LIRIOMYZA LEAFMINERS, ASSOCIATED PARASITOIDS AND INSECTICIDE EVALUATION IN SOUTH TEXAS

A Thesis

by

RICARDO HERNANDEZ MORENO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Tong-Xian Liu
	Marvin Harris
Committee Member,	Kevin Crosby
Head of Department,	Kevin Heinz

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ABSTRACT

Liriomyza Leafminers, Associated Parasitoid and Insecticide Evaluation in South Texas.

(May 2009)

Ricardo Hernandez Moreno, B.S., New Mexico State University

Co-Chairs of Advisory Committee: Dr. Tong-Xian Liu
Dr. Marvin Harris

In the Lower Rio Grande Valley of Texas, dipterous leafminers cause damage to pepper crop by destroying small plants (excessive mining), reduction of yield, and by vectoring plant diseases. The objectives of the present research were to identify leafminers species, which cause damage to peppers in South Texas, their associated parasitoid guilds and to evaluate the efficacy of abamectin, novaluron, spinetoram and lambda-cyhalothrin against leafminers as well as their effects on the parasitoid complex.

Field surveys were conducted on various pepper varieties in different cities of South Texas. Insecticide evaluation was carried out on field plots in Weslaco TX using the different insecticide treatments and water. To determine the insecticides' lethal effects on adult leafminer parasitoids, *Neochrysocharis formosa* and *Ganaspidium nigrimanus,* laboratory bioassays, such as topical insecticide application, pesticide intake and residual effects were performed.

The surveys suggested that the leafminers causing the most damage to pepper crops in South Texas is *Liromyza trifolii*, which represents more than 99% of the collected and identified species. Twenty parasitoid species, of four different families, were found to be attacking *L. trifolii* on pepper plants in the field. Novaluron was the most effective insecticide in controlling *L. trifolii*, followed by spinetoram and abamectin. Lambda-cyhalothrin was the least effective, showing *L. trifolii* tolerance to the compound. In field evaluation novaluron showed the lowest parasitoid: leafminer larvae ratio and parasitoid diversity index. In contrast, novaluron had the least impact on adult parasitoids in laboratory bioassays compared with other treatments (abamectin, spinetoram, lambda-cyhalothrin). The lambda-cyhalothrin showed negative effects only to *Ganaspidium nigrimanus* in topical assays, but in the residual assays it had negative effects on *G. nigrimanus* as well as *N. formosa*. On the other hand, abamectin showed negative effects on *N. formosa* and *G. nigrimanus* in the topical and intake bioassays and negative effects on *G. nigrimanus* but no-effect on *N. formosa* in the residue bioassay. Furthermore spinetoram showed negative effects on *N. formosa* and *G. nigrimanus* in all bioassays carried out in the laboratory. Leafminer species, parasitoid species composition, efficacy of insecticides, effects of insecticides on parasitoids and development of tolerance to lambda-cyhalothrin by *L. trifolii* and *N. formosa* were discussed.

DEDICATION

To God, my wife and my family

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CHAPTER I

INTRODUCTION

Integrated Pest Management (IPM) is a comprehensive technique used to reduce pests below tolerance levels using multiple pest control tactics that are effective, economically feasible and ecological compatible, and that meet the needs of agricultural growers and society (Pedigo 2002, Norris et al. 2003). IPM is a desirable technique to control agricultural pests that cause crop losses from 20 to 50 percent in important agricultural commodities around the world (Norris et al. 2003). Among agricultural pests, arthropods including insects, can cause economic, environmental, and health-safety impacts (Norris et al. 2003). One of the components of an IPM program is the use of pesticides.

Pesticides are chemicals that directly influence pests by toxic means and there are advantages and disadvantages to their use. Some of the advantages are the control of pests that are impossible to manage with any other IPM tactic, their low cost when compared to other management sources, and the rapid control of target pests, among others. However, they also have disadvantages; for example, effects on untargeted organisms, residue and drift, food contamination, human and animal toxicity, and creation of other pest problems (Norris et al. 2003). Another IPM tactic is biological control. This is defined as the use living organisms like predators, parasites, antagonists and diseases to maintain tolerance levels of pests and lessen damage (Hajek 2004).

This thesis follows the style of Journal of Economic Entomology.

It is imperative to use IPM tactics such as pesticides and biological control in order to manage pests that cause economic damage to agricultural crops. Examples of such pests are the *Liriomyza* leafminers.

THE PEST

Leafminers in the family Agromyzidae (Diptera) are composed of about 1800 species and ~75% produce mines on leaves (Bader 2006). Twenty three of ~300 species in the genus *Liriomyza* are capable of causing economic damage to agricultural and horticultural crops (Spencer 1973, Parella 1987). Six of the important economic species are polyphagous: *Liriomyza sativae* (Branchard), *L. trifolii* (Burgess), *L. huidobrensis* (Branchard), *L. bryoniae* (Kaltenbach), *L. strigata* (Meigen) and *L. longei* (Frick); they all occur world-wide (Spencer 1973, Parella and Keil 1984, van-der-Linden 1990, Lanzoni et al. 2002, Bader 2006, Liu et al. 2009). *Liriomyza* species can develop host-plant specialization, which might be explained by pre-imaginal adult experience or the presence of cryptic species (Scheffer 2000, Facknath and Wright 2006).

In the Lower Rio Grande Valley of Texas (LRGV), *L. sativae* and *L. trifolii* are the economically important species attacking vegetable crops and are targeted by as many as 10 insecticide applications per season in South Texas (Chandler 1981, 1984a, 1985b, 1987, 1988). The damage of *L. sativae* is relative to the plant's size and maturity (age). Although leafminer density is low from the seedling stage to first bloom, such damage is considered important since leaf area is also low. A significant increase in leafminer density occurs from plant bloom to harvest (Chandler 1984a). *Liriomyza trifolii* is the most abundant species on bell pepper (*Capsicum annuum*) (Chandler 1985b) and can also be found on weeds in the LRGV (Chandler 1988). This crop/weed relationship has also been found elsewhere in the world (Schuster et al. 1991, Rauf et al. 2000, Chen et al. 2002).

Liriomyza sativae and *L. trifolii* adult females injure the plant using their ovipositor by puncturing the leaf with a series of thrusts forming a fan or tubular pattern; the exudates from the wounds are fed on by the female. Tubular wounds are also used for oviposition (Dimetry 1971, Bethke and Parella 1985, Parella 1987). The eggs are oval and elongated in shape, with a cream/white color, and measure ~0.25 mm in length and ~0.10 mm in width (Dimetry 1971). Eggs are inserted in the palisade leaf tissue, and they increase in size by imbibing plant fluids (Tilden 1950, Dimetry 1971). Larvae hatch and feed in the palisade mesophyll using their mouthhooks (Dimetry 1971, Parella et al. 1985) and complete their four larval instars while mining between the upper and lower epidermis (Dimetry 1971). The larval developmental period is completed after 147.5 degree-days above 10.1 ^{o}C (Miller and Isger 1985) and larval instars may be determined either by mine or mouthhook size (Tauber and Tauber 1968, Webb and Smith 1969). The sated larva exits the leaf and pupates in the soil (Dimetry 1971, Parella 1987, Malais and Ravensberg 1992). Temperature variations affect the developmental time of the egg (Dimetry 1971), larval (Fagoonee and Toory 1984, Liebee 1984, Parella 1987), and pupal (Oatman and Michelbacher 1959, Parella 1987) stages.

Liriomyza leafminers, including *L. trifolii* and *L. sativae*, have highly mobile adults with a high reproductive potential that give rise to rapidly maturing immatures growing in protected plant tissue followed by pupation in the soil (Parella 1987); these factors contribute to their pestiferous capabilities. *Liriomyza* has also demonstrated the capacity to express resistance to insecticides (Genung 1957, Wolfenbarger 1958, Stegmaier 1966, Parella and Keil 1984, Parkman and Pienkowski 1989).

Leafminers inflict stress to plants by several means: transmitting plant diseases (Zitter and Tsai 1977); killing or slowing down the development of young seedlings by excessive mining (Elmore and Ranney 1954); reducing photosynthetic activity and hence crop yields (Wolfenbarger 1954, Ledieu and Heyler 1985, Weintraub and Horowitz 1995); and causing leaf drop from the top of developing fruit that could lead to "sunburning" of the fruit (IPM Manual Group 1985, Parella 1987); thereby reducing aesthetics of ornamental plants (Parella et al. 1985).

CONTROL TECHNIQUES

Resistance

Research has focused on different control tactics to suppress *Liriomyza.* Host monogeneic plant resistance to *Liriomyza* in melons (*Charentais*) has been researched (Dogimont et al. 1999) and genetic resistance in some chrysanthemum cultivars (*C. pacificum*) has been reported (De Jong and Van De Vrie 1987), and some antixenotic and antibiotic resistance has been found in *Apium* species (Trumble and Quiros 1988).

Cultural practices are also used to control leafminers, including, the use of clean stock (cuttings) free of leafminers; elimination of host weeds in the crop environment (Price and Harbaugh 1981), exclusion of leafminers from greenhouse growing areas by physical barrier (mesh protection) (Schuster and Harbaugh 1979), adequate fertilization eliminating excessive nitrogen that may favor leafminer development (Woltz and Kelsheimer 1958, Poe et al. 1976, Poe and Overman 1977); and the use of gravel as a substrate in the greenhouse to reduce leafminer survival (Oetting 1983).

Insecticides

The most common tactic to control *Liriomyza* densities is insecticidal control. Nicotine was among the first insecticides used to control leafminer, killing the adult by contact and larvae by osmosis through the leaf epidermis (Sanders 1912, Smulyan 1914, Mesnil and Marcel 1935, Miles and Cohen 1936). The use of nicotine for leafminer control was popular from 1900 to 1940 (Spencer 1973). After World War II, however, DDT was adopted as the main insecticide to control leafminer around the world (Hely 1947, Speyer and Parr 1948, Venugopal and Venkataramani 1954, Ali 1957) including the United States, i. e., Florida (Kelsheimer 1948, Wolfenbarger 1958), California (Wilcox and Howland 1952), Arizona (Hills and Taylor 1951), and Texas (Wene 1953, 1955).

Harding and Wolfenbarger (1963) tested several granular systemic insecticides on cucumbers and southern peas against *Liriomyza sativae* in Texas, finding successful control with Di-syston, and phorate. They based their insecticide selection on insect control and predator-parasite protection.

In recent years, avermectins and cyromazine have been the most successful insecticides to control leafminers (Trumble 1984, Hara 1986, Mujica et al. 2000) on horticultural crops (Hara 1986) and vegetables (Schuster and Everftt 1983, Trumble 1984, Civelek and Weintraub 2003). Neem-based insecticides like azadirachtin are also expanding the spectrum of compounds available to control *Liriomyza* (Weintraub and Horowitz 1997, Civelek and Weintraub 2003). In addition, some fruit extracts such as

Melia azedarach (Meliaceae) have also been investigated for their effects on the control of leafminers (Banchio et al. 2003).

Liriomyza leafminers have shown resistance to several pesticides (Hills and Taylor 1951, Wene 1955, Oatman 1959, Spencer 1973, Musgrave et al. 1976, Oatman and Kennedy 1976, Johnson et al. 1980b, Parrella 1983, Trumble and Toscano 1983, Keil and Parrella 1990, Ferguson 2004) including dichloro-diphenyl-trichloroethane (DDT) and Lindane (BHC) (Spencer 1973).

In addition, leafminers have shown resistance to carbamate, organophosphate, pyrethroids and insect growth regulators (Hills and Taylor 1951, Wene 1955, Oatman 1959, Musgrave et al. 1976, Oatman and Kennedy 1976, Johnson et al. 1980b, a, Parrella 1983, Trumble and Toscano 1983). Colonies of *L. trifolii* from Florida and California were shown to be resistant to DDT, cypermethrin, permethrin, methyl parathion and methamidophos (Keil and Parrella 1990). More recently, populations of *L. trifolii* have shown resistance to abamectin, spinosad and cyromazine (Ferguson 2004).

NATURAL ENEMIES

Liriomyza leafminers are attacked by a variety of natural enemies, including predators, entomopathogenic nematodes, entomopathogens and parasitoids (Liu et al. 2009). Parasitoid guilds are the most common category of natural enemies of leafminers and many species have been reported around the world. Liu et al. (2009) compiled a list of the species of parasitoids reported around the world including 23 species in the Nearctic region, 14 from Florida, 72 from South America, 28 from Japan, 14 in China, 11 in Indonesia, eight species in Malaysia, 18 species in Vietnam and several from Europe and Turkey. These species belong to the Braconidae, Cynipidae, Pteromalidae, Figitidae and Eulophidae families of Hymenoptera. Some of these natural enemies are used to manage leafminers and have kept pest densities under control in several countries including Senegal (Neuenschwander et al. 1987), Indonesia (Rauf et al. 2000), Iran (Talebi et al. 2005), Malaysia (Sivapragasam et al. 1999), Vietnam (Tran et al. 2005b, Tran et al. 2006), Japan (Yano 2004), China (Xu et al. 1999, Wen et al. 2002), and the U.S.A. (Zehnder and Trumble 1984). Biological control using parasitoids is mainly used in protected horticultural systems (Chow and Heinz 2005, van-der-Linden 2005), and vegetable production systems (Trumble 1990) and to a lesser extent in field crops (Reitz et al. 1999).

Conservation biological control

Conservation biological control is the modification of existing farming practices, in order to protect natural enemies and reduce the effect of the pest (Barbosa, 1998), and has been used for the control of leafminers. The most important strategy is the use of pesticides that exclude or have minimal impact on leafminer parasitoids (Liu et al. 2009). For example in Mexico, a reduction in leafminer density was shown to be associated with using IPM strategies in comparison with a conventional pesticide program (Trumble and Alvarado-Rodriguez 1993). Similarly in Califonia, natural enemies kept the leafminer density below threshold level in tomatoes and celery fields (Trumble and Alvarado-Rodriguez 1993, Trumble et al. 1997, Reitz et al. 1999).

Classical biological control, which is the practice of introducing non-indigenous parasitoids for the control of leafminers has had some success under field conditions (Liu et al. 2009). In Hawaii, *G. utilis* Baerdsley, *N. diastatae* and *C. oscinidis* were released for the control of *L. trifolli* and *L. sativae* on watermelon, celery, tomato, pumpkin, bean, and Irish potato with great success (Johnson et al. 1983, Johnson and Hara 1987, Johnson 1993, Liu et al. 2009).

Augmentation biological control, which relies on routine rearing and periodic release of mass reared parasitoids (Yano 2004), has been used principally under protected agriculture. Two approaches are used, innundative augmentation and inoculative augmentation biological control.

Inundative augmentation

The application of high densities of parasitoids to achieve a high initial control (Yano 2004) has been used in greenhouse high value crops (Liu et al. 2009). *Diglyphus isaea* is commercially available for the control of *Liriomyza*, and has been reported to be effective against *L. trifolii* (Cabitza et al. 1993, Ulubilir and Sekeroglu 1997, Ozawa et al. 1999) and *L. bryoniae* (Boot et al. 1992, Ushchekov 1994, Sampson and Walker 1998) in several crops including tomatoes and nursery plants. Another example is *Diglyphus begini,* which is not commercially available, however research has shown its effectiveness; for example in marigolds *L. trifolii* was controlled with time-releases of *D. begini* reducing leafminer densities to near zero (Heinz et al. 1988, Heinz and Parrella 1990). Similar success was achieved with greenhouse-grown chrysanthemum (Parrella et al. 1992).

Dacnusa sibirica in combination with *Diglyphus isaea* is also used in inundative biological control. *Liriomyza trifolii* has been controlled by releasing these two parasitoids in greenhouses producing tomatoes and cucumbers (Ozawa et al. 1993, Matsumura et al. 2001, Abd-Rabou 2006); the combination of these parasitoids is available commercially.

Hemiptarsenus varicornis has also been effective in controlling *L. trifolii* by inundative conditions in greenhouse cherry tomatoes (Ozawa et al. 2004). *Neochrysocharis formosa* is also effective in controlling *L. trifolii* on egg plant (Shimomoto 2005, Hondo et al. 2006), and *Opius pallipes* improved the control of *L. bryoniae* (Van Schelt and Altena 1997).

Inoculative biological control

In this method, the release of small numbers of parasitoids anticipates they will reproduce and their offspring will continue with the control of the pest for a longer period of time (Van Driesche and Bellows 1996), and is also used against *Liriomyza* leafminers. In Europe, *L. bryoniae* was suppressed with inoculative releases of *Diglyphus isaea* (Boot et al. 1992), and in Japan *L. trifolli* was controlled with inoculative releases of *D. isaea* and *D. sibirica* (Ozawa et al. 2001). *Opius dissitus* was also established in Senegal for the control of *L. trifolii* (Neuenschwander et al. 1987).

