculated by placing adults into 26 ± 0.06°C (16:8 light:dark photoperiod and 75% humidity) environmental chambers right after eclosion and feeding them a 10% sugar solution or honey water *ad libitum* on cotton balls. The adults' lifespans were recorded in days and converted into degree-days using the Baskerville-Emin method and 12.1°C was used as the lower developmental threshold. The different estimates for a given event in the life cycle were averaged, and the mean, range and source of information were listed for each stage.

Statistics. A repeated measures analysis with first order autoregressive correlation among the time points was used to evaluate the fit of the degree-day model. Asparagus miner adult abundance was used as the dependent variable and trap height, growing degree-days, year of data collection and field were used as independent variables in the linear mixed model with a generalized least squares function (R Project for Statistical Computing, http://www.r-project.org/). A Kenward-Roger correction was applied to the degrees of freedom. The residuals were analyzed to evaluate assumptions of normality and the data were log-transformed to meet assumptions of homogeneity of variances and normal distribution around a mean of zero.

While there has been much debate on the proper calculation of the lower developmental threshold (Preuss, 1983), we evaluated both nonlinear (Wagner et al., 1984) and linear methods (Wilson and Barnett, 1983). The model yielding the lowest combined AIC, BIC and R^2 (or Efron's Pseudo-R in the case of nonlinear models; Efron, 1978) was selected. The linear approximation had the best fit to our data and this model was then used to calculate the lower

developmental threshold. As a result, the effect of temperature on pupal duration (d) and developmental rate (1/d) against temperature was analyzed with a linear regression.

Two linear regressions were performed to assess the accuracy of the degree-day model for the asparagus miner. First, the predicted phenological event accumulated GDD from 2011 was compared to the observed phenological event accumulated GDD in all other years with linear regression. Second, just a subset of this data, from 2012, was used to validate the degree-day model's predictions from the previous year. To assess the sensitivity of the model to location of data collection, the deviation between each predicted and observed phenological event was calculated, and a one-way ANOVA was performed (see locality sensitivity analysis (LSA), Supplemental Table S2). Before the analysis, the residuals were inspected to ensure that the assumptions of normality were fulfilled; no transformation of the data was necessary.

Results

Degree-day model development. Overall, the growing degree-day totals were associated with a significant amount of variability in asparagus miner abundance (Repeated Measures ANOVA: $F_{47,4422}=47.30$, $P<0.01$). Beginning of adult flight occurred around 100 GDD, with the first adult population peak at 490 GDD and second population peak at 1530 GDD. Asparagus miner adult flight ended at 1850 accumulated GDD (Table 3.2). The first pupae and larvae appeared about 280-390 GDD after the beginning of adult flight (Table 3.2). It then took another 360-450 GDD before the immature stages reached peak abundance in the field. As a reference, asparagus harvesting took place between 222-666 accumulated GDD, depending on the field and grower.

Lower Developmental Threshold. The lower developmental threshold was 12.1°C (Figure 3.1.). It took pupae a mean of 207±15 d to develop at 12°C, while it only took 14±0.5 d to develop at 26°C. However, no asparagus miners emerged at 8, 9, 10, 11 or 11.5°C, even after running the experiment for over 545 d. The asparagus miner developed at significantly different rates among the various temperatures, and the rate increased in a roughly linear manner as temperature increased (Figure 3.1.; Adj. $R^2 = 0.84$, $P<0.01$). As temperature increased, pupal duration also linearly decreased (Figure 3.1.; Adj. $R^2 = 0.93$, $P<0.01$).

Farm-level variation in voltinism. There was significant variability in adult population dynamics among different fields (Repeated Measures ANOVA: $F_{4,5.5}=21.99$, $P<0.01$). While we consistently recorded two immature peaks in both 2011 and 2012 (Figure 3.2.A, B), the results were more variable for the adults. In 2011, we observed one adult generation with a minimal or non-existent second generation in some fields (Figure 3.3.C), while in others, there were two pronounced generations of asparagus miner adults in a season (Figure 3.3.A, B). In 2012, with a more rapid overall accumulation of growing degree-days, we observed two generations in most fields (Figure 3.3.E, G), though some again had a reduced second generation (Figure 3.3.F).

Comparison to previous years' asparagus miner abundance. There is a strong and significant correlation between the predicted accumulated growing degree-days (AGDD) for the 2011 phenological events in the life cycle of the asparagus miner and the observed AGDD for previous years' (Adj. $R^2=0.93$, $P<0.01$; Figure 3.4.). Moreover, 2011 had 2.5 times more asparagus miners overall on the traps than in 2012. Location of data collection also affected the accuracy of the degree-day model. The average GDD deviation from predicted phenological events is 111.2 ± 13 for miner populations in Michigan, 266.9 ± 66 for those in Massachusetts, and 510.5 ± 131 for those in the United Kingdom. Thus, the most accurate predictions are for

observations in Michigan, while observations in Massachusetts are 2.5 times less accurate, and those in Europe are about 4.5 times less accurate than in Michigan (ANOVA, deviation from expectations by location: $F_{2,41}=11.44$, $P<0.01$).

Validation of degree-day model. There was a strong correlation between the predictions from 2011 for observed events in 2012 (Adj. $R^2=0.94$, $P<0.01$). The mean (±SEM) deviation from the expected GDD for the phenological events in 2012 was 142 ± 37 GDD, with the most accurate prediction for the second generation adult peak and the least accurate prediction for the end of adult flight (Table 3.2.). Predictions for adult stages were about 1.5 times more accurate than predictions for immature stages in 2012.

Developmental time budget. According to estimates derived from this study and previously published literature (Supplemental Table S3), a generation should last on average 731.1±30 GDD from deposition of egg to the death of the asparagus miner adult (Figure 3.5.). Because the asparagus miner is usually bivoltine, the total life cycle should take on average 1462 GDD (95% CI: 1000-1925 GDD). The egg is the most ephemeral stage, while the larval and adult stages are similar in length, each composing about a quarter of the life cycle for the asparagus miner. When considering Julian days, the pupal stage accounts for a little less than two-thirds of the total life cycle (Supplemental Table S4).

Table 3.1. Summary of information about the five commercial fields from Oceana County, MI that were used in sampling between 2010-2012.

ID	Area (ha)	Year Planted	Nearest Enviro-weather Station (km)	End of Harvest		No. Insecticide Sprays	
				2011	2012	2011	2012
Field 1	3.54	2009	5.7	5/31/11	6/12/12	6	5
Field 2	1.82	2008	9.9	6/6/11	6/19/12	5	4
Field 3	3.77	2008	7.3	7/6/11	6/7/12	7	4
Field 4	6.06	2009	9.8	6/30/11	na	5	na
Field 5	3.66	2010	10.85	6/30/11	na	5	na

Table 3.2. Summary of the predicted accumulated growing degree-days from the 2011 field season in Oceana Co., MI for different adult and immature phenological events and the deviation from expected in 2012 using the Baskerville-Emin method. We used a biofix date of 1 March, and a base of 12.05°C in combination with weather data from the MSU EnviroWeather Hart Station.

Predicted GDD	Phenological Event	Deviation from Predicted in 2012 (GDD)
Adults		
100	Beginning of flight	57.2
490	1st peak	83.1
940	Beginning of 2nd generation	48.3
1530	2nd peak	43.9
1850	End of flight	350.2
Immatures		
380	First appearance of larvae	na[a]
490	First appearance of pupae	na[a]
670	1st larval peak	96.9
940	1st pupa peak	216.7
1530	2nd larval peak	104.7
1600	2nd pupal peak	274.8

[a] Estimates for these two events were considered too biased to include in the analysis because longer harvesting of older fields delayed data collection.

Figure 3.1. The mean±SEM developmental rate (open triangles, dashed line, left axis) and pupal duration (closed squares, solid line, right axis) of the asparagus miner in environmental chambers at various temperatures with a 16:8 L:D cycle and 75% humidity. There was no miner emergence at 8°C, 9°C, 10°C, 10.5°C, 11°C, and 11.5°C, despite running the experiment about 545 days. The linear model fit the data best and was significant for the developmental rate ($y=0.0054x-0.065$, Adj $R^2=0.84$, $P<0.01$) and pupal duration ($y=-11.53x+307.4$, Adj $R^2=0.93$, $P<0.01$).

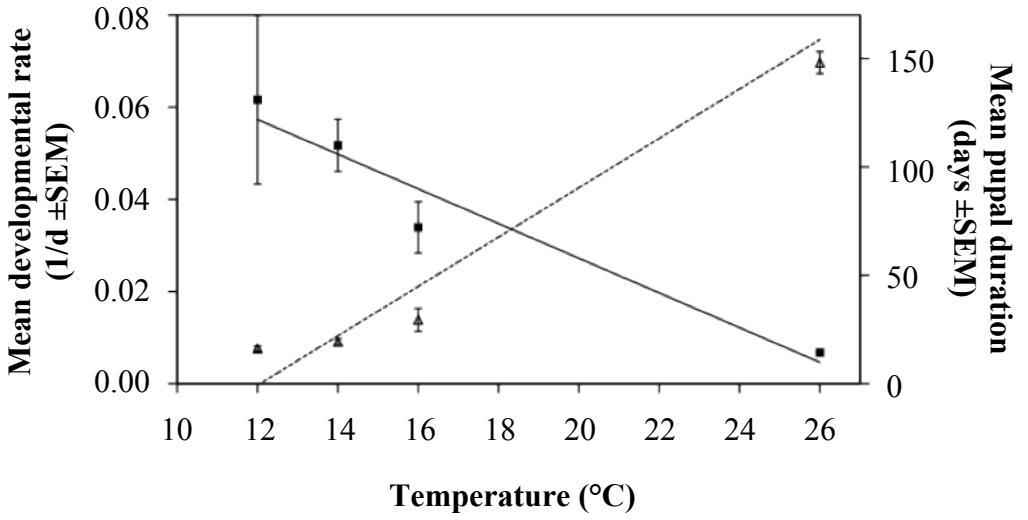


Figure 3.2. Mean±SEM asparagus miner abundance from commercial fields in Oceana Co., for immature stages (pupae – solid line; larvae – dashed line) for A) 2011 and B) 2012. Accumulated degree-days are calculated based data from the MSU Enviroweather station located in Hart, MI with a biofix date of 1 March, a base temperature of 12.1C, and the Baskerville-Emin method calculation method.

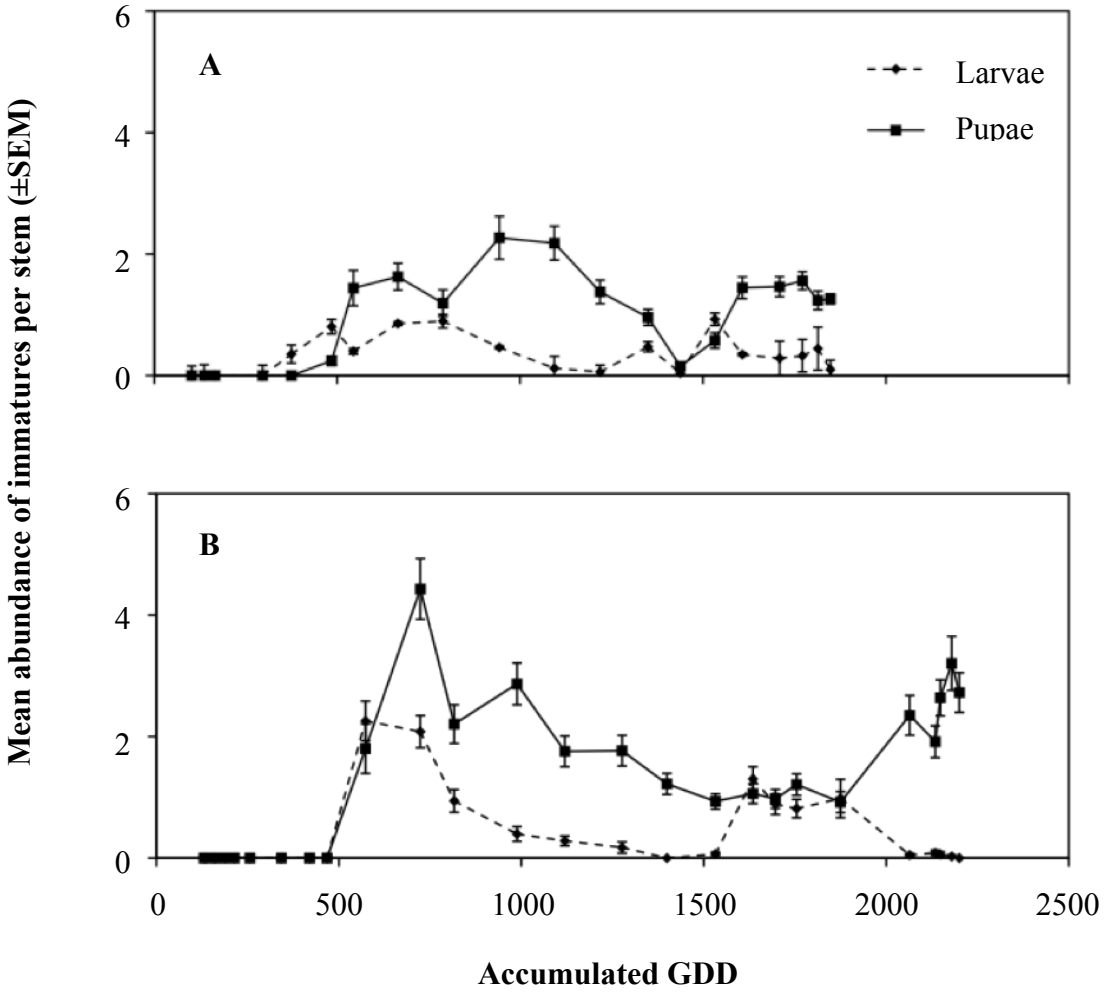


Figure 3.3. Mean±SEM asparagus miner adult population abundance from Oceana Co., MI in 2011 (left column) and 2012 (right column) in field 1 (A, E), field 2 (B, F), field 3 (C, G) and overall (D, H). Degree-days were calculated as in 3.2, while arrows indicate when harvesting stopped. Arrows indicate when spear harvesting stopped.

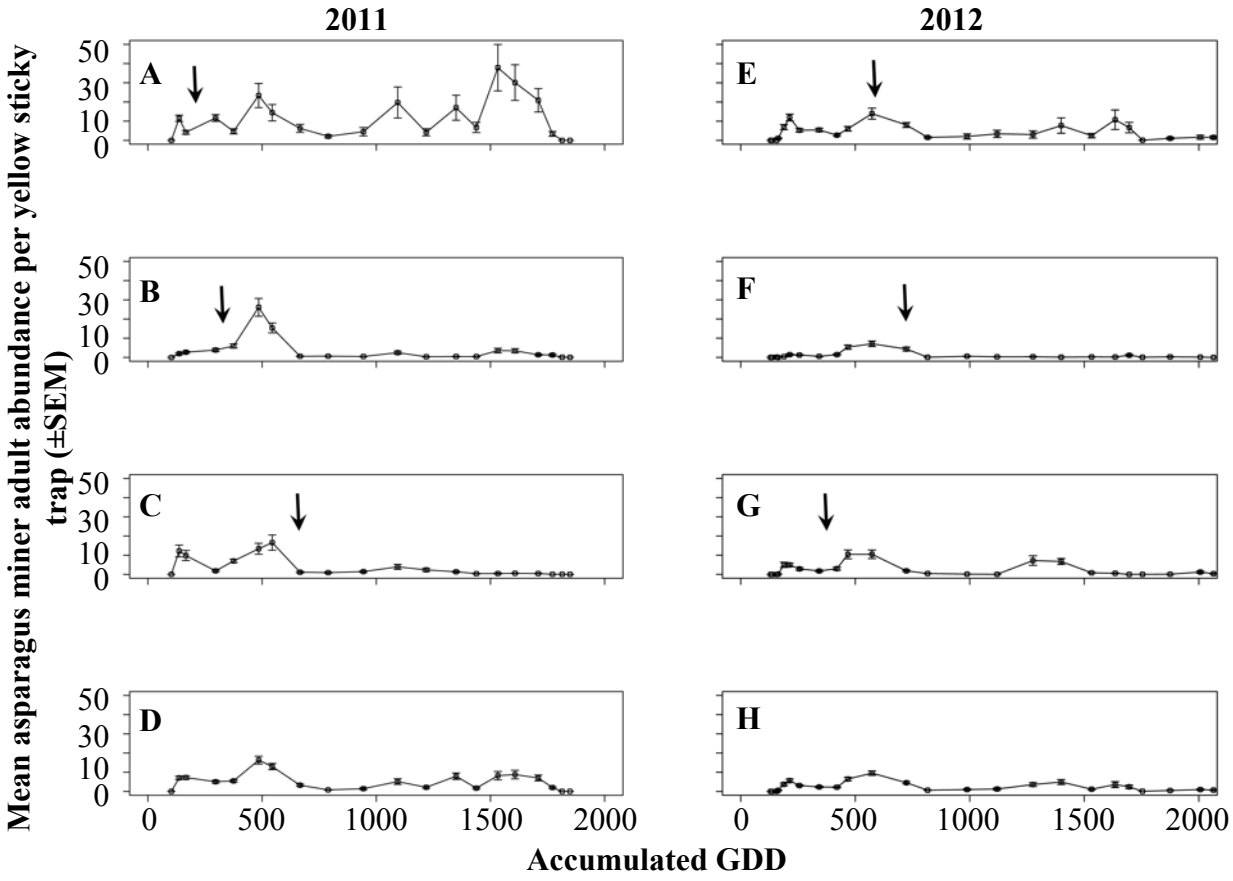


Figure 3.4. Linear regression for the predicted accumulated GDD from 2011 for important phenological events against the observed accumulated GDD for the same events in the asparagus miner life cycle from 1974, 2001, 2010, and 2012. Degree-days were calculated as in 3.2. The model has a significant slope (y=1.13x-44.35, P<0.01, with an adjusted R^2=0.93)

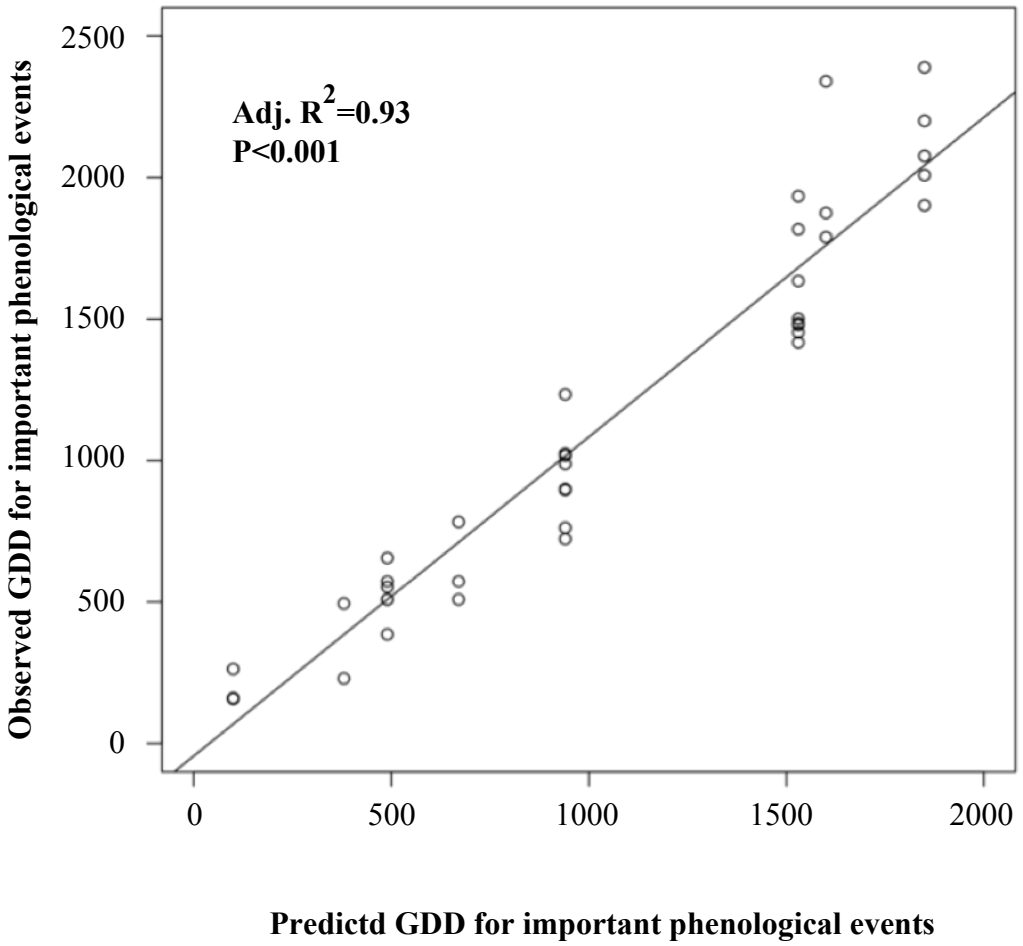
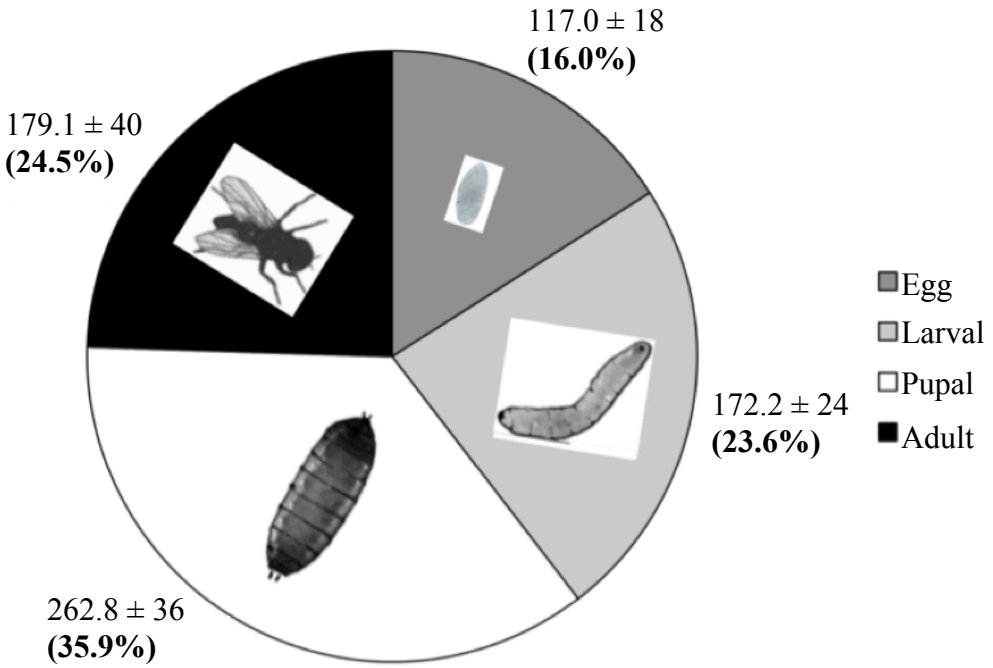


Figure 3.5. A developmental time budget based on mean±SEM degree-days to complete each life stage of the asparagus from previous estimates in the literature and the current study, with mean developmental time in DD outside each slice, and the percent of the total in the parentheses. Egg duration is shortest, while the pupa is the longest-lasting stage. Illustrations by Marlene Cameron, MSU.



