EVALUATION OF ORGANIC INSECTICIDES TO CONTROL HARLEQUIN BUG, *MURGANTIA HISTRIONICA* (HAHN), AND YELLOWMARGINED LEAF BEETLE, *MICROTHECA OCHROLOMA* STÅL, ON LEAFY GREENS

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CHAPTER I

INTRODUCTION

Heading Brassica crops (broccoli, cabbage, cauliflower) are grown in Oklahoma primarily for the fresh market (Kahn et al., 2007). Leafy Brassica crops (turnips, kale, mustard, collards) are grown in Oklahoma for both the fresh market and processing industry (Kahn et al., 2007; Motes et al., 2007). A significant acreage of leafy Brassica crops is grown in the south-central plains, including Oklahoma, Texas, and Arkansas, mostly for the processing market (J. V. Edelson, personal communication). Crops grown for the processing industry are marketed under strict guidelines as regulated by the United States Food and Drug Administration. These guidelines allow for minimal contamination of produce including damage, excrement, and/or insect parts. When the crop is grown for the fresh market, consumers demand high quality product with little to no visible damage. Crops grown for fresh produce are regulated by the United States Department of Agriculture (USDA), and regulatory standards differ from that of processed crops.

The USDA has recently created a National Organic Program (NOP) as mandated by the Organic Foods Protection Act of 1990. The NOP allows organic producers to become certified and sets standards that allow for organic labeling. Regulations require non-synthetic materials in the production and handling of products for organic certification and labeling (Public Law 101-624, 1990).

The materialization of the concept of Organics began with literature of J. I. Rodale in the 1930's with publication of his magazine "Organic Farming and Gardening." His work instituted the philosophy of organic agriculture and currently the magazine is called "Organic Gardening." J. I Rodale was enlightened by the literature of Sir Albert Howard, who officially began the "organic movement" (Rodale et al., 1999).

Today, the National Organic Standards Board (NOSB) defines organics as "an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony." The NOSB was organized to aid the United States Department of Agriculture with laws concerning organic agriculture (Kuepper and Gegner, 2004).

The afore-mentioned definition to "off-farm inputs" refers to use of pesticide applications, such as botanical insecticides. Instead, organic producers rely on natural factors, such as natural enemies and healthy plants and soil, to suppress pests (Geier, 2000). However, some instances warrant the need to apply pesticides as a "rescue" of the marketable crop (J. V. Edelson, personal communication).

Common insect pests of both heading and leafy Brassica crops include aphids, flea beetle, seed-corn maggot, cabbage looper, diamondback moth, imported cabbage worm, armyworm, and harlequin bug (Maynard and Hochmuth, 1997; Motes et al., 2007). The yellowmargined leaf beetle is a pest of leafy Brassica (Brees, 2007).

The harlequin bug, *Murgantia histrionica* Hahn, (HB) is a pest of crops of the genus Brassica, including broccoli, Brussels sprouts, and cauliflower (Maynard and Hochmuth, 1997). The HB is native to the southern tropics of North America and was first recorded present in Texas and Louisiana during the late 1800's. It has been found as far north as Minnesota (Hodson and Cook, 1960; Webster and Webster, 1896). In Oklahoma, the HB is considered a key pest of cabbage, cauliflower, and broccoli (Kahn et al., 2007). Although this Pentatomid has been reported to feed on numerous vegetable and fruit crops, it prefers Brassica species (McPherson and McPherson, 2000).

The HB damages the plant through its feeding behavior in which it inserts its piercing-sucking mouthparts into plant tissue resulting in discolored blotches and, under heavy infestation, plant death (Metcalf and Metcalf, 1993). In the southern United States, the HB was once considered a serious pest; however, with the creation and widespread use of effective synthetic insecticides, the reported pest status of the HB has declined (Metcalf and Metcalf, 1993; McPherson and McPherson, 2000).

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål. (YMLB) is native to South America and was first reported in the U. S. in Louisiana in 1945. The first observation of large numbers of this species infesting Brassica was reported in Alabama three years later (Chamberlin and Tippins, 1948). The YMLB has been further noted in Arkansas, Florida, Mississippi, North Carolina, and Texas and feeds primarily on collards, turnips, and mustard (Ameen and Story, 1997; Oliver and Chapin, 1983; Staines, 1999). The YMLB damages the plant by chewing leafy material and can consume the entire plant until only vascular tissue remains.

Recently, the HB and other agriculturally injurious Pentatomids have gained new attention due to outbreaks in cropping systems with reduced synthetic insecticide use (McPherson and McPherson, 2000). Edelson and Mackey (2006), have shown synthetic insecticides are effective, available and labeled for controlling HB populations; however there are no reports in the literature for methods of organic control.

The YMLB is a pest of leafy Brassica crops in Texas where conventional insecticides, such as carbaryl, are effective in controlling this pest (Brees, 2007). In Florida, organic producers suffer great monetary losses due to this pest (Bowers, 2003).

Because there is a dearth of information available for managing the HB and YMLB, and other significant Heteroptera (formerly Hemiptera) and Coleopteran pests on economically important crops utilizing organic control methods, we initiated research to:

1. Determine whether organic pesticides are toxicologically active against the HB and YMLB in laboratory toxicity studies.
2. Evaluate those pesticides showing a response in the toxicity study to determine efficacy of applied field rates to these pests.

The insecticides evaluated are approved for use in certified organic cropping systems and include: two botanically derived insecticides, azadirachtin [Neemix® 4.5], and pyrethrum [Pyganic® EC 1.4]. The final insecticide evaluated was a bacterially derived macrocyclic lactone that has a novel mode of action, spinosad [Entrust® Naturalyte® Insecticide].

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CHAPTER II

REVIEW OF LITERATURE

Organic Crop Production

The USDA created the National Organic Program (NOP) as part of the Organic Foods Protection Act (OFPA) of 1990. The NOP allows organic producers to become certified and sets standards that allow for organic labeling. Regulations require non-synthetic materials in the production and handling of products for organic certification and labeling. Synthetics include any chemicals or materials that are not products of “naturally occurring biological processes,” i.e. have not been altered in their chemical structure. The NOP standards are set by an appointed board of 15 that include individuals from all levels; producers, wholesalers, the general public and an organic certifying agent (Public Law 101-624, 1990). For a complete and up to date list of inputs, including prohibited natural and allowed synthetics inputs, refer to the NOP website (NOP, 2007). Other inputs, such as formulated insecticides, into the production or handling system may also be reviewed by the Organic Materials Review Institute (OMRI) that compiles a list of restricted and allowed inputs. However, this review process is to the discretion of the producer of the said product since the NOP also reviews inputs (OMRI, 2007).

As prescribed by the OFPA, the Secretary of Agriculture created a certification program for production and handling of goods marketed and labeled as "certified organic." Agents are accredited by the NOP and a current list of accredited agents can be found on the NOP website www.ams.usda.gov/NOP/indexIE.htm. A farm that has $5,000 or less in annual organic sales is not required to become certified by an accredited agent to market their product as organically produced (NOP, 2007; Public Law 101-624, 1990).

In 2005, the U.S. had over three million hectares of land used for certified organic production, including farm, crop, and rangeland. Of that, nearly 40,000 hectares were used for growing vegetables (USDA-ERS, 2007).

Vegetable Crops

Leafy greens are vegetables grown throughout the United States and include spinach, lettuce, collards, turnips, mustards, and broccoli. Vegetable growers grossed over two billion in sales of leafy crops in 1996 (Lucier, 1998). Although leafy green crops have been grown for decades, there has been a general increase in the production and sales of these crops recently, speculated due to greater awareness of their nutritional value. Leafy vegetable crops are high in vitamins A and C, and minerals such as iron and calcium (Lucier, 1998). Studies have indicated that consumption of antioxidants obtained from leafy greens and vegetables in general can lower cancer risks (Larson and Christensen, 2007; Seifried et al., 2007).