Effect of insecticides on natural enemies

Natural enemies are in close association with leafminers in the field, and any chemical treatment used against *Liriomyza* will directly or indirectly affect its associated natural enemies. The effects of different insecticides used against leafminers and natural enemies have been reported throughout time. In the literature, Darvas and Polgar (1998) alleged that insecticides that disrupt the parasitoid food chain by killing leafminers would always have a negative effect on their natural enemies. A field trial showed that methomyl could encourage pest growth through the disturbance of the parasitoid guilds, methomyl could encourage pest development through the disturbance of parasitoid guilds, and that methamidophos was safer than methomyl to the natural enemies (Trumble and Toscano 1983). In contrast to methomyl and methamidophos, abamectin showed higher densities of the leafminer parasitoid *Diglyphus isaea* on treated potatoes compared to cyromazine (Weintraub 2001). Prijono et al. (2004), however, have shown that abamectin was not the safest insecticide against *Hemiptarsenus varicornis* Gerault, *Opius* sp., *Gronotoma micromorpha* Perkins, *Hemiptarsenus varicornis* and *D. isaea*. Similarly, Kaspi and Parrella (2005) studied the compatibility of the widely used abamectin insecticide with the commercially available parasitoid *D. isaea*. Topical applications greatly affected parasitoid survival. Abamectin residue on plants also negatively affected *D. isaea* survival up to 5 days after application. Parasitism of treated leafminer larvae was lethal for the natural enemy. However, application of insecticide after the leafminer was parasitized and parasitoid larvae started feeding, did not affect *D. isaea* emergence and longevity.

The susceptibility of the insecticides imidacloprid, pymetrozine and lufenuron to the leafminer parasitoid *Neochrysacharis formosa* (Westwood) were investigated using glass vials coated with different insecticide solutions (Tran et al. 2005a). The sublethal effects on longevity showed that imidacloprid and pymetrozine reduced longevity and that lufenuron did not have an effect on longevity of the parasitoid.

Hidrayani et al. (2005) studied the effects of profenofos, carbosulfan, and abamectin. They observed that profenofos, and carbosulfan reduced parasitism by *Hemiptarsenus varicornis, Opius chromatomyiae* and *C. humilis*, and abamectin did not reduce parasitism.

Bjorksten and Robinson (2005) tested larval and pupal mortality and sublethal effects of abamectin, cyromazine and mancozeb on the two important parasitoids of Australian *Hemiptarsenus varicornis* (Girault) and *Diglyphus isaea* (Walker). Abamectin caused mortality to the larvae and pupae of the two species; cyromazine and mancozeb did not. In addition, cyromazine and mancozeb did not cause a reduction in longevity and progeny production compared to the control, and abamectin had a significantly higher mortality in the first 3 days and females dying within these 3 days did not produce any progeny. Females surviving these three days produced progeny similar to the control. They concluded that cyromazine and mancozeb were compatible in an IPM program in Australia, and that abamectin should be used with caution.

The effects of neem (azadirachtin), abamectin and spinosad to *Neochrysocharis formosa* and *Opius chromatomyiae* were summarized by Hossain and Poehling (2006). Neem-Azal-U is used for soil applications, and it caused low mortality on *O. chromatomyiae* when the parasitized *L. sativae* pupae came in contact with the insecticide; the longevity of the emerged parasitoids was unaffected. The foliar formulations of azadirachtin, spinosad and abamectin were all highly toxic to *O. chromatomyiae*. The application of spinosad and abamectin to parasitized leafminer larvae by *N. formosa* had strong negative effects on its emergence. However, NeemAzal-T/S (azadirachtin) had no detrimental effects on the parasitoid observed.

PROJECT FOCUS

Vegetable production in South Texas covers approximately 26,300 hectares (Johnson 1997). This region is heterogeneous in its farming techniques, crops, and insect pests. Although the use of natural enemies as a sole technique for controlling dipterous leafminers is the ideal model, the complex interactions of other pests and economic thresholds may require the incorporation of insecticides in the pest control program. Insecticides that conserve natural enemies may be integrated into an IPM program to successfully control *Liriomyza*.

Liriomyza species composition and parasitoid guilds were determined by sampling different pepper growing areas in the Lower Rio Grande Valley (LRGV). In addition, current commercially used insecticides like abamectin, novaluron, spinetoram and lambda-cyhalothrin were tested to record their effects on the parasitoid complex attacking *Liriomyza* and leafminer species.

The overall objective of this research was to develop methods and strategies to manage *Liriomyza* utilizing both insecticides and biological control agents. Pepper, one of the most important cash crops and hosts of the leafminers, was used as the research crop. This model system for the management of leafminers could also be applied to other crops.

The research addressed the following objectives:

- To determine the composition of leafminer species attacking vegetables;
- To identify the native, larval, larval-pupal and pupal parasitoid complexes attacking the leafminer species;

- To determine which currently used insecticides are effective in controlling leafminers;
- To summarize the effects of different insecticides on the parasitoid complexes and make suggestions for leafminer management.

Field and laboratory experiments were conducted in order to achieve the objectives. Pepper-growing areas in South Texas were sampled for leafminer and parasitoid species composition. Experimental plots were established on the research farm, Texas AgriLife Research at Weslaco, where insecticides were applied and their efficacy in controlling *Liriomyza* and effects on the parasitoids were determined. Laboratory research used two colonies of native parasitoids that were established and insecticide bioassays were used to investigate the insecticide effects on the natural enemies.

CHAPTER II

LIRIOMYZA AND PARASITOID SPECIES COMPOSITION

INTRODUCTION

Leafminers of the family Agromizidae (Diptera) are composed of about 1800 species and 75% of them produce mines in leaves (Bader 2006). Twenty three species in the genus *Liriomyza* (~ 300 species) are capable of inflicting economic damage on agricultural and horticultural crops (Spencer 1973, Parella 1987). Six of the economically important species are polyphagous: *L. sativae* (Branchard), *L. trifolii* (Burgess), *L. huidobrensis* (Branchard), *L. bryoniae* (Kaltenbach), *L. strigata* (Meigen) and *L. longei* (Frick) (Spencer 1973, 1981, Morgan et al. 2000b, van-der-Linden 2005, Liu et al. 2009). *Liriomyza* species are considered a secondary pest but the reduction in natural enemies due to indiscriminate use of insecticides has lead the insect to develop as a major pest of several crops (Oatman and Kennedy 1976, Trumble and Toscano 1983, Schuster and Wharton 1993). *Liriomyza* leafminers damage plants in several different ways; however the aggressive feeding-tunneling behavior causes the death of young seedlings, reduction of photosynthesis and yield loss (Elmore and Ranney 1954, Wolfenbarger 1954, Ledieu and Heyler 1985, Weintraub and Horowitz 1995). In the LRGV, peppers (*Capsicum annum*) are an important crop which are severely attacked by *Liriomyza* species which has triggered several insecticide applications to control the pests (Chandler 1981, 1984a). In LRGV, *L. sativae* (Blanchard) and *L. trifolli* (Burguess) were reported to damage vegetable crops ~20 years ago (Chandler 1984a, 1985a, 1987, 1988).

Interactions among different *Liriomyza* species could cause displacements among them. For example, *L. huidobrensis* is capable of displacing *L. trifolii* from different hosts and therefore *L. trifolii* could not get established onto a particular host plant (Reitz and Trumble 2002). In addition, the displacement of *L. sativae* by *L. trifolii* has also been documented on gypsophila (Price and Stanley 1982) and tomato (Schuster and Everett 1982) in Florida, and celery in California (Zehnder and Trumble 1984).

Liriomyza leafminers are attacked by hymenopterous parasitoids of different families including Braconidae, Figitidae, Pteromalidae, and Eulophidae and they are considered an imperative factor for their control (Liu et al. 2009). Several parasitoids were recorded in South Texas attacking *L. sativae* and *L. trifolli* including *Chrysonotomyia* spp., *Chrysocharis ainsliei* (Crawford), *Closterocerus cinctipennies* (Ashmead), *Diglyphus intermedius* (Girault), and *Zagromosoma americanum* (Girault), all from the family Eulophidae. Unidentified *Opius* spp. from the family Braconidae; *Cothonaspis* spp. from the family Eucolidae; and *Halticoptera circulus* (Walker) from the family Pteromalidae (Chandler 1982). In another study *Chrysocharis* spp., and *Disorygma* spp. were also reported (Chandler 1985b). All these species were collected from the same pepper growing area in South Texas (Weslaco) from cantaloupe and bell pepper varieties.

The objective of this chapter was to identify and update the species composition of the leafminers present in the LRGV attacking peppers. In addition, parasitoids associated with the pest were also collected and identified as components of the parasitoid complex in South Texas.

MATERIALS AND METHODS

Study sites and crop

Chili peppers (*Capsicum annuum*) were selected as the research crop for the collection of leafminer species and parasitoid guilds in the LRGV for several reasons. Throughout the LRGV, the pepper fields in South Texas are distributed on irrigated farmland. Pepper varieties planted in the LRGV differ among fields and they are grown in both the spring and fall, making year-round research on them feasible.

During the Fall of 2007, five sites were established for the subsequent survey of parasitoid guilds and leafminers on pepper plants. Study site locations were distributed throughout the LRGV in Edinburg, San Juan, Weslaco, La Feria, and Brownsville. In the spring of 2008, the study sites were located in Weslaco, Pharr, and La Feria cities of Texas (Table 1). Locations differed in pepper varieties grown, size of farm, planting dates and farming practices.

Table 1. Study site descriptions Fall 2007 and Spring 2008

City	**County**	**Season**	**Location**	**Variety**	**Area**
Edinburg, TX	Hidalgo	Fall 2007	26^{o} 21’58.70” N, 98^{o} 12’02.79” W	Cuban Hots	5 hectares
San Juan, TX	Hidalgo	Fall 2007	26^{o} 08’50.2” N, 98^{o} 08’45.12” W	Serrano pepper	8 hectares
Weslaco, TX	Hidalgo	Fall 2007	26^{o} 09’33.01” N, 97^{o} 57’32.67” W	Jalapeno M	1 hectare
La Feria, TX	Cameron	Fall 2007	26^{o} 07’44.20” N, 97^{o} 50’37.73” W	Tam Veracruz	11.3 hectares
Brownsville, TX	Cameron	Fall 2007	25^{o} 58’50.47” N, 97^{o} 36’25.58” W	Tam Veracruz	24 hectares
Weslaco, TX	Hidalgo	Spring 2008	26^{o} 09’33.01” N, 97^{o} 57’32.67” W	Jalapeno M	1 hectares
La Feria, TX	Cameron	Spring 2008	26^{o} 12’17.38” N, 97^{o} 48’38.49” W	Magnum 45 Cayenne 408	0.5 hectares
Pharr, TX	Hidalgo	Spring 2008	26^{o} 14’11.55” N, 98^{o} 11’41.04” W	Tormenta	3 hectares

Infested foliage collection

Mined leaves with larvae were collected and inspected. Sampling began when the crop was in the fifth true leaf and continued bi-weekly until the crop was harvested. Sampling consisted of collecting leaves from different areas within the field; they were inspected for leafminer larvae development. Leaves containing visible leafminer larvae were collected; groups of 10 infested leaves were placed in one-gallon plastic zipper storage bags for a total of fifty infested leaves in five different bags per field. The bags were placed in an icebox during the field collection process. The material was transported to the laboratory where the total number of mines and larvae were recorded. Consequently, leaves were arranged on a piece of paper towel avoiding contact among them, and the paper towel was placed back inside the zipper plastic bag with a cup in the middle to keep the top part of the bag from touching the leaves.

The bags were closed with the zipper, and cotton balls were used to allow air diffusion. The bags were held in an insectary at 28^{o}C, at a photoperiod of 11:13 (L:D) hours; the bags were checked for adults emergence every ten days (30 days total) and leafminer adults, pupae, larvae, and parasitoids were collected. Leafminers were identified to species; parasitoids of the leafminers were identified to genus. After specimen separation, the specimens were sent to the appropriate experts for further identification.

Tray pupae collection

In addition to larval collection, pupal collection was also performed in order to collect potential parasitoid species that attack pupae directly in the soil. Pupae were collected from the same study sites as those utilized for larvae collection (Table 1). Foam trays of 26 x 4.6 x 5.1 cm (Genpak 10k tray) were used for the pupal collection. The trays were modified from the Johnson et al. (1980b) tray collection technique. Each tray contained a rectangular slit at the bottom covered with mesh to allow for water drainage in case of rain and irrigation. A layer of beach sand ~2.5 cm in thickness was placed in each tray to mimic field soil conditions. A total of 20 trays per location were placed under plant canopy and on top of the row to avoid damage by farming practices. The trays were fixed to the soil by a 12 cm nail, flagged and maintained for two weeks.

After this period, trays were collected / replaced and transported to the laboratory where pupae were extracted using a 650 µm mesh siever. Pupae were held in aerated plastic cups until adult emergence. Adults emerging from pupae were identified and preserved. Species diversity and abundance was recorded throughout the season.

Specimen identification

Five taxonomy experts assisted with the specimen identifications:

Dr. Robert Wharton, Entomology Department, Texas A&M University, College Station TX. (Braconidae).

Dr. Chao-Dong Zhu, Institute of Zoology Chinese Academy of Sciences, Beijing, China (Eulophidae).

Dr. Matthew Buffington. Systematic Entomology Lab, USDA/ARS NMNH, Smithsonian Institution, Washington DC (Figitidae).

Dr. Steve Heydon R.M. Bohart Museum, Department of Entomology, University of California, Davis (Pteromalidae).

Dr. Zhongren Lei, Institute of Plant Protection, Chinese Academy of Agricultural Science Beijing, China (*Liriomyza* spp).

RESULTS

Leafminer species composition

One predominant leafminer species was collected in the LRGV. In Fall 2007, *L. trifolii* accounted for 93.9% (1076) of the individuals collected, *L. sativae* accounted for 0.3% (3) and 5.9% (67) of the specimens collected were unidentifiable due to their poor condition (Table 2). In Spring 2008, the leafminer infestation was significantly lower; *Liriomyza trifolii* was the only species collected with 85.8% (247), and 14.2% (35) were unidentified due to the lack of morphological characters (Table 3).

Table 2. *Liriomyza* species Fall 2007

Sampling method	Leafminer Family	Genus	Species	No. of specimens	% of total
Infested foliage sampling	Agromyzidae	*Liriomyza*	*L. trifolii*	811	70.8
			L. sativae	3	0.3
Tray pupae sampling	Agromyzidae	*Liriomyza*	*L. trifolii*	265	23.1
			L. sativae	0	0.0

Table 3. Liriomyza species Spring 2008

Sampling method	Leafminer Family	Genus	Species	Number of specimens	Percent of total
Infested foliage sampling	Agromyzidae	*Liriomyza*	*L. trifolii*	242	84.0
			L. sativae	0	0.0
Tray pupae sampling	Agromyzidae	*Liriomyza*	*L. trifolii*	5	1.7
			L. sativae	0	0.0

Parasitoid guild composition

In the two seasons, parasitoid species from four different families were collected: Eulophidae, Braconidae, Figitidae, and Pteromalidae. In the family Eulophidae eight different species were found; seven from the family Braconidae; four from Figitidae, and one from Pteromalidae (Tables 4 and 5). The species composition from Fall 2007 and Spring 2008 is similar with minimal variability.

Fall 2007 and Spring 2008: Eulophidae species were the same except for *Cirrospulus* spp and *Asecodes* spp that were only found in the Fall (Table 4). In the family Braconidae, *Opius dissitus, O. dimidiatus, O*. nr *browsvillensis* and *Opis* spp. *2* were found in both seasons (Table 4, 5). In addition, an unidentified *Opius* species (*Opius* spp *1*) and *O. thoracosema* were found only in Fall 2007 (Table 4), and *O. bruneipes* was found only in Spring 2008 (Table 5). In the family Figitidae, all four species were found in both seasons (Table 4, Table 5). *Halticoptera* nr. *circulus* spp (Pteromalidae) was found in both Fall 2007 (Table 4) and Spring 2008 (Table 5). Samples of specimens were placed in the Texas A&M University insect museum, voucher number 673.

