## ABSTRACT

AMBROSE, MARGERY LEE. Characterization of the insecticidal properties of acetamiprid under field and laboratory conditions. (Under the direction of Dr. J.R. Bradley, Jr. and Dr. J. W. Van Duyn.)

Acetamiprid, a member of the neonicotinoid insecticide family, is a fairly new insecticide that has recently entered the market place. Its unique mode of action offers control against many important pests that had previously evolved resistant strains to most insecticides. Acetamiprid is very selective and provides outstanding control of sucking pests such as aphids and whiteflies without having detrimental effects on non-targets. Also, laboratory and greenhouse testing revealed acetamiprid's ovicidal activity against many pest species, including the bollworm, *Helicoverpa zea* (Boddie).

Field trials were conducted to evaluate the ovicidal properties of acetamiprid (Intruder® 70WP) and other various neonicotinoids, against *Helicoverpa zea* (Boddie) under field conditions in Washington, Perquimans and Nash counties, North Carolina during 2001 and 2002. Newly laid (white) bollworm eggs were collected from cotton foliage within respective treatments at 0, 1, and 2 days after application and evaluated in the laboratory for ovicidal and eclosion mortality. During 2001, acetamiprid initially (0 day) provided mortality of bollworm eggs comparable to that obtained with two ovicide standards, thiodicarb and lambda-cyhalothrin. For eggs collected at one day after application, ovicidal activity of acetamiprid had declined more than that observed for the two standard ovicides; only the highest rate of acetamiprid provided comparable ovicide activity. There was no significant ovicidal activity with any of the insecticides for eggs deposited two nights after application (2 DAT). During 2002, the neonicotinoids acetamiprid, imidacloprid, and thiamethoxam provided mortality for eggs present on the plants at application (0 DAT),

comparable to that obtained with thiodicarb and lambda-cyhalothrin. Mortality of eggs deposited the night after application and collected 24 hr after application (1 DAT) dropped precipitously in all insecticide treatments, except thiodicarb. Egg mortality at 1 DAT was comparable for all neonicotinoids. For eggs collected at two day after application, ovicidal activity of the neonicotinoids had ceased and egg mortality in the standard treatments had declined to ca. 25%. These studies confirmed ovicidal activity of all tested neonicotinoids under field conditions; however ovicidal activity of neonicotinoids was ephemeral and not comparable to that of the ovicide standards.

Comparative effects of various insecticide residues on populations of certain beneficial and pest arthropod species in cotton were examined in two field studies. Treatment effects were evaluated by population assessment through sweep net sampling at 1, 4, 7 and 14 days after application. Acetamiprid, spinosad, indoxacarb, and methoxyfenozide had no adverse impact on numbers of beneficial or pest species population levels when data were averaged over sampling dates and test locations. The lack of an observed treatment effect for any of the insecticides tested, particularly at 1 and 4 DAT, confirmed the selective toxicity profiles of the compounds.

Acetamiprid, imidacloprid, lambda-cyhalothrin, spinosad, and thiodicarb were evaluated to access their toxicity to preimaginal *Trichogramma exiguum* in a laboratory study and in a field study to determine the toxicity of residues on adult *T. exiguum*. Lambda cyhalothrin and spinosad caused very high mortalities of preimaginal *T. exiguum* when applied to host eggs; imidacloprid caused intermediate mortality, and acetamiprid and thiodicarb were not toxic. Acetamiprid was the only insecticide that was not toxic to *T. exiguum* adults when exposed at 1 DAT to residues on leaves of treated cotton plants in the field.  None of the insecticides were toxic to adult *T. exiguum* at 6 DAT under field conditions.

**CHARACTERIZATION OF THE INSECTICIDAL**

**PROPERTIES OF ACETAMIPRID UNDER**

**FIELD AND LABORATORY CONDITIONS.**

by

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# CHAPTER I

# LITERATURE REVIEW

Since the 1970's three chemical classes, the organophosphates, the carbamates and the pyrethroids have dominated the insecticide market. Insect resistance to these chemical classes has developed in recent years resulting in a decrease in their effectiveness to suppress pest populations (Leonard *et al*. 1998, Plapp and Campanhola 1986, Roof and DuRant 1998). Over the past decade, a spate of new insecticides with new chemistries have entered the market place; these include the neonicotinoids. Due to a different mode of action, neonicotinoids control many important pests that have evolved strains resistant to most other insecticides. Since the introduction of the first neonicotinoid, imidacloprid, the neonicotinoids have become the largest selling insecticide class worldwide; they are marketed today in more than one hundred and twenty countries for use on more than one hundred and forty crops.

**Nicotine**

Nicotine has been used for centuries to control sucking insect pests of many crops, despite its relative low efficacy against pests and high toxicity to humans. Nicotine was used as an insecticide as early as 1746 when the infusion of tobacco leaves was used for treating aphids, but it was not isolated until 1828. Its greatest use was in the 1930's and 40's, just prior to the synthetic insecticide era, when 1.2 million pounds were produced and sold commercially. Nicotine activates post-synaptic acetylcholine receptors in the insect central nervous system, which results in violent convulsions followed by paralysis and death. The success of nicotine was due to its rapid knockdown of susceptible insects. However, its success was limited because of its rapid degradation in the environment, lack of selectivity, and its toxicity to vertebrates made nicotine impractical in most agricultural situations.

## Discovery of neonicotinoids

The nicotinic acetylcholine receptors (nAChRs), located in the insect central nervous system (CNS), are agonist gated ion channels responsible for neurotransmission. Acetylcholine mediates excitation on all nAChRs which are widely expressed in the insect central nervous system on synaptic nerve terminals of motor neurons, interneurons, and sensory neurons (Breer 1988). The great abundance and essential physiological function of nAChRs within the insect brain make them good target sites for the development of insecticides. Nicotine is an agonist to these receptors and was simplified by Yamamoto (1965) to 3-pyridylmethylamines, but this had no practical insecticidal value. The subsequent addition of a 3-pyridylmethyl group increased the insecticidal activity and further optimization resulted in the invention of the first neonicotinoid, imidacloprid (Shiokawa *et al.* 1986). Imidacloprid was found to be an agonist to the nAChRs (Bai *et al.* 1991, Tomizawa and Yamamoto 1992, 1993, Liu and Casida 1993). Nicotine and imidacloprid share the same structural moiety, the same mode of action, and essentially the same structure activity relationships (Tomizawa and Yamamoto 1993, Liu *et al.* 1993, Tomizawa 1994). Several analogs, including acetamiprid, thiamethoxam and thiacloprid, have since been discovered and have been shown to have the same mode of action as imidacloprid (Tomizawa and Yamamoto 1993, Liu *et al.* 1993, Yamamoto *et al.* 1995). Having nitroimine, cyanoimine, or nitromethylene as an essential moiety, they can be collectively called neonicotinoids (Tomizawa and Yamamoto 1993).

## Mode of Action of Neonicotinoids

Binding of acetylcholine, the major excitatory neurotransmitter in insects (Pitman 1971), to the nAChRs opens the ion pore of the receptor and induces a depolarization of the nerve cell membrane which can trigger an action potential (nerve impulse). The neonicotinoids bind agonistically to the nAChRs in the CNS of insects (Bai *et al.* 1991, Liu and Casida 1993, Nauen *et al.* 1996, 2001, Lind *et al.* 1999, Zhang *et al.* 2000). During this process they mimic acetylcholine and induce abnormal excitement in the insect by interrupting the normal synaptic transmission. ACh- esterase, which degrades the natural transmitter ACh, does not affect the neonicotinoids so they continue to cause additional nerve excitement. Consequently, the insect suffers from excitation and paralysis, followed by death. They are effective on contact and via stomach action. This mode of action is unique to the neonicotinoids so cross-resistance to conventional insecticides is nonexistent.

**Characteristics**

The neonicotinoids possess common characteristics that distinguish then from conventional insecticides (Yamamoto 1965, Iwata and Takase 1993, Kashiwada 1996, Matsuda and Takahashi 1996). They are effective against a broad insect spectrum (Elbert *et al.* 1990, Takahashi *et al.* 1992), offering excellent control of aphids, whiteflies and other insects, especially homopteran pest species, worldwide (Mullins 1993, Wang *et al.* 1995, Nauen *et al.* 1998). Neonicotinoids are effective through several different modes of application, including foliarly and as a seed or soil treatment (Elbert *et al.* 1998). They have low hydrophobicity, which is related to their excellent systemic and translaminar activity. The systemic behavior of neonicotinoids has been studied in several crops, such as wheat, cotton and sugar beet (Stein-Dönecke *et al.* 1992, Westwood *et al.* 1998). These studies demonstrated that neonicotinoids are mainly acropetally transported in the xylem. This systemic property allows the chemical to become evenly distributed in the young, growing plant (Elbert *et al.* 1998). A correlation has been found between the method of application and the systemic efficacy of neonicotinoids. Imidacloprid tends to be more effective systemically after soil application while acetamiprid, a first generation neonicotinoid developed by Aventis CropScience, was superior after foliar application (Horowitz *et al.* 1998). Not only does application play a part, but the crop species also affects the systemic efficacy of the active ingredient. Imidacloprid's penetration and translocation in cotton leaves is less pronounced than in cabbage (Bucholz and Nauen 2001). Translaminar movement is also an important attribute that allows the insecticide to control pests on both the sprayed leaf surface and the opposite side. This is important when treating pests such as aphids and whiteflies that live and feed predominantly on the underside of leaves. Having characteristics such as systemic and translaminar activities makes the neonicotinoids particularly effective on sucking pests such as aphids, leafhoppers, and whiteflies (Natwick 2001, Parrish et al 2001).

