TOXICITY OF INSECTICIDES FOR MANAGING THE DIFFERENTIAL GRASSHOPPER IN LEAFY GREEN VEGETABLE CROPS

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Introduction

Background and significance

The vegetable crop production industry is held to quality standards implemented by the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA). Growers must prevent the occurrence of insect pest infestations to avoid detectable insect damage and contaminants in the harvested products.

Marketing of leafy greens such as kale, collards, mustard, spinach, and cabbage is of two types. First, the crops may be harvested as fresh produce which is not processed or changed in any meaningful manner. These products fall under the regulatory guidelines of USDA. Secondly, processed products in which the leaves are changed in a meaningful manner fall under the regulatory guidelines of the FDA. There are strict standards on the level of contaminants allowed in processed foods including leafy greens packaged in 'cello packs' of fresh cut greens, canned greens, and frozen greens (Gecan et al. 1990). FDA has regulatory authority to inspect the produce prior to processing, during processing and after completion of processing. Contamination may occur during the production phase of growing such as that from infestation by insects, insect parts, insect wastes and insect skins.

The primary insect pests that occur on and damage leafy greens include the green peach aphid; lepedopteran larvae such as the cabbage looper, armyworm and diamondback moth; and various species of grasshoppers (Sweeden 1996). Of these insects, grasshoppers do not necessarily reproduce and spend their entire life cycle in the

same fields. They are more abundant in weeds, soybean, or surrounding vegetation than in leafy greens (Sweeden 1996).

Literature on grasshoppers on vegetable crops in the south central United States is scarce. In 1962, Coppock (1962) conducted a study of grasshoppers in Oklahoma and cataloged species of grasshoppers belonging to the family Acrididae. The differential grasshopper *Melanoplus differentialis nigricans* Cockrell was one of the most common and destructive grasshoppers that has adapted to agricultural environments of its range. Its habitat range is very general, occurring in cultivated crops as well as waste areas, roadsides, field borders, and grasslands (Coppock 1962). It probably does more damage to cultivated crops than any other species of grasshopper in Oklahoma (Coppock 1962). According to Harvey et al. (1993), the differential grasshopper causes damage to corn, wheat, and alfalfa in central parts of the United States. Sweeden (1996) studied the species composition and distribution of grasshoppers within and around fields of leafy greens adjacent to soybean fields in the Arkansas River Valley. He found that the Acrididae grasshoppers, *Schistocerca americana* (Drury), the red legged grasshopper *Melanoplus femurrubrum* (De Geer), the differential grasshopper, *M. differentialis* (Thomas), and the Carolina grasshopper, *Dissosteira carolina* (Linnaeus) were common in crop fields and surrounding vegetation.

Management of grasshopper populations in leafy green vegetables is important to the quality of the final product. This is critical late in the growing season when grasshoppers move into the field just prior to harvest. They move into the actively growing crops when other crops are senescing. The tolerance for insects, insect parts, and contaminants for leafy green crops marketed in United States is practically zero.

Therefore, populations of pests must be maintained at very low levels. The major tool for controlling insect pests has been the application of insecticides, mostly organophospates and carbamates because they have broad-spectrum activity and have been shown to be very effective (Ware 2000). Grasshoppers can be managed either by killing the migrating and feeding grasshoppers or repelling them from the field. The control strategies must be fast-acting to stop insects moving into the field just prior to harvest and must have residual activity so that control can be achieved at a reasonable interval prior to harvest and yet continue to be active until harvest.

The implementation of the Food Quality Protection Act (FQPA) in 1996 may result in loss of registration and/or restriction of use of many pesticides including organophospates, carbamates, and Group A, B and C classes of carcinogenic pesticides currently used by growers to produce leafy greens for processing. Under these circumstances, there is a need for determining the efficacy of new insecticides that could be used in the management of insect pests of leafy greens. Many of these have been newly registered or are currently under consideration.

Leafy green vegetables in the south-central United States are grown from August through May and thus the crops and insects are subject to a wide range of temperatures varying between 5°C and 35°C. Temperature is a major factor affecting insecticide efficacy (Johnson 1990). Toxicity effects mediated by temperature can be either positive or negative (Ware 2000). These response relationships depend on the target species, method of application and quantity of insecticide ingested or contacted (Johnson 1990). Different classes of insecticides can react differently at varying temperatures. Therefore

it is important to determine the effects of different temperatures on the efficacy of insecticides targeted for grasshoppers damaging leafy green vegetables.

The objectives of this research were to determine toxicity of selected insecticides as they affect grasshoppers and to determine how toxicity may be mediated by temperature.

Objectives

A. Evaluate and compare the efficacy and residual activity of different classes of insecticides including: an insect growth regulator, diflubenzuron [Dimilin]; a botanically derived insect growth regulator, azadirachtin [Neemix 4.5]; a microbial insecticide, *Beauveria bassiana* GHA [Mycotrol]; a microbial-derived insecticide, spinosad [Spintor] to currently recommended insecticides for insect pest control in leafy greens (cyclodine, endosulfan [Thiodan]; pyrethroid, esfenvalarate [Asana]; and organophosphate, naled [Dibrom]) for control of the differential grasshopper.

B. Evaluate the effect of temperature on the efficacy of the insect growth regulator, diflubenzuron [Dimilin]; the botanically derived insect growth regulator, azadirachtin [Neemix 4.5]; the microbial insecticide, *Beauveria bassiana* GHA [Mycotrol]; the microbial-derived insecticide, spinosad [Spintor]; and the pyrethroid, esfenvalerate [Asana] for the control of differential grasshoppers damaging leafy greens.

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Chapter II

Literature Review

Leafy green vegetables

Leafy green vegetables are classified as minor crops by the United States Department of Agriculture due to the low acreage grown and low production value relative to agronomic crops such as corn and cotton. However they are important components of our diet. Most of the important leafy green vegetables such as kale, collards, mustard, turnips, chinese cabbage, and cabbage belong to the Cruciferae, which is commonly called the Mustard family (Ware et al. 1980). The above mentioned crucifers belong to the Genus *Brassica* (Ware et al. 1980) and are often called brassica greens. Spinach belongs to the genus *Spinacia* within the family Chenopodiaceae which is commonly called the Goosefoot family (Ware et al. 1980).

Insect pests of leafy greens

Leafy vegetables such as collards, kale, mustard, and turnip greens are susceptible to attack by a wide variety of insects and other pests while in the field (Gecan et al. 1990). Because of the physical form of greens leaves and the numerous pests that may become entrapped among the leaves, these raw products must be thoroughly washed before processing. They are usually soaked in tanks of water combined with agitation and sprays, followed by a series of high-pressure water sprays to remove strongly adhering soil and insects (Gecan et al. 1990). In spite of processors' best efforts, many insect pests such as adult and larval moths, beetles, flies, ants, wasps, aphids, scale

insects, thrips, psocids, and mites are found as minute contaminants in canned processed greens (Gecan et al. 1990).

The primary insect pests that occur on and damage leafy greens in temperate North America include the green peach aphid, cabbage aphid, and turnip aphid; lepedopteran larvae such as the cabbage looper, armyworm species, and diamond black moth and various species of grasshoppers (Sweeden 1996).

Aphids. Aphids are small soft bodied insects belonging to the family Aphididae and are frequently found in large numbers feeding on stems or leaves (Borror et al. 1989). Groups of aphid often occur that include individuals in all stages of development. The members of this family can usually be recognized by their characteristic pearlike shape, a pair of cornicles at the posterior end of the abdomen, and fairly long antennae (Borror et al. 1989). They have numerous parasites and predators. The principal parasites of aphids are braconid and chalcidoid wasps and the most important predators are ladybird beetles and lacewings (Borror et al. 1989). Aphid species such as the green peach aphid, *Myzus persicae* (Sulzer) can be serious pests of cultivated plants (Borror et al. 1989). It is one of the most important aphid species damaging leafy greens including spinach, primarily because it is a potential contaminant of processed products (Sweeden 1996), and when abundant may attract other insect predators and parasites to the crop that then serve as contaminants.

Lepidopteran larvae. Of the important lepidopteran larvae that damage leafy greens, the cabbage looper, *Trichoplusia ni* (Hubner) and armyworm belong to the family

Noctuidae (Borror et al. 1989). The Noctuidae is the largest family of the order Lepidoptera and has moths with nocturnal habits. Another important Lepidoptera that damages leafy greens is the diamondback moth, which belongs to family Plutellidae (Borror et al. 1989). The diamondback moth is a serious pest of cabbage and other cruciferous plants. The larvae eat holes in the leaves and pupate in silken cocoons attached to the leaves and thus act as contaminants (Borror et al. 1989).

Grasshoppers. Grasshoppers belong to the order Orthoptera, which contains two sub-orders, Ensifera and Caelifera (Preston-Mafham 1990). The suborder Ensifera contains the families, Gryllidae (crickets), Gryllotalpidae (mole crickets), Gryllocrididae and Tettigonidae (katydids). The sub-order Caelifera is comprised of the Acrididae (short horned grasshoppers) and Tetrigidae (pigmy grasshoppers) (Preston-Mafham 1990). The family Acrididae is further subdivided into several sub families, Melonoplinae (spur throated grasshoppers), Gompahocerinae (slant faced grasshoppers) and Oedipodinae (band winged grasshoppers) that are of economic importance (Pfadt 1994).

Research literature on grasshoppers in the South Central United States is scarce. Coppock (1962) studied and cataloged acridid species in the region. The Acrididae is one of the most widely distributed groups of insects in the world. They are found in every continent and are especially adapted to a grassland environment (Coppock 1962).