Table 4. Parasitoid species Fall 2007

Sampling method	Parasitoid Family	Genus	Species	No. of specimens	% of total
Infested Foliage Sampling	Eulophidae	*Neochrysocharis* Kurdjumov	*N. formosa*	574	60.8
		Closterocerus Westwood	*C. cinctipennis*	73	7.7
		Diglyphus Walker	*D. isaea*	20	2.1
		Cirrospilus variegatus group	*Cirrospilus* spp.	46	4.9
		Asecodes Förster	*Asecodes* spp.	4	0.4
		Pnigalio Schoranx	*Pnigalio* spp.	2	0.2
		Zogrammosoma Ashmead	*Zogrammosoma* spp.	1	0.1
		Chrysocharis Förster	*Chrysocharis* spp.	1	0.1
	Braconidae	*Opius* Wesmael	*O. dissitus*	37	3.9
			O. dimidiatus	3	0.3
			O. thoracosema	2	0.2
			Opius spp *1*	1	0.1
			O. nr *browsvillensis*	7	0.8
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	55	5.8
			G. nigrimanus	61	6.5
		Disorygma Foerster	*D. pacifica*	5	0.5
		Agrostocynips	*A. robusta*	4	0.4
	Pteromalidae	*Halticoptera* nr. *circulus* Walker	*Halticoptera nr. circulus* spp.	28	3.0
Tray Pupae Sampling	Braconidae	*Opius* Wesmael	*O. dissitus*	25	2.6
			Opius spp *2*	2	0.2
			O. nr *browsvillensis*	1	0.1
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	10	1.1
			G. nigrimanus	6	0.6
		Disorygma Foerster	*D. pacifica*	1	0.1
		Agrostocynips	*A. robusta*	2	0.2
	Pteromalidae	*Halticoptera* nr. circulus Walker	*Halticoptera nr. circulus* spp.	9	1.0

Table 5. Parasitoid species Spring 2008

Sampling method	Parasitoid Family	Genus	Species	No. of specimens	% of total
Infested Foliage Sampling	Eulophidae	*Neochrysocharis* Kurdjumov	*N. formosa*	538	60.3
		Closterocerus Westwood	*C. cinctipennis*	30	3.4
		Diglyphus Walker	*D. isaea*	45	5.0
		Pnigalio schoranx	*Pnigalio* spp.	15	1.7
		Zogrammosoma Ashmead	*Zogrammosoma spp.*	5	0.6
		Chrysocharis Förster	*Chrysocharis* spp.	11	1.2
	Braconidae	*Opius* Wesmael	*O. dissitus*	87	9.8
			O. dimidiatus	9	1.0
			Opius spp 2	5	0.6
			O. bruneipes	22	2.5
			O. nr *browsvillensis*	12	1.3
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	49	5.5
			G. nigrimanus	19	2.1
		Disorygma Foerster	*D. pacifica*	10	1.1
		Agrostocynips	*A. robusta*	1	0.1
Tray Pupae Sampling	Braconidae	*Opius* Wesmael	*O. dissitus*	25	2.8
			O. bruneipes	4	0.4
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	4	0.4
	Pteromalidae	*Halticoptera* nr. circulus Walker	*Halticoptera* nr. *circulus* spp.	1	0.1

DISCUSSION

Liriomyza *species composition*

Liriomyza trifolii is the predominant leafminer attacking peppers in the LRGV. *L. trifolii* and *L. sativae* were reported present in the LRGV (Chandler 1982, 1984b, 1985b, 1987) in the 1980's and *Liriomyza sativae* was found in high numbers attacking pepper (Chandler 1984b). Our results indicate a displacement of *L. sativae* from pepper by *L. trifolii* has occurred in the LRGV. This phenomenon had been reported before (Price and Stanley 1982, Schuster and Everett 1982, Zehnder and Trumble 1984, Reitz and Trumble 2002). The potential cause for leafminer species displacement is host preferences and host specialization (Morgan et al. 2000a, Reitz and Trumble 2002). Thus, *Liriomyza trifolii* may be better adapted to pepper species than *L. sativae* and other *L. trifolii* populations. Zhao and Kang (2003) reported that among olfactory responses to host plants by *L. sativae*, bell pepper was classified among those with the smallest response. In another study *Capsicum annuum* ovipositional deterrent compounds deterred *L. trifolii* females from laying eggs on peppers (Kashiwagi et al. 2005). In California, two geographically separated species of *L. trifolii* were tested for host preferences: the central California species more successfully reproduced on bell pepper than other crops, and the southern *L. trifolii* did not successfully reproduce on bell pepper (Reitz and Trumble 2002). Polymerase Chain Reaction (PCR) was used to study speciation between the two *L. trifolii* populations in California, however the lack of *Liriomyza* infestation on the same host prevented obtaining a clear answer (Morgan et al. 2000a). The *L. trifolii* attacking peppers in the LRGV may be a specialist on this host or may consist of a cryptic species.

Parasitoid species composition

Other parasitoid surveys in the LRGV (Weslaco TX) were performed in 1979 (Chandler 1982), 1981, 1982, and 1984 (Chandler 1985b) on cantaloupe and bell pepper.

Cantaloupe samples yielded eight different species of parasitoid from *L. trifolii* and *L. sativae*: *Chrysonotomyia* spp., *Chrysocharis ainsliei* (Crawford), *Closterocerus cinctipennies* (Ashmead), *Diglyphus intermedius* (Girault), and *Zagromosoma americanum* (Girault) (Family: Eulophidae). Unidentified *Opius* spp. (Family: Braconidae); *Cothonaspis* spp. (Family: Figitidae); and *Halticoptera circulus* (Walker) (Family Pteromalidae) (Chandler 1982). In bell pepper, attacking *L. trifolii,* six parasitoid species were collected: *Chrysocharis* spp., *Chrysonotomyia* spp., *Zagromosoma americanum* (Girault) (Eulophidae); *Opius* spp. (Family: Braconidae); and *Disorygma* spp. (Family: Figitidae) (Chandler 1985b).

In the present study two of the species identified in previous samples were found again (*Closterocerus cinctipennies* (Ashmead), *Halticoptera circulus* (Walker). In addition, 15 new records of species in South Texas attacking *L. trifolii* were made, including: *D. isaea* (65), *Cirrospilus* spp. (46), *Asecodes* spp. (4), *Pnigalio* spp. (17) from Eulophidae family. *Opius dissitus* (174), *O. dimidiatus* (12), *Opius* spp *1* (1), *Opius* spp *2* (7), *O. bruneipes* (26), *O.* nr *browsvillensis* (20), *O. thoracosma* (2) from Braconidae family. *Ganaspidium pusillae* (118), *G. nigrimanus* (86), *D. pacifica* (16), and *A. robusta* (6) from the Figitidae family.

The most abundant parasitoid in both seasons was *N. formosa* (Eulophidae), this parasitoid accounted for ~60 % of the total collected (Tables 4 and 5). *Neochrysocharis formosa* is an endoparasitoid of *Liriomyza* leafminers reported from many parts of the world (Harding 1965, Ozawa et al. 2002, Noyes 2004). *Neochrysocharis formosa* is also reported to be a successful biological control agent (Shimomoto 2005, Hondo et al. 2006) and it has also been reported to be a hyperparasitoid of *D. isaea* (Ozawa et al. 2002). The specimens collected from this species showed a high variability in morphological characters that all key out to *N. formosa*.

Opius dissitus was the most abundant parasitoid of the family Braconidae with 9.5% of the collected species (Tables 4 and 5). This parasitoid is a larval-pupal endoparasitoid that attacks several species of leafminers. Research shows it has potential for biological control of leafminer pests (Neuenschwander et al. 1987).

In the family Figitidae the most abundant species are *G. nigrimanus* 4.7% and *G. pusillae* 6.4%. *Ganaspidium nigrimanus* was more abundant in Fall 2007 and *G. pusillae* was more abundant in Spring 2008 (Tables 4 and 5). *G. nigrimanus*, which is currently a synonym of *G. utilis* (Buffington 2004) has been used successfully in biological control programs (Johnson et al. 1983, Johnson and Hara 1987, Liu et al. 2009).

To conclude, *Liriomyza trifolii* is the leafminer responsible for economic damage to pepper crops in the LRGV. It has displaced *L. sativae* from this host. The molecular comparison of this particular *L. trifolii* species with another population of *L. trifolii* in the country may answer the question of potential development of biotypes or cryptic species. An extensive survey in other agricultural and weed hosts is needed, in order to obtain more complete data on the *Liriomyza* species found in the LRGV.

A total of 20 parasitoid species were recorded, including new records for the LRGV. High diversity of parasitoid species was found attacking *L. trifolii* pepper crops in South Texas compared to other surveys in the United States. For example, in Florida 20 parasitoid species attacking *L. trifolii* and *L. sativae* were found on tomato (Schuster et al. 1991, Schuster and Wharton 1993) and eight different weed species and in Weslaco, Texas, nine different parasitoid species reared from *L. trifolii* and *L. sativae* attacking peppers and cantaloupe (Chandler 1982, 1984a, 1985b).

The number of *Liriomyza* specimens in Spring 2008 (288) was significantly lower than Fall 2007 (1146); however the same ratio difference was not recorded in parasitoid specimens. Parasitoids collected in Fall 2007 were 944 in comparison with 892 in Spring 2008. A higher amount of parasitoid to leafminer ratio was found in Spring 2008. This may account for the reduction of the leafminer population in Spring 2008 compared to Fall 2007.

Tray sampling method was originally established to monitor any potential parasitoid species attacking leafminers directly in the soil. Parasitoid species collected using this method, were not different from parasitoid species collected with infested foliage sampling. The presence of parasitoids specializing in pupae in the soil is not ruled out by this work, but direct evidence of this occurring was not obtained.

Neochrysocharis formosa was present as the predominant parasitoid species collected in the survey (60%). The morphological characters of these specimens showed a high variation however they all fit into a *N. formosa* description. Molecular studies for these specimens will reveal and clarify the existence of potential new *Neochrysocharis* species.

A detailed survey of other agricultural crops and weeds will potentially increase the parasitoid species recorded in South Texas. A high diversity of parasitoid species contributes to the proper balance between pest and natural enemies, the conservation of biodiversity is essential to keep outbreaks from occurring (Altieri 1999, Landis et al. 2000, Cai et al. 2007). Careful studies should be performed on the effects of current

Liriomyza management techniques in the LRGV, such as insecticide effects on natural enemies, and habitat conservation. In order to maintain and foster parasitoid biodiversity that contributes to leafminer pest management.

CHAPTER III

FIELD EVALUATION OF INSECTICIDES ON *LIRIOMYZA TRIFOLII* AND ITS NATURAL ENEMIES ON PEPPERS

INTRODUCTION

Liriomyza trifolii leafminer can inflict damage to plants at two stages of its life cycle, adult and larval stage. The adult female can injure plant tissue using its ovipositor, which could be used for feeding and egg oviposition (Dimetry 1971, Bethke and Parella 1985, Parella 1987). The eggs are inserted into the leaf tissue and after hatching the larvae start tunneling primarily on the palisade mesophyll and complete the feeding period of four larval instars in the leaf (Dimetry 1971, Parella et al. 1985). The tunneling causes damage to the plant by reducing the plant's photosynthesis capacity; abscission of leaves (Parella 1987); death of young seedlings (Elmore and Ranney 1954); reduced aesthetics (Parella et al. 1985); transmission of diseases (Zitter and Tsai 1977); and decline of crop yields (Wolfenbarger 1954, Ledieu and Heyler 1985, Weintraub and Horowitz 1995).

Liriomyza trifolli has an extensive host range, including the families of Umbelliferae, Solanaceae, Malvaceae, Liliaceae, Leguminosae, Curcurbitaceae, Compositae, and Chenopodiaceae (Stegmaier 1966). Similarly in South Texas *Liriomyza* leafminers attack different agricultural crops, including celery, tomato, melon, cucumber, watermelon, cotton, and in particular, pepper. The only method for the control of *Liriomyza* leafminers in the Lower Rio Grande Valley (LRGV) is insecticidal application. Several formulations and mode of action insecticides are use to control *L. trifolli*, including pyrethroids, avermectins, cyromazine, permethrin, methamidophos, spinosyn and neem-based insecticides (azadirachtin) (Schuster and Everett 1983, Trumble 1984, Hara 1986, Keil and Parrella 1990, Weintraub and Horowitz 1997, Mujica et al. 2000, Civelek and Weintraub 2003, Ferguson 2004). Several insecticide applications each season are applied to pepper in the LRGV to control *Liriomyza* (Chandler 1981, 1984a).

Extensive use of insecticides has resulted in development of *Liriomyza* resistance to most of the formulations such as pyrethroids, permethrin, methamidophos (Keil and Parrella 1990), avermectins, cyromazine, and spinosyn (Keil and Parrella 1990, Ferguson 2004). In addition, insecticide applications have negatively affected survival and fitness of *L. trifolii* natural enemies (Trumble and Toscano 1983, Darvas and Polgar 1998, Weintraub 2001, Prijono et al. 2004, Bjorksten and Robinson 2005, Hidrayani et al. 2005, Kaspi and Parrella 2005, Tran et al. 2005a, Hossain and Poehling 2006).

Some of the commonly used insectides on the LRGV are novaluron, spinetoram, abamectin and lambda-cyhalothrin.

Novaluron (1-[chloro-4-(1,1,2-trifluoro-methoxyethoxy) phenyl]-3-(2,6-difluorobenzoyl) which is a benzoylphenyl urea that inhibits chitin development (Ishaaya and Casida 1974), causes unsuccessful endocuticular deposition and disruptive molting (Mulder and Gijswijk 1973). It acts primarily by ingestion and contact, and it also has translaminar activity (Ishaaya et al. 2003).

Spinetoram (mixture of spinosyn A and spinosyn D) is a nicotinic acetylcholine receptor agonist (mimic). This insecticide belongs to the family of spinosyns and it causes disruption of the central nervous system by hyperexcitation (Sparks et al. 2001).

Abamectin (avermectin B1a and avermectin B1b) belongs to the avermectin insecticides, and targets the nervous system as a chloride channel activator. Avermectins bind to GABA on chloride channels blocking stimulation of the nervous system resulting in the insect's death (Brown 2005).

Lambda-cyhalothrin ([1a(*S**),3a(Z)]-(±)-cyano-(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2- dimethylcyclopropanecarboxylate) is a pyrethroid insecticide which acts on the nervous system as a sodium channel modulator. This axonic poison prevents sodium channels from closing, causing the insect to over-excite and subsequently paralyze (Narahashi 1971).

The objective of this research is to evaluate the different insecticides commonly used for the control of *Liriomyza trifolii*. Their efficacy on controlling field leafminer populations and their effects on their natural enemies are of particular importance.

MATERIALS AND METHODS

Plot design

Research field plots were established at the Research Farm, Texas AgriLife Center at Weslaco, TX (26° 09'33.01" N, 97° 57'32.67" W) in the Fall 2007 and Spring 2008 growing seasons. Plots were in a complete randomized block design and the size of each plot was 9.14 x 20.42 m for a total of 15 plots in Fall 2007 (three replications) and 20 plots in the Spring 2008 (four replications). Two rows of sorghum were planted as windbreak between the plots to avoid insecticide drift between the treatments. The pepper variety used was Jalapeño M (Seminis Vegetable Seeds, Oxnard, CA) and the seeds were planted inside a greenhouse with a management program similar to the one used by farmers. After the plants reached the fourth true leaf stage they were transplanted in a single row to the plots at spacing of 12 cm. The plots were drip-irrigated and fertilized and no chemical insecticide was used except for the experimental treatments.

Treatments

In Fall 2007 and Spring 2008, four insecticides (abamectin, novaluron, spinetoram and lambda-cyhalothrin) were applied to peppers as treatments; the treatments were replicated three times on a total of 15 plots in Fall 2007 and four times for a total of 20 plots in the Spring 2008. Plots were treated with insecticides using specific company recommended rates (Table 2).

Table 6. Treatments, general mode action and application rates

Treatment	**General Mode of Action**	**Rate Used**
Abamectin 0.15 EC	Chloride channel activator	4.25 g ai/ac
Lambda-Cyhalothrin	Sodium channel modulator	9.07 g ai/ac
Novaluron 0.83 EC	Chitin synthesis inhibitor (CSI)	23.53 g ai/ac
Spinetoram	Nicotinic acetylcholine receptor agonist (mimic)	30 g ai/ac
Untreated Control	None	Water

In order to avoid insecticide drift, wind was monitored before application with a hand wind speed sensor and no application was made if the wind speed was greater than 4.5 kph. In addition, the plots were surrounded by two rows of sorghum. The chemical sprayer was calibrated to deliver 76 liters per acre (GPA), with 13 nozzles separated at 51 cm (TeeJet 8002VS) delivering 372 ml/30 sec/nozzle. Applications were made according to leafminer infestation pressure. In Fall 2007, two applications were made, one on 26 October 2007, and a second one on 25 November 2007. In Spring 2008 the leafminer infestation was less severe requiring only one application on 8 April 2008.

Data collection

In order to describe insecticide efficacy on controlling leafminers and its effects on parasitoids, two methods were used for sampling the plots. The first one (a) helped to decide which insecticide was better at controlling leafminers. The leafminer density per treatment throughout time was calculated. In addition, insecticide efficacy compared to untreated control was also calculated. The second sampling method (b) helped to compute the number of parasitoids per leafminer larvae and compared the parasitoid species diversity from the different treatments in order to make conclusions of the effects of treatments to natural enemies.

Random sampling method (a)

In order to monitor the efficacy of each insecticide, 30 randomly selected leaves from the bottom (10), median (10), and top (10) portion of the plant were collected per plot bi-weekly for a total of 450 leaves per collection. The samples were taken on each crop leaving the margins un-sampled to avoid any "edge effect"; the sampling started 2 m from the borders of the plots. This space was in addition to the row of sorghum separating the plots (1 m). The leaves were examined and quantified for the total amount of occupied mines, empty mines and larvae. The leaves were placed in 1 gal zipper bags in groups of 10. The bags were labeled according to treatment and transported to the laboratory, and processed for emergence of specimens. In addition, total number of leaves from 20 randomly selected plants was counted bi-weekly to monitor plant phenology.