Neonicotinoids have also demonstrated ovicidal activity against many crop pests (Horowitz *et al.* 1998, Elbert *et al.* 2001, Parrish *et al.* 2001). Horowitz *et al.* (1998) reported ovicidal activity of neonicotinoids against the cotton whitefly, *Bemisia tabaci*, while Elbert *et al.* (2001) reported ovicidal activity of thiacloprid, a neonicotinoid developed by Bayer, against *Cydia pomonella.* Elzen (1997) and All *et al.* (2001) reported that imidacloprid possessed ovicidal activity on tobacco budworm, *Heliothis virescens*. Parrish *et al.* (2001) reported ovicidal activity of neonicotinoids against a variety of crop pests including the tobacco budworm, bollworm, cabbage looper, green stinkbug and Colorado potato beetle in greenhouse tests.

With the ability to control sucking pests, including vectors of plant pathogenic viruses, neonicotinoids reduce infection rate and spread of many crop viruses. Imidacloprid does not cause rapid knockdown of sucking pests; however, it does possess properties that cause a cessation in feeding (Dewar 1992, Knaust and Poehling1992, Bethke *et al.* 2001) thereby, reducing plant damage and virus infection during the premortality phase. After uptake of imidacloprid by sucking pests, feeding ceases and an avoidance behavior results (Dewar 1992, Dewar and Read 1990, Knaust and Poehling 1992, Tatchell 1992, Mason *et al.* 2000). Dewar and Read (1990) suggested this avoidance behavior could be a repellent effect, however others believe it may be due to a strongly delayed antifeedant effect (Knaust and Poehling 1992). Whatever the mechanism, neonicotinoids have substantially reduced virus infections in several field crops. This is partially due to a reduction in virus transmission to treated plants because of the effects of neonicotinoids on insect mortality and behavior (Bethke *et al.* 2001, Tacthell 1992). Lengthy residual activity contributes to their success in reducing transmission of viruses (Bethke *et al.* 2001, Buchholz and Nauen 2001, Elbert et al 2001, Mason *et al.* 2000, Knaust and Poehling 1992). Application method can be a factor in the efficacy of neonicotinoids against virus transmission. Oleander leaf scorch transmission by *Homalodisca coagulata* (Say) was blocked by applications of foliar-applied acetamiprid, as well as soil-applied imidacloprid and thiamethoxam (Bethke *et al.* 2001). Drench application of thiamethoxam to tomato plants provided protection from tomato yellow leaf curl for up to twenty-two days, while foliar treatment was only effective for eight days (Mason *et al.* 2000). Tomato spotted wilt virus was reduced in tobacco fields by applying imidacloprid in the plant house, while applying the same dosage in transplant water was less effective (Rudolph and Rogers 2001).

Neonicotinoids have also been useful for the control of white grubs (Coleoptera: Scarabaeidae) through disrupting normal nerve function causing behavior modification.

Imidacloprid and thiamethoxam have been found to alter diapause behavior of *Popillia japonica* (Grewal *et al.* 2001) through preventing downward movement of overwintering grubs at the onset of winter. Thus, grub mortality increased indirectly from freezing, by being more available to predation by vertebrates such as birds and raccoons, and by increasing susceptibility of treated grubs to arthropod natural enemies and pathogens. Also, Koppenhoffer *et al.* (1991, 1998) found that grubs treated with imidacloprid were more susceptible to entomopathogenic nematodes.

**Environmental Fate**

Agricultural land is no longer seen only as a crop production site, but is also regarded as an integral part of a larger ecosystem. Thus interventions of any kind must be evaluated for their effects on the complex of plants, animals, soil, and water. Before any chemical is accepted for use in crop protection its short and long term impacts on the environment must be known. Environmental behavior of chemicals depends on physico-chemical and chemical properties defined principally by the chemical structure. Acetamiprid and imidacloprid possess the same physico-chemical properties, but acetamiprid happens to be more hydrophilic (Buchholz and Nauen 2001).

Climate differences along with diversities of soils must also be taken into account when characterizing the behavior of chemicals in the environment. Research is conducted to describe the environmental behavior of insecticides. The goal of insecticide discovery is to find compounds with market potential that do not present residual problems in the near or long term. Protection of ground water resources from pesticide contamination is essential. Any proportion of an insecticide being applied foliarly that is not intercepted by vegetation will eventually reach the soil surface. The rate of degradation of imidacloprid on soil surfaces is accelerated by sunlight (Krohn 2001, Krohn and Hellpointer 2002). However, it is not completely broken down and some chemical will enter the soil where it will be subject to chemical processes and undergo metabolism that hopefully ends with complete metabolization to carbon dioxide. If the rate of metabolism is too slow, the chemical may persist and accumulate in the soil, particularly after repeated applications. This is not the case for the neonicotinoids as they are thoroughly metabolized to carbon dioxide and do not persist in the soil (Krohn 2001, Krohn and Hellpointner 2002). The half-life of imidacloprid in soil is <200 days, depending on rate and the amount of ground cover (Scholz and Spiteller 1992). Long term dissipation studies have confirmed that imidacloprid does not accumulate in the soil and that it will actually reach a plateau concentration despite multiple applications (Krohn and Hellpointer 2002). Soil half-life for thiacloprid, measured under field conditions, ranged from nine to sixteen days (Krohn 2001) and did not adversely affect microbial processes in soil when used as recommended (Schmuck 2001).

Weather, topography, vegetation and soil texture affect the mobility of a chemical in the soil. Imidacloprid has a strong affinity for organic matter and strongly binds to soil. Thus, the overall effect is that the compound remains in the upper root zone (Krohn 2001) as leaching to deeper-lying soil zones is negligible. Thiacloprid has been classified as being only slightly mobile in soil and hence it has potential for leaching into deeper soil horizons and into ground water (Krohn and Hellpointer 2002).

Though neonicotinoids used for agriculture are not intended for use in water, small amounts may reach water bodies by spray drift or by runoff after application. Therefore it is necessary to investigate their fate in the aquatic environment. Several investigators (Krohn 2001, Krohn an Hellpointer 2002) have reported that neonicotinoids are not stable in aqueous environments and are rapidly degraded by exposure to sunlight. Rapid degradation occurs in all natural surface waters such as rivers and lakes, and in aerosols like fog and rainwater.

The use of neonicotinoids in agriculture does not entail known harmful environmental effects, as they have been found to dissipate rapidly from soil, water and air.

**Non-Targets**

Arthropod natural enemies have the ability to suppress insect pest populations, so their conservation is a valuable component of integrated pest management (IPM) programs. Through suppressing pest populations, natural enemies also play a part in reducing insecticide use, thereby delaying insecticide resistance evolution (Greene *et al.* 1995). However, natural enemies may not suppress populations of insect pests below their respective economic threshold levels, so insecticide treatments often are necessary to control pest infestations and protect crop yields (Newsom *et al.* 1980, Baldwin *et al.* 1997). Croft (1990) noted that beneficial arthropod species typically have a greater susceptibility to insecticides than their hosts or prey species. Natural enemies are subject to insecticide poisoning by three routes of exposure: (1) direct contact, (2) consumption of prey species previously exposed to insecticides, and (3) residual contact. Primary pest release and resurgence, and increases in populations of secondary pests may occur as a result of the destruction of natural enemy populations by chemical pesticides. Pest release and resurgence have been widely reported as a consequence of pesticide use or over-use (Michelbacher *et al.* 1946, Doutt 1948, DeBach and Bartlett 1951, Lingren and Ridgway 1967). Any pesticide applied during the growing season has the potential to disrupt biological control. New insecticide chemistries, with novel modes of action, may have more desirable selectivity profiles. Thus, they have greater impacts on target pests than on natural enemies, thereby conserving biological control agents in agricultural environments. In any crop system it is important to determine which insecticides are not compatible with key biological control agents and to identify the possible disruptive effects of each. The impact of neonicotinoids on beneficial arthropods varies depending upon species. Elzen (2001) evaluated the effects of insecticides on two insect predators, the insidious flower bug, *Orius insidiosus* (Say) and big-eyed bug *Geocoris punctipes* (Say). The predators were given insecticide-treated *Helicoverpa zea* eggs to consume and toxicity was recorded. Of the insecticides tested, imidacloprid produced the highest toxicity to female *O. insidiosus,* but was the least toxic to male *G. punctipes*. Consumption of treated *H. zea* eggs by *G. punctipes* was significantly lower in imidacloprid treatments when compared with the control, suggesting an antifeedant effect due to the neonicotinoids. Other hemipteran predators, including *Geocoris* and *Nabis* spp., had poor response or lack of coordination when probed after contact with insecticide (Boyd and Boethel 1998). These symptoms are similar to those observed by De Cock *et al.* (1996) with spined soldier bug adults and nymphs. Boyd and Boethel (1998) observed that imidacloprid caused moderate contact toxicity after hemipteran predators were exposed to treated foliage. De Cock *et al.* (1996) demonstrated that imidacloprid's greatest toxicity to spined soilder bugs occurred after nymphs and adults were exposed to treated glass plates. Overall, Boyd and Boethel (1998) found that newer insecticides were less toxic than older standards to hemipteran predators following exposure to treated foliage.