Sweeden (1996) studied the species composition and distribution of grasshoppers within and around an actively growing field of leafy greens adjacent to soybean fields in the Arkansas River Vally. According to this study, acridid grasshoppers, *Schistocerca americana* (Drury); the red legged grasshopper, *Melanoplus femurrubrum* (De Geer); the

differential grasshopper, *Melanoplus differentialis* (Thomas); and the Carolina grasshopper, *Dissosteira carolina* (Linnaeus) were common in leafy greens fields and surrounding vegetation. Of these *S. americana, M. femurrubrum* and *M. differentialis* belong to the sub-family Melanoplinae and *D. carolina* to the sub family Oedipodinae. This study will focus on *M. differentialis* because it is abundant and more damaging to cultivated crops.

Melanoplus differentialis (Thomas)

There are two sub-species of *M. differentialis* (Thomas) according to the listing of Roberts (1942). They are *M. differentialis differentialis* (Thomas) and *M. differentialis nigricans* Cockerel. These two subspecies can be separated only on the basis of internal male genetalia (Coppock 1962). Of the two subspecies, only *M. differentialis nigricans* Cock. is found in Oklahoma, while *M. differentialis differentialis* (Thomas) is not found any closer to Oklahoma than eastern Arkansas (Coppock 1962).

M. differentialis is one of the most common and destructive grasshoppers among the acridids (Coppock 1962). It has adapted to the local agricultural environments of its range better than any other acridid (Coppock 1962). Its range of habitat is very general, occurring in cultivated crops as well as waste areas, roadsides, field borders, and grasslands. Additionally, *M. differentialis* does more damage to cultivated crops than any other species of grasshopper in the state of Oklahoma. It is a severe pest of small grains, corn, alfalfa, soybeans, cotton, various vegetables, and deciduous fruit trees (Pfadt 1994). According to Pfadt (1994) *M. differentialis* is responsible for much damage to alfalfa, corn, and cotton in southern states including Oklahoma and Missouri.

The differential grasshopper is a polyphagous insect feeding on both grasses and forbs although it prefers forbs to grasses (Pfadt 1994). Both in its nymphal and adult stages, *M. differentialis* is a mobile insect. After hatching from eggs, nymphs concentrate in groups along field boarders and roadsides (Pfadt 1994). The third and older instars move into barley, wheat, and alfalfa by crawling and hopping (Pfadt 1994). In contrast, adults fly upwind in short, low flights in search for green, actively growing crops (Pfadt 1994).

The nymphs are inactive at night and rest on vegetation. Nymphs descend to the ground and bask in the sun on clear mornings when temperatures reach 18°C. They start to feed when temperatures reach 20 –23°C and continue (Pfadt 1994) feeding until air temperatures reach 32°C and soil surface is around 44°C (Pfadt 1994). At higher temperatures, they climb vegetation to seek shade in order to escape from heat. Usually nymphs are on the ground from 6000 to 1100 hour. The nymphs remain inactive if the skies are cloudy, irrespective of the temperature (Pfadt 1994).

Adults and nymphs of the differential grasshopper rest high on plants descending when the air temperature exceeds 20°C. They respond to light striking the ground, and begin to feed. Feeding slackens when temperature reach 30°C and cease at temperatures above 32°C and/or soil surface temperatures exceed 44°C. They seek shade or rise in flight (Pfadt, 1994).

Characteristics. *M. differentialis* is a large grasshopper with a body color that varies from yellowish brown to light-brown dorsally, and bright to dull yellow ventrally (Coppock 1962). The chevron-like markings on the hind femur and shape of the male

cercus are diagnostic characteristics of the differential grasshopper (Pfadt 1994). There are three oblique, dark bars in the inner faces of hind femur (Coppock 1962). The cercus is one of the characteristics that identify the adult male. Both male and female can be identified by the black chevrons in the hind femur (Pfadt 1994).

Development from eggs to adult. Differential grasshoppers have one generation annually. Eggs are the over-wintering stage of the life cycle. In late spring, eggs which were deposited in the previous summer start to hatch (Pfadt 1994). Nymphs grow rapidly with the exposure to the high temperatures of early summer and become adults after about thirty-two days (Pfadt 1994). In the following several weeks they feed and increase in weight. During this time their ovaries and testes mature. Pairs form in the morning and copulation may continue for 20 to 24 hours (Pfadt 1994). For oviposition, females seek adjacent grassland or rank weeds. When ready to lay eggs, a female may brace herself in a vertical position against a grass or weed stalk and work her ovipositor down into the soil and deposits 45 to 194 eggs among the roots and in the form of a large pod for their protection (Pfadt 1994). The egg pods are curved and 2.54 to 3.81 cm in length and 0.635 cm in diameter. The eggs are olive and 0.44 to 0.51 cm in length (Pfadt 1994).

Insecticides

The conventional insecticides currently recommended for the control of aphids and caterpillars damaging leafy green vegetables are dimethoate, malathion, diazinon, and endosulfan (Extension Agents' Handbook 2002). In addition, neem extracts, pyrethrins, insecticidal soap, *Beauveria bassiana*-based products, and *Bacillus*

thuringiensis-based products are recommended as organic insecticides for insect pest control in leafy greens (Extension Agents' Handbook 2002). None of these insecticides are specifically recommended for grasshopper control in leafy green vegetables.

This study was conducted to evaluate insecticides that may serve as alternatives to organophosphate and carbamate insecticides for control grasshoppers. The insecticides selected were from different categories of insecticides i.e. an insect growth regulator, diflubenzuron [Dimilin]; a botanical insect growth regulator, azadirachtin [Neemix 4.5]; a microbial insecticide, *Beauveria bassiana* GHA [Mycotrol] and a microbial derived insecticide, spinosad (Spintor). In addition to these, several currently used insecticides for insect control in leafy green vegetables were included for comparison of efficacy. They were the organophosphate; naled (Dibrom), the pyrethroid; esfenvalerate (Asana), and the cyclodine; endosulfan (Thiodan).

Reduced risk insecticides

The Environmental Protection Agency's (EPA) uses various criteria to grant reduced-risk status to insecticide registrations (Pesticide Regulation [PR] Notice 97-3, 1997). These include human health effects such as very low mammalian toxicity, lower toxicity than alternatives, displacement of chemicals that pose potential human health concerns [e.g. organophosphate insecticides, probable carcinogens] and reduced risk of exposure by mixers, loaders, applicators and reentry workers; reduced risk to non-target organisms such as birds, fish, honey bees, and beneficial insects; groundwater effects such as low potential for ground water contamination, low drift and runoff potential; and finally the criteria such as lower use rate than alternative insecticides, low pest resistance

potential (i.e., new mode of action), compatibility with integrated pest management and the efficacy (Pesticide Regulation (PR) Notice 97-3, 1997). Of these criteria, the most important are human health effects, non-target organism effect to birds and fish, potential groundwater impact, displacement of higher risk alternatives and the efficacy (Pesticide Regulation (PR) Notice 97-3, 1997).

Diflubenzuron (Dimilin). Diflubenzuron belongs to the class of benzoylphenylureas (Environmental Health Criteria 184, 1996). Benzylphenylureas act as insect growth regulators (Ware 2000). They are different from typical nerve poisons that attack insect nervous system in that they interfere with chitin synthesis and/or deposition in the cuticle (Environmental Health Criteria 184, 1996). They are taken up more by ingestion than by contact and are important in the control of caterpillars and beetle larvae (Ware 2000).

Diflubenzuron is the only benzoylphenylurea currently registered in U.S. It was first registered for gypsy moth, cotton boll weevil, forest caterpillars, soybean caterpillars, and mushroom flies and now has a broader range of targets (Ware 2000). It is an effective stomach and contact insecticide and acts on larval stages of most insects by inhibiting or blocking the synthesis of chitin. Typical effects on developing larvae are the rupture of a malformed cuticle or death by starvation (Ware 2000). Thus all stages of insects that form a new cuticle should be susceptible to diflubenzuron exposure. It has no systemic activity and does not penetrate plant tissue thus making it generally non-effective for sucking insects (Environmental health Criteria 184, 1996).

Spinosad. Spinosad is a fermentation derived insect control agent and belongs to perhaps the newest class of insecticides, the spinosyns (Sparks et al. 1999). Spinosad is a product of fermentation metabolites of the soil inhibiting actinomycete *Saccharoplyspora spinosa* (Ware 2000). It has a novel molecular structure and mode of action that results in insect mortality typically associated with synthetic insecticides. It acts by disrupting the binding of acetylcholine in nicotinic acetylcholine receptors at the postsynaptic cell (Salgado 1997).

Spinosad was first registered for use on cotton in 1997 (Ware 2000). Spinosad is a mixture of spinosyn A and D thus making its name spinosAD. These molecules belong to a family of new, unique macrolides which are molecules containing macrocyclic lactones (Sparks et al. 1999). Spinosad has broad-spectrum activity against most caterpillar pests at very low active ingredient rates per acre (Ware 2000). It has both contact and stomach poison activity against lepidopteran larvae, leafminers, thrips and termites with long residual activity. In the U.S., it is currently registered for use on cotton, vegetables, tree fruits, and ornamentals. Spynosyn A acts on the insect nervous system to increase spontaneous activity, leading to involuntary muscle contractions and tremors. This increased excitation appears to result from persistent activation of nicotinic acetylcholine receptors and prolongation of acetylcholine responses. (Sparks et al. 1999). In addition, the spinosyns can also alter the functions of GABA-gated chloride channels in a manner distinct from all known insect control agents making the mode of action of spinosad unique (Sparks et al. 1999).