Leaves were arranged on a piece of paper towel avoiding contact among them. The paper towel was placed back inside the zipper plastic bag with a cup in the middle to prevent the top part of the bag from touching the leaves. The bags were sealed with the zipper, and cotton balls were used to allow air diffusion. The bags were held in an insectary at 28° C, at a photoperiod of 11:13 (L:D) hours. Bags were checked for adult emergence every 10 days (30 days total) and leafminer and parasitoids were collected. Leafminers and parasitoids were identified and separated by species.

Infested foliage sampling (b)

Infested foliage was sampled to monitor the number of parasitoids per leafminer larvae. Parasitoids per leafminer larvae (mean number of parasitoids/mean number of leafminer larvae) were calculated in order to monitor the effects of insecticides to the parasitoid larvae. This ratio was calculated instead of percent parasitism in order to avoid mis-representation by gregarious species.

Furthermore, the leafminer species attacking pepper, parasitoid species composition, and the biodiversity among treatments was also recorded.

Ten mined-leaves with larvae were collected from each plot at 2-week intervals. The leaves were placed in the plastic zipper bags and processed as mentioned above for adult emergence and identification. The number of larvae, mines, emerged leafminers, and emerged parasitoids were recorded. Specimens were sent to different experts for identification (see chapter II).

DATA ANALYSIS

Random sampling

The calculation of leafminer density was made using leafminers collected and plant number of leaves. Analysis of variance (ANOVA) using GLM procedure (alpha 0.1) was used to compare leafminer density among treatments on each sampling date. Total efficacy was calculated using Abbott formula (density of control – density of treatment / density of control) and analysis of variance (ANOVA) (GLM procedure alpha 0.1) was used to compare insecticide season efficacy per treatment from Fall 2007 and Spring 2008 samples (SAS institute 2000).

Infested foliage sampling

The number of parasitoids per leafminer larva (ratio) was calculated to determine the effects of insecticides to larval parasitoid species (mean number of parasitoids/mean number of leafminer larvae). Analysis of variance (ANOVA) (GLM procedure alpha 0.1) was used to compare parasitoid per leafminer larvae among different treatments each sampled date. Furthermore, Shannon-Wiener diversity index (*H'*) was calculated per treatment to analyze the effects of different insecticides on parasitoid diversity. Analysis of variance (ANOVA) (GLM procedure alpha 0.1) was used to compare *H'* among treatments (SAS institute 2000).

RESULTS

Insecticide evaluation for leafminer management

In Fall 2007, novaluron showed lower leafminer larvae density per plant than control seven and 17 days from first application. After a second application a similar trend followed on 30 November 2007 and on 13 December 2007 novaluron showed the lowest larvae per plant among treatments. Novaluron was the only insecticide showing significant lower density than control 25 and 35 days from second application (Figure 1) (Table 7). In Spring 2008, novaluron reduced leafminer density after the first application (8 April 2008). Novaluron showed numerical differences from the control 22 days from application, but not statistical differences. No statistical difference was shown on subsequent samplings (Figure 2) (Table 8).

In Fall 2007, spinetoram showed lower leafminer larvae density per plant than the control seven days from first application. Similarly, spinetoram showed lower larvae per plant than control, abamectin and lambda-cyhalothrin after the second application on 30 November 2007 and 13 December 2007. Furthermore, larvae density in the spinetoram treated plants increased from the previous sample from 13 December 2007 to 20 December 2007 ($F = 14.16$; $df = 7, 3$; $P < 0.0001$). Spinetoram showed density reduction following application; however density increased on the following samples compared to novaluron (Figure 1) (Table 7). In Spring 2008 season, spinetoram reduced leafminer density compared to control after the first application on 8 April 2008. No statistically significant differences were shown on subsequent samplings (Figure 2) (Table 8).

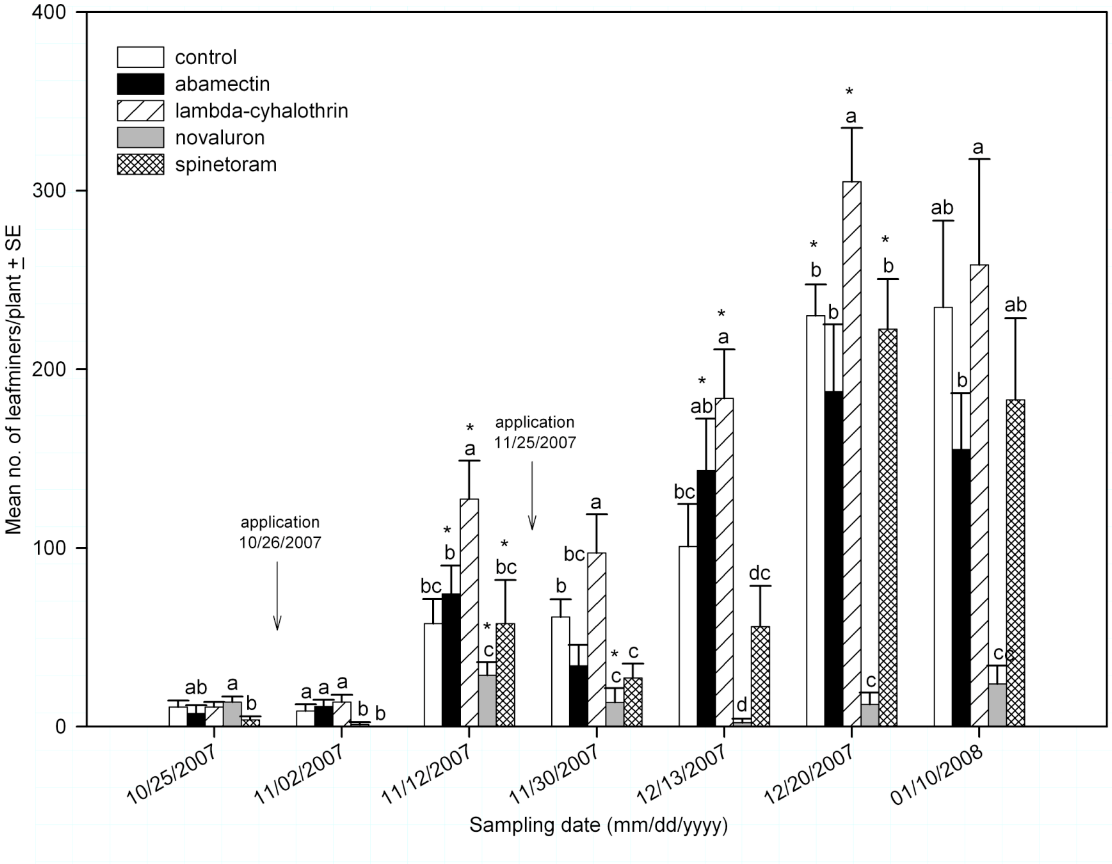

Figure 1. Leafminer larvae density per plant Fall 2007. Arrows represent application date. Different letters represent statistical differences among treatments. Star (*) represents statistical differences from previous sample date within treatments .

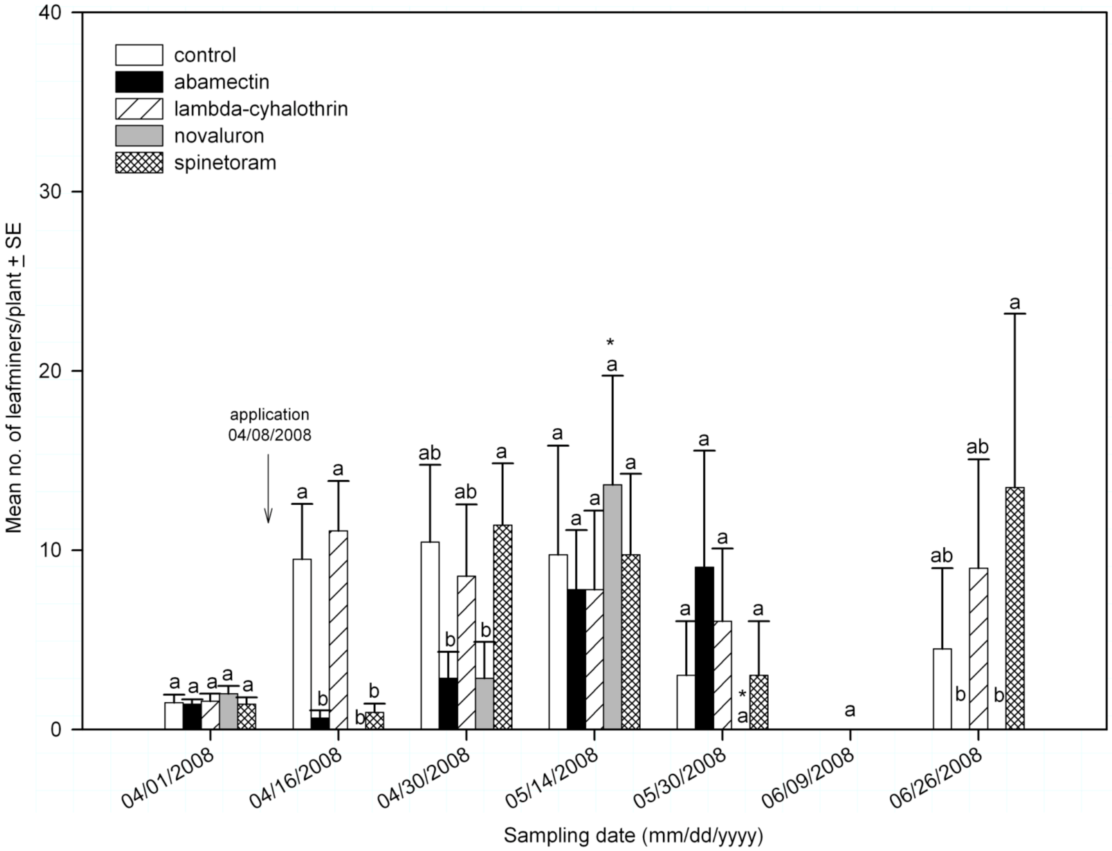

Figure 2. Leafminer larvae density per plant Spring 2008. Arrows represent application date. Different letters represent statistical differences among treatments. Star (*) represents statistical differences from previous sample date within treatments.

Table 7. Leafminer density statistics Fall 2007

Date	F-value	Pr<F	df
10/25/07	1.37	0.2621	4
11/02/07	4.07	0.0074	4
11/12/07	4.25	0.0059	4
11/30/07	6.61	0.0004	4
12/13/07	9.5	0.0001	4
12/20/07	17.02	0.0001	4
01/10/08	4.68	0.0034	4

Table 8. Leafminer density statistics Spring 2008

Date	F-value	Pr<F	df
04/04/08	0.39	0.8145	4
04/16/08	8.21	0.0001	4
04/30/08	1.61	0.1859	4
05/14/08	0.23	0.9212	4
05/30/08	0.77	0.5501	4
06/09/08	.	.	4
06/26/08	1.14	0.3473	4

Abamectin did not show statistical differences from control the entire Fall 2007 sampling season. In addition an increase of leafminer density was recorded with abamectin (*F* = 12.17; *df* = 7, 3; *P* < 0.0001) from sample day 30 November 2007 to sample day 13 December 2007 (Figure 1) (Table 7). In spring 2008, after the first application on 8 April 2008 abamectin reduced leafminer density compared to the control. Abamectin showed numerical differences from the control 22 days from application, but not statistical differences. No statistically significant differences were found on subsequent samplings (Figure 2) (Table 8).

In Fall 2007, lambda-cyhalothrin did not show leafminer reduction compared to the control seven days from first application. Lambda-cyhalothrin showed a significantly higher density than all treatments including control 17 days after first application. Lambda-cyhalothrin was statistically higher than control after a second application on 30 November 2007 and 13 December 2007. In addition an increase of leafminer density was recorded in the lambda-cyhalothrin treatment from sample day 30 November 2007 to sample day 13 December 2007 (*F* = 17.56; *df* = 7, 3; *P* < 0.0001). On 20 December 2007, lambda-cyhalothrin showed a higher density than untreated control and in 10 January 2008 no difference from the control. (Figure 1) (Table 7). In Spring 2008, lambda-cyhalothrin did not have an effect on leafminer density compared to the control (Figure 2) (Table 8).

In addition to leafminer density, total insecticide efficacy was calculated in Fall 2007 and Spring 2008 using the Abbott formula (density of control – density of treatment / density of control). In Fall 2007, total insecticide efficacy showed that the most effective insecticide was novaluron followed by spinetoram. Abamectin did not show any effect on leafminers compared to the control. Lambda-cyhalothrin increased leafminer density with a potential hormoligosis effect (*F* = 19.11; *df* = 3, 3; *P* < 0.0001) (Figure 3). In Spring 2008, novaluron was the treatment with the highest efficacy, and was significantly higher than lambda-cyhalothrin (*F* = 1.38; *df* = 3, 3; *P* < 0.2790) (Figure 4).

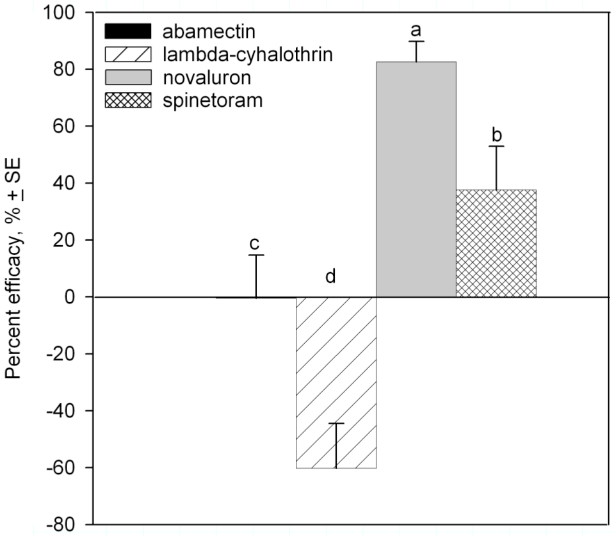

Figure 3. Percent efficacy compared to control on Fall 2007.
Different letters represent statistical differences among treatments.

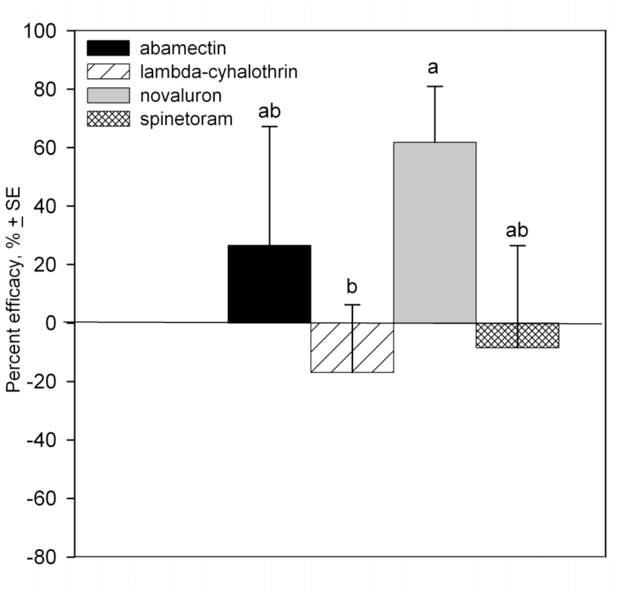

Figure 4. Percent efficacy compared to control on Spring 2008.
Different letters represent statistical differences among treatments.

Notes on insecticide effects to natural enemies

In Fall 2007, novaluron and spinetoram showed statistical significant lower parasitoids per leafminer larvae than the control 16 days after the first application (12 November 2007). Abamectin and lambda-cyhalothrin did not show differences from the control (F = 6.38; df = 4, 3; $P < 0.0081$) (Figure 5). Novaluron showed lower parasitoid per leafminer larvae after the second application on 13 December 2007 (F = 3.64; df = 4, 3; $P < 0.0442$), 20 December 2007 (F = 4.48; df = 4, 3; $P < 0.0247$), and 10 January 2008 (F = 1.20; df = 4, 3; $P < 0.3697$).