Coccinellids are also affected by neonicotinoids, either directly through contact or through ingestion of treated prey, indirectly through a shift in foraging area as a result of a decimated prey population, or a combination of both (Wells *et al.* 2001) Coccinellids, *Hippodamia convergens* and *Scymnus spp*., exhibited a population decrease after exposure to imidacloprid. It is hard to say if this reduction in the population was due to mortality induced by contact with the insecticide or because of dispersal into areas of higher prey concentrations. It is likely that both factors played a role in the reduction of the coccinellid population in the imidacloprid treatments. Imidacloprid was one of the most disruptive insecticides to coccinellid populations examined in South Carolina cotton (Duffie *et al.* 1998). Mizell and Sconyers (1992) found that imidacloprid was toxic to coccinellid adults and larvae under laboratory testing as well.

Epperlein and Schmidt (2001) tested the effects of pelleting seed with imidacloprid on arthropods under field conditions. They believed that applying the insecticidal active ingredient in this way avoids whole-area treatment and may be less toxic the beneficial arthropods. They found that pelleting seed with imidacloprid did not have any adverse effects of beneficials living on the soil surface such as spiders, millipedes, and rove beetles. Eclector traps placed to catch flying insects caught the highest numbers of insects in the imidacloprid plots which suggested that the mobility of ground beetles and rove beetles was not affected.

When tested for contact toxicity on two parasitoids, *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), imidacloprid was found to have high acute toxicity, but short residual toxicity as one day residues were found to be nontoxic (Brunner *et al.* 2001).

Many systemic insecticides are considered fairly safe to beneficials because no direct exposure takes place unless the insects feed on plant tissue. However, systemic insecticides can potentially contaminate floral and extrafloral nectar when systemically distributed throughout the plant (Lord *et al.* 1968) and cause high mortality to nectar feeding parasitoids after insecticide application (Cate *et al.* 1972). Upon evaluation of imidacloprid-treated cotton the foraging ability, flight response and longevity of *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) was affected for two, four and ten days respectively. Though imidacloprid did affect the parasitoids, the time periods observed for imidacloprid were the shortest when compared to the other insecticides tested. These sublethal effects of imidacloprid may ultimately cause beneficial insects to be less effective as biological control agents.

Perhaps one-third of our total diet is dependent, either directly or indirectly, upon insect-pollinated plants, and fruit quality and agricultural efficiency are also largely influenced by insect pollination (McGregor 1976). Thus, the effects of any new insecticide on insect pollinators is of concern to growers who depend on bees and other insects for crop pollination. Schmuck (2001) evaluated the effects of thiacloprid on honeybees and concluded that thiacloprid has a favorably low toxicity to honeybees. He found that it would be safe to use thiacloprid at the proposed application rates even during crop flowering without posing an unreasonable risk to bees. Imidacloprid is highly toxic to bees if used as a foliar application, especially during flowering, but is not considered a hazard to bees when used as a seed treatment (Kidd and James 1994).

With respect to birds, thiacloprid spray used in ornamentals are considered acceptable (Schmuck 2001). Imidacloprid was found to be toxic to upland game birds (Meister 1995, Kidd and James 1994). In studies with red-winged blackbirds and brown-headed cowbirds, it was observed that birds learned to avoid imidacloprid treated seeds after experiencing transitory gastrointestinal distress and loss of coordination. Based on these studies, imidacloprid appears to have potential as a bird repellent seed treatment (Avery *et al.* 1994, Avery *et al.* 1993).

While most insecticides are toxic in some way to non-targets, integrated pest management programs seek to employ least toxic insecticides and to use insecticides in ways that are less hazardous. Though neonicotinoids have adverse effects on beneficals, as a group they are much safer than older insecticides such as organophosphates and carbamates (Boyd and Boethel 1998, Brunner *et al.* 2001, Elzen 2001).

## Mammalian Toxicity

Active ingredients to be used in plant protection must undergo extensive testing to establish the toxicological hazard potential they might have to humans as users of the formulations and as consumers of the treated crops. Selective toxicity, involving low hazard for mammals and high potency to pests, are essential requirements for safe and effective pesticides. Nicotine was used for centuries to control sucking insects despite relatively low efficacy and high toxicity to mammals. The neonicotinoids are more toxic to insects and less toxic to mammals, providing an excellent example of selective toxicity (Kagabu 1997, Yamamoto and Casida 1999). Neonicotinoids have been found to have low mammalian toxicity (Schmuck 2001). Imidacloprid is classified by the U.S. Environmental Protection Agency (EPA) as both a toxicity class II and class III agent. It must be labeled with the signal word “warning” or “caution” (Meister 1995). Tolerances for residues of imidacloprid and its metabolites on food/feed additives range from 0.02 ppm in eggs, to 3.0 ppm in hops (U.S. EPA 1995). Imidacloprid was found to be moderately toxic with the oral dose $LD^{50}$ being 450 mg/kg body weight in rats (Meister 1995). The 24-hour dermal LD 50 of imidacloprid in rats is greater than 5000 mg/kg. It was found to have practically no acute dermal toxicity and low acute inhalation toxicity. It is not irritating to skin or eyes and is not a skin sensitizer (Kidd and James 1994). Imidacloprid exhibits no genotoxic or mutagenic potential. It is categorized by EPA as a "Group E" carcinogen (U.S. EPA 1995). Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces in humans. Thiacloprid spray uses are considered acceptable with respect to risk on mammals (Schmuck 2001).

**Resistance**

Strategies must be developed to maintain the effectiveness of insecticides and decrease the rate of insect resistance evolution. In many agricultural systems worldwide, *Bemisia tabaci* has developed high resistance levels to conventional insecticides such as organophosphates, carbamates, and pyrethroids. Since their introduction 1991, neonicotinoids have been the most encouraging innovation in whitefly control. Monitoring results published in 1996 revealed the first signs of resistance to imidacloprid in *Bemisia* populations in the Almeria region of southern Spain (Cahill *et al.* 1996, Elbert *et al.* 1996). Other studies have shown that whitefly populations in Arizona cotton growing areas became slightly less susceptible to imidacloprid from 1995 to 1997 (Dennehy and Denholm 1998). Studies conducted by Elbert and Nauen (2000) found that *B. tabaci* from Almeria, Spain, clearly demonstrated a steady decline in susceptibility to neonicotinoids. Also, testing indicated strong cross-resistance between imidacloprid, thiamethoxam, and acetamiprid. Resistance studies conducted with *Aphis gossypii* showed that resistance to imidacloprid gradually and steadily increased producing an 8-fold resistance ratio after 13 generations (Wang *et al.* 2002). A japanese strain of a tobacco-feeding form of *Myzus persicae* has become tolerant to imidacloprid (Nauen *et al.* 1996, Nauen and Elbert 1997, Elbert and

Nauen 2000). These studies demonstrate potentially serious implications for growers who rely heavily on imidacloprid, because cross resistance to other neonicotinoids is very likely to occur. This evidence should encourage farmers to work together to sustain the efficacy of neonicotinoids by applying resistance management strategies such as rotation of compounds and crops, as well as employment of other resistance management strategies.

**General Use Patterns**

The neonicotinoids are used in many crops to control agricultural pests. Imidacloprid has soil, seed and foliar uses and is recommended for control of sucking insects including aphids and whiteflies, as well as thrips, termites, beetles, etc. It is most commonly used on rice, cereals, maize, potatoes, vegetables, sugar beets, fruit, cotton, grapes, canola, pecans, hops and turf, and is especially systemic when used as a seed or soil treatment. Imidacloprid is available in many formulations including as a dustable- powder, granular, seed dressing, soluble concentrate, suspension concentrate, or wettable powder (Meister 1995). Typical application rates range from 0.05-0.125 lb. ai/acre and are considerably lower than use rates for older, traditional insecticides. It has also been used commercially in the USA since 1996 as a veterinary medicinal product for flea control on cats and dogs. The commercial products Admire®, Advantage®, Confidor®, Gaucho®, Premier®, Premise®, Provado® and Marathon® all contain imidacloprid as their active ingredient.

Acetamiprid is a second-generation chloronicotinyl insecticide with contact and systemic activity via foliar applications. Acetamiprid was manufactured by Aventis (now Bayer) and has been registered for use on leafy vegetables, cole crops, fruiting vegetables, pome fruits, cotton, citrus, stone fruits, and ornamentals for control of many of the same insects as imidacloprid. It has been sold commercially as Assail®, Intruder®, Mosiplan®, Rescate® and Pristine®. Acetamiprid application rates range from 0.025 to 0.15 lb. ai/a. It controls several pests in cotton including aphids, fleahoppers, plant bugs, whiteflies, and also is an ovicide for bollworms.

The neonicotinoids have demonstrated ovicidal activity for bollworm and tobacco budworm (Parrish *et al.* 2001, Elzen 1997, All *et al.* 2001). Elzen (1997) reported that laboratory assays demonstrated that imidacloprid was ovicidal to field and lab strains of *Heliothis virescens*. All *et al.* (2001) reported that imidacloprid provided residual ovicidal activity for *H. zea* up to 48 hours after application under greenhouse testing. However, under field conditions the ovicidal activity of imidacloprid significantly decreased after 24 hr. Imidacloprid had no ovicidal activity 72 hours after spraying (All *et al.* 2001). Parrish *et al.* (2001) reported ovicidal activity of acetamiprid for bollworm and budworm under greenhouse testing. They reported acetamiprid to be effective as a direct spray and as a residual. Thiodicarb and lambda-cyhalothrin are considered ovicide standards for heliothines as they possess excellent initial ovicidal activity and have demonstrated residual activity for up to four days after application (Allen *et al.* 1997, Bradley and Agnello 1988, Pitts and Pieters 1980, Newell *et al.* 1991, Harden *et al.* 1988, Scott and Snodgrass 1988, DuRant and Moore 1989, DuRant 1991). Their efficacy is not just due to their ability to kill eggs, but they also possess larvicidal properties (Harden *et al.* 1988, DuRant and Moore 1989). For an insecticide to be successful as an ovicide in North Carolina, where bollworm infestations are typically high, it is important for it to have residual activity and larvicidal activity as well.