Azadirachtin. Oil extracts from neem tree seeds (*Azadirachta indica*: Meliaceae) contain the active ingredient azadirachtin, a nortriterpenoid belonging to the lemonoids (Ware 2000). Azadirachtin has insecticidal, fungicidal and bactericidal properties including insect growth regulator qualities. It disrupts molting by inhibiting biosynthesis or metabolism of ecdysone, the juvenile molting hormone (Ware 2000). It is sold as a stomach/contact insecticide for use in green house and ornamental crops (Ware 2000). Neem extracts function primarily as insect growth regulators (IGR), but also as a behavior-modifying substance, deterring feeding and /or oviposition in certain pest species (Isman 1999). Of equal importance, neem has minimal toxicity to vertibrates, has minimal effect on natural enemies and pollinators and degrades rapidly in the environment. It has non-neurotoxic modes of action (Isman 1999). It does not disrupt foraging by honeybees and other pollinators. These properties suggest that neem as an insecticide is compatible with integrated pest management in many crop ecosystems (Isman 1999).

Azadirachtin acts as an insect growth regulator (IGR) effecting the hormonal system of insects and, preventing them from normal development (Walter 1999). Azadirachtin is structurally similar to the natural insect hormone ecdysone which regulates the development of insects. Any disruption in its balance causes improper development (Walter 1999). Azadirachtin interferes with the production and reception of ecdysone during an insect's growth and molting, thus blocking the molting cycle and, causing the insect to die (Walter 1999).

Because of its IGR effect, azadirachtin does not immediately kill insects and does not kill adult insects. Immature insects die during their development, thereby reducing

the overall population over a period of time. The length of time depends mostly on the species of insect, age of insect, and the size of the population (Walter 1999). Mortality can be seen as quickly as 1-2 days, to as long as a few weeks. Therefore Azadirachtin has its greatest effect on the early instars (Walter 1999).

One drawback of many IGR's is that they do not immediately kill the insect pest, thus leaving the insect to cause further damage or contaminate the harvested product until it succumbs to the IGR. In the case of azadirachtin, additional modes of action help protect the plants from damage while the IGR works on the insect. For example many insects exposed to azadirachtin will stop feeding shortly after exposure which in effect, stops further damage to the plants even though the insects are still present (Walter 1999).

***Beauveria bassiana* strain GHA (Mycotrol).** The application of insect pathogens may offer an environmentally sound method for managing grasshoppers and locusts. Hypomycete fungi are the most promising candidates because in addition to causing diseases in many insects, they are not pathogenic to non-target organisms (Johnson et al. 1990). They can penetrate directly through the cuticle and do not necessarily need to be ingested in order to initiate disease. Grasshoppers are highly susceptible to some of the isolates of the entomopathogenic fungus *B. bassiana* (Johnson et al.1990).

According to Moore & Earlandson, 1988 *B. bassiana* infects orthopterans and has been demonstrated to be pathogenic to grasshoppers under laboratory conditions. *B. bassiana* strains have been isolated from *Melanoplus* spp. (Moore et al. 1988). Adult males, females, and emerging nymphs of *Melanoplus sanguinipes* are susceptible to infection by *B. bassiana* in sand under controlled environments (Inglis et al. 1995). *B.*

bassiana and other entomopathogenic fungi are being examined as potential biological agents for insect control. Present knowledge of fungal pathogenesis in insects indicates that it occurs via a series of integrated, systematic events progressing from spore attachment to germination, penetration, growth, and proliferation within the body of the host, interaction with insect defense mechanisms and finally reemergence from the cadaver (Hegedus et al. 1996). Environmental conditions such as high temperature and exposure to UV radiation can adversely affect this pathogenesis of *B. bassiana* (Inglis et al. 1997).

High-Risk Insecticides

According to EPA's criteria, insecticides with very high mammalian toxicity, higher toxicity than alternative insecticides, broad-spectrum activity; high toxicity to birds, fish and beneficial insects; and high potential for ground water contamination are considered as high-risk insecticides (Pesticide Regulation [PR] Notice 97-3, 1997). In addition they have high pest resistance potential and less compatibility with integrated pest management (Pesticide Regulation [PR] Notice 97-3, 1997). Insecticides such as organophosphates, carbamates, cyclodines, and organochlorines fall under this category.

Organophosphate Insecticides. Organophosphate insecticides are derived from phosphoric acid and are generally the most toxic of all pesticides to vertebrate animals. They are related to the nerve gases sarin, soman and tabun (Ware 2000). The organophosphate insecticides have two distinctive features. Firstly, they are generally much more toxic to vertebrates than the organochlorine insecticides, and secondly they

are chemically unstable or non-persistent compared to persistent organochlorines (Ware 2000).

Organophosphate insecticides exert their toxic action by inhibiting cholinesterases which are important enzymes of the nervous system. At the nerve synapse, an impulse is transmitted by acetylcholine which is then destroyed by the cholinesterase enzyme so the synapse will be cleared for another transmission (Ware 2000). These chemical reactions happen within microseconds and continue constantly, as needed, under normal conditions. Organophosphates prevent the cholinesterase enzyme from clearing away the acetylcholine transmitter. This inhibition results in the accumulation of acetylcholine, which interferes with the neuromuscular junction in mammals, producing rapid twitching of voluntary muscles and finally resulting in paralysis and death due to respiratory failure. Symptoms in insects follow the same pattern of nerve poisoning with restlessness, hyper-excitability, tremors and convulsions and paralysis (Ware 2000).

Carbamate Insecticides. Carbamate insecticides are derivatives of carbamic acid (Ware 2000). Like the organophosphate insecticides, the mode of action of carbamates is inhibition of the enzyme cholinesterase. The carbamates mimic the molecular shape of acetylcholine. They are broad spectrum in effectiveness. Carbamates have both contact and systemic activity (Ware 2000). They are used as insecticides, miticides and molluscicides (Ware 2000).

Cyclodiene Insecticides. Cyclodiene insecticides are also known as the diene – organochlorine insecticides. Cyclodienes are generally persistent and stable in soil and

relatively stable to the ultraviolet action of sunlight. Because of their persistence, their use on crops was restricted as undesirable residues remained beyond the time for harvest (Ware 2000).

The toxicity of cyclodienes increases with temperature (Ware 2000). They act on the inhibitory mechanism that is naturally active in the insect nervous system, the GABA (gamma-aminobutyric acid) receptor, which operates by increasing chloride ion permeability into neurons. Cyclodienes prevent chloride ions from entering the neurons, and thereby antagonize the calming effects of GABA. They are thus known as GABA-gated chloride channel antagonists (Ware 2000).

Pyrethroid Insecticides. The basis for synthetic pyrethroid insecticides is pyrethrum a natural extract from the flowers of chrysanthemum which causes the immediate paralysis of insects (Ware 2000). Pyrethrum has mostly been used as a household insecticide. Because of its cost and instability in sunlight, it has seldom been used for agricultural purposes. Unlike pyrethrum, synthetic pyrethrum-like materials termed "synthetic pyrethroids" are stable in sunlight and effective against most of agricultural insect pests (Ware 2000). The structure of pyrethroid insecticides are similar. They also share similar modes of action, resembling that of DDT, by keeping open the sodium channels in neuronal membranes. There are two types of pyrethroid insecticides, Type I and Type II. Type I pyrethroids are more effective when temperature is lowered. In contrast, Type II, pyrethroids have increased activity with increase in temperature (Ware 2000). Pyrethroids are axonic poisons which act as sodium channel blockers. The axon of a nerve cell or neuron is an elongated extension of the cell body and is especially important

in the transmission of nerve impulses from the region of the cell body to an other cell. Virtually all axonic transmission of impulses is electrical. Axonic chemicals are those that in some way affect this impulse transmission in the axon. All DDT type chlorinated insecticides and pyrethroids are considered axonic poisons (Ware 2000).

Pyrethroid insecticides affect both peripheral and central nervous system of the insect. They initially stimulate nerve cells to produce repetitive discharges and eventually cause paralysis. Such effects are caused by their action on the sodium channel, a tiny hole through which sodium ions are permitted to enter the axon to cause excitation.

Effect of Temperature on activity of insects and insecticides

One of the most important factors affecting biological processes in all living organisms is temperature. Temperature affects metabolic rate, locomotion, rate of water loss, food consumption, growth, maturation, and habitat selection of grasshoppers (Chappell et al. 1990). Temperature also affects insecticide efficacy (Johnson 1990) and toxicity (De Vries 1978). The relationship between temperature and toxicity can be either positive or negative, depending on the target species, method of application, and quantity of insecticide ingested or contacted (Johnson 1990). DDT and the pyrethroid insecticides have a negative temperature coefficient, i.e. higher mortality with decreasing temperature. Carbamate insecticides may have a slightly negative coefficient and organophosphate insecticides a slightly positive temperature coefficient (De Vries 1978). The toxicity of organophosphate insecticides (e.g. chlorpyrifos, diazinon, malathion and parathion) to the onion maggot (*Delia antiqua*) has been positively correlated with post-

treatment temperature, while temperature has little effect on naled toxicity (Turnbull et al. 1986). In contrast, the toxicity of the pyrethroids such as fenvalerate, deltamethrin, permethrin and cypermethrin were negatively correlated with temperature for *D. antiqua* (Turnbull et al. 1986). Studies conducted with boll weevils using the organophosphates, methyl parathion and malathion also indicated positive temperature coefficients of toxicity (Norment et al. 1970).

Results of experiments conducted using second instar nymphs of the grasshopper *Melanoplus sanguinipes* (Fab.) indicated that the pyrethroid insecticides deltamethrin, fenvalerate and cyfluthrin have negative temperature coefficients (Hinks 1985). When used at lower dosages, cypermethrin, was shown to increase mortality of second instar nymphs of the grasshopper *Melanoplus sanguinipes* (Fab.) with increasing temperature from 15° to 30°C, and then leveled off above 30°C (Ewen et al. 1984). At higher dosages, mortality increased slightly with increasing temperature from 15° to 20°C and then declined at higher temperatures. (Ewen et al.1984). The carbamate insecticide, methomyl was more toxic to larvae of *Spodoptera littoralis* (Boisd.) when the post treatment temperature was increased from 20° to 35°C (Riskallah 1983).