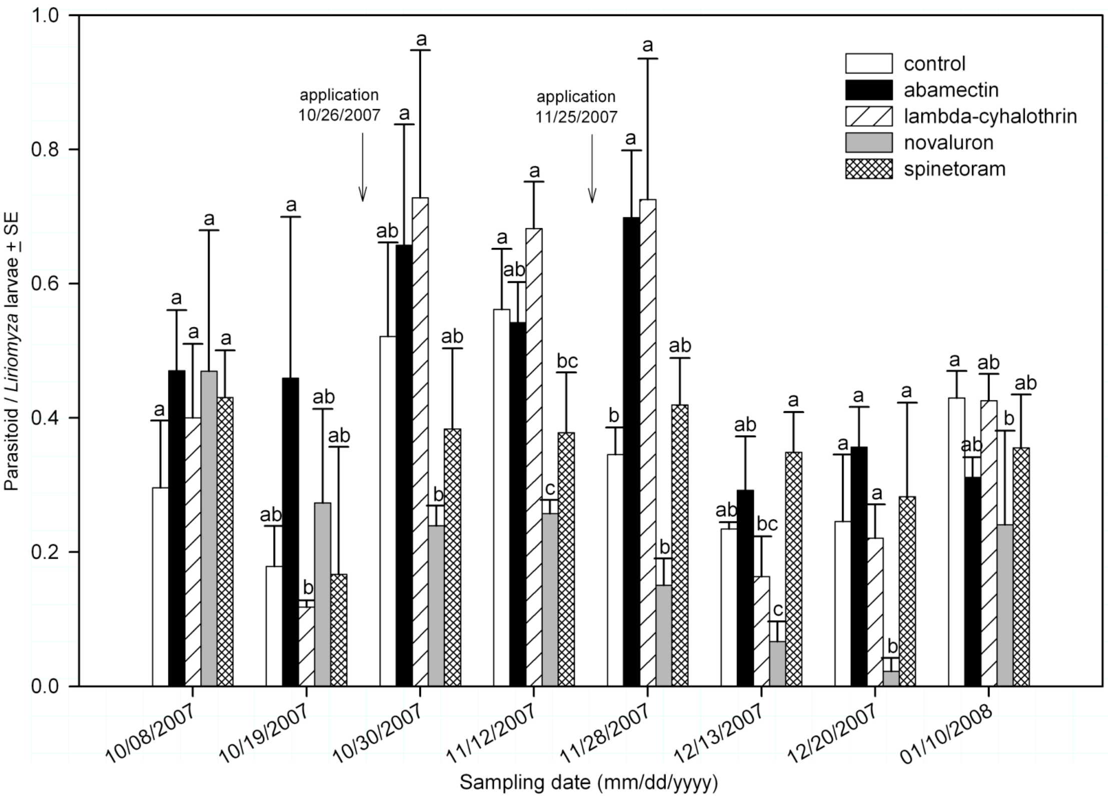

Figure 5. Ratio of parasitoids per leafminer larvae Fall 2007. Arrows represent application date. Different letters represent statistical differences among treatments.

In Spring 2008, novaluron showed a lower parasitoid leafminer ratio 8 days after the first application (16 April 2008) (*F* = 2.65; *df* = 4, 3; *P* < 0.0747) (Figure 6). Twelve days after application (30 April 2008) spinetoram and novaluron showed lower parasitoids per leafminer larvae (*F* = 1.60; *df* = 4, 3; *P* < 0.2245).

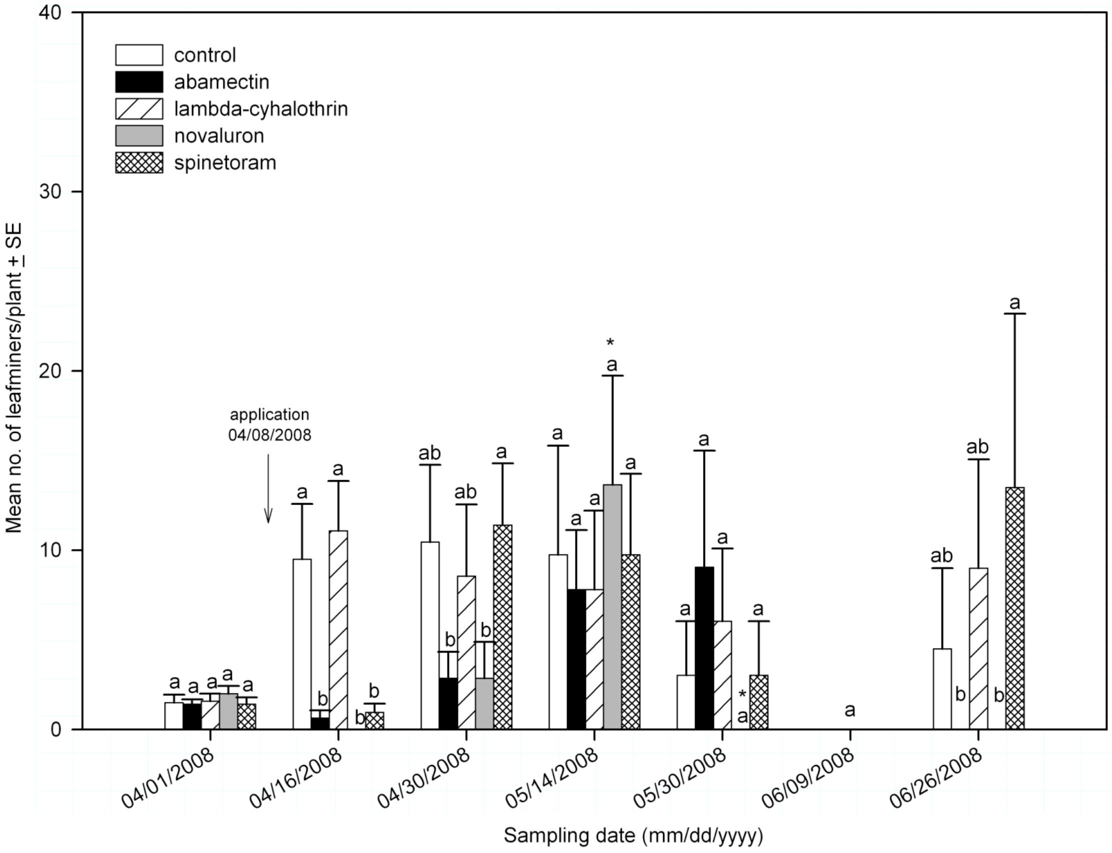

Figure 6. Ratio of parasitoids per leafminer larvae Spring 2008. Arrows represent application date. Different letters represent statistical differences among treatments.

Natural enemy diversity is an important factor for conservation biological control (Altieri 1999, Landis et al. 2000, Cai et al. 2007).

In Fall 2007, the control had the highest Shannon-Wiener diversity index (*H'*) (Table 9). In addition, spinetoram and novaluron were the only two insecticides with statistically lower indecies compared to the control. Novaluron had the lowest diversity index in Fall 2007 ($F = 5.37$; $df = 4, 3$; $P < 0.0029$).

Table 9. Shannon-Wiener diversity index Fall 2007

Treatment	Mean H'	StdErr
Control	0.6161 (a)	0.02
Abamectin	0.5607 (ab)	0.06
Lambda -cyhalothrin	0.4369 (ab)	0.07
Spineto ram	0.3774 (b)	0.13
Novaluron	0.1516 (c)	0.07

F= 5.37, df = 4, P< 0.0029

In Spring 2008, the *H'* in the untreated control was numerically higher than other treatments (Table 10). However no significant difference was found among treatments ($F = 0.80$; $df = 4, 3$; $P < 0.5349$).

Table 10. Shannon-Wiener diversity index Spring 2008

Treatment	Mean H'	StdErr
Control	0.5596 (a)	0.09
Abamectin	0.5375 (a)	0.09
Lambda -cyhalothrin	0.4673 (a)	0.06
Spineto ram	0.4165 (a)	0.05
Novaluron	0.4122 (a)	0.08

F= 0.80, df = 4, P< 0.5349

Liriomyza *and parasitoid species composition*

The *Liriomyza* species composition in the Fall 2007 was found to be 454 specimens of *L. trifolii*, one *L. sativae,* while 33 specimens were unidentifiable due to the lack of morphological characters. In spring 2008, 144 specimens belonged to *L. trifolii* and 23 specimens were unidentifiable. *Liriomyza sativae* was not found in Spring 2008.

Several parasitoid species were found in the Jalapeño M insecticidal plots. In Fall 2007, 13 species were found: *Neochrysocharis formosa* (278), *Closterocerus cinctipennis* (41), *Diglyphus isaea* (12), *Cirrospilus* spp. (30), and *Asecodes* spp. (3) from the Eulophidae family. *Opius dissitus* (21), *O. dimidiatus* (3), *O. browsvillensis* (7), and *Opius* spp *1* (1), from Braconidae family. *Ganaspidium pusillae* (4), *G. nigrimanus* (39), and *Agrostocynips robusta* (3), from the Figitidae family and *Halticoptera circulus* (20) from Pteromalidae. The total number of specimens collected in this season was 438 (Table 11).

In Spring 2008, 15 species were found: *Neochrysocharis formosa* (351), *Closterocerus cinctipennis* (28), *Diglyphus isaea* (32), *Pnigalio spp.* (13), *Zogrammosoma* spp. (2), *Chrysocharis* spp (10), from the Eulophidae family. *Opius dissitus* (59*), O. dimidiatus* (9), *O. browsvillensis* (8), *O. bruneipes* (15), and *Opius* spp *2* (4), from the Braconidae family. *Ganaspidium pusillae* (19), *G. nigrimanus* (17), *Agrostocynips robusta* (1), and *Disorygma pacifica* (6), from the family Figitidae. The total number of specimens collected in Spring 2008 was 620 (Table 12).

Table 11. Insecticide evaluation parasitoid species composition Fall 2007

Sampling method	Parasitoid Family	Genus	Species	No. of specimens	% of total
Infested Foliage Sampling	Eulophidae	*Neochrysocharis* Kurdjumov	*N. formosa*	278	63.5
		Closterocerus Westwood	*C. cinctipennis*	41	9.4
		Diglyphus Walker	*D. isaea*	12	2.7
		Cirrospilus variegatus group	*Cirrospilus* spp.	30	6.8
		Asecodes Förster	*Asecodes* spp.	3	0.7
	Braconidae	*Opius* Wesmael	*O. dissitus*	21	4.8.
			O. dimidiatus	3	0.7
			Opius spp *1*	1	0.2
			O. nr *browsvillensis*	7	1.6
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	4	0.9
			G. nigrimanus	39	8.9
		Agrostocynips	*A. robusta*	3	0.7
	Pteromalidae	*Halticoptera* nr. circulus Walker	*Halticoptera nr. circulus* spp.	20	4.6

Table 12. Insecticide evaluation parasitoid species composition Spring 2008

Sampling method	Parasitoid Family	Genus	Species	No. of specimens	% of total
Infested Foliage Sampling	Eulophidae	*Neochrysocharis* Kurdjumov	*N. formosa*	351	56.6
		Closterocerus Westwood	*C. cinctipennis*	28	4.5
		Diglyphus Walker	*D. isaea*	32	5.2
		Cirrospilus variegatus group	*Cirrospilus* spp.	46	7.5
		Pnigalio Schoranx	*Pnigalio* spp.	13	2.1
		Zogrammosoma Ashmead	*Zogrammosoma* spp.	2	0.3
		Chrysocharis Förster	*Chrysocharis* spp.	10	1.6
	Braconidae	*Opius* Wesmael	*O. dissitus*	59	9.5
			O. dimidiatus	9	1.5
			O. bruneipes	15	2.4
			Opius spp *2*	4	0.6
			O. nr *browsvillensis*	8	1.3
	Figitidae	*Ganaspidium* Weld	*G. pusillae*	19	3.1
			G. nigrimanus	17	2.7
		Disorygma Foerster	*D. pacifica*	6	1.0
		Agrostocynips	*A. robusta*	1	0.2

DISCUSSION

The main difference between the two season trials was the leafminer pressure, reaching 300 leafminers per plant in Fall 2007 in contrast to ~ 15 leafminers per plant in Spring 2008. Both season results agreed that novaluron was effective against leafminers, while lambda-cyhalothrin increased leafminer density or had no effect. Spinetoram was also efficacious against leafminers, while efficacy of abamectin varied in both seasons.

Novaluron was the insecticide showing lower leafminer density as well as the highest efficacy in Fall 2007 and Spring 2008. This compound provided control to high densities of leafminers throughout Fall 2007 and in Spring 2008 after application. Resistance to it has not been found so far.

Some resistance of *Liriomyza* species to spinosad has been documented. (Ferguson 2004), however no resistance was detected in this field evaluation. Spinetoram reduced *Liriomyza* field populations after applications in Fall 2007 and Spring 2008. However its control persistence appears to be lower than novaluron. In Fall 2007 spinetoram leafminer density increased from 13 December 2007 to 20 December 2007 (Figure 1) and in Spring 2008, leafminer density in the treatment of spinetoram was higher on 30 April 2008 compared to novaluron on 30 April 2008 (Figure 2). This lower control persistence may be influenced by the effects of spinetoram on the natural enemies, or lower plant residue for the control of emerging leafminers.

In Fall 2007, overall efficacy of abamectin was nil, showing zero efficacy compared to control. In spring 2008 when leafminer density was lower, abamectin reduced leafminer density after application and appeared efficacious. *Liriomyza* resistance to abamectin has been documented (Ferguson 2004). Abamectin is a widely used insecticide in South Texas primarily for the control of mites and on a lesser extend for the control of *Liriomyza* leafminers. More careful studies need to be done in order to make conclusions of abamectin resistance and efficacy in the LRGV.

Lambda-cyhalothrin, as with other pyrethroids, has a documented resistance in several agricultural pests (Rodriguez et al. 2001, Ahmad et al. 2002).

A developed insecticide tolerance by *L. trifolii*, in addition to its negative effects on natural enemies (lethal, sublethal, repellant), may be the reason for the increase in *Liriomyza* density compared to the control in Fall 2007 (Figure 1) and no reduction on density in Spring 2008 (Figure 2) as well as its low efficacy in both seasons (Figure 3, Figure 4). Studies on the resistance to lambda-cyhalothrin by *L. trifolii* from South Texas will help to explain the increase of *Liriomyza* density in the Lower Rio Grande Valley. Lambda-cyhalothrin results are consistent in both seasons. This insecticide did not cause reduction of the leafminer population and did have a positive effect on the population by increasing the numbers of leafminers. Leafminers in the LRGV are tolerant to lambda-cyhalothrin.

A rotation of insecticides such as novaluron, spinetoram and abamectin could help in the management of the pest. Lambda-cyhalothrin should be avoided for the control of *Liriomyza*.

Notes on insecticide effects on natural enemies

Based on the field evaluation data, novaluron had negative effects on immature parasitoid stages. Novaluron toxicity may directly or indirectly affect parasitoid species because it was effective against the *Liriomyza* leafminers. No research has been done on this insecticide's effects on natural enemies. Spinetoram also negatively affect parasitoid immature stages. Previous research has shown that this insecticide is harmful to natural enemies (Hossain and Poehling 2006).

Lambda-cyhalothrin showed no effect on parasitoid species in this study, the tolerance of *Liriomyza* leafminers to this compound may affect the parasitoid susceptibility. In the parasitoid survey *N. formosa* was the most abundant parasitoid. This parasitoid is a larval parasitoid and has a close association with leafminer larvae biology at the larval stage. This association could potentially have resulted in parasitoid tolerance to the compound. *N. formosa* represented 60% of the total collected parasitoid species and a developed tolerance of *N. formosa* to lambda-cyhalothrin could explain the lack of a difference on parasitoid abundance and diversity between control and lambda-cyhalothrin in the research plots.

Species composition

Liriomyza trifolii was the species that caused damage to Jalapeño M in the insecticide plots. It accounted for 99 percent of the identified specimens.

In this evaluation, 19 different parasitoid species were collected from the families of Eulophidae, Braconidae, Figitidae and Pteromalidae. The number of leafminers collected in Fall 2007 was 488 in contrast with 167 in Spring 2008. The number of parasitoids collected in Fall 2007 was 438, in contrast with 620 in Spring 2008. The number of parasitoids per leafminer ratio was much higher in Spring 2008 than Fall 2007. The higher abundance of parasitoids in Spring 2008, potentially contributed to the lower amount of leafminers in the experimental plots during this season.

Using the results of this study, a diversity of hymenopterous parasitoids of *Liriomyza* is present in the LRGV and it appears they were able to control *Liriomyza* infestations, I will recommend to avoid the application of any insecticide and allow this natural enemies to establish and control the pest. If insecticide application is required to control *L. trifolii* outbreaks, one time application of novaluron should be used. If infestation requires more applications, a sustainable insecticide program should be planed to avoid resistance development. Lambda-cyhalothrin should be avoided for the management of *L. trifolii* due to the tolerance of this pest to the compound.

CHAPTER IV

INSECTICIDAL EFFECTS ON TWO ADULT PARASITOIDS OF *LIRIOMYZA TRIFOLII*: *GANASPIDIUM NIGRIMANUS* (FIGITIDAE) AND *NEOCHRYSOCHARIS FORMOSA* (EULOPHIDAE)

INTRODUCTION

Liriomyza trifolii causes damage to plants by its adult and larval feeding behavior. Adult females pierce the leaf cuticle for feeding and oviposition. Hatched larvae feed by making tunnels into the leaf mesophyll and complete the larval stages (Dimetry 1971, Parella et al. 1985). At the last larval instars, the larvae exit the mine and usually pupate in the soil (Dimetry 1971, Parella 1987, Malais and Ravensberg 1992). Leafminers such as *L. trifolii* damage plants by excessive mining and feeding wounds. This behavior causes a reduction of plant photosynthesis and yield (Wolfenbarger 1954, Ledieu and Heyler 1985, Weintraub and Horowitz 1995), killing of small seedlings (Elmore and Ranney 1954), transmitting of plant diseases (Zitter and Tsai 1977), reducing quality of ornamental crops and creating quarantine issues (Parella et al. 1985).