**Conclusion**

From reviewing the literature, the question is do neonicotinoids posses ovicidal activity against bollworms under field conditions in cotton comparable to that of current standards. Imidacloprid has shown ovicidal activity against *Helicoverpa zea* under greenhouse and field testing (All *et al.* 2001), while acetamiprid has shown ovicidal control under laboratory and greenhouse conditions (Parrish *et al*. 2001). If neonicotinoids are to be used solely as an ovicide they need to possess long residual ovicidal activity against eggs and contain larvicidal properties. Having characteristics such as ovicidal activity plus control of most sucking insect pests could increase the benefits of using acetamiprid in cotton fields.

Natural field populations of insect predators and parasitoids, if undisturbed, may substantially reduce heliothine pest populations in cotton. Estimates that 50-90% of heliothine eggs and larvae in cotton were consumed or parasitized by natural populations of insect predators and parasitoids have been reported (Bell and Whitcomb 1964, Ridgway and Lingren 1972). Parasitism by natural populations of *Trichogramma* wasps have been found to be as high as 59-92% in cotton (Johnston 1985, Segers *et al.* 1984). However, natural enemies may not suppress populations of insect pests below their respective economic threshold levels in all cases, so insecticide treatments are often necessary to reduce pest populations and protect crop yields (Newsom *et al.* 1980, Baldwin *et al.* 1997). Information on the toxicities of cotton insecticides to beneficial arthropods is important for selection of compounds that will minimize mortality of beneficial arthropods.

The research reported in this thesis was conducted to assess the ovicidal properties of neonicotinoids and to assess the effects of acetamiprid and other selected insecticides on beneficial arthropods to determine the detrimental effects on beneficals.

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# CHAPTER II

# COMPARATIVE OVICIDAL ACTIVITY OF NEONICOTINOIDS AND CURRENT STANDARDS AGAINST BOLLWORM, *HELICOVERPA ZEA*, ON COTTON IN A FIELD ENVIRONMENT

**Abstract**

Field trials were conducted to evaluate the ovicidal properties of acetamiprid (Intruder® 70WP) and various other neonicotinoids, against *Helicoverpa zea* (Boddie) under field conditions in Washington, Perquimans and Nash counties, North Carolina during 2001 and 2002. Newly laid (white) bollworm eggs were collected from cotton foliage within respective treatments at 0, 1, and 2 days after application and evaluated in the laboratory for ovicidal and eclosion mortality. During 2001, acetamiprid initially provided mortality of bollworm eggs comparable to that obtained with two ovicide standards, thiodicarb and lambda-cyhalothrin on the day of application (0 DAT). For eggs collected at one day after application (1 DAT), ovicidal activity of acetamiprid had declined more than that observed for the two standard ovicides; only the highest rate of acetamiprid provided comparable ovicide activity. There was no significant ovicidal activity with any of the insecticides for eggs deposited two nights after application (2 DAT). During 2002, the neonicotinoids acetamiprid, imidacloprid, and thiamethoxam provided mortality for eggs present on the plants at application (0 DAT), comparable to that obtained with thiodicarb and lambda-cyhalothrin. Mortality of eggs 1 DAT dropped precipitously in all insecticide treatments, except thiodicarb. Egg mortality at 1 DAT was similar for all neonicotinoids. Ovicidal activity of the neonicotinoids had ceased and egg mortality in the standard treatments had declined to ca. 25% by 2 DAT. These studies confirmed ovicidal activity of all tested neonicotinoids under field conditions; however, ovicidal activity of neonicotinoids was ephemeral and not comparable to that of the ovicide standards.

Virtually every cotton field in North Carolina requires some level of control annually for the bollworm, *Helicoverpa zea* (Boddie), to prevent yield loss. Bollworm control is achieved either through the planting of Bollgard® cotton varieties or through the application of insecticides or both. Pyrethroid insecticides have been the leading insecticide products of choice for bollworm control since the late 1970's. Pyrethroids have been used successfully because they are active against both egg and larval stages of heliothines (DuRant 1990) and they are cost effective. While recent studies have found that bollworm has become more tolerant to pyrethroids (Roof and DuRant 1998), they remain the most economically effective insecticides for control of bollworm. Thiodicarb is a carbamate insecticide that has also been used to control bollworm in cotton and other crops and has demonstrated ovicidal activity against heliothines (Brickle et al. 2001, Bradley and Agnello 1988, Leonard et al. 1990).

The neonicotinoids were recently introduced as a novel class of insecticidal compounds and have been widely adopted in many commercial market niches throughout the world. The success of neonicotinoids is due to their unique chemistry and biological properties, including their mode of action, low application rates, broad insecticidal spectrum, excellent systemic and translaminar properties, and low environmental and ecological risk concerns. The neonicotinoids have exhibited outstanding control of sucking pests of cotton and other crops, such as aphids and whiteflies (Natwick 2001, Parrish 2001). Acetamiprid is a representative of the neonicotinoid class that has shown excellent ovicidal activity against heliothines and other lepidoptera species in the laboratory and greenhouse (Parrish *et al.* 2001). Imidacloprid, another neonicotinoid, has also shown ovicidal activity against *Heliothis virescens* under greenhouse and field testing (All *et al.* 2001).

In order to understand the ovicidal properties of the neonicotinoids, we conducted a two year study with the following objectives: 1) to determine if acetamiprid would provide effective ovicidal control of bollworm under field conditions similar to that reported in greenhouse studies, 2) to compare the ovicidal activity of acetamiprid with that of current standards, 3) to determine if the neonicotinoids imidacloprid and thiamethoxam had ovicidal activity against bollworm under field conditions comparable to that of acetamiprid, 4) to compare the ovicidal activity of the three neonicotinoids with that of current standards.

**Materials and Methods**

Two field trials were conducted during the summer of 2001, one each in Perquimans and Washington counties, North Carolina. Cotton variety's FM958 and ST4793R were planted on 4 May and 16 July in Perquimans and Washington counties respectively. Fields were divided into six blocks of 8 rows x 21.33 meters in length. Treatments were applied on 15 August and 30 August in Perquimans and Washington counties, respectively, once adequate numbers of *H. zea* eggs were meet. Insecticides were sprayed using a $CO_2$-backpack sprayer fitted with a single TX-12 hollow cone nozzle per row delivering 113 liters per hectare at a $CO_2$ pressure of 3.94 kilograms per square centimeter. The six middle rows of each block were sprayed with either acetamiprid (Intruder®, Aventis CropScience, Research Triangle Park, NC) at 0.028, 0.056, and 0.112 kg a. i./ha, thiodicarb (Larvin® 4F, Aventis CropScience, Research Triangle Park, NC) at 0.225 kg a .i./ha, or lambda-cyhalothrin (Karate Z® 2.08 CS, Syngenta Crop Protection, Inc., Greensboro, NC) at planting at 0.028 kg a. i./ha. An untreated control was also included. Thrips control was accomplished by applying aldicarb (Temik® 15G, Aventis Cropscience, Research Triangle

Park, NC) at 3.36 kg a. i./ha in Perquimans county. No foliar insecticides had been applied to field prior to treatment.

The 2002 field test was conducted in Nash County, North Carolina. Cotton (cv DP50) was at peak flower at time of test initiation and plants were ca. 101.6 cm in height. No foliar insecticides had been applied to the test site. Control of thrips had been accomplished through application of aldicarb (Temik® 15G, Aventis CropScience, Research Triangle Park, NC) at 0.69 kg a. i./ha in-furrow. Plots of 6 rows x 25.91 meters were established in a randomized complete block design with four replications. Rows were spaced 91.44 cm apart. Insecticide treatments were applied on 29 July using a $CO_2$-backpack sprayer fitted with a single TX-12 hollow cone nozzle per row delivering 93.5 liters per hectare at 4.22 kilograms per square centimeter psi. The four middle rows of each plot were sprayed with either acetamiprid (Intruder® 70 WP, Aventis CropScience, Research Triangle Park, NC) at 0.056 kg a. i./ha, imidacloprid (Trimax® 4 SC, Bayer, Kansas City, MO ) at 0.056 kg a. i./ha, thiamethoxam (Centric® 25 WG, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.056 kg a. i./ha, thiodicarb (Larvin® 3.2F, Aventis CropScience, Research Triangle Park, NC) at 0.231 kg a. i./ha, or lambda-cyhalothrin (Karate Z® 2.08 CS, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.028 kg a. i./ha. An water-sprayed control was also included. No foliar insecticides had been applied to field prior to treatment.