Insecticide application decisions should include knowledge of efficacy as affected by temperature (Edelson et al. 1997). This is especially important in crop production systems that encounter large variations in temperatures when pesticides are likely to be applied. Previous research results indicate that for brassica crop production in south-central United States, permethrin and other similar pyrethroid insecticides work best for diamondback moth control when applied under low temperature conditions (≤10°C) (Edelson et al. 1997). In contrast methamidophos and similar organophosphate

insecticides are most effective when applied under high temperature conditions (≥35°C). Within intermediate temperature regimes (20°–30°C) the two materials provide similar control (Edelson et al. 1997).

Temperature may also affect toxicity of microbial-based insecticides. Recent evidence suggests that environmental conditions limit the efficacy of *B. bassiana* in the field (Inglis et al. 1996). Reduced efficacy of *B. bassiana* against rangeland grasshoppers has been a result of unfavorable temperature and light conditions and not the result of inadequate pathogen virulence (Inglis et al. 1997a). In addition, high temperatures and thermo-regulation can adversely affect *B. bassiana* mycosis of grasshoppers and may be the cause of reduced efficacy observed in some field experiments (Inglis et al.1996). The activity of *B. bassiana* on mortality of *Melanoplus sanguinipes* was increased at low temperatures in comparison to high temperatures (Inglis et al.1997b).

The influence of post-treatment temperature on the insecticidal activity of *Azadirachta indica* (A. Juss) a seed extract, against the dessert locust, *Schistocerca gregaria* (Forskal) was reported by Kabaru et al. 2000. In tests on adults, the toxicity of crude *A. indica* seed extracts increased by about 10-fold when the post-treatment temperature was raised from 22°C to 40°C. This temperature dependent toxicity was observed in insects treated tropically or via injection (Kabaru et al. 2000).

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Chapter III

Effect of temperature on toxicity of insecticides applied to control the differential grasshopper in leafy green vegetable crops

Abstract

This study was conducted to determine the effect of temperature on activity of possible alternative insecticides for replacing the use of organophosphate and carbamate insecticides for controlling grasshoppers in leafy green vegetables. The research focused on different classes of insecticides such as: an insect growth regulator, diflubenzuron [Dimilin]; a botanically derived insect growth regulator, azadirachtin [Neemix 4.5]; a microbial insecticide, *Beauveria bassiana* GHA [Mycotrol]; a microbial-derived insecticide, spinosad [Spintor] and a pyrethroid, esfenvalarate [Asana] to control third instars of the differential grasshopper *Melanoplus differentialis* (Thomas).

Esfenvalarate provided excellent efficacy in controlling third instar *M. differentialis* and its activity at labeled use rates is not temperature dependent. The microbial-derived insecticide spinosad was equally effective but efficacy increased with time after application and at increasing treatment temperature of 10°C, 15°C, 25°C and 35°C. The activity of *B. bassiana* is more pronounced at 25°C and was adversely affected by high and low temperatures. The insecticide diflubenzuron provided good activity at high temperatures. The activity of azadirachtin is moderate in regard to mortality of *M. differentialis* and its activity is temperature dependent.

Introduction

Leafy green vegetables such as kale, collards, mustard, turnips and spinach are grown in the south central United States from August through May. Thus the crops and insects encounter temperatures that vary between 5°C and 35°C. The important insect pests of leafy green crops in the south central region include aphids, cucumber beetle species, seed maggots, and larvae of several lepidopteran species including the diamondback moth and armyworm. In addition, grasshoppers have been identified as a significant contaminant problem in leafy green fields. They are often difficult to control due to their large size and mobility. Due to the strict standards limiting insect parts and feeding contamination of processed vegetable crops, the number of grasshoppers tolerated in fields of leafy greens grown for the processing market is practically zero. Therefore, management and control of grasshoppers is a critical issue. The application of insecticides to kill grasshoppers is the main tool for managing populations. Due to the variation of temperatures during the time period in which the leafy greens are grown, both insecticides and insects are exposed to a broad range of temperatures.

One of the most important factors affecting biological processes in all living organisms is temperature. Thus, temperature influences metabolic rate, locomotion, rate of water loss, food consumption, growth, maturation and habitat selection of grasshoppers (Chappell et al. 1990). Temperature also is a major factor affecting insecticide efficacy (Johnson 1990 and Scott 1995) and toxicity (De Vries 1978). Temperature effects on toxicity can be either positive or negative. These response relationships depend on the target species, method of application, and quantity of

insecticide ingested or contacted (Johnson 1990). Different categories of insecticides can react differently at varying temperatures.

Insecticides such as DDT and various pyrethroids have been shown to have negative temperature coefficients, ie they are more toxic as temperature decreases. Carbamate insecticides may have a slightly negative coefficient and organophosphate insecticides may show a slightly positive temperature coefficient (De Vries 1978). This study focussed on the effect of temperature on toxicity of different insecticides representing different insecticide classes to the differential grasshopper.

Materials & Methods

Insecticides. The insecticides used were as follows: diflubenzuron (Dimilin 2L, Uniroyal Chemical Comp., INC, Middleburg, CT 06749) 0.07kg a.i./ha, appl. rate, 0.28 kg/ha; azadirachtin (Neemix 4.5, Certis USA, Columbia, MD 21046) 0.048 kg a.i./ha, appl. rate, 1.121 kg/ha; *Beauveria bassiana* GHA (Mycotrol, Mycotech Corp. Butte, MT 597022) 0.179 kg a.i. /ha. appl. rate, 1.592 kg/ha; esfenvalerate (Asana XL, E. I. Du Pont de Nemours & Comp., INC, Wilmington, DE 19898) 0.055 kg a.i./ha, appl. rate 0.673kg/ha; spinosad (Spintor 2SC, Dow Agro Sciences LLC, Indianapolis, IN 46268) 0.175 kg a.i./ha, appl. rate, 0.7 kg/ha.

Third instar *Melanoplus differentialis* (Thomas) were collected from a pasture in southeast Oklahoma in June and July 2001 and June 2002 using a 38cm diameter sweep net (Bioquip®). The third instars were identified by the general color of pale yellow to tan, with body length in the range of 9.4 to 12.4 mm and a black stripe of the hind femur occupying the center of the medial area (Pfadt 1994). The Systematic Entomology

Laboratory, USDA, Beltsville, MD identified and verified the samples of the third instars and adults as *M. differntialis nigricans* (Lot No.0107781). The collected grasshoppers were transported to the entomology laboratory at the Wes Watkins Agricultural Research and Extension Center (WWAREC), Lane, Oklahoma and kept in aluminum wire mesh mosquito cages of 45x45x45cm (Bioquip®). Cages were covered on the bottom with a layer of fine sand of 3cm thickness. Fresh collard leaves and water were provided each day. Cages were maintained under a 14-hour photoperiod and a room temperature of 26.7 ± 0.5°C throughout the duration of the experiment.

Collard seeds (*Brassica oleraceae* L. var. *acephala* cultivar "Champion') were planted in plastic pots (10cm in height and 11cm in diameter) containing a soil-less medium (Horticultural vermiculite [45%] and Canadian sphagnum moss [55%]) and 6g of Osmocote (controlled releasing fertilizer with 14:14:14 NPK). They were raised in a greenhouse for 6 weeks at 30°C.

Individual leaves with a mean leaf area of 27.2 ± 1.5 cm^2 were selected from the plants grown in the greenhouse and were treated with the insecticides. Each insecticide solution was prepared as a 100 ml solution using distilled water and stirred for 10 minutes using an electrical stirrer. Individual leaves were dipped in specific insecticides, removed and dried at 25°C for 30 minutes. The cut end of the leaf petioles were covered with moist cotton plugs and placed in plastic cages containing a third instar *M. differentialis* nymph that had been starved for 24 hours. The containers were then transferred to environmental temperature chambers and held at a 14-hour photoperiod and a temperature of either 10°C, 15°C, 25°C, and 35°C. After 48 hours the treated leaves were replaced with untreated leaves. Insecticide efficacy was determined based on

mortality of grasshoppers over a ten-day observation period. Mortality was determined by either lack of movement, the insect lying on its side, or inability to respond to a slight shaking of the cage with a coordinated movement. The cadavers in cages containing leaves treated with *B. bassiana* were kept in an environmental temperature chamber at 25°C for 14 days for determination of mycelium growth. Mortality due to *B. bassiana* was determined when mycelia of *B. bassiana*, were visible on the integument through a microscope. The experimental design was completely randomized with 6 replications and with a water-treated control. One cage each with a third instar was used per treatment. The experiment was repeated with the addition of the insecticide spinosad (Spintor 2SC) with 5 replications, in July 2001 and June 2002. SAS (SAS Institute 1999) software was used for the data analysis. The CATMOD procedure was used to find the experiment versus treatment interactions at a 0.1 significance level. The data for all three experiments (Experiment 1, 2001, Experiment 2, 2001 and Experiment 3, 2002) were pooled and Proc Freq in SAS with Fisher's Exact Test was used to determine the treatment effect for each temperature and the effect of temperature for each treatment. We determined the efficacy of insecticides as insect mortality and used $P<0.1$ significance level. Pooled data of spinosad represents the data from Experiment 2 and 3 only.

Results

We used the pooled data to determine the treatment effect for each temperature and effect of temperature for each treatment. The treatments were compared at 10°C,

15°C, 25°C and 35°C at the intervals of 1 and 10-DAT (Days After Treatments). No mortality of nymphs was observed in water-treated controls in all temperatures.

For each treatment except the water and *B. bassiana*, results indicated that efficacy increased with an increase in temperature. For *B. bassiana* efficacy was low at low and high temperatures.

Treatment effect at each temperature (Table 3. 01).