Leafy greens include varieties from the plant families Brassicaceae (kale, mustards, collards, broccoli), Chenopodiaceae (spinach, beets), and Asteraceae (lettuce, dandelion) (Pierce, 1987). These crops are considered “cool season crops” and in fact kale may acquire better flavor after a slight freeze. Thus, leafy greens are grown throughout the year in the eastern U.S., although California is the leading producer of fresh market greens (Lucier, 1998). Further, some leafy varieties are traditionally grown in the south and include Brassica, or crucifer crops, such as kale, collard and mustards although they also are grown and savored in northeastern states (Lucier, 1998).

Insect pests of Brassica greens vary by state and type of crop, for instance, whether it is heading or non-heading. In Oklahoma, predominant insect pests of heading Brassica such as broccoli and cabbage include the grub worm, wireworm, cutworm, cabbage looper, diamondback moth larva, imported cabbageworm, beet armyworm, and aphids. Less predominant insect pests include thrips, flea beetles, harlequin bugs, and spider mites (Kahn et al., 2007). Predominant insect pests of non-heading Brassica such as collard, turnip and kale include aphids, cabbage loopers, diamondback moth larvae, imported cabbageworms, armyworms, flea beetles, and the seed-corn maggot (Motes et al., 2007). In Georgia, the harlequin bug is a problem in cabbage and collard, while the chinch bug, false chinch bug, and yellowmargined leaf beetle are pests of turnip and mustard crops (Adams, 2000). In North Carolina, the harlequin bug is predominantly a pest of collards (Sanders, 2001).

Insect damage decreases the marketability of both heading and non-heading cultivars. Lepidoptera larvae feed on the leafy portion of collards and mustards resulting in “holes” and further reducing the value with subsequent waste material and pupae (Motes et al., 2007). Lepidoptera larvae feed on the leafy portion and tunnel into heads of cabbage and broccoli that are the marketed end-product of these crops. Damage to the head or leafy portion of heading Brassica causes non-marketable produce and monetary losses (Kahn et al., 2007). Foliage feeding, induced by the yellowmargined leaf beetle, results in leafy fresh produce that are unattractive to consumers. The inevitable insect waste and remains contaminate produce destined for the processing industry, thus resulting in contaminated canned or frozen goods. The same is true for sucking-piercing insects including aphids and the harlequin bug. The feeding behavior of the HB leaves discolored blotches on leafy portions of the plant. Aphids have potential of causing plant growth distortions (Kahn et al., 2007; Motes et al., 2007).

In addition to insect pests, diseases are common on Brassica crops. In Oklahoma, fungal diseases include damping-off, white and black leaf spot, anthracnose, downy mildew, and white rust. Bacterial diseases include black rot, bacterial leaf spot and peppery leaf spot. As is the case with insect pests, bacterial and fungal pathogens cause disease-induced crop damage, thus lowering the marketability of the crop (Duthie et al., 2007).

Synthetic pesticides are available, effective and labeled for controlling pests and diseases of Brassica. The HB is effectively reduced with conventional pesticides including pyrethroids (cypermethrin, cyhalothrin, cyfluthrin) (Edelson and Mackey, 2006a), and nicotinoids (imidiacloprid, acetamiprid, thiamethoxam) (Edelson and Mackey, 2006b). For control of the YMLB, carbamates (carbaryl) and organophosphates (mevinphos) are effective (Brees, 2007). Other insects, including the green peach aphid, cabbage looper, and diamondback moth, are effectively controlled by pyrethroids and nicotinoids (Edelson and Mackey, 2005; Walgenbach and Schoof, 2006). In addition, studies indicate that spinosad may provide sufficient control of the HB and other important pests on heading and non-heading Brassica (Overall et al., 2007; Walgenbach and Schoof, 2006).

Harlequin bug, *Murgantia histrionica*

The harlequin bug, *Murgantia histrionica* (Hahn), (Heteroptera: Pentatomidae) was first reported in Texas and Louisiana in the late 1800's (Walsh, 1866). The harlequin bug (HB) has been considered a pest of Brassica crops starting as early as the 1890's with L. O. Howard's USDA circular no. 10 (1895). HB adults are typical shield-shaped stink bugs with distinctive reddish to orange and black coloration (Chittendon, 1908). The nymphs resemble the adults in coloration but they lack wings and require five molts before maturity. The eggs are laid in groups of 12, on average, and each egg has distinct black markings on a white background (Streams and Pimentel, 1963; Canerday, 1965; Chittenden, 1908). Paddock (1918) reported six instars for this insect; however, this is the only account of an additional instar and could be attributed to the fact that he is the sole investigator to study the biology of the HB under field conditions.

In general, the entire life cycle of the HB ranges from 30 to 50 days depending on temperature (Canerday 1965; Chittenden 1908) while it has been reported that females lived over 80 days (Streams and Pimentel 1963). The egg stage existed for seven days under a controlled temperature of 22 ºC. Under the same temperature, the first instar is reported to exist for four days, the second exists nine days, followed by eight, nine, and 15 days for the third, fourth, and fifth instars, respectively (Streams and Pimentel, 1963). When the temperature is increased to 25 ºC, the first instar exists three days and the fifth exists 13 days (Canerday 1965). Chittenden, (1908) and his colleagues studied the biology of the HB under office conditions of 20 to 21 ºC and reported the egg stage to exist for 11 days.

The HB is multivoltine, i.e. multiple generations per year, with more generations per year in warmer climates. It is known to survive warm winters in the south with diapause or inactivity occurring in extremely cold winters. The HB adults will over-winter within grasses or cabbage and emerge in early spring. Upon becoming active again the HB adults move to wild Brassica to begin their first generation (Chittendon, 1908; Paddock, 1918).

The HB preferentially consumes both cultivated and wild *Brassica* sp. However, this pest has been observed to feed on potatoes, pigweed, ragweed, okra, orange, cowpeas, squash, and grapes (Chittenden, 1908; Paddock, 1918).

The HB has been considered void of significant predation (Howard, 1895), possibly due to its warning coloration (Paddock, 1918), secretion of chemicals (Aliabadi et al., 2003), or uptake of glucosinolates (distasteful chemicals) from the plants it feeds upon (Aldrich et al., 1996). There have been two egg parasitoids reported, *Trissolcus murgantiae* Ashm. (Hymenoptera: Scelionidae) and *Oöencyrtus johnsoni* How. (Hymenoptera: Encyrtidae) in Texas, North Carolina, Virginia, and Georgia (Chittendon, 1908; Huffaker, 1941; Ludwig and Kok, 1998; Miller, 1971). The current investigators have further observed two egg parasitoid species in southeastern Oklahoma and a polyphagous predaceous stink bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) feeding on early instars. One of the egg parasitoids was identified as *Trissolcus brochymenae* Ashmead (Hymenoptera: Scelionidae) by Matthew Buffington, Research Entomologist, Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture.

The HB is a pest of cultivated Brassica that include collards, turnips, and cabbage. It damages the leafy portion through its feeding behavior of these crops resulting in unmarketable fresh produce and contaminated product for the processing industry.

The HB is effectively controlled with synthetic insecticides, including pyrethroids and nicotinoids (Edelson and Mackey, 2006a, Edelson and Mackey, 2006b). However, when systems are void of synthetic inputs the HB will occur in large numbers and will lower marketability of the crop.

Yellowmargined leaf beetle *Microtheca ochroloma*

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål. (Coleoptera: Chrysomelidae) is native to South America and was first reported in the U. S. in Louisiana during the mid-1900's (Chamberlin and Tippins, 1948). The species is a significant pest of *Brassica* sp, such as mustards and turnips, especially in organic cropping systems (Bowers, 2003). It is widely distributed in the U.S. where Brassica crops are grown (Ameen and Story, 1997)

The yellowmargined leaf beetle (YMLB) adults are black to brownish with yellowish markings along the distal margins of the elytra and have a reported size of 5 by 2.5 mm (Chamberlin and Tippins, 1948). The eggs are yellow-orange and extremely ovulate deposited singly or in groups on "plant stems" or "under fallen leaves or on the soil surface." The larvae are brownish with typical campodieform features (Bowers, 2003). Under a temperature of 27 ± 1 ºC the entire life cycle of the YMLB requires 22 to

23 days with eggs hatching in five days after oviposition. Under the same temperature, the first instar exists for three days, the second instar exists for four days, and the third instar exists for five to six days and spins the cocoon the final day using “anal” excretions. The pupal instar exist for five to six days with the young adults occupying the light to dark brown “course-mesh fibrous” cocoon until they darken and hardened, which takes a couple of days (Oliver and Chapin, 1983).