One of the methods to manage the pest is by chemical control. Several compounds are being used for the management of *Liriomyza*, such as abamectin, spinetoram, lambda-cyhalothrin and novaluron.

Abamectin (avermectin B1a and avermectin B1b) belongs to avermectin insecticides and it targets the nervous system as a chloride channel activator (Brown 2005). This insecticide is commonly used to control sucking pests such as spider mites, broad mites, thrips, chewing insects such as *Plutella xylostella*, and *Liriomyza* leafminers.

Spinetoram (a mixture of spinosyn A and spinosyn D) is a nicotinic acetylcholine receptor agonist (mimic) causing disruption of the central nervous system by hyperexcitation (Sparks et al. 2001). It has been effectively used to control armyworms, thrips, leafminers, and Colorado potato beetle among others.

Lambda-cyhalothrin belongs to synthetic pyrethroids group. It acts on the nervous system as a sodium channel modulator by preventing sodium channels from closing causing the insect to over-excite and paralyze (Narahashi 1971) and is labeled to control many insect species including leafminers, aphids, armyworms, grasshoppers, mites, thrips, and whiteflies among others.

Novaluron is a benzoylphenyl urea and inhibits chitin formation (Ishaaya and Casida 1974) causing suppression of endocuticular deposition and molting (Mulder and Gijswijk 1973). This insecticide is labeled to control leafrollers, loopers, Colorado potato beetle, armyworms, whiteflies, cucumber beetles, and leafminers, among others.

Biological control is a naturally occurring practice in most agricultural systems and can be enhanced by manipulation of environment and farming techniques (conservation biological control) (Barbosa 1998). It can also be highly specialized as classical biological control and augmentative biological control (Yano 2004). In addition to chemical control, biological control is another less recurrent management practice for leafminers. Parasitoid species are commonly associated with leafminer on crops around the world helping in the management of the pest (Zehnder and Trumble 1984, Neuenschwander et al. 1987, Sivapragasam et al. 1999, Xu et al. 1999, Rauf et al. 2000, Yano 2004, Talebi et al. 2005, Tran et al. 2006). Among parasitoids, two species of parasitoids commonly found in surveys and successfully used for biological control programs are *Neochrysocharis formosa* and *Ganaspidium nigrimanus*.

Neochrysocharis formosa is an endoparasitoid, and prefers second and early third instar leafminers (Wang et al. 2007). Host killing by feeding and oviposition in laboratory conditions can reach up to 317 leafminers in their whole life (Chien et al. 2005). *N. formosa* has been used as a biological control agent on several crops including bean, eggplant and tomatoes (Arakaki and Kinjo 1998, Maryana 2000, Shimomoto 2005, Hondo et al. 2006). *N. formosa* was also the most abundant parasitoid collected on South Texas (Chapter II).

Ganaspidium nigrimanus is a synonym of *G. utilis* (Buffington 2004), which is a solitary larval-pupal endoparasitoid. The efficacy of *Ganaspidium nigrimanus* is independent of temperature, for example it can oviposit on ~ 20 *L. trifolii* larvae in adult life over a wide range of temperatures (Kafle et al. 2005). This parasitoid has been used for several biological control programs against *Liriomyza* species in Guam, Hawaii, Marianas, Tonga, and Taiwan (Lai and Funasaki 1986, Johnson 1993, Kafle et al. 2005). *G. nigrimanus* was also one of the most abundant larval-pupal parasitoid collected in South Texas (Chapter II).

Chemical and biological control tactics can be integrated to achieve better success, but harmful effects of widely used synthetic insecticides can disrupt natural biological control (Bjorksten and Robinson 2005, Hidrayani et al. 2005, Kaspi and Parrella 2005, Tran et al. 2005a, Hossain and Poehling 2006). To achieve an integrated pest management (IPM) approach for the control of *Liriomyza* pest, the effects of commonly used insecticides on natural enemies should be addressed for a better management recommendation. Insecticides can affect both immature and adult stages. The objectives of the present studies were to determine the lethal impact of abamectin, spinetoram, lambda-cyhalothrin and novaluron on the adult stage of the two important parasitoids of *Liriomyza* species: *Neochrysocharis formosa* and *Ganaspidium nigrimanus*.

MATERIALS AND METHODS

Parasitoid colonies

Liriomyza trifolii was collected from pepper fields located at Weslaco Texas (26° 09'33.01" N, 97° 57'32.67" W) in January and February 2008 due to *L. trifolli* high specialization on this host in South Texas (see Chapter II). Adults collected were released and reared on Jalapeño M peppers (Seminis Vegetable Seeds, Oxnard, CA) in cages (61 x 66 x 61 cm) held in an insectary at 28°C, at a photoperiod of 11:13 (L:D) hours.

Neochrysocharis formosa and *G. nigrimanus* were collected from *L. trifolii* from Weslaco, Texas (26° 09'33.01" N, 97° 57'32.67" W) in February 2008 and they were introduced to cages with second and third instar leafminer larvae. After the colonies were established, introduction of fresh peppers, infested with *L. trifolii* larvae was done bi-weekly for maintaining the population.

To obtain sufficient *N. formosa* specimens of the same age for experiments, pepper plants from the greenhouse were transferred to *L. trifolii* cages for 12 hours to allow oviposition. The plants were then placed in clean cages for 3-5 days to allow larval development to the second and third larval instars. These plants were transferred to parasitoid cage to allow parasitoid oviposition and development. After ~ 15 days the leaves from the plants were excised and placed on styrofoam trays in lidded cylindrical containers (25.4 cm diameter, 30.5 cm height) with a removable clear plastic cup. These containers were placed at a controlled temperature in an insectary at 28^{o}C, with a photoperiod of 11:13 (L:D) hours. Every day emerged parasitoids were collected, separated by sex (using abdominal structures) into culture tubes and fed with 20% honey water when appropriate.

To obtain *G. nigrimanus* specimens of the same age for experiments, the parasitoids were reared as described above. Furthermore, parasitoids were allowed to oviposit. The cages used for this parasitoid species had a removable laminated paper at the bottom of the cage. After leafminer larvae exited from the leaves and pupated on the paper, the paper was removed from the cage and the pupae were collected by running water through the paper, washing the pupae onto a sieve. Pupae were then transferred to a Petri dish to allow parasitoid emergence. Every day, emerged parasitoids were collected, separated by sex using antennal segments (15 males, 13 females) into glass tubes (13 x 100 mm) and were fed with 20% honey water when appropriate.

Treatments

Abamectin, novaluron, spinetoram, lambda-cyhalothrin and water were used as treatments using specific company recommended rates (Table 11).

Table 13. Insecticide treatments

Treatment	**General Mode of Action**	**Rate Used**
Abamectin 0.15 EC	Chloride channel activator	4.25 g ai/ac
Lambda-cyhalothrin	Sodium channel modulator	9.07 g ai/ac
Novaluron 0.83 EC	Chitin synthesis inhibitor (CSI)	23.53 g ai/ac
Spinetoram	Nicotinic acetylcholine receptor agonist (mimic)	30 g ai/ac
Untreated Control	None	Water

Direct insecticide application on adult N. formosa *and* G. nigrimanus

Neochrysocharis formosa and *G. nigrimanus* males and females (1-2 days old) were placed individually in Petri-dishes (8.9 cm diameter, 1.27 cm height). The Petri dishes were place under the sprayer (Potter precision laboratory spray tower) and the top lead was removed and adults were sprayed with 2 ml of abamectin ($n_{male} = 20$, $n_{female} = 20$), lambda-cyhalothrin ($n_{male} = 20$, $n_{female} = 20$), novaluron ($n_{male} = 20$, $n_{female} = 20$), spinetoram ($n_{male} = 20$, $n_{female} = 20$), and water ($n_{male} = 40$, $n_{female} = 40$) in addition an untreated control ($n_{male} = 20$, $n_{female} = 20$) was placed as treatment. After treatment, the treated parasitoids were transferred to Petri dishes with a perforated lid and another aperture covered with a small plug for feeding. Petri dishes were placed randomly in an insectary (28^oC, 11:13 (L:D) and were provided with feeding solution (1:1 honey/water) as needed. *N. formosa* and *G. nigrimanus* survival was monitored daily for 30 days.

Effects of insecticide intake by N. formosa *and* G. nigrimanus

Male and female *N. formosa* and *G. nigrimanus* were starved for 24 hours after emergence and placed separately in Petri dishes (8.9 cm diameter, 1.27 cm height). The treatments consisted of parasitoid feeding on honey:water (1:1) solution ($n_{male} = 20$, $n_{female} = 23$), honey: abamectin (1:1) solution ($n_{male} = 20$, $n_{female} = 20$), honey: lambda-cyhalothrin (1:1) solution ($n_{male} = 20$, $n_{female} = 20$), honey: novaluron (1:1) solution ($n_{male} = 19$, $n_{female} = 24$), and a honey: spinetoram (1:1) solution ($n_{male} = 20$, $n_{female} = 20$). Feeding time of individual parasitoids was recorded. After feeding ceased, parasitoids were transferred to Petri dishes with a perforated lid and another aperture covered with a small plug for feeding. Petri dishes were placed randomly in an insectary (28^oC, 11:13

(L:D)) and were provided with feeding solution (1:1 honey/water) as needed. *N. formosa* and *G. nigrimanus* survival was monitored daily for 30 days.

Effects of insecticide leaf residue on N. formosa *and* G. nigrimanus

Pepper plants (var. Jalapeño M) (n = 12 per treatment) were sprayed to run off using a hand sprayer (The-bottle-crew ppg-32) with water or insecticide treatments. After 24 hours from application, clip cages (2.5 cm diameter, 3.8 cm height) were fastened to the treated leaves. *N. formosa and G. nigrimanus* females were individually introduced into the cages: water ($n_{\text{female}} = 24$), abamectin ($n_{\text{female}} = 24$), lambda-cyhalothrin ($n_{\text{female}} = 24$), novaluron ($n_{\text{female}} = 24$), and spinetoram ($n_{\text{female}} = 24$). Plants were arranged randomly in an insectary (28^{o}C, 11:13 L:D) and parasitoids were provided with a honey water solution (1:1). *N. formosa* and *G. nigrimanus* mortality was recorded for 20 days.

DATA ANALYSIS

Parasitoid survival differences among treatment were calculated using Kaplan-Meier survival curves. The curves were compared for statistical differences using Log-rank (Mantel-Cox) and Breslow (generalized Wilcoxon) tests (alpha 0.05) (SPSS 14.0).

RESULTS

Direct insecticide application on adult N. formosa *and* G. nigrimanus

For *N. formosa,* no significant difference was found between water treatment and untreated (1) (numbers correspond to Table 12). In addition, novaluron and lambda-cyhalothrin did not show negative effects compared to water (2, 3 respectively). Lambda-cyhalothrin showed statistical differences compared to untreated (4). Spinetoram showed negative survival effects compared to untreated (5), water (6), novaluron (7) and lambda-cyhalothrin (8). The most harmful insecticide in terms of parasitoid mortality by direct application was abamectin that showed lower cumulative survival compared to untreated (9), water (10), novaluron (11), lambda-cyhalothrin (12) and spinetoram (13) (Figure 7, Table 9).

On males, untreated control showed statistical differences from water control (14). Novaluron and lambda-cyhalothrin showed no significant difference from water (15, 16, respectively). Similarly, spinetoram showed negative effects on females compared to untreated control (17), water control (18), novaluron (19), and lambda-cyhalothrin (20). Abamectin was the most harmful insecticide compared to all the treatments: untreated (21), water (22), novaluron (23), lambda-cyhalothrin (24) and spinetoram (25) (Figure 7, Table 12).

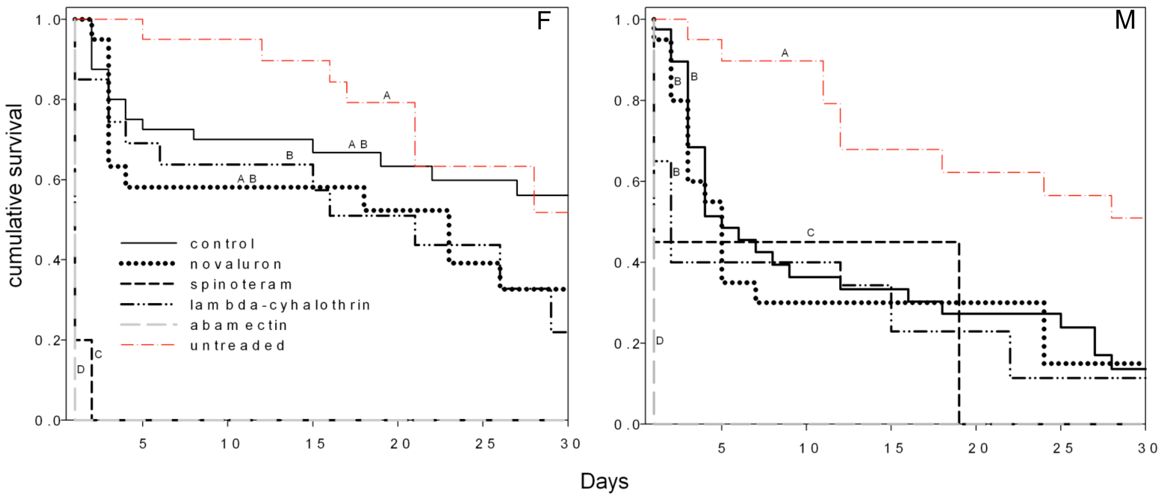

Figure 7. Effects of topical insecticide applications on *N. formosa.* Females (F) and males (M). Different letters represent statistical differences among treatments.

Table 14. Log-rank and Wilcoxon for effects of topical insecticide applications on *N. formosa*

1	log rank: $\chi^2 = 0.192$, *df* = 1, *P*< 0.661; Wilcoxon: $\chi^2 = 1.253$, *df* = 1, *P*< 0.263
2	log rank: $\chi^2 = 1.761$, *df* = 1, *P*< 0.184; Wilcoxon: $\chi^2 = 1.151$, *df* = 1, *P*< 0.283
3	log rank: $\chi^2 = 2.674$, *df* = 1, *P*< 0.102; Wilcoxon: $\chi^2 = 1.394$, *df* = 1, *P*< 0.238
4	log rank: $\chi^2 = 4.336$, *df* = 1, *P*< 0.037; Wilcoxon: $\chi^2 = 5.119$, *df* = 1, *P*< 0.024
5	log rank: $\chi^2 = 43.620$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 37.944$, *df* = 1, *P*< 0.0001
6	log rank: $\chi^2 = 58.882$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 56.765$, *df* = 1, *P*< 0.0001
7	log rank: $\chi^2 = 41.083$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 36.735$, *df* = 1, *P*< 0.0001
8	log rank: $\chi^2 = 30.770$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 24.883$, *df* = 1, *P*< 0.0001
9	log rank: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001
10	log rank: $\chi^2 = 59.000$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 59.000$, *df* = 1, *P*< 0.0001
11	log rank: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001
12	log rank: $\chi^2 = 28.826$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 28.826$, *df* = 1, *P*< 0.0001
13	log rank: $\chi^2 = 4.333$, *df* = 1, *P*< 0.037; Wilcoxon: $\chi^2 = 4.333$, *df* = 1, *P*< 0.037
14	log rank: $\chi^2 = 10.569$, *df* = 1, *P*< 0.001; Wilcoxon: $\chi^2 = 11.329$, *df* = 1, *P*< 0.001
15	log rank: $\chi^2 = 0.231$, *df* = 1, *P*< 0.631; Wilcoxon: $\chi^2 = 0.471$, *df* = 1, *P*< 0.493
16	log rank: $\chi^2 = 1.960$, *df* = 1, *P*< 0.161; Wilcoxon: $\chi^2 = 6.373$, *df* = 1, *P*< 0.012
17	log rank: $\chi^2 = 40.064$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 36.126$, *df* = 1, *P*< 0.0001
18	log rank: $\chi^2 = 51.826$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 48.671$, *df* = 1, *P*< 0.0001
19	log rank: $\chi^2 = 27.768$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 25.382$, *df* = 1, *P*< 0.0001
20	log rank: $\chi^2 = 7.263$, *df* = 1, *P*< 0.007; Wilcoxon: $\chi^2 = 4.931$, *df* = 1, *P*< 0.026
21	log rank: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 39.000$, *df* = 1, *P*< 0.0001
22	log rank: $\chi^2 = 54.786$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 54.786$, *df* = 1, *P*< 0.0001
23	log rank: $\chi^2 = 35.286$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 35.286$, *df* = 1, *P*< 0.0001
24	log rank: $\chi^2 = 18.778$, *df* = 1, *P*< 0.0001; Wilcoxon: $\chi^2 = 18.778$, *df* = 1, *P*< 0.0001
25	log rank: $\chi^2 = 11.323$, *df* = 1, *P*< 0.001; Wilcoxon: $\chi^2 = 11.323$, *df* = 1, *P*< 0.001

On female *G. nigrimanus* untreated and water did not show any statistical differences (26) (numbers correspond to Table 13). Novaluron survival curve was statistically lower than untreated (27) and water (28). Lambda-cyhalothrin survival was statistically lower than untreated (29), water (30) and novaluron (31). Abamectin showed negative effects compared to untreated (32), water (33) and novaluron (34). Abamectin and lambda-cyhalothrin showed no statistical difference on survival (35). Spinetoram showed the shortest survival compared to untreated (36), water (37), novaluron (38), lambda-cyhalothrin (39) and abamectin (40) (Figure 8) (Table 13).