10°C. At 10°C and 1-DAT treatment with esfenvalarate resulted in 100% mortality of nymphs and spinosad treatment resulted in 40% mortality. Both were significantly greater than the control. At 10-DAT each treatment resulted in mortality of nymphs. Mortality ranged from 100% with esfenvalarate to18% with *B. bassiana* and azadirachtin which were not significantly different from the control. Results indicate that the treatment with esfenvalarate resulted in the greatest mortality.

15°C. At 15°C at 1-DAT, application of esfenvalarate resulted in 100% mortality and spinosad treatment with 40% mortality. Both were significantly greater than the control. Both *B. bassiana* and diflubenzuron resulted in mortality of 6% and were not significantly different from the control. At 10-DAT each treatment resulted in mortality of nymphs. Mortality ranged from 100% with esfenvalarate to 18% with azadirachtin which was not significantly different from the control. As at 10°C esfenvalarate resulted in the greatest mortality followed by the treatment with spinosad. Results indicate that the treatments with esfenvalarate resulted in the greatest mortality followed by the treatment with spinosad.

25°C. At 25°C, treatment with esfenvalarate resulted in 81% mortality of nymphs and spinosad resulted in 60% mortality. Both were significantly greater than the control. Application of diflubenzuron resulted in 13% mortality but was not significantly different from the control. Mortality increased with time for each treatment and at 10-DAT it ranged from 100% mortality with esfenvalarate and spinosad to 31% with azadirachtin which was significantly greater than the control. *B. bassiana* treatment resulted in 63% mortality. Results indicate that the treatments with esfenvalarate and spinosad resulted in greatest mortality of nymphs.

35°C. At 35°C, esfenvalarate treatments resulted in 94% mortality of nymphs and spinosad resulted in 80% mortality at 1-DAT. Both were significantly greater than the control. The treatment with diflubenzuron resulted in 6% mortality and was not significantly different from the control. Mortality increased with time for each treatment except *B. bassiana*. AT 10-DAT, the treatments with esfenvalarate and spinosad resulted in 100 percent mortality and were significantly greater than the control. Treatment with diflubenzuron resulted in 94% mortality and azadirachtin treatment with 38% mortality of nymphs and were significantly greater than the control. Results indicate that the greatest mortality was recorded for the treatments with esfenvalarate, spinosad and diflubenzuron and their activity is not statistically different.

Temperature effect for each treatment (Table 3. 02).

There was no mortality noted for the water-treated control in all 4 treatment temperatures of 10°C, 15°C, 25°C and 35°C.

B. bassiana. At 1-DAT, 6% of the nymphs were killed when exposed to treatment with *B.bassiana* at 15°C. Mortality increased over time at all temperatures except 35°C where we found no mortality across all dates. At 10-DAT, mortality was significantly higher at 15°C and 25°C than at 10°C and 35°C. Results indicate that the activity is reduced at by high (35°C) and low (10°C) temperatures.

Spinosad. The treatment with spinosad resulted in an increase of mortality of nymphs ranging from 30 to 80% with increasing treatment temperatures at 1-DAT. Mortality increased over time at each temperature and at 10-DAT, it ranged from 100% for both temperatures at 25°C and 35°C and 60 percent at 10°C. There were no significant differences among mortality at 15°C, 25°C and 35°C. Results indicate that there is a trend of increasing mortality with increasing temperatures over time for spinosad but was not statistically significant.

Diflubenzuron. At 1-DAT, with diflubenzuron, the mortality of nymphs ranged from 13% at 25°C and 6% at 15°C and 35°C and were not significantly different among the temperatures. Mortality increased over time and at 10-DAT ranged from 94% at 35°C and 25% with 15°C. Mortality at 35°C was significantly greater than the mortality at 10°C, 15°C and 25°C. Results indicate that the activity increases with increasing (35°C) temperature.

Azadirachtin. For the treatment with azadirachtin, there was no mortality of nymphs at 1-DAT. Mortality increased slightly over time and at 10-DAT ranged from 38% at 35°C and 19% at 10°C and 15°C and were not significantly different among the temperatures. Results indicate that there was a trend of increasing mortality with increasing temperatures but was not statistically significant.

Esfenvalarate. At 1-DAT, for the treatment with esfenvalarate, the mortality of nymphs ranged from 100% for 10 °C and 15°C to 81% and 94% for 25°C and 35°C, and were not significantly different. Mortality at 25°C and 35°C increased to 100% at 2-DAT. Results show that the activity is not influenced by temperature at the concentration used in this research.

Table 3.01 Percent mortality of *M. differentialis* (Thomas) due to insecticide treatments across time by temperature using pooled data from Experiment 1, Experiment 2 and Experiment 3.

Temp(°C)	Treatment	Percent Mortality Days after treatment					
		1	2	4	6	8	10
10	control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	B.bassiana	0.0a	0.0a	12.5a	12.5a	12.5ab	18.75ab
	spinosad	40.0b	50.0b	50.0b	50.0b	60.0c	60.0c
	diflubenzuron	0.0a	0.0a	12.5a	18.75a	25.0b	31.25bc
	azadirachtin	0.0a	0.0a	0.0a	12.5a	12.5ab	18.75ab
	esfenvalarate	100.0c	100.0c	100.0c	100.0c	100.0d	100.0d
15	control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	B.bassiana	6.25ab	6.25ab	12.5a	18.75a	25.0b	50.0c
	spinosad	30.0b	30.0b	70.0b	80.0b	80.0c	90.0d
	diflubenzuron	6.25ab	6.25ab	18.75a	18.75a	25.0b	25.0bc
	azadirachtin	0.0a	6.25ab	12.5a	18.75a	18.75ab	18.75ab
	esfenvalarate	100.0c	100.0c	100.0c	100.0b	100.0c	100.0d
25	control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	B.bassiana	0.0a	18.75a	31.25c	50.0b	50.0b	62.5c
	spinosad	60.0b	60.0b	90.0d	100.0c	100.0c	100.0d
	diflubenzuron	12.5a	12.5a	25.0bc	25.0b	31.25b	37.5bc
	azadirachtin	0.0a	0.0a	6.25ab	25.0b	25.0b	31.25b
	esfenvalarate	81.25b	93.75c	100.0d	100.0c	100.0c	100.0d
35	control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	B.bassiana	0.0a	0.0a	0.0a	0.0a	0.0a	6.25a
	spinosad	80.0b	90.0c	100.0c	100.0d	100.0c	100.0c
	diflubenzuron	6.25a	25.0b	50.0b	62.5c	87.5c	93.75c
	azadirachtin	0.0a	0.0a	12.5a	31.25b	37.5b	37.5b
	esfenvalarate	93.75b	100.0c	100.0c	100.0d	100.0c	100.0c

For each insecticide percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Table 3.02 Percent mortality of M. *differentialis* (Thomas) due to insecticide treatments across time by treatments using the pooled data from Experiment 1, Experiment 2, and Experiment 3.

		Percent Mortality					
Treatment	Temp(°C)	Days after treatment					
		1	2	4	6	8	10
B.bassiana	10	0.0	0.0	12.5ab	12.5a	12.5ab	18.75a
	15	6.25	6.25	12.5ab	18.75a	25.0bc	50.0b
	25	0.0	18.75	31.25b	50.0b	50.0c	62.5b
	35	0.0	0.0	0.0a	0.0a	0.0a	6.25a
spinosad	10	40.0a	50.0a	50.0a	50.0a	60.0a	60.0a
	15	30.0a	30.0a	70.0ab	80.0ab	80.0ab	90.0ab
	25	60.0ab	60.0ab	90.0bc	100.0b	100.0b	100.0b
	35	80.0b	90.0b	100.0c	100.0b	100.0b	100.0b
diflubenzuron	10	0.0	0.0a	12.5a	18.75a	25.0a	31.25a
	15	6.25	6.25ab	18.75a	18.75a	25.0a	25.0a
	25	12.5	12.5ab	25.0ab	25.0a	31.25a	37.5a
	35	6.25	25.0b	50.0b	62.5b	87.5b	93.75b
azadirachtin	10	0.0	0.0	0.0	12.5	12.5	18.75
	15	0.0	6.25	12.5	18.75	18.75	18.75
	25	0.0	0.0	6.25	25.0	25.0	31.25
	35	0.0	0.0	12.5	31.25	37.5	37.5
esfenvalarate	10	100.0	100.0	100.0	100.0	100.0	100.0
	15	100.0	100.0	100.0	100.0	100.0	100.0
	25	81.25	93.75	100.0	100.0	100.0	100.0
	35	93.75	100.0	100.0	100.0	100.0	100.0

For each insecticide, percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Discussion

We observed significant differences in activity among treatment insecticides within set temperatures at 1-DAT but with time the difference in levels of activity gradually decreased and at 10-DAT there were few significant differences among treatments. Variation in temperatures did not result in mortality of the third instar M. *differentialis*. This was indicated by the zero mortality recorded for the control treatment in all four temperatures during the experiment.

Esfenvalarate was the most effective insecticide at 1-DAT at each treatment temperature. Pyrethroid insecticides have quick killing action compared to other classes of insecticides (Ware 2000). Temperature did not affect mortality of grasshoppers exposed to esfenvalarate. Other pyrethroid insecticides have been shown to respond negatively or positively to temperature (Scott 1995), however the temperature effect for the activity of esfenvalarate for grasshoppers has not been previously studied. Therefore, this information is important for the use of this pyrethroid in field conditions, at varying environmental temperatures.

Spinosad treatment provided comparatively better activity at 1-DAT, compared to other treatments except esfenvalarate at 25°C and 35°C. At 10-DAT, no differences in activity were observed between spinosad and esfenvalarate except at 10°C. This efficient and quick activity of spinosad may be due to its unique mode of action affecting nicotinic acetylcholine and GABA receptors in insect nervous system (Sparks et al. 1999). The activity of spinosad increased with increasing temperature but was not statistically different from each other except at 10°C. Mortality was significantly greater than the control at 10°C and 15°C but activity was comparatively higher at 25°C and 35°C

temperatures. Mortality reached 100% 4- DAT at 35°C and at 6-DAT at 25°. This suggests that spinosad is active quickly at high temperatures. Other than the pyrethroid esfenvalarate, spinosad is the only insecticide with significant activity at all temperatures tested. Due to its quick killing action at varying temperatures, spinosad is a possible choice of insecticide for use in grasshopper control in leafy green vegetables.