YMLB’s live longer and are more productive when they consume mustard or turnips compared to when they consume collards or cabbage. Both larvae and adults feed on Brassica foliage (Ameen and Story, 1997).

Adults aestivate in the hot summer months, emerging in October (Brees, 2007). Bowers, (2003) recovered aestivating adults in Florida from a field. After exposing these adults to shorter day length, they began to reproduce and then oviposit eggs. From these studies and other observations, it can be surmised that the YMLB “over-summers” or enters a quiescent state in the adult stage during the summer months. In general, diapause behavior is unknown.

The YMLB has not been a serious pest possibly due to traditional crop pest control programs that use effective synthetic insecticides. In Florida, this beetle has become a major concern for organic farmers (Bowers, 2003). In Texas, the YMLB has recently become a pest to growers of mustards, turnips, Chinese cabbage, and collards (Brees, 2007). In Georgia, the YMLB has been known to infest mustards (Adams, 2000). Although there are no reports of natural enemies in the literature, the current investigators observed a predaceous stink bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae), feeding upon both adults and larvae of the YMLB.

The YMLB is successfully controlled using synthetic insecticides (Brees, 2007); however, in organic cropping systems this pest has potential to occur at damaging levels (Webb, 2006).

Insecticides

In the present study three Organic Materials Review Institute, (OMRI™), approved insecticides were evaluated for HB and YMLB control. These insecticides included the botanically derived neem [Neemix® 4.5], the microbial derived macrocyclic lactone, spinosad [Entrust® Naturalyte® Insecticide], and botanically derived pyrethrum [Pyganic® EC 1.4].

Pyrethrum

Pyrethrum is the general term that describes the natural substance extracted from the fruiting structures of *Chrysanthemum cinerariaefolium* Vis. flowers. The chemicals that constitute pyrethrum are pyrethrin esters (Crombie and Elliott, 1961) and are cinerin I and II, pyrethrin I and II, and jasmoline I and II (Crombie et al., 1976). Each ester is characterized by a three-member ring linked to a five-member ring by an ester (Holmstead and Soderland, 1978). Each is capable of producing multiple stereoisomers (Crombie and Elliott, 1961).

The physical effects of pyrethrum on insects are characterized by a flux of uncontrolled nerve impulses followed by “decreased excitability and fatigue” (Burt and Goodchild, 1971). The flux causes a quick “knockdown” of the insect followed by death when sufficient insecticide is applied. When the amount of insecticide is not enough to elicit death, the insect is able to recover. It is speculated that pyrethrum acts on insects' nerves (Camougis and Davis, 1971). More specifically, pyrethrum compounds "block voltage-gated sodium channels in nerve axons" causing the symptoms discussed above (Isman, 2006).

The breakdown of pyrethrins is temperature dependent in crop storage systems (Atkinson et al., 2004). Further, pyrethrins have a half-life less than 24 hours and residues may fall to undetectable levels shortly after application (Angioni et al., 2005).

Recent studies have found pyrethrum to be effective in controlling a Dipteran pest of blueberries (Barry et al., 2005). Other studies have found pyrethrum to have deterrent properties towards the sweetpotato whitefly (Toscano et al. 1997) and pyrethrum applications provided control of *Dysaphis* aphids in apple orchards (Kehrli and Wyss, 2001). Simmonds et al., (2002) found pyrethrum to be detrimental to a glasshouse whitefly and its parasitoid.

Azadirachtin

The active component of neem, azadirachtin, is derived from seeds of the neem tree *Azadiracta indica* A. Juss (Butterworth and Morgan, 1971). Neem tree derivatives are rich in proteins and numerous other chemicals whose structures have been isolated but are new to science. One such group is tetracyclic triterpenoids that include the well-known azadirachtin (AZA). Other novel compounds similar to AZA include nimbin, nimbinin, meliantriol, azadirachtol, salannin, and azadirone that are all characterized by multiple ring structures (Koul et al., 1990). Analyses of stability of these compounds concluded that salannin and nimbin are more stable than AZA when heat treated. In addition AZA is most stable at pH 6 with stability decreasing with increasing alkalinity. Storage of AZA is possible in organic solvent. The half-lives of AZA vary from 11 hours to 6 days in water and methanol, respectively (Jarvis et al., 1998).

The physical insecticidal actions of AZA include deterrence of feeding and action on the development of insect growth. The actual mode of action of feeding deterrence is largely unknown. The action on development of insects is better understood and the effects seen are inhibition and aberration of the molting process (Mordue and Blackwell, 1993). A current study concludes that AZA acts on *Ostrinia furnacalis* fat bodies "by interfering with its protein synthesis and secretion ability and finally indirectly regulating lipid metabolism" (Huang et al., 2007).

AZA has been shown to disrupt development in the insect order Thysanaptera (Premachandra et al., 2005) and to provide full control of insects in orders Heteroptera (Abudulai et al., 2003; Mitchell et al., 2004), Coleoptera (Weathersbee et al., 2002), and Diptera (Barry et al., 2005). In addition AZA does not harm parasitoids (Mitchell et al., 2004; Simmonds et al., 2002) or predators (Abudulai et al., 2004; Medina et al., 2004). Further, AZA does not negatively effect pollination of crops by the honey bee, *Apis mellifera* L. (Elzen et al., 2004).

Feeding deterrence has been demonstrated in Coleoptera ((Musabyimana et al., 2001; Showler et al., 2004), Heteroptera (Riba et al., 2003), Homoptera (Toscano et al., 1997). However, adult Lepidoptera pests were not deterred from oviposition on cabbage (Liu and Liu, 2006).

Although neem may be relatively safe for some natural enemies, one study suggested that AZA caused significant mortality to a parasitoid and predators

(Neuroptera and Coleoptera) of a Dipteran pest while controlling that pest (Li et al., 2003). Another study found that ingested neem negatively affected aphid predators in orders Coleoptera, Diptera, and Neuroptera (Ahmad et al., 2003). Studies have also shown that freshwater invertebrates may be susceptible to neem (Kreutzweiser et al., 2002; Kreutzweiser et al., 2004; Thompson et al., 2004).

Spinosad

The bacterium *Saccharopolyspora spinosa* was first isolated from a soil sample collected from an abandoned still in the Virgin Islands (Mertz and Yao, 1990). From this soil bacterium a fermentation product of its metabolism was isolated and was proven to be insecticidal. The compound is known as spinosad (Thompson et al., 2000). "SpinosAD" refers to spinosyns A and D that constitute this compound. They are classified as macrocyclic lactones due to the lactone present in their structures (Crouse et al., 2001). In addition to the most abundant insecticidal components, spinosyn A and D, there are spinosyns C through Y. A number of these exhibit insecticidal activities along with synthesis of spinosyn-like compounds (Sparks et al., 2001)

The physical effects of spinosad on insects are characterized by a nervous system boost, "leading to involuntary muscle contractions, prostration with tremors, and paralysis" (Thompson et al., 2000). The biochemical background to this involves alteration of the function of *gamma*-aminobutyric acid-gated and nicotinic receptors on insect neurons (Salgado et al., 1998; Sparks et al., 2001; Watson, 2001). Up until now, the precise mechanism of spinosad action on these receptors is uncertain (Sparks et al., 2001).

Spinosad formulations have been shown to be effective in controlling numerous Lepidopteran larvae (Pineda et al., 2004; Wanner et al., 2000; Wanner et al., 2002) including common pests of Brassica crops, such as the cabbage looper (Liu et al., 1999). Additionally, spinosad has been shown to provide control of pests in the orders Coleoptera, Thysanoptera, Hymenoptera, Isoptera, and Diptera (Blanc et al., 2004; Bret et al., 1997; Ludwig and Oetting, 2001; Vargas et al., 2003). Recently, spinosad has been shown to be effective in controlling stored grain pests (Blanc et al., 2004; Daglish and Nayak, 2006).