On male *G. nigrimanus,* novaluron showed no statistical differences compared to untreated (41) and water (42). Abamectin showed negative effects compared to untreated (43), water (44), and novaluron (45). Lambda-cyhalothrin showed statistical differences compared to untreated (46), water (47) and novaluron (48). However, lambda-cyhalothrin did not show differences with abamectin (49) and spinetoram (50). Spinetoram showed negative effects compared to untreated (51), water (52), novaluron (53) and abamectin (54) (Figure 8, Table 13).

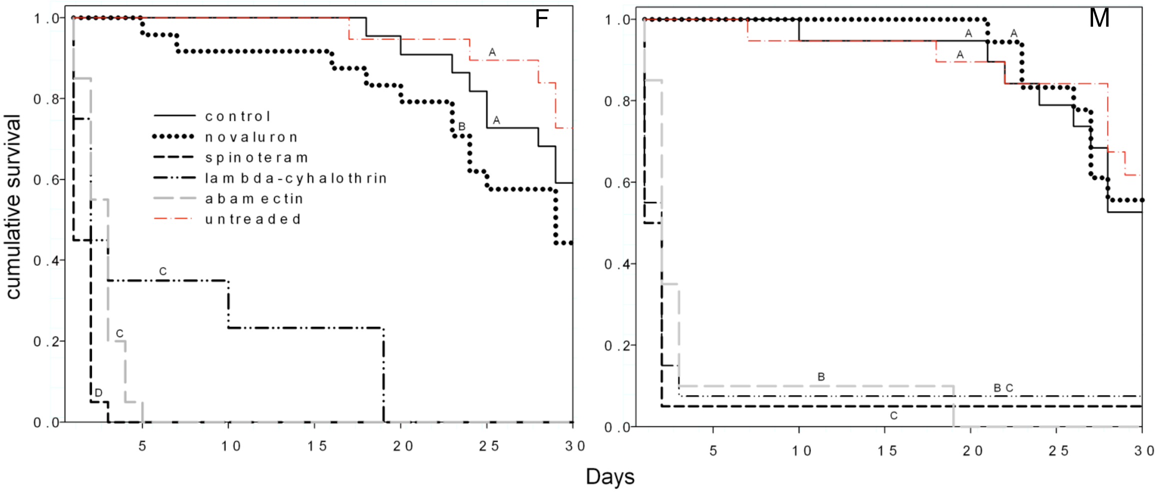

Figure 8. Effects of topical insecticide applications on *G. nigrimanus.* Females (F) and males (M) Different letters represent statistical differences among treatments.

Table 15. Log-rank and Wilcoxon for effects of topical insecticide applications on *G. nigrimanus*

26	log rank: χ^2 = 0.531, *df* = 1, *P*< 0.466; Wilcoxon: χ^2 = 0.653, *df* = 1, *P*< 0.425
27	log rank: χ^2 = 18.027, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 15.678, *df* = 1, *P*< 0.0001
28	log rank: χ^2 = 12.126, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 9.793, *df* = 1, *P*< 0.002
29	log rank: χ^2 = 25.088, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 22.703, *df* = 1, *P*< 0.0001
30	log rank: χ^2 = 24.781, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 24.103, *df* = 1, *P*< 0.0001
31	log rank: χ^2 = 15.717, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 19.389, *df* = 1, *P*< 0.0001
32	log rank: χ^2 = 43.319, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 36.021, *df* = 1, *P*< 0.0001
33	log rank: χ^2 = 48.732, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 41.602, *df* = 1, *P*< 0.0001
34	log rank: χ^2 = 49.631, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 42.890, *df* = 1, *P*< 0.0001
35	log rank: χ^2 = 1.593, *df* = 1, *P*< 0.207; Wilcoxon: χ^2 = 0.005, *df* = 1, *P*< 0.944
36	log rank: χ^2 = 42.458, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 36.598, *df* = 1, *P*< 0.0001
37	log rank: χ^2 = 46.953, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 41.005, *df* = 1, *P*< 0.0001
38	log rank: χ^2 = 48.436, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 42.461, *df* = 1, *P*< 0.0001
39	log rank: χ^2 = 10.151, *df* = 1, *P*< 0.001; Wilcoxon: χ^2 = 7.791, *df* = 1, *P*< 0.005
40	log rank: χ^2 = 13.751, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 12.625, *df* = 1, *P*< 0.0001
41	log rank: χ^2 = 3.591, *df* = 1, *P*< 0.058; Wilcoxon: χ^2 = 1.691, *df* = 1, *P*< 0.193
42	log rank: χ^2 = 0.028, *df* = 1, *P*< 0.867; Wilcoxon: χ^2 = 0.027, *df* = 1, *P*< 0.869
43	log rank: χ^2 = 42.174 *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 35.188, *df* = 1, *P*< 0.0001
44	log rank: χ^2 = 42.922, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 35.758, *df* = 1, *P*< 0.0001
45	log rank: χ^2 = 40.510, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 33.587, *df* = 1, *P*< 0.0001
46	log rank: χ^2 = 23.157, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 25.951, *df* = 1, *P*< 0.0001
47	log rank: χ^2 = 26.533, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 27.962, *df* = 1, *P*< 0.0001
48	log rank: χ^2 = 25.496, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 26.171, *df* = 1, *P*< 0.0001
49	log rank: χ^2 = 1.969, *df* = 1, *P*< 0.161; Wilcoxon: χ^2 = 4.206, *df* = 1, *P*< 0. 040
50	log rank: χ^2 = 0.579, *df* = 1, *P*< 0.447; Wilcoxon: χ^2 = 0.341, *df* = 1, *P*< 0.560
51	log rank: χ^2 = 35.176, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 32.022, *df* = 1, *P*< 0.0001
52	log rank: χ^2 = 35.891, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 32.965, *df* = 1, *P*< 0.0001
53	log rank: χ^2 = 33.797, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 30.669, *df* = 1, *P*< 0.0001
54	log rank: χ^2 = 8.259, *df* = 1, *P*< 0.004; Wilcoxon: χ^2 = 8.108, *df* = 1, *P*< 0.004

Effects of insecticide consumption by N. formosa *and* G. nigrimanus

Novaluron (55) and lambda-cyhalothrin (56) (numbers correspond to Table 14) showed no statistical differences from control on female *N. formosa*. Abamectin showed negative effects compared to control (57), novaluron (58), and lambda-cyhalothrin (59). Abamectin and spinetoram showed no statistical differences (60). Spinetoram showed negative effects compared to control (61), novaluron (62), and lambda-cyhalothrin (63) (Figure 9) (Table 14).

A similar trend was present on male *N. formosa* feeding effects, where control showed no difference from novaluron (64), and lambda-cyhalothrin (65). Abamectin showed statisticant differences compare to control (66), novaluron (67) and lambda-cyhalothrin (68). Similarly to females, abamectin showed no differences from spinetoram (69). Spinetoram showed negative effects compared to control (70), novaluron (71) and lambda-cyhalothrin (72) (Figure 9, Table 14).

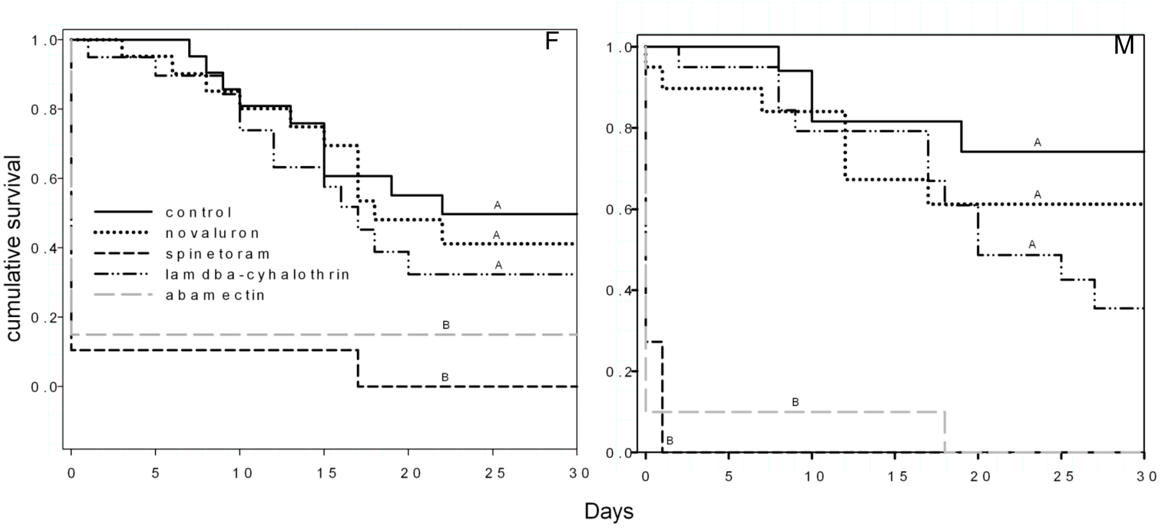

Figure 9. Effects of insecticide intake on adult *N. formosa*. Females (F) and males (M). Different letters represent statistical differences among treatments.

Table 16. Log-rank and Wilcoxon for effects of insecticide intake by *N. formosa*

55	log rank: χ^2 = 0.195, *df* = 1, *P*< 0.659; Wilcoxon: χ^2 = 0.140, *df* = 1, *P*< 0.708
56	log rank: χ^2 = 1.160, *df* = 1, *P*< 0.281; Wilcoxon: χ^2 = 0.973, *df* = 1, *P*< 0.324
57	log rank: χ^2 = 46.492, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 39.860, *df* = 1, *P*< 0.0001
58	log rank: χ^2 = 45.485, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 39.501, *df* = 1, *P*< 0.0001
59	log rank: χ^2 = 42.656, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 37.221, *df* = 1, *P*< 0.0001
60	log rank: χ^2 = 0.360, *df* = 1, *P*< 0.548; Wilcoxon: χ^2 = 0.190, *df* = 1, *P*< 0.663
61	log rank: χ^2 = 38.374, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 35.532, *df* = 1, *P*< 0.0001
62	log rank: χ^2 = 38.374, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 35.532, *df* = 1, *P*< 0.0001
63	log rank: χ^2 = 36.383, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 34.114, *df* = 1, *P*< 0.0001
64	log rank: χ^2 = 0.826, *df* = 1, *P*< 0.363; Wilcoxon: χ^2 = 1.054, *df* = 1, *P*< 0.305
65	log rank: χ^2 = 3.156, *df* = 1, *P*< 0.076; Wilcoxon: χ^2 = 2.231, *df* = 1, *P*< 0.135
66	log rank: χ^2 = 42.247, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 37.485, *df* = 1, *P*< 0.0001
67	log rank: χ^2 =37.167, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 33.392, *df* = 1, *P*< 0.0001
68	log rank: χ^2 = 43.412, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 38.669, *df* = 1, *P*< 0.0001
69	log rank: χ^2 = 1.979, *df* = 1, *P*< 0.160; Wilcoxon: χ^2 = 1.979, *df* = 1, *P*< 0.160
70	log rank: χ^2 = 40.830, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 34.818, *df* = 1, *P*< 0.0001
71	log rank: χ^2 = 34.508, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 29.466, *df* = 1, *P*< 0.0001
72	log rank: χ^2 = 42.221, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 36.140, *df* = 1, *P*< 0.0001

On *G. nigrimanus* female feeding, novaluron did not show differences compared to control (73) (numbers correspond to Table 15). Lambda-cyhalothrin did not show significant difference from control (74), however it showed a significant difference compared to novaluron (75). Abamectin showed negative effects compared to control (76), novaluron (77) and lambda-cyhalothrin (78). Spinetoram was the most harmful insecticide on female ingestion, showing negative effects compared to control (79), novaluron (80), lambda-cyhalothrin (81) and abamectin (82) (Figure 10) (Table 15).

The effects on *G. nigrimanus* male feeding were similar to those for females. Novaluron did not show differences from control (83). Abamectin showed negative effects compared to control (84), and novaluron (85). Abamectin showed no differences compared to lambda-cyhalothrin (86). As with females, spinetoram showed negative effects compared to control (88), novaluron (89), lambda-cyhalothrin (90) and no difference compared to abamectin (87): (Figure 10, Table 15).

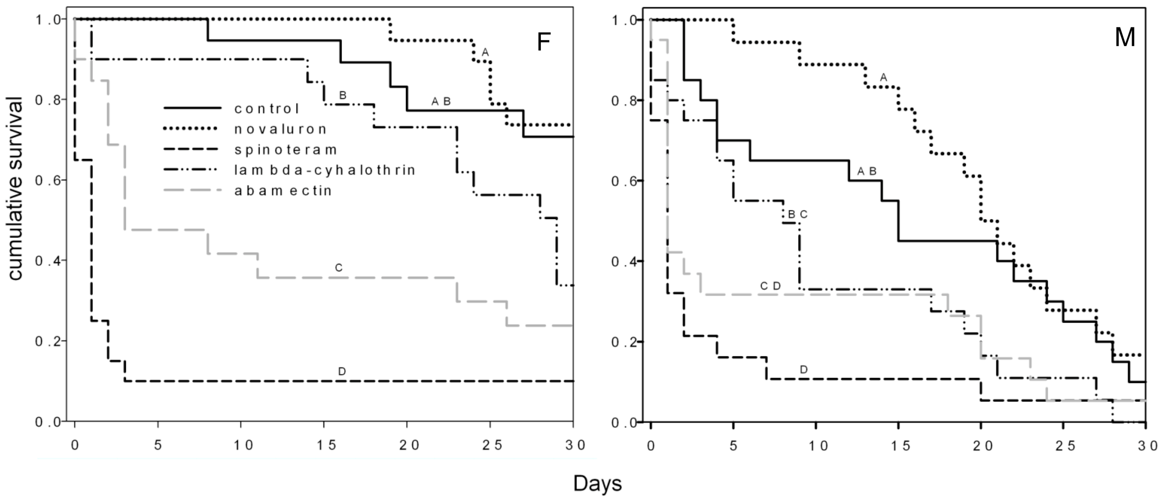

Figure 10. Effects of insecticide intake on adult *G. nigrimanus.* Females (F) and males (M). Different letters represent statistical differences among treatments.

Table 17. Log-rank and Wilcoxon for effects of insecticide intake by *G. nigrimanus*

73	log rank: χ^2 = 0.158, *df* = 1, *P*< 0.691; Wilcoxon: χ^2 = 0.339, *df* = 1, *P*< 0.560
74	log rank: χ^2 = 3.788, *df* = 1, *P*< 0.052; Wilcoxon: χ^2 = 3.223, *df* = 1, *P*< 0.073
75	log rank: χ^2 = 6.216, *df* = 1, *P*< 0.013; Wilcoxon: χ^2 = 6.480, *df* = 1, *P*< 0.011
76	log rank: χ^2 = 11.697, *df* = 1, *P*< 0.001; Wilcoxon: χ^2 = 13.403, *df* = 1, *P*< 0.0001
77	log rank: χ^2 = 14.198, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 15.868, *df* = 1, *P*< 0.0001
78	log rank: χ^2 = 3.267, *df* = 1, *P*< 0.071; Wilcoxon: χ^2 = 5.719, *df* = 1, *P*< 0.017
79	log rank: χ^2 = 26.575, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 27.447, *df* = 1, *P*< 0.0001
80	log rank: χ^2 = 27.521, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 27.752, *df* = 1, *P*< 0.0001
81	log rank: χ^2 = 15.050, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 20.239, *df* = 1, *P*< 0.0001
82	log rank: χ^2 = 7.862, *df* = 1, *P*< 0.005; Wilcoxon: χ^2 = 11.271, *df* = 1, *P*< 0.001
83	log rank: χ^2 = 0.696, *df* = 1, *P*< 0.404; Wilcoxon: χ^2 = 1.769, *df* = 1, *P*< 0.183
84	log rank: χ^2 = 4.713, *df* = 1, *P*< 0.030; Wilcoxon: χ^2 = 9.626, *df* = 1, *P*< 0.002
85	log rank: χ^2 = 6.496, *df* = 1, *P*< 0.011; Wilcoxon: χ^2 = 11.811, *df* = 1, *P*< 0.001
86	log rank: χ^2 = 0.114, *df* = 1, *P*< 0.736; Wilcoxon: χ^2 = 1.412, *df* = 1, *P*< 0.235
87	log rank: χ^2 = 1.655, *df* = 1, *P*< 0.198; Wilcoxon: χ^2 = 2.243, *df* = 1, *P*< 0.134
88	log rank: χ^2 = 11.120, *df* = 1, *P*< 0.001; Wilcoxon: χ^2 = 18.545, *df* = 1, *P*< 0.0001
89	log rank: χ^2 = 15.846, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 22.609, *df* = 1, *P*< 0.0001
90	log rank: χ^2 = 3.599, *df* = 1, *P*< 0.058; Wilcoxon: χ^2 = 7.172, *df* = 1, *P*< 0.007

Effects of insecticide leaf residue on N. formosa *and* G. nigrimanus

There were no residual effects of abamectin (91) or novaluron (92) (numbers correspond to Table 16) on *N. formosa* compared to the control. Novaluron did show a small difference with abamectin (93). Spinetoram showed negative effects compared to control (94), novaluron (95), and abamectin (96) and no difference compared to lambda-cyhalothrin (97). Lambda-cyhalothrin showed negative effects compared to control (98), novaluron (99) and abamectin (100) (Figure 11, Table 16).