Beauveria bassiana treatments resulted in a moderate mortality levels at 15°C and 25°C, at 10-DAT and the activity was not significantly different from that of esfenvalarate, spinosad, diflubenzuron and azadirachtin. *Beauveria bassiana* treatments resulted in a temperature-mortality relationship with increasing mortality, from 15°C and 25°C. At 25°C, *B. bassiana* resulted in a high mortality of 83%. At 35°C, *B. bassiana* was completely inactivated. This relationship is supported by the work of Inglis et al. (1999) who showed that the migratory grasshopper *Melanoplus sanguinipes* treated with *B. bassiana* had the greatest number of fungal colony forming units at 25°C. At temperature above 25°C, populations of *B. bassiana* decreased (Inglis et al. 1999). It was determined that the upper thermal limit for conidial germination of *B. bassiana* is approximately 35-38°C and mycosis in grasshoppers treated with *B. bassiana* was inhibited by constant exposure to high temperature above 25°C (Inglis et al.1999). The arrest of conidial germination and disease development is likely the reason for low mortality from *B. bassiana* at 35°C. The slow activity of *B. bassiana* is due to the time required for conidia to germinate, penetrate through the cuticle, and cause disease.

Diflubenzuron and azadirachtin treatment results did not differ from each other at 10 DAT at all 4-treatment temperatures. Both diflubenzuron and azadirachtin are insect growth regulators and their activity is increases over with time. This probably explains

the low activity during the first few days of the experiment, which increased at latter stages of the experiments.

Mortality of diflubenzuron-treated grasshoppers increased with increasing temperature and time. Mortality was significant P=0.1 at 35°C compared to 10°C, 15°C and 25°C. The mortality was associated with molting deformities. The effect of temperature on the activity of diflubenzuron has not been noted previously. In storage, at 50°C for one week and 100°C for one day it was found that there was no significant decomposition of diflubenzuron and it is stable to sunlight (Environmental Health Criteria 1996). During the experiment, the third instar *M. differentialis* were observed to feed more and molt quickly at 35°C than in the 3 lower treatment temperatures irrespective of the treatment insecticide. This observation supports the maximum feeding rates recorded for *M. sanguinipes* nymphs at 35°C (Lactin et al. 1995). Diflubenzuron is an insect growth regulator, affecting chitin synthesis and translocation and thus the effect is more pronounced during molting (Ware 2000). This can be the cause for molting deformities observed in these experiments. The fast growth and early molting of the third instar *M. differentialis* at 35°C facilitated the increased mortality caused by diflubenzuron. Results suggest that its use for grasshopper control may be warranted during periods when temperatures are high.

Mortality of azadirachtin-treated grasshoppers increased with temperature although it was not statistically significant. Azadirachtin acts as an insect growth regulator which inhibits biosynthesis and metabolism of ecdysone hormone during molting (Isman 1999). In addition it acts as a behavior-modifying substance, deterring feeding (Isman 1999). Third instar grasshoppers tend to eat more, grow rapidly and molt

quickly at higher temperatures. Since azadirachtin is active at several sites the exact mode of action has not been determined. It may be a combination of all its activities causes the increase of mortality with increasing temperature.

To summarize, the pyrethroid insecticide esfenvalarate provided excellent efficacy in controlling third instar *M.differentialis* and its activity at labeled use rates is not temperature dependent. The microbial-derived insecticide spinosad has equally good efficacy as esfenvalarate for *M.differentialis* but efficacy increases with time after application and at increasing temperature. The activity of *B. bassiana* is greatest at 25°C and is negatively affected by high and low temperatures. The insecticide diflubenzuron provided good activity at high temperatures. The activity of azadirachtin is moderate in regard to mortality of *M.differentialis* and its activity is temperature dependent.

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Chapter IV

Efficacy and residual effect of insecticides for managing the differential grasshopper in leafy green vegetable crops

Abstract

This study was conducted to determine activity of possible alternative insecticides for replacing the use of organophosphate and carbamate insecticides for controlling grasshoppers in leafy green vegetables. Data on efficacy and residual activity of different classes of insecticides such as: an insect growth regulator, diflubenzuron [Dimilin]; a botanically derived insect growth regulator, azadirachtin [Neemix 4.5]; a microbial insecticide, *Beauveria bassiana* GHA [Mycotrol]; a microbial-derived insecticide, spinosad [Spintor] were collected and compared to similar data on currently recommended insecticides including endosulfan (cyclodiene, [Thiodan]; esfenvalarate pyrethroid, [Asana]; and organophosphate, naled [Dibrom]) to control the third instar differential grasshopper *Melanoplus differentialis* (Thomas).

Spinosad, diflubenzuron, endosulfan and esfenvalrate provided significant levels of control of third instar *M. differentialis* (Thomas). Activity of diflubenzuron was increased with time relative to the other three insecticides. The activity of these insecticides decreased with increasing time of exposure to the high summer environmental temperatures. The activity of naled was more contact oriented and had a short residual period. *Beauveria bassiana* was susceptible to inactivation under high

temperature and intense sunlight as occurs in the summer. The activity of azadirachtin was more pronounced when it made direct contact with the nymphs.

Introduction

Leafy green crops including kale, collards, mustard, turnip, and spinach are grown throughout temperate North America for the fresh and processing markets. Those crops grown for the processing market are typically harvested mechanically using cutter bars mounted in front of conveyer belts that transfer cut leaves to a holding container for transfer to the processing plant. Cut leaves are delivered to the processing plant in bulk and are processed into whole or cut leaf products that are canned or frozen. Processed raw ingredients are subject to stringent regulatory standards set by the United States Food and Drug Administration (FDA). The FDA has stringent standards that limit contaminants including insects, insect parts and fecal material.

Key pests of leafy green crops in the south central region of the United States include aphids, cucumber beetles, seed maggots, and larvae of several lepidopteran species. These pests are generally attracted to the crops and feed and reproduce on plants in production fields. Grasshoppers can be a significant contaminant of leafy greens (Gecan et al.1990). Generally, grasshoppers do not reproduce within fields of leafy greens but rather move into them from adjacent rangeland, pastures, or other crops (Sweeden, 1996). The grasshoppers do feed on leafy greens and may reduce productivity. However the main problem is that due to their large size, they serve as significant sources of contamination in the harvested product.

Grasshoppers are often difficult to control due to their large size and mobility (Sweeden, 1996). Populations may be less susceptible to insecticides in comparison to smaller insects such as aphids or lepidopteran larvae simply due to their larger body mass. Due to their mobility, they can leave a treated field quickly, then return quickly and/or re-infest a treated area several days when residual activity has decreased due to exposure to rain or sunlight.

Due to the strict standards limiting insect parts and feeding contamination of processed vegetable crops, grasshoppers thresholds in fields of leafy greens destined for the processing market are practically zero. Therefore, management and control of insect pests and especially of grasshoppers is a critical issue (Gecan et al. 1990). Insecticides are the main tool used for managing grasshopper populations and considerable research has been conducted to determine insecticide efficacy and residual activity for controlling grasshoppers on various crops. However, due to implementation of the Food Quality Protection Act in1996, several organophosphate, carbamate and pyrethroid insecticides are under regulatory review and may lose their registration. Registered insecticides that are currently under review by EPA for possible de-registration include diazinon, dimethoate, chlorpyriphos, malathion, methomyl, permethrin, carbaryl, and dibrom. Diazinon, dimethoate, methomyl, dibrom, and permethrin are effective, commonly used insecticides for controlling insect pests on leafy green crops (Extension Agents' Handbook, 2002). Possible replacements include spinosad, esfenvalerate, diflubenzuron, neem extracts, and biological insecticides based on the fungus *Beauveria bassiana*.

The following research was conducted to determine activity of possible alternative insecticides for replacing the use of organophosphate and carbamate

insecticides for controlling grasshoppers. The research focussed on developing efficacy data including residual activity.

Materials and Methods

Insecticides. The following insecticides were obtained as formulated materials as noted and used at the rates as stated: diflubenzuron (Dimilin 2L, Uniroyal Chemical Comp., INC, Middleburg, CT 06749) 0.07kg a.i./ha, appl. rate, 0.28 kg/ha; azadirachtin (Neemix 4.5, Certis USA , Columbia, MD 21046) 0.048 kg a.i./ha, appl. rate,1.121 kg/ha; *Beauveria bassiana* GHA (Mycotrol, Mycotech Corp. Butte, MT 597022) 0.179 kg a.i. /ha. appl. rate, 1.592 kg/ha; esfenvalerate (Asana XL, E. I. Du Pont de Nemours & Comp., INC, Wilmington, DE 19898) 0.055 kg a.i./ha, appl. rate 0.673kg/ha; spinosad (Spintor 2SC, Dow Agro Sciences LLC, Indianapolis, IN 46268) 0.175 kg a.i./ha, appl. rate, 0.7 kg/ha; endosulfan (Thiodan 3EC FMC Corp., Philadelphia, PA 19103) 1.118 kg a.i./ha, appl. rate,2.981 kg/ha; and naled (Dibrom8, Amvac Chemical Corp. Los Angeles, CA 90023) 2.102 kg a.i./ha, appl. rate, 2.242 kg/ha.