To determine if spinosad is safe for non-target insects, studies have been conducted to determine if spinosad adversely affects the activity and livelihood of honeybees that are essential pollinators of food plants. Honeybees are highly susceptible to spinosad when they come into contact with or ingest it. However, contact with or ingestion of dried residues of spinosad is less toxic to honeybees (Mayes et al., 2003; Miles et al., 2002). In addition, Morandin et al., (2005) found that spinosad did not adversely affect bumble bees, *Bombus* sp. when recommended spray rates were used.

With respect to all other non-target insects, results vary depending upon application method, and species of natural enemy. Throughout the literature, over fifty different species, including both predators and parasitoids, were analyzed. Of those, 71% of the predators were not harmed by spinosad in laboratory assays. These include species in orders Coleoptera, Hemiptera, and Neuroptera. However, Dermapteran predators and Hemiptera, *Podisus* sp., were moderately harmed by spinosad. Spinosad was more toxic to parasitoids compared to the predators with over 75% of Hymenopteran parasitoids harmed by spinosad applications (Williams et al., 2003).

Environmental conditions affect activity of insecticides. Cleveland et al., (2002) found spinosyns decompose when exposed to sunlight with a half-life of less than 24 hours. Further, spinosyns decomposed in salt and fresh waters with half-lives of a couple of hours in direct sunlight (Liu and Li, 2004).

Both the YMLB and HB are effectively controlled using synthetic insecticides; however, there exists little to no studies evaluating control of these pests for organically produced leafy greens in the south central plains. Because of the absence of studies pertaining to organic control of the YMLB and HB, we initiated this study.

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CHAPTER III

TOXICITY OF THREE ORGANIC REGISTERED INSECTICIDES TO THE HARLEQUIN BUG, *MURGANTIA HISTRIONICA* (HAHN), AND YELLOWMARGINED LEAF BEETLE, *MICROTHECA OCHROLOMA* STÅL

Abstract

The harlequin bug, *Murgantia histrionica* (Hahn) (Heteroptera: Pentatomidae), and yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chysomelidae), are important pests of Brassica crops that include turnips, collards, broccoli, and cauliflower in the eastern U. S. Both are effectively controlled with synthetic insecticides including pyrethroids, nicotinoids, carbamates and organophosphates. Currently, organic growers rely on cultural and mechanical methods to control both pests. The following study was undertaken to estimate a concentration-response for three organic insecticides used to manage populations of the harlequin bug and yellowmargined leaf beetle feeding on collards and turnips. Leaf dip bioassays were used and mortality was recorded at 24 and 48 hours after treatment. After 48 hours, spinosad was more effective in inducing mortality in the harlequin bug and yellowmargined leaf beetle than was pyrethrum.

Introduction

Estimation of concentration-response or dose-response for pesticides is invaluable to biologists and provides information that enables them to estimate safe, effective concentrations for field application rates (Cordero et al., 2007). It also helps biologists track insecticide resistance in insect populations (Prabhaker et al., 2006; Snodgrass et al., 2005; Willrich et al., 2003; Zhao et al., 2002). A dose-response is also known as a "quantal' or 'all-or-nothing" response that describes an outcome of a test subject that has been exposed to a measurable toxin or toxicant (Finney, 1971). Robertson and Preisler (1992), refer to this biological assay as a "binary response with one explanatory variable."

In order for accurate estimation of lethal insecticidal concentrations or doses, at least 120 samples are necessary. Further, insect test subjects must be standardized to reduce experimental variation and selection of subjects for treatments must be random (Robertson et al., 1984). Prior to the design of software, such as PoloPlus, exhaustive mathematical analyses were required to estimate dose-mortality responses (Finney, 1971). Along with PoloPlus and its previous versions, other statistical analyses programs that are regularly implemented include Generalized Linear Interactive Modeling (GLIM) and SAS (Pineda et al., 2007; Robertson and Preisler, 1992; Snodgrass et al., 2005). The various methods have minimal variations in output "except at the extreme ends of the probability distribution" (Robertson and Preisler, 1992). The "extreme ends" refer to the LC_{99}, for instance, that is essential in quarantine programs to ensure zero tolerance for pest occurrence.

The methodology of dose-response relationships involves a regression analysis to create a fit of the mortality as a function of the dose. In order to better interpret the data, the x-axis (dose) is converted to a logarithmic scale producing a sigmoid curve. In order to achieve a straight line, the y-axis is converted to probit or logit units assuming a normal distribution (Robertson and Preisler, 1992).

There are numerous methods of application of a dose to measure toxicity of a substance, including direct injection of insecticide into the insects or insect contact with an insecticide-coated surface (Prabhaker et al., 2006; Robertson and Preisler, 1992; Tillman, 2006). Studebaker and Kring, (2003) hypothesize that insect mortality may result from the application method of the insecticide or toxicant, such as direct injection into the insect. Further, contact bioassays involving application of insecticide on glass vial or Petri dish may not represent actual field conditions. Commonly, assays will compare methods, such as leaf-dip and glass-vial methods, to estimate insecticide-induced responses in insects (Prabhaker et al., 2006). Finally, a commonly used method that better mirrors that occurring in the field is leaf dip bioassays (Studebaker and Kring, 2003).

The current investigation utilized leaf dip bioassays to estimate $LC_{50,90}$ values for three organic registered insecticides used to induce mortality of the HB and YMLB feeding on leafy greens. The results will establish baseline toxicity data for the tested insecticides and insects.

Materials and Methods

Insecticides

The insecticides used were Organic Materials Review Institute (OMRI®) Listed and were obtained formulated as follows: Microbial derived spinosyns A and D (Entrust® Naturalyte® Insect Control, Dow AgroSciences LLC, Indianapolis, IN); botanically derived IGR, azadirachtin, (Neemix 4.5®, Certis USA, LLC, Columbia, MD); and botanically derived pyrethrins (Pyganic® Crop Protect. EC 1.4, McLaughlin Gormley King Company, Minneapolis, MN).

Insects

Harlequin bugs, *Murgantia histrionica* (Hahn), used in the laboratory studies were collected from untreated collard fields in April 2006 and 2007 and insects were caged in wooden boxes with mesh-covered openings in a laboratory maintained at 30 ± 1 °C and 68 ± 8% relative humidity (RH) under 16-h photoperiod at the Wes Watkins Agriculture Research and Extension Center, Lane, OK. Bugs were supplied fresh collard and turnip plants as needed. In both years, nymphs were also obtained from untreated collard and turnip plants in the field to supplement those obtained from the laboratory colonies.

In summer 2007, large numbers of leaf beetles were observed consuming entire turnip plants and were identified by the Plant Disease and Insect Diagnostic Laboratory at Oklahoma State University to be the yellowmargined leaf beetle, *Microtheca ochroloma* Stål. Due to their abundance and availability, this beetle was included in the toxicology studies and adult females were collected from untreated collard plants as needed.

Voucher specimens for both the YMLB and HB were collected and deposited in K.C. Emerson Entomology Museum, 127 Noble Research Center, Oklahoma State University.

Bioassays

To estimate $LC_{50,90}$ values for the insecticides, leaf dip bioassays were used. For the HB study, leaf disks with an area of 3.14 cm^2 were cut from collard leaves, *Brassica oleraceae* L. (variety *acephala* “Champion”), with average size of 11 cm by 14 cm. For the YMLB, turnip leaf disks, *Brassica rapa* L., (“Topper”), of the same area were taken from leaves with an average size of 9 cm by 17 cm. Leaf disks were then placed for three seconds in the prepared insecticide concentrations and then removed and placed on a clean paper towel to allow drying at 20 ºC for 30 minutes.