Freshly laid bollworm eggs were collected from the upper 25% of the canopy of cotton plants at 2 (0 DAT), 24 (1 DAT), and 48 (2 DAT) hours after application in all tests. Only newly laid eggs were chosen, which were identified by their pearly white color and the absence of any darkening or ring formation. At least 100 eggs were collected from each treatment on each sample date. A small amount of cotton foliage bearing each egg was collected, placed in labeled paper bags, and transported in an insulated cooler containing ice to the laboratory. Small sections of leaf tissue bearing each egg were cut from the leaves and placed singly into #1 gelatin capsules and held at 26°C. Mortality assessments were conducted four days after each egg collection date to ensure that egg hatch was complete. Each egg was categorized and recorded as hatched normally, failed to hatch, or that the larva died partially eclosed from the egg. Normally hatched eggs were those in which the larvae hatched and emerged completely from the eggshell. Ovicidal and eclosion mortalities were combined for the mortality values presented in Tables 1 and 2.

All treatment mortality data were corrected for control mortality according to Abbott's formula (1925) and were then subjected to ANOVA using PROC GLM (SAS Institute 1990); means for each treatment were separated ($P \leq 0.05$) using Fisher's Protected Least Significant Difference test in SAS.

**Results**

During the summer of 2001, all insecticide treatments were significantly higher in egg mortality compared to the untreated controls when averaged across two locations and compared to the untreated control at 0 days after application (F=23.5; df=5,5; $P$=0.0012) (Table 1). Corrected mortality ranged from 59 to 83% among insecticide treatments. The two highest rates of acetamiprid were comparable to the two standard ovicides, thiodicarb and lambda-cyhalothrin, at 0 DAT. Eggs collected 1 DAT were eggs laid the night following insecticide application. Mortality due to all rates of acetamiprid fell dramatically in 1 DAT eggs (F=6.08; df=5,5; $P$=0.0285) (Table 1); all three rates of acetamiprid were statistically similar to the control. The highest rate of acetamiprid was not statistically different from the two standard ovicides, while the standard ovicides were still significantly higher than the control at 1 DAT. None of the insecticides tested provided adequate control of eggs that were laid two nights after application (F=0.99; df=5,5; *P*=0.7886) (Table 1).

During 2002, at 0 DAT acetamiprid and lambda-cyhalothrin provided the highest levels of egg mortality; imidacloprid and thiamethoxam were intermediate (F=8.86; df=5,15; *P*=<0.0001) (Table 2). Egg mortalities for the three neonicotinoids dropped dramatically for eggs collected 1 DAT, while the two standard ovicides were still significantly higher than the control (F=2.28; df=5,15; *P*=0.0399) (Table 2) . At 2 DAT the neonicotinoids no longer exhibited ovicidal activity; however, the ovicide standards, particularly lambda-cyhalothrin, continued to show ovicidal activity(F=6.86; df=5,15; *P*=0.0004) (Table 2). Larval identification from subsamples of eggs collected from the control confirmed that 98% were bollworm.

## Discussion

The mode of insecticidal action in insect eggs is not well understood and at least two types of mortality have been associated with death of the developing insect. The embryo in the egg may be killed and further development (embryogenesis) halted or the larva dies as it feeds on the chorion during eclosion. Bradley and Agnello (1988) reported substantial mortality from thiodicarb in which the larvae died partially eclosed from the egg. They concluded that this mortality was due to sublethal doses of insecticide incapable of halting embryo development. Leonard *et al.* (1990) reported both types of ovicidal activity for heliothine eggs exposed to lambda-cyhalothrin. We observed both types of mortality in this study, but no attempt was made to differentiate between the two.

During 2001, acetamiprid exhibited high initial ovicidal activity against bollworm eggs under field conditions in North Carolina, however, acetamiprid was not as persistent at lower rates compared to the ovicide standards, thiodicarb and lambda-cyhalothrin. Only the highest rate of acetamiprid demonstrated ovicidal activity comparable to that of standard ovicides with respect to eggs laid one night after application. Ovicidal activity of all three ovicides tested declined significantly for eggs deposited two nights after application. During 2002, all the neonicotinoids tested in the study exhibited ovicidal activity against bollworm eggs under field conditions. Their ovicide effects where more ephemeral than the ovicide standards thiodicarb and lambda-cyhalothrin. These 2002 results were similar to those observed from 2001, with the exception that overall ovicide mortalities recorded in 2001 were higher. Obviously, field environments (e.g. plant size, temperatures, humidity) vary from test to test with respect to factors critical to insecticide efficacy. The results from these tests confirm that the use of insecticides that exhibit only ovicidal activity against bollworm is unrealistic under North Carolina conditions where bollworm infestations are typically high. In contrast, lambda-cyhalothrin and thiodicarb have been demonstrated to provide highly effective bollworm control because they possess both ovicidal and larvicidal activities.

Neonicotinoids provided ovicidal control of bollworm eggs only through direct contact, no significant residual activity was observed. It may be practical to use neonicotinoids as an ovicide only on Bollgard cotton where supplemental control is all that is required. Such a situation may arise where threshold levels of cotton aphid or some other sucking insect pest occur during a time when bollworm moths are depositing eggs on cotton. Having additional characteristics such as ovicidal activity against bollworms may increase the benefits of using neonicotinoids in situations where multiple pests occur simultaneously.

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Table 1. Percent mortality[a] (SE) of bollworm (*Helicoverpa zea*) eggs collected from cotton plants at 0, 1, and 2 days after treatment with insecticides averaged across two locations, Perquimans and Washington counties, N.C., 2001.

| Insecticide | Rate (kg AI/ha) | 0 DAT[b] | 1 DAT[b] | 2 DAT[b] |
|---|---|---|---|---|
| acetamiprid | 0.028 | 58.7 (7.2) b | -1.8 (0.7) b | -14.7 (11.9) a |
| acetamiprid | 0.056 | 69.6 (10.1) ab | 7.7 (5.2) b | -21.7 (44.3) a |
| acetamiprid | 0.112 | 83.2 (4.3) a | 26.8 (0.3) ab | -3.9 (5.6) a |
| thiodicarb | 0.225 | 77.0 (1.1) ab | 45.8 (22.9) a | -2.5 (8.3) a |
| lambda-cyhalothrin | 0.028 | 65.2 (2.1) ab | 54.2 (11.1) a | 9.1 (5.1) a |
| control | -- | 0.0 (0.0) c | 0.0 (0.0) b | 0.0 (0.0) a |

[a]Treatment mortality data were corrected for control mortality using Abbott's formula (1925).

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD, ($P \leq 0.05$).

Table 2. Percent mortality[a] (SE) of bollworm (*Helicoverpa zea*) eggs collected from cotton plants at 0, 1, and 2 days after application of insecticides, Nash county, N. C., 2002.

| Insecticide | Rate (kg AI/ha) | 0 DAT[b] | 1 DAT[b] | 2 DAT[b] |
|---|---|---|---|---|
| lambda-cyhalothrin | 0.028 | 71.8 (7.3) a | 32.8 (6.4) a | 46.8 (18.2) a |
| acetamiprid | 0.056 | 62.0 (4.9) ab | 25.5 (34.0) a | 7.0 (3.8) bc |
| thiamethoxam | 0.056 | 46.5 (5.0) bc | 25.8 (23.4) a | -7.6 (3.6) c |
| imidacloprid | 0.056 | 38.6 (5.5) c | 19.5 (5.4) a | -6.5 (3.0) c |
| thiodicarb | 0.231 | 32.1 (11.5) c | 58.3 (7. 8) a | 26.5 (5.2) ab |
| control | - | 0.0 (0.0) d | 0.0 (0.0) a | 0.0 (0.0) c |

[a]Treatment mortality data were corrected for control mortality using Abbott's formula (1925).

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD, ($P \leq 0.05$).

# CHAPTER III

# COMPARATIVE EFFECTS OF ACETAMIPRID AND SELECTIVE INSECTICIDES ON POPULATIONS OF SOME BENEFICIAL AND PEST ARTHROPODS IN COTTON

## Abstract

Comparative effects of various insecticide residues on populations of certain beneficial and pest arthropod species in cotton were examined in two field studies. Treatment effects were evaluated by population assessment through sweep net sampling at 1, 4, 7 and 14 days after application. Acetamiprid, spinosad, indoxacarb, and methoxyfenozide had no adverse impact on numbers of beneficial or pest species population levels when data were averaged over sampling dates and test locations. The lack of an observed treatment effect for any of the insecticides tested, particularly at 1 and 4 DAT, confirmed the selective toxicity profiles of the compounds.

## Introduction

Arthropod natural enemies have the ability to suppress insect pest populations, which makes their conservation a valuable component of integrated pest management (IPM) programs (Funderburk *et al*. 1993, Nuessly and Sterling 1994, Ruberson *et al*. 1994). They have the ability to make a significant contribution to the economics of cotton production (Peterson and Sprenkel 1999). Through suppressing pest populations, natural enemies also play a part in reducing insecticide use, thereby delaying insecticide resistance evolution (Greene *et al.* 1995). However, natural enemies do not suppress populations of insect pests below their respective economic threshold levels in all cases; thus, insecticide treatments are often necessary to control pest infestations and protect crop yields (Newsom *et al.* 1980, Baldwin *et al.* 1997). Croft (1990) noted that beneficial arthropod species are typically more susceptible to insecticides than prey species. Even the development of newer, more target-specific insecticides may have profound adverse side effects (Croft 1990). A objective of IPM is the selection of the appropriate materials, when insecticides are warranted, that have the lowest impact on the environment and beneficial organisms. Risk reduction and safer pesticide policies of EPA have increased the potential for industry to register safer products at an accelerated pace (Hall 1999). Thus, pesticide development has entered a transitional phase where efficacy against pests is only one of several factors that are considered. The crop protection industry also evaluates new active ingredients on the basis of reduced risk factors, including: IPM compatibility and flexibility, low use rates, human and environmental safety, high biological efficacy, and safety to beneficial and non target insects (Carroll 1999).