Third instar *Melanoplus differentialis* nymphs were collected from a pasture in southeast Oklahoma using a 38cm diameter sweep net (Bioquip®). The third instars were identified by the general color of pale yellow to tan, with body length in the range of 9.4 to 12.4 mm and a black stripe on the hind femur occupying the center of the medial area (Pfadt, 1994). The Systematic Entomology Laboratory, USDA, Beltsville, MD identified and verified the samples of the nymphs and nymphs reared to adults as *M.*

differntialis nigricans (Lot No.0107781). The collected grasshoppers were immediately transported to the entomology laboratory at the Wes Watkins Agricultural Research and Extension Center (WWAREC), Lane, Oklahoma and kept in aluminum wire meshed mosquito cages of 45x45x45cm (Bioquip®). A 3cm layer of sand was placed on the bottom of the cages. Fresh collard leaves and water were provided each day. Insects were maintained at a 14-hour photo-period and room temperature of 26.7 ± 0.5°C.

The experimental site was a research field at the Wes Watkins Agricultural Research and Extension Center (WWAREC), Lane, Oklahoma. Before planting, fertilizer was applied to the soil at the rate of 112.08 kg/ha of 17:17:17 NPK. Collards (*Brassica oleraceae* L. var. acephala, *cultivar* "Champion") seeds were direct seeded into raised beds, 1.8m wide with 2 rows with row spacing of 0.9m on 16 May 2001 using a tractor-mounted planter at the rate of 1 inch between seeds within rows. A pre-emergent herbicide, Treflan, was applied to the soil 3 days before planting at the rate of 0.84 kg/ha. Irrigation was provided through an overhead sprinkler system. The experimental design was a randomized complete block with 5 blocks. Each plot was 1 bed wide and 6m long, bordered on each side by an untreated bed and on each end by a 4.5m – long fallow area.

Forty, third instar *M. differentialis* nymphs were selected from the rearing cages. Nymphs of similar size were isolated in groups and starved for 24 hours in the laboratory at a temperature of 26.7±0.5°C. Each was placed in an aluminum wire meshed cage 30cm in length and 45cm in perimeter. Cloth sleeves were glued to each end to facilitate access to the cage from both ends. The sleeves were secured with a tie-on strip of material.

After the 24-hour starvation period, the cages with third instars were sealed from both sides and transferred to the experiment site. A plant was selected from each plot and one leaf from the upper plant canopy with a mean leaf area of 265.1 ± 8.5 cm^2 was carefully inserted in to each cage by opening one end of the cloth sleeve and tied closed. Cages were placed as one per each plot. Each insecticide treatment except *B. bassiana* was sprayed using a tractor-mounted sprayer with 6 hollow cone nozzles per bed applying 332 Liter/ha solution on July 6, 2001 (Experiment 1). *B. bassiana* was sprayed on the evening of July 5, 2001 to avoid exposure to high environment temperature soon after the treatment.

After 24 hours, each cage was checked for insect mortality for a ten-day period. Mortality of grasshoppers was determined by monitoring for lack of movement, insects lying on their sides, or inability to have a coordinated response to a slight shaking of the cage. The cadavers of the nymphs in *B. bassiana* treatments were kept in a temperature chamber at 25°C for 14 days for mycelium growth. Mortality from *B. bassiana*, was counted only when mycelium was visible on the integument through microscopic examination. To assess residual activity, another set of 40 cages, each containing one 24-hour starved third instar *M. differentialis* were placed in each plot 24 hours after the initial treatment. Grasshopper mortality in this study was recorded at 24 hours intervals over a ten-day period. This experiment was repeated on July 19, 2001 (Experiment 2), and the cages were placed in the field at one hour and 24 hours after spraying. The mortality was recorded as described above.

This experiment was repeated in the summer of 2002 (Experiment 3). The plots were direct seeded on April 26, 2002 and insecticide treatments applied on June 20, 2002.

The experimental design was a completely randomized with 5 replications. Cages were placed in plots before treatment, 1 hour after treatment and 24 hours after treatment. Mortality of grasshoppers was recorded as indicated previously.

Leaf Bio-assays. A fully expanded leaf with mean leaf area of 262.1 ± 8.5 cm^2 was collected from the top of the plant canopy after 24 hours, on the 4^{th} and 7^{th} day after spraying, from each plot. Leaves were taken to the laboratory and separately placed into plastic cages. The cut end of the leaf petioles were plugged with moistened cotton balls in order to delay drying. A single leaf was placed in a cage containing a third instar *M. differentialis* nymphs that had been starved for 24 hours. Grasshopper mortality was determined at 24-hour intervals over a 10-day period as described above. Leaf bioassays were performed for all 3 experiments as previously described.

SAS (SAS Institute 1999) software was used for the data analysis. The CATMOD procedure was used to find the experiment and treatment effects at the $P<0.1$ significance level. The data for all three experiments were pooled and Proc Freq in SAS with Fisher's Exact Test was used to determine the treatment effect for the caged grasshoppers as well as for the leaf bioassays. In these experiments we determined the efficacy of insecticides as insect mortality and used a 0.1 significance level.

Results

Treatment effect for cages placed before treatment. There was no mortality of nymphs for the water- treated control (Table 4. 01). One hundred percent mortality of nymphs

resulted from treatment with naled at 1-DAT and it was significantly greater than the water-treated control (Table 4.01). Endosulfan treatment resulted in 80% mortality and the treatment with esfenvalarate and spinosad resulted in 70% mortality and were significantly greater than the water-treated control. Mortality increased over time with spinosad, diflubenzuron, azadirachtin, esfenvalarate and *B. bassiana*. At 10-DAT mortality ranged from 100% with naled to 20% with *B. bassiana* which were not significantly different from the water-treated control (Table 4.01). Results indicate that spinosad, endosulfan,, naled, and esfenvalarate applications resulted in greater mortality than the water-treated control at 1-DAT and that and azadirachtin and diflubenzuron resulted in greater mortality at 10-DAT.

Treatment effect for cages placed 1 hour after treatment. There was no mortality of the nymphs with the water-treated control (Table 4.02). At 1-DAT, 40% of the nymphs died after being treated with spinosad and 30% with naled. These mortality rates were significantly greater than the water-treated control. Twenty percent of the nymphs died after exposure to treatments with endosulfan and esfenvalarate and these were not significantly different from the water-treated control. Mortality increased over time for each treatment. At 10-DAT, mortality ranged from 80% with spinosad to 20% with *B. bassiana* but were not significantly different from the water-treated control. Results indicate that the 1-hour old residues of esfenvalarate, naled and endosulfan resulted in low mortality 1-DAT. However, 1-hour old residues of spinosad and esfenvalarate did provide significant levels of mortality 10-DAT.

Treatment effect for cages placed 24 hours after treatment. Esfenvalarate was the only treatment that resulted in grasshopper mortality when cages were placed on plants 24 hours after treatment (Table 4.03). At 2-DAT, 13% mortality was noted for spinosad and 7% mortality for azadirachtin, diflubenzuron, endosulfan and esfenvalarate. They were not significantly different from the water-treated control. Mortality increased over time for spinosad, diflubenzuron and azadirachtin treatments. At 10-DAT, 47% of the nymphs died from treatment with spinosad and 60 percent died from treatment with diflubenzuron and 27% mortality was noted for azadirachtin treatment. These were significantly greater than the water-treated control. Results indicate that only treatments with spinosad, diflubenzuron and azadiractin resulted in mortality when grasshoppers were exposed to 24-hour-old residues.

Bioassay of leaves removed 1hour after treatment. AT 1-DAT, 10 percent of the nymphs died from treatment with naled and 20 % mortality was noted for treatments with spinosad and endosulfan and these were not significantly different than the water-treated control (Table 4.04). Esfenvalarate treatment resulted in 30 % mortality and was significantly more toxic than the water-treated control. Mortality gradually increased with time for each treatment. At 10-DAT, mortality ranged from 70 % with esfenvalarate to 20 percent with diflubenzuron and naled but were not significantly different from the water-treated control. Results show that esfenvalarate, endosulfan and spinosad treatments resulted in better residual effect after exposure to the environment for 1 hour.

Bioassay of leaves removed 24 hours after treatment. At 1-DAT, (Table 4.05) 20% of the nymphs were dead resulting from treatment with *B. bassiana*, and 7% were dead from the treatment with spinosad, endosulfan and esfenvalarate and were not significantly different from the water-treated control. Mortality increased with time for spinosad, azadirachtin, and esfenvalarate treatments. At 10-DAT, 27% mortality resulted from treatment with esfenvalarate and was significantly greater than the water-treated control. Results show that the residual activity of insecticides decreases with time and exposure to the environment, and that esfenvalarate may be the only insecticide that provide significant levels of mortality with 24 hour old residues.

Bioassay for leaves removed 4 days and 7 days after treatment. None of the treatments resulted in any mortality when leaves were removed 4 days and 7 days after treatment.

Table 4.01 Percent mortality over time with each treatment for *M. differentialis* (Thomas) for the cages placed on plants before treatment.

Treatment	Percent Mortality Days After Treatments (DAT)					
	1	2	4	6	8	10
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
spinosad	70.0bc	90.0b	90.0c	90.0ef	90.0ef	90.0ef
diflubenzuron	0.0a	10.0a	30.0b	30.0bc	30.0bc	60.0cde
azadirachtin	0.0a	10.0a	20.0ab	40.0c	40.0c	40.0bc
endosulfan	80.0cd	80.0b	80.0c	80.0def	80.0def	80.0def
naled	100.0d	100.0b	100.0c	100.0f	100.0f	100.0f
esfenvalarate	70.0bc	80.0b	80.0c	80.0def	80.0def	80.0def
B.bassiana	0.0a	10.0a	10.0a	10.0ac	10.0ac	20.0ab

Percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Table 4.02 Percent mortality over time with each treatment for *M. differentialis* (Thomas) for the cages placed on plants 1 hour after treatment.

Treatment	Percent Mortality Days After Treatments (DAT)					
	1	2	4	6	8	10
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
spinosad	40.0c	60.0e	80.0e	80.0e	80.0f	80.0f
diflubenzuron	0.0a	10.0abc	20.0abcd	30.0bcd	70.0ef	70.0ef
azadirachtin	0.0a	0.0ab	10.0abc	20.0abcd	30.0bcd	30.0bcd
endosulfan	20.0abc	30.0bcde	30.0bcd	30.0bcd	30.0bcd	30.0bcd
naled	30.0bc	40.0cde	40.0cd	40.0cd	40.0cde	40.0cde
esfenvalarate	20.0abc	50.0de	50.0de	50.0de	50.0def	60.0def
B.bassiana	0.0a	0.0a	10.0abc	10.0abc	10.0abc	20.0abc

Percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Table 4.03 Percent mortality over time with each treatment for *M. differentialis* (Thomas) for the cages placed on plants 24 hours after treatments.

Treatment	Percent Mortality Days After Treatments (DAT)					
	1	2	4	6	8	10
Control	0.0	0.0	0.0	0.0a	0.0a	0.0a
spinosad	0.0	13.3	26.7b	46.7c	46.7cd	46.7cd
diflubenzuron	0.0	6.7	20.0ab	33.3bc	53.3d	60.0d
azadirachtin	0.0	6.7	6.7ab	20.0abc	26.7bcd	26.7bc
endosulfan	0.0	6.7	6.7ab	6.7a	6.7ab	6.7ab
naled	0.0	0.0	0.0a	0.0a	0.0a	0.0a
esfenvalarate	6.7	6.7	6.7ab	6.7a	6.7ab	6.7ab
B.bassiana	0.0	0.0	0.0a	0.0a	0.0a	0.0a

Percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Table 4.04 Percent mortality over time with each treatment for *M. differentialis* (Thomas) using a leaf bioassay of tissue removed 1 hour after treatment.

Treatment	Percent Mortality Days After Treatments (DAT)					
	1	2	4	6	8	10
control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
spinosad	20.0ab	20.0ab	50.0cd	60.0cd	60.0de	60.0de
diflubenzuron	0.0a	0.0a	0.0a	10.0a	10.0ab	20.0abc
azadirachtin	0.0a	0.0a	0.0a	0.0a	0.0a	30.0bcd
endosulfan	20.0ab	20.0ab	40.0bcd	50.0bcd	50.0cde	50.0cde
naled	10.0ab	10.0ab	10.0ab	10.0a	10.0ab	20.0abc
esfenvalarate	30.0b	40.0b	60.0d	70.0d	70.0e	70.0e
B. bassiana	0.0a	0.0a	0.0a	0.0a	30.0bcd	30.0bcd

Percent mortality followed by the same letter in a column for each date (DAT) are not significantly different ($P > 0.1$, Fisher's Exact Test)

Table 4.05 Percent mortality over time with each treatment for *M. differentialis* (Thomas) using a leaf bioassay of tissue removed 24 hours after treatment.

Treatment	Percent Mortality Days After Treatments (DAT)					
	1	2	4	6	8	10
control	0.0	0.0	0.0	0.0a	0.0a	0.0a
spinosad	6.7	6.7	13.3	13.3ab	13.3ab	13.3ab
diflubenzuron	0.0	0.0	0.0	0.0a	0.0a	0.0a
azadirachtin	0.0	0.0	0.0	13.3ab	13.3ab	13.3ab
endosulfan	6.7	6.7	6.7	20.0ab	20.0ab	20.0ab
naled	0.0	0.0	6.7	6.7ab	6.7ab	6.7ab
esfenvalarate	6.7	6.7	20.0	26.7b	26.7b	26.7b
B. bassiana	20.0	20.0	20.0	20.0ab	20.0ab	20.0ab

Percent mortality followed by the same letter in a column for each date (DAT) are not significantly different (P >0.1, Fisher's Exact Test)

Discussion

None of the nymphs treated with water died in any of the experiments. Therefore, nymph mortality was caused only by insecticides and not by the environmental factors common to all treatments.

Except for *B. bassiana*, all the other insecticide treatments resulted in control of the third instars of *M. differentialis* (Thomas) at 10-DAT for the cages placed before the treatments so that insects were directly exposed to insecticides. High mortality of nymphs resulted from both insect growth regulators, diflubenzuron and azadirachtin with time. Mortality was greater with spinosad, endosulfan, naled and esfenvalarate treatment at 1-DAT. These insecticides are nerve toxins with quick killing action compared to the microbial insecticides and insect growth regulators (Ware 2000). The activity of insecticides decreased with time of exposure to the environment for endosulfan, naled and esfenvalarate. None of these insecticide treatments resulted in significant activity when grasshoppers were exposed to residues at 24 hours or greater time after treatment.

In contrast to the activity of the nerve toxins, only the treatment with spinosad resulted in significant activity over time. This efficient and rapid activity of spinosad may be due to its unique mode of action, affecting nicotinic acetylcholine and GABA receptors in insect nervous system (Sparks et al. 1999).

The insect growth regulators, diflubenzuron and azadirachtin did not result in mortality at 24 hours for the cages placed before treatment, or in cages placed one and 24 hours after treatment. Insect growth regulators result in mortality over time. Diflubenzuron must be ingested by the nymphs to initiate its activity on chitin synthesis

and translocation in the insect cuticle (Ware 2000). These activities are initiated at the time of molting and insects need to molt in order to exhibit the effects of diflubenzuron such as molting deformities (Environmental health Criteria 184 1996). Thus, the significant mortality resulting from treatment with diflubenzuron at 10-DAT was expected. Diflubenzuron resulted in high mortality for grasshoppers in cages placed before treatment, and 1 and 24 hours after treatment. This indicates its stability under ambient temperature and sunlight compared to the other treatments. This supports the long photo stability previously reported for diflubenzuron Environmental health Criteria 184 1996).

Azadirachtin is a stomach and contact active insecticide with a complex mode of action (Ware 2000). In addition to its activity as an insect growth regulator which affects biosynthesis and metabolism of ecdysone hormone, azadirachtin also acts as a behavior modifying substance deterring feeding in some insects (Isman 1999). Thus, the low mortality noted may be due to the low consumption of treated leaves by the nymphs observed during the experimental period.

In the leaf bioassay experiments, esfenvalarate and endosulfan resulted in increased effect compared to naled for both assays of leaf tissues removed 1 and 24 hours after treatment. This may be due to exposure to a constant temperature of 26°C maintained inside the laboratory compared to the insecticides being exposed to high temperature (Appendix A), radiation, and sunlight under field conditions. Spinosad treatment resulted in similar mortality for both cages under field condition and for the leaf bioassay of 1 hour. But its activity in the field after 24 hours was greater than the

activity indicated with leaf bioassays of 24 hours. This indicates that spinosad may be more active at higher temperatures than under the moderate laboratory temperature.

In general, the treatment with *B. bassiana* resulted in slightly better activity using leaf bioassays than indicated in the field cages. From the leaf bioassays its activity was more pronounced for the leaf tissue removed 1-hour after the treatment application compared with tissue removed 24 hours after. *B. bassiana* is sensitive to high temperatures and intense sunlight and the literature indicates that more colony forming units occur around 25°C (Inglis et al. 1999). This may be a cause for its inactivity under the field conditions with exposure to the high summer temperatures (Appendix A).

In summary, spinosad, diflubenzuron, endosulfan and esfenvalrate provided significant levels of control of third instar *M. differentialis*. The effect of diflubenzuron was more pronounced with time than the other three insecticides. The activity of these insecticides decreased with increasing time of exposure to the summer environment. The activity of naled was contact oriented with a short residual period. Indications are that *B. bassiana* is susceptible to inactivation under high temperature and intense sunlight as occurs in the summer. The activity of azadirachtin was more pronounced when it made direct contact with the nymphs.

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Conclusions

The pyrethroid insecticide esfenvalarate provided excellent efficacy in controlling third instar *M. differentialis* (Thomas) and its activity at labeled use rates is not temperature dependent. The microbial-derived insecticide spinosad has equally good efficacy as esfenvalarate for *M.differentialis* but efficacy increases with time after application and at different temperatures. The activity of *B. bassiana* is more pronounced at 25°C and is affected by high and low temperatures and susceptible to inactivation under high temperature and intense sunlight as occurs in the summer. The insecticide diflubenzuron provided good activity at high temperatures and its effect is more pronounced with time. The activity of azadirachtin is moderate in regard to mortality of *M. differentialis* and its activity is temperature dependent. Endosulfan provided significant level of control of third instars. The activity of naled was more contact oriented.

APPENDIX A. Maximum daily temperature recorded at Lane, Oklahoma for the months of June and July 2001 and June 2002 (Mesonet climatological data summary-Oklahoma Climatological survey).

Day	Maximum Temperature °C		
	June 2001	July 2001	June 2002
1	28.33	30.56	31.67
2	32.22	31.11	32.22
3	31.67	32.78	31.67
4	32.22	33.89	31.11
5	31.11	33.89	27.78
6	26.67	35.0	28.33
7	30.56	34.44	29.44
8	31.67	33.89	30.0
9	32.22	35.56	31.67
10	32.22	35.56	31.67
11	32.78	35.56	32.22
12	32.78	37.22	32.78
13	33.33	34.44	30.56
14	32.22	26.67	28.33
15	31.11	31.11	28.89
16	32.22	34.44	27.78
17	32.22	35.56	29.44
18	32.78	35.56	29.44
19	32.78	35.0	32.22
20	32.78	35.56	32.22
21	30.0	36.67	32.78
22	30.0	37.22	32.22
23	31.67	37.22	32.78
24	32.22	37.22	31.67
25	31.67	36.67	30.56
26	31.67	36.11	30.56
27	30.56	36.11	31.11
28	26.11	35.56	32.22
29	26.67	33.33	30.56
30	30.56	36.11	29.44
31		36.67	

VITA 2

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