Prior to each treatment application, HB fourth instars and adult YMLB females were individually placed in 29.6 ml Dixie® cups for 24 hours. The insects received no food during the 24 hours. Ten HB’s or YMLB’s were randomly selected for each treatment and the trials were replicated three times. The insects were then placed on the treated collard or turnip leaf disks which in turn were placed with the abaxial surface down upon 10 ml of nutrient agar medium within 29.6 ml Dixie® cups. Insects were kept at 30 ± 1 ˚C and 68 ± 8% RH under 16-h photoperiod and were observed at 24 and 48 hours. They were noted as dead if they were unable to stand upright and coordinate in a normal manner after slight touch with a dissecting probe (Snodgrass et al., 2005). Evidence of feeding and wet weight of treatment groups were noted after 48 hours.

The initial concentrations applied to both the YMLB’s and HB’s were prepared by serial dilutions of insecticides with water to concentrations of 10,000, 1,000, 100, 10, 0 ppm active ingredient (AI). From the results of each initial assay, we determined a more narrow range of concentrations within which to examine more closely (Tables 3.01 and 3.02). However, azadirachtin at full strength (45,000 ppm AI) was required to induce 90% mortality in HB's and was therefore excluded from further replicates and was excluded from YMLB bioasssays.

Analysis

Concentration-response data were analyzed using the computer software program PoloPlus (LeOra Software, 2003). Natural response was selected as a parameter when mortality in controls occurred, along with transformation of concentrations to logarithms using the probit mathematical model. Assays were significant if the t-ratio was greater that 1.96 ($P < 0.05$). Further the data fit the model if the heterogeneity (χ^2/df) was less that one.

Results and Discussion

Harlequin bug

The initial $\log_{10}$ concentrations for estimating $LC_{50,90}$ values for spinosad, pyrethrum, and azadirachtin against the harlequin bug were 10,000, 1,000, 100, 10, and 0 ppm active ingredient (AI) (n=120). The results were used to determine a narrow range of concentrations within which to look at effects and were: 1,500, 1,000, 100, 10, 1, and 0 ppm AI (n=150) for spinosad. The estimated LC_{50} was 184 ppm AI (95% Confidence Interval (CI): 89 - 390) and LC_{90} was 2,444 ppm AI (95% CI: 951 - 15,228) with a slope of 1.14 ± 0.17 and chi-square (χ^2) value of 18.54 with 13 degrees of freedom (df). This assay was significant ($P < 0.05$), however, did not fit the probit model.

For pyrethrum, narrow concentrations were: 8,000, 6,000, 5,000, 4,000, 3,000, 2,000, 1,000 and 1 ppm AI (n=190). The estimated LC_{50} was 2,964 ppm AI (95% CI: 2,484-3,517) and the LC_{90} was 8,615 ppm AI (95% CI: 6,609-13,179) with slope of 2.77 ± 0.38 and chi-square value of 15 with 17 df.

For azadirachtin, the narrow concentrations were: 45,000, 35,000, 25,000, 15,000, 0 ppm AI (n=80) (Table 3.01).

Table 3.01 Concentration-mortality data for fourth instar harlequin bugs exposed to spinosad, pyrethrum, and azadirachtin (AZA) (LC values are ppm AI, CI = Confidence Interval)

Insecticide	n	slope ± SE	LC_{50} (95% CI)	LC_{90} (95% CI)	$\chi 2$ (df)
Spinosad	150	1.14 ± 0.17	184 (89 - 390)	2,444 (951 - 15,228)	18.54 (13)
Pyrethrum	190	2.77 ± 0.38	2,964 (2,484 - 3,517)	8,615 (6,609 - 13,179)	15.0 (17)
AZA	80	9.92 ± 3.45	36,343 (28,910 - 40,627)	48,940 (43,066 - 82,814)	0.54 (2)

Results obtained in this study indicated that AZA was not effective in inducing mortality of the harlequin bug using leaf dip bioassays. A study by Riba et al. (2003), which involved topical application of AZA to an economically important Pentatomid, *Nezara viridula* L., concluded that AZA at high doses, 200 to 500 ng/insect, induced mortality in nymphs, caused aberrations to normal moulting resulting in death, or resulted in adults with deformities such that nymph-like characteristics were retained. However, lower applied doses, 2 and 50 ng/insect, of AZA to this stink bug did not induce large percentages of mortality. In the present study, 100% mortality was observed with the undiluted formulation of AZA and the lower concentrations caused deformities in the adults. Further, an insect in the order Hemiptera, *Clavigralla scutellaris,* was dipped in AZA and the estimated LC_{50} was 220 ppm AI (Mitchell et al., 2004). This LC_{50} value is noticeably lower than observed in the present study, which is 36,000 ppm AI. The difference in results could be attributed to differences in application method of insecticide. It is possible that physically dipping the insect in the toxin allows for greater contact with insect surface area, including entry through spiracles, thus resulting in greater mortality. In addition to variability from application method, a study concluded that different species in the family Pentatomidae vary in their mortality response to the same synthetic insecticide (Willrich et al., 2003).

Comparing the toxicity of organic insecticides with synthetic insecticides, the LC_{50} estimated for the adult stink bugs treated with a pyrethroid (Permethrin) and organophosphate (Malathion) were 9.28 and 19.5 mg/vial (1 ppm=1 mg/kg=1 mg/L), respectively (Snodgrass et al., 2005). Although the investigators used glass vial bioassays, the LC_{50} values are lower than estimated for pyrethrum and azadirachtin from the present study.

Comparing results obtained from pyrethrum and spinosad in Table 3.01, spinosad has much lower $LC_{50,90}$ values than that of pyrethrum used to induce mortality in HB's. This suggests that spinosad is more toxic than pyrethrum to HB's in leaf-dip bioassays.

Yellowmargined leaf beetle

Now discussing the YMLB, the initial $\log_{10}$ doses for estimating $LC_{50,90}$ values for spinosad and pyrethrum to induce mortality were 10,000, 1,000, 100, 10, and 0 ppm AI (n=120). The results were used to determine a narrow range within which to look at effects. The narrow concentrations of spinosad used to induce mortality in YMLB were: 32, 16, 8, 4, 2, 0 ppm AI (n=150). The estimated LC_{50} was 1.80 ppm (95% CI: 0.22-3.30) and the LC_{90} was 11 ppm (95% CI: 7-64) with slope of 1.59 ± 4.52 and chi-square of 23.74 with 15 df (Table 3.02).

For pyrethrum, the narrow concentrations used to induce mortality in YMLB were: 2,000, 1,500, 1000, 500, 100, 0 ppm A.I. (n=200). The estimated LC_{50} was 224 ppm (95% CI: 162-290) with slope of 2.32 ± 0.28 and chi-square equal to 12.77 with 18 df (Table 3.02).

Table 3.02 Concentration-mortality data for yellowmargined leaf beetles exposed to spinosad and pyrethrum (LC values are ppm AI, CI=Confidence Interval)

Insecticide	N	slope ± SE	LC_{50} (95% CI)	LC_{90} (95% CI)	$\chi 2$ (df)
Spinosad	150	1.59 ± 4.52	1.80 (0.22 - 3.30)	11 (7 - 64)	23.74 (13)
Pyrethrum	200	2.32 ± 0.28	224 (162 - 290)	801 (610 - 1,145)	12.77 (18)

A leaf dip bioassay study of spinosad applied to the eggplant flea beetle, *Epitrix fuscula* (Chrysomelidae), indicated that the LC_{50} and LC_{90} values estimated were 9.8 and 65.4 ppm AI, respectively (McLeod et al., 2002). Although the values were slightly higher, they are similar to those obtained in the present study.

Azadirachtin toxicity was not evaluated against the YMLB. This decision was based upon results obtained from previous studies with the HB. Further studies should be conducted to evaluate the toxicity of AZA on Chrysomelidae pests similar to the YMLB. Azadirachtin (Neemix 4.5) is labeled as a contact and stomach poison (specimen label) and may provide adequate control of the leaf beetle since it would consume the toxin.