Since synthetic insecticides are often required to decrease insect pest numbers and associated crop damage and losses, knowledge of the non-target effects of each choice is essential for selection of the most appropriate insecticide for the situation. Selectivity information may be generated only through properly designed laboratory and field studies. Boyd and Boethel (1998) observed that some of the newly registered insecticides (e.g. spinosad, imidacloprid, emamectin benzoate) were much more selective than older insecticides (e.g. acephate, methyl parathion, permethrin) when evaluated in a soybean system. Duffie *et al.* (1998) and Tillman *et al.* (1998) reported that spinosad, indoxacarb, and methoxyfenozide demonstrated a high degree of selectivity when used in a cotton system.

The study described herein was conducted in a cotton system to compare acetamiprid to certain insecticides previously proven to be have minimal adverse effects on beneficial arthropods with respect to impact on populations of selected pest and beneficial species. While acetamiprid has demonstrated outstanding activities against aphids (Natwick 2001, Parrish 2001), whiteflies (Natwick 2001, Parrish 2001), and other homopterous pests, its non-target effects are poorly described.

## Materials and Methods

Two field studies were conducted during the summer of 2001 in Johnston and Washington counties, North Carolina. Deltapine 5690RR cotton variety was planted in Johnston County on 16 May, 2001 and in Washington County on 6 June, 2001. Both tests utilized the randomized complete block design with four replications. Plots in Washington county were 8 rows wide by 12.19 meters length, while in Johnston county plots were 10 rows wide by 12.19 meters in length. At both sites weed control, fertilization, and plant growth regulation were achieved as recommended by the North Carolina Agricultural

Extension Service (North Carolina Cooperative Extensive Service 2002). No foliar insecticides, except the test substances, were applied for insect control to either test site.

Insecticides evaluated at both sites included spinosad (Tracer® 4SC, Dow AgroSciences LLC, Indianapolis, IN) at 0.071 kg a.i./ha, indoxacarb (Steward® 1.25 SC, DuPont Agricultural Products, Wilmington, DE) at 0.124 kg a.i./ha, methoxyfenozide (Intrepid® 2 SC, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.168 kg a.i./ha, and acetamiprid (Assail™ 70WP, Aventis CropScience, Research Triangle Park, NC) at 0.056 kg a.i./ha. A $CO_2$ –powered backpack sprayer fitted with one TX-12 hollow cone nozzle per row delivering 113.2 liters per hectare at a $CO_2$ pressure of 3.9 kilograms per square centimeter was used to apply test insecticides. Insecticide treatments were applied to the center six and eight rows of each plot in Washington and Johnston counties, respectively. At each site an untreated control was also included in the experimental design.

Insect populations were assessed through sweep net samples (38 cm diameter sweepnet) consisting of 50 sweeps from two rows in each plot taken at 1, 4, 7 and 14 days after application. Alternate rows were used on each sample date to ensure sampling methods did not disrupt arthropod populations. Samples were placed in gallon-sized Zip Loc® (S. C. Johnson, Racine, Wisconsin) bags and transported on ice to the laboratory. Samples were then placed in the freezer until arthropod totals could be tallied.

All data was subjected to ANOVA using PROC GLM (SAS Institute 1990); and means for each treatment were separated ($P \leq 0.05$) using Fisher's Protected Least Significant Difference test in SAS.

## Results

**Effects on pest species** Sweep net samples indicated no differences among insecticide treatments when averaged across sampling dates and locations for tarnished plant bugs, *Lygus lineolaris,* (F=2.17; df=4,72; *P*=0.683) (Table 1) and potato leaf hoppers, *Empoasca fabae,* (F=3.83; df=4,72; *P*=0.269) (Table 2). Soybean nodule fly, *Rivellia quadrifasciata*, was the only other insect pest species found in sufficient numbers (Plymouth site only) to include in the analysis. While soybean nodule fly is not known as a pest of cotton, it was considered to be an indicator of insecticidal activity. There were significant treatment effects at 1 (F=4.40; df=4,12; *P*=0.0039) and 4 DAT (F=4.38; df=4,12; *P*=0.0381) (Table 3). On both dates, only the acetamiprid treatment contained significantly higher numbers of soybean nodule fly adults compared to the CONTROL. Soybean nodule fly adult numbers did not differ among other treatments, including the CONTROL, on both dates; however, at 4 DAT, spinosad and methoxyfenozide treatments contained similar numbers of soybean nodule fly adults to that of acetamiprid. No significant differences in numbers of soybean nodule fly adults were evident among treatments at the 7 and 14 DAT evaluations.

**Effects on predator species** Predaceous insects present at population levels sufficient for treatment comparisons were limited to coleopteran and hemipteran species; species within each insect order were combined for analysis because of marginal numbers for individual species. Coleopteran species included *Hippodamia convergens* (Guerin), *Harmoni axyridis* (Pallas), *Coleomegilla maculata* (De Geer), *Coccinella septempunctata* (Linnaeus), and *Notoxus monodon* (Fabricius). Hemipteran species included of *Geocoris punctipes* (Say), *Nabis roseipennis* (Reuter), *Orius insidiosus* (Say), and Reduviidae.

A location by treatment interaction was detected for numbers of coleopteran predators (F=3.49; df=4.72; *P*=0.0020); therefore, site specific analyses were conducted for those species. A date by treatment interaction was detected for coleopteran predators at the Clayton location (F=2.11; df=12,48; *P*=0.0249) (Table 5), but there were no significant differences among treatments on any sample date. At the Plymouth location no differences were evident among treatments when averaged across sample dates (F=1.90; df=12,48; *P*=0.4942) (Table 6). There were no significant treatment effects on numbers of hemipteran predators averaged over two locations and four sampling dates (F=2.02, df=4,72, *P*=0.0825)(Table 7).

## Discussion

Selective toxic action is a desirable attribute for modern insecticides to possess because it allows insecticides to be utilized for pest population regulation with minimal disruptive impacts. In this study, insecticides that had known selectivity profiles were compared to a newly developed insecticide, acetamiprid, for impact on populations of certain pest and predaceous insect species. None of the insect species monitored were negatively affected by acetamiprid or the other insecticides at post-treatment sampling times ranging from 1-14 DAT. Even though numbers of individual predator species were low, the combined numbers of coleopterans and hemipterans were adequate to detect any negative treatment effect. Immigration of insects from refuge areas outside treated plots may have impacted the overall results, but recolonization should have been a gradual process with negligible impact on insect numbers for the early sampling dates. Thus, this study confirms that acetamiprid is a highly selective insecticide that may be used in IPM programs to achieve control of aphids and other susceptible insect pests with minimum disruptive effects on non-target species.

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Table 1. Mean (SE) numbers of adult and nymph tarnished plant bugs, *Lygus lineolaris*, per 50 sweeps in cotton plots oversprayed with various insecticides averaged across two locations and four sample dates in NC, 2001.

| Insecticide | Rate (kg AI/ha) | Mean[a] |
|---|---|---|
| spinosad | 0.071 | 3.25 (0.644) a |
| indoxacarb | 0.124 | 3.03 (0.560) a |
| methoxyfenozide | 0.168 | 3.0 (0.362) a |
| acetamiprid | 0.056 | 2.34 (0.358) a |
| control | -- | 2.97 (0.461) a |

[a]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 2. Mean (SE) numbers of potato leaf hoppers, *Empoasca fabae*, per 50 sweeps in cotton plots oversprayed with various insecticides averaged across two locations and four sample dates in NC, 2001.

| Insecticide | Rate (kg AI/ha) | Mean[a] |
|---|---|---|
| spinosad | 0.071 | 3.56 (0.813) a |
| indoxacarb | 0.124 | 2.16 (0.475) a |
| methoxyfenozide | 0.168 | 2.63 (0.544) a |
| acetamiprid | 0.056 | 2.44 (0.557) a |
| control | -- | 2.97 (0.532) a |

[a]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 3. Mean (SE) numbers of soybean nodule flies, *Rivellia quadrifasciata*, per 50 sweeps in cotton plots oversprayed with insecticides for each sample date at the Tidewater Research Station, Plymouth, NC, 2001.

| Insecticide | Rate (kg AI/ha) | 1 DAT[a] | 4 DAT[a] | 7 DAT[a] | 14 DAT[a] |
|---|---|---|---|---|---|
| spinosad | 0.07 | 28.2 (5.91) b | 14.00 (2.58) a | 13.0 (3.00) a | 3.25 (1.11) a |
| indoxacarb | 0.12 | 22.3 (2.56) b | 10.75 (1.11) b | 15.75 (2.14) a | 2.25 (0.479) a |
| methoxyfenozide | 0.18 | 19.5 (1.26) b | 15.00 (2.48) a b | 12.25 (2.87) a | 3.5 (0.289) a |
| acetamiprid | 0.06 | 48.0 (6.58) a | 18.00 (1.83) a | 13.0 (2.65) a | 2.25 (0.854) a |
| control | -- | 32.0 (2.16) b | 10.50 (2.90) b | 14.5 (3.86) a | 4.0 (0.577) a |

[a]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 4. Mean (SE) numbers of soybean nodule flies, *Rivellia quadrifasciata,* per 50 sweeps in cotton plots oversprayed with various insecticides for each sample date at the Central Crop Research Station, Clayton, NC, 2001.

| Insecticide | Rate (kg AI/ha) | Mean[a] |
|---|---|---|
| spinosad | 0.071 | 1.31 (0.326) a |
| indoxacarb | 0.124 | 1.06 (0.281) a |
| methoxyfenozide | 0.168 | 0.56 (0.203) a |
| acetamiprid | 0.056 | 0.75 (0.250) a |
| control | -- | 1.06 (0.295) a |

[a]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 5. Mean (SE) numbers of coleopteran predators [a] per 50 sweeps in cotton plots oversprayed with various insecticides for each sample date at the Central Crop Research Station, Clayton, NC, 2001.

| Insecticide | Rate (kg AI/ha) | 1 DAT[b] | 4 DAT[b] | 7 DAT[b] | 14 DAT[b] |
|---|---|---|---|---|---|
| spinosad | 0.071 | 8.5 (2.66) a | 4.5 (1.44) a | 15.25 (2.59) a | 12.25 (2.87) a |
| indoxacarb | 0.124 | 9.75 (1.25) a | 2.75 (1.32) a | 11.25 (3.47) a | 10.0 (1.35) a |
| methoxyfenozide | 0.168 | 10.25 (1.70) a | 6.5 (1.04) a | 8.25 (1.97) a | 16.0 (2.74) a |
| acetamiprid | 0.056 | 18.75 (3.71) a | 5.25 (1.38) a | 11.5 (1.44) a | 5.0 (1.00) a |
| CONTROL | -- | 10.75 (2.18) a | 4.5 (1.26) a | 10.25 (4.50) a | 10.5 (2.39) a |

[a]Species included *Hippodamia convergens, Notoxus monodon, Harmonia axyridis, Coleomegilla maculata, Coccinella septempunctata* etc.

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 6. Mean (SE) numbers of coleopteran predators[a] per 50 sweeps in cotton plots oversprayed with various insecticides averaged across four sample dates at the Tidewater Research Station, Plymouth, NC, 2001.

| Insecticide | Rate (kg AI/ha) | Mean[b] |
|---|---|---|
| spinosad | 0.071 | 3.75 (0.560) a |
| indoxacarb | 0.124 | 3.69 (0.840) a |
| methoxyfenozide | 0.168 | 4.00 (0.665) a |
| acetamiprid | 0.056 | 2.75 (0.581) a |
| CONTROL | -- | 3.75 (0.642) a |

[a]Species included *Hippodamia convergens, Notoxus monodon, Harmoni axyridis, Coleomegilla maculata, Coccinella septempunctata* etc.

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

Table 7. Mean (SE) numbers of hemipteran predators[a], per 50 sweeps in cotton plots oversprayed with various insecticides averaged across two locations and four sample dates in NC, 2001.

| Insecticide | Rate (kg AI/ha) | Mean[b] |
|---|---|---|
| spinosad | 0.071 | 6.44 (0.857) a |
| indoxacarb | 0.124 | 4.81 (0.904) a |
| methoxyfenozide | 0.168 | 5.78 (0.758) a |
| acetamiprid | 0.056 | 5.34 (0.503) a |
| CONTROL | -- | 4.66 (0.597) a |

[a]Species included *Geocoris punctipes*, *Nabis rosipiennis*, Reduviidae spp., *Orius insidiosus*, etc.

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected Least Significant Difference Test ($P \leq 0.05$).

# CHAPTER IV

# TOXICITY OF ACETAMIPRID AND SELECTED INSECTICIDES ON PREIMAGINAL AND ADULT *TRICHOGRAMMA EXIGUUM* (TRICHOGRAMMATIDAE: HYMENOPTERA)

## Abstract

Acetamiprid, imidacloprid, lambda-cyhalothrin, spinosad, and thiodicarb were evaluated to assess their toxicity to preimaginal *Trichogramma exiguum* in a laboratory study, their toxicity to adult *T. exiguum* was evaluated in a field study. Lambda-cyhalothrin and spinosad caused very high mortalities of preimaginal *T. exiguum* when applied to host eggs; imidacloprid caused intermediate mortality, and acetamiprid and thiodicarb were not toxic. Acetamiprid was the only insecticide that was not toxic to *T. exiguum* adults exposed at 1 DAT to residues on leaves of treated cotton plants in the field. None of the insecticides were toxic to adult *T. exiguum* at 6 DAT under field conditions.

Since the eradication of the bollworm, *Anthonomus grandi,s* in North Carolina, heliothines, predominantly the bollworm, *Helicoverpa zea* (Boddie), have emerged as the primary mid-to-late season insect pests in cotton (Bacheler 2002). Natural field populations of insect predators and parasitoids alone, if undisturbed, can substantially reduce heliothine pest populations in cotton. Estimates that 50-90% of heliothine eggs and larvae in cotton were consumed or parasitized by natural populations of insect predators and parasitoids have been reported (Bell and Whitcomb 1964, Ridgway and Lingren 1972). Parasitism by natural populations of *Trichogramma* wasps have been found to be as high as 59-92% in cotton (Johnston 1985, Segers *et al.* 1984, Suh *et al.* 1998). However, natural enemies may not suppress populations of insect pests below their respective economic threshold levels in all cases, so insecticide treatments are often necessary to control pest infestations and protect crop yields (Newsom *et al.* 1980, Baldwin *et al.* 1997).

Foliar insecticides applied to conventional cotton in North Carolina are mostly aimed at controlling the heliothine complex, *H. zea* and tobacco budworm, *Heliothis virescens.* Historically these insecticides have been predominantly broad-spectrum compounds that kill pests and beneficial arthropods alike. Recently greater emphasis has been placed on developing more environmentally sound products that selectively kill pests and have minimal effects on beneficial and other nontarget species. Numerous laboratory and field studies have shown that *Trichogramma* spp. wasps are highly susceptible to broad-spectrum insecticides (Bull and Coleman 1985). The studies reported herein were conducted to assess the toxicity of acetamiprid and certain other insecticides on preimaginal *T. exiguum* and to determine the toxicity of insecticide residues on adult *T. exiguum*. Insecticides tested were selected on the basis of their utility or potential utility for insect control in North Carolina cotton.

## Materials and Methods

***Trichogramma exiguum* source.** The *T. exiguum* colony used in all experiments originated from parasitized *H. zea* and/or *H. virescens* eggs collected in August 2002 from cotton fields located near Plymouth, NC. Parasitized eggs (indicated by black, shiny appearance of the egg chorion) were held individually in glass vials (15ml) containing a small streak of 50% honey solution. *Trichogramma* wasps were reared on irradiated *H. zea* eggs (North Carolina State University Insectary, Raleigh NC) for three generations at which time identity of each isoline was confirmed using Plinto's (1998) key to the species of North American *Trichogramma*. Isolines of *T. exiguum* were combined and maintained in glass cylindrical containers (8.5 cm height x 2.0 cm diameter) until initiation of the study. There after, the parasitoids colony was reared on irradiated *H. zea* eggs, and provided 50% honey solution as a food source throughout remainder of the study. Suh *et al.* (1998) found there was no apparent reduction in the quality of *T. exiguum* wasps after being maintained under laboratory conditions for up to one year.

**Preimaginal toxicity study.** *H. zea* eggs oviposited on cheesecloth sheets were obtained from the North Carolina State University Insectary (Raleigh, NC). Eggs (<24 h-old) were washed off cloths with a 0.03% sodium hypochlorite (Clorox®) solution and then filtered onto a paper coffee filter. Eggs were allowed to dry for approximately 2h and then exposed to UV radiation(Philips G36T6L, 70 microwatts @ 1m) 0.3 m from the source for 4 h to prevent hatching. Once irradiated, *H. zea* eggs were immediately exposed to mated *T. exiguum* females.

Parasitized eggs were partitioned into groups, each containing at least 35 eggs. Each egg group was placed on a single filter paper disc (9.0 cm diam.; Fisherbrand Qualitative P5) pressed into the bottom of a petri dish, then exposed to an insecticide solution. Insecticide solutions and concentrations used are listed in Table 1.

All insecticide solutions were prepared with 100 ml of distilled water in a 400 ml beaker and thoroughly mixed. The concentrations of each insecticide solution reflected recommended field applications rates. Eggs within a dish were concentrated in the middle of the paper and exposed to insecticides by pipetting 100 µl of the insecticide solution directly onto the eggs. This procedure ensured that all eggs within dishes were sufficiently and similarly exposed to insecticides, with excess solution being absorbed by the filter paper. The control group was treated with distilled water only. Filter papers and eggs were allowed to dry for 1 hour, then eggs from each insecticide treatment and control were transferred individually into #1 gelatin capsules. Capsules containing eggs were held at 25°C, 80% RH, and a photoperiod of 16:8 (L:D) h. Capsules were monitored daily for emerged parasitoids, and eggs were visually inspected for emergence holes. Eggs with dead adults incompletely emerged, or eggs with partially chewed exit holes with dead adults remaining in eggs were categorized as partially emerged.

All data were subjected to ANOVA using PROC GLM (SAS Institute 1990), means for each treatment were separated ($P \leq 0.05$) using Fisher's Protected Least Significant Difference test in SAS.

**Field Residue bioassay.** The study site at the Tidewater Research Station near Plymouth, NC was planted with cotton variety SG521R on May 26, 2002. The test was managed as recommended by the North Carolina Agricultural Extension Service(2002) with the exception that no insecticides were applied prior to the test substances. The five insecticide treatments tested for residual affects on *T. exiguum* adults are listed in Table 1.

Insecticides were applied with a $CO_2$-backpack sprayer fitted with a single TX-12 nozzle per row delivering 91.7 liters per hectare at 4.22 kilograms per square centimeter. Insecticides were applied on 5 October using recommended field rates.

Twenty-four hours (1 DAT) after applying insecticides, 12 leaves were excised from plants in each treated and control row. Only fully expanded leaves in the upper 1/3 portion of the cotton canopy at time of spraying were collected. Two sections (5 x 1 cm each) were removed from each leaf and individually placed in 15 ml vials containing approximately 15-30 *T. exiguum* adults (<24h-old) and a streak of 50% honey solution. Vials were placed on their sides to ensure that *Trichogramma* adults frequently contacted leaf sections. Vials were held at 25°C, 80% R.H. and a photoperiod of 16:8 (L:D) h. The number of dead females and males were counted 24 h after exposure, and vials were frozen until the total number of individuals was counted to determine % mortality. This process was repeated 6 days post-treatment.

All treatment mortality data were corrected for control mortality according to Abbotts formula (1925) and were then subjected to ANOVA using PROC GLM (SAS Institute 1990); Means for each treatment were separated ($P \leq 0.05$) using Fisher's Protected Least Significant Difference test in SAS.

## Results

**Preimaginal bioassay.** Lambda-cyhalothrin, spinosad, and imidacloprid significantly reduced the percent of *H. zea* eggs yielding adult *T. exiguum* (F=104.8; df=5,30; $P$=<0.0001) (Table 2). Less than 10% of parasitized eggs treated with lambda-cyhalothrin and spinosad produced adults. Imidacloprid had significantly lower emergence than the control but was not comparable to lambda-cyhalothrin and spinosad. The percentage of host eggs yielding *T. exiguum* adults in thiodicarb and acetamiprid treatments were similar to that of the control.

Mean numbers of emerged *T. exiguum* adults per host egg were dramatically decreased by lambda-cyhalothrin and spinosad treatments (F=38.51, df=5,30, *P*=<0.0001) (Table 2). Imidacloprid significantly reduced number of *T. exiguum* per host egg, whereas thiodicarb and acetamiprid yielded similar numbers of *T. exiguum* adults per host egg as the control.

**Residue Bioassay.** Adult *T. exiguum* survival was significantly reduced by insecticide residues of spinosad and thiodicarb at 1 DAT (F=3.67; df= ,5,30; *P*= 0.0012) (Table 3). Percent mortality for thiodicarb was the highest 79, followed by spinosad 46. Lambda-cyhalothrin, imidacloprid, and acetamiprid were all comparable to the control. None of the insecticides had significantly higher mortality of *T. exiguum* adults than the control at 6 DAT (F=3.24; df=5,20; *P*=0.0960) (Table 3).

## Discussion

Preimaginal stages of *Trichogramma* developing within host eggs appear to be well protected from insecticide exposure, while adults are very susceptible (Bull and House 1983, Bull and Coleman 1985, Brar *et al.* 1991). These studies indicated that organophosphates and carbamates adversely effected preimaginal development while pyrethroids generally had a slight to moderate effect. Other more selective compounds appeared to have no measurable effect on adult emergence form insecticide- treated host eggs.

In our study, lambda-cyhalothrin and spinosad severely effected emergence of adult *Trichogramma* and decreased the number of adults that emerged per host egg. Imidacloprid had a slight, but significant adverse effect, while thiodicarb and acetamiprid had no effect on adult numbers or emergence. These results coincide with those reported by Suh *et al.* (2000) for the effects of lambda-cyhalothrin and spinosad. The level of emergence obtained with lambda-cyhalothrin was lower than that previously reported for pyrethroids by Brar *et al.* (1991) and Kring and Smith (1995). These observed differences could be related to the species of *Trichogramma*, the host eggs used, and concentrations tested in each study. The size of the host egg and number of *Trichogramma* wasps that emerge tend to effect the overall fitness of the adult wasp (Bai *et al.* 1992). *Trichogramma* wasps that emerge from smaller hosts tend to be less fit and smaller (Bigler *et al.* 1987), and therefore more susceptible to insecticides. *T. exiguum* was the species used in this study along with *H. Zea* eggs as a host, which are a relatively large natural host. Spinosad is generally regarded as having low adverse effects on beneficial insects (Elzen 2001, Hendrix *et al.* 1997, Murray and Lloyd 1997), however, our results along with Suh *et al.* (2000) confirm that spinosad has detrimental effects on adult *Trichogramma* emergence. In contrast, thiodicarb and acetamiprid had no effect on the emergence of *Trichogramma* adults and imidacloprid only had a slight negative effect.

In our field residue study, thiodicarb was the most toxic insecticide to *T. exiguum* adults at 1 DAT with 79 percent mortality. These findings are similar to Brunner *et al.* (2001) who reported that carbamate residues were highly toxic at 1 DAT to *T. platneri.* Hendrix *et al.* (1997) reported that once spinosad dried *Trichogramma* wasps were no longer susceptible, but this report conflicts with our results. Our data, along with those reported by Suh *et al.* (2000), indicate that adult wasps are susceptible to spinosad residues at 1 DAT. Residue studies have found pyrethroids to have relatively low residual toxicity to species of *Trichogramma* (Navarajan and Agarwal 1989) and our results agree as lambda-cyhalothrin along with imidacloprid, and acetamiprid exhibited no toxic effects to *T. exiguum* at 1 DAT. At 6 DAT none of the insecticides included in our test displayed toxic effects to *Trichogramma* adults.

In conclusion, our data confirm that thiodicarb and spinosad residues are highly toxic to adult *Trichogramma* wasps. We found that more selective insecticides, such as acetamiprid, are less toxic and better suited for conserving natural or released populations of *Trichogramma* wasps. Acetamiprid also had no adverse effect on preimaginal development, indicating that this compound is compatible with *T. exiguum* wasps. Eggs in the preimaginal study were completely drenched with insecticide solutions, which is uncharacteristic with field conditions. Under field conditions eggs receive a much lower dose and many eggs are oviposited on the underside of leaves where they escape insecticide exposure. Thus, many eggs could produce viable adult *Trichogramma*. Furthermore, since augmentation of *Trichogramma* wasps has been found to be ineffective in cotton (Suh *et al.* 1998), the emphasis of further research should be placed on conserving or enhancing naturally occurring populations of *Trichogramma* as well as other natural enemies.

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Table 1. Insecticide formulations and concentrations tested against *T. Exiguum* in preimaginal and residue studies.

| Common name | Formulation | Concentration (µ [AI]/ml)[a] | Rate (kg AI/ha)[b] |
|---|---|---|---|
| lambda cyhalothrin | Karate Z 2.08 CS (Syngenta Crop Protection, Inc., Greensboro, NC) | 305.7 | 0.028 |
| thiodicarb | Larvin 4 F (Aventis CropScience, Research Triangle Park, NC) | 3057.0 | 0.281 |
| spinosad | Tracer 4 SC (Dow AgroSciences LLC, Indianapollis, IN) | 733.8 | 0.067 |
| imidacloprid | Trimax 4 SC (Bayer, Kansas City, MO) | 611.5 | 0.056 |
| acetamiprid | Intruder 70 WP (Aventis CropScience, Research Triangle Park, NC) | 611.0 | 0.056 |

[a] Stock concentrations used in preimaginal study.

[b] Rate applied in field residue study.

Table 2. Mean (SE) *Helicoverpa zea* host eggs yielding *T. exiguum* adults and emerged *T. exiguum* adults per host egg after exposure to insecticides. Raleigh, NC, 2003.

| Insecticide | Concentration (μ [AI]/ml)[a] | Rate (kg AI/ha)[b] | % host eggs yielding *T. exiguum* adults[c] | Mean # of emerged *T. exiguum* adults/host egg[c] |
|---|---|---|---|---|
| acetamiprid | 611.0 | 0.056 | 90.3 (2.17) a | 2.36 (0.16) a |
| thiodicarb | 3057.0 | 0.281 | 87.8 (2.14) a | 2.37 (0.09) a |
| control | -- | -- | 86.7 (2.29) a | 2.31 (0.19) a |
| imidacloprid | 611.46 | 0.056 | 71.8 (5.06) b | 1.86 (0.22) b |
| spinosad | 733.8 | 0.067 | 9.43 (2.98) c | 0.21 (0.08) c |
| lambda cyhalothrin | 305.7 | 0.028 | 8.73 (1.60) c | 0.13 (0.03) c |

[a] Stock concentrations used in preimaginal study.

[b] Rate applied in field residue study.

[c] Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD, ($P \leq 0.05$).

Table 3. Percent mortality[a] (SE) of adult *Trichogramma exiguum* after exposure to insecticide residues on treated cotton leaves at 1 and 6 DAT. Plymouth, NC, 2002.

| Insecticide | Rate (kg AI/ha) | 1 DAT[b] | 6 DAT[b] |
|---|---|---|---|
| thiodicarb | 0.281 | 79.1 (11.7) a | 0.5 (23.2) a |
| spinosad | 0.067 | 45.8 (20.8) ab | 31.8 (12.8) a |
| imidacloprid | 0.056 | 31.0 (5.93) bc | -16.3 (19.8) a |
| lambda-cyhalothrin | 0.028 | 22.2 (23.1) bc | 22.2 (15.4) a |
| control | -- | 0.0 (0.00) c | 0.0 (0.00) a |
| acetamiprid | 0.056 | -4.59 (12.8) c | -12.4 (15.7) a |

[a]Treatment mortality data were corrected for control mortality using Abbott's formula (1925).

[b]Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD, ($P \leq 0.05$).