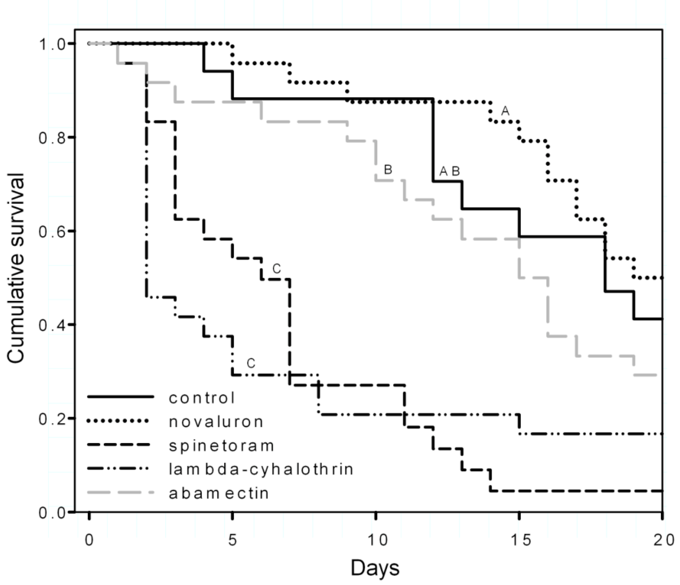

Figure 11. Effects of insecticide residue on adult *N. formosa* females (F) and males (M). Different letters represent statistical differences among treatments.

Table 18. Log-rank and Wilcoxon for effects of leaf residue to *N. formosa*

91	log rank: χ^2 = 1.120, *df* = 1, *P*< 0.290; Wilcoxon: χ^2 = 1.488, *df* = 1, *P*< 0.222
92	log rank: χ^2 = 0.470, *df* = 1, *P*< 0.493; Wilcoxon: χ^2 = 0.690, *df* = 1, *P*< 0.406
93	log rank: χ^2 = 3.542, *df* = 1, *P*< 0.060; Wilcoxon: χ^2 = 4.342, *df* = 1, *P*< 0.037
94	log rank: χ^2 = 21.181, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 18.160, *df* = 1, *P*< 0.0001
95	log rank: χ^2 = 35.463, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 30.255, *df* = 1, *P*< 0.0001
96	log rank: χ^2 = 17.990, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 14.145, *df* = 1, *P*< 0.0001
97	log rank: χ^2 =0.001, *df* = 1, *P*< 0.979; Wilcoxon: χ^2 = 2.176, *df* = 1, *P*< 0.140
98	log rank: χ^2 = 11.262, *df* = 1, *P*< 0.001; Wilcoxon: χ^2 = 18.312, *df* = 1, *P*< 0.0001
99	log rank: χ^2 =15.197, *df* = 1, *P*< 0.0001; Wilcoxon: χ^2 = 21.237, *df* = 1, *P*< 0.0001
100	log rank: χ^2 = 6.330, *df* = 1, *P*< 0.012; Wilcoxon: χ^2 = 11.556, *df* = 1, *P*< 0.001

With *G. nigrimanus*, the control and novaluron showed no significant differences for residual effects (101) (numbers correspond to Table 17). Abamectin had a negative effect compared to control (102) and novaluron (103). Spinetoram also showed a negative effect on the survival compared to the control (104) and novaluron (105), and no difference compared to abamectin (106). Lambda-cyhalothrin showed the highest negative effect to parasitoids compared to all treatments: control (107), novaluron (108), abamectin (109) and spinetoram (110) (Figure 12) (Table 17).

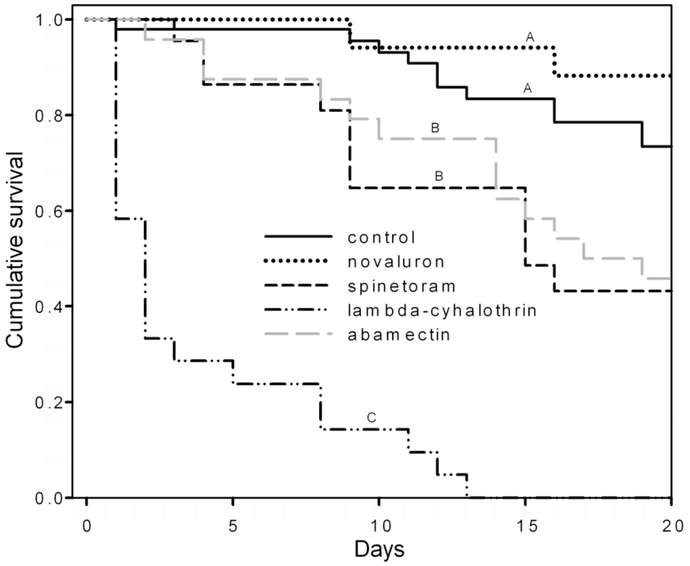

Figure 12. Effects of insecticide residue on adult *G. nigrimanus* females (F) and males (M). Different letters represent statistical differences among treatments.

Table 19. Log-rank and Wilcoxon for effects of leaf residue to *G. nigrimanus*

101	log rank: $\chi^2 = 1.506$, $df = 1$, $P < 0.220$; Wilcoxon: $\chi^2 = 1.461$, $df = 1$, $P < 0.227$
102	log rank: $\chi^2 = 5.556$, $df = 1$, $P < 0.018$; Wilcoxon: $\chi^2 = 5.587$, $df = 1$, $P < 0.018$
103	log rank: $\chi^2 = 7.664$, $df = 1$, $P < 0.006$; Wilcoxon: $\chi^2 = 7.542$, $df = 1$, $P < 0.006$
104	log rank: $\chi^2 = 6.891$, $df = 1$, $P < 0.009$; Wilcoxon: $\chi^2 = 7.613$, $df = 1$, $P < 0.006$
105	log rank: $\chi^2 = 8.518$, $df = 1$, $P < 0.004$; Wilcoxon: $\chi^2 = 8.557$, $df = 1$, $P < 0.003$
106	log rank: $\chi^2 = 0.066$, $df = 1$, $P < 0.797$; Wilcoxon: $\chi^2 = 0.120$, $df = 1$, $P < 0.729$
107	log rank: $\chi^2 = 72.553$, $df = 1$, $P < 0.0001$; Wilcoxon: $\chi^2 = 61.279$, $df = 1$, $P < 0.0001$
108	log rank: $\chi^2 = 45.786$, $df = 1$, $P < 0.0001$; Wilcoxon: $\chi^2 = 34.723$, $df = 1$, $P < 0.0001$
109	log rank: $\chi^2 = 36.150$, $df = 1$, $P < 0.0001$; Wilcoxon: $\chi^2 = 30.819$, $df = 1$, $P < 0.0001$
110	log rank: $\chi^2 = 31.193$, $df = 1$, $P < 0.0001$; Wilcoxon: $\chi^2 = 27.624$, $df = 1$, $P < 0.0001$

DISCUSSION

Research on the lethal and sublethal effects of novaluron to natural enemies is limited. The insect growth regulator appears to be safe for natural enemies (Alston and Lindstrom 2002, Chappell et al. 2005). Alston and Lindstrom (2002) showed an increase in predator densities in novaluron-treated plants compared with other insecticides. They found that higher densities of the aphid parasitoid *Aphelinus mal* were present on novaluron treatments compared with control and other treatments.

In the present studies, novaluron appears to be the safest insecticide to *N. formosa* and *G. nigrimanus* male. However, direct application of novaluron to *G. nigrimanus* females had significantly higher mortality than the controls. In the intake experiment (novaluron/honey solutions) cumulative survival of *N. formosa* and *G. nigrimanus* males and females was not affected compared with the controls. The direct consumption of honey and insecticides suggest that novaluron would be the safest pesticide when sprayed on floral structure in the field. In addition, leaf residue studies imply that novaluron residues are the safest for the parasitoids when the insecticide is applied in the field to control leafminer.

Spinetoram has a very low mammalian toxicity (Breslin et al. 2000) and fast environment breakdown (Cleveland et al. 2002). It also has a low impact on predator populations (Williams et al. 2003). However a literature review on the effects of the insecticide to parasitoids, rates spinosad as a class 3 and 4, the latter being the most harmful class against parasitoids (Williams et al. 2003). In the present study spinetoram showed harmful effects and reduction of cumulative survival to *N. formosa* and *G. nigrimanus* males and females compared with controls on topical applications, feeding and leaf residue. Field and greenhouse adult parasitoid populations may be eliminated if sprayed with this insecticide, in addition parasitoid species feeding from contaminated honey sources will also perish. Parasitoids entering a zone after spinetoram application will also face mortality due to its residue. This insecticide was the most harmful to adult parasitoids in this study. Similarly to spinosad, spinetoram also showed harmful effects on adult parasitoids and it should be cautiously used in pest management systems.

Reports on abamectin effects on natural enemies vary in the literature. In field conditions abamectin treatments appear not to affect parasitoid populations on celery (Trumble 1984, Weintraub 1999), and chrysanthemum (Hara 1986) and peppers (see chapter III). In contrast, under laboratory conditions abamectin showed negative effects on parasitoid species. Schuster (1994) recounted high percentage mortality of *Diglyphus* species. My findings showed a high mortality of *N. formosa* and *G. nigrimanus* males and females by topical application of abamectin. Furthermore, abamectin also showed high mortality when *N. formosa* and *G. nigrimanus* males and females that were fed with honey/abamectin solution. These results support previous research that showed high toxicity of direct application and feeding of abamectin (Kaspi and Parrella 2005). In the residual effects study, abamectin showed negative effects on *G. nigrimanus*. However, no-significant harmful effects were present on *N. formosa* compared to control. The results suggest that abamectin should only be used with caution in IPM programs to avoid high mortality of parasitoid species.

Lambda-cyhalothrin has been reported to have harmful effects on natural enemies (White et al. 1990, Prasifka et al. 2005, Devotto et al. 2007). In the present studies however, topical application showed no harmful effect on *N. formosa.* In contrast, it had negative effects on males and females of *G. nigrimanus.* In the direct consumption, the insecticide was safe to both parasitoids species. The residual study gave opposite results and had negative impacts on survival of both parasitoids species.

It has been reported that lambda-cyhalothrin topical applications and residue are harmful to natural enemies. For example, Tillman and Mulrooney (2000) showed that topical applications of lambda-cyhalothrin negatively affected natural enemies *Coleomegilla maculata* and *Hippodamia convergens* (Coleoptera: Coccinellidae), *Geocoris punctipes* (Hemiptera: Lygaeidae), and *Bracon mellitor* (Hymenoptera: Braconidae). In the same study, lambda-cyhalothrin residue was toxic to *B. mellitor, C. nigriceps, C. maculata,* and *G. punctipes* after treatment.

Lambda-cyhalothrin topical application differences between *N. formosa* (no harmful effects) and *G. nigrimanus* (harmful effects) found in this study may be related to a potential development of *N. formosa* resistance to this compound. *Neochrysocharis formosa* is a larval endoparasitoid completing all its development in close association with *Liriomyza* larvae, in contrast to *G. nigrimanus,* a larval pupal parasitoid that starts its development on *Liriomyza* pupal stage. *Liriomyza* from field investigations appear to be resistant to lambda-cyhalothrin, potentially leading to resistance to this parasitoid species.

In addition, *N. Formosa* was the most abundant parasitoid present on peppers in South Texas ~ 60% of the collected specimens; this could also be influenced by its potential resistance to pyrethroid insecticides.

The results of present findings on insecticidal effects on the adult parasitoids *N. formosa* and *G. nigrimanus* showed that novaluron had no lethal effects to adult parasitoid species, lambda-cyhalothrin, abamectin and spinetoram should be used cautiously because they could disrupt natural enemies' populations. In order to have a better understanding of these compounds and their compatibility to biological control it is imperative to research the sub-lethal effects on adults and on immature parasitoid stages.

CHAPTER V

GENERAL CONCLUSIONS

Liriomyza leafminers on South Texas are causing damage to vegetable crops particularly in pepper. According to this study *L. trifolii* is the only leafminer causing economic damage to pepper fields showing specialization on this host.

Liriomyza leafminers in South Texas are attacked by a large diversity of parasitoid species. In our survey, 20 different parasitoid species were collected from *Liriomyza* feeding on peppers. The parasitoids belong to the hymenopterous families of Eulophidae, Braconidae, Figitidae, and Pteromalidae. The most abundant parasitoid was *N. formosa* with 60 % of the total collected specimens. In addition, 15 species were recorded for the first time in South Texas attacking *L. trifolii*: *D. isaea* (65), *Cirrospilus* spp. (46), *Asecodes* spp. (4), *and Pnigalio spp.* (17) from Eulophidae family. *Opius dissitus* (174), *O. dimidiatus* (12), *Opius* spp *1* (1), *Opius* spp *2* (7), *O. bruneipes* (26), *O. browsvillensis* (20), and *O. thoracosema* (2) from Braconidae family. *Ganaspidium pusillae* (118), *G. nigrimanus* (86), *D. pacifica* (16), and *A. robusta* (6) from the Figitidae family.

In order to have a better understanding of the *Liriomyza* and parasitoid populations present in the LRGV, a survey of other agricultural and weeds hosts should be performed.

Insecticidal control is the main management method for *Liriomyza* pests in South Texas. A variety of chemical compounds are available on the market for the control of leafminers. Among them, novaluron, spinetoram, abamectin and lambda-cyhalothrin are commonly used in South Texas and their efficacy, as well as their effects on natural enemies, was investigated.

According to our field evaluation, novaluron was the most effective insecticide for the management of *L. trifolii* pest, followed by spinetoram and abamectin. Lambda-cyhalothrin showed no efficacy on the control of *L. trifolii.* A potential development of resistance to lambda-cyhalothrin in *L. trifolii* populations in the LRGV may be the reason for the lack of insecticide efficacy. It is recommended to avoid the use of this compound in South Texas.

In field evaluations, novaluron showed the least amount of parasitoid per leafminer:larvae ratio and the smallest diversity index among treatments. This low parasitoid ratio and low biodiversity index may be explained by a potential toxicity of novaluron to the immature stages of leafminer parasitoids and it may be directly correlated to its efficacy on controlling *Liriomyza trifolii.*

In order to increase the understanding of insecticide effects on natural enemies, adult parasitoids *Neochrysocharis formosa* (Eulophidae) and *Ganaspidium nigrimanus* (Figitidae) were used for insecticide bioassays using novaluron, spinetoram, abamectin and lambda-cyhalothrin. Among the treatments, spinetoram showed the greater negative effects on *N. formosa* and *G. nigrimanus* adult parasitoids followed by abamectin and lambda-cyhalothrin. Novaluron did not show any lethal effects on adult parasitoids of the two species. Lambda-cyhalothrin did not show negative effects on *N. formosa* directly applied with this compound. In contrast, it showed harmful effects on *G. nigrimanus*. The survival of *N. formosa* exposed to lambda-cyhalothrin may be explained by a potential development of resistance of this parasitoid to this compound.

In order to better understand the insecticidal effects on the natural enemies it is necessary to investigate the sub-lethal effects of these compounds on adult parasitoids and the effects on immature parasitoid stages.

To conclude, parasitoid populations are present in the LRGV helping on the control of *Liriomyza* pest, and the management programs currently used in this area should be redefined in order to incorporate the important contribution of this natural occurring pest management tool. My advice will be to allow these natural enemies to develop and establish on *L. trifolii* infested fields and avoid the used of insecticides. If insecticide application is require, one application of novaluron per season can be done.

Insecticides and natural enemies are important tool in IPM. Understanding their compatibility will help improve IPM in vegetables. Results in this study indicate a complex interaction is occurring including differences of natural enemy's responses and possibly resistant effects of insecticides on adult and larval populations. Further studies of these interactions will help refine IPM programs in vegetables.

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VITA

Name: Ricardo Hernandez Moreno

Address: Department of Entomology, Texas A&M University
TAMU 2475, College Station, TX 77843-2475

Email Address: ricahm@gmail.com

Eduction: B.S. Agronomy 2005. Crop Consulting, New Mexico State University at Las Cruces, NM

M.S. Entomology 2009. Biological Control & IPM, Texas A&M University at College Station, TX