Comparing the results from the YMLB and HB toxicology studies, differences exist between amounts of toxin required to induce mortality of 50% test subjects (Tables 3.01 and 3.02). As indicated in Table 3.01, the estimated LC_{50} value of pyrethrum inducing mortality in the HB is 2,964 ppm AI, whereas 224 ppm AI pyrethrum induced 50% mortality in YMLB. The estimated LC_{50} value of spinosad inducing mortality in the HB is 184 ppm AI, while the LC_{50} estimate was 1.80 ppm AI spinosad inducing mortality in YMLB's. The differences are great and might be explained by the different feeding behaviors of the two pests. For instance, the HB pierces plant tissue to extract the plant nutrients and therefore contact toxicity would prevail in the cause of mortality whereas the YMLB chews and consumes leafy plant material, therefore directly ingesting the toxicant. This hypothesis needs evaluation through comparison of results from HB's that have ingested a known amount of toxin or toxicant with YMLB that have been exposed to the toxin using glass-vial bioassays.

Conclusion

In summation, spinosad proved to be more effective in inducing mortality to HB's than either pyrethrum or azadirachtin. However, pyrethrum is the sole insecticide in this study specifically labeled for control of the HB. The $LC_{50,90}$ values estimated for the HB treated with spinosad were 184 ppm AI (95% CI: 89 - 390) and 2,444 ppm AI (95% CI: 951 – 15,228), respectively. For pyrethrum, the $LC_{50,90}$ values were 2,964 ppm AI (95% CI: 2,484-3,517) and 8,615 ppm AI (95% CI: 6,609-13,179), respectively. Both regression analyses were significant ($P < 0.05$) signifying presence of a linear dose-response. However, the spinosad assay does not fit the probit model.

Likewise, spinosad and pyrethrum were effective in inducing mortality of the YMLB. The estimated $LC_{50,90}$ values for spinosad inducing mortality were 1.80 ppm AI (95% CI: 0.22-3.30) and 11 ppm AI (95% CI: 7 - 64), respectively. For pyrethrum, the $LC_{50,90}$ values were 224 ppm AI (95% CI: 162 – 290) and 801 ppm AI (95% CI: 610 - 1,145), respectively. Although both regression analyses are significant ($p < 0.05$), the spinosad assay does not fit the probit model.

These results will be used to compare with efficacy of field application rates to control the HB and YMLB on collards and turnips in southern Oklahoma and to establish baseline toxicity data for organic insecticides against the YMLB and HB.

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CHAPTER IV

FIELD EVALUTION OF THREE ORGANIC REGISTERED INSECTICIDES TO CONTROL THE HARLEQUIN BUG, *MURGANTIA HISTRIONICA* (HAHN), AND YELLOWMARGINED LEAF BEETLE, *MICROTHECA OCHROLOMA* STÅL, ON LEAFY GREENS IN SOUTHERN OKLAHOMA

Abstract

Research was conducted over two successive years to compare effectiveness of registered organic insecticides for managing leafy green pests in the southern United States. Two major pests are the harlequin bug (HB), *Murgantia histrionica* (Hahn), (Heteroptera: Pentatomidae) and the yellowmargined leaf beetle (YMLB), *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae). These insects were chosen as models because they have different feeding behaviors. The YMLB feeds by chewing leafy material while the HB uses a piercing-sucking feeding method. Treatments evaluated were Organic Materials Review Institute Listed® and included Neemix® 4.5, Entrust® Naturalyte® Insecticide, and Pyganic® EC 1.4 at 0.73 L (AI)/ha, 207.5 g (AI)/ha and 4.68 L (AI)/ha, respectively. Field trials were conducted at the Wes Watkins Agricultural Research and Extension Center, Lane, OK. Plots of turnips and collards were treated using an all-terrain vehicle (ATV) mounted sprayer. In 2007, results indicated that

Entrust and Pyganic significantly reduced YMLB adult numbers 96% and 63%, respectively, compared to the untreated plots one day post treatment. In 2006, Entrust and Pyganic significantly reduced HB 61% and 73%, respectively, compared to the untreated plots one day post treatment. These results are being used to develop IPM strategies for producing organically grown leafy greens.

Introduction

Recently, the USDA created the National Organic Program (NOP) as part of the Organic Foods Protection Act (OFPA) of 1990 (Public Law 101-624, 1990). The NOP allows organic producers to become certified and sets standards that allow for organic labeling. Regulations require non-synthetic materials in the production and handling of products for organic certification and labeling. Synthetics include any chemicals or materials that are not derived from unmodified biological organisms or products thereof or materials that have been chemically transformed in some way, such as radiation treatment (NOP, 2007).

These guidelines restrict the use of synthetic insecticides, such as pyrethroids, nicotinoids, and carbamates that have efficiently controlled pests in vegetable crops including leafy greens or Brassica crops (Brees, 2007; Edelson and Mackey, 2006a; Edelson and Mackey, 2006b). Pest management in organic cropping systems vary from that seen in traditional practices, such that use of organic insecticides are considered an indication of unbalances in the natural processes that would otherwise control or reduce pest outbreaks. However, pest control measures are necessary under circumstances of sporadic pest outbreaks to save the marketable portion of the crop.

Leafy green vegetables are grown throughout the United States and include spinach, lettuce, collards, turnips, mustards, and broccoli. Although these crops have been grown for decades, there has been a general increase of the production and sales of leafy greens, speculated due to greater awareness of their nutritional value (Lucier, 1998). Brassica crops include both heading and non-heading cultivars and common insect pests are diamondback moth larvae, cabbage loopers, aphids, imported cabbage worm, and harlequin bug (Kahn et al., 2007; Motes et al., 2007). Flea beetles and yellowmargined leaf beetles are pests of the non-heading Brassica varieties including collards and turnips (Adams, 2000; Sanders, 2000).

Synthetic insecticides are available, effective and labeled for controlling pests and diseases of Brassica. The harlequin bug (HB) is effectively reduced with conventional pesticides including pyrethroids (cypermethrin, cyhalothrin, cyfluthrin) (Edelson and Mackey, 2006a), and nicotinoids (imidiacloprid, acetamiprid, thiamethoxam) (Edelson and Mackey, 2006b). For control of the yellowmargined leaf beetle (YMLB), carbamates (carbaryl) and organophosphates (mevinphos) are effective (Brees, 2007). Other insects, including the green peach aphid, cabbage loopers, and diamondback moths, are effectively controlled by pyrethroids and nicotinoids (Edelson and Mackey, 2005; Walgenbach and Schoof, 2006).

The harlequin bug (HB), *Murgantia histrionica*, and the yellowmargined leaf beetle (YMLB), *Microtheca ochroloma*, are important pests of Brassica crops in North America. In conventional leafy green production, pests are managed through use of synthetic insecticides including pyrethroids, carbamates, and organophosphates. However, both are potentially serious pests in organic cropping systems that are void of synthetic insecticides. Currently, organic producers have no reliable method of managing populations of these pests. This study was undertaken to compare efficacy of three organic registered insecticides at the recommended field rates to control the HB and YMLB on leafy greens in southern Oklahoma**.**

Materials and Methods

Insecticides

The insecticides used were Organic Materials Review Institute (OMRI®) Listed and were obtained formulated as follows: Microbial derived spinosyns A, D (Entrust® Naturalyte® Insect Control, Dow AgroSciences LLC, Indianapolis, IN), 207.5 g [AI]/ha; botanically derived IGR, azadirachtin, (Neemix® 4.5, Certis USA, LLC, Columbia, MD), 0.73 L [AI]/ha; and botanically derived pyrethrins (Pyganic® Crop Protect. EC 1.4, McLaughlin Gormley King Company, Minneapolis, MN), 4.68 L [AI]/ha. The insecticides were prepared using the labeled application rates for cole crops or leafy greens and were diluted in deionized water. The application rates determined for the sprayer were 329 and 346 liters per hectare for 2006 and 2007, respectively. The total spray volume was determined through successive measurements of each nozzle output under controlled conditions.

Field Plots

Collard, *Brassica oleraceae* L. (variety *acephala* “Champion”) and turnip, *Brassica rapa* L., (“Topper”) were direct seeded using a tractor mounted planter with a 3.2 cm (7.6 cm in 2007) spacing on raised beds with 91 cm centers 14 March 2006 and 19 March 2007 at the Wes Watkins Agricultural Research and Extension Center, Lane, OK. The plots were pre-treated with the herbicide, Treflan® E.C., at 1.2 liters per hectare and were fertilized with 17:17:17 % nitrogen-phosphorous-potassium ($N-P_2O_5-K_2O$) at 673 kg per hectare.

