PHENOLOGY, NATURAL ENEMIES, AND MANAGEMENT OF LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

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PHENOLOGY, NATURAL ENEMIES, AND MANAