Chemical Ecology and Management of Yellowmargined Leafbeetle *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae)

by

Rammohan Balusu

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Approved by

Henry Fadamiro, Chair, Associate Professor of Entomology
Arthur Appel, Alumni Professor of Entomology
David Held, Assistant Professor of Entomology
Joseph Kloepper, Professor of Plant Pathology

Abstract

Cruciferous vegetable production is an important industry in Alabama and other parts of the southern United States (U.S.). Many farmers in the region grow various kinds of cruciferous crops (e.g., turnip, radish, mustard, napa cabbage, cabbage, collards, arugula, and Japanese leafy vegetables, such as mizuna and mibuna) as mixed cropping systems in the spring and fall using organically acceptable practices. The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) is the most damaging pest of organic cruciferous crop production in the region. The goals of this project are to investigate the ecology of *M. ochroloma* and develop alternative and organically acceptable management practices, in particular biorational insecticides and attractant-based strategies for managing *M. ochroloma* in the southern U.S. In chapter II, I investigated the mechanism of host plant selection and preference in *M. ochroloma*. The host plants investigated were napa cabbage (*Brassica rapa* subsp. *pekinensis* cultivar Minuet F1), collards (*Brassica oleracae* var. *acephala* cultivar champion), cabbage (*Brassica oleracea* var. *capitata* cultivar Farao F1), and turnip (*Brassica rapa* var. *rapa* cultivar purple top white globe). The results showed that turnip and napa cabbage are highly preferred by *M. ochroloma* while cabbage and collards are less preferred host plants. In chapter III, I examined the headspace volatile profiles of four host plants that were tested in host preference study in chapter II using in situ dynamic headspace collection and analytical techniques. The headspace volatile profiles of the two highly preferred host plants(turnip and napa cabbage) were different from that of the less preferred host plants (cabbage and collards), suggesting that host preference is likely mediated by differences in volatile profiles of the host plants. Further analysis by GC-EAD

showed that one peak, which was unique to the highly preferred host plants, elicited significant GC-EAD activity in female beetles. This compound (a novel isothiocyanate) was later identified by GC-MS as a putative host plant attractantfor *M. ochroloma*.

In chapter IV, I carried out laboratory experiments to evaluate the susceptibility of larvae and adults of *M. ochroloma* to some botanical and microbial insecticide formulations using leaf-dip bioassays. Insecticides evaluated included OMRI (Organic Material Review Institute) approved formulations such as PyGanic® (pyrethrum), Entrust® (spinosad), Mycotrol O® (*Beauveria bassiana* strain GHA), and NOFLY® (*Paecilomyces fumosoroseus* strain FE 9901). Others were MBI-203 (an experimental organic formulation of *Chromobacterium subtsugae*) and BotaniGard® 22WP (a conventional formulation of *Beauveria bassiana* strain GHA). The insecticides were first evaluated at the field recommended rate against *M. ochroloma* larvae and adults, followed by multiple-concentration assays to determine the LC_{50} (median lethal concentrations) and LT_{50} (lethal time to kill 50% of test insects) for promising formulations. At the field recommended rate, all tested formulations were toxic to the larvae compared to the untreated control, whereas only Entrust® and PyGanic® were effective against the adults. These two most effective formulations caused 100% mortality to the larvae and adults just after 24 h of exposure. The LC_{50} values of Entrust® and PyGanic® were 200 ×and 15 × less than the actual field recommended rate, respectively. MBI-203 was effective against the larvae (100% mortality after 5 days) but not the adults. All three entomopathogenic fungal formulations, Mycotrol®, NOFLY®, and BotaniGard®, caused significantly higher larval mortality than the untreated control after 5 days of exposure, butnone was effective against the adults. In chapter V, I conducted field experiments over four growing seasons (2007-2010) in Alabama to evaluate some botanical and microbial insecticides evaluated in Chapter IV against *M.* o*chroloma* in

organically grown crucifer crops. Insecticides evaluated included OMRI approved formulation such as PyGanic®, Aza-Direct®, Entrust®, Mycotrol O®, and NOFLY®. Others were Novodor® (*Bacillus thuringiensis* subspecies *tenebrionis*) and two experimental organic formulations, Tick-Ex (*Metarhizium anisopliae* strain F52) and MBI-203. The insecticides were applied as stand-alone treatments at recommended field rates on a weekly schedule. The results showed that Entrust® consistently performed well in suppressing *M. ochroloma* adults, larvae and crop damage. PyGanic® was the second best treatment. PyGanic® or NOFLY™ can be applied in rotation with Entrust® for effective management of *M. ochroloma*. Entrust® applied weekly or in alternation with PyGanic® and NOFLY™ provided acceptable control of *M. ochroloma* in organic crucifer production. The findings of this study have given the base line information towards the development of attractant-based management strategies, such as detection and monitoring, attract and kill, and push-pull strategies, against *M. ochroloma* in organic crucifer vegetable production.

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Dedication

This work is dedicated to my father and mother

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Production and Uses of Vegetables

Cruciferous vegetable crops (Cole crops) have an estimated annual market value of about $3.5 billion in the U.S. (USDA-NASS 2009). Nationally, Alabama ranks 12th in commercial crucifer vegetable production with an annual value of over $10 million (USDA, NASS 2009). The most common cruciferous crops in the southern U.S. are broccoli, cabbage, cauliflower, turnip, collards, radish, mustard and Japanese leafy vegetables. Since the enactment of the Organic Foods Production Act (OFPA) in 1990, the production and development of standards for U.S. organic products have improved considerably (Dimitri and Oberholtzer 2005). This also improved the production of organic cruciferous crops which were included in the Specialty Crop Competitiveness Act of 2004 signed by President Bush on December 21, 2004. The Act was to help promote increased production and consumption of specialty crops and increase the competitiveness of specialty crop producers. Although cruciferous crops are traditionally produced using conventional production practices, organic crucifer vegetable production is an emerging industry in Alabama and much of the southern U.S. Many cruciferous crops are usually grown organically as mixed cropping systems in the spring and fall in the region.

Vegetables crops including crucifers are a vital source of essential minerals, vitamins and dietary fiber. More recently these crops have been recognized as a vital source for

phytochemicals. Van Duyn and Pivonka (2000) reported that vegetables can reduce the risk of cancer and coronary heart disease. This underscores the importance of vegetables in the diet of people across the world.

1.2. Organic Crucifer Vegetable Production

Although organic production has currently emerged as one of the ways to reduce the use of synthetic inputs in agriculture in many parts of the U.S., particularly the northern states, organic crucifer vegetable production is just emerging in the southern U.S. Cruciferous crops which are grown organically in most parts of the U.S. include turnip, radish, mustard, napa cabbage, cabbage, collards, arugula, and Japanese leafy vegetables such as mizuna and mibuna.These crops are usually grown as mixed cropping systems in the spring and fall in the region (Figure 1). In the U.S. growers and the acreage dedicated to organic vegetable farming has increased considerably. However, organic vegetable production in the southern U.S. is far behind other regions (Green and Kremen 2001). The reason for this lower rate of adoption of organic vegetable production in the region is due to production the challenges posed by insects pests. Conditions such as a warm humid climate and relatively mild winters favor year-round development and persistence of insect pests, diseases and weeds, which subsequently harm organically produced crops.

1.3. Key Insect Pest Challenges of Crucifer Vegetable Growers

Cruciferous crops like many other crops have a wide range of insect pests that attack them and make their production very difficult. Among the pests that attack these crops are diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), imported cabbageworm,

Pieris rapae (Linnaeus) (Lepidoptera: Pieridae), centre grub, *Hellula hydralis* (Guenee) (Lepidoptera: Pyralidae), cabbage cluster caterpillar, *Crocidolomia pavonana* (Fabricius) (Lepidoptera: Pyralidae), cluster caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), *Helicoverpa* spp. (Lepidoptera: Noctuidae), armyworms (*Spodoptera* spp.), cutworms (*Agrotis* spp.), and flea beetles (*Phyllotreta spp.*) (ATTRA 2006, Southeastern U.S vegetable crop hand book, 2008, Heisswolf et al. http://web.entomology.cornell.edu/shelton/diamondbackmoth/pdf/1996papers/1996DBM39.pdf).

However, yellowmargined leaf beetle (*Microtheca ochroloma*) is has become an important pest that is currently responsible for the damage in organic crucifer production in the southeastern U.S. This insect has consistently been ranked the most limiting factor in organic vegetable production in the region. Among southern growers, the insect is commonly known as turnip bug. Chamberlin and Tippins (1948) first reported that the insect came to the U.S. through Mobile, AL., in 1947 from South America and has now spread to all major southern regions, particularly where organic vegetables are popular (Ameen and Story 1997a,b,c, Story et al. 1997, Bowers 2003, Overall and Edelson 2007). Despite its economic importance and wide distribution in the southern U.S., there is very little research on the biology and management of *M. ochroloma*.

1.4. Description, Biology, and Ecology of *Microtheca ochroloma*

Despite its economic importance and wide distribution in the southern U.S., little is known about the biology and ecology of *M. ochroloma*. The adult is approximately 0.2 inch long with a black head and brown wing covers (Figure 2). The beetle owes its name to the conspicuous pale-yellow border surrounding the wing covers on each of which are four rows of deep pits or

punctures. It overwinters presumably as adults, which become active during early spring. Eggs (Figure 2) are laid under foliage of crucifer plants and the larvae (Figure 2) pass through four instars. The pupae (Figure 2) are commonly found above ground attached to leaves or debris (Chamberlin and Tippins 1948). Total developmental period under laboratory conditions is ~ 27 days and the beetle has multiple generations per year. Adult *M. ochroloma* is long-lived with longevity of ~105 days on radish (Ameen and Story 1997a, b). There are anecdotal reports of higher field population densities of *M. ochroloma* on turnip and mustard relative to the other crucifer plants (Oliver and Chapin 1983). Hence the popular view that turnip, mustard, and radish are better hosts than collard and cabbage. However, there are presently no quantitative data to support this notion.

It is not known how *M. ochroloma* locates its host plants. Many pest insects have been shown to use host-specific semiochemical odors (kairomones) as cues to locate their hosts. For instance, adult Colorado potato beetle, *Leptinotarsa decemlineata* (Say) is attracted to potato plant volatiles (Landolt et al. 1999). All crucifer plants (family Brassicaceae) contain characteristic secondary plant metabolites called glucosinolates, the precursor of mustard oils. Glucosinolates, upon hydrolysis by myrosinase, are broken down into biologically active compounds such as isothiocyanates, nitriles, and organic cyanides, and are responsible for the characteristic bitter and sharp taste of plants such as mustard, cabbage, and horse-radish. Glucosinolates are generally considered to have defense function against herbivores. However, pests of crucifer plants have apparently evolved to use these compounds to locate their host plants. In fact, several synthetic glucosinolate compounds are known attractants for various pests of crucifer plants (Finch 1986). For instance, laboratory and field studies have demonstrated attraction of flea beetles (*Phyllotreta spp*) to various glucosinolate derivatives (as single compounds or mixtures)

including allyl isothiocyanate, benzyl isothiocyanate, ethyl isothiocyanate, butenyl isothiocyanate, methyl-4-isothiocyanate, n-butyl isothiocyanate, and butenyl thiocyanate (e.g., Liblikas et al. 2003). It is likely that *M. ochroloma* uses crucifer-specific volatiles for host location and selection. However, the nature and identity of such volatile compounds mediating host location and preference remain unknown. Given the potential applications of semiochemicals in IPM, isolation and identification of compounds mediating host location will likely contribute immensely towards the development of organically acceptable IPM strategies (e.g., monitoring, mass trapping, and attract-kill) for *M. ochroloma*.

Currently, there are no published studies on efficacy of organically acceptable management tactics against *M. ochroloma*. Pest management tactics and formulations approved by the Organic Materials Review Institute (OMRI) and that could potentially be used to control *M. ochroloma* in organic and low-input vegetable production include applications of botanical insecticides, insecticidal soaps, biopesticides, microbials (including entomopathogenic fungi and nematodes), and semiochemicals. However, these tactics need to be evaluated against this beetle in Alabama organic vegetable fields.

1.5. Integrated Management of *Microtheca ochroloma*

Microtheca ochroloma pressure is usually not intense in most commercial production systems but could be devastating in an organically managed vegetable farm. Integrated pest management, which is a decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner, is the only strategy that can effectively deal with many pest problems.

Although the control of most pests of vegetables has relied extensively on the use of insecticides, the situation has changed recently and that emphasis and philosophy toward research on control of vegetable pests have instead been geared towards the use of integrated pest management rather than over-dependence on chemicals. The reason for this change is due largely to consumer perceptions about safety and also the fact that many insect pests developed resistance to these insecticides. This resistance required that insecticide volumes had to be increased, resulting in many adverse effects such as health problems to man, non-target organisms, contamination of water bodies etc. The emphasis of pest management through IPM was shifted to understanding pest and host plant biology, monitoring, population dynamics, cultural control, and biological control. Host plant resistance with insecticides has been the last resort when all control options fail.

1.5.1 Monitoring (sampling)

Pest monitoring is a critical component and usually the major step in finding for a control strategy for a given pest. Monitoring using traps can assist in detecting the insect pests, timing of control measures, risk assessment, and population density estimates (Alston 1996). Monitoring can also help in establishing a quantitative relationship between trap captures of a particular pest and the plant damage caused by it. Monitoring systems have enabled more effective targeting of major pest control tactics including use of pesticides and biopesticides.

Currently there is no trapping program developed for *M. ochroloma* because no effective trap and attractant are available. Methods that have commonly been used for other related species of chrysomelid such as Colorado potato beetle, *Leptinotarsa decemlineata* (Say), can potentially be used for monitoring *M. ochroloma* is by the use of direct visual plant counts or

sweep nets (Senanayake and Holliday 1988). Pitfall traps and different types of barriers are also available to estimate or monitor the walking population of this related species (Voss and Ferro 1990, Noronha and Cloutier 1999) which can be exploited for *M. ochroloma*, but to date none of these have been tested for *M. ochroloma*.

1.5.2 Cultural Control

Cultural control practices modify the environment, making it less favorable to pest invasion, reproduction, survival, or dispersal (Flint and Gouveia 2001). However, some cultural practices which include organic mulch, reduced pest populations, increased soil moisture, suppression of weeds, and increased crop yield (Greer and Dole 2003), were shown to be ineffective in managing *M. ochroloma* (Manrique et al. 2010).

Another cultural management practice that has high potential to manage *M. ochroloma* is the removal of neglected or wild hosts. These plants usually serve as alternate host of *M. ochroloma* and other insect pests. Also, planting, some plants in the borders as refuge or a "trap crop," could reduce migration into organic fields. However, to date none of these practices have been tested against *M. ochroloma*.

1.5.3 Arthropod and other Natural Enemies

No comprehensive studies have been done to determine the number of biological agents that directly or indirectly attack *M. ochroloma*. Since vegetables particularly the cruciferous crops, are produced annually, the long-term continuity of overwintering sites and habitats for both pests and natural enemies show some degree of instability. The possibility of directly utilizing biological control agents is generally not encouraged because of the short-term

plantings. Little information is available in the literature about natural enemies of *M. ochroloma* (Montemayor and Cave 2009). No classical biological control involving the introduction of natural enemies from the host insect's place of origin has been attempted because the species until recently was not a problem in commercial vegetable production. Several authors have provided a list of three generalist predators, *Podisus maculiventri* (Say), *Hippodamia convergens* (Say), and *Chrysoperla rufi labris* (Burmeister), which have been observed preying on the *M. ochroloma* in the field and in the laboratory (McPherson 1980, Weeden et al. 1993, Flint and Dreistadt 1998, Montemayor and Cave 2009).

Hough-Goldstein et al. (1993) also provided a list of some of the important natural enemies reported to attack some species of the family Chrysomelidae. These include mites, phalangid, spiders, and insects that have been studied as potential biological control agents of the Colorado potato beetle and other related chrysomelid beetles. Insects such as lacewings (Neuroptera: Chrysopidae), predatory stink bugs (Hemiptera: Pentatomidae, subfamily Asopinae), parasitic flies (Diptera: Tachinidae), predatory beetles (Coleoptera: Coccinelidae, Cicindelidae, Staphylinidae, and Carabidae), and parasitic and predatory Hymenoptera can also serve as effective biocontrol agents of most chrysomelid beetles.

1.5.4 Chemical Control

Microtheca ochroloma has traditionally been managed in conventional vegetable production systems in the southern U.S. using multiple applications (sometimes weekly) of synthetic, broad-spectrum, foliar insecticides such as chlorpyrifos (Lorsban) and diazinon (Story et al. 1997). Repeated applications of these insecticides coupled with the short generation time of *M. ochroloma* (~25 days under favorable conditions) may facilitate resistance development and

have negative impacts on beneficial arthropods. Furthermore, many of the available conventional insecticides are being restricted as a result of the Food Quality Protection Act (FQPA 1996). Currently, there are no effective control options available to organic vegetable farmers. Pest management tactics and formulations approved by OMRI, which could potentially be used to control *M. ochroloma* in organic and low-input vegetable production include applications of botanical insecticides, insecticidal soaps, and microbials; however, there are no published studies on their efficacy against *M. ochroloma*.

Generally, botanicals insecticides, which contain plant extracts as active components, are environmentally friendlier than synthetic insecticides (Dadang et al. 2009). They are also considered safer compounds because they degrade more rapidly than most conventional pesticides In addition; they are less likely to kill beneficial insects than insecticides with longer residual activity. In spite of their advantages, there are other limitations that make them less attractive to growers. For example, they have to be applied more often in addition to higher costs of production, which usually mak botanicals more expensive to use than synthetic insecticides. Although botanicals are considered as a "natural" product, most of them are not labeled for all crops and not all are allowed by organic certification standards. The toxicity of botanicals to other organisms is variable; although as a group, they tend to be less toxic to mammals (with the exception of nicotine and rotenone) than non-botanicals (Weinzierl 2000, Pottorff 2010). Examples of some botanicals that have been used in pest management include pyrethrum, rotenone, sabadilla, neem, etc.

Pyrethrum is the dried flower of a Chrysanthenum plant, *Chrysanthemum cinerariaefolium,* which grows mainly in East Africa particularly Kenya and Tanzania (http://www.coopext.colostate.edu/4dmg/VegFruit/organic.htm). The insecticidal chemical that

is obtained from the flower of this plant is called pyrethrins. Generally, pyrethrins act as nerve poisons causing immediate paralysis to most insects. Many insects eventually recover from pyrethrum paralysis so its efficacy is boosted by addition of a synergist, piperonyl butoxide (PBO) (Weinzierl 2000, Pottorff 2010). Pyrethrins break down very quickly in sunlight. Pyrethroids are synthetic insecticides, chemically similar to pyrethrins, but more toxic and longer lasting. They are not allowed in certified organic programs (Weinzierl 2000, Pottorff 2010).

Rotenone is extracted from the roots of several tropical legumes such as the Cube plant (*Lonchocarpus* spp.*)* grown in Peru. Originally used as a fish poison by the Indians, it is highly toxic to fish and moderately toxic to humans. A broad spectrum poison mainly used to control leaf-eating beetles and caterpillars, rotenone breaks down quickly in sunlight or when mixed with soaps or lime (Weinzierl 2000, Pottorff 2010).

Sabadilla is an alkaloid derived from the seeds of a tropical American lily. It is most effective against true bugs such as harlequin bugs and squash bugs (Weinzierl 2000, Pottorff 2010). Sabadilla degrades quickly. The purified form is highly toxic to mammals. The dust is moderately toxic to mammals, but is highly toxic to honey bees.

Ryania is an alkaloid derived from the stems of a South American shrub. A slow-acting stomach poison with moderate toxicity to mammals, it has longer residual activity than most botanicals (Weinzierl 2000).

Nicotine, derived from tobacco, is extremely toxic and fast-acting on mammals. Most organic certification programs do not allow the use of nicotine. The most common use is in greenhouses and to control soft bodied insects such as aphids and mites (Pottorff 2010).

Neem, derived from the neem tree of arid tropical regions, contains many active compounds that act as feeding deterrents and as growth regulators. The main active ingredient

is azadirachtin, which is said to be effective on 200 types of insects, mites, and nematodes. It has low toxicity to mammals (Weinzierl 2000, Pottorff 2010).

1.6 Justification

Increasing consumer interest in organically-grown vegetables has made organic vegetable production one of the fastest growing segments of U.S. agriculture. However, organic vegetable production has lagged in the southern U.S. (Green and Kremen 2001). Pest management in organic and low-input systems is a major challenge nationwide and is a major factor limiting the growth of organic vegetable production, particularly, in the hot and humid climate of the southern U.S. (Green and Kremen 2001). *Microtheca ochroloma* is presently regarded by many southern farmers as the most devastating pest of organic crucifer crops and an emerging pest in conventional vegetable production systems. Crop profiles or pest management strategic plans (PSMPs) have not been developed for organic cruciferous vegetable production in the South. However, *M. ochroloma* is listed in several southern states' crop profiles, including Crop Profile for Leafy Greens in Georgia (Guillebeau 2001) and Crop Profile for Leafy Brassicas in Florida (Aerts and Mossler 2005). *M. ochroloma* is also listed as one of the major local problems in "Growing Small Farms" in North Carolina (http://www.ces.ncsu.edu/chatham/ag/SustAg/yelmarginleafbeetle.html). The impact of *M. ochroloma* on organic vegetable production in Alabama and other southern states has been documented in other ways, including at grower meetings and workshops.

1.7 Dissertation Outline, Goals and Objectives

The goal of this dissertation is to develop an effective IPM program for *M. ochroloma* in organic and low-input crucifer vegetable production based on attractants and effective biorational insecticides. The main aim is to overcome the challenges posed by insect pests by developing appropriate National Organic Program (NOP) approved IPM solutions for organic crucifer vegetable production in the southern U.S, in particular Alabama.

Specific objectives are as follows:

1). Investigate mechanism of host plant selection and preference in yellowmargined leaf beetle (*M. ochroloma*)

2). Identify the semiochemical cues used by *M. ochroloma*to locate crucifer host plants

3). Evaluate efficacy of organically-acceptable (OMRI-approved) biopesticides and botanicals against *M. ochroloma*

In chapter II, I investigated the mechanism of host plant selection and preference in *M. ochroloma*. The host plants investigated were: napa cabbage (*Brassica rapa* subsp. *pekinensis* cultivar Minuet F1), collards (*Brassica oleracae* var. *acephala* cultivar Champion), cabbage (*Brassica oleracea* var. *capitata* cultivar Farao F1), and turnip (*Brassica rapa* var. *rapa* cultivar Purple top white globe). The results showed that *M. ochroloma* preferred turnip and napa cabbage compared with the other test host plants. A follow-up study was conducted in the laboratory (Chapter III) to identify semiochemical cues used by *M. ochroloma* to locate crucifer host plants using analytical and electrophysiological techniques. Results showed that volatiles profiles of preferred host plants were qualitatively different from less preferred host plant. Furthermore, biologically active compound detected in host plant was present only in preferred

hosts. Probable explanations for results and proposals for further investigations are discussed. In chapter IV, laboratory evaluation of microbial and botanical insecticides was conducted. Results showed that three insecticidal formulations, Entrust®, PyGanic®, and MBI-203, were effective against both larval and adult *M. ochroloma*. These insecticides were further evaluated under field conditions (Chapter V) and showed consistently similar results.

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Figure Legend

Figure 1. Typical vegetable field with mixed crucifer crops in rows typical of organic growers farm in Alabama

Figure 1. Life stages of *M. ochroloma*: Eggs (a); Larvae (b); Pupae (c); Adults (d)

Figure 2. Feeding damage caused by *M. ochroloma* to napa cabbage (a); and turnip (b).

Figure 3. A row of organically-managed napa cabbage field in south Alabama severely damaged by *M. ochroloma* (a); close-up view showing severe defoliation of napa cabbage by *M. ochroloma* (b)

Figure 1.

Figure 2.

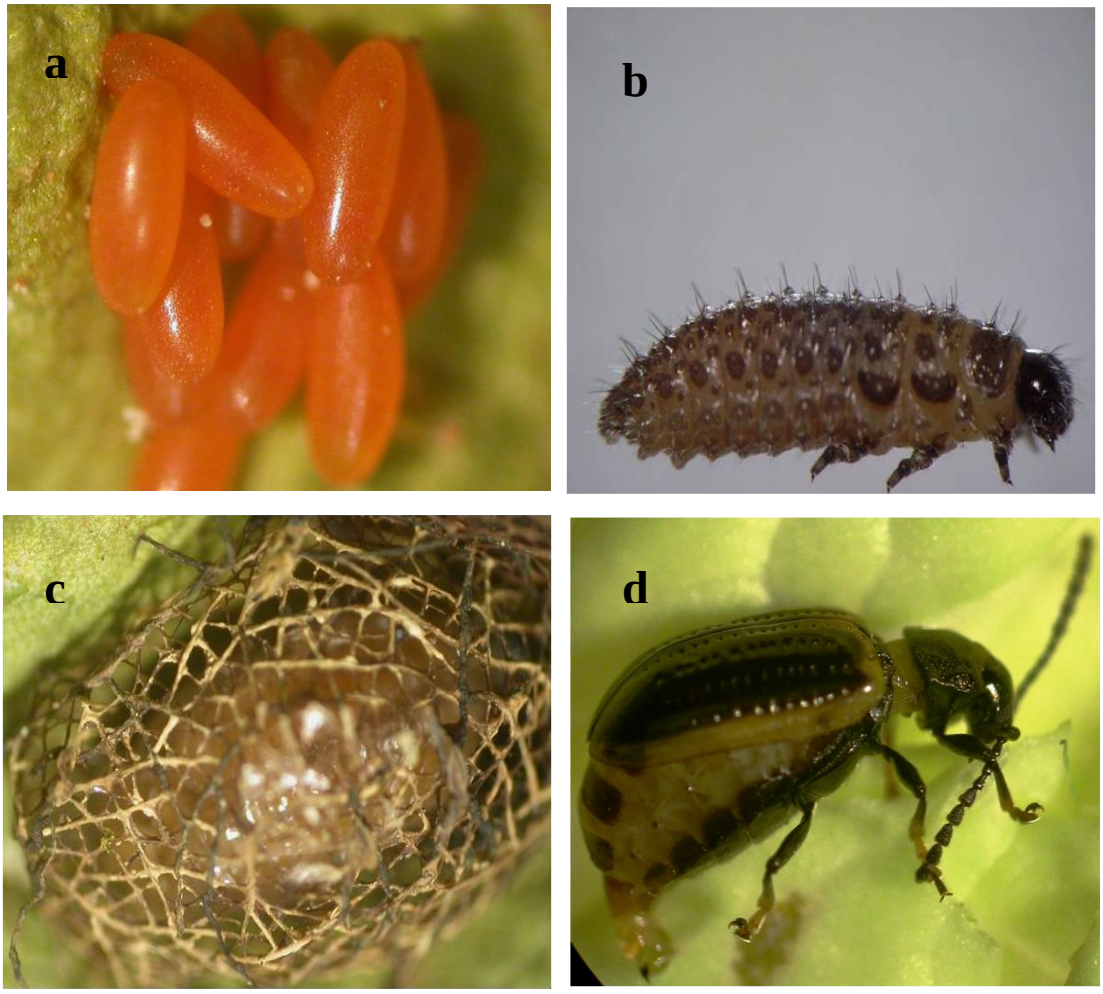

Figure 3.

Figure 4.

CHAPTER 2

HOST FINDING AND ACCEPTANCE PREFERENCE OF THE YELLOWMARGINED LEAF BEETLE, *MICROTHECA OCHROLOMA* (COLEOPTERA: CHRYSOMELIDAE), ON CRUCIFEROUS CROPS

2.1 Introduction

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) is a major pest of cruciferous crops in the southeastern United States. Native to South America, *M. ochroloma* was first reported in the United States in Mobile, Alabama, in 1947 on young cabbage plants (Chamberlin and Tippins 1948). It is now widely distributed in the southeastern U.S. with major field infestations reported in Alabama, Florida, Louisiana, Mississippi, South Carolina and Texas (Rohwer et al. 1953, Oliver 1956, Woodruff 1974, Balsbaugh 1978, Ameen and Story 1997a). Cruciferous crops (Brassicacea) attacked by *M. ochroloma* include cabbage (*Brassica oleracea* var. *capitata*), collard (*B. oleracea* var. *acephala* L.), mustard (*B. juncea* Cosson), napa cabbage [*B. pekinensis* (Lour.) (a type of Chinese cabbage)], Japanese leafy vegetables such as mizuna and mibuna, radish (*Raphanus sativus* L.), turnip (*B. rapa* L.), and watercress (*Nasturtium officinale* L.) (Chamberlin and Tippins 1948, Racca Filho et al. 1994, Ameen and Story 1997b, Bowers 2003). These crops are typically grown using conventional production practices however organic production of cruciferous crops is an emerging industry in the southeastern U.S. Many cruciferous crop species are usually grown organically as mixed cropping systems in the spring and fall in Alabama and much of the region. Adults and larvae of *M. ochroloma* often feed in clusters on leaves of cruciferous crops with

potential for major economic loss. In particular, *M. ochroloma* poses a major threat to organic production of cruciferous crops in the southeastern U.S. since only a few effective organically acceptable management tactics have been identified for *M. ochroloma* (Balusu and Fadamiro 2011). \

Despite its economic importance and impact on organic vegetable production, very little research has been conducted on the biology and ecology of *M. ochroloma.* In Alabama, *M. ochroloma* is a multivoltine cool season pest that typically occurs in vegetable fields from October to May. Fall activity usually commences in early October when adult beetles migrate in mass numbers from summer aestivation sites (wild mustard plants) into cruciferous crops (R. Balusu and H. Fadamiro, personal observation). Although *M. ochroloma* is known to feed on a wide range of cruciferous plants, it appears to show preference for certain cruciferous crops over others in field conditions (Chamberlin and Tippins 1948, Haeussler 1951, Rohwer et al. 1953, Anonymous 1976, Oliver and Chapin 1983). Chamberlin and Tippins (1948) reported that most of the heavy field infestations of *M. ochroloma* were confined to turnip and occasionally found on mustard and collards. Our field observations also indicate that *M. ochroloma* prefers certain cruciferous crops over others (R. Balusu and H. Fadamiro, unpublished data). However, no systematic study has been conducted to investigate host preference in *M. ochroloma.* Thus, the present study was conducted to evaluate host finding and acceptance preferences of *M. ochroloma* on select cruciferous crops and determine the cues that mediate its host preferences. Understanding host plant preferences may provide crucial information necessary for the development of alternative and organically-acceptable management strategies, such as trap cropping, host plant resistance, and attractant-based strategies against *M. ochroloma*.

2.2 Materials and Methods

2.2.1 Host Plants. Four known host plants of *M. ochroloma* were compared in the study: cabbage (*B. oleracea* var. *capitata* cultivar Farao F1), collard (*B. oleracae* var. *acephala* cultivar Champion), napa cabbage (*B. rapa* subsp. *pekinensis* cultivar Minuet F1), and turnip (*B. rapa* var. *rapa* cultivar Purple top white globe). These host plants were selected based on their importance as locally grown cruciferous crops, as well as their known association with ***M. ochroloma*** (Chamberlin and Tippins 1948, Haeussler 1951, Rohwer et al. 1953, Oliver and Chapin 1983, Ameen and Story 1997a, 1997b). Seedlings were raised from seeds purchased from Johnny's Selected Seeds (Winslow, ME, USA) in 60-well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2ºC and 55 ±5 % RH). Seedlings (three to four weeks old) were transplanted into pots in Sunshine potting mixture #8 consisting of 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum, and wetting agent (SunGro Horticulture, Washington, USA). Plants were irrigated daily and fert-irrigated twice a week with Scotts® peat lite special fertilizer (Scotts-Sierra Horticultural Product Company, Marysville, OH, USA), a 20-10-20 water soluble NPK fertilizer mixture with micronutrients. Plants were grown using organic pest management practices, and no pesticides were applied. Plants tested in the experiments were about five weeks old after transplanting. Similar methodology was used to raise all host plants to rear insect colony.

2.2.2 Insects. Adult *M. ochroloma* collected from a commercial organic farm in central Alabama in October 2006 were used to start laboratory colonies that were supplemented annually by field-collected adults. Adults were reared in clear plastic Petri dishes (150 mm

diameter × 30 mm height) lined with paper towels (Bounty®, Procter & Gamble, Cincinnati, OH) on fresh leaves from greenhouse grown cruciferous plants. Twenty pairs of beetles were enclosed per dish. Petri dishes were cleaned, remnants of food and frass were removed, and old leaves were replaced with fresh leaves daily. To limit the effect dietary history could possibly had on the response of test insects, the beetles were reared on a mixture of four host plants (cabbage, napa cabbage, collard, and turnip) by offering a different type of host plant daily. Bounty® paper towels were used as oviposition substrates since the beetles preferred to lay eggs on this brand than on most other common brands or leaves of host plants. The dishes were cleaned daily with soap and 10% Clorox® and then rinsed with water to maintain disease free colony. The colony was maintained at 25 ± 2ºC 50 ±10% RH, and a photoperiod of 14:10 (L:D).

2.2.3 Greenhouse Experiment. A multiple choice experiment was conducted in the greenhouse (Auburn University Plant Sciences Research Center) to evaluate host preference of *M. ochroloma* by comparing host finding (attraction) and acceptance (oviposition and feeding) behavior of adult beetles on four host plants: cabbage, napa cabbage, collard, and turnip. The test was conducted in cages (122 ×122 × 91 cm) made of PVC pipe (2 cm diameter) frame and covered with mosquito netting (SCS Ltd, Lake Ariel, PA, USA) (Figure 4). The cages were arranged on a bench in the greenhouse at 25 ± 2ºC, 50 ±10% RH, and a photoperiod of 14:10 (L:D). The four legs of the bench were placed in a tray filled with water to prevent ants and other insects from climbing into the cages.

The horizontal surface of each cage was virtually divided into four corner sections and a single potted plant (~ 5 weeks old) of each of the four host plant species (treatments) was placed in each corner section (i.e. the four host plants were simultaneously tested in each cage). The position of each treatment in the cage was determined randomly and rotated during each

replication. A group of 25 pairs of newly-emerged (~2-5 days old), starved adult beetles was placed in a Petri dish (150 mm diameter × 30 mm high) and transferred from the laboratory to the greenhouse 1 h prior to the start of the experiment for acclimation. The beetles were then released at the center of the cage by opening the Petri dish lid. The experiment was replicated four times over time. The replication and rotation schemes ensured that each treatment was located in each of the four corners of the cage once.

Each cage was examined daily for a period of 19 d to evaluate host plant preference by using three parameters. First, the number of beetles on each host plant was recorded daily as a measure of host finding preference (attraction). The number of larvae on each host plant was also recorded daily as a measure of host acceptance and oviposition preference. Finally, using a method modified after Maletta et al. (2004) each plant was rated for *M. ochroloma* feeding damage (also a measure of host acceptance) on a scale of 1 to 6 as follows: 1 = very light defoliation with < 10% of damage; 2 = light defoliation (10-30%); 3 = moderate defoliation (30-50%); 4 = heavy defoliation (50-70%); 5 = very heavy defoliation (70-90%) and 6 = complete (total) defoliation (> 90%). Data on number of adults, larvae, or damage rating, were not normally distributed and thus were square-root transformed and then analyzed using analysis of variance followed by the Tukey-Kramer HSD comparison test ($P<0.05$; JMP® 7.0.1, SAS Institute 2007).

2.2.4 Laboratory Experiment. A four-choice olfactometer bioassay was used to determine the cues that mediate host plant preference of *M. ochroloma,* using methods modified after Pettersson (1970), Kalule and Wright (2004), and Chen et al. (2009). Briefly, the olfactometer (Analytical Research Systems, Gainesville, FL) consists of a central chamber (30 cm long × 30 cm wide × 6 cm high) with orifices or "arms" (17 cm long × 7 cm diameter) at the

four sides and a central orifice where mixing of the airflow from the arms occurs. The orifices were connected through Teflon-glass tube connectors to four glass chambers (22.8 cm diameter × 40.6 cm high) with lids, which housed the test host plants for headspace volatile collection. Each glass chamber was provided with an inlet at the bottom and an outlet at the opposite top and connected through Teflon® tubing and ChemTred® (8mm I.D.) connectors to a flow meter on an air delivery system (ARS, Inc., Gainesville, FL), which was in turn connected to an air source fitted with charcoal filter. The inlet air was further purified (using a second set of charcoal filter placed between the flow meters and the glass chambers) and was pushed at a constant rate of 200 ml/min through the headspace of the test host plants in the glass chambers into the orifices and removed by suction via a vacuum pump through the central orifice of the olfactometer at the rate of 900 ml/min. The olfactometer apparatus was placed in a cardbox box (82 cm long × 82 cm wide × 61 cm high), lined with white paper, and positioned under a fluorescent light source (~100 lux) for uniform lighting.

An individual test host plant (in 1-gal pot with the soil covered with aluminum foil paper) was placed in a glass chamber for headspace volatile collection. The four host plants were tested in two separate experiments in which a set of three host plants was compared with the control (glass chamber containing a pot covered with aluminum foil). In the first experiment, napa cabbage, turnip, cabbage and control were compared. Napa cabbage, turnip, collards and control were compared in the second experiment.

For each experiment, 10 female or male beetles (2 to 4-d old) were released at the bottom of the central chamber. The beetles were allowed a maximum of 40 min to make a choice among the four air fields, and those found in each arm were counted and removed. Beetles that did not walk into any of the arms within 40 min were scored as “nonresponders” and were not included

in the analysis. After each test, the olfactometer was cleaned with hexane and acetone, and the arms were rotated (90°) to minimize positional effect. Each experiment was replicated at least 21 times per sex. All tests were conducted at 25±1°C and 50 ± 10% RH. For each experiment, data on number of beetles attracted were first square-root transformed and then analyzed using analysis of variance followed by the Tukey-Kramer HSD comparison test ($P<0.05$; JMP® 7.0.1, SAS Institute 2007).

2.3 Results

2.3.1 Greenhouse Experiment. The host finding and acceptance preference of *M. ochroloma* were evaluated by simultaneously presenting adult beetles with four host plant species: cabbage, napa cabbage, collards, and turnip. The results showed that *M. ochroloma* actively discriminated among the four host plants (Fig. 1A). Significantly higher numbers ($F = 21.61$; d.f = 3, 18; $P < 0.001$) of adult beetles were recorded on turnip (50% of the beetles) and napa cabbage (35%) than on collards (7%) and cabbage (3%) one day after they were released in the cage. Although not significantly different, a higher number of adults were recorded on turnip than on napa cabbage. A similar trend was observed on day 2 to day 6 after release. However, on day 7 when ~30% of the turnip plant had been defoliated, the beetles began to move from turnip to napa cabbage. This culminated in a significantly higher number of beetles on napa cabbage compared to turnip on day 11 ($F = 169.3$; d.f = 3, 12; $P < 0.001$). This trend continued throughout the remaining observation periods: significantly higher numbers of beetles were recorded on napa cabbage than on turnip on day 19 ($F = 30.26$; d.f = 3, 12; $P < 0.001$) (Figure 1A).

The number of larvae on each plant (Figure 1B) was recorded as a measure of host plant acceptance and oviposition preference. No larvae were recorded on any of the test plants on days 1-8 after release of adult beetles, since the eggs laid by the adults were yet to hatch into larvae. Very few larvae were recorded on day 9, and the numbers were not significantly different among the host plants ($F = 2.02$; d.f = 3, 12; $P < 0.164$). Significant differences in larval density were recorded among the test plants starting on day 10 ($F = 3.80$; d.f = 3, 12; $P < 0.039$), with higher numbers of larvae recorded on turnip and napa cabbage than on cabbage or collards. This trend continued until d15 ($F = 7.75$; d.f = 3, 12; $P < 0.039$). However, significantly higher numbers of larvae were recorded on napa cabbage than on the remaining host plants starting on day 16 ($F = 15.3$; d.f = 3, 12; $P < 0.0002$) and continuing through day 19 ($F = 39.16$, d.f = 3, 12; $P < 0.001$). The reduction in the number of larvae on turnip relative to napa cabbage from day 16 was possibly due to pupation and complete defoliation of turnip. It was observed that when turnip had been completely defoliated, some of the larvae began to feed on the root while others started to move to napa cabbage (Fig. 1B).

Plant damage ratings were generally low and not significantly different among the tested host plants on days 1-3 (Fig. 1C). However, significant differences in damage ratings were recorded among the host plants beginning on day 4 when a significantly higher ($F = 5.14$; d.f = 3, 12; $P < 0.016$) damage rating was recorded on turnip than on cabbage and collard. On day 5, significantly higher ($F = 25.33$; d.f = 3, 12; $P < 0.0001$) damage ratings were recorded for turnip and napa cabbage compared to the other two plants. This trend continued throughout the remaining observation periods (days 6-19) (Fig. 1C).

2.3.2 Laboratory experiment. The results of the four-choice olfactometer experiment 1, comparing the response of adult *M. ochroloma* to headspace volatiles of napa cabbage, turnip,

cabbage and control, showed significant differences in the behavioral response of females (F = 38.12; df = 3, 80; P = 0.0001) and males (F = 71.17; df = 3, 80; P = 0.0001) to the treatments (Fig. 2). Both sexes were significantly more attracted to napa cabbage than to the remaining treatments, with turnip being the second most attractive treatment. However, cabbage was not more attractive than control. The four-choice olfactometer experiment 2 which compared napa cabbage, turnip, collards, and control showed significant differences in the response of female (F = 11.49; df = 3, 80 ; P = 0.0001) and male (F = 30.64; df = 3,80; P = 0.0001) *M. ochroloma* to the treatments. Females were significantly more attracted to napa cabbage than to collards or control (Fig. 3). Males also showed significantly greater attraction to napa cabbage and turnip compared to collards or control.

2.4 Discussion

The results from both the greenhouse and laboratory experiments clearly demonstrated the ability of *M. ochroloma* to discriminate among the tested host plants, preferring turnip and napa cabbage over cabbage or collards. Data from the greenhouse multiple choice experiment, which simultaneously evaluated host finding preference (i.e., attraction) and host acceptance (i.e., feeding and oviposition preference), showed that turnip was the most preferred among the tested host plants followed by napa cabbage. Although *M. ochroloma* is known to attack several cruciferous crops other than those tested in the present study (e.g., mustard, radish, Japanese leafy vegetables), our results demonstrated its preference for certain cruciferous plants such as turnip and napa cabbage. Several species of oligophagous leaf beetles (Coleoptera: Chrysomelidae) have also been reported to show preference for some host plants over others. For example, the striped flea beetle, *Phyllotreta striolata* (F.), was shown to discriminate among its

host plants in the family Brassicaceae, prefering some hosts such as *Brassica oleracea*, *B. napus*, and *B. Campestris* over *B. juncea* (L.) Czern and *B. nigra* (L.) Koch (Lamb and Palaniswamy 1990, Anderson et al. 1992). The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), also demonstrated host preference among its host plants, preferring potato, *Solanum tuberosum* L., over tomato, *Lycopersicon esculentum* L., and eggplant, *Solanum melongena* L. (Hitchner et al. 2008).

Insect host plant selection and preference is a complex phenomenon that is governed by a variety of cues, in particular olfactory and visual cues (De Wilde et al. 1969, Visser 1976, Zehnder and Speese 1987). Even though the host plant preference experiment conducted in the greenhouse can reveal useful information on host preference, it is difficult to determine precisely the cue(s) mediating host preference with this method. Therefore, the laboratory four-choice olfactometer experiments, in which the role of visual cues in host location is eliminated (McIndoo 1926, Visser and Piron 1998), allowed a reliable evaluation of the role of plant volatiles in host preference. The results showed that host finding and acceptance preference of *M. ochroloma*, which was observed in the greenhouse experiment, is mediated primarily by host plant volatiles. Specifically, the olfactometer results demonstrated strong attraction of both sexes of *M. ochroloma* to headspace volatiles of napa cabbage followed by turnip, generally in agreement with the results of the greenhouse experiment.

Oligophagous insects typically use plant odors as informational cues to recognize their host plants (Visser 1986). The odor may consist of general odor components (green leaf volatiles) such as alcohols, aldehydes, fatty acid derivatives and terpenoids, but specific odor components such as isothiocyanates, sulfides, and benzaldehyde are important signature compounds (Visser 1986, Jermy et al. 1988, Dickens 2000). All crucifer plants have

characteristic secondary plant metabolites called glucosinolates, which are hydrolyzed by the enzyme myrosinase into isothiocyanates. Most crucifer-specific insect pests use different types of isothiocyanates as olfactory cues to locate their host plants (Louda and Mole 1991, Evans and Allen-Williams 1994). For example, the crucifer-specific seed weevil, *Ceutorhynchus assimilis* Payk, uses 3-butenyl and 4-pentenyl isothiocyanates for host-plant recognition (Free and Williams 1978, Bartlet et al. 1993). Feeny et al. (1970) showed that the flea beetles, *Phylotreta cruciferae* and *P. striolata*, use allyisothiocyanate to locate their host plants from long range. The cabbage maggot, *Delia radicum* (L.) is also attracted by allyisothiocyanate (Finch and Skinner 1982). Preliminary headspace volatile analyses of the host plants tested in this study suggest that the volatile profiles of napa cabbage and turnip are similar but qualitatively and quantitatively different from the profiles of cabbage and collards (R. Balusu and H. Fadamiro, unpublished data), further confirming that host preference of *M. ochroloma* is mediated primarily by host plant volatiles. Recent follow-up studies have also resulted in preliminary identification of an isothiocyanate as a putative attractant for *M. ochroloma* (R. Balusu and H. Fadamiro, unpublished data).

Our results on host preference of *M. ochroloma* are in agreement with field observations of crop damage by the pest. Chamberlin and Tippins (1948) first reported no evidence of damage by *M. ochroloma* on young, tender cabbage crop adjacent to a heavily infested turnip field in a commercial vegetable farm in Alabama. Similarly, many workers have reported field observations of higher population densities of *M. ochroloma* on turnip and mustard crop compared to other crucifer crops (Haeussler 1951, Rohwer et al. 1953, Oliver and Chapin 1983). In addition, our personal observations in commercial organic vegetable farms in southern and central Alabama since 2005 also showed higher population densities of *M. ochroloma* on turnip

and napa cabbage plantings relative to adjacent cabbage or collards. Ameen and Story (1997b) showed in a laboratory Petri dish study that *M. ochroloma* adults and larvae prefer to feed more on turnip and mustard foliage than on collard or cabbage. The authors also demonstrated in a related study that while *M. ochroloma* can successfully develop on various host plants including cabbage, collard, mustard, radish, and turnip, higher fecundity was recorded on turnip than on the other tested host plants (Ameen and Story 1997c).

In general, our laboratory results, which showed preference of *M. ochroloma* for napa cabbage and turnip, are fairly consistent with the results of the greenhouse experiment. However, turnip was the most preferred host in the greenhouse experiment, whereas the beetles were more attracted to napa cabbage in the olfactometer bioassays. This subtle but important difference may be related to relative differences in host acceptance of turnip versus napa cabbage. The greenhouse experiment was a measure of host finding or location (i.e., chemical and/or visual attractiveness) and acceptance (feeding and oviposition), whereas the olfactometer experiment simply evaluated chemical attractiveness of the host plants. The results suggest that napa cabbage was more attractive chemically, whereas turnip was relatively more acceptable. Host acceptance is based on many factors including nutrient composition and balance, secondary plant metabolites, morphological factors such as texture, pubescence, and color. Thus, it is possible that the relatively higher acceptance of turnip compared to napa cabbage, as observed in the greenhouse experiment, is related to factors other than differences in volatile profile. Clearly, further studies are necessary to determine the basis for the higher preference and acceptance of *M. ochroloma* for turnip.

In conclusion, our results showed that turnip and napa cabbage are two preferred host plants of *M. ochroloma*, whereas cabbage and collards are non-preferred. Preference is mediated

primarily by quantitative and/or qualitative differences in chemical volatile profiles of the host plants, but other factors appear to contribute to host acceptance. Ongoing studies on chemical analyses of various host plants and complete identification of host plant attractants may support the development of an efficient trap crop system and other attractant-based strategies for managing *M. ochroloma* in organic and conventional crucifer production systems.

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Figure legend

Figure 1. Mean (± SE) number of *M. ochroloma* recorded daily on four host plants (cabbage, collards, napa cabbage, and turnip) in a multiple choice greenhouse cage experiment (A) adults, (B) larvae, and (C) damage ratings. Twenty-five pairs of newly emerged starved adult beetles were released per test and replicated four times. Means within each date having no letter in common are significantly different ($P < 0.05$, Tukey-Kramer HSD test).

Figure 2. Response of *M. ochroloma* in a four-choice olfactometer to headspace volatiles of three host plants (cabbage, napa cabbage and turnip) versus control. Figure shows mean (± SE) number of female or male beetles attracted per 40 min. Ten beetles of either sex were released per test and replicated 21 times. Means for the same sex having no letter in common are significantly different ($P < 0.05$, Tukey-Kramer HSD test).

Figure 3. Response of *M. ochroloma* in a four-choice olfactometer to headspace volatiles of three host plants (collards, napa cabbage and turnip) versus control. Figure shows mean (± SE) number of female or male beetles attracted per 40 min. Ten beetles of either sex were released per test and replicated 21 times. Means for the same sex having no letter in common are significantly different ($P < 0.05$, Tukey-Kramer HSD test).

Figure 4. Host preference experiment setup with four-host plants under greenhouse conditions.

Figure 1

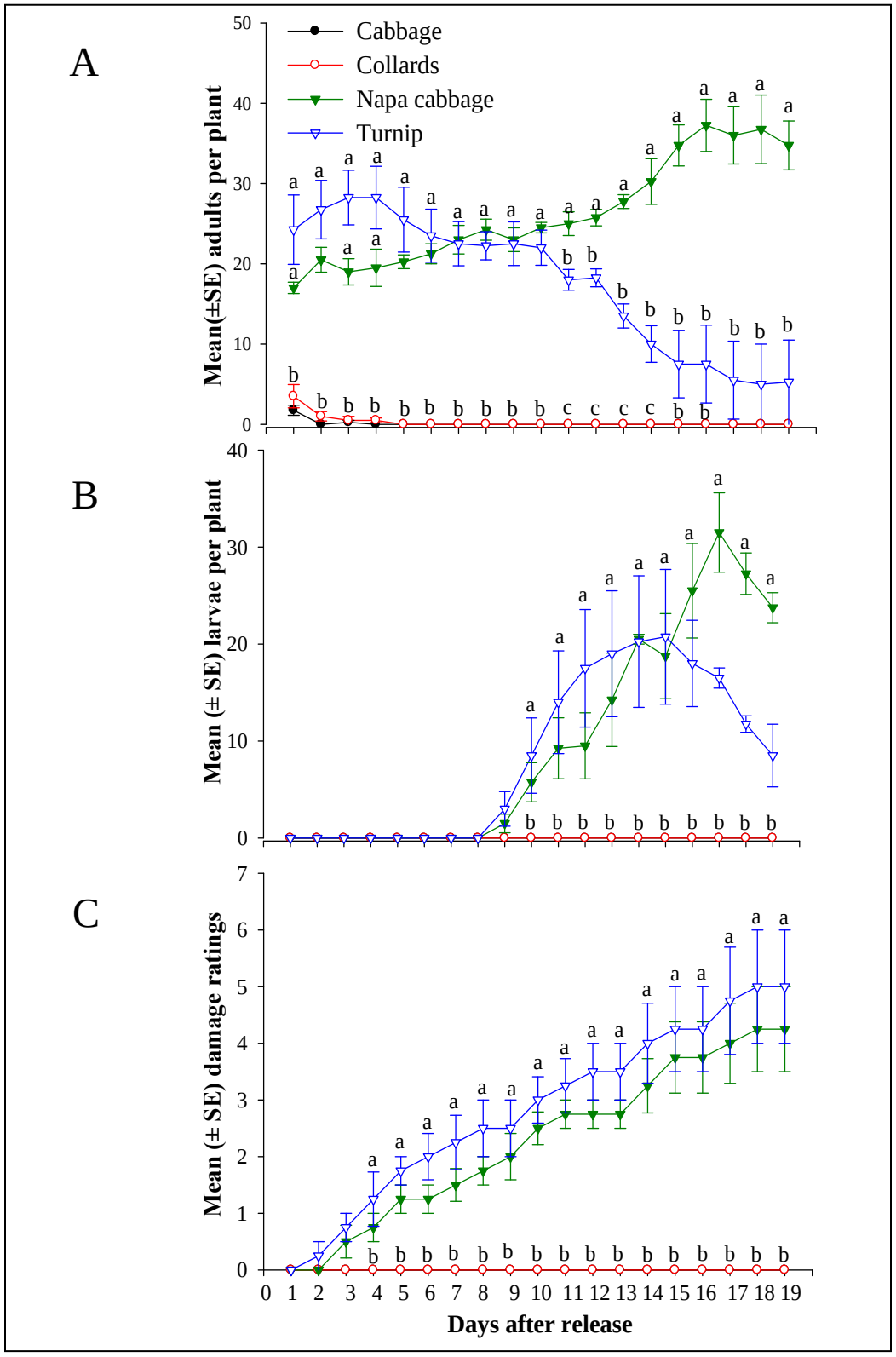

A
B
C
Cabbage
Collards
Napa cabbage
Turnip
Mean(±SE) adults per plant
Mean (± SE) larvae per plant
Mean (± SE) damage ratings
Days after release

Figure 2

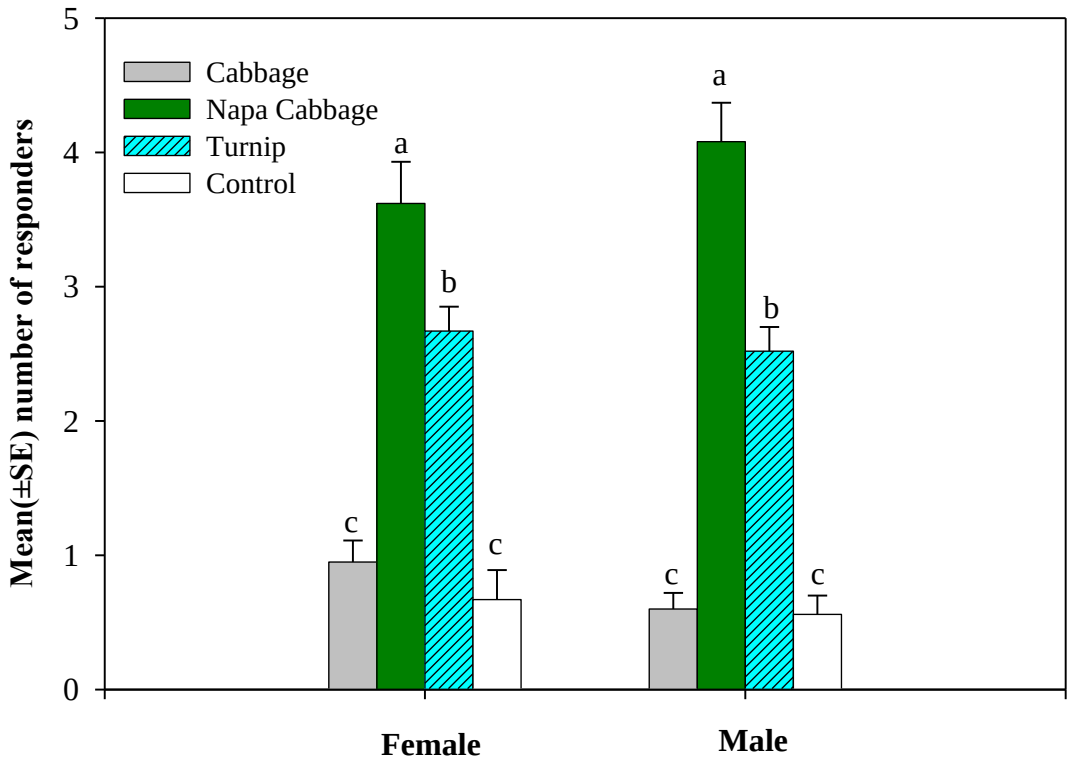

Cabbage
Napa Cabbage
Turnip
Control
Mean(±SE) number of responders
0
1
2
3
4
5
a
b
c
a
b
c
c
c
Female
Male

Figure 3

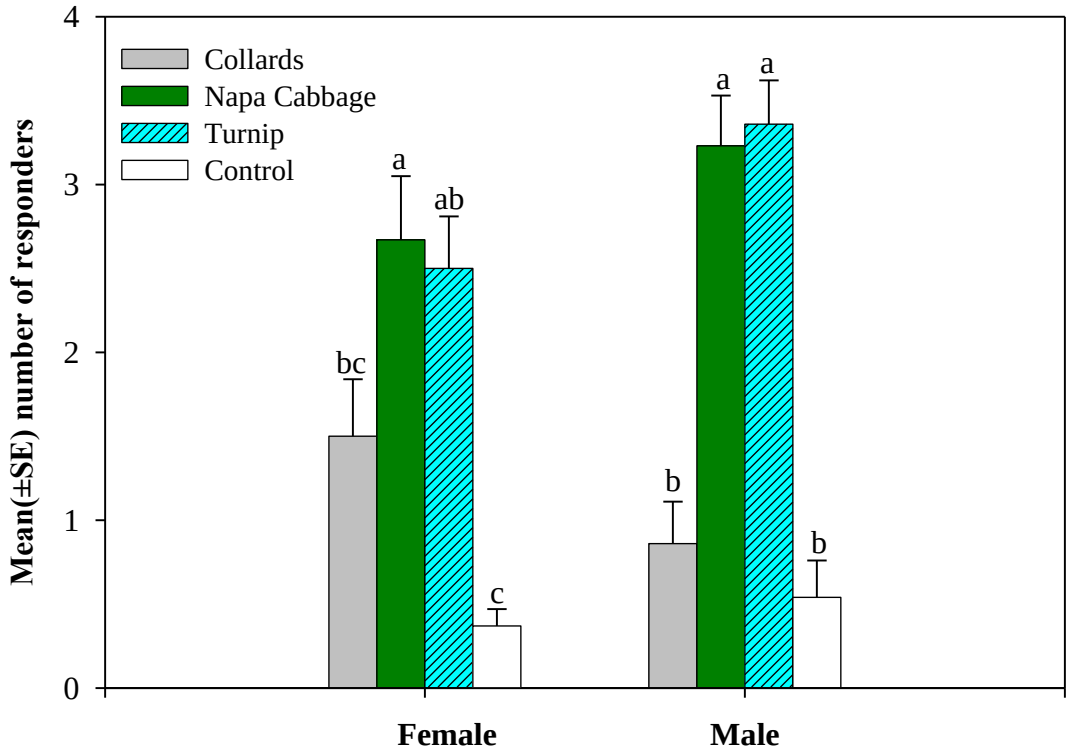
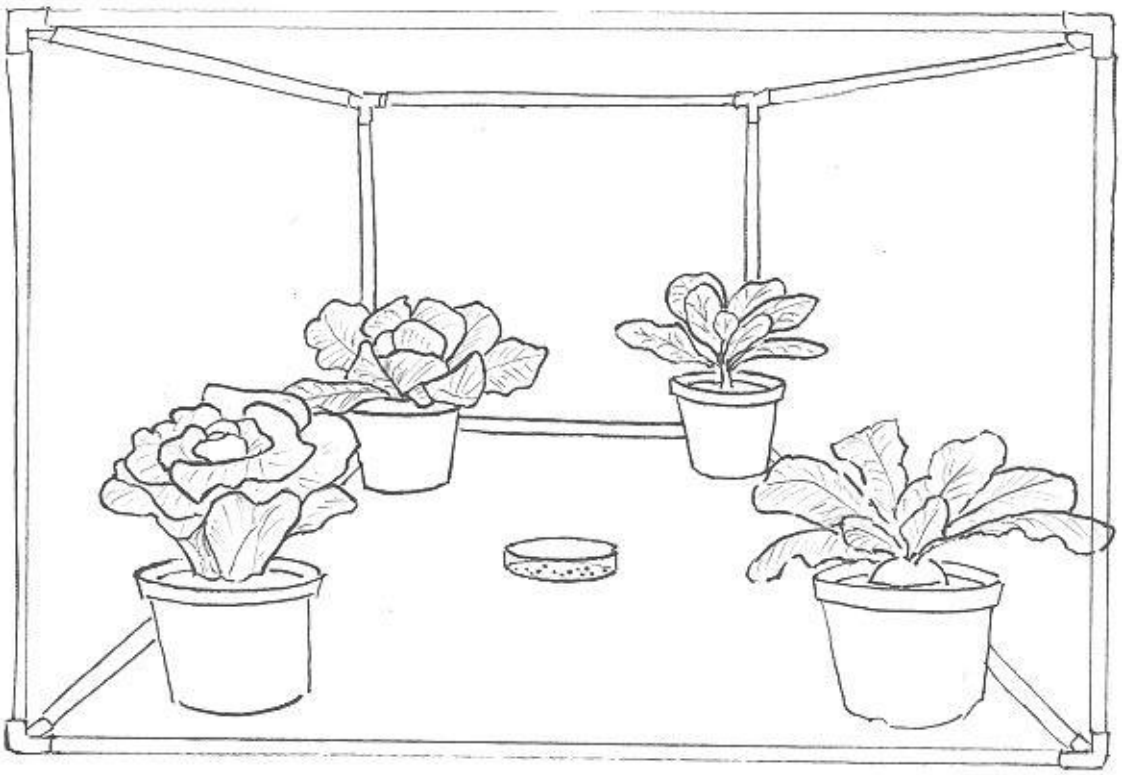

Collards
Napa Cabbage
Turnip
Control
Mean(±SE) number of responders
0
1
2
3
4
bc
a
ab
c
b
a
a
b
Female
Male

Figure 4



CHAPTER 3

IDENTIFICATION OF SEMIOCHEMICAL CUES USED BY YELLOWMARGINED LEAF BEETLE (YMLB) TO LOCATE CRUCIFER HOST PLANTS

3.1 Introduction

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) is a major pest of cruciferous crops in the southeastern United States (U.S.). This beetle was accidentally introduced into the U.S. from South America. It is now widely distributed in the southeastern U.S with major field infestations reported in Alabama, Florida, Louisiana, Mississippi, South Carolina, and Texas (Rohwer et al. 1953, Oliver 1956, Woodruff 1974, Balsbaugh 1978, Ameen and Story 1997a). Cruciferous crops (Brassicacea) attacked by *M. ochroloma* include cabbage (*Brassica oleracea* var. *capitata*), collard (*B. oleracea* var. *acephala* L.), mustard (*B. juncea* Cosson), napa cabbage [*B. pekinensis* (Lour.) (a type of Chinese cabbage)], Japanese leafy vegetables such as mizuna and mibuna, radish (*Raphanus sativus* L.), turnip (*B. rapa* L.), and watercress (*Nasturtium officinale* L.) (Chamberlin and Tippins 1948, Ameen and Story 1997b, Bowers 2003, Racca Filho et al. 1994). These crops are typically grown using conventional production practices; however, organic production of cruciferous crops is an emerging industry in the southeastern U.S. Many cruciferous crop species are usually grown organically as mixed cropping systems in the spring and fall in Alabama and much of the region.

Adults and larvae of *M. ochroloma* often feed in clusters on leaves of cruciferous crops with potential for major economic loss. In particular, *M. ochroloma* poses a major threat to organic production of cruciferous crops in the southeastern U.S. since only very few effective organically-acceptable management tactics have been identified for *M. ochroloma* (Balusu and Fadamiro 2011).

Despite its economic importance and impact on organic vegetable production, very little research has been conducted on the biology and ecology of *M. ochroloma.* In Alabama, *M. ochroloma* is a multivoltine cool season pest which typically occurs in vegetable fields from October to May. Fall activity usually commences in early October when adult beetles migrate in mass numbers from summer aestivation sites (wild mustard plants) into cruciferous crops (R. Balusu and H. Fadamiro, personal observation). *M. ochroloma* therefore needs to find a host plant while migrating from their aestivation site.

Plant odors are one of the primary cues that insects use for finding host plants. Attraction of *M. ochroloma* to odors of its host plants has been shown in chapter 2. All members of family Brassicaceae (formerly known as Cruciferae) have characteristic secondary plant metabolites called glucosinolates. Myrosinase, an enzyme stored in special cells in the tissue of crucifer plants (Rask et al. 2000), enhances the hydrolysis of non-volatile glucosinolates to volatile biologically-active isothiocyabates, thiocyanates, and nitriles (Vaughn and Berhow 2005). The composition of glucosinolates varies among Brassicacae plants (Sorensen 1991). Isothiocyanates are known to attract various crucifer specific feeding insects (Visser 1986). Verschaffelt (1911) was the the first to demonstrate the effectiveness of glucosinolates as feeding stimulants for specialist crucifer-feeding insects by showing that *Pieris brassicae* and *P. rapae* L. would eat previously rejected plants if the plants were wetted with crucifer juice or sinigrin. Other

researchers have since reported that glucosinalates or their cleavage products, isothiocyanates, act as attractants, feeding and oviposition stimulants for more than 25 insect species in the Coleoptera, Lepidoptera, and Diptera that are specialized on cruciferous plants (Chew 1988, Louda and Mole 1991, Traynier and Truscott 1991, Hopkins et al. 2009). For instance, isothiocyanates, specifically 3-butenyl, 4-pentenyl, and 2-phenylethy, are known attractants for cabbage seed weevil, *Ceutorhynchus assimilis* (Smart and Blight 1997). Also, Neilson (1978) reported that glucosinolates and their hydrolysis products, allyl glucosinolates are strong feeding stimulants for flea beetle *Phyllotreta armoraciae* (Koch). Similarly, Alan et al. (2006) reported that certain isothiocyantates (3-methylsulfinylpropyl and 4-methylsulfinyl-3-butenyl) are oviposition stimulants for diamondback moth, *Plutella xylostella*. However, the identity of the chemicals that acts as attractants, feeding and oviposition stimulants for *M. ochroloma* remains unknown.

Several scientists have reported preference of *M. ochroloma* among crucifer host plants based on field observation (Chamberlin and Tippins 1948, Haeussler 1951, Rohwer et al. 1953, Anonymous 1976, Oliver and Chapin 1983). In Chapter 3, it was demonstrated that host plant preference in *M. ochroloma* is mediated mainly by host plant semiochemicals. However, the identities of the host plant semiochemicals are unknown. Thus, the present investigation was conducted to compare the volatile profiles of various host plants of *M. ochroloma*, with the goal of identifying the chemicals mediating its host preference. Results of this study may provide crucial information necessary for the development of semiochemical based, organically acceptable management strategies for *M. ochroloma*.

3.2 Materials and Methods

3.2.1 Host plants. Four known host plants of *M. ochroloma* were compared in the study: cabbage (*B. oleracea* var. *capitata* cultivar Farao F1), collard (*B. oleracae* var. *acephala* cultivar Champion), napa cabbage (*B. rapa* subsp. *pekinensis* cultivar Minuet F1), and turnip (*B. rapa* var. *rapa* cultivar Purple top white globe). These host plants were selected based on their importance as locally grown cruciferous crops as well as their known association with *M. ochroloma* (Haeussler 1951, Rohwer et al. 1953, Oliver and Chapin 1983, Chamberlin and Tippins 1948 and Ameen and Story 1997a, b). Seedlings were raised from seeds purchased from Johnny's selected seeds (Winslow, ME, USA) in 60 well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2ºC and 55 ±5 % RH). Seedlings (3-4 weeks old) were transplanted into pots in Sunshine potting mixture #8 consisting of 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum and wetting agent (SunGro Horticulture, Washington, USA). Plants were irrigated daily and fert-irrigated twice a week with Scotts® peat lite special fertilizer (Scotts-Sierra Horticultural Product Company, Marysville, OH, USA), a 20-10-20 water soluble NPK fertilizer mixture with micronutrients. Plants were grown using organic practices and no pesticides were applied. Plants tested in the experiments were about five weeks old after transplanting. Similar methodology was used to raise all the four host plants.

3.2.2 Insects. Adults of *M. ochroloma* collected from a commercial organic farm in central Alabama in October 2006 were used to start laboratory colonies, which were supplemented annually by field-collected adults. Adults were reared in clear plastic Petri dishes (150 mm diameter × 30 mm height) lined with paper towels (Bounty®, Procter & Gamble, Cincinnati, OH) on fresh leaves from greenhouse grown cruciferous plants. To limit the potential

effect of dietary history on the response of test insects, the beetles were reared on a mixture of four host plants (cabbage, napa cabbage, collard and turnip) by offering a different type of host plant daily. Twenty pairs of beetles were enclosed per dish. Petri dishes were cleaned, remnants of food and frass were removed, and old leaves were replaced with fresh leaves daily. Paper towels were used as oviposition substrates since the beetles preferred to lay eggs on this brand than on most other common brands or leaves of host plants. The dishes were cleaned daily with soap and 10% bleach (Clorox®, Oakland, CA) and then rinsed with water to maintain disease free colony. The colony was maintained at 25 ± 2ºC 50 ±10% RH, and a photoperiod of 14:10 (L:D).

3.2.3 Collection and GC Analysis of Headspace Volatiles

The methodology and protocols used for volatile collection were similar to those reported by Turlings et al. 1998), but with some modifications. Volatile collection chamber consisted of vertically placed, open bottom glass cylinder (9 cm I.D., 60 cm high) and glass plate (2in × 2 in) that was placed against open bottom of glass cylinder. The glass plate consisted of two equal halves with 2-cm-diameter hole at the center. The two halves of the plates could be pushed together in a "guillotine-like" wooden frame that helped in holding the two half of glass plate together (Fig.. 1). The two halves were pushed together around the lower part of plant's stem in such a way that the stem fits in a central hole of the plate which allowed most of the plant and all of its leaves inside the glass jar, while the pot remained outside. This setup ensured consistent volatile profiles with no background noise from potting soil or other external sources. A volatile collection trap was attached ~ 2.5 cm above the base of the cylinder with the aid of 8 mm I.D. ChemTred®. The collection traps were glass tubes (8 cm long, 6 mm diam.) containing 30 mg of

80/100 mesh Super Q adsorbent (Analytical Research Systems, Gainesville, FL, USA). A purified (activated charcoal) air at the constant rate of 500 ml/min was blown over the plant that was enclosed in open bottom glass cylinder. The air was drawn at constant rate of 400 ml/min through a Super-Q adsorbent trap, while the rest of the air vented through the hole in the bottom, thus preventing external, impure air from entering the collection chamber. The odor delivery system was used to control air flow (Analytical Research Systems, Gainesville, FL, USA). All the connections were done with Teflon® tubing and the system was purged for 2 h at an airflow of 500 ml/min to remove volatile contaminant. Subsequent volatiles were drawn through the Super Q traps for 24 h and eluted with 200 µl of methylene chloride. The resulting extracts were stored in a freezer (at −20°C) until use.

3.2.4 Analysis of headspace volatiles

One µl of each headspace volatile extract was injected into a Shimadzu GC-17A equipped with a flame ionization detector (FID). The dimension of capillary column used was as follows: Rtx-1MS, 0.25 mm I.D., 0.25µm film thickness (Restek, Bellefonte, PA, USA). Helium was used as carrier gas at a flow rate of 1 ml/min. The GC oven was programmed as follows: inject at 40°C, hold at 40°C for 2 min, and then increase by 5°C/min to 200°C for a total of 40 min. The temperature of both injector and detector was set at 200°C. The chromatogram was acquired with Syntech® GC-EAD pro (Syntech®, Hilversum, The Netherlands) software.

3.2.5 Coupled gas chromatography-electroantennogram detection (GC-EAD)

The headspace volatiles were subjected to coupled gas chromatography-electroantennogram detection (GC-EAD) analyses with female beetles to detect biologically

active peaks (components). GC-EAD analyses were conducted with samples of headspace volatiles from two preferred host plants (turnip and napa cabbage) and two less preferred host plants (cabbage and collards) (Balusu and Fadamiro, in review; Chapter 3). The GC-EAD techniques used were similar to those described by Fadamiro et al. (2010). Briefly, the system was based on a Shimadzu GC-17A equipped with a FID and coupled to an EAG detector. The dimension of the GC capillary column was the same as described above. The GC oven was programmed as follows: inject at 40°C, hold at 40°C for 2 min, and then increase by 10°C/min to 200°C for a total of 20 min. The temperature of both injector and detector was set at 200°C. The column effluent was split 1:1, with one part going to the FID of the GC and the other through a heated (220°C) transfer line (Syntech®, Hilversum, The Netherlands) into a humidified airstream (1,000 ml/min) directed at the antenna preparation (EAG detector). The antenna preparation and EAG techniques were the same as previously described (Chen and Fadamiro 2007). Glass capillaries (1.1 mm I.D.) filled with Ringer solution was used as electrodes. Beetles were first anaesthetized by chilling, and the head was isolated. The reference electrode was connected to the neck of the isolated head, while the recording electrode was connected to the antennal tip (with the last segment of antenna cut off). Chlorinated silver-silver chloride junctions were used to maintain electrical contact between the electrodes and input of a 1× preamplifier (Syntech®). The analog signal was detected through a probe (INR-II, Syntech®), captured and processed with a data acquisition controller (IDAC-4, Syntech®), and later analyzed with software (GCEAD Pro, Syntech®, Hilversum, The Netherlands) on a personal computer. A 3-µl aliquot of each sample was injected for a GC-EAD run. Four successful GC-EAD recordings were obtained for each treatment, and traces were overlaid on the computer

monitor to determine which GC peaks consistently yielded EAD responses. GC-EAD active peaks were identified with GC-MS analysis as below.

3.2.6 GC-MS Analyses

The GC-EAD active peaks were identified by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890A GC coupled to a 5975C Mass Selective Detector, with an HP-5 ms capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). One μl of each headspace extract was injected into the GC in splitless mode and under the GC conditions described above for headspace volatile analysis. The chromatographic profiles were similar to those obtained from GC-EAD recordings, thus making it possible to match the peaks. Mass spectra were obtained by using electron impact (EI, 70 eV). Identification of GC-EAD active peaks was done by using NIST 98 library (National Institute of Standards and Technology, Gaithersburg, Maryland) and by comparing with published mass spectrums of isothiocyanate compounds (Botting et al. 2002, Choi et al., 2004, Kim et al. 2004). The structures of the identified compounds were confirmed by using commercial custom synthesized standard with purity >97% (as indicated on the labels) obtained from ISCA® Technologias, Ltd. (Riverside, CA, USA).

3.3 Results

Analysis of headspace host plant volatiles revealed both qualitative and quantitative differences among the tested host plants with a total of 16 detectable peaks (Fig.2). At least three of these peaks (2, 5, and 11) were detected consistently in the headspace volatile profiles of all four host plants. More interestingly, the headspace volatile profiles of the two preferred host plants (turnip and napa cabbage) were strikingly similar but very different from the headspace

volatile profiles of the two less preferred host plants (cabbage and collards). Peaks 10 and 14 were uniquely detected in the preferred host plants. In contrast, peaks 4, 7, 9, and 11 were present in the less preferred host plants, but absent in the preferred host plants (Fig. 2).

Although the headspace volatile profiles of the two preferred host plants were qualitative similar, some quantitatively differences were noted. For instance, peaks 10 and 15 were detected in higher amounts in napa cabbage compared to turnip (Figs. 2C and 2D). Further analysis byGC-EAD showed that only peak 15, which was unique to the preferred host plants, elicited significant GC-EAD activity in female beetles (Fig. 3). The mass spectrum of biologically active compound was identified and compared with synthetic compound.

3.4 Discussion

The results show that the headspace volatile profiles of the two preferred host plants (turnip and napa cabbage) were different from those of the less preferred host plants (cabbage and collards) suggesting that host preference is likely mediated by differences in volatile profiles of the host plants. Further analysis by GC-EAD showed that one peak, which was unique to the preferred host plants, elicited significant GC-EAD activity in female beetles. In general, these results support the findings for many insect species that specialize on crucifer plants in which glucosinolates (secondary plant metabolites) or their volatile products, have been shown to serve as cues for location and recognition of their host-plants (Harborne 1988, Hopkin et al. 2009). For instance, isothiocyanates were shown to serve as attractants to crucifer specific insects (Evans and Allen-Williams 1993, Smart et al. 1996, Hopkin et al. 2009). Glucosinolate profiles are highly variable among crucifer plant species (Sorensen 1991) and over 120 different glucosinolates have been identified (Fahey et al. 2001). Therefore, crucifer specialists may

discriminate between crucifer plant species on the basis of species-specific glucosinolate profiles (Rodman and Chew 1980, Louda and mole 1991). For example, the cabbage seed weevil, *Ceutorhynchus assimilis*, is attracted to 3-butenyl isothiocyanate, but not to 2-phenylethyl isothiocyanate (Evans and Allen-Williams 1998). Pivnick et al, (1992) also found that allylisothiocyanate and 3-methylthiopropyl isothiocyanates attract the flea beetles *Phyllotreta cruciferae* (Goeze) and *P. striolata* (F.).

Because glucosinolates are ubiquitous in Brassicaceae, the sensitivity to these specific compounds or mixtures may help specialist feeders recognize particular plant species of this family. For example, single cell recording revealed a specialist olfactory cell in Pollen beetle, *Meligethes sp* (Blight et al. 1995). As well as attracting crucifer specialist pests, glucosinolate metabolites such as isothiocyanates also induce feeding and/or oviposition behavior. For example, Alan et al. (2006) showed that certain isothiocyanates, specifically 4-methylsulfinyl-3-butenyl isothiocyanate and 4-methylsulfinyl-3-butenyl isothiocyanate, act as oviposition stimulants for diamondback moth, *P. xylostella*.

Our results which showed remarkable differences in the headspace volatile profiles of the preferred versus less preferred host plants of *M. ochroloma* support this view; volatile profiles of the preferred host plants (turnip and napa cabbage) were strikingly similar but very different from volatile profiles of the less preferred host plants (cabbage and collards).

In summary, the results provide insight into how *M. ochroloma* can distinguish between crucifer plant species, even though all of them have glucosinolates as the main signature compounds. The data suggest that differences between preferred plants (turnip and napa cabbage) and less preferred plants (cabbage and collard) is mainly due to few compounds. Although we have identified a novel isothiocyanate as a putative host plant attractant for *M.*

ochroloma, further behavioral and analytical studies are necessary to confirm our findings and develop an effective host plant attractant for monitoring and management of *M. ochroloma* in organic crucifer production.

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Figure legend

Figure 1. Dynamic headspace volatile collection set-up

Figure 2. Gas chromatograph (GC) analysis of headspace volatile profiles of two less preferred host plants of *M. ochroloma*, cabbage (A) and collards (B) vs. two highly preferred host plants,napa cabbage (C) and turnips (D).

Figure 3. GC-EAD response of female *M. ochroloma* to headspace volatiles of napa cabbage (a highly preferred host plant). * indicates the main GC-EAD peak (putative crucifer attractant).

Figure 1

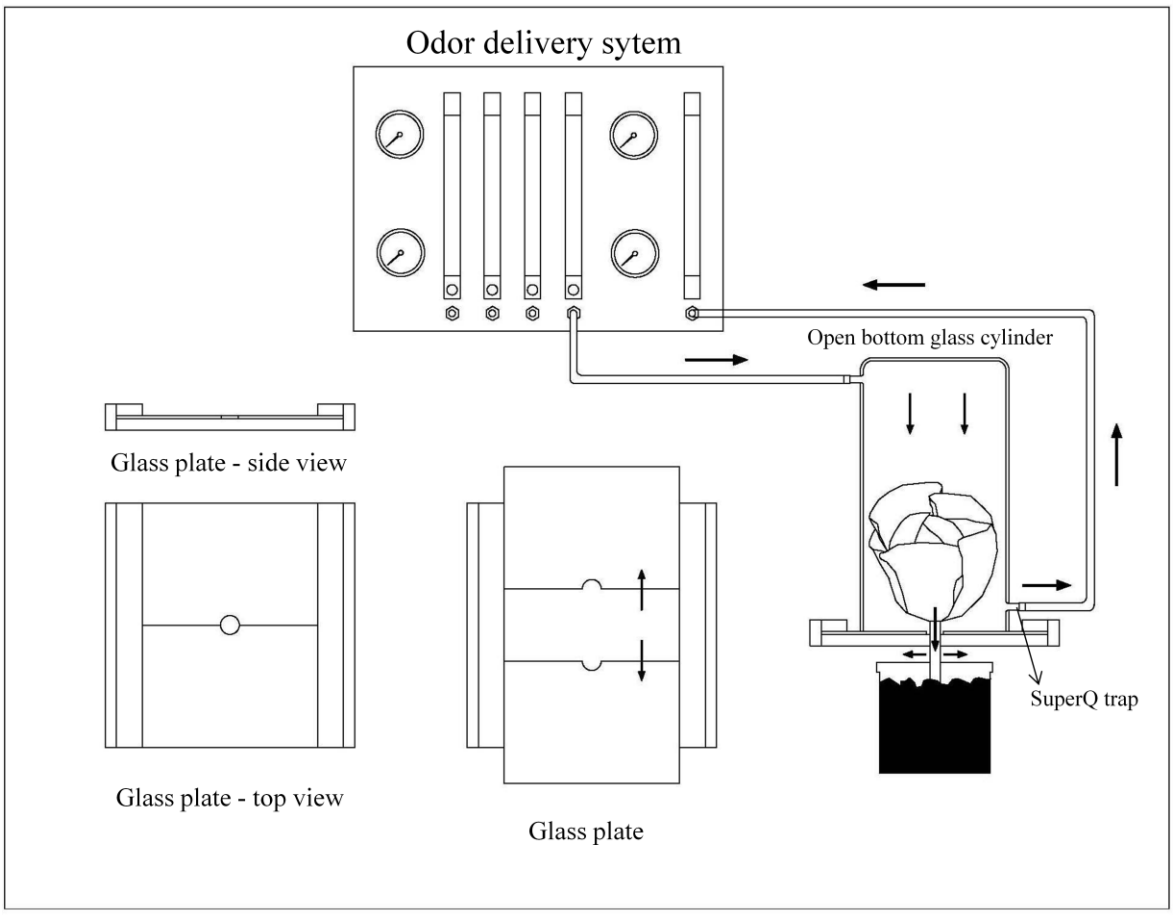
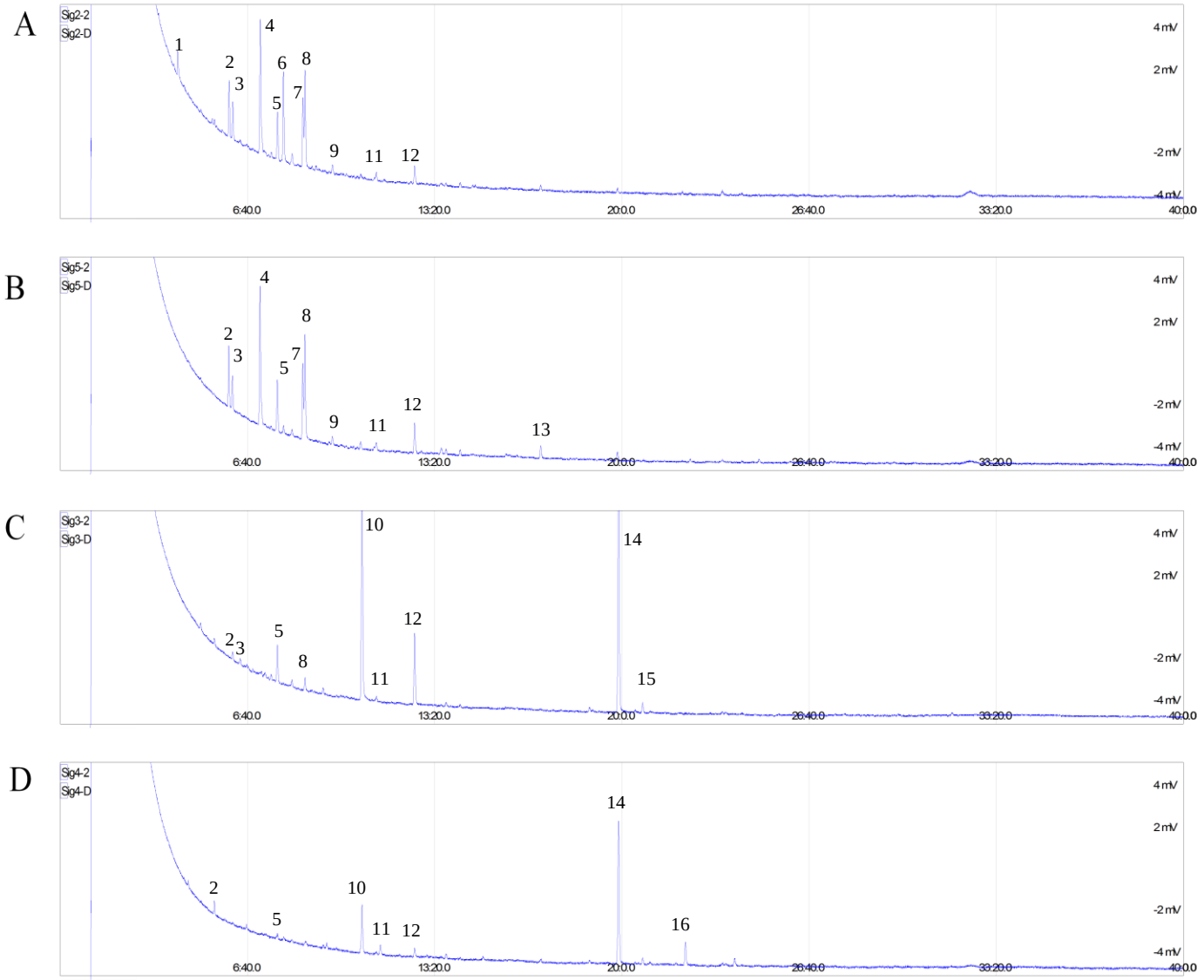

Odor delivery sytem
Open bottom glass cylinder
Glass plate - side view
Glass plate - top view
Glass plate
SuperQ trap

Figure 2.


A
Sig2-2
Sig2-D
1
2
3
4
5
6
7
8
9
11
12
4 mV
2 mV
-2 mV
-4 mV
6:40.0
13:20.0
20:0.0
26:40.0
33:20.0
40:0.0
B
Sig5-2
Sig5-D
2
3
4
5
7
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9
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C
Sig3-2
Sig3-D
2
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5
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2
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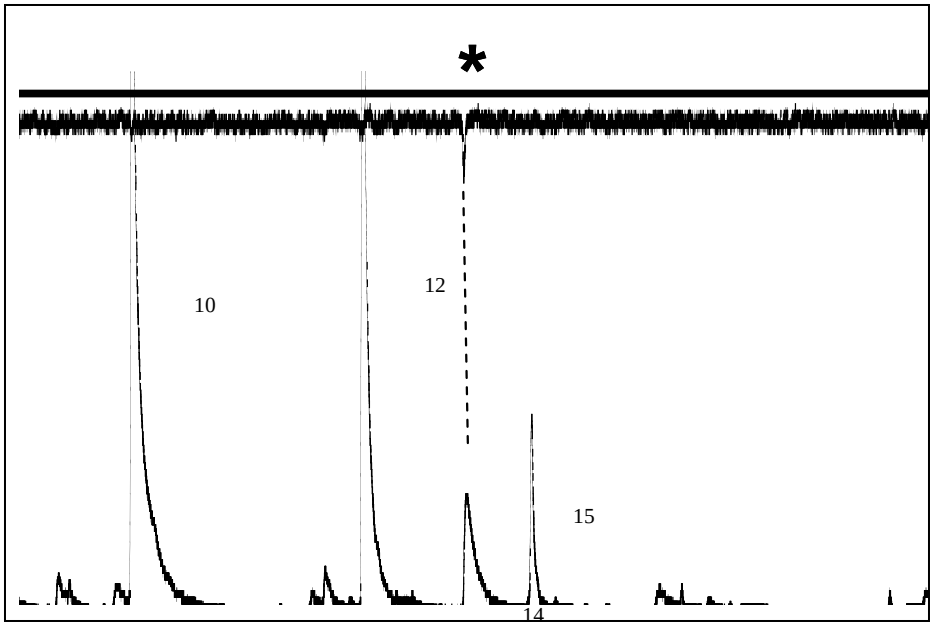
Figure 3



CHAPTER 4

SUSCEPTIBILITY OF *MICROTHECA OCHROLOMA* (COLEOPTERA: CHRYSOMELIDAE) TO BOTANICAL AND MICROBIAL INSECTICIDE FORMULATIONS

4.1 Introduction

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), is a major pest of cruciferous vegetables in the southern United States (U.S) (Chamberlin and Tippins 1948, Ameen and Story 1997a, Bowers 2003). This beetle, which was accidentally introduced into the USA from South America in the 1940s (Chamberlin and Tippins 1948), is now widely distributed in the southern U.S with major field infestations reported in Alabama, Florida, Louisiana, Mississippi, South Carolina, North Carolina, and Texas (Ameen and Story 1997b). Both larvae and adults of *M. ochroloma* feed in groups on foliage and may cause complete defoliation of crucifers. Although *M. ochroloma* is often not a major problem in conventional crucifer production due to its susceptibility to synthetic foliar insecticides (Bowers 2003), the beetle poses a major threat to organic vegetable production since organic farmers cannot use effective synthetic insecticides. Organic production of crucifers is presently an emerging industry in Alabama and other parts of the southern U.S, and *M. ochroloma* is often the predominant pest and a major factor limiting the growth and expansion of the industry

Several years of field studies by our group and others have identified only a few effective OMRI (Organic Material Review Institute) approved formulations, specifically Entrust® (spinosad) against *M. ochroloma* (Overall 2008, Balusu and Fadamiro 2011). PyGanic® (pyrethrum) was moderately effective, while other tested insecticides including some entomopathogenic fungal formulations showed very little or no efficacy against larvae or adults (Balusu and Fadamiro 2011).

To understand the basis for the poor performance of most insecticides tested in our field trials and establish baseline toxicity of the insecticides against *M. ochroloma*, the present study was conducted to measure the susceptibility of *M. ochroloma* larvae and adults to various botanical and microbial formulations under laboratory conditions. The materials evaluated (Table 1) included OMRI (Organic Material Review Institute) approved formulations such as PyGanic® (pyrethrum), Entrust® (spinosad), Mycotrol O® (*B. bassiana* strain GHA), and NOFLY® (*Paecilomyces fumosoroseus* strain FE 9901). Others were MBI-203 (experimental organic formulation of *Chromobacterium subtsugae*) and BotaniGard® 22WP (conventional formulation of *Beauveria bassiana* strain GHA). Entrust® WP (spinosad), the most effective treatment in our field trials, was evaluated as positive control, whereas BotaniGard® 22WP was evaluated as a conventional control for the microbial formulations. Many of the above insecticides are known to be effective against other coleopteran insect pests (Jones 1999, Andersen et al. 2006, Igrc Barcic et al. 2006, Isman 2006, Padilla-Cubas et al. 2006) and thus are expected to show activity against *M. ochroloma.*

Ultimately, it is hoped that this study will identify additional effective organically acceptable insecticides that can be applied as stand-alone treatments or in rotation with Entrust® for effective management of *M. ochroloma* in organic crucifer production. Furthermore,

knowledge of baseline susceptibility of *M. ochroloma* to various insecticides can be used to determine changes in susceptibility over time and the onset of resistance development.

4.2 Materials and Methods

4.2.1 Plants. Turnip (*Brassica rapa* var. *rapa* cultivar Purple top white globe) seedlings were raised from seeds purchased from Johnny's selected seeds (Winslow, ME, USA) in 60 well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2ºC and 55 ±5 % RH). Seedlings (3 weeks old) were transplanted into 0.5 L pots in Sunshine potting mixture #8 consisting of 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum and wetting agent (SunGro Horticulture, WA, USA). Plants were irrigated daily and fert-irrigated twice a week with Scotts® peat lite special fertilizer (Scotts-Sierra Horticultural Product Company, Marysville, OH, USA), a 20-10-20 water soluble NPK fertilizer mixture with micronutrients. Plants were grown using organic practices, and no pesticides were applied. Plants used for the bioassays and insect rearing were about 5-6 weeks old after transplanting.

4.2.2 Insects. Adults of *M. ochroloma* collected from a commercial organic farm in central Alabama in October 2006 were used to start laboratory colonies, which were supplemented annually with field-collected adults. *M. ochroloma* was reared on foliage of turnip plants (grown in the greenhouse as described above) in clear plastic Petri dishes (150 mm diameter × 30 mm height) lined with paper towels (Bounty®, Procter & Gamble, Cincinnati, OH). The colony was maintained at 25 ± 2ºC, 50 ±10% RH, and a photoperiod of 14:10 (L:D).

4.2.3 Toxicity Bioassays.

Toxicity of the insecticides (Table 1) against *M. ochroloma*, larvae and adults, was determined in two experiments. Single (field recommended rate) concentration screening assays were first carried out, and the most promising treatments were evaluated further in multiple-concentration assays. Solutions of each insecticide were made in distilled water. All bioassays were performed using the leaf-dip method at ambient conditions, 26ºC, 14:10 (L:D), and 50 % relative humidity. Briefly, intact leaves on a turnip plant were directly immersed in each insecticide solution for 5 s. The leaves were then removed and air dried for ~ 4 h to remove excess solution on leaf surface. A leaf disc (~ 90 mm) cut out of an excised treated leaf was placed in a 100-mm-diameter Petri dish lined with moist filter paper. To maintain higher humidity levels than the ambient relative humidity, the Petri dishes were covered with damp paper towel and placed inside a large plastic container (25 by 45 cm). Mortality of insects was recorded daily for 14 d (or until pupation of larvae). Insects that failed to move when probed with a dissection needle were recorded as dead and removed from the Petri dish. For the entomopathogenic fungal formulations, dead insects were removed and incubated separately in Petri dishes lined with damp filter paper. The Petri dishes were placed in a desiccator and inspected for the presence of mycelium on cadavers (mycosis) starting on day 7.

4.2.3.1 Toxicity at field recommended rates

In the first experiment, all insecticide formulations were evaluated at field recommended rates (i.e., single concentration assays) for efficacy against *M. ochroloma* larvae and adults. Test solutions of field recommended rates of the insecticides (Table 1) were prepared in distilled water. A group of 20 larvae (1-day-old) from the same batch was placed in a Petri dish

containing a treated leaf disc. Similarly, a group of 20 adults (4-5 days old) from the same batch was placed in a Petri dish that contained a treated leaf disc by using a fine camel's hair brush (#00). The experiment was replicated five times per insecticide and insect mortality was determined as described above.

4.2.3.2 Multiple-concentration assays

Promising treatments in the first experiments were selected for further evaluation in multiple-concentration assays to determine the LC_{50} (lethal concentration that kills 50% of test insects or median lethal concentration) and LT_{50} (lethal time to kill 50% of test insects). Based on the results of the first experiment, all six insecticides were evaluated against the larvae but only two (Entrust® and PyGanic®) were evaluated against the adults. Each insecticide was tested at five concentrations plus distilled water control for a total of six rates. The concentration range for each insecticide was determined based on the results of preliminary bioassays that gave mortality ranges of 10 to 90%. For each concentration, a group of 20 adults (4-5 days old) or 20 larvae (1 day old) from the same batch was placed in a Petri dish containing a treated leaf disc. The experiment was replicated five times per concentration and insect mortality was determined as described above.

4.2.4 Data analysis. Mortality data were tested using non-parametric test because the actual and arcsin sqrt (x + 0.001) data did not meet the assumptions of ANOVA. The data was further run using the ordinary F-test after which the results were compared with the non-parametric test. When both procedures give similar results, the ANOVA assumptions were assumed to be satisfied reasonably well, and the standard ANOVA is satisfactory

(http://faculty.evansville.edu/ch81/bio415f02/BIO415Topic7.pdf) then the means were separated with Tukey's honestly significant difference (HSD) test. The LC_{50} values expressed in parts per million (ppm), LT_{50} values (in days), 95% fiducial limits (FL), and regression slopes were estimated by probit analysis (Finney 1991) using POLO PLUS software for windows (LeOra software 2007). Tests of parallelism of probit regression lines for all treatments were conducted using chi-square goodness-of-fit tests (POLO PLUS, LeOra software 2007).

4.3 Results

4.3.1 Toxicity at field recommended rates

There was a significant effect of insecticide treatment at the field recommended rate on mortality of *M. ochroloma* larvae (F=1251.2; df = 6, 28; $P < 0.0001$) as early as 24 h of exposure to treated leaf discs (Fig. 1). Entrust® and PyGanic® resulted in 100% larval mortality after 24 h. MBI-203 also caused significantly greater mortality than the untreated control starting at 24 h however, 100% mortality was not attained until day 5. In contrast, no significant differences were recorded between the entomopathogenic fungal formulations (i.e., Botanigard®, Mycotrol O®, and NOFLY®) and untreated control on days 1-4. On day 5 the fungal formulations caused significantly higher mortality than untreated control (F=492.9: df = 6, 28: P<0.0001); however, no fungal formulation resulted in more than 50% larval mortality throughout the exposure period (Fig. 1). The average survival time for larvae treated with Entrust®, PyGanic®, MBI-203, Botanigard®, and Mycotrol O® and NOFLY® at field recommended rate was 1, 1, 5, 8, 8, and 7 days after treatment, respectively (Fig. 1).

For adults, Entrust® and PyGanic® were the most effective treatments with 100% mortality after 24 h, which was significantly greater than the other treatments or the untreated

control (F=1974; df=6,28; P<0.0001) (Fig. 2). No significant differences were recorded between the other treatments and the untreated control on days 1-7. On day 8, Botanigard® (14% mortality) and Mycotrol O® (12% mortality) were significantly better than the untreated control (F=979.1; df = 6, 28; P<0.0001), whereas mortalities caused by MBI-203 (2%) and NOFLY® (2%) were not significantly different from the untreated control (Fig. 2). The average survival time for adults treated with Entrust®, PyGanic®, Botanigard®, and Mycotrol O® at field recommended rates were 1, 1, 9, and 9 d, respectively (Fig. 2).

In general, the fungal formulations were slow-acting and relatively less toxic to *M. ochroloma*. None resulted in more than 50% larval or 14% adult mortalities over the 9-day exposure period. Comparing the two life stages, the larvae were more susceptible to the insecticides than adults.

4.3.2 Multiple-concentration assays

The LC_{50} and LT_{50} values, 95% fiducial limits, slope, and chi-square values for the insecticides tested are presented in Table 2. All chi-square values were not significant ($\alpha = 0.05$) in Pearson's goodness-of-fit test on the probit model, indicating a good fit of regression line. Entrust® and PyGanic® had the lowest LC_{50} values against the larvae (Table 2), indicating higher toxicity. However, Entrust® (LC_{50} = 0.1 ppm) was ≈ 100 times more toxic to the larvae than PyGanic® (LC_{50} = 10.9 ppm). All other treatments (Mycotrol O®, NOFLY®, MBI-203 and BotaniGard®) were not significantly different after 24 h of exposure. Significant concentration-mortality responses of the larvae were observed for all insecticides tested, as indicated by the positive slope values (Table 2). MBI-203 had the highest slope (6.21 ± 0.66), followed by PyGanic® (2.86 ± 0.23), Mycotrol O® (1.99 ± 0.25), BotaniGard® (1.96 ± 0.27), NOFLY® (1.28

± 0.29), and Entrust® (1.15 ± 0.08); higher slopes indicate greater concentration-mortality response. The second measure of efficacy was the LT_{50} values that were calculated for the field recommended rates (Table 3). LT_{50} values were not estimated for Entrust® and PyGanic® since both treatments caused complete mortality of larvae after 24 h at the field recommended rate. Among the remaining treatments, MBI-203 had significantly lower LT_{50} (2 days) than BotaniGard® (9 days), Mycotrol O® (10 days), or NOFLY® (12 days) (Table 3).

Since Entrust® and PyGanic® were the only effective treatments against the adults at the field recommended rate (experiment 1), LC_{50} values were estimated only for these two formulations against the adults (Table 4). Entrust® (LC_{50} =2.4 ppm) was ≈ 10 times more toxic to the adults than PyGanic® (LC_{50} =24.1 ppm). LT_{50} values were not estimated since both insecticides caused complete mortality after 24 h of exposure.

4.4 Discussion

The results of this laboratory study demonstrated varying levels of susceptibility of *M. ochroloma* to the tested insecticides. Among the formulations, Entrust® was the most effective causing 100% larval and adult mortality after 24 h, as well as having the lowest LC_{50} values and survival time. PyGanic®, the second best treatment, also caused complete mortality of both life stages after 24 h but was ≈ 100 fold and 10 fold less toxic than Entrust® to the larvae and adults, respectively, The results further showed that the LC_{50} values of Entrust® and PyGanic® were only a fraction, 200 × and 15 ×, less than the actual field recommended rate, respectively. MBI-203 (experimental organic formulation of *C. subtsugae*) was effective against the larvae but not against the adults. At the field recommended rate, the entomopathogenic fungal formulations

(Mycotrol®, NOFLY®, and BotaniGard®) were comparatively less toxic to the larvae and showed no efficacy against the adults.

The efficacy of Entrust® or its active ingredient (spinosad) has also been documented against some other beetles in the same family (Chrysomelidae) as *M. ochroloma*, including flea beetles, *Phyllotreta* spp. in cruciferous crops (Andersen et al. 2006), *Epitrix tuberis* in potato (Chu et al. 2006), and Colorado potato beetle, *L. decemlineata* in potato (Igrc Barcic et al. 2006). Entrust® was also effective against lepidopteran pests of crucifer crops in Alabama (Maxwell and Fadamiro 2006). The efficacy of Entrust® recorded in the present study may be attributed to its broad spectrum activity (Cisneros et al. 2002), multiple modes of entry (Eger and Lindenberry 1998, Liu et al. 1999), and/or and residual effect (Igrc Barcic et al. 2006). The active ingredient in Entrust® is both a contact and stomach poison (Eger and Lindenberry 1998, Liu et al. 1999). The efficacy of PyGanic®, a botanical insecticide with pyrethrum as the active ingredient, against *M. ochroloma* was not surprising, since pyrethrum is known for its rapid knockdown effect on insects. PyGanic® has also been reported as effective against other insect pests including Colorado potato beetle, *L. decemlineata* (Igrc Barcic 2006), and harlequin bug, *Murgantia histrionic* (Hahn) (Overall 2008). In general, the results of this laboratory study are in agreement with our field data that identified Entrust® as the only effective treatment and PyGanic® as moderately effective (Balusu and Fadamiro 2011). The high activity of the experimental formulation, MBI-203, against *M. ochroloma* larvae is very encouraging and suggests that (if registered) this insecticide may play a role in the management of *M. ochroloma*. *Chromobacterium subtsugae*, the active ingredient in MBI-203, has been reported to be toxic to several insects including larvae of Colorado potato beetle, *L. decemlineata* (Martin et al. 2004), as well as adults of corn rootworms, *Diabrotica undecimpunctata howardi* Barber and

Diabrotica virgifera virgifera LeConte, and southern green stink bug, *Nezara viridula* (L.) (Martin et al. 2007).

The entomopathogenic fungal formulations were only slightly effective against *M. ochroloma* larvae and did not work against the adults. Although some studies have reported, the efficacy of entomopathogenic fungal formulations against some Colorado potato beetle, *L. decemlineata* (Poprawski et al. 1997), and some other chrysomelid beetle species (Butt et al. 1992), our laboratory results which also agree with our field data (Balusu and Fadamiro 2011) suggest that these fungal formulations are relatively non-toxic to *M. ochroloma*. The lack of efficacy of the fungal formulations against *M. ochroloma* may be attributed to the fungitoxic nature of isothiocyanates that are associated with *Brassica* species. Inyang et al. (1999) demonstrated the inhibitory activity of isothiocyanates, specifically, phenylethyl-, 2-chlorophenyl- and allyl-isothiocyanates, on entomopathogenic fungal conidia germination and its ability to infect the insects. Nevertheless, we observed some sub-lethal effects of the fungal formulations on *M. ochroloma*, in terms of reduced feeding and fecundity. If confirmed, these sub-lethal effects can be as valuable as direct insect mortality (Liu and Bauer 2008) in decreasing the pest status of *M. ochroloma,* and thus are worthy of further investigation.

The results showed that *M. ochroloma* larvae were more susceptible than the adults to the tested insecticides. For instance, the LC_{50} value of Entrust® against the larvae (0.1 ppm) was 20× lower than that of the adults (2.36 ppm). This difference in susceptibility may be attributed to the differential feeding rate of both life stages; larvae are more voracious feeders than adults. Another possible explanation for the differential susceptibility observed between the two life stages may be difference in the ability of the insecticides to penetrate through the cuticle or difference in the composition of the cuticle. Christie and Wright (1990) attributed marked

differences in relative toxicity of the insecticide abamectin between larval instars of *Spodoptera littoralis* (Boisduval) to differences in the insecticides penetration rates.

In summary, this study has identified some promising OMRI-acceptable biopesticides and effective rates against *M. ochroloma* larvae and adults. Entrust® and PyGanic® were the most effective insecticides followed by MBI-203. The data also showed that the actual lethal concentrations of Entrust® and PyGanic® were only a fraction of the field recommended rates. Additional studies are necessary to further evaluate the field activity of the promising treatments identified in this study, in particular in rotation with Entrust® for effective management of *M. ochroloma* in organic crucifer production.

4.5 Acknowledgments

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Table 1. Insecticides tested against *M. ochroloma*

Registered name	Active ingredient	Type	Company	Field recommended rate/acre
PyGanic® 1.4 EC	Pyrethrum	OMRI approved	McLaughlin Gormley King Company, Minneapolis, MN	2 quarts
Entrust® WP	Spinosad	OMRI approved	Dow AgroSciences LLC, , Indianapolis, IN	2 oz
Mycotrol O® ES	*Beauveria bassiana* strain GHA	OMRI approved	Laverlam International Corporation, Butte, MT	1 quart
NOFLY® WP	*Paecilomyces fumosoroseus* strain FE 9901	OMRI approved	Natural Industries, Inc. Houston, Houston, TX	2 lb
MBI-203 SC	*Chromobacterium subtsugae*	Experimental	Marrone Bio Innovations, Davis, CA	2 quart
BotaniGard® 22WP	*Beauveria bassiana* strain GHA	Conventional	Laverlam International Corporation, Butte, MT	2 lb

Table 2. Concentration-mortality response of *M. ochroloma* larvae exposed to various insecticide formulations in leaf-dip assays.

Treatment	No. insects	Slope ± SE	LC_{50} (ppm of a.i)	95% Fiducial limits (ppm)	χ^2
				Lower - Upper	
PyGanic®	121	2.86 ± 0.23	10.9^{b}	8.48 - 14.03	8.85
Mycotrol O®	118	1.99 ± 0.25	$6.67\times10^{2\ a}$	534.9 - 922.2	2.46
NOFLY®	114	1.28 ± 0.29	$4.08\times10^{3\ a}$	2018.5 - 25038	0.78
MBI-203	118	6.21 ± 0.66	$5.21\times10^{3\ a}$	4064.2 - 6417.2	8.55
Entrust®	118	1.15 ± 0.08	0.1^{c}	0.061 - 0.18	10.81
BotaniGard®	117	1.96 ± 0.27	$1.3\times10^{3\ a}$	829.4 - 5938.6	6.1

Table 3. Probit analysis of time-mortality response of bioassay with test formulation against *M. ochroloma* larvae.

Treatment	No. insects	Slope ± SE	LT_{50} (days)	95% Fiducial limits (days)	χ^2
				Lower - Upper	
Mycotrol O®	108	2.54 ± 0.25	10.44[a]	7.82 - 21.52	34.88
NOFLY®	114	4.77 ± 0.68	12.26[a]	10.77 - 15.27	3.09
MBI-203	112	5.34 ± 0.31	2.21[b]	1.94 - 2.58	23.9
BotaniGard®	111	5.88 ± 0.58	9.03[a]	8.52 - 9.76	0.67

Table 4. Concentration-mortality response of *M. ochroloma* adults exposed to various insecticide formulations in leaf-dip assays

Treatment	No. insects	Slope ± SE	LC_{50} (ppm of a.i)	95% Fiducial limits (ppm)		χ^2
				Lower-	Upper	
PyGanic®	117	3.24±0.27	24.14[a]	19.71	31.22	8.85
Entrust®	118	1.15±0.08	2.36[b]	1.30	4.29	10.81

List of figures:

Figure. 1

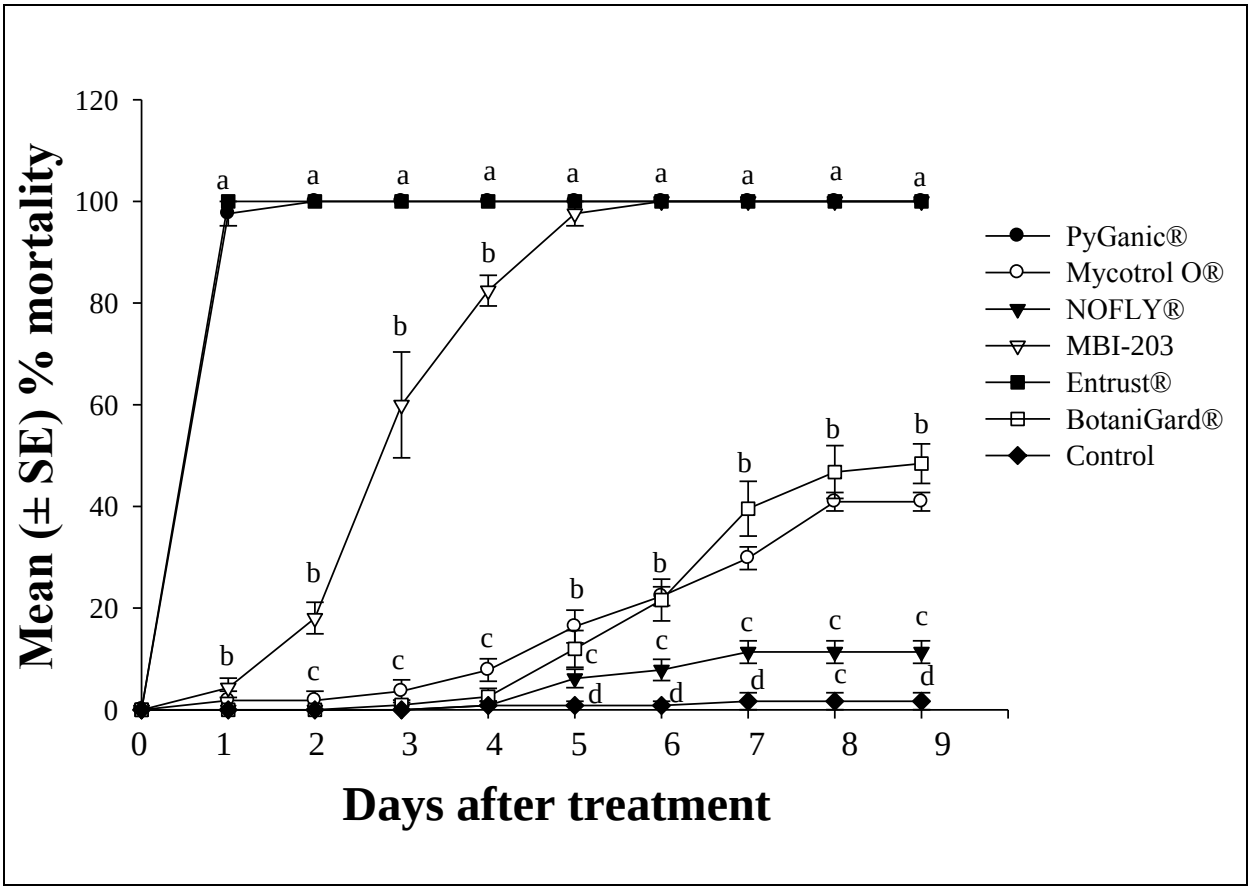

Mean (± SE) % mortality
Days after treatment
PyGanic®
Mycotrol O®
NOFLY®
MBI-203
Entrust®
BotaniGard®
Control

Figure. 2

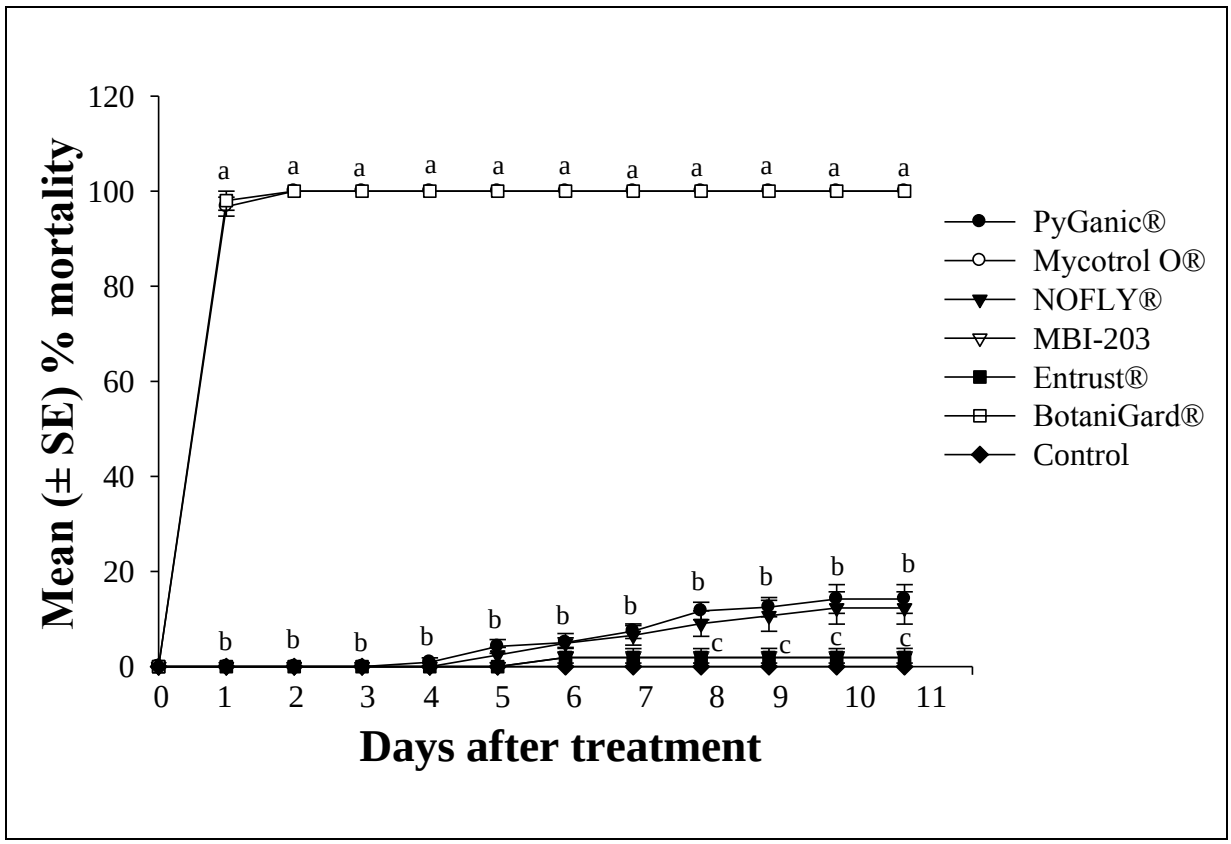
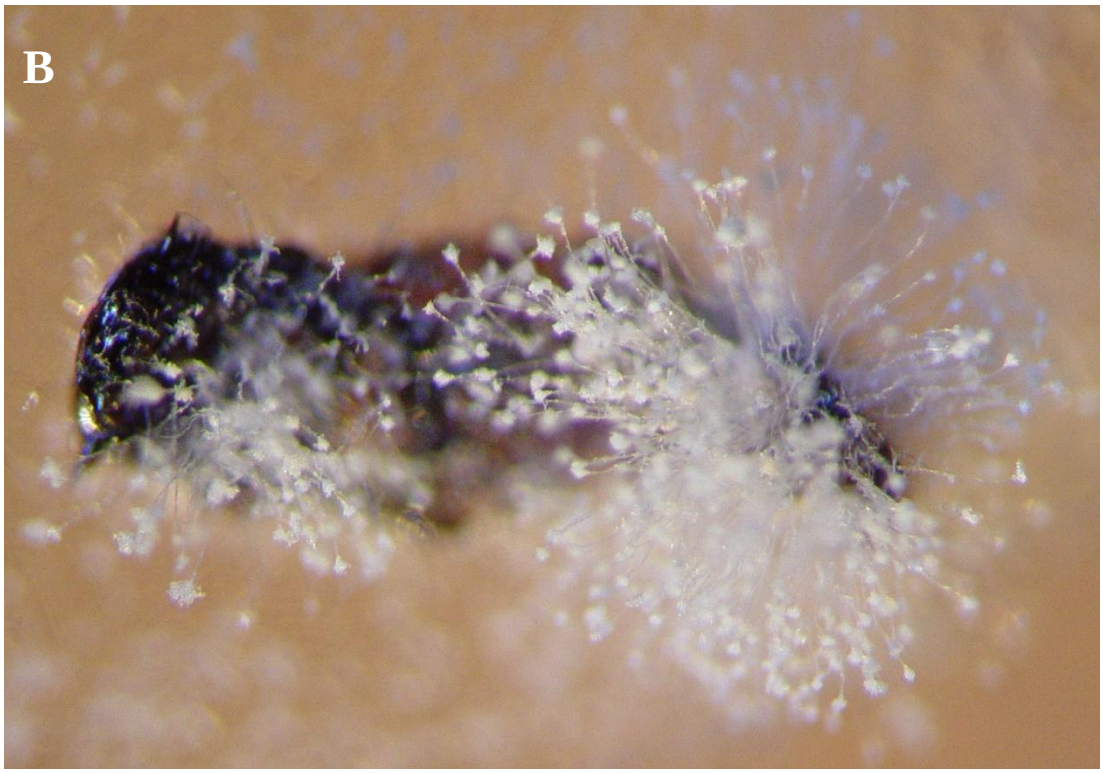

Mean (± SE) % mortality
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11
Days after treatment
PyGanic®
Mycotrol O®
NOFLY®
MBI-203
Entrust®
BotaniGard®
Control

Figure 3.



CHAPTER 5

EVALUATION OF ORGANICALLY ACCEPTABLE INSECTICIDES AS STAND-ALONE TREATMENTS AND IN ROTATION FOR MANAGING YELLOWMARGINED LEAF BEETLE*, MICROTHECA OCHROLOMA* (COLEOPTERA: CHRYSOMELIDAE) IN ORGANIC CRUCIFER PRODUCTION

5.1 Introduction

Cruciferous vegetable production is an important industry in Alabama and other parts of the southern United States (U.S.). Many farmers in the region grow various kinds of cruciferous crops (e.g., turnip, radish, mustard, napa cabbage, cabbage, collards, arugula, and Japanese leafy vegetables, such as mizuna and mibuna) as mixed cropping systems in the spring and fall using organically-acceptable practices.

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), is arguably the most damaging pest of organic cruciferous crop production in the region (Chamberlin and Tippins 1948, Ameen and Story 1997b, Bowers 2003). Indigenous to South America, this beetle was accidentally introduced into the U.S from South America. Inspectors of the Bureau of Entomology and Plant Quarantine detected the first specimen of *M. ochroloma* in North America in 1945 at the port of New Orleans on grapes from Argentina (Balsbaugh 1978). The first field population of the beetle was later recorded in Mobile, Alabama in 1947 (Chamberlin and Tippins 1948). The beetle was subsequently reported in Mississippi (Rohwer et al. 1953), Louisiana (Oliver 1956), Florida (Woodruff 1974), and Texas (Balsbaugh

1978) and is now widely distributed in the southern U.S. with major field infestations reported in Alabama, Florida, Louisiana, Mississippi, South Carolina, North Carolina, and Texas (Ameen and Story 1997a).

Both adult and larval stages of *M. ochroloma* often feed in groups on foliage of cruciferous crops with potential for major economic loss. When feeding on its host plants, *M. ochroloma* make small, irregularly-shaped holes in the leaves and feed upon the leaf margins. *M. ochroloma* is rarely a major problem in conventional vegetable production systems due to its susceptibility to synthetic foliar insecticides (Bowers 2003). However, it poses a major threat to organic vegetable production since organic farmers cannot use effective synthetic insecticides. *M. ochroloma* is the predominant and often the only key pest detected in local organic vegetable fields (personal observation) in Alabama. Currently, there are no published studies on the efficacy of organically acceptable management tactics against *M. ochroloma*. Pest management tactics and formulations approved by the Organic Materials Review Institute (OMRI), which could potentially be used to manage *M. ochroloma* in organic and low-input vegetable production systems, include botanical insecticides, microbials, insecticidal soaps, and semiochemicals.

The objective of this study was to evaluate the efficacy of some OMRI listed and experimental formulations of botanical and microbial insecticides for management of *M. ochroloma* in organically-grown crucifer crops. The ultimate goal was to identify organically acceptable treatments effective against *M. ochroloma* for recommendation to organic vegetable growers in the southern U.S. The materials evaluated at recommended field rates included OMRI listed formulations such as PyGanic® 1.4 EC (2 quarts/acre, McLaughlin Gormley King Company, Minneapolis, MN), Aza-Direct® EC (2 pints/acre, Gowan Company LLC, Yuma,

AZ), Entrust® WP (2 oz/acre, Dow AgroSciences LLC, Indianapolis, IN), Mycotrol O® ES (1 quart/acre, Laverlam International Corporation, Butte, MT), Novodor® FC (4 quarts/acre, Valent BioScience Corporation, Libertyville, IL), and NOFLY® WP (2 lb/are, Natural Industries, Inc. Houston, TX). In addition, two experimental organic formulations were also evaluated: Tick-Ex EC (3.65 quart/acre, Novozymes Biologicals, Inc. Salem, VA) and MBI-203 SC (2 quart/acre, Marrone Bio Innovations, Davis, CA).

PyGanic® 1.4 EC is an OMRI listed formulation of pyrethrum derived from the flowers of *Chrysanthemum* spp. (Weinzierl 2000). Pyrethrum, which is well known for its quick knockdown effect, is the predominant botanical insecticide in use, perhaps accounting for 80% of the global botanical insecticide market (Isman 2005). It has been shown to be effective against several insect pests including Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Igrc Barčić 2006), and harlequin bug, *Murgantia histrionic* (Hahn) (Overall 2008). Aza-Direct® EC is an OMRI-listed formulation of Azadirachtin, a tetranotriterpenoid derived from seed kernels of neem trees, *Azadiracta indica* (Spollen and Isman 1996). It is well known as an insect growth regulator that affects feeding and molting in a wide variety of insects (Isman 2006). Entrust® WP is a natural insect control product formulated for organic crop production. The active ingredient, spinosad, is developed from a fermentation by-product of the soilborne actinomycete bacterium, *Saccharopolyspora spinosa* (Thompson et al. 1997, Dow AgroSciences 2001). The efficacy of Entrust® or its active ingredient, spinosad, has been demonstrated against several chrysomelid beetles (Andersen et al. 2006, Chu et al. 2006, Igrc Barcic et al. 2006) and lepidopteran pests (Maxwell and Fadamiro 2006). Mycotrol O® ES is an organic formulation of the entomopathogenic fungus, *Beauveria bassiana* strain GHA. It has been reported as effective against *L. decemlineata* (Jones 1999). Novodor® is a biological insecticide containing the active

protein crystal produced by *Bacillus thuringiensis* subspecies *tenebrionis (Btt)*. It is effective against the larval stages of several chrysomelid beetles including *L. decemlineata* (Hilbeck et al. 1998). NOFLY® WP is a microbial formulation that contains live blastospores of the naturally occurring fungus, *Paecilomyces fumosoroseus* strain FE 9901. It has been shown to be effective against whiteflies and other insects (Padilla-Cubas 2006). Tick-Ex EC is an experimental organic formulation of the entomopathogenic fungus, *Metarhizium anisopliae* strain F52, which was shown to be effective against black vine weevil, *Otiorhynchus sulcatu*s (Fabricius) (Bruck 2005). MBI-203 SC is an experimental formulation of the bacterium, *Chromobacteria subtsugae*, which was reported to be toxic to *L. decemlineata* (Martin et al. 2004).

We hypothesized that most of the above formulations will be effective against *M. ochroloma* since they are known to be effective against other Coleoptera. The formulations were evaluated in different sets (i.e. not all formulations were evaluated in all years) at multiple locations over four field seasons (spring 2007, spring 2008, fall 2009, and fall 2010) in Alabama. In the first three seasons, the formulations were evaluated as stand-alone treatments. In fall 2010, some formulations were evaluated in rotation (alternation) with Entrust®, which was identified in the previous seasons as the most effective treatment.

5.2 Materials and Methods

This study was conducted over four growing seasons; spring 2007, spring 2008, fall 2010, and fall 2010 at three different locations in Alabama; Red Root Herb and Vegetable Farm (Banks), Snow's Bend Farm (Tuscaloosa), and E.V. Smith Research Center (Shorter). The study was not repeated in 2009 due to an unusually low field population of *M. ochroloma* in Alabama, possibly because of some weather related factors. Experimental plots consisted of long single

rows of turnip (*Brassica rapa* var. *rapa*) plants of 10.68 m (35 ft) by 0.76 m (2.5 ft) with plants spaced at ~ 0.12 m (0.4 ft) apart within a row and 1.52 m (5 ft) between the rows for a total of ~90 plants per plot. Treatments were arranged in a randomized complete block design with three replicates in spring 2007 and four replicates in the remaining three field seasons. Organic certified seeds of "Purple top white globe" turnip (Johnny's Selected Seed, Winslow, ME, USA) were established in all the trials and maintained using standard organic crop production practices. All insecticide treatments were evaluated at the recommended field rates, and each trial included an untreated control.

In spring of 2007, the experiment was conducted at the Banks location from 18 May to 5 June 2007. The following four materials were evaluated as stand-alone treatments: PyGanic®, Aza-Direct®, Entrust®, and Mycotrol O®. The spring 2008 trial was conducted at the Tuscaloosa location from 9 May to 27 May 2008 and included five insecticide treatments: PyGanic®, Entrust®,Mycotrol O®, Novodor®, and Tick-Ex. The fall 2008 trial was conducted at the Banks location from 28 October to 12 November 2008. Four insecticide treatments were compared: PyGanic®, Aza-Direct®, Entrust®, and Mycotrol O®. In fall of 2010, the experiment was conducted at the Shorter location from 1 November to 22 November 2010. Treatments were modified to include only those that performed well in the previous seasons (i.e. PyGanic® and Entrust®) and two new materials: NOFLY™ and MBI-203 (an experimental formulation). These four materials were evaluated as stand-alone treatments. In addition, two insecticide rotation/alternation treatments were evaluated: Entrust® alternated with PyGanic® and Entrust® alternated with NOFLY™. In both alternated treatments, Entrust® was applied first followed by application of the alternate insecticide (i.e., PyGanic® or NOFLY™) one week later, followed again by application of Entrust®.

In all seasons, foliar applications of treatments were made weekly with a pressurized, hand operated, knapsack sprayer (Solo®, Newport News, VA), which was calibrated to deliver 44 gpa at 35-40 psi. A total of three weekly sprays were made per season starting from the onset of beetle activity in the field. Plots were evaluated twice a week (every 3-4 days) by visually sampling five randomly selected plants per plot for *M. ochroloma* larvae and adults. The five plants were also rated for *M. ochroloma* feeding damage using a method modified after Maletta et al. (2004). In this method plants were rated on a scale of 1 to 6 as follows: 1 = very light defoliation with < 10% of damage; 2 = light defoliation (10-30%); 3 = moderate defoliation (30-50%); 4 = heavy defoliation (50-70%); 5 = very heavy defoliation (70-90%) and 6 = complete (total) defoliation (> 90%).

For each season, mean number of *M. ochroloma* larvae and adults and mean damage ratings were calculated for each treatment. Data were transformed by the square-root method ($\sqrt{x}+ 0.5$) and analyzed for significant treatment effects by analysis of variance (ANOVA) with the replicates considered as blocks. Means were compared by using the Tukey-Kramer HSD test (JMP version 7.0.1, SAS Institute, 2007). Significant differences were established at the 95% confidence level ($P < 0.05$).

5.3 Results

Significant numbers of *M. ochroloma* were recorded in all the locations and field seasons. In general, no significant block (replicate) effects were detected on any of the key variables, suggesting that the blocks were similar in *M. ochroloma* density and treatment efficacy. Other crucifer pests were either not recorded (i.e., caterpillars, leaf beetles, and harlequin bug) or recorded in very low numbers (i.e., aphids) in the experimental plots during the four seasons.

Thus, we were unable to assess the effect of the treatments on other crucifer pests. Beneficial insects (i.e., lady beetles and tiger beetles) were observed in low numbers in the experimental plots. Tiger beetles were observed feeding on larvae of *M. ochroloma,* but their effect was not quantified.

Very low numbers of *M. ochroloma* larvae were recorded in spring 2007 at the Banks location. This was possibly because the trial was commenced (on 18 May), several weeks after the onset of *M. ochroloma* activity. Thus, only the data collected on the number of adults and damage ratings were presented. No significant differences in adult counts were recorded among the treatments in the pre-treatment samples collected on 18 May ($F = 1.02$; df = 4, 68; $P = 0.4034$). However, significant differences in adult counts were recorded among the treatments on 21 May, ($F = 88.44$; df = 4, 68; $P < 0.0001$), 25 May, ($F = 86.77$; df = 4, 68; $P < 0.0001$), 29 May, ($F = 36.43$; df = 4, 68; $P < 0.0001$), 1 June, ($F = 20.49$; df = 4, 68; $P < 0.0001$), and 5 June, ($F = 11.87$; df = 4, 68; $P < 0.0001$). On most of the sampling dates, significantly fewer *M. ochroloma* adults were recorded in Entrust® treated plots compared with the untreated (control) plots or plots treated with the other insecticides (Fig. 1A). PyGanic® also resulted in significant suppression of *M. ochroloma* adults on most dates compared to Aza-Direct®, Mycotrol O® or the untreated control. Similar results were obtained for damage ratings: Entrust® and PyGanic® produced significantly lower damage ratings than the control or other treatments (Fig. 1B).

The results obtained in spring 2008 at the Tuscaloosa location were generally similar to those of spring 2007 in terms of treatment efficacy. Pre-treatment sampling on 9 May showed no significant differences among the treatments in adult counts ($F = 1.75$; df = 4, 92; $P = 0.14$, Fig. 2A), larval counts ($F = 0.24$; df = 4, 92; $P = 0.9149$, Fig. 2B), and damage ratings ($F = 1.36$; df =4, 92; $P = 0.25$, Fig. 2C). However, significant differences in adult counts were recorded among

the treatments on 13 May (F = 9.07; df = 4, 92; P < 0.0001), 16 May (F = 31.16; df = 4, 92; P < 0.0015), 20 May (F = 3.71; df = 4, 92; P =0.0075), and 27 May (F = 2.98; df = 4, 92; P = 0.0231) (Fig. 2A). No signigicant differences in larval counts were recorded among the treatments on 23 May (F = 0.64; df = 4, 92; P= 0.6372). Similarly, significant differences in larval counts were recorded among the treatments on 13 May, (F = 73.29; df = 4, 92; P < 0.0001), 16 May, (F = 176.31; df = 4, 92; P < 0.0001), 20 May, (F = 195.11; df = 4, 92; P < 0.0001), 23 May, (F = 207.88; df = 4, 92; P < 0.0001), and 27 May, (F = 54.56; df = 4, 92; P < 0.0001) (Fig. 2B). Significant differences were also recorded among the treatments in mean damage ratings on 16 May, (F = 31.16; df = 4, 92; P <0.0001), 20 May, (F = 154.16; df = 4, 92; P < 0.0001), 23 May, (F = 256.16; df = 4, 92; P < 0.0001), and 27 May, (F = 980.94; df = 4, 92; P < 0.0001) (Fig. 2C). In general, adult counts, larval counts and damage ratings were significantly lower in plots treated with Entrust® compared to the other treatments on most sampling dates. PyGanic® also resulted in lower larval counts on some dates compared to the other treatments (Fig. 2).

In fall 2008 at the Banks location, pre-treatment data collected on 28 October showed fairly uniform distribution of *M. ochroloma* adults (F = 2.30; df = 5, 111; P = 0.0493, Fig. 3A) and larvae (F = 1.36; df = 5, 111; P = 0.2444, Fig. 3B) in all experimental plots. Very low damage ratings were also recorded in all the plots (F = 0.60; df = 5, 111; P = 0.6972, Fig. 3C). Significant differences in adult counts were recorded among the treatments on 31 October (F = 78.16; df = 5, 111; P < 0.0001), 3 November (F = 115.00; df = 5, 111; P < 0.0001), 6 November (F = 78.25; df = 5, 111; P < 0.0001), 9 November (F = 12.47; df = 5, 111; P < 0.0001), and 12 November (F = 3.67; df = 5, 111; P = 0.0042) (Fig. 3A). Also, significant differences in larval counts were recorded among the treatments on 31 October (F = 101.08; df = 5, 111; P <

0.0001), 3 November (F = 233.17; df = 5, 111; P < 0.0001), 6 November (F = 297.89; df = 5, 111; P < 0.0001), 9 November (F = 101.82; df = 5, 111; P < 0.0001), and 12 November (F = 13.51; df = 5, 111; P < 0.0001) (Fig. 3B). Similarly, damage ratings were significantly different among the treatments on 31 October (F = 8.30; df = 5, 111; P < 0.0001), 3 November (F = 46.04; df = 5, 111; P < 0.0001), 6 November (F = 134.57; df = 5, 111; P < 0.0001), 9 November (F = 973.45; df = 5, 111; P < 0.0001), and 12 November (F = 883.21; df = 5, 111; P = 0.0042) (Fig. 3C). In general, the lowest adult counts, larval counts and damage ratings were recorded in plots treated with Entrust® or PyGanic®. Novodor® and Mycotrol O® also produced lower larval counts compared to the control.

Promising materials identified in the previous seasons, and two new treatments, were evaluated as stand-alone treatments and in rotation with Entrust® during fall of 2010 at the Shorter location. Pre-treatment data collected on 1 November showed that adult counts (F = 0.60; df = 6,130; P = 0.7276, Fig. 4A), larval counts (F = 1.94; df = 6,130; P = 0.0789, Fig. 4B), and damage ratings (F = 1.42; df = 6,130; P = 0.2117, Fig. 4C) were similar in all the experimental plots. However, significant differences in adult counts were recorded among the treatments on 5 November (F = 19.75; df = 6, 13; P < 0.0001), 9 November (F = 21.87; df = 6, 130; P < 0.0001), 13 November (F = 44.77; df = 6, 130; P < 0.0001), 17 November (F = 30.78; df = 6, 130; P < 0.0001), and 22 November (F = 87.07; df = 6, 130; P < 0.0001) (Fig. 4A). Larval numbers were also significantly different among the treatments on 5 November (F = 276.80; df = 6, 130; P < 0.0001), 9 November (F = 439.57; df = 6, 130; P < 0.0001), 13 November (F = 958.23; df = 6, 130; P < 0.0001), 17 November (F = 193.81; df = 6, 130; P < 0.0001), and 22 November (F = 76.55; df = 6, 130; P < 0.0001) (Fig. 4B). Similarly, significant differences in damage ratings were recorded among the treatments on 5 November (F = 4.91; df

= 6, 130; $P = 0.0001$), 9 November ($F = 25.90$; df = 6, 130; $P < 0.0001$), 13 November ($F = 462.69$; df = 6, 130; $P < 0.0001$), 17 November ($F = 122.60$; df = 6, 130; $P < 0.0001$), and 22 November ($F = 473.30$; df = 6, 130; $P < 0.0001$) (Fig. 4C). Compared to the control the following four treatments resulted in significant suppression of *M. ochroloma* larvae, adults and damage on most sampling dates; Entrust® stand-alone, PyGanic® stand-alone, Entrust® alternated with PyGanic®, and Entrust® alternated with NOFLY™. MBI-203 (experimental formulation) was effective only against the larvae. NOFLY™ stand-alone treatment was not effective against *M. ochroloma* larvae or adults.

5.4 Discussion

The goal of this study was to identify effective OMRI approved insecticides for managing *M. ochroloma* in organic crucifer vegetable production systems. Data from the four field seasons in multiple locations confirmed that *M. ochroloma* is indeed a major constraint to organic crucifer production in Alabama. Of all the various insecticides tested, which included botanical and microbial formulations and weekly sprays of Entrust®, a formulation of spinosad for organic crop production, consistently performed well in suppressing *M. ochroloma* adults, larvae and crop damage. PyGanic®, a botanical insecticide with quick knockdown effect, was the next best treatment. A few of the materials such as Novodor® (*Bacillus thuringiensis* subspecies *tenebrionis*), Mycotrol O® (*Beauveria bassiana* Strain GHA) and MBI-203 (an experimental formulation of *Chromobacterium subtsugae*) showed some efficacy in some seasons against *M. ochroloma* larvae but did not sufficiently suppress the adults or crop damage. The other tested materials including Aza-Direct® (a botanical insecticide with Azadirachtin as active ingredient), NOFLY™ (*Paecilomyces fumosoroseus* strain FE 9901), and Tick-Ex (an experimental organic

formulation of *Metarhizium anisopliae* Strain F52) showed no efficacy against *M. ochroloma,* and ultimately did not suppress crop damage by the pest. Additionally, the results of the fall 2010 trials demonstrated that application of Entrust®, in rotation or alternation with PyGanic® or NOFLY™, was as effective as the Entrust® stand alone treatment.

The efficacy of Entrust® or its active ingredient (spinosad) has also been documented against some other beetles in the same family (Chrysomelidae) as *M. ochroloma*, including flea beetles, *Phyllotreta* spp., in cruciferous crops (Andersen et al. 2006), and *Epitrix tuberis* in potato (Chu et al. 2006), and Colorado potato beetle, *L. decemlineata,* in potato (Igrc Barcic et al. 2006). Furthermore, a recent study in Alabama reported the efficacy of Entrust® against lepidopteran pests of cole crops (Maxwell and Fadamiro 2006). The efficacy of Entrust® recorded in the present study and others listed above may be attributed to its broad spectrum activity (Cisneros et al. 2002), multiple modes of entry (Eger and Lindenberry 1998; Liu et al. 1999), and residual effect (Igrc Barcic et al. 2006). The active ingredient in Entrust® is both a contact and stomach poison (Eger and Lindenberry 1998, Liu et al. 1999). Our results, which showed that weekly sprays of Entrust® were highly effective, suggest that its residual effect in the field may be longer than one week, contrary to the report by McLeod et al. (2002) which indicated that activity of the Entrust® degraded within one week in the field. Our results, which showed that application of Entrust® in rotation/alternation with NOFLY™ (which was not effective as a stand-alone treatment) was as effective as weekly sprays of Entrust®, alone further suggest that the residual activity of Entrust® is over one week and perhaps up to two weeks or more, given that Entrust® was applied at two week intervals in the rotation treatments.

PyGanic®, the most commonly used botanical insecticide by local organic growers, was the second best treatment, but not as effective as Entrust®. The rapid knockdown effect of its

active ingredient, pyrethrum, may have contributed significantly to its efficacy against *M. ochroloma*, particularly against the larvae. The rapid colonizing behavior and destructive capacity of *M. ochroloma* is possibly an important factor limiting field efficacy of relatively slow acting formulations such as Aza-Direct® (Azadiractin), Mycotrol O® (*Beauveria bassiana* Strain GHA), Novodor® (*Btt*), NOFLY™ (*Paecilomyces fumosoroseus* Strain FE 9901), and Tick-Ex (experimental organic formulation of *Metarhizium anisopliae* Strain F52). Aza-Direct®, a slow acting botanical with antifeedent activity and effect on molting, was also shown to be ineffective against some other chrysomelid species, including flea beetles in cruciferous crops (Andersen et al. 2006) and *L. decemlineata* (Igrc Barcic et al. 2006). Similarly, the entomopathogenic fungal formulations such as Mycotrol O®, NOFLY™, and Tick-Ex were ineffective possibly because of their slow activity and unfavorable environmental conditions such as high temperatures and low humidity under the short turnip crop canopy. Interestingly, most of these formulations were effective against *M. ochroloma* in laboratory trials (unpublished data) therefore their inefficacy in the field trials may be related to unfavorable field conditions. Long et al. (2000) observed an inverse relationship between *B. bassiana* Strain GHA induced mortality of *L. decemlineata* and temperatures ranging from 15 to 30ºC. Lacey et al. (1999) reported improved control of *L. decemlineata* larvae following row (canopy) closure and suggested that this coincided with higher humidity and increased protection from sunlight. Wraight and Ramos (2002) showed that *B. bassiana* Strain GHA as a stand-alone product was ineffective against *L. decemlineata* larvae under field conditions.

In summary, this study has identified promising OMRI-acceptable biopesticides for managing *M. ochroloma* in organic crucifer vegetable production systems in the southern U.S. Entrust® was the most effective insecticide followed by PyGanic®. Furthermore, our results also

showed that some insecticides, such as PyGanic® and NOFLY™, can be applied in rotation with Entrust® for effective management of *M. ochroloma* and possibly other pests of organic crucifer production and thus limit the potential for development of resistance to Entrust®. Our data, which showed that MBI-203 (experimental formulation of *Chromobacterium subtsugae*) was effective against *M. ochroloma* larvae, are also encouraging and suggest that this formulation may be used in rotation with Entrust® or PyGanic®. A proactive approach to reduce the potential for development of resistance to Entrust® in organic vegetable production is prudent given that many pests have been reported to show resistance to its active ingredient, spinosad in conventional production systems (Sayyed et al. 2004). Rotation with other insecticides is a viable insecticide resistance management strategy since spinosad has not been reported to share cross resistance mechanisms with any other group of insecticides (Liu and Yue 2000, Wei et al. 2001). Further studies are necessary to determine the efficacy of bi-weekly sprays of Entrust® and PyGanic® and to identify other potential rotational products.

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Figure Legend

Figure 1 Mean (± SE) number of *M. ochroloma* adults (A) and damage ratings (B) of turnip plants in plots treated with different insecticide formulations during spring 2007 in Banks, Alabama. For each date, means having no letter in common are significantly different (ANOVA, Tukey Kramer HSD, $P < 0.05$). Arrows indicate treatment dates.

Figure 2 Mean (± SE) number of *M. ochroloma* adults (A), larvae (B), and damage ratings (C) of turnip plants in plots treated with different insecticide formulations during spring 2008 in Tuscaloosa, Alabama. For each date, means having no letter in common are significantly different (ANOVA, Tukey Kramer HSD, $P < 0.05$). Arrows indicate treatment dates.

Figure 3 Mean (± SE) number of *M. ochroloma* adults (A), larvae (B), and damage ratings (C) of turnip plants in plots treated with different insecticide formulations during fall 2008 in Banks, Alabama. For each date, means having no letter in common are significantly different (ANOVA, Tukey Kramer HSD, $P < 0.05$). Arrows indicate treatment dates.

Figure 4 Mean (± SE) number of *M. ochroloma* adults (A), larvae (B), and damage ratings (C) of turnip plants in plots treated with different insecticide formulations and rotations during fall 2010 in Shorter, Alabama. For each date, means having no letter in common are significantly different (ANOVA, Tukey Kramer HSD, $P < 0.05$). Arrows indicate treatment dates.

Figure 5. Turnip plants damaged by *M. ochroloma* in (A) untreated control plot and (B) Entrust®-treated plot

Figure. 1

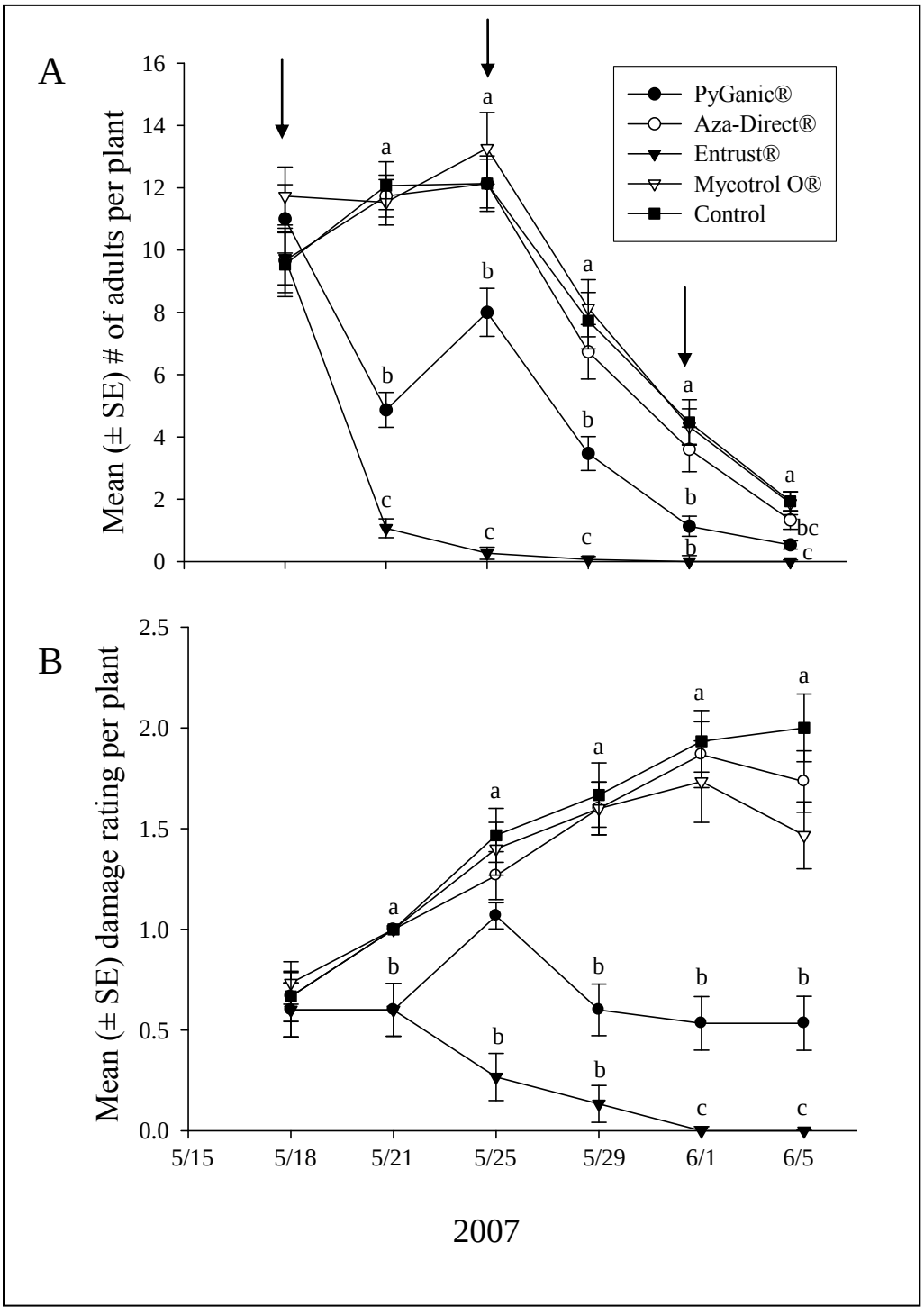
A
Mean (± SE) # of adults per plant
PyGanic®
Aza-Direct®
Entrust®
Mycotrol O®
Control
B
Mean (± SE) damage rating per plant
5/15
5/18
5/21
5/25
5/29
6/1
6/5
2007

Figure.2

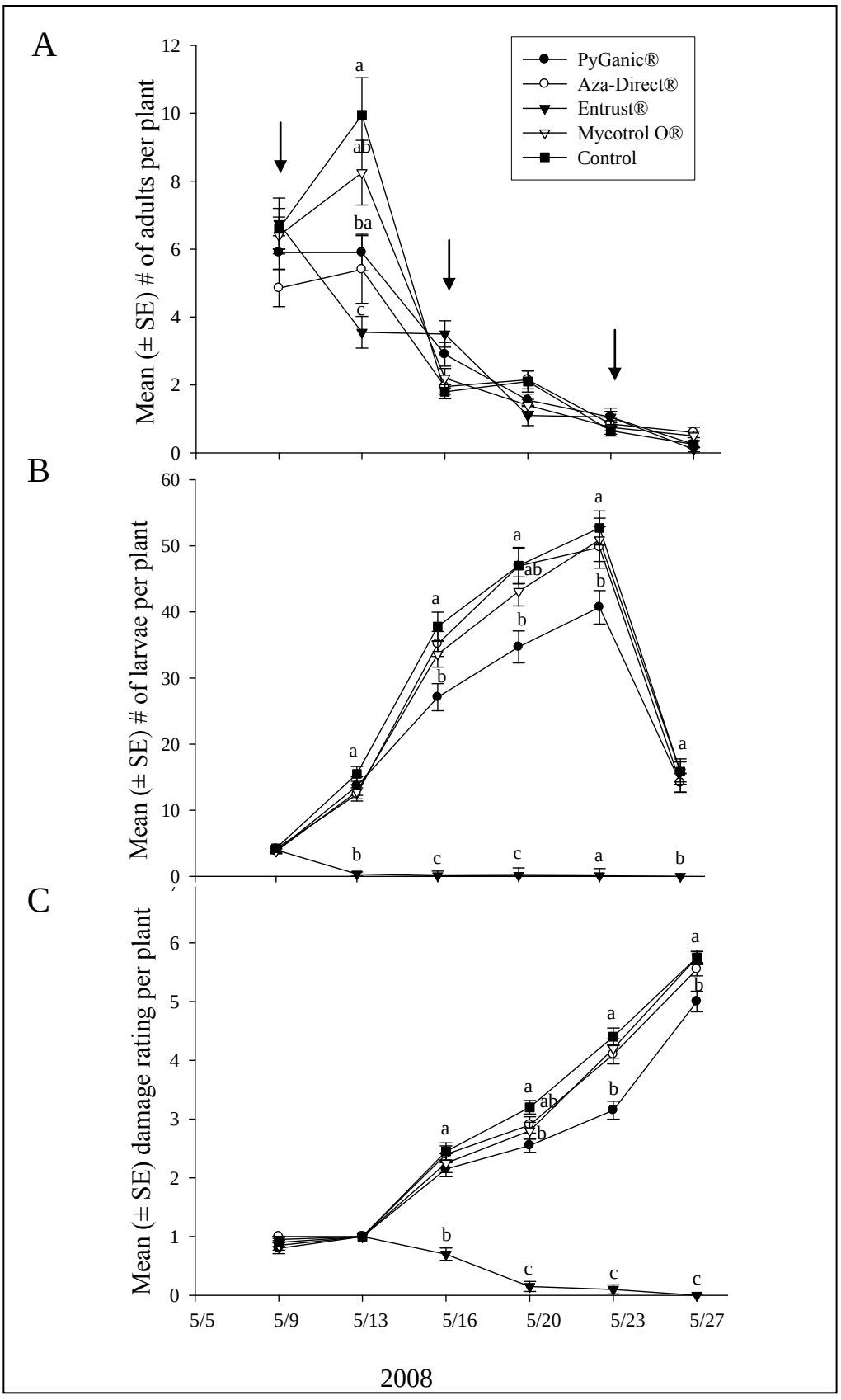
A
B
C
PyGanic®
Aza-Direct®
Entrust®
Mycotrol O®
Control
Mean (± SE) # of adults per plant
Mean (± SE) # of larvae per plant
Mean (± SE) damage rating per plant
5/5
5/9
5/13
5/16
5/20
5/23
5/27
2008

Figure 3.

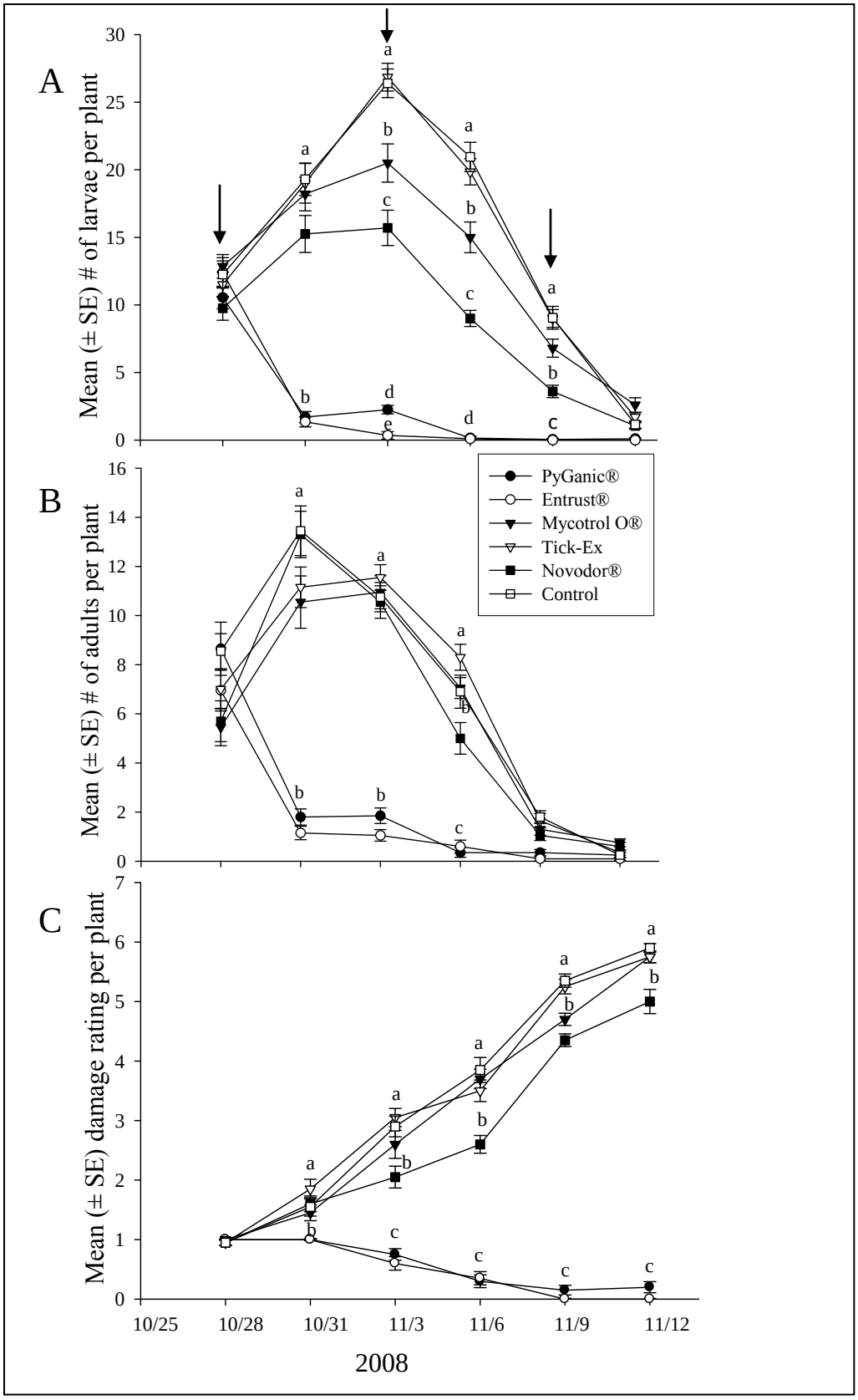
A
Mean (± SE) # of larvae per plant
B
Mean (± SE) # of adults per plant
C
Mean (± SE) damage rating per plant
PyGanic®
Entrust®
Mycotrol O®
Tick-Ex
Novodor®
Control
10/25
10/28
10/31
11/3
11/6
11/9
11/12
2008

Figure 4.

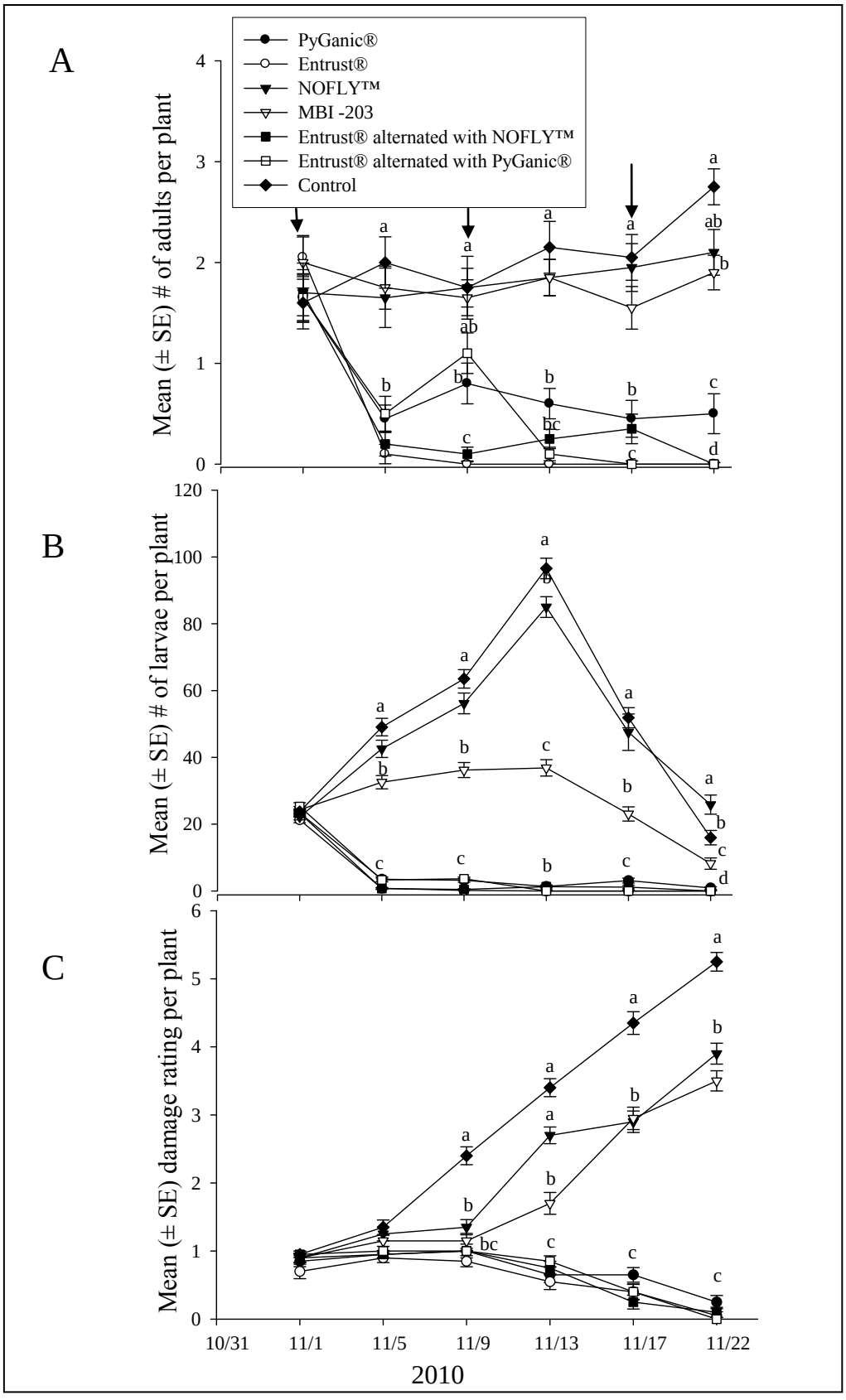
A
B
C
PyGanic®
Entrust®
NOFLY™
MBI -203
Entrust® alternated with NOFLY™
Entrust® alternated with PyGanic®
Control
Mean (± SE) # of adults per plant
Mean (± SE) # of larvae per plant
Mean (± SE) damage rating per plant
10/31
11/1
11/5
11/9
11/13
11/17
11/22
2010

Figure 5