The experimental design was a complete randomized block with four treatments and six replicate blocks. Plots were 1.83 m wide and 6.1 m in length with minimum of 6.1 m alleys and plots were comprised of one row of collards and one row of turnips. Treatments were Pyganic® EC 1.4 at 4.68 L/ha, Entrust® at 207.5 g/ha, Neemix® 4.5 at 0.73 L/ha, and a control treated with water. An ATV mounted sprayer with a single nozzle over the top of each row and nozzles on drops to each side of the row of plants was used to treat the plots. The sprayer was operated at 3.16 kg/cm^2 and delivered 329 and 346 liters per hectare for 2006 and 2007, respectively.

Plots were treated on 18 and 25 May, and 1 and 8 Jun 2006. In 2007, plots were treated 16 May, and 6, 13, and 25 June. For both years, plots were sampled 1, 4, and 6 days after treatment (DAT). Five collard and five turnip plants per plot were visually inspected for presence of yellowmargined leaf beetle (YMLB) adults, YMLB larvae, harlequin bug (HB) adults, HB nymphs, and HB egg masses. In 2007, large populations of YMLB, *Microtheca ochroloma* Stål were abundant, completely consuming turnip plots before the second insecticide application. Therefore, only collard plants were monitored in 2007. The leaf beetle was sent to the OSU Plant Disease and Insect Diagnostics Lab in Stillwater, OK where it was identified as *Microtheca ochroloma* Stål (yellowmargined leaf beetle).

Analysis

The design was a split-split plot in a randomized complete block and analyzed using PROC-MIXED. The first split factor was period and the second split factor was the period and day after treatment (DAT) interaction. Period in the analysis refers to the time following each of the eight insecticide applications across both years, i.g., the first period represents the time following the first insecticide application in 2006 and period eight follows the final insecticide application in 2007. Means were compared by pairwise t-tests and were protected by the slice option within the days after treatment (SAS institute, 2003). Voucher specimens of both HB and YMLB are deposited in the K. C. Emerson Entomology Museum, 127 Noble Research Center, Oklahoma State University.

Results and Discussions

The tables following report the results after the PROC MIXED analysis. The periods represent insecticide applications and range from period 1 through 8. The first four periods occurred in 2006 and period 5 through period 8 occurred in 2007. However, counts were not made 6 days after treatment (DAT) when insect numbers were evidently increasing. Only those insecticide application periods that resulted in significant reductions in insects are included. YMLB's were counted only in 2007. In addition heavy precipitation prevented counts 6 DAT in most treatment periods (Table 4.03, 4.04).

In 2006, spinosad significantly reduced HB adult numbers 75% compared to the control six DAT, period two (Table 4.01). The following period, spinosad significantly reduced HB adult numbers 61% compared to the control one DAT. Pyrethrum significantly lowered HB adults by 73% one DAT in period three compared to the control (Table 4.01).

Harlequin Bug Results

Table 4.01 Mean numbers of HB adults per 10 plants, 2006 (means followed by different letters are significantly different, P ≤ 0.05)

	Period			Period			Period		
	2			3			4		
	Days after Treatment			Days after Treatment			Days after Treatment		
Treatment	1	4	6	1	4	6	1	4	6
Control	1.89a	1.5a	2.57a	3.22a	2.07a	#	1.37a	4.23a	8.97a
Spinosad	0.6ae	0.54ac	0.63bc	1.25b	1.23a	#	1.27a	3.87a	9.07a
AZA	2.73,abd	1.97ab	2.47a	2.47a	2.13a	#	1.67a	5.17a	9.05a
Pyrethrum	0.87ac	1a	1.23ac	0.88b	1.5a	#	0.93a	4a	7.03a

Table 4.02 Mean number HB nymphs per 10 plants, 2006 (means followed by different letters are significantly different, $P \leq 0.05$)

	Period			Period			Period		
	2			3			4		
	DAT			DAT			DAT		
Treatment	1	4	6	1	4	6	1	4	6
Control	14.54a	12.98a	19.24a	18.92a	7.5a	#	7.1a	12.13a	20.53a
Spinosad	12.95a	11.65a	16.39a	10.72b	3.0a	#	2.43a	2.87b	8.8b
AZA	16.91a	16.95a	22.55a	14.93a	9.37a	#	9.1a	11.9a	15.97a
Pyrethrum	13.62a	17.4a	21.3a	20.62a	10.10a	#	7.53a	13.63a	16.47a

Spinosad significantly reduced HB nymphs 43% one DAT in period three compared to the control (Table 4.02). In period four, spinosad reduced HB nymphs 76% and 57% four and six DAT, respectively, compared to the controls (Table 4.02).

Yellowmargined Leaf Beetle Results

In 2007 (Table 4.03 and Table 4.04) the treatment of spinosad and pyrethrum mixed was evaluated to control both the YMLB and HB. There were no significant differences among treatments against both HB adults and nymphs observed. Insecticide mixtures may lower susceptibility of insects to develop resistance to insecticides and some insecticides may potentially act synergistically although antagonism may also be seen (Attique et al., 2006).

In 2007 (Table 4.03) results indicated that spinosad significantly reduced YMLB adult numbers 96% and 91% one and four DAT, respectively, in period six compared to the untreated plots. In the same period, pyrethrum significantly reduced YMLB adults 63% and 74% one and four DAT, respectively, compared to the control. In addition, pyrethrum, azadirachtin, and the mixture of pyrethrum and spinosad all significantly reduced YMLB adults both one and four DAT in period six compared to the control (Table 4.03).

Table 4.03 Mean number of YMLB adults per five plants, 2007 (means followed by different letters are significantly different, P ≤ 0.05)

	Period		Period		Period		Period	
	5 DAT		6 DAT		7 DAT		8 DAT	
Treatment	1	4	1	4	1	4	1	4
Control	1.75a	#	17.67a	51.63a	17.83a	29.8a	3.03a	#
Spinosad	1.32a	#	0.7b	4.63b	0.7c	6.21b	2.6a	#
Neem	1.78a	#	4.63b	13.97b	11.6ab	26.3a	3.7a	#
Pyrethrum	1.63a	#	6.9b	13.47b	6.23bc	21.3a	2.9a	#
Mixture	2.27a	#	3.57b	11.4b	0.7c	9.8b	2.13a	#

Table 4.04 Mean number of YMLB larvae per five plants, 2007 (means followed by different letters are significantly different, P ≤ 0.05)

	Period		Period		Period		Period	
	5 DAT		6 DAT		7 DAT		8 DAT	
Treatment	1	4	1	4	1	4	1	4
Control	0a	#	10.93a	15.57a	1.9a	0.17a	0a	#
Spinosad	0a	#	0.07b	0.2c	0a	0.07a	0a	#
Neem	0a	#	8.57a	3.17b	0.47a	0a	0a	#
Pyrethrum	0a	#	10.43a	2.4c	0.67a	0.03a	0a	#
Mixture	0a	#	0.57b	0.6c	0a	0a	0a	#

In period seven, pyrethrum significantly reduced YMLB adults 65% compared to the control one DAT. In the same period and one DAT, spinosad and the mixture reduced YMLB adult numbers 96% compared to the control. Four DAT, spinosad and the mixture reduced YMLB adults 79% and 67%, respectively, compared to the control (Table 4.03).

Significant differences among treatments and control were seen in period six only. Spinosad alone and the mixture of spinosad and pyrethrum significantly reduced YMLB larvae 99% and 94%, respectively, compared to the control one DAT. Four DAT, all treatments had significantly fewer HB larvae present than the control (Table 4.04). Further, an evaluation of efficacy of the same formulation implemented in the present study to control flea beetles (Chrysomelidae) similarly found that spinosad was more effective in reducing damage than both azadirachtin and pyrethrum (Anderson et al., 2006).

YMLB and HB have different feeding behaviors thus allowing differing modes of action of the insecticide to be compared. The YMLB chews and ingests insecticide treated plant tissues thus providing the active components faster entry into the insect. On the contrary, the HB feeds on internal leaf fluids and therefore, the HB is mostly poisoned through contact with residues from its mouthparts or other appendages. All insecticides significantly reduced YMLB adults in 2007; whereas, in 2006 HB adults were significantly reduced in the spinosad treated plots.

In organic cropping systems, the use of botanical or microbial control methods is warranted when all other methods, such as cultural and mechanical methods are ineffective. Mechanical methods such as row covers can cost $1000/acre for the material and this does not include the labor expense (Anderson et al., 2006). Unfortunately, the organic approved insecticides are expensive as well. The cost of applying spinosad can reach $81 per acre to control pests on Brassica crops. Pyrethrum can cost as much as $98 per acre and neem can cost as much as $65 per acre. For controling both the YMLB and HB, spinosad would be the most economical choice in terms of effectiveness.

Further, organic grown fruits and vegetables are more expensive than conventionally grown vegetables (USDA-ERS, 2007). The processing industry pays on average $95 per ton and the average harvest is eight to nine tons per acre (= $760/acre if 8 tons/acre are harvested) for conventionally grown produce destined for processing industry (Anonymous source, 2007). The value is $2,800 per acre for conventionally grown collards and turnips destined for the fresh market (Anonymous, 2007). Organic fresh produce will have a price premium as great as 30 percent over conventional grown fresh fruits and vegetables (Dimitri and Greene, 2002).

Conclusion

Concluding, in all spray periods AZA did not provide significant control of HB adults or nymphs compared to the controls. However, AZA significantly reduced YMLB adults 74% compared to the control one DAT in period six. In the same period and DAT, AZA reduced YMLB larvae by 21% compared to that control.

Pyrethrum provided more control of HB adults than that of AZA. In period three, one DAT, pyrethrum reduced HB adults 73% compared to that control. However, pyrethrum did not significantly reduce HB nymphs in any spray periods. Pyrethrum did provide control of YMLB adults and larvae.

Compared to AZA efficacy, spinosad provided more control of both HB and YMLB. In period three, one DAT, spinosad significantly reduced HB adults and nymphs by 61% and 43% compared to the controls. With regards to the YMLB, spinosad provided excellent control of both the adults and larvae. In period six, the spinosad treated plots had 96% and 91% fewer adult YMLB than the control one and four DAT, respectively. In the same spray period, spinosad treated plots had 99% fewer YMLB larvae compared to the control both one and four DAT.

Finally, a mixture of spinosad and pyrethrum was evaluated to control both the HB and YMLB on leafy greens. Data are only available for 2007 and the results from the HB counts were insignificant for the spray periods in 2007. In period six, one DAT, the mixture of spinosad and pyrethrum significantly reduced YMLB adults and larvae 80% and 95%, respectively, compared to the controls.

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CHAPTER V

DISCUSSION

The North American continent has led the world in consumers' demand for organic produce with over $14 billion in sales of organic products in the United States two years ago (Yussefi, 2006). In the near future, choices of organically produced foods will be available in fast food, dining, hospital, and school sectors (Haumann, 2006)

Why have organic agriculture and the subsequent products become popular in recent years? The answer to the increase in the number of certified organic production operations is evident; to meet the demands of consumers. However, the reasons consumers demand more "organic" foods and products are less evident. The consumers' palate does not detect noticeable differences in likeness and tastes between organically and conventionally grown vegetables (Zhao et al., 2007). Research has been minimal and conflicting in concluding that organically produced foods are more nutritious (Trewavas, 2004). However, there is evidence that organic foods have higher antioxidant levels (Brenbook, 2005). A survey of Americans found that they purchase organic products because they were fresh and nutritious and to ensure they are not consuming pesticides or genetically modified foods. More than 50% of respondents felt that they were making environmentally friendly decisions (Haumann, 2006).

Unfortunately, organic produce may have trace amounts of insecticides that are allowed in the production (Zang et al., 1998) and not allowed in the production (Gonzales et al., 2005). On one extreme hand, Trewavas, (2004) asserts that fruits and vegetables contain insecticides naturally that are as harmful as the synthetic insecticides.

Regardless of the method of growing a crop in the United States, the commodity must meet Government regulations. The regulating agencies differ depending upon the end product. With regards to the fresh market, i.e. fruits and vegetables sent directly to market without additional processing, the USDA sets grades. For instance, fresh collard and broccoli greens are assigned Grade 1 classification or are unclassified. The unclassified label suggests that the produce has not been assigned a grade. The Grade 1 produce meets standards such that the produce is “fresh, fairly, tender, fairly clean, well trimmed, and of characteristic color for the variety.” Fresh turnips will be assigned a Grade 1 or Grade 2 according to similar standards set for fresh collards (USDA-AMS, 2007).

The FDA regulates the fruit and vegetable processing industry and qualify the produce in terms of overall fitness, contamination of insect parts, and/or presence of rodent hairs and will either process or not process the produce with respect to the extent of contamination. Whereas, the fresh market is more flexible and driven by what is available. For instance, if the produce is destined for the processing industry and according to FDA guidelines will not be processed due to number of damaged leaves. However, if the destination is the fresh market, the USDA grade will be lowered or the produce will not be graded according to the damage. The unclassified or lowered Grade produce can still be marketed allowing profit for the grower. For instance, Community

Supported Agriculture (CSA) grown vegetables are more expensive than that of conventionally grown vegetables and the buyer happily consumes damaged, contaminated produce (Kris Giles, personal communication).

In conclusion, popularity of organic agriculture is evidently growing worldwide (Yussefi, 2006). The U. S. sector is meeting the demands of its citizens with export and import with other countries. In 2002, imports reached $1.5 billion while exports were over $100 million. The increased demand on organic agriculture will continue to grow. Recently, the USDA supported organic agriculture research with over $4 million (Haumann, 2006).

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This study was undertaken to evaluate laboratory and field efficacy of organic registered insecticides to control the harlequin bug, *Murgantia histrionica* (Hahn) (Heteroptera: Pentatomidae) and yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) on leafy greens in southern Oklahoma. Both insects are common pests of Brassica crops (collards, turnips, mustards, Broccoli) in the eastern United States and both are effectively controlled using conventional synthetic insecticides such as pyrethroids and carbamates. However, in the absence of conventional insecticides, both the harlequin bug (HB) and the yellowmargined leaf beetle (YMLB) have potential of occurring in large numbers and reducing the marketable portions of the crops. Currently, organic producers of leafy greens lack reliable means to control sporadic outbreaks of these pests. Laboratory toxicity studies using leaf dip bioassays were implemented to estimate a concentration-response relationship. To compare the results with field application rates, a field trial followed. Insecticides evaluated were spinosad, azadirachtin, and pyrethrum.

The $LC_{50,90}$ values estimated for the HB treated with spinosad were 398 ppm active ingredient (AI) (95% CI: 223-791) and 4,763 ppm AI (95% CI: 1,948-25,204), respectively. For Pyrethrum, the $LC_{50,90}$ values were 2,964 ppm AI (95% CI: 2,484-3,517) and 8,615 ppm AI (95% CI: 6,609-13,179), respectively. Likewise, spinosad and pyrethrum were effective in inducing mortality of the YMLB. The estimated $LC_{50,90}$ for spinosad inducing mortality were 1.80 ppm AI (95% CI: 0.22-3.30) and 11 ppm AI (95% CI: 7 - 64), respectively. For pyrethrum, the $LC_{50,90}$ values were 224 ppm AI (95% CI: 162 – 290) and 801 ppm AI (95% CI: 610 - 1,145), respectively.

In 2007, results indicated that spinosad and pyrethrum significantly reduced YMLB adult numbers 96% and 63%, respectively, compared to the untreated plots one day post treatment. In 2006 and 2007, spinosad and pyrethrum significantly reduced HB adults 61% and 73%, respectively, compared to the untreated plot one day post treatment. These results are being used to develop IPM strategies for producing organically grown leafy greens.

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