Discussion

This is the first study to develop and validate a degree-day model for management of the asparagus miner in Michigan. The key phenological events for timing the currently available management methods against the asparagus miner are the first and the second peak adult flights that are the points at which the asparagus miner is most vulnerable to currently available pesticides. Reduced-risk foliar pesticides could be most efficiently timed shortly before both adult population peaks to minimize oviposition into stems and subsequently reduce crop damage (e.g. 350 GDD for first population and 1350 GDD for the second peak). In future research, the degree-day model's predictions will be employed in a commercial field setting to evaluate whether its use results in decreased asparagus miner abundance.

Our predicted accumulated growing degree-days for various phenological events in the life cycle of the asparagus miner deviated from Tuell's (2003) study by a mean of 86 ± 21 GDD for all phenological events. On the other hand, there was a mean deviation of 109 ± 26 GDD and 267 ± 66 GDD for the bionomic data collected by Lampert et al. (1984) and Ferro and Gilbertson (1982), respectively. The GDD observed by Ferro and Gilbertson may have deviated more because that data was recorded in Hadley, MA. Taken together with the fact that there was far more deviation from expected events in the United Kingdom than either Massachusetts or Michigan, this indicates that the degree-day model should be validated in other regions before it is recommended for grower adoption outside the midwestern and eastern United States.

Although lower developmental thresholds for two closely related species leaf-mining flies are already known, these values can vary greatly among species, and therefore, they cannot be used as a starting point for the asparagus miner. For example the lower developmental threshold for pupae of *Liriomyza huidobrensis* is 7.3°C, while for *L. trifolii* it is 10.7°C (Lanzoni et al.,

2002). Both species are cosmopolitan and occur at the same latitudes as the asparagus miner, but *L. huidobrensis* is also found at higher elevations. Our study is the first investigation of the lower developmental threshold for asparagus miner pupae, which our data shows is 12.1°C. In some cases, there may be varying developmental thresholds for different life stages of the same species (Jarošik et al., 2002), although some related miner species have an isomorphic developmental rate (i.e.: *L. trifolii* and *L. huidobrensis*: Lanzoni et al., 2002). However, isomorphy must be evaluated on a species-by-species basis because stage-specific lower developmental thresholds affect the overall rate of insect development. This will be the focus of future work.

In our study, the average age of the sampled fields was 1-2 years at the beginning of our study, and since miners prefer recently planted fields, these were representative of young, vulnerable fields. In some fields we detected two population peaks, while in others the second peak was lacking or reduced, which could be due to local biotic and abiotic conditions, management actions by the growers, or the age of the asparagus field. In addition, the duration of asparagus harvest is different for young and mature fields: harvest may take 550 GDD in mature fields after the plants begin developing at ca. 100 GDD, and this overlaps with the first adult peak. Previous reports have suggested that the normal six-week harvest for mature fields acts as an impediment to asparagus miner oviposition (Bishop et al., 2004). In our study, fields with longer harvest times (e.g. field 3 in 2011 and field 2 in 2012) had a reduced second generation of asparagus miners, whereas those with shorter harvests (e.g. field 1 and 2 in 2011; field 1 and 3 in 2012) had a more pronounced second generation of adults. However, this may just be the case for the particular fields that we sampled in the current study, because when a greater number of fields were included in another study (Morrison III and Szendrei, 2013), this trend was contradicted. Moreover, extending the harvest season longer than necessary has been shown to

deplete the carbohydrate stores of young asparagus plants and reduce yields in subsequent years (Peterson, 2005), so this strategy should not be recommended to growers as a way to manage miners. While there is a clear and consistent first population peak regardless of field, our data highlights the need to pair the degree-day model with scouting in the field during the second asparagus miner adult peak to verify the presence of insects in order to appropriately target management strategies.

The appearance of the first adult asparagus miners ranged between 102-136 GDD in 2011 and 157-163 GDD in 2012, depending on the field. This variability in emergence may be due to the distance of the field from the weather station that was used to calculate the degree-days. Fields 1 and 2 were located close to the weather station while field 3 was farther away, so it is possible that the reference weather station was less representative, and as a result, the difference between the actual GDD was relatively larger. Regardless, such spatial effects are likely minor compared to the overall accuracy of the degree-day model, and may not significantly affect management decisions, at least in this bioregion.

It takes about 280 GDD between the emergence of the first adults (at 100 GDD) to the appearance of the first larvae (at 380 GDD) in the field, and 390 GDD between the first adults and pupae. The former is important to know since younger larvae may sometimes be easier to kill with pesticides than older larvae. For example, other agromyzid larvae have been shown to be more susceptible to insecticides at earlier instars than later ones (Parrella et al., 1982). Moreover, if larvae are killed soon after eclosion, they will not be able to inflict significant damage on the photosynthetic ability of the asparagus plant, and will not render the plant vulnerable to species of *Fusarium*. Up to this point, asparagus growers have not been able to kill asparagus miner larvae, as they remain protected within the stem. However, research is currently

ongoing to test systemic insecticides that may be able to target larvae (Szendrei Z, unpublished data). If effective chemistries are found, growers will then be able to use the time between the appearance of first adults and first larvae to guide decisions about when to apply reduced-risk systemic or translaminar insecticides.

According to the developmental time budget described in this study, the total life cycle of the asparagus miner lasts a mean of 1462 DD, with an upper limit of about 1925 DD. According to our degree-day model, which was calculated separately from the compilation of information from the literature about the length of different stages of the asparagus miner, the end of flight in a season for the asparagus miner should occur around 1850 DD. There are two putative explanations for the disjunction in our finding. It may be possible that the DD budget systematically underestimates the duration of the life stages for the asparagus miner. This could happen, for example, if the food we used to rear the asparagus miner adults was not as ideal as the food they have access to under natural conditions. Second, it may be possible that because the estimates for the DD budget were derived from various geographic locations around the world, there is an amount of inherent uncertainty (Andresen et al., 2001). On the other hand, the DD model estimate was uniformly developed from Michigan data, which provides greater certainty and consistency in comparing similar circumstances to each other. Regardless of the exact cause, it is likely that the full life cycle of the asparagus miner requires upwards of 2000 DD to complete.

We found that the egg stage was the shortest-lasting stage, while the pupa was the longest-lasting stage. Previous studies with closely related organisms have also found that the egg stage is usually the shortest, and the pupal stage is often the longest, for example in *Liriomyza*

bryoniae (Kaltenbach) (Diptera: Agromyzidae) (Minkenberg et al., 1990) and in *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Minkenberg, 1988).

Overall, the information presented here can be used as part of an integrated pest management program for minimizing economic damage by the asparagus miner and the non-target effects associated with pesticides. The results from this study will be incorporated into the MSU Enviro-weather website for use by asparagus growers. Overall, our results are expected to contribute to the long-term sustainability of asparagus miner management, minimize costs for growers, as well as fill in knowledge gaps about the biology of the asparagus miner.

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CHAPTER 4

The natural enemies of the asparagus miner

(Diptera: Agromyzidae)

Introduction

Natural enemies are a vital part of agroecosystems because they provide important biological control services (Gurr et al. 2003). The goal of conservation biological control programs is to maximize the abundance or efficacy of the endemic natural enemy population in order to suppress pest populations in a field or greenhouse (Barbosa 2003, Landis et al. 2000). A prerequisite of such programs is the identification and understanding of the basic biology of the natural enemies present in the system so that their biocontrol impact can be maximized through specific techniques, such as the provisioning of required resources. Much previous work in perennial agroecosystems has examined plant species or combination of species whose flowers attract and maintain a vibrant natural enemy community (Fiedler 2007, Tscharntke et al. 2007, Isaacs et al. 2009; Walton and Isaacs 2011a, b). Among the various criteria for ranking flowering plants for supporting ecosystem services, are (Fiedler et al. 2008): (1) flowers should attract the most effective natural enemy species of the pests (Maingay et al. 1991, Patt et al. 1997), (2) flowers should be accessible to the natural enemies (Baggen et al. 1999), (3) the plant should not attract other pests into the field (Kehrli and Wratten 2011), (4) the plant should be commercially available (Hickman and Wratten 1996), and (5) resources for the natural enemies should be provided throughout the entire growing season (Rebek et al. 2005, Stephens et al. 1998). As a result, plant selection is often a multi-factorial process.

The asparagus miner, *Ophiomyia simplex* Loew (Diptera: Agromyzidae), is a pest of asparagus, *Asparagus officinalis* (L.) (Asparagaceae), potentially spreading pathogenic fungi such as *Fusarium* spp. (Morrison III et al. 2011, Tuell 2003), making it a major concern to the asparagus industry (pers. comm. J. Bakker, 2013). The asparagus miner is bivoltine and its larvae bore through cortical tissue in the plant (Eichmann 1943), creating undulating mines along the asparagus stem. The miner has the potential to decrease the vigor of asparagus fields during the post-harvest period and can cause early decline of fields due to the introduction of diseases, which may reduce the economic lifespan of a field by 5–8 years.

The asparagus miner overwinters as pupae (Barnes 1937), and in Michigan, the first generation adults usually emerge in mid-May. First peak adult abundance occurs around mid-June and the second generation peaks in late July or August (Lampert et al. 1984, Morrison et al. 2013). Adequate control measures are difficult to develop because the adult flight is prolonged and the larvae are impervious to foliar insecticide sprays, because they remain protected within the asparagus stems.

The natural enemies of the asparagus miner remain poorly studied, especially in continental North America where the only recorded parasitoids are two species originally described from Europe, *Chorebus rondanii* (Giard) (Braconidae) and *Pediobius epigonus* (Walker) (Eulophidae) (Supplemental Table S5). Mailloux et al. (2004) provided an illustrated identification guide to the parasitoids and predators found in asparagus fields in Canada, but this work is not specific to the asparagus miner and, except for one pest and parasitoid species, no information is given on abundance, behavioral or ecological data of the natural enemies. Further, the photograph given for *P. epigonus*, identified as *Pleurotropis epigonus* (Mailloux et al. 2004, Figure 6), is a female of the family Pteromalidae (Hymenoptera) and not a eulophid. In Europe,

in addition to *C. rondanii* and *P. epigonus*, *Neochrysocharis moczari* Szelényi (Eulophidae) was described as a parasitoid of the asparagus miner in Hungary (Szelényi 1973). Barnes and Walton (1934) also reared *P. epigonus, Dacnusa bathyzona* Marsh (Braconidae), and *Sphegigaster* sp. (Pteromalidae) from asparagus miner pupae in the United Kingdom. Finally, *Centrodora xiphidii* Perkins (Aphelinidae) is recorded as a parasitoid of the asparagus miner in Hawaii (Bianchi 1941). A few other surveys of the natural enemies of asparagus pests other than the asparagus miner have also been conducted. In a survey of the natural enemies of the asparagus aphid, *Brachycorynella asparagi* Mordvilko (Hemiptera: Aphididae) in New Jersey, the most commonly identified predators were Coccinellidae (Coleoptera) and Chrysopidae (Neuroptera), though Syrphidae (Diptera), Anthocoridae, and Nabidae (Hemiptera) were also found in low abundance along with some other taxa (Angalet and Stevens 1976). The most common parasitoid found in this and another study was *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) (Angalet and Stevens 1976, Stary 1990). In a study of the common asparagus beetle, *Crioceris asparagi* L. (Coleoptera: Chrysomelidae), *Tetrastichus coeruleus* (Nees) (identified as *Tetrastichus asparagi* Crawford) (Hymenoptera: Eulophidae) was described as a parasitoid (Capinera and Lilly 1975, van Alphen 1980).

The goals of the current study were to determine for commercial asparagus fields in Michigan: (1) the identity of pupal parasitoids of the asparagus miner, (2) the effect of different diets and floral resources on the lifespan of adult asparagus miners and their pupal parasitoids in order to determine the potential for the use of floral resources in managing the parasitoid community, (3) how parasitism affects damage on asparagus stems, and (4) the field-level abundance of predators, parasitoids and pests.

Materials and Methods

Study site. Three commercial farms were used in 2010, 2011 and 2012, and two farms in 2013. Multiple fields were used at some of the farms so there was a total of five field sites in the first two years, three in 2012, and four in 2013. Fields were located 1.21–16.14 km apart in Oceana County, MI (Supplemental Table S6), the major asparagus-producing region of Michigan. The asparagus fields were bordered by habitats characteristic of Michigan (Morrison and Szendrei 2013).

Insect collections. Immature asparagus miner stages were collected as per Morrison and Szendrei (2013). Specifically, 8–53 stems were collected from each field (mean ± SEM: 33.8 ± 11 stems) every 6–10 d throughout the growing season from 1–5 fields per sampling date. The dates of collection ranged from 22 July to 17 September in 2010, 31 May to 3 October in 2011, 14 June to 7 October in 2012, and 18 June to 5 September in 2013. Asparagus stems were collected at least 30 m into the field by cutting them 5 cm below the soil surface, and then again at the height of the longest mine. Stems were sealed in plastic bags and placed in a cooler with ice packs until transported to the laboratory. Samples were stored at 5°C in the laboratory, and the stems were processed within 1–14 d. A razor blade was used to carefully peel the epidermis from the asparagus stem to reveal the immature stages. For each stem, we recorded: the number of larvae, pupae, castings (puparia), as well as the length of the longest mine, and the percent mining damage within the bottom 5 cm of the stem. Damage measures were taken to evaluate the effects of parasitism on parasitized versus unparasitized stems. Pupae were placed individually in cups (3 x 5.5 cm H:D) in 2010, 2011, and 2013 or Petri dishes in 2012 (1.5 x 6 cm H:D) and stored at 5°C until used in the diet assay or floral assay (described below). Sets of 60 pupae were placed in an environmental chamber at a constant 26°C (16:8 L:D cycle, 75% RH) or on a lab

bench (16:8 L:D, 23.7±0.2°C) every week during the experiments to allow adults to emerge. Emergence of adult asparagus miners or parasitoids was checked daily, asparagus miners were sexed (only from pupae collected in 2013), the family of parasitoid was recorded, and pupal duration was calculated.

Parasitoid identification. All parasitoids from all years and experiments were identified to family using an authoritative key to the Nearctic Chalcidoidea (Gibson et al. 1997), and other keys for Hymenoptera (e.g. Marshall 2006, Evans 1978). Parasitoids not used in other experiments in 2010–2012 were sent for species identification to the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) in Ottawa, Ontario. Consequently, species identifications were not made for some 2010–2012 individuals, but were made for all individuals reared in 2013. Voucher specimens are deposited in the CNC and at the Michigan State University A.J. Cook Arthropod Research Collection (voucher #2014-01) in East Lansing, MI.

Diet assay. Upon emergence from pupae, adult asparagus miners or parasitoids were fed one of five different diets in random order as they emerged on different days. Bioassays were conducted from 2010-2013 with pupae collected from the field during the same period. Adults were fed only one kind of diet *ad libitum* over their lifespan. The diets included: no food (N=41 individuals), water only (N=89), sugar solution (20.4% m/v; N=91), honey solution (10% v/v; N=120), or asparagus spears (fresh, snapped at the tip; N=17). The water, sugar and honey solutions were added to a piece of cotton ball and presented to the adults in their containers. Adults were kept either on a lab bench or in an environmental chamber at a constant 26°C temperature, and the temperature was monitored with a digital thermometer daily. Adults were

checked daily, and the lifespan was calculated by subtracting the date of death from the date of emergence.

Floral resource assay. Some of the newly emerged adult asparagus miners or parasitoids from the pupal collections were used in this experiment, spanning 27 July 2012 to 10 October 2013. Each adult was placed in a mesh cage (47.5 x 47.5 x 93 cm L:W:H from Megaview Science Co., Ltd., Taichung, Taiwan) with one of four different plants and provided with supplemental water plus a negative control of an adult insect with water only. Water was provided from a screw cap vial (2 dram from Bioquip Products, Rancho Dominguez, CA, USA) via a folded wipe (11 x 21 cm W:L from Kimberly-Clark Professional, Roswell, GA, USA) sealed with Parafilm (Parafilm "M", Pechiney Plastic Packaging, Menasha, WI, USA) to prevent evaporation and drowning of the adult. Adults were kept in the laboratory on a 16:8 L:D cycle with full-spectrum lights (120 W, Agro-Lite BR40, Philips Lighting Company, Somerset, NJ) suspended above the cages, at 23.7 ± 0.33˚C. Though there were not always enough adults emerging on a given day to run the full set of treatments, whenever two or more adults emerged, one control treatment was concurrently included. The total lifespan and sex of the adult was recorded in 2013. Sex ratio was about 1:1.

The four different plants used were *Fagopyrum esculentum* (Polygonaceae) (buckwheat, exotic, annual), *Lobularia maritima* (Brassicaceae) (sweet alyssum, var. carpet of snow, exotic, annual), *Vicia faba* (Fabaceae) (faba bean, var. broad Windsor, exotic, annual), and *Oligoneuron ridellii* (Asteraceae) (Riddell's goldenrod, native, perennial). The negative control consisted of adults with water only without any potted plants. Plants were selected based on previously published literature on attraction of natural enemies to each plant species (e.g. Fiedler and Landis, 2007a, b), ability of parasitoids/asparagus miner adults to use flowers, and commercial

availability. The annual plants were raised in the greenhouse from seed (Eden Brothers, Asheville, NC, USA). A single seed was planted in a pot (10.5 x 10.5 x 12.5 cm L:W:H) into potting soil (SureMix Perlite, Michigan Grower Products, Inc., Galesburg, MI, USA). Riddell's goldenrod was obtained from WildType Nursery (Mason, MI, USA) from seed planted in 2011. Experiments were carried out in a laboratory at Michigan State University's campus (East Lansing, MI). At least seven individuals of each annual species were planted weekly, and all plants were watered daily during the cool season and twice daily during the summer. Plants were kept on a 16:8 L:D cycle throughout the year, except Riddell's goldenrod (a perennial), which was allowed to senesce outside in ambient temperatures and light conditions during the winter. Fertilizer (20-20-20 N:P:K with micronutrients, J. R. Peters, Allentown, PA, USA) was delivered to plants in liquid form (1.2% v/v).

Pests and natural enemies in the field. Sticky traps were deployed from 14 May to 5 September 2013 in four commercial fields. A single yellow sticky card (7.62 x 12.7 cm W:L from Great Lakes IPM, Vestaburg, MI, USA) was placed on three edges of a field, and changed every 6–8 days during the growing season. From each trap, the identity and number of asparagus pests and natural enemies were recorded to the lowest possible taxonomic unit in consultation with online resources and Marshall (2006).

Statistical analysis. The data from the diet assay were analyzed by a 2-way factorial ANOVA with unequal sample sizes in R Software (R Core Development Team 2013). The response variable was the lifespan (days) of adult parasitoids or asparagus miners, while the two explanatory factors were diet type and insect taxonomic (family) identity. The residuals did not conform to the assumption of normality, so the data were natural log-transformed. Pairwise tests

were performed using Tukey's HSD test. Our critical value for significance for all tests was $\alpha=0.05$.

The data from the floral resource assay were analyzed with a one-way factorial ANOVA with unequal sample sizes. The response variable was lifespan of asparagus miner adults. Flower type was the explanatory variable. The residuals from the data were inspected and conformed to a normal distribution, so no transformation was required. Pairwise post-hoc comparisons were performed with Tukey's HSD.

In order to evaluate how parasitism of asparagus miner pupae affects damage in the field, Welch t-tests and multiple regression were performed on damage data recorded from collected stems that were linked with whether they had parasitized pupae. Specifically, three t-tests were performed that examined whether the average percent stem mined (within 5 cm of the base of the stem), the average longest mine, or average asparagus miner pressure (composite measure including asparagus miner pupae, larvae, and puparia) were significantly different between stems that either had or had not experienced parasitism. The average percentage stem mined was arcsine-transformed in order to theoretically give the variable more freedom to vary so that it can fulfill the requirements of a t-test, namely that the variable is not bounded by limits. The residuals from the average percent stem mined were normally distributed with homogenous variance. In addition, the residuals from the other t-tests were inspected to confirm assumptions of normality, but variances were unequal. As a result, the Welch degrees-of-freedom correction was applied. To understand what proportion of asparagus miner pupae were parasitized in a stem by different parasitoid families, a Kruskal-Wallis test was performed with the percent of miner pupae parasitized as the response variable and the number of pupae parasitized from a given family as an explanatory variable (classified as a factor). This test was used because parametric

assumptions were not fulfilled, even after transformation. Multiple comparisons among the parasitoid families were performed using the Steel-Dwass algorithm after a significant result from the Kruskal-Wallis test. In addition, multiple regression was performed with the amount of damage per stem (longest mine) as the response variable and the asparagus miner pressure as the continuous explanatory variable separately for stems that were either parasitized or unparasitized.

Because we were primarily interested in what may be eating the asparagus miner or competing with it during each of the asparagus miner's life stages (see Morrison III et al. 2013), a Wilk's MANOVA test identified differences in the abundance of pests, predators, and parasitoids (all coded as response variables) between the two generations (explanatory variable) of the asparagus miner. None of the response variables conformed to the assumptions of normality, and so the predators and parasitoids were log-transformed, while the pest numbers were inverse square root-transformed. Inspection of residuals afterwards indicated that assumptions of normality were fulfilled. The first generation was defined as the period between 121.5–653.3 GDD (N=96 yellow sticky traps; base 12.1°C, Mar 1st biofix, Baskerville-Emin calculation method) (Baskerville and Emin 1969, Morrison III et al. 2013), whereas the second generation was defined as the period between 653.3–1557.5 GDD (N=108) in 2013. With a significant MANOVA, sequential univariate ANOVAs were performed for each response variable to determine differences among means.

Results

Parasitoid identity and abundance. A total of twelve hymenopterous parasitoid species were reared from asparagus miner pupae, including *Chorebus rondanii* (Braconidae) and at least ten species in three families of Chalcidoidea (Eulophidae, Eupelmidae and Pteromalidae) plus

one species in a family of Chrysidoidea (Bethylidae). The pteromalids were *Cyrtogaster vulgaris* Walker, *Merismus megapterus* Walker, *Sphegigaster cracentis* Heydon & LaBerge, *Thinodytes cephalon* (Walker), *Spaniopus dissimilis* Walker, *Halticoptera* sp., and *Trichomalopsis viridascens* (Walsh). The eupelmids were *Eupelmus vesicularis* (Retzius) and a species questionably identified as *Brasema allynii* (French). There was also a single eulophid that was identified as either *Neochrysocharis formosus* (Westwood) or *N. diastatae* (Howard). The bethylid was *Laelius utilis* Cockerell (Table 4.1.; Figure 4.1.). All of the chalcids and the bethylid represent new parasitoid records for the asparagus miner.

All of the parasitoids were solitary except for the eulophid, which had an average of 9.3±0.8 individuals per pupa. Most of the reared parasitoids belong to Pteromalidae, which parasitized about 17% of the asparagus miner pupae collected (Table 4.2.). Pteromalidae had a 3-fold greater abundance than the next most common family, Braconidae. Eulophidae accounted for less than 1.4% of total parasitism, and Eupelmidae and Bethylidae was the lowest at 0.8 and 0.1% of all the pupae, respectively. Overall, the single most prevalent parasitoid was *Thinodytes cephalon*, parasitizing 7.2% of the total asparagus miner pupae, while the second most abundant parasitoid was *Chorebus rondanii*, which parasitized 6.0% of the total asparagus miner pupae. Total parasitism by all species ranged from about 40% in 2010 to 15.5% in 2013. Overall parasitism for all years and species reached over 25%.

Diet assay. The diet that asparagus miner or parasitoid adults were fed significantly affected their lifespan (ANOVA: $F_{4,80} = 86.74$, $P<0.001$; Figure 4.2.), as did the family identity (including the families to which the pest and parasitoids belonged, ANOVA: $F_{2,168} = 18.63$, $P<0.001$). Specifically, adults fed a sugar-rich diet (e.g. honey or sugar solution) had a significantly greater life span compared with adults fed water only, or snapped asparagus spears

(Figure 4.2.). Adult parasitoids fed a sugar-rich diet lived about twice as long as asparagus miner adults fed on the same diet, with a lifespan of about 13.8 d compared to 7.3 d for the asparagus miner. Relative to the control (e.g. water only), the life span of asparagus miner adults increased by a factor of 5.2, whereas the life span of parasitoid adults increased by a factor of 7.7 when fed sugar-rich diets.

Floral resource assay. The flower species that an asparagus miner adult had access to significantly influenced its longevity (ANOVA: $F_{4,70} = 38.06$, $P<0.001$). Specifically, miners which were enclosed with Riddell's goldenrod lived about twice as long as those that had access to water only (Figure 4.3.). The asparagus miner life span on buckwheat and faba bean was similar to that in the control. Sweet alyssum resulted in intermediate life spans for the asparagus miner, with adults living about 2.5 d on average.

Asparagus miner damage and parasitism. There was a significant positive correlation between the number of pupae parasitized on a stem and the total number of asparagus miner pupae found on the stem (Adj. $R^2=0.883$, $P<0.001$; Figure 4.4.). The highest number of pupae that were parasitized on a stem was 4 (Figure 4.4.), though it was much more common to find stems with 1 or 2 parasitized pupae (Morrison, unpublished data). Moreover, there was a significant positive relationship between the amount of damage on the stem and the amount of asparagus infestation (combined count of asparagus miner pupae, larvae and puparia) when stems were unparasitized (Adj. $R^2=0.20$, $m=1.09$, $P<0.001$; Figure 4.5.). However, there was no significant relationship for stems that had parasitized pupae (Adj. $R^2 = 0.02$, $m=0.568$, $P<0.313$). When a stem was parasitized by braconids, only 40% of the asparagus miners in a given stem were parasitized on average (Kruskal-Wallis: $\chi^2=18.35$, $df=3$ $P<0.001$; Table 4.3.). Eupelmids attacked asparagus miner pupae when there was only a single individual located on a stem

(Morrison, unpublished data). Eulophids parasitized twice as many pupae per stem, relative to the braconid parasitoids (Table 4.3.). Overall, stems that were parasitized had 1.4 times longer mines, 1.2 times greater proportion of the stem mined, and almost twice as many asparagus miner life stages (AM Pressure) than unparasitized stems (Table 4.4.).

Pests and natural enemies in the field. Abundance between the first and second generation for natural enemies and pests were significantly different (MANOVA: $F_{1,199}$=56.99, P<0.001). Parasitoids were generally caught in low frequencies in the field on the yellow sticky traps (Table 4.5.), with a higher abundance during the first than the second asparagus miner generation (ANOVA: $F_{1,199}$=53.08, P<0.001). The most commonly caught parasitoids on yellow sticky traps belonged to Ichneumonidae (Hymenoptera) and Tachinidae (Diptera). Predators were 8.5-fold more abundant than parasitoids during the first asparagus miner generation, and 13-fold more abundant in the second generation (Table 4.5.). There were a total of 23 predator taxa, and of those, Asilidae (Diptera) was the most abundant, constituting a little more than 70% of the individuals in the first generation, and over 45% of the predators in the second generation. Numerically, the abundance of most predator groups increased from the first to the second generation, except asilids, which declined in abundance from the first to the second generation by 41%, though this trend was not significant (ANOVA: $F_{1,199}$=0.067, P=0.796). Pests were significantly more abundant in the second generation compared with the first (ANOVA: $F_{1,199}$=120.98, P<0.001). In terms of abundance of various pest groups, the asparagus miner outnumbered the next most common pest, the tarnished plant bug, by about 80 to 1. The least abundant pest was the asparagus beetle, followed next by Scarabaeidae (Coleoptera).

Table 4.1. Summary of life history parameters for parasitoids emerging from asparagus miner pupae in Oceana Co., MI during 2010-2013.

Species	Known hosts	Diet breadth	Distribution	Stage parasitized	Parasitoid type	Aggregation	Gen. (year^{-1})	Citations
Pteromalidae								
Halticoptera sp. Spinola	27 species of Agromyzidae, 6 species from 3 other Dipteran families (Cecidiomyiidae, Chloropidae & Opomyzidae)	Specialist on agromyzids and other stem and leaf-mining Diptera	Holarctic, neotropical & afrotropical	Larvae	Primary	Solitary	Un-known	Hagvar et al., 1998; Lynch and Johnson, 1987; Noyes, 2013
Merismus megapterus Walker	5 species of Agromyzidae, 4 species of Elachistidae (Lepidoptera)	Specialist on concealed feeders	Holarctic			Solitary	Un-known	Kamijo, 1996
Spaniopus dissimilis Walker	12 species in 4 orders: Diptera (mainly *Phytophaga destructor*), Hymenoptera, Araneae and Lepidoptera	Polyphagous	Holarctic	Eggs?/ pupae	Primary or rarely a hyper-parasitoid	Solitary	Un-known	Bouček, 1972; Noyes, 2013
Sphegigaster cracentis Heydon	unknown, poss. Cecidiomyiidae	Unknown	Nearctic	Pupae	Unknown	Solitary	~1-2	Heydon and Laberge, 1988
Cyrtogaster vulgaris Walker	21 hosts in Agromyzidae, other hosts in 14 families across 5 orders: Diptera, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera	Polyphagous	Holarctic	Pupae	Primary/fac-ultative hyper-parasitoid (in a single report for a eulophid)	Solitary	1-2	Guppy and Meloche, 1989; Noyes, 2013

Table 4.1 (cont'd)

Species	Known hosts	Diet breadth	Distribution	Stage parasitized	Parasitoid type	Aggregation	Gen. (year^{-1})	Citations
Thinodytes cephalon (Walker)	*Phytophaga destructor* *Meromyza americana* *Liriomyza trifoliearum* *Oscinella frit*	Specialist on leaf and grass-stem mining Diptera	Nearctic & neotropical	Larvae	Pupal?	Solitary	2	Heydon, 1995; Allen and Pienkowski, 1973
Trichomalopsis viridascens (Walsh)	Hosts in 16 families from 4 orders: Diptera, Hymenoptera, Lepidoptera and Coleoptera	Highly poly-phagous	Nearctic	Pupae	Braconid & ich-neumonid hyper-parasitoid, always primary parasitoid for Diptera	Solitary	Un-known	Gibson and Floate, 2001
Eupelmidae								
Eupelmus vesicularis (Retzius)	Over 100 hosts in: Diptera, Coleoptera, Hymenoptera, and Lepidoptera	Highly poly-phagous	Holarctic	Pupae	Primary or hyperparasito id	Solitary	Un-known	Gillespie et al., 2006; Ellis and LeRoux, 1964
Brasema allynii (French)	Hosts in 28 families spread across 6 orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera)	Highly poly-phagous	Nearctic	Pupae	Primary or hyperparasito id	Solitary	4	French, 1884; Schuster and Lidell, 1990; Thompson and Solomon, 1986

Table 4.1. (cont'd)

Species	Known hosts	Diet breadth	Distribution	Stage parasitized	Parasitoid type	Aggregation	Gen. (year^{-1})	Citations
Brasema allynii (French)	Hosts in 28 families spread across 6 orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera)	Highly poly-phagous	Nearctic	Pupae	Primary or hyperparasitoid	Solitary	4	French, 1884; Schuster and Lidell, 1990; Thompson and Solomon, 1986
Braconidae								
Chorebus rondanii (Giard)	*Ophiomyia simplex* Loew	Host-specific	Holarctic	Pupae	Primary	Solitary	Un-known	Giard, 1904; Barnes and Walton, 1934
Eulophidae								
Neo-chrysocharis[b] sp. -*N. formosus* (Westwood) or *N. diastatae* (Howard)	12 species in Agromyzidae, 16 other species spread across 4 orders (Diptera, Coleoptera, Hymenoptera, Lepidoptera)	Poly-phagous	Holarctic & neotropic	Larvae/ pupae	Primary	Gregarious	~2	Johnson, 1987; Schuster and Wharton, 1993
Bethylidae								
Laelius utilis Cockerell	5 species, all dermestids (Coleoptera)	Dermestid specialist[c]	Nearctic	Pupae	Primary	Solitary	~7[d]	Mertins, 1985; Evans, 1964; Evans, 1978

[a] Information based on a very tentative identification of *H. circulus*. However *Halticoptera* sp. are unrevised in North America, so the exact species may be different.

Table 4.1. (cont'd)

[b] Information for *Neochrysocharis* spp. is derived from species data relating to *N. diastatae* (Howard), however *N. formosus* (Westwood) is also polyphagous.

[c] Bethylidae is commonly found associated with hosts living in cryptic situations, such as soil, plant stems, and other concealed locations.

[d] For indoor locations.

Table 4.2. Number of parasitoids and percent parasitism of asparagus miner pupae collected between 2010 and 2013 in six commercial asparagus fields in Oceana County, MI.

	2010[d]		2011[e]		2012[f]		2013[g]		Total[h]	
	Abund	% Parasitism	Abund	% Parasitism	Abund	% Parasitism	Abund	% Parasitism	Abund	% Parasitism
Braconidae										
Chorebus rondanii	3	1.6	22	5.5	20	15.7	16	5.2	61	6.0
Overall Braconidae[a]	**3**	**1.6**	**22**	**5.5**	**20**	**15.7**	**16**	**5.2**	**61**	**6.0**
Pteromalidae										
Pteromalidae morph[b]	40	21.5	27	6.8	7	5.5	0	0.0	74	7.2
Cyrtogaster vulgaris	2	1.1	7	1.8	3	2.4	5	1.6	17	1.7
Halticoptera sp.	0	0.0	0	0.0	0	0.0	1	0.3	1	0.1
Merismus megapterus	0	0.0	1	0.3	0	0.0	1	0.3	2	0.2
Spaniopus dissimilis	0	0.0	0	0.0	0	0.0	1	0.3	1	0.1
Sphegegaster cracentis	0	0.0	2	0.5	0	0.0	0	0.0	2	0.2
Thinodytes cephalon	26	14.0	28	7.0	7	5.5	13	4.2	74	7.2
Trichomalopsis viridascens	0	0.0	1	0.3	0	0.0	1	0.3	2	0.2
Overall Pteromalidae[b]	**68**	**36.6**	**66**	**16.5**	**17**	**13.4**	**22**	**7.1**	**173**	**16.9**
Eupelmidae										
Brasema ?allynii	0	0.0	0	0.0	1	0.8	1	0.3	2	0.2
Eupelmus vesicularis	0	0.0	2	0.5	2	1.6	2	0.6	6	0.6
Overall Eupelmidae[b]	**0**	**0.0**	**2**	**0.5**	**3**	**2.4**	**3**	**1.0**	**8**	**0.8**
Eulophidae										
Neochrysocharis sp.[c]	2	1.1	6	1.5	0	0.0	6	1.9	14	1.4
Overall Eulophidae[b]	**2**	**1.1**	**6**	**1.5**	**0**	**0.0**	**6**	**1.9**	**14**	**1.4**
Bethylidae										
Laelius utilis	0	0.0	0	0.0	0	0.0	1	0.3	1	0.1
Overall Bethylidae	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.3**	**1**	**0.1**
Total parasitism	**73**	**39.2**	**96**	**24.0**	**40**	**31.5**	**48**	**15.5**	**257**	**25.1**

[a] Combined abundance and parasitism rates for all the morphs/species within a family.

[b] Morph designation indicates that the individual was only taken to the family level because species-level identification was not possible at time of emergence and specimen was since used for the diet and floral assays.

[c] Identified to either *N. formosus* (Westwood) or *N. diastatae* (Howard)

[d] N=186 pupae collected in 2010, and the total number of asparagus miners that emerged was 113 adults.

[e] N=400 pupae collected in 2011, and the total number of asparagus miners that emerged was 304 adults.

Table 4.2. (cont'd)

[f] N=127 pupae collected in 2012, and the total number of asparagus miners that emerged was 87 adults.

[g] N=309 pupae collected in 2013, and the total number of asparagus miners that emerged was 261 adults.

[h] N=1022 pupae collected over four years, and the total number of asparagus miners that emerged was 765 adults.

Table 4.3. Summary of the average percent of asparagus miner pupae parasitized per asparagus stem by parasitoid family from pupae collected in Oceana Co., MI from 2011-2013

		Mean/stem			
Family	**N**	**% AM[a] pupae parasitized**	**±**	**SEM[b]**	
Braconidae	36	40	±	0.06	b
Eulophidae	9	81	±	0.18	a
Pteromalidae	65	46	±	0.03	ab
Eupelmidae	3	100	±	0.01	a

[a] Abbreviation: AM – asparagus miner

[b] Rows with shared letters are not significantly different from one another from Steel-Dwass multiple comparisons: Kruskal-Wallis, χ^2=18.35, df=4, P<0.001.

Table 4.4. Summary of damage and of asparagus miner pupal abundance on parasitized or unparasitized asparagus stems.

	Parasitized			Unparasitized			Between group test		
Measure	N	Mean	SEM	N	Mean	SEM	t	df[e]	P
Longest mine[a]	113	14.8	± 0.61	3534	10.8	± 0.12	-6.34	118.1	<0.0001
% Stem mined[b]	113	97.2	± 0.09	3534	81.4	± 0.58	-13.57[d]	118.1	<0.0001
AM[c] pressure	113	6.06	± 0.03	3534	3.24	± 0.05	-8.77	117.3	<0.0001

[a] in centimeters

[b] This is a measure of the amount of the stem surface that has been girdled within the bottom 5 cm of the asparagus stem.

[c] AM (=asparagus miner) pressure is a composite measure which additively combines the number of pupae, larvae, and castings (puparia) for a given stem.

[d] Before testing, this variable was arcsine transformed to fulfill the requirements of a t-test.

[e] Welch correction applied to degrees of freedom.

Table 4.5. Summary of abundance of the major natural enemy and pest taxa found in asparagus fields on yellow sticky traps from Oceana Co., MI during 2013.

	First generation[d]				Second generation			
	Mean	±	SEM	Total	Mean	±	SEM	Total
Natural enemies[a]								
Parasitoids								
Braconidae	0.10	±	0.03	10	0.05	±	0.00	5
Eupelmidae	0.01	±	0.01	1	na	±	na	0
Pteromalidae	0.01	±	0.01	1	0.01	±	0.01	1
Eulophidae	na	±	na	0	0.04	±	0.00	4
Bombylidae	0.03	±	0.09	3	0.04	±	0.04	4
Tachinidae	0.07	±	0.03	7	0.14	±	0.15	15
Ichneumonidae	0.26	±	0.25	25	0.02	±	0.00	2
Chrysididae	0.07	±	0.07	7	0.06	±	0.00	6
Overall number of parasitoids	**0.56**	**±**	**0.10**	**54**	**0.34**	**±**	**0.06**	**37**
Predators								
Anisoptera	na	±	na	0	0.01	±	na	1
Asilidae	2.99	±	0.49	287	1.77	±	0.41	191
Cantharidae	0.02	±	0.90	2	0.09	±	0.06	10
Carabidae	0.11	±	0.03	11	0.07	±	0.06	8
Coccinellidae	0.11	±	0.03	11	0.11	±	0.03	12
Dolichopodidae	0.02	±	na	2	0.20	±	0.06	22
Lacewing	0.02	±	0.00	2	0.11	±	0.05	12
Minute Pirate Bug	0.01	±	na	1	0.39	±	0.10	42
Nabidae	0.05	±	0.04	5	0.02	±	0.00	2
Staphylinidae	0.04	±	0.05	4	0.05	±	0.04	5
Scoliidae	0.13	±	0.22	12	0.23	±	0.08	25
Spiders	0.09	±	0.06	9	0.03	±	0.00	3
Syrphidae	0.17	±	0.05	16	0.31	±	0.15	33
Vespidae	0.03	±	0.00	3	0.06	±	0.04	7
Zygoptera	0.02	±	0.00	2	0.01	±	na	1
Overall number of predators	**3.82**	**±**	**0.44**	**367**	**3.46**	**±**	**0.40**	**374**
Pests								
Asparagus miner	14.90	±	1.59	1430	12.60	±	1.38	1361
Other pests								
Asparagus beetle	0.02	±	0.00	2	na	±	na	0
Flea beetle	na	±	na	0	0.24	±	0.15	26
Plant bugs	0.03	±	0.06	3	0.03	±	0.20	6
Scarabaeidae[b]	0.01	±	0.01	1	0.02	±	0.01	2
Tarnished plant bug	0.21	±	0.16	20	0.14	±	0.14	15
Overall number of pests[c]	**0.27**	**±**	**0.07**	**26**	**0.45**	**±**	**0.09**	**49**

[a] Other groups than those listed in this table were counted, but if the total number of individuals was ≤2, then they were excluded from this list. These groups included: Apidae, Berytidae, *Oulema melanopus*, Colletidae, Coreidae, Melyridae, Reduviidae, Sphecidae, and Cicindelinae.

Table 4.5. (cont'd)

[b] This family includes the following species: *Strigoderma arboricola*, *Popillia japonica*.

[c] Overall averages of pests do not include asparagus miner numbers so as to not unduly influence the measure.

[d] This is the overall number of traps through the season. First generation of the asparagus miner is N=96 and lasts from 121.5-653.3 GDD (49 Julian days), while second generation of the asparagus miner is N=108 and lasts from 653.3-1557.5 GDD (65 Julian days).

Figure 4.1. Habitus images of adult parasitoids that emerged from asparagus miner pupae collected from stems in commercial plantings of asparagus from Oceana Co., MI during 2010-2013: (1) *Thinodytes cephalon* (male and female), (2) *Cyrtogaster vulgaris* (male and female), (3) *Sphegigaster cracentis* (male and female), (4) *Trichomalopsis viridascens* (female and male), (5) *Merismus megapterus* (female), (6) *Spaniopus dissimilis* (female), (7) *Halticoptera* sp. (female), (8) *Eupelmus vesicularis* (female), (9) *Brasema ?allynii* (female and male), (10) *Chorebus rondanii* (female), (11) *Neochrysocharis* sp. (female), and (12) *Laelius utilis* (female).

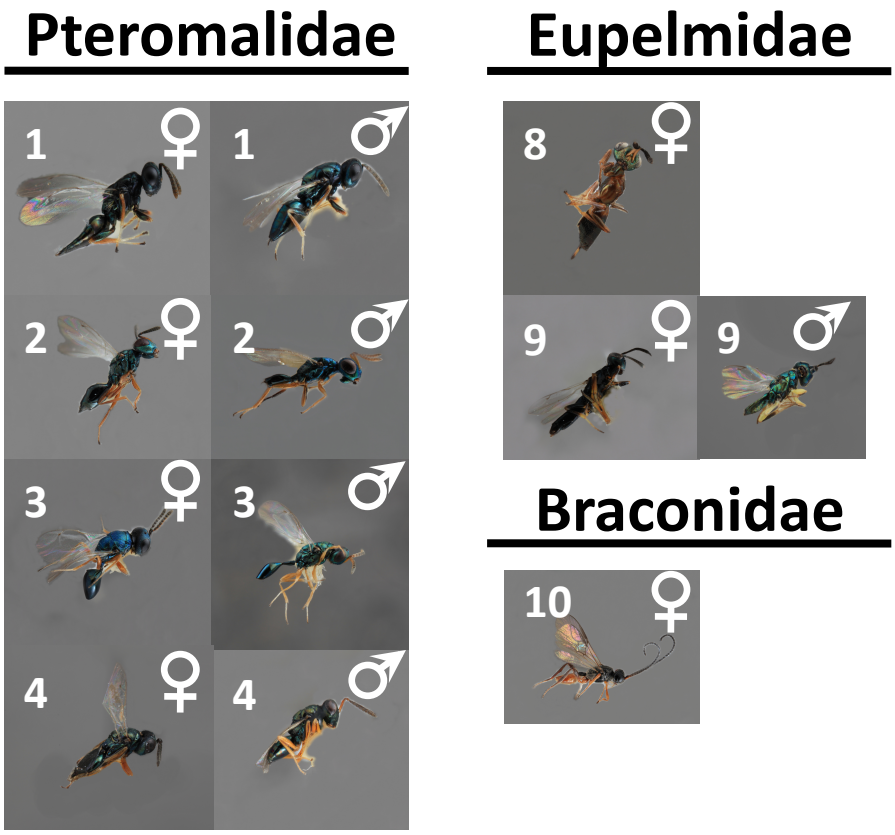


Figure 4.1 (cont'd)

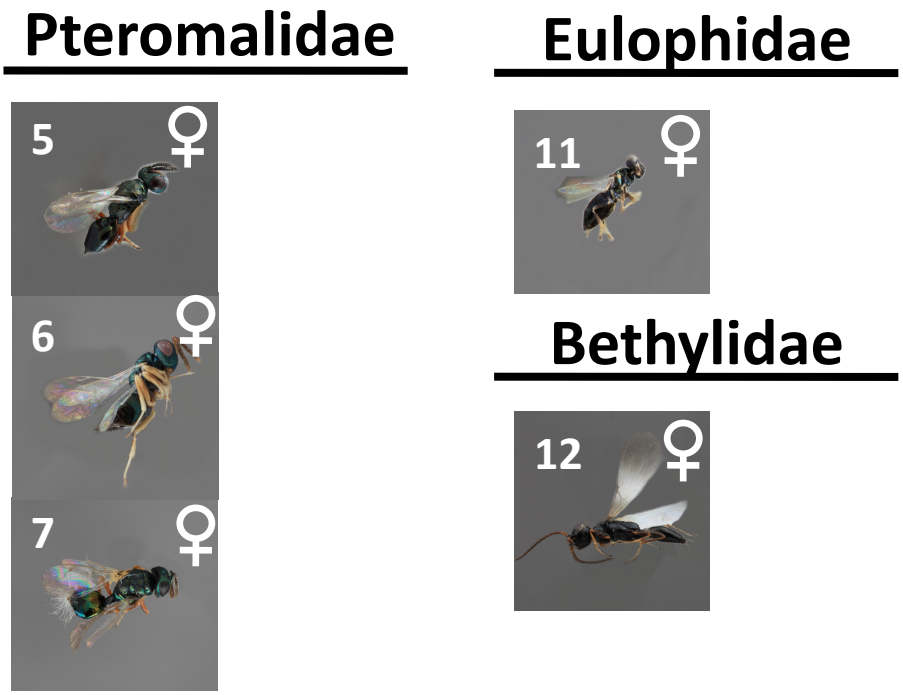


Figure 4.2. Longevity of asparagus miner adults and two groups of parasitoids (Braconidae and Pteromalidae) on various diets. Adults were fed ad libitum during their lifespan. Diets (across species) with shared and capitalized letters are not significantly different from one another (Tukey's HSD). Lower case letters represent comparisons within a diet, among species (Tukey's HSD).

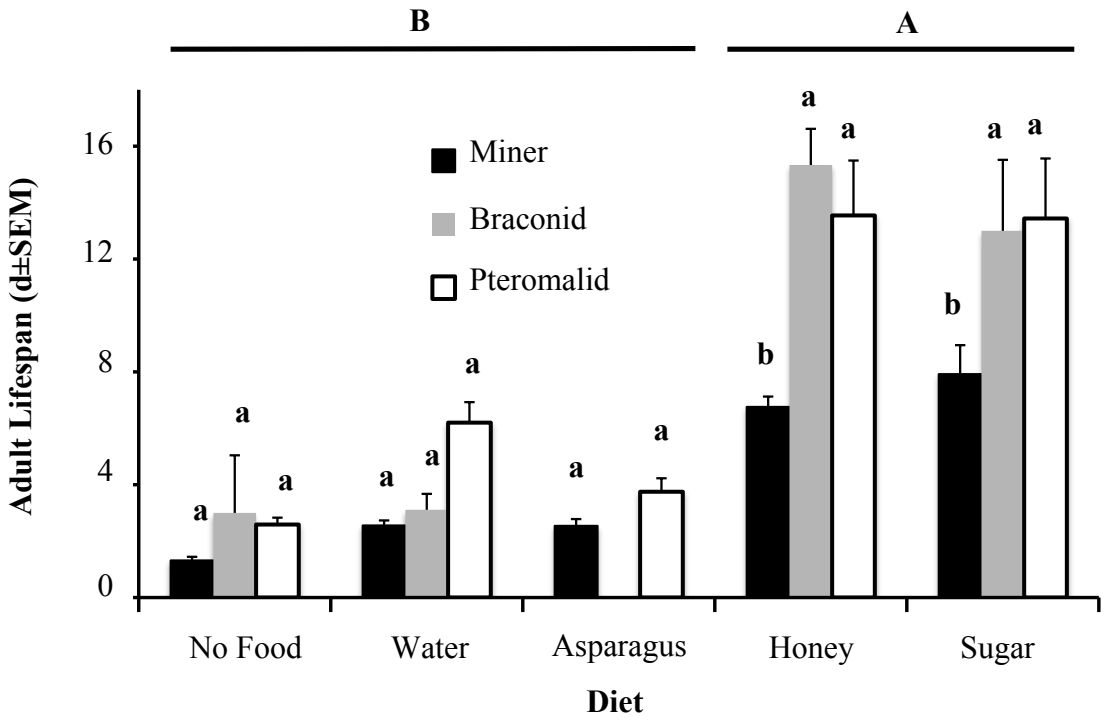


Figure 4.3. Longevity of asparagus miner adults fed on various flowers. Species with a shared capital letter are not significantly different from one another (Tukey's HSD). The sample size of adults tested is above each bar.

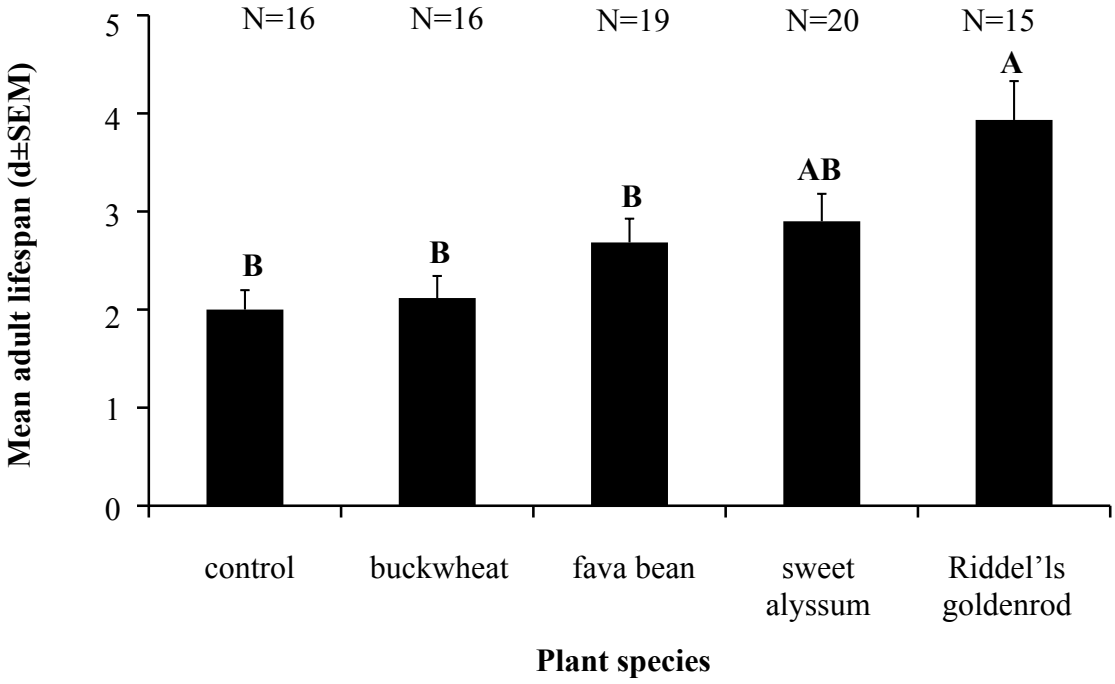


Figure 4.4. Relationship between the number of parasitized asparagus miner pupae per stem and the mean number of asparagus miner pupae per stem (±SEM) for stems collected from Oceana Co., MI between 2011 and 2013. There is a significant positive correlation between parasitism and the number of pupae per stem (R^2=0.883, P<0.0001, y = 3.39x – 0.0068).

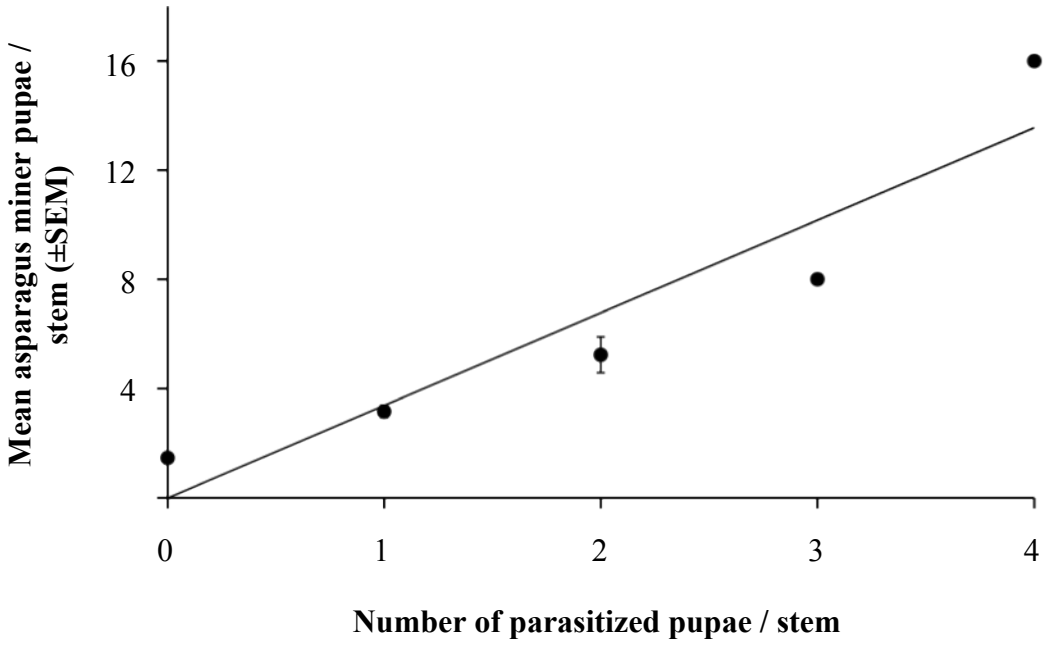
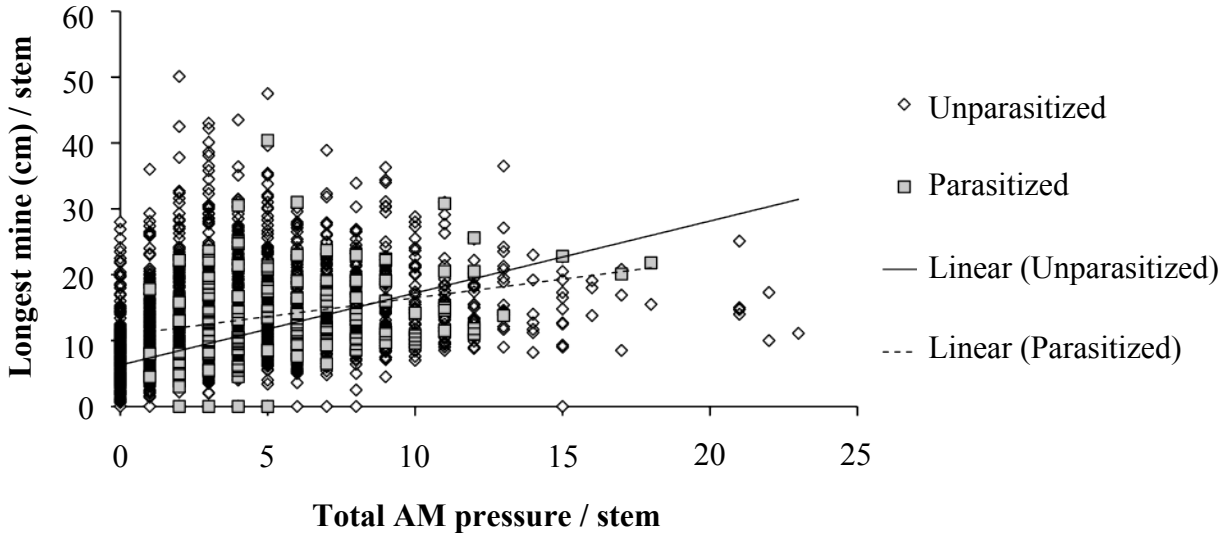


Figure 4.5. Relationship between the amount of damage on a stem (mine length) and the total abundance of various life stages of the asparagus miner (AM pressure: asparagus miner pupae, larvae, and castings) for stems that are unparasitized (open diamonds) or parasitized (grey squares). Asparagus stems were collected from commercial fields in Oceana Co., MI from 2011-2013. Multiple regression was performed for unparasitized (solid line; R^2=0.201, P<0.0001; y = 1.09x + 6.27) and parasitized (dotted line; R^2 = 0.02, N.S.; y = 0.568x + 10.78) stems.



Discussion

Of the parasitoids reared from the asparagus miner, only *Chorebus rondanii* was reported previously as a parasitoid of the asparagus miner, once in Massachusetts (Krombein et al. 1979) and in Europe (Giard 1904). It is a host-specific parasitoid of the asparagus miner (Griffiths 1968) and was the second-most common parasitoid reared. By far the most common parasitoid reared was *Thinodytes cephalon* and its parasitism rate probably is underestimated because most of the "Pteromalidae morphs" (Table 4.2.) were likely also this species. Prior host records of *T. cephalon* include Cecidomyiidae and Chloropidae (Diptera) (Table 4.1.), but because of the numbers reared from pupae of the asparagus miner, this species, along with *C. rondanii*, may be effective natural enemies of the asparagus miner and good candidates for conservation biological control of this pest in Michigan. The species assemblages of parasitoids for the asparagus miner in other regions of the United States should be assessed to evaluate whether these two species are also good candidates for biological control elsewhere, as the dominant parasitoids for a particular host can vary depending on region especially in large countries like the United States or Canada (Mason et al. 2011).

Most of the other chalcid parasitoid species identified in this study are omnivorous or polyphagous and parasitize a variety of hosts. However, even some of these polyphagous species such as *C. vulgaris*, *M. megapterus* and species of *Halticoptera* are known to have Agromyzidae hosts, and *T. cephalon*. *S. dissimilis* and *T. viridascens* are known to parasitize Chloropidae and Cecidomyiidae (Table 4.1.). This suggests that these species are parasitoids of leaf or stem miners and thus were able to switch from their native hosts in the Nearctic to the asparagus miner when it was introduced from Europe. The host of *S. cracentis* is unknown. An unidentified

species of *Sphegigaster* Spinola was reported as a parasitoid of the asparagus miner in Europe (Barnes 1937, Barnes and Walton 1934), but it is likely different from the species we reared.

Based on known biology, at least two of the parasitoids, *Eupelmus vesicularis* (Retzius) and *Brasema* ?*allynii* (French) could be either primary parasitoids of asparagus miner or hyperparasitoids through one of the other parasitoid species. The impact of these two species on biological control services in the field likely is minimal because of their low numbers (Table 4.2.). Hyperparasitism may constrain biological control in simplified environments, such as monocultures (Rosenheim et al. 1995), where hosts are easy to locate, especially if primary hyperparasitoids are abundant. However, the outcome of intraguild predation often depends on crop specific conditions (Müller and Brodeur 2002).

We reared only a single specimen of *Laelius utilis.* This rare event for the species has never been recorded for any hosts other than beetles of the family Dermestidae. However, with only a single rearing within four years of data collection, asparagus miners are certainly not typical hosts for this species.

Sugar-rich diets increased the lifespan of the pteromalid and braconid parasitoids, indicating that the addition of nectar and floral resources could benefit biological control in this system. Of concern, however, is the observation that the provisioning of sugar-rich diets also increased the life span of the asparagus miner. This empirical observation suggests that it is important to provide flowers in the field that the natural enemy community can exploit without inadvertently benefiting the pest species (Kehrli and Wratten 2011), since the agroecosystem should be managed in such a way that does not extend the lifespan of the asparagus miner more than it would otherwise be. During asparagus bloom, the abundantly available flowers may provide resources to both the asparagus miner (Ferro and Gilbertson 1982) and the parasitoids.

At other times of the year, the majority of flowers are on the field margins, with the asparagus field being relatively depauperate of floral resources. It may be most important to supply the parasitoid community with floral resources during these periods.

The asparagus miner had the shortest lifespan when individuals were allowed access to buckwheat, while they had the longest lifespan on Riddell's goldenrod (native Michigan species). Despite the fact that the goldenrod has been shown to attract chalcids and braconids (Fiedler and Landis 2007a, Landis et al. 2013), we would not recommend it for use in floral plantings or conservation strips for asparagus because of its potential beneficial impact on the asparagus miner lifespan. Buckwheat is an exotic plant from central Asia, and it has also been shown to attract chalcids and braconids in Michigan (Fiedler and Landis 2007a), the families to which *T. cephalon* and *C. rondanii* belong. On the other hand, the same study showed that buckwheat also attracts pests such as *Lygus* spp. (Hemiptera) and the Japanese beetle, *Popillia japonica* Newman (Scarabaeidae) (Landis et al. 2013), which are pests in asparagus fields. Attracting *Lygus* spp. may not cause problems for asparagus production because asparagus plants can recover, even at a young age, from infestations by this pest (Grafius and Morrow 1982), though long-term potential for damage may still exist.

Our study showed a positive correlation between the number of pupae parasitized on the stem, the amount of damage on the asparagus stem, and the total number of asparagus miner pupae. Therefore, pupal parasitoids may have a preference for stems that are already damaged by asparagus miner pupae. This could be due to the cues emanating from damaged stems—usually the higher the concentration of host kairomones cues, the greater the attractiveness of a patch to the parasitoid (van Alphen et al. 2003). Among other signals, possible kairomones that may be used by the parasitoids of the asparagus miner are presence of mining damage, exposure of

pupae where the skin of the mine has been broken, and alteration of the volatile bouquet emitted by the plant or flowers (see Dannon et al. 2010, Geervliet et al. 1996). The chemical ecology of the asparagus miner, the crop and its parasitoids deserve special attention in follow-up studies to develop alternative control approaches for this pest. Additionally, we found support for models of optimal searching behavior, which indicate that when prey distribution is variable (as in the case of the asparagus miner since some stems had one unparasitized miner pupa, whereas others had up to 15), then parasitoids are expected to incrementally increase their time at a patch with each oviposition (Iwasa et al. 1981).

Predictably, we found a positive correlation between the amount of damage on a stem and the number of asparagus miner immature life stages per stem, but in parasitized stems the correlation was not significant. In contrast with our finding that parasitoids may prefer more damaged, miner-infested stems, this finding suggests that parasitoids may be able to reduce the damage inflicted by asparagus miners in the field. In other systems, parasitoids have been shown to increase fitness of plants (van Loon et al. 2000), and decrease damage by pests (Norton and Welter 1996). Though we do not know the impact of the parasitoids on plant fitness, the fact that the parasitoids were able to uncouple the relationship between damage and pest infestation is a promising sign for future biological control efforts.

Interestingly, more parasitoids were found during the first generation of the asparagus miner when the miner was more abundant, indicating that parasitoid abundance may be synchronized with that of its host. Biological control is predicted to work optimally when natural enemy populations are in synchrony with their host populations (Hassell 2000). Predators were equally abundant during the first and second generation of the asparagus miner, which perhaps shows a lack of synchrony or predation of other hosts. Some of the trapped parasitoids, such as

Chrysididae, and many of the predators likely were not using the asparagus miner specifically as a host but rather were in or simply passing through the asparagus field to feed on different hosts. However, the sheer magnitude of robber flies on traps is suggestive that they may be using the asparagus miner, the most common pest, as prey. This unresolved question deserves further attention in future work.

Our study suggests that native chalcid generalist parasitoids of dipterous and other leaf or stem miners will include the asparagus miner as a host and assist in biological control of this pest. Of the parasitoids found, *C. rondanii,* a host-specific parasitoid, and *T. cephalon* and may be the best candidates for conservation biological control of the asparagus miner based on observed parasitism rates. Buckwheat or faba bean were the best candidates for providing a flowering resource, though there may be other alternatives that were not examined. Further research is specifically needed into the following areas before a biological control program can be implemented in asparagus: 1) investigation of whether buckwheat or faba bean attracts and aids the natural enemy community of the asparagus miner in the field, 2) understanding the chemical ecology in interactions between the asparagus miner, its natural enemies and the crop, and 3) ensuring floral resources in the margins of asparagus fields do not benefit other non-target pests in the field.

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CHAPTER 5

Identification and evaluation of asparagus semiochemicals and their ecological interactions with its arthropod community

Introduction

There is increasing interest in manipulating the chemical ecology of agricultural plants to enhance biological control (Khan et al. 2008; Turlings and Ton 2006; Åhman et al. 2010), and manipulate herbivore behavior (De Moraes et al. 2001) to provide environmentally safe pest management solutions. Little is known about the plant volatiles asparagus (*Asparagus officinalis* L.) emits and their interactions with associated arthropods. Globally, 62 countries produce approximately 494,000 acres of asparagus worth $450 million (Benson 2009). Despite increasing demand for asparagus (Huang and Huang 2007), early decline of asparagus fields is a problem around the world (Keulder, 1999), due in part to increased prevalence of pests and the "replant problem" (Grogan and Kimble 1959; Morrison III et al. 2011). To date, only two published studies have investigated the chemicals emitted by asparagus, one using ground whole spear preparations that were first frozen, then heated to 50°C to evaporate the volatiles onto a trap (Sun et al. 2001), and the other using 2-hr cooked asparagus (Ulrich et al. 2001). The first study found the major plant volatiles to be hexanal, trans-2-hexenal, and 1-octen-1-ol, depending on the asparagus cultivar. Other volatiles found in much lower quantities include ketones, alkenes and terpenes. The second study found a total of 36 compounds, and analyzed their contribution to human odor perception of asparagus, but did not investigate their quantity (Ulrich et al. 2001). However, no study has examined the headspace of asparagus and its interaction with insects.

Identifying the quality and quantity of asparagus headspace volatiles using currently available research tools and methods could aid in the development of management strategies for pests in this system. This may be in the form of genetic manipulation of the asparagus resulting in cultivars with upregulated priming ability (Aharoni et al. 2006; Dudareva and Pichersky 2008), deployment of traps baited with herbivore-induced plant volatiles for attracting natural enemies to suppress pests, or sprays that induce plants to become primed or to produce volatiles (Bruinsma et al. 2009) that attract biological control agents (Thaler 1999).

Asparagus is attacked by a suit of pests, including the common (*Crioceris asparagi* L.; Coleoptera: Chrysomelidae) and spotted (*Crioceris duodecimpunctata* L.; Coleoptera: Chrysomelidae) asparagus beetles, black cutworm (*Agrostis ipsilon* (Hufnagel); Lepidoptera: Noctuidae), asparagus aphid (*Brachycorynella asparagi* (Mordvilko); Hemiptera: Aphididae), tarnished plant bug (*Lygus* spp.; Hemiptera: Miridae), Japanese beetle (*Popillia japonica* Newman; Coleoptera: Scarabaeidae), and the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae). The black cutworm, is a generalist and feeds on the asparagus fern and stem in the larval stage. This insect is problematic at the beginning of the growing season when plants are young, whereas asparagus miners are present throughout the season and feed on the stem internally. Herbivore-induced plant volatiles can be herbivore-specific (Takabayashi et al. 1995), thus different volatiles may be emitted by asparagus plants depending on the specific pest. The elicitors in caterpillar saliva (Turlings et al. 2000), for example, might induce asparagus in a different way than induction by the internal feeding of asparagus miner larvae.

The asparagus miner is host-specific (Barnes 1937), and as a result, has likely evolved to recognize volatile cues from its only host plant species (Schoonhoven et al. 1998; Szendrei and Rodriguez-Saona 2010). The adult females seek out asparagus and oviposit under the epidermis

of the stem near the base of the plant (Eichmann 1943), where larvae hatch and feed. Females may be important targets for manipulation with plant volatiles because they are often more responsive than males, since they rely on constitutive plant volatile cues for recognizing appropriate oviposition sites (Szendrei and Rodriguez-Saona 2010). Insect pests may also be repelled by induced plant volatiles, because these may signal that plants produce toxic secondary compounds, that potential competitors for food and oviposition may be present, or that the plant may be now attractive to predators and parasitoids (Bernasconi et al. 1998). However, some studies have found that the plant is a more important determinant of volatile emissions than the herbivore (e.g. Takabayashi et al. 1991). Numerous generalist predator and parasitoid species (Morrison et al. in review) also present in asparagus fields are unlikely to have fixed responses to herbivore species-specific volatiles from asparagus. Their response to asparagus volatiles will probably be based on constitutive or general herbivore induced volatiles.

The primary aims of the current study were to 1) identify and compare headspace volatiles of asparagus in healthy, mechanically-damaged or herbivore (black cutworm) damaged plants; 2) investigate the response of asparagus miner adults to healthy or asparagus miner-damaged asparagus stems in an olfactometer; and 3) evaluate the response of arthropods (pests, predators and parasitoids) to individual asparagus plant volatile components in the field.

Methods

Plants. Asparagus used in the headspace collection was grown in the greenhouse from var. Millenium 1-year-old crowns from a nursery in Oceana Co., MI. Crowns were stored at 5°C in a cold room under complete darkness until needed for planting in the greenhouse. Plants were potted in 3.79 l pots (27.9 cm x 29.2 cm H:D) with a mixture of 75% washed play sand (Kolorscape, Oldcastle, Inc., Atlanta, GA, USA) with 25% potting soil (SureMix Perlite, Michigan Grower Products, Inc., Galesburg, MI, USA). Sand was used to simulate the sandy soils that asparagus prefers. Plants were watered in the greenhouse once daily during the warm season, once weekly during the cool season, and kept on a 16:8 L:D cycle throughout the year. Fertilizer (20-20-20 N:P:K with micronutrients, J. R. Peters, Allentown, PA, USA) was delivered to plants in liquid form (1-2% v/v) as plants were watered.

Headspace collection. To evaluate how the asparagus headspace varies with plant damage, plant volatiles were collected from asparagus plants that were either intact (healthy), mechanically-damaged or black cutworm-damaged plants. This pest was chosen to cause herbivore damage because asparagus miners were not available in colony in the laboratory. The mechanical damage consisted of grinding three separate 5 cm segments of cladophylls between gloved fingers with about 5 g silicon carbide powder (120 grit, Alfa Aesar, Lancs, UK) for approximately 10 s, and was performed immediately before headspace collection. Black cutworm eggs (Benzon Research, Inc., Carlisle, PA, USA) were reared on an artificial diet in plastic containers (12 x 7 x 5 cm L:W:H) until the third instar, then they were transferred to asparagus plants to feed. Five black cutworms were placed on an individual asparagus plant, and were allowed to feed prior to headspace collection for at least 5 hours. The caterpillars were removed 10-30 min prior to headspace collection. The treatments were replicated over time, and

each treatment was represented at least once in each replication. In addition to the three plant treatments, a negative control consisting of an empty glass chamber was also included. At least 6 asparagus crowns were planted at a time in the greenhouse for each replication to ensure uniformity of soil and abiotic conditions for the plants.

For headspace collection, plants were covered by 4 l glass chambers (36 x 20 cm H:D). Using a push-pull system, air was pumped through a flow meter and a charcoal filter for purification. Purified air entered through a valve near the top of each chamber at 2 l/min, and volatiles were collected in Alltech SuperQ adsorbent traps (30 mg/trap; Analytical Research Systems, Gainesville, FL) by pulling air from the chambers at a rate of 1 l/min (Szendrei et al. 2009). The positive pressure assured that ambient air did not enter the headspace equipment during collection. The collected volatiles from the SuperQ traps were eluted with dichloromethane (150 µl), and 400 ng of tetradecane (Sigma-Aldrich, St. Louis, MO) was added as an internal standard.

Plants were spaced approximately 60 cm apart, and metal guillotines (23 x 31 cm W:L) were clamped around the stems of the plants at the base of the glass chambers. The opening of the guillotine was blocked with cotton balls around the stem of the plant. The guillotines and glass chambers were elevated above the pots of the asparagus plants.

A round of headspace collection lasted on average 6 hrs, typically from 0900 to 1500 hrs, coinciding with the photosynthetically active stage of asparagus. After headspace collection, the aboveground plant biomass was weighed, and all glassware, SuperQ traps, and surfaces were washed and wiped with methanol, then hexane.

GC-MS headspace analysis. The volatile extracts were run on an Agilent 6890 N gas chromatograph (GC) equipped with an FID and an Agilent Durabond DB-5 column (10 m length,

100 μm diameter and 0.34 μm film thickness, He as the carrier gas at constant 5 ml/min flow, and 39 cm/s velocity) coupled with an Agilent 5975B inert XL mass spectrometer (MS). Compounds were separated by injecting 1.0 μl of sample into the GC/MS. The program consisted of 40°C for 1 min followed by 14°C min^{-1} to 180°C for 8 min, and 40°C min^{-1} to 300°C for 2 min. After a solvent delay of 3 min, mass ranges between 50-550 atomic mass units were scanned. Compounds were identified by comparison of spectral data with those from the NIST library and by GC retention index (Adams 2009) and confirmed by comparing their retention times with those of commercially available compounds.

Insect collection and Y-tube assay. Asparagus stems were collected weekly from 1-5 commercial asparagus fields in Oceana Co., MI from 2010-2013 (Morrison et al. 2014). Stems were cut 5 cm below the soil surface and again at the height of the longest mine, transported to the laboratory in a cooler, and placed at 5°C until dissection. Pupae were dissected from the stems within 1-14 d of collection, and placed individually in plastic cups or petri dishes where they were allowed to develop at 23±0.03°C under a 16:8 L:D cycle. Pupae were checked daily, and emerged adults were sexed and used for the Y-tube olfactometer assays.

In order to assess the asparagus miners' preference for the different asparagus headspace a Y-tube olfactometer (two 7 cm and one 13 cm arms; 1.5 cm diameter, ground glass joints; glass blower, Michigan State University, East Lansing, MI) was employed. Air was first humidified, then filtered through activated charcoal, and was subsequently split into two 1 l/min air streams, regulated by flow meters. Afterwards, each air stream was delivered to a volatile chamber (16.5 cm x 8.5 cm H:D), which was connected to the olfactometer by inert Teflon tubing. Odor sources included 65-222 g (avg ± SEM: 157.7 ± 4.8 g) of asparagus stems that were either asparagus miner-damaged or healthy. Empty chambers served as the negative control. Upon addition of the

plant material, deionized water was filled to 3 cm level within the chamber to keep the stems hydrated. All plant material came from 5 commercial fields (all var. Millennium) in Oceana Co., Michigan and was collected between May-August from 2012-2013. Before the beginning of the assays, plant material was stored at 5°C in a sealable bag for 1-4 d. Prior to placement in the volatile chamber, the asparagus was rinsed with deionized water to remove any sand, dirt or other foreign substrate.

Adult asparagus miners were used in the Y-tube olfactometer on the same day they eclosed to ensure uniformity of age among tested adults. At the beginning of the assays, asparagus miners were placed individually at the bottom of the Y and were observed until a choice was made, or for a maximum of 15 min. A decision was made by the insect when the individual moved past the mid-point of one of the arms containing a volatile source. Non-responding individuals were marked as such and excluded from the subsequent statistical analysis. After every second run, the position of the odor sources was randomized to eliminate the possibility of positional bias. When possible, treatment type was changed after every second run. Because of limitations in obtaining the correct combination of fresh asparagus miner-damaged stems, asparagus miner pupae and timing of asparagus miner emergence, not all treatment combinations were tested in every set of assays during the beginning of the growing season (generally May and the beginning of June). After every insect, the Y-tube was rinsed with methanol, then hexane and heated in a drying oven for at least 10 min at 60°C to dry. Glassware was allowed to cool down for 3 min before using again in assays. Each treatment was replicated 22-40 times from 4 May 2012 to 12 August 2013. All experiments were performed between 0800-1600 hrs at 22.8 ± 0.3°C and 600-700 lux, under laboratory conditions.

Baiting field experiment. A variety of volatiles was deployed in 2011, 2012, and 2013 in Oceana Co., MI in 3-5 commercial asparagus fields in baits. There were several criteria for selecting volatiles for this experiment. One of these was based on data from preliminary headspace analysis which indicated that a specific volatiles was present in ≥2 times the quantity in one headspace treatment (e.g. intact, mechanically-damaged, or herbivore-damaged) when compared in a pairwise fashion to the others, indicating that it may be acting as a signal to other trophic levels. Volatiles were also potentially included if they were present in all the treatments in roughly equal amounts (constitutive volatiles), or if the volatile has known biological activity in the tritrophic interactions of other systems (James 2003; James 2005; Mallinger et al. 2011; Ozawa et al. 2000; Thaler 1999). Volatiles varied in different years, but the negative control was always mineral oil (light oil, Sigma-Aldrich, Milwaukee, WI, USA) only. In 2011 and 2012, six volatiles were tested in addition to the mineral oil, while five were tested in 2013 (Table 5.1). The average ± SEM release rate across baits was 70.8 ± 12 ng/day, individual volatile release rates are given in Table 5.2. In 2012, we examined several new volatiles singly and retained several from the previous year. In addition to testing each volatile alone, the following combinations were deployed in 2013 with a ratio of 0.35:1 with mineral oil for each compound: hexanoic acid with (*Z*)-3-hexen-1-ol, hexanoic and methyl salicylate, and hexanoic acid together with methyl salicylate and (*Z*)-3-hexen-1-ol. Volatiles were added to 1.7 ml (2011, 2012) or 2.0 ml (2013) plastic centrifuge tubes with snap caps, and were punctured once on the side near the top of the tube with a dissecting needle to allow diffusion of the volatiles into the surrounding air. In 2011, 500 µl of volatiles were added to 1000 µl of mineral oil, while in 2012, 350 µl of volatiles were added to 700 µl of mineral oil. In 2013, total volume was kept constant while 350

µl of volatiles—either singly or in combination—was added to 300-1000µl of mineral oil, depending on how many compounds were in a bait.

Five, three, and four commercial asparagus fields were used in 2011, 2012, and 2013, respectively for testing the baits. Baited centrifuge tubes were affixed to the base of yellow sticky traps (7.6 cm x 12.7 cm W:L, Great Lakes IPM, Vestaburg, MI, USA) with gardening wire (plastic jacketed), attached to metal stakes, and the traps were set at the top of 1 m long pieces of hollow metal conduit (1.9 cm diameter) at each sampling point. One (2011) or three (2012 and 2013) transects of baited traps were placed on three randomly chosen edges of a field, with 7 traps (2011 and 2012) or 8 traps (2013) spaced 10 m apart within the transect at the asparagus crop edge, since this is where the highest abundance of asparagus miner adults are located (Morrison and Szendrei 2013). Traps were changed every 6-10 d during the growing season, and a volatile's position along the transect was randomized each time, from 6 July to 3 October 2011, 11 April to 1 October 2012, and 7 May to 5 September 2013. Traps were brought to the laboratory and the abundance of asparagus miner adults was recorded (2011, 2012 and 2013), while natural enemy abundance (common parasitoid and predator families) and other asparagus pests were recorded on the traps in 2013 only. Parasitoid and predators were identified to family using a combination of (Marshall 2006) and online resources, including (Gardiner 2013).

Statistical analyses. Peak areas were quantified from the gas chromatograms using MSD ChemStation v.2.00 software (Agilent Technologies, Inc., Santa Clara, CA). These were transformed into units of ng of volatiles per g of fresh plant biomass per hour, using the ratio of the given volatile's peak area to that of the known internal standard, the mass of plants from which each headspace of volatiles originated and the sampling duration of the headspace

collection. Background compounds found in the control (no plant) that were also present in the asparagus plant samples were discarded from the analysis. In addition, compounds that were only found in two or fewer samples were also disregarded. Using ng per g fresh tissue per h values for individual volatiles, pairwise Bray-Curtis similarities were calculated among treatments, and non-metric multi-dimensional scaling (NMDS) was used to visualize the differences. Stress values for NMDS procedure were <0.1, indicating that good interpretation was possible. To assess significance of differences, an analysis of similarity (ANOSIM) with 1,000 permutations was employed. The 95% confidence ellipses based on the centroid of each treatment was calculated. These statistical tests and all others, except where otherwise noted, were carried out in R Software (R Core Development Team 2013) with $\alpha=0.05$.

In order to understand the relative contribution of each volatile compound to the overall similarity or dissimilarity of the headspace from each treatment (healthy, mechanically damaged, or black-cutworm damaged), a similarity percent (SIMPER) procedure was implemented using Primer E v.6.1.6, whereby each compound's contribution to the similarity (within a treatment) or dissimilarity (between treatments) was calculated. The cutoff for inclusion of compounds was when the overall cumulative average similarity within a group or dissimilarity between groups reached at least 90%. In addition, Tukey's HSD were performed on compounds contained within the headspace between the different treatments to assess differences in quantities.

A G-test for goodness of fit coupled with William's correction for the P-value (Sokal and Rohlf 1995) was performed to assess the significance of asparagus miner preference for volatiles in the Y-tube, with the null hypothesis that asparagus miners would choose both sides of the olfactometer with equal probability.

Because different volatiles were assayed for asparagus miners in different years and at different concentrations, single mixed model, repeated measures analysis of variances (ANOVAs) were performed for 2011-2013, using the asparagus miner abundance as the response variable, volatile as the independent, field as a random variable, and time as a fixed factor (and its interaction with volatile). A first order autoregressive correlation structure was used to model the correlation matrix, since the expectation was that sampling dates further apart would be more dissimilar. The subject of the repeated measures was set as the location of a trap on a given side of a specific field. Asparagus miner abundance did not conform to the expectations of a normal distribution in any of the years, so the abundance in each year was log transformed. The resulting residuals from preliminary models (containing fixed factors only) were inspected and found to conform to the assumptions of a normal distribution, and natural log-transformed data was used for all subsequent analyses. When a significant result was found with the ANOVA, pairwise Tukey's HSD comparisons were performed to separate treatment means.

In 2013, the influence of volatiles on other pests, predators and parasitoids was examined with a repeated measures multivariate analysis of variance (MANOVA), with each feeding guild combined as an aggregate response variable. The remainder of the model had the same form as above. Field was a random variable, and volatile, time, and a volatile by time interaction were fixed independent variables. The subject of the repeated measures was the location of a sticky trap on a given side of a specific field. Upon inspection of the residuals from the preliminary model, assumptions of normality were violated, so parasitoid and predator abundance were log-transformed, while pest abundance was inverse square root-transformed. After transformation, the variables conformed to the assumptions of normality and homogeneity of variances. With a

significant result from the MANOVA, individual sequential repeated measures ANOVAs (same model as above) were performed on each response to test for treatment differences. For those response variables with significant results from the ANOVAs, pairwise comparisons using Tukey's HSD were done, which addressed differences in the overall abundance of each response relative to volatiles. However, to assess species composition difference among the insect communities attracted to each volatile, NMDS was used to visualize the data, while ANOSIM was employed to assess statistical differences among volatile baits in the community composition (for elaboration on these methods, see Clarke and Warwick 2001). The NMDS plots were constructed based on Bray-Curtis similarity index values, which were generated for pairwise distances between samples. Stresses for the NMDS ordinations were <0.1, indicating that they are of sufficient quality that interpretation of the plots is possible. The ANOSIM gives an R-value between 0-1, where greater values indicate greater dissimilarity among treatments. The test was run with 1,000 permutations.

Results

Headspace collection. Twenty-five compounds were identified from intact asparagus plants, 16 from mechanically damaged plants, and 18 from herbivore-damaged plants (Table 5.1.). The volatile headspace emitted by asparagus differed significantly among treatments, depending on whether it was intact, mechanically damaged or damaged by herbivores (ANOSIM: R=0.308, n = 33, P<0.001; Figure 5.1.). Asparagus headspace compounds include 5- and 6-chained carbon compounds, such as alcohols, alkenes and aldehydes, as well as higher order carbon-compounds, such as 1-octadecene (Table 5.1.). The intact plants emitted significantly more (*Z*)-3-hexen-1-ol than did mechanically- or herbivore-damaged plants (Table 5.1., $F_{2,30}$ =3.83, P < 0.05). The

headspace of intact plants contained about half the amount of (*E*)-3-hexenyl acetate ($F_{2,30}$ = 0.61, *P*=0.55) and 1-hexadecene ($F_{2,30}$ = 1.89, *P*=0.17), 13 times more (*Z*)-3-hexen-1-ol, and 50% less pentadecane ($F_{2,30}$ = 11.33, $P < 0.001$) when compared to the headspace of herbivore-damaged plants. By contrast, the headspace of black cutworm-damaged plants on average, contained numerically about 50% more (*E*)-3-hexenyl acetate, hexanoic acid, and decanal, and four times more pentadecane ($F_{2,30}$= 11.33, *P*<0.001) than did headspace from mechanically-damaged asparagus. Additionally, mechanically-damaged headspaces contained significantly more neryl acetone ($F_{2,30}$ = 5.90, *P*<0.01) and pentadecanol ($F_{2,30}$ = 5.50, *P*<0.01) than the other two treatments, while herbivore-damaged headspaces had significantly more cis-threo-davanafuran than the other two treatments ($F_{2,30}$ = 5.46, *P*<0.05). Intact headspace had significantly more of an unidentified compound (unknown3: $F_{2,30}$ = 4.81, *P*<0.05), though the quantity was less than 2% of the total headspace emission.

Between 5-7 compounds accounted for over 90% of the similarity in asparagus headspace within a treatment, but only 2-4 of these were typical of each treatment (Table 5.3.), as indicated above. The amount of the volatile compound present in the headspace was important, as many of the same volatiles acted as reliable discriminators between the treatment groups, including (*E*)-3-hexenyl acetate (Table 5.4.).

Y-tube assay. The asparagus miner was significantly attracted to healthy asparagus stems over air ($G_{adj.}$=4.19, df=1, *P*=0.0411), while there was no preference for asparagus miner-damaged asparagus stems over air ($G_{adj.}$=0, df=1, *P*=1; Figure 5.2.). When the two types of stems were offered at the same time, asparagus miners significantly preferred miner-damaged stems over healthy asparagus ($G_{adj.}$=18.02, df=1, *P*<0.001).

Baiting field experiment. The volatile baits significantly affected the number of asparagus miners caught in 2011 (Repeated Measures ANOVA: $F_{6,19}$=13.19, P<0.001). Specifically, the (*Z*)-3-hexen-1-ol bait caught over 5 times (avg: 1.67 miners per trap) as many asparagus miners as the control (avg: 0.31 miners per trap), whereas the other volatiles were not significantly different from the control (Tukey's HSD, Figure 5.3.A). The abundance of asparagus miners significantly fluctuated over the course of the season ($F_{1,19}$=34.60, P<0.001), pattern of trap catch was qualitatively and quantitatively consistent over the season (volatile x time interaction: $F_{6,19}$=0.542, P=0.745).

Similarly, the identity of the volatile significantly affected asparagus miner abundance on yellow sticky traps in 2012 (Repeated Measures ANOVA: $F_{6,54}$=5.89, P<0.001, Figure 5.3.B) and 2013 ($F_{7,78}$=19.43, P<0.001, Figure 5.3.C). Asparagus miner adults were attracted to (*Z*)-3-hexen-1-ol in 2011, but not in 2012 or 2013, relative to the other volatile treatments. Methyl salicylate-baited traps caught significantly more asparagus miner adults than the decanal, (*Z*)-3-hexen-1-ol, pentadecane or 1-hexadecene baited ones in 2012, but methyl salicylate-baited traps performed similarly to the control. When methyl salicylate was combined with hexanoic acid in 2013, it was significantly more attractive to the asparagus miner compared to other volatiles. In contrast, decanal reduced the asparagus miner catch, resulting in over 25% fewer adults in 2012, and almost a third fewer adults in 2013 (Figure 5.3.B and C). The effect of volatile attraction did not significantly vary through the season in 2012 (volatile x time interaction: $F_{6,54}$=0.032, P=0.999) or 2013 (volatile x time interaction: $F_{7,78}$=0.947, P=0.469).

Pests (excluding miners) and natural enemies caught on yellow sticky traps in 2013 totaled 7,975. Of these, 87% were natural enemies, while 13% were pests. It is notable that there were over 35 times as many asparagus miners (20,408) as all other pest combined. Together,

Miridae (53.2%) and Chrysomelidae (42.2%) comprised over 95% of the remaining pests. These families primarily included tarnished plant bugs and both species of asparagus beetles, though they also contained other species to a lesser extent. Of the natural enemies, 90% were predators, while 10% were parasitoids. The three largest predator groups were Asilidae (65.1% of predators), Syrphidae (10%), and Anthocoridae (4.4%), while the largest parasitoid groups were Tachinidae (30.6% of parasitoids), Ichneumonidae (27.7%), and Bombylidae (10.2%). The volatile baits significantly impacted the abundance of the pest and natural enemies caught on the traps (Repeated Measures MANOVA: $F_{21,234}$=5.614, P<0.001). Specifically, the baits influenced the abundance of parasitoids (Repeated Measures ANOVA: $F_{7,78}$=6.60, P<0.001) and pests ($F_{7,78}$=13.86, P<0.001), but not predators ($F_{7,78}$=1.49, P=0.183). Methyl salicylate was the most attractive volatile to the parasitoid community, while hexanoic acid was the least attractive, and (*Z*)-3-hexen-1-ol was intermediate (Figure 5.4.). The attractiveness of volatiles did not vary through the season for the parasitoids (volatile x time interaction: $F_{7,78}$=2.04, P=0.06). In terms of pest abundance, (*Z*)-3-hexen-1-ol was the most attractive bait, recruiting almost twice as many pests as the least attractive volatile, hexanoic acid with (*Z*)-3-hexen-1-ol and methyl salicylate. The influence of these volatiles on the pest community fluctuated throughout the season (volatile x time interaction: $F_{7,78}$=21.01, P<0.001), indicating that implications from the main effects are not conclusive. However, the various volatiles recruited unique species communities for pests (ANOSIM: R=0.83, P<0.001; Figure 5.5.), but not for parasitoids (ANOSIM: R=0, P=0.86) or predators (ANOSIM: R=0, P=0.76). For example, (*Z*)-3-hexen-1-ol attracted the most scarabids, and chrysomelids, while ranking second only to hexanoic acid in the number of mirids attracted. On the other hand, the baits with the following combinations of volatiles, HOA+MSA, HOA+CHL+MSA, and HOA+CHL, attracted the fewest scarabids, chrysomelids and mirids,

respectively. The volatile combination least similar to the control for pest species composition contained hexanoic acid, methyl salicylate and (*Z*)-3-hexen-1-ol together. In contrast, the bait most similar in species community of pests to the control was hexanoic acid, while the volatile most similar to the control was hexanoic acid.

Table 5.1. Volatiles identified by GC-MS from var. Millennium asparagus plants that were either left intact (healthy), mechanically-damaged by rubbing silicon carbide along the stem, or herbivore-damaged by black cutworm

		Intact[a]				Mechanically-damaged				Herbivore-damaged			
	Compound	Mean[b]	± SE	% total		Mean	± SE	% total		Mean	± SE	% total	
1	Hexanoic acid	16.2	± 9.3	4.8		19.2	± 4.0	8.46		28.30	± 11	6.31	
2	Mesitylene	0.95	± 0.6	0.3		0.00	± 0.0	0.00		0.00	± 0.0	0.00	
3	(Z)-3-Hexen-1-ol	33.6	± 14	9.9	*a*[*]	0.00	± 0.0	0.00	*b*	0.00	± 0.0	0.00	*b*
4	(E)-3-Hexenyl acetate	50.1	± 10	14.7		59.1	± 15	26.1		112.1	± 39	25.0	
5	Limonene	2.17	± 1.3	0.64		0.00	± 0.0	0.00		1.02	± 0.7	0.23	
6	(Z)-β-Ocimene	7.11	± 4.9	2.09		25.2	± 25	11.1		0.00	± 0.0	0.00	
7	5-Methylhexanoic acid	6.10	± 6.3	1.79		12.3	± 5.1	5.40		2.00	± 1.3	0.45	
8	Methyl salicylate	4.27	± 2.6	1.26		0.00	± 0.0	0.00		0.00	± 0.0	0.00	
9	Decanal	26.5	± 5.0	7.81		18.9	± 3.1	8.35		33.1	± 11	7.37	
10	Undecanal	1.12	± 1.0	0.33	*a*	2.16	± 0.9	0.95	*b*	2.61	± 2.3	0.58	*ab*
11	Unknown3	4.07	± 1.2	1.20		0.00	± 0.0	0.00		2.68	± 2.1	0.60	
12	Decanoic acid	2.00	± 2.1	0.59		0.71	± 0.5	0.32		1.14	± 0.8	0.25	
13	1-Tetradecene	2.01	± 1.6	0.59	*ab*	0.00	± 0.0	0.00	*b*	4.71	± 2.7	1.05	*a*
14	cis-threo-Davanafuran	11.4	± 8.6	3.34		0.44	± 0.4	0.20		17.67	± 10.2	3.94	
15	(E)-Caryophyllene	1.42	± 1.2	0.42	*b*	1.07	± 1.1	0.47	*a*	2.70	± 2.7	0.60	*b*

Table 5.1. (cont'd)

		Intact				Mechanically-damaged				Herbivore damaged			
	Compound	Mean[b]	±	SE	% total	Mean	±	SE	% total	Mean	±	SE	% total
16	Neryl acetone	5.15	±	3.4	1.51	6.45	±	2.0	2.84	0.00	±	0.0	0.00
17	Unknown4	4.66	±	3.5	1.37 *b*	0.00	±	0.0	0.00 *b*	3.27	±	3.2	0.73 *a*
18	Pentadecane	18.33	±	5.8	5.39	5.97	±	1.9	2.63	45.5	±	15	10.2
19	Unknown5	4.31	±	2.6	1.27	0.00	±	0.0	0.00	0.00	±	0.0	0.00
20	1-Hexadecene	17.48	±	8.5	5.14	0.00	±	0.0	0.00	39.5	±	16	8.81
21	Unknown6	0.00	±	0.0	0.00	0.79	±	0.6	0.35	4.83	±	2.6	1.08
22	Unknown7	6.04	±	4.0	1.78 *b*	0.00	±	0.0	0.00 *a*	0.00	±	0.0	0.00 *b*
23	n-Pentadecanol	0.00	±	0.0	0.00	3.50	±	2.1	1.54	0.00	±	0.0	0.00
24	Unknown8	14.05	±	6.9	5.43	0.00	±	0.0	0.00	0.00	±	0.0	0.00
25	1-Octadecene	10.06	±	7.0	2.96	0.00	±	0.0	0.00	8.78	±	6.8	1.96
26	Hexadecyl acetate	0.00	±	0.0	0.00	37.7	±	36	16.6	0.00	±	0.0	0.00
27	Unknown9	3.71	±	2.6	1.09	4.01	±	1.5	1.77	9.27	±	5.0	2.07
28	Unknown11	4.31	±	3.3	1.7	0.00	±	0.0	0.0	2.20	±	5.0	0.7
	Total	**257**	**±**	**104**	**100**	**197**	**±**	**99**	**100**	**321**	**±**	**173**	**100**

[a] Numbers are based on headspace collected between 0800-1500 hrs for N=15 intact plants, N=7 mechanically-damaged plants, and N=11 herbivore-damaged plants

[b] Mean±SE units are ng volatile/g plant tissue/h

[*] Letters denote significant differences among treatments for a compound; treatments with shared letters are not significantly different from one another Tukey's HSD (α=0.05)

Table 5.2. Summary of volatiles used in baited yellow sticky traps in commercial asparagus fields from 2011-2013 in Oceana Co., MI

Compound	Identified from or role	Concentration (µL volatile/µL MO[a])		Mean release rate (ng/day±SEM)		Years tested				
		2011 & 2012	2013	2011 & 2012	2013	2011	2012	2013	Purity[b]	Supplier
Mineral oil	Negative control	-	-	0 ± 0.0	0 ± 0.0	X	X	X	light	Sigma-Aldrich[c]
(Z)-3-Hexen-1-ol	Healthy plants	0.5	0.35	94 ± 12	66 ± 8.1	X	X	X	>98	Sigma-Aldrich
Methyl salicylate	Common attractant	0.5	0.35	32 ± 4.2	22 ± 3.0	X	X	X	trap/>95	Ag Bio[d]/Sigma-Aldrich
6-Methyl-5-hepten-1-ol	Other systems	0.5	-	179 ± 52	- - -	X			>98	Sigma-Aldrich
β-Caryophyllene	Other systems	0.5	-	13 ± 5.8	- - -	X			>80	Sigma-Aldrich
(E)-3-Hexenyl acetate	Herbivore damage	0.5	-	477 ± 313	- - -	X			natural	Sigma-Aldrich
Nonanal	Other systems	0.5	-	281 ± 247	- - -	X			>95	Sigma-Aldrich
Pentadecane	Herbivore damage	0.5	0.35	3.3 ± 0.9	- - -		X		>98	Sigma-Aldrich
(Z)-β-Ocimene	Mechanical damage	0.5	-	231 ± 37	- - -		X		>90	Sigma-Aldrich
1-Hexadecene	Herbivore damage	0.5	-	8.6 ± 3.0	- - -		X		>94	Alfa Aesar[e]
Decanal	Constitutive volatile	0.5	0.35	32 ± 7.5	22 ± 5.2		X	X	>96	Alfa Aesar
Hexanoic acid	Constitutive volatile	-	0.35	- - -	48 ± 17			X	>98	Sigma-Aldrich

[a] Abbreviations: MO - mineral oil

[b] Initial stock concentration of volatiles, v/v%

[c] Location: Milwaukee, WI, USA

[d] Location: USA

[e] Location: Ward Hill, MA, USA

Table 5.3. Compounds in asparagus bouquets from intact, mechanically damaged, or herbivore-damaged plants that collectively contribute at least 90% cumulatively to the average Bray-Curtis similarity within each treatment

Average similarity (27.38) Intact, healthy plants			Average similarity (42.68) Mechanically-damaged plants			Average similarity (29.78) Herbivore-damaged plants[a]		
No.[b]	Compound[c]	% cumulative similarity	No.	Compound	% cumulative similarity	No.	Compound	% cumulative similarity
4	(E)-3-Hexenyl acetate	37.66	4	(E)-3-Hexenyl acetate*	38.45	4	(E)-3-Hexenyl acetate	30.73
9	Decanal*	62.50	1	Hexanoic acid*	60.66	18	Pentadecane*	56.18
18	Pentadecane*	72.82	9	Decanal*	79.09	1	Hexanoic acid	73.84
3	(Z)-3-Hexen-1-ol	81.53	7	5-Methylhexanoic acid	85.70	9	Decanal*	87.72
1	Hexanoic acid	86.18	18	Pentadecane*	90.64	20	1-Hexadecene	94.44
20	1-hexadecene	89.61						
24	Unknown8	91.96						

[a] Black cutworm larvae were used as the herbivore for this treatment.

[b] Numbers correspond to those found in Table 5.1.

[c] Compound identifications are tentative based on spectra library for essential plant oils, insert citation

* These compounds reliably typify the treatment group (e.g. the average contribution to the overall similarity is large compared to the standard deviation for that compound: avg. contribution/SD $\geq$ 1.0)

Table 5.4. Compounds in asparagus bouquets from intact, mechanically damaged, or herbivore-damaged plants that collectively contribute at least 90% cumulatively to the Bray Curtis disimilarity between each pair of treatments

Average dissimilarity (71.32) Between IN & MD[a]			Average dissimilarity (74.39) Between IN & HD			Average dissimilarity (70.35) Between MD & HD		
No.[b]	Compound[c]	Avg. % cumulative dissimilarity	No.	Compound	Avg. % cumulative dissimilarity	No.	Compound	Avg. % cumulative dissimilarity
4	(E)-3-Hexenyl acetate*	15.72	4	(E)-3-Hexenyl acetate*	23.00	4	(E)-3-Hexenyl acetate*	28.05
3	(Z)-3-Hexen-1-ol*	25.55	1	Hexanoic acid	31.87	18	Pentadecane*	37.00
1	Hexanoic acid	33.77	3	(Z)-3-Hexen-1-ol*	40.61	1	Hexanoic acid*	45.52
26	Hexadecyl acetate	40.54	18	Pentadecane*	48.63	9	Decanal*	53.53
6	(Z)-β-Ocimene	46.66	20	1-Hexadecene*	56.64	20	1-hexadecene	60.58
7	5-Methylhexanoic acid	52.45	9	Decanal	64.54	26	Hexadecyl acetate	67.43
9	Decanal*	57.99	14	cis-threo-Davanafuran	69.00	7	5-Methylhexanoic acid	72.49
20	1-Hexadecene	62.37	24	Unknown8	72.21	6	(Z)-β-Ocimene	76.78
18	Pentadecane*	66.70	25	1-Octadecene	74.71	14	cis-threo-Davanafuran	80.44
24	Unknown8	70.28	7	5-Methylhexanoic acid	77.08	27	Unknown9	83.66
16	Neryl acetone*	73.10	27	Unknown9	79.38	16	Neryl acetone	86.13
27	Unknown9	77.74	6	(Z)-β-Ocimene	83.43	21	Unknown6	87.68
14	cis-threo-Davanafuran	79.86	17	Unknown4	85.05	23	n-Pentadecanol	88.93
25	1-Octadecene	81.73	8	Methyl salicylate	86.58	10	Undecanal	90.18
8	Methyl salicylate	83.42	28	Unknown11	87.92			
17	Unknown4	84.94	12	Decanoic acid	89.22			
28	Unknown11	86.15	11	Unknown3	90.40			
22	Unknown7	87.33						
23	n-Pentadecanol	88.51						
11	Unknown3	89.66						
12	Decanoic acid	90.71						

[a] Abbreviations: IN - intact plants, MD - mechanically-damaged plants, HD - herbivore damaged plants (black cutworm)

Table 5.4. (cont'd)

[b] Numbers correspond to those found in Table 1.
[c] Compounds are tentative identifications based on reference to spectra library for plant essential oils, insert citation.

* These compounds act as reliable discriminating compounds between the two groups (e.g. the average contribution to the overall dissimilarity between the pair is large compared to the standard deviation for that compound: avg. contribution/SD ≥ 1.0)

Figure 5.1. Non-metric multi-dimensional scaling plot of the differences in the headspace between asparagus plants assigned to one of three different treatments: intact, healthy (black, solid), mechanically-damaged (black, dotted), or black-cutworm damaged (grey, solid). The ellipses of the treatment colors indicate the 95% confidence interval around the centroid of each group. Blends of volatiles were significantly different from one another among the treatments (ANOSIM: R=0.308, n=32, P<0.0001).

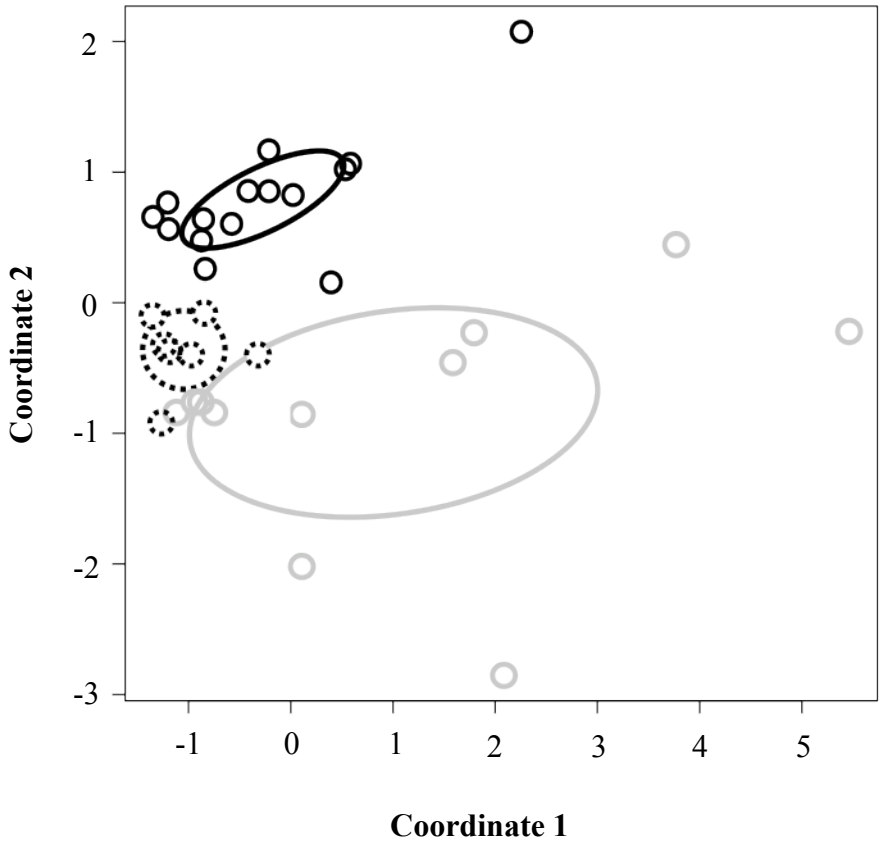


Figure 5.2. Results of the behavioral assay in a y-tube olfactometer to assess attraction of 1 d old asparagus miner adults to various asparagus headspaces depending on health condition of stems. The N/As represent individuals that failed to respond after 15 min and were excluded from the analysis. Headspaces affected the behavior of the asparagus miner: n.s. = not significant, * = P<0.05, ** = P<0.001.

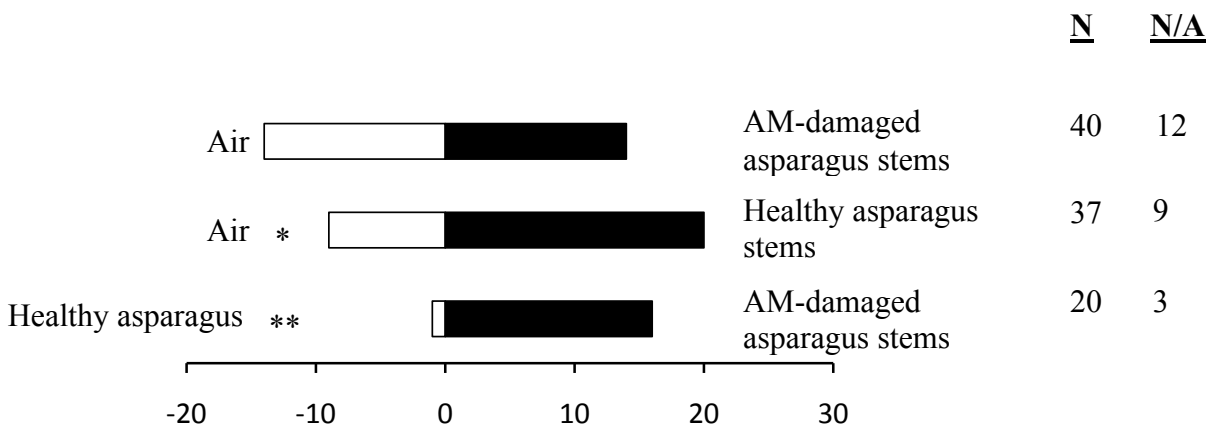


Figure 5.3. Mean (± SEM) seasonal abundance of asparagus miner adults on yellow sticky cards baited with different volatiles (singly or in combination), which were deployed in commercial asparagus fields in Oceana Co., MI from A) 2011: N=60 per treatment, B) 2012: N=222, and C) 2013: N=204. Abbreviations: CHL – cis-3-hexen-1-ol, HOA - hexanoic acid, MSA – methyl salicylate. Treatments with shared letters are not significantly different from one another (Tukey's HSD, α=0.05).

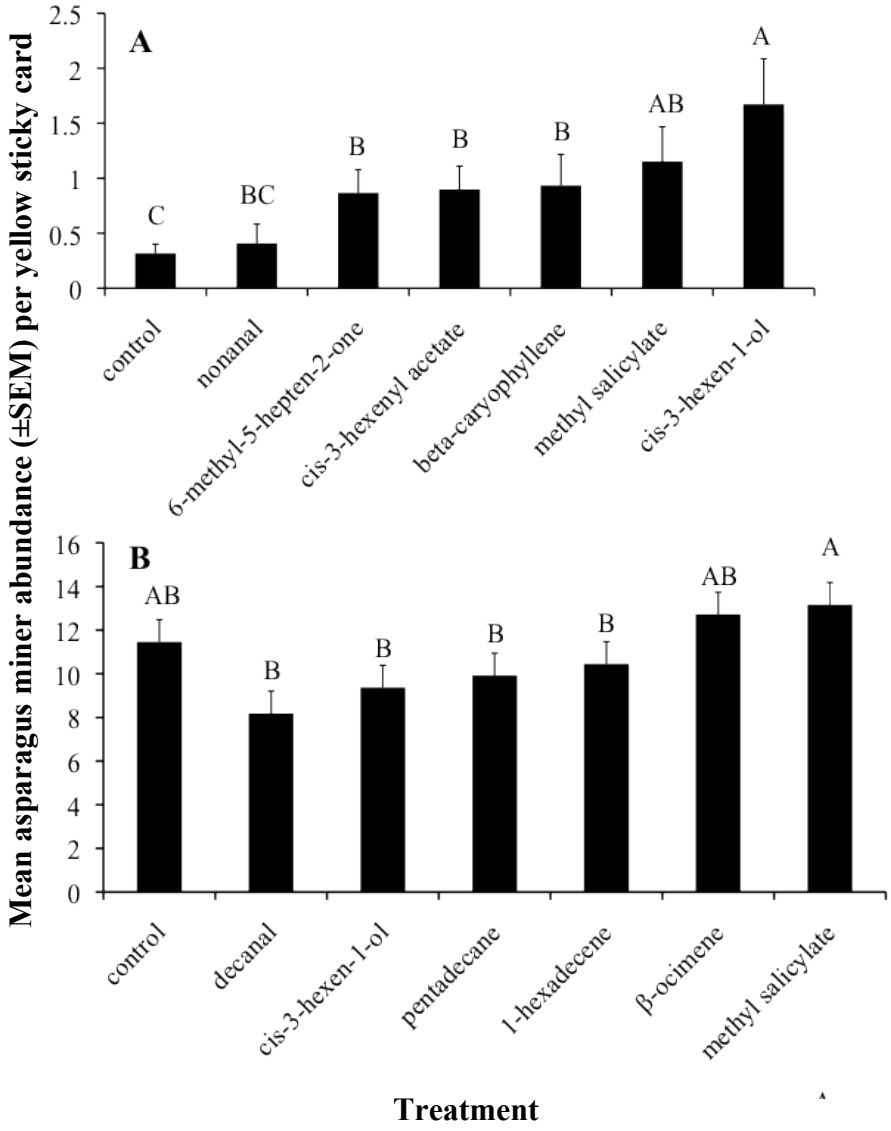


Figure 5.3. (cont'd)

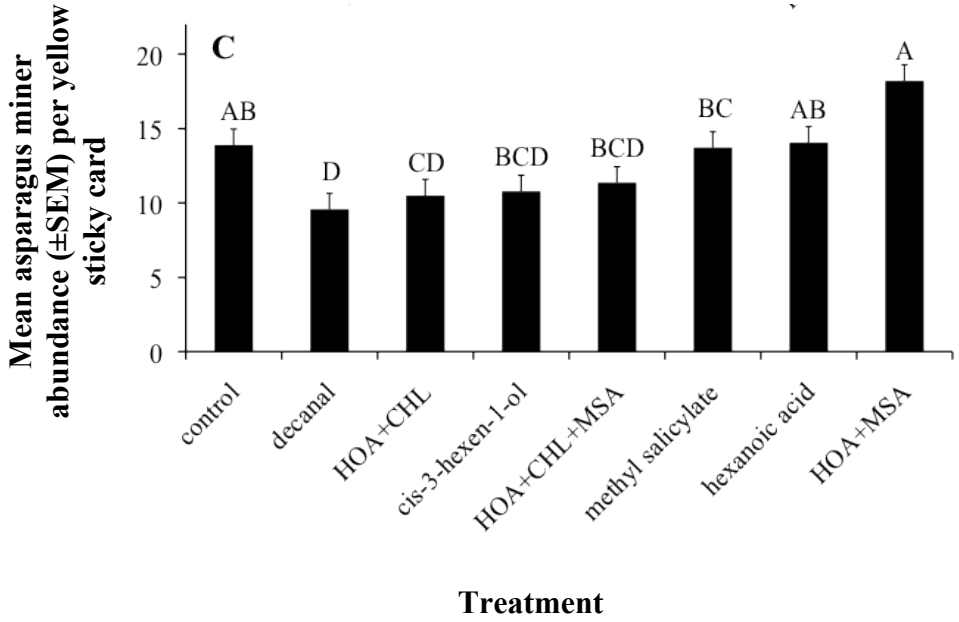


Figure 5.4. The abundance of pests (black) or parasitoids (white) in 2013 on yellow sticky traps (N=204 per volatile and sampling date) placed in four commercial asparagus fields in Oceana Co., MI. Upper case and lower case letters represent pairwise Tukey's HSD (α=0.05) comparisons between the seasonal mean number of parasitoids and pests, respectively. Shared letters between treatments indicate that there are no significant differences between the groups.

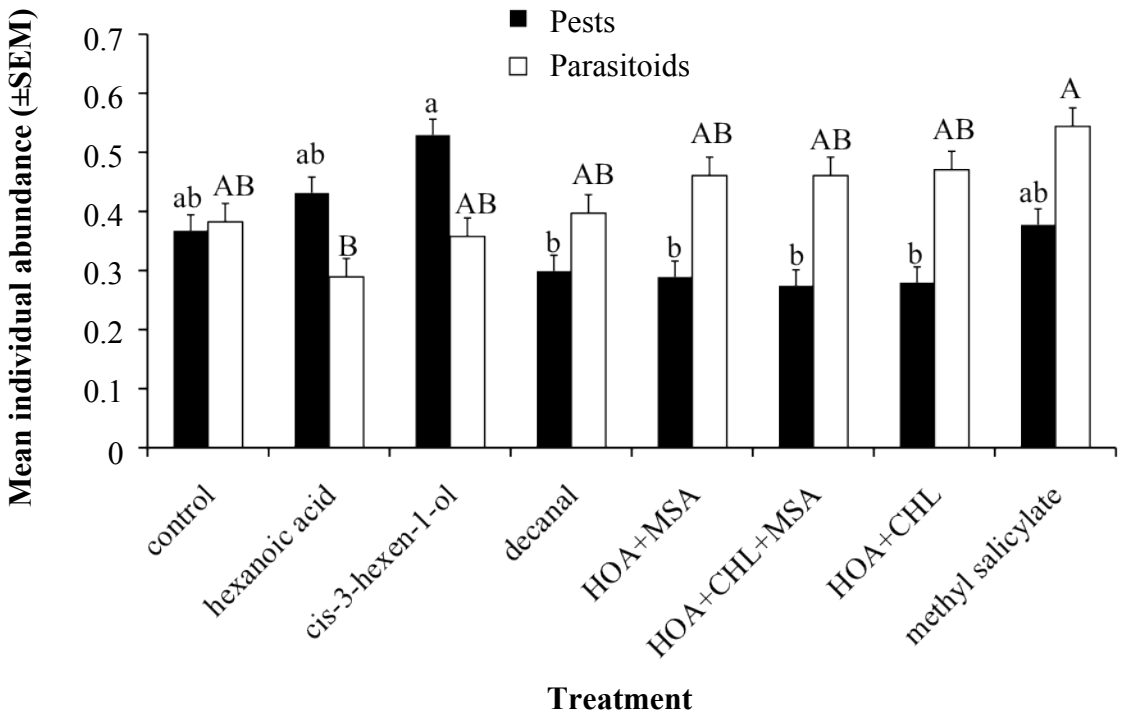
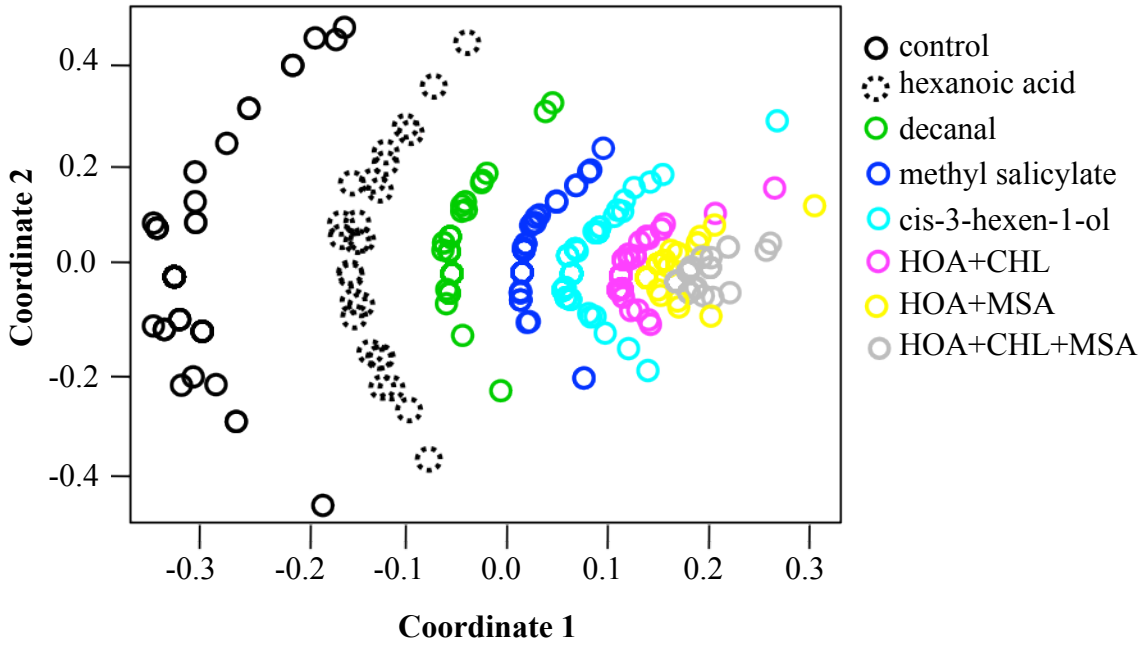


Figure 5.5. Non-metric multi-dimensional scaling ordination of species communities for a given volatile on a specific sampling date for pest families caught on yellow sticky traps during 2013 in four commercial asparagus fields in Oceana Co., MI. The greater the distance between two circles, the more dissimilar the species composition (ANOSIM: R=0.83, P<0.001). Abbreviations: MSA – methyl salicylate, CHL – cis-3-hexen-1-ol, HOA – hexanoic acid.



Discussion

This study is the first to detail the headspace volatiles emitted by asparagus and how it may influence its associated arthropod community. Induced asparagus produces a qualitatively and quantitatively different volatile headspace than intact plants. Asparagus volatile baits placed in the field affected the behavior of both pests and natural enemies indicating that these may be useful in future integrated pest management programs.

Our headspace collection found aldehydes, alkenes, alkanes and alcohols to be characteristic of asparagus headspace. Previously, Sun et al (2001) identified hexenal and 1-octene-3-ol as the most abundant compounds in ground up asparagus samples. We identified green leaf volatiles as major components, including hexenyl acetate and (*Z*)-3-hexen-1-ol. The main compounds associated with the headspace from mechanically and herbivore damaged asparagus plants in our study included: pentadecane, pentadecanol, (*Z*)-β-ocimene, hexadecyl acetate, and (*E*)-3-hexenyl acetate. Common induced volatiles by caterpillars of various species in other systems include (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene, and various terpenes in cotton (Röse et al. 1996) as well as those same volatiles plus (*Z*)-3-hexen-1-ol and β-caryophyllene in tobacco (De Moraes et al. 2001). The compounds induced by the black cutworms in our study are possibly produced by the jasmonic acid-signaling pathway, since some of the same compounds were found to be associated with this pathway and feeding by caterpillars in a previous study with lima beans (Ozawa et al. 2000). In a study that examined the odor components of 2-hr cooked asparagus, similar compounds were found as some of the components in our headspace collection, for example many 5-10 carbon alkenes, alkanes, alcohols, and aldehydes (Ulrich et al. 2001). Specifically, some of the volatiles included 1-pentanol, hexenal, 1-octen-3-ol, furan-containing, and sulfur-containing compounds (Ulrich et al.

2001). As a result, many of the headspace compounds we found in asparagus are in alignment with findings for compounds identified in other systems in the literature.

In olfactometer assays, we found that the asparagus miner is attracted to healthy asparagus stems over clean air. Asparagus miners seek out newly planted fields (Tuell 2003) that enter the fern stage before production fields and are probably more attractive because of their relatively larger amount of biomass. In a study on *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), odor cues were found to be important for host-location of the crop, and these same cues also served as an aggregation cue for these leafmining flies (Zhao and Kang 2003). However, in the presence of healthy stems, the asparagus miner prefers volatiles coming from the asparagus miner-damaged stems over healthy ones. This highlights the importance of the background context for modulating the ecological role of specific volatiles. Indeed, Webster et al. (2010) has documented how certain attractive volatile cues in a blend cease to be attractive when presented alone (e.g. in a different volatile context). In a field setting, the asparagus miner probably encounters damaged stems in a volatile backdrop of healthy stems, suggesting that the asparagus miner may seek out already impaired stems in which to oviposit its eggs and/or from which to feed. One potential evolutionary reason for this may be because greater numbers of larvae per stem could reduce probability that any individual larva is parasitized (e.g. Côté and Poulin 1995).

The pest complex in asparagus is relatively diverse, having specialist aphids and stem-miners, along with generalist caterpillars and true bugs impacting plants. The (*Z*)-3-hexen-1-ol baits attracted the most and greatest diversity of pests, including Japanese beetles, asparagus beetles and tarnished plant bugs. This compound is a general green leaf volatile (Paré and Tumlinson 1999), and many insects respond to this group of plant volatiles (Bruce et al. 2005).

In our 2011 field experiment the (*Z*)-3-hexen-1-ol bait attracted the most asparagus miners, and this compound is generally attractive to Agromyzidae (James 2005). On the other hand, the same study has shown that (*Z*)-3-hexen-1-ol is also attractive to various natural enemies such as minute pirate bugs, syrphids and micro-hymenoptera, including Braconidae (James 2005). The fact that it was attractive to asparagus pests may make it a suitable component in attract-and-kill baits, though it would need to be combined with a synergist in order to improve catches to appreciable levels. In contrast to the other asparagus pests, the asparagus miner was most attracted to baits containing methyl salicylate singly or in combination with other volatiles during most of the sampled years. When aromatic compounds, including methyl salicylate, are paired with green leaf volatiles, there may be a synergistic effect on attracting pests (Dickens 2000; Piñero and Dorn 2007). This was the case for Colorado potato beetle (*Leptinotarsa decemlineata* Say; Coleoptera: Chrysomelidae) in potato, and oriental fruit moth (*Cydia molesta* (Busck); Lepidoptera: Tortricidae) in peach. The only other described leafmining fly attracted to methyl salicylate specifically is *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae), which had more than double the abundance on methyl salicylate-baited sticky traps in greenhouse tomatoes (Būda and Radžiutė 2008). A related species of mining fly, the serpetine leafminer (*Liriomyza sativae*) had the greatest antennal responses to a variety of green leaf volatiles, including (*Z*)-3-hexen-1-ol, and (*E*)-3-hexenyl acetate, in the headspace of lima beans (Zhao and Kang 2002). A review of the effect of methyl salicylate on various taxa in agricultural settings provided evidence that positive taxis towards methyl salicylate may be genetically conserved among Diptera (Rodriguez-Saona et al. 2011).

Previous research has indicated that certain induced volatiles can repel pests, because these compounds may signal danger for herbivores as a result of the plant's "cry for help" (Dicke

2009). Indeed, in contrast to methyl salicylate, decanal resulted in decreased numbers of both the asparagus miner and other pests in all the years it was included in the field experiment. Compared to the control, decanal-baited yellow sticky traps caught a third fewer asparagus miners. This may signify to herbivores that there is a change in the physiology of the plant that is detrimental in some way to them, as decanal baited traps did not seem to affect the abundance of the third trophic level. Further refinement and combination of decanal with other repellent volatiles may lead to a commercially viable bait that asparagus growers may use to protect newly planted asparagus fields from the asparagus miner. In a review of 34 published studies, including over 50 herbivores, Szendrei and Rodriguez-Saona (2010) found that plant volatile baits were most attractive when they contained multiple compounds. While some plant volatile baits with multiple components attracted greater numbers of asparagus miners, baits with greater than one compound often resulted in equal or decreased numbers of individuals caught relative to the control for the parasitoids and pests. However, this may be due to the identity or concentration of the specific volatile in question. In fact, some studies have found that blends as complex as those containing five compounds are needed to maintain or increase attraction to a bait equal to that found in the natural headspace of a host plant (Piñero and Dorn 2007). In the future, if a bait was to be used to monitor the asparagus miner, those volatiles showing biological activity for this insect should be combined into a bait with multiple components in the ratios present in the plant (Szendrei and Rodriguez-Saona 2010).

The asparagus miner also has an active natural enemy community with 12 known species of parasitoids that cause about 20-30% pupal mortality in commercial fields, as well as a suit of predators (Morrison et al. submitted). Similarly to the asparagus miner, parasitoids in asparagus fields were most attracted to the methyl salicylate baits. Methyl salicylate has been extensively

documented to be attractive to natural enemies in many different cropping systems, including grapes and hops (James and Price 2004), soybean (Mallinger et al. 2011), strawberries (Lee 2010), lima bean (de Boer and Dicke 2004), and tomato (Ament et al. 2010), among others. Taxa that have been attracted to traps placed in these crops include braconid wasps, minute pirate bugs, flower flies (James and Price 2004), green lacewings, brown lacewings, chalcids, lady beetles (Zhu and Park 2005), and predatory mites (Ament et al. 2010; de Boer and Dicke 2004), among others. Likewise, we found that braconid wasps were most attracted to methyl salicylate in our study by a factor of almost 2:1 compared to the control. Parasitoids and natural enemies may also be manipulated in asparagus fields with induced plant volatiles, which could lead to decreases pest pressure. However, formulations need to be devised that do not concurrently attract the asparagus miner.

Overall, our research contributes to basic and applied knowledge for an integrated pest management program for the asparagus miner. Outstanding topics to be explored further include 1) understanding the identity and role of asparagus miner pheromones in mediating the behavior of the asparagus miner among conspecfics, 2) using GC-EAD with the asparagus miner to identify biological activity of the volatiles described in this study, and 3) elucidating the influence of asparagus volatiles on potential conservation biological control candidates for the asparagus miner (e.g. *Thinodytes cephalon* and *Chorebus rondanii*: Morrison et al., submitted).

Acknowledgement of Prior Submission

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CHAPTER 6

Conclusions and Future Directions

Asparagus is threatened by a diverse array of insect and fungal pathogens. Foremost on this list is the asparagus miner, which may be a vector for the causative agent of Fusarium crown and root rot. The research in this dissertation focused on three main insect-plant interactions, namely the spatiotemporal distribution of the asparagus miner, the natural enemy community of the pest, and the chemical ecology of asparagus. Specifically, this dissertation investigated how the population dynamics of the asparagus miner are affected by temperature, as well as how management may be affected by the spatiotemporal distribution of pest individuals. In addition, the pupal parasitoids and natural enemies of the asparagus miner, and the longevity of both on various dietary resources were investigated. Finally, the patterns of asparagus emissions based on health status, and the repercussions that these volatiles have for other arthropods in the asparagus field was analyzed.

In terms of spatial distribution, results indicate that asparagus miners are uniform, then clustered around field edges in the first and second generations, respectively. Neighboring habitats to field also had an impact on the abundance of asparagus miners. However, adult movement on a landscape-level is still poorly understood. I found asparagus miners up to 35 m outside of asparagus fields. Future studies should track asparagus miner movement in different parts of the season over a wider area to understand how widely asparagus miners are found outside of asparagus fields, and when the greatest adult movement happens. The clustering near the edges in the second generation suggests that asparagus miner movement increases later in the

season as ovipositional sites become scarce in focal fields. Moreover, it would also be interesting to evaluate landscape-level effects on the abundance of asparagus miners in a field in a more systematic pattern, employing GIS to discover the key landscape cover variables determining abundance. For example, I observed a loose connection between neighboring orchards and a greater abundance of asparagus miner adults in a field. These provide ample areas to follow-up on the research undertaken in this study.

I found that the degree-day model could reliably predict important phenological events in the life cycle of the asparagus miner. This model will be available to asparagus growers on MSU EnviroWeather's website and will undoubtedly help them in knowing when to spray for the asparagus miner. However, future work needs to determine specific intervals for spraying that will yield the maximum level of control for adult asparagus miners. Because the model takes into account both adult and immature life stages, intervals for applying systemic insecticides may also be developed for the immature stages when effective chemistries are discovered.

Chorebus rondanii and *T. cephalon* are the best candidates for a conservation biological control program in Michigan. There was substantial evidence that sugar-rich resources can increase the lifespan of both the asparagus miner and its parasitoids, and that certain floral resources are more favorable to the asparagus miner than others. In future work, the flowers found to be unfavorable to the asparagus miner, buckwheat and fava bean, need to be tested to determine their impact on the lifespan of the two conservation biological candidates. If these are found to increase their lifespan, the flowers can be planted in the margins of asparagus fields to determine whether they can increase biological control in commercial asparagus fields. These or other flowers may be useful in a conservation biological control program for the asparagus miner without the need to rear the parasitoids in the lab However, if an adequate rearing procedure for

the parasitoids can be developed, then a program using the biological candidates in augmentative releases could also be evaluated for suppression of the asparagus miner. In addition, this study mostly evaluated the pupal parasitoids of the asparagus miner, but there are likely also egg parasitoids (at least one in this study has been described as a egg parasitoid on other concealed feeders) as well as many predators in the field that use the asparagus as a host or prey item. If a colony of asparagus miners can be established in the lab, then eggs could be deployed in a sentinel study for egg parasitoids. This study evaluated other predators that are present in asparagus fields, but not predators of the asparagus miner per se. As a result, molecular gut content analysis could be employed to determine which other predators in the environment are actively consuming the asparagus miner. This will provide valuable ecological information to support a biological control program. Once predation is assessed, the relative contribution of predators or pupal parasitoids to the mortality of the asparagus miner could be determined.

In the third part of this dissertation, I evaluated the chemical ecology of asparagus. I found that the headspace of asparagus changes both quantitatively and qualitatively, depending on whether asparagus plants are healthy, mechanically-damaged or herbivore-damaged. Overall, there were 20+ compounds in the headspace of asparagus, but only a fraction of these were tested based on preliminary data and documentation of biological activity in other systems. As a result, a logical next step would be to assay the asparagus miner using GC-EAD to assess the remaining volatiles described in the asparagus headspace from this study. This would allow researchers to identity those that have biological activity with the asparagus miner, and the ones resulting in the greatest response could be deployed in various combinations in a baiting experiment. I found that methyl salicylate-containing baits not only attracted the most parasitoids, but also attracted the greatest abundance of asparagus miners, suggesting that baits containing

this compound should not be used in asparagus fields. Likewise, cis-3-hexen-1-ol resulted in the greatest numbers of pests, and should also be avoided in the future. Follow-up research may likely yield important information about baits for the asparagus miner to use in managing pest populations.

Overall, this research contributes significantly to an integrated pest management program, combining different approaches towards the single goal of controlling asparagus miner populations. Future research into this pest and agricultural system may be expected to yield even greater benefits by building on the foundation laid out in this dissertation.

APPENDICES

APPENDIX 1.1

Supplementary Material

Supplementary Table S1. Monthly normal mean temperatures (°C)/total precipitation (mm) and observed departures from normal, March through October, for Hart, Michigan, 2010, 2011, and 2012[37]

	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	MAR-OCT
2010				1.9/86.1	3.4/41.1	3.5/-4.3	0.9/-12.9	2.1/-45.0	2.7/65.0
2011	-0.7/-18.3	0.1/56.6	1.4/-37.8	1.3/11.4	3.5/7.1	2.0/14.0	1.0/7.6	2.2/11.9	1.4/52.5
2012	8.8/51.8	0.5/-6.6	3.2/-14.7	2.4/-9.4	4.7/33.5	1.4/-49.5	1.3/-29.2	0.8/96.3	2.9/72.2
Normal 1981-2010	-0.4/53.8	6.1/77.7	11.7/95.8	16.8/82.5	19.2/77.5	18.6/84.1	14.4/90.9	8.3/89.4	11.8/651.7

Supplementary Table S2. Summary of the data used from previous studies in various analyses within the current publication, with degree-days calculated for each publication from the weather stations indicated, using a base of 12.05C, a biofix date of 1 March and the Baskerville-Emin calculation method.

Year	City	Locale	Used in Analysis	Source of Weather Info	GPS Coordinates	Publication
1912	Geneva	NY	DTB[a]	Cornell Agricultural Experiment Station	42°52'45.57"N, 77°01'54.33"W	Fink, 1913
1934	Harpenden	UK	LSA, DTB	Rothamsted Experimental Station	51°48'39.36"N, 0°22'36.95"W	Barnes, 1937
1935	Harpenden	UK	LSA, DTB	Rothamsted Experimental Station	51°48'39.36"N, 0°22'36.95"W	Barnes, 1937
1940	Prosser	WA	DTB	Western Regional Climate Center	46°15‘00.00”N, 119°45’0.00”W	Eichmann, 1943
1975	East Lansing	MI	LSA, RV, DTB	Horticultural Research Farm, MSU	42°40'24.24"N, 84°29'13.20"W	Lampert et al., 1975
1980	Hadley	MA	LSA, RV, DTB	Weather Station at in Chicopee, MA	42°20'09.28"N, 72°55'00.63"W	Ferro & Gilbertson, 1980
2001	Hart	MI	LSA, RV	Enviro-Weather Station, Hart	43°44'11.33"N, 86°21'31.48"W	Tuell, 2003
2002	Hart	MI	LSA, RV	Enviro-Weather Station, Hart	43°44'11.33"N, 86°21'31.48"W	Tuell, 2003
2010	Hart	MI	LSA, RV, DTB	Enviro-Weather Station, Hart	43°44'11.33"N, 86°21'31.48"W	Current
2011	Hart	MI	LSA, RV, DTB	Enviro-Weather Station, Hart	43°44'11.33"N, 86°21'31.48"W	Current
2012	Hart	MI	LSA, RV, DTB	Enviro-Weather Station, Hart	43°44'11.33"N, 86°21'31.48"W	Current

[a]Abbreviations: LSA - locality sensitivity analysis, RV - regression for validation, DTB - developmental time budget

Supplementary Table S3. Various estimates that were used in the developmental degree-day budget, their sources, calculation methods, and corresponding Julian Date estimation.

Stage	Type	Year	Source	Calculation Method	Julian Days	DD Estimate[a]
Adult	EC[b]	2011	Current study	Adult lifespan with sugar diet	8	213
Adult	EC	2012	Current study	Adult lifespan with sugar diet	13	343
Adult	EC	2011	Current study	Adult lifespan with honey diet	7	188
Adult	EC	2012	Current study	Adult lifespan with honey diet	5	153
Adult	Insectary	1940	Eichmann, 1943	Adults raised on unknown diet	4	51
Adult	Insectary	1940	Eichmann, 1943	Adults raised on unknown diet	10	127
Egg	Field	1934	Barnes, 1937	First eggs to first larvae	17	64
Egg	Field	1912	Fink, 1913	Lower limit of egg duration	12	100
Egg	Field	1912	Fink, 1913	Upper limit of egg duration	18	166
Egg	Insectary	1934	Barnes, 1937	Lower limit of egg duration	14	108
Egg	Insectary	1934	Barnes, 1937	Upper limit of egg duration	21	147
Larval	Field	2011	Current study	Diff. btw. first larval & pupal appearance	7	110
Larval	Field	2011	Current study	Diff. btw. first larval & pupal peak	14	277
Larval	Field	2011	Current study	Diff. btw. second larval and pupal peak	6	76
Larval	Field	2012	Current study	Diff. btw. first larval and pupal peak	7	150
Larval	Field	2012	Current study	Diff. btw. second larval and pupal peak	14	240
Larval	Field	1980	Ferro & Gilbertson, 1980	Diff. btw. first larval and pupal appearance	14	156
Larval	Field	1980	Ferro & Gilbertson, 1980	Diff. btw. first larval and pupal peak	7	254
Larval	Field	1975	Lampert et al., 1975	Diff. btw. first larval and pupal appearance	7	162
Larval	Field	1975	Lampert et al, 1975	Diff. btw. first larval and pupal peak	6	116
Larval	Field	1975	Lampert et al., 1975	Diff. btw. second larval and pupal peak	14	288
Larval	Insectary	1934	Barnes, 1937	From egg hatch to pupation	7	65
Pupal	Field	2011	Current study	First appearance of adults	77	103
Pupal	Field	2012	Current study	First appearance of adults	48	157
Pupal	Field	2002	Tuell, 2003	First appearance of adults	81	158
Pupal	Field	2001	Tuell, 2003	First appearance of adults	90	263
Pupal	Field	1980	Ferro & Gilbertson, 1980	First appearance of adults	76	161
Pupal	Field	1912	Fink, 1913	Time of pupation	17	395
Pupal	EC	2011	Current study	Time to pupal hatch	11	276
Pupal	EC	2012	Current study	Time to pupal hatch	16	410
Pupal	Insectary	1940	Eichmann, 1943	From pupation to hatch	22	330
Pupal	Insectary	1940	Eichmann, 1943	From pupation to hatch	25	375

[a] DD estimates made from a model with a base of 12.1°C, biofix date of 1 Mar, and Baskerville-Emin method of calculation

[b] EC = environmental chamber, constant temp (26°C), 75% humidity, 16:8 L:D

Suplementary Table S4. Summary of Julian day and degree-day estimates for stages in the life cycle of the asparagus miner derived from previous research in Supplementary Table S3.

Stage	N	Avg. Julian Days		SE	% of Total	Avg. DD[a]		SE	% of Total
Egg	5	16.4	±	1.6	21%	117.0	±	18.1	16%
Larval	11	9.4	±	1.1	12%	172.2	±	24.2	24%
Pupal	10	46.3	±	10.0	58%	262.8	±	35.7	36%
Adult	6	7.9	±	1.3	10%	179.1	±	39.9	25%
Total[b]	32	79.9	±	9.0		731.1	±	30.1	

[a] DD estimates based on a model with a base of 12.1°C, biofix date of 1 Mar, and using the Baskerville-Emin method of calculation

[b] Total duration for 1 generation. There are usually two generations per year.

Supplementary Table S5. Summary of known parasitoids of the asparagus miner (*Ophiomyia simplex* Loew) from the published literature.

Species	Family	Locality	Citation
Chorebus rondanii (Giard)	Braconidae	United Kingdom, United States (Massachusetts)	Barnes and Walton, 1934
Pediobius epigonus Walker	Eulophidae	United Kingdom	Barnes and Walton, 1934
Neochrysocharis moczari Szelényi	Eulophidae	Hungary	Szelényi, 1973
Dacnusa bathyzona Marsh	Braconidae	United Kingdom	Barnes and Walton, 1934; Barnes, 1937
Sphegigaster sp.	Pteromalidae	United Kingdom	Barnes and Walton, 1934; Barnes, 1937
Centrodora xiphidii Perkins	Aphelinidae	United States (Hawaii)	Bianchi, 1941

Supplementary Table S6. Summary information of variety Millennium asparagus field sites located in Oceana Co., MI.

Field ID	Farm	Area (ha)	Year Planted	Sampled	Nearest field (km)[a]	Farthest field (km)[a]
Farm 1	Farm 1	6.06	2009	2010, 2011, 2012, 2013	0.73	17.00
Farm 2	Farm 2	3.77	2009	2010, 2011,	3.18	14.18
Farm 3	Farm 3	3.54	2009	2010, 2011, 2012, 2013	1.35	16.28
Farm 4	Farm 2	1.82	2008	2010, 2011	5.60	16.14
Farm 5	Farm 1	3.66	2010	2010, 2011, 2012, 2013	0.73	17.56
Farm 6	Farm 3	6.66	2012	2013	1.35	17.56

[a] Nearest distance to the neighboring field, and distance to the most distally located field from the focal field in the experimental area.

APPENDIX 1.2

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: 2014-01

Author and Title of thesis
Author: William Robert Morrison III
Title: Investigation of the tritrophic interactions of the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) and the influence of temperature on its population dynamics

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

Specimens:
**If lowest taxonomic level is above family, lowest classification used for arthropod is indicated

Supplementary Table S7. Voucher specimens deposited at the Albert J. Cook Arthropod Research Collection (Michigan State University).

Family	Genus-Species	Life Stage	Quantity	Preservation
Agromyzidae	*Ophiomyia simplex*	adult / female	10	pinned
Agromyzidae	*Ophiomyia simplex*	adult / female	10	pinned
Agromyzidae	*Ophiomyia simplex*	larvae	10	Weak KAAD/EtOH
Braconidae	*Chorebus rondanii*	adult	5	pinned
Eulophidae	*Neochrysocharis* sp.	adult	5	pinned
Eupelmidae	*Eupelmus vesicularis*	adult	1	pinned
Eupelmidae	*Brasema ?allynii*	adult	1	pinned
Pteromalidae	*Thinodytes cephalon*	adult	5	pinned

Supplementary Table S7 (cont'd)

Family	Genus-Species	Life Stage	Quantity	Preservation
Pteromalidae	*Cytogaster vulgaris*	adult	2	pinned
Pteromalidae	*Sphegigaster cracentis*	adult	2	pinned
Pteromalidae	*Merismus megapterus*	adult	1	pinned
Pteromalidae	*Halticoptera* sp.	adult	1	pinned
Pteromalidae	*Trichomalopsis viridascens*	adult	1	pinned
Totals				
5 families	11 species	2 stages	54 individuals	

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LITERATURE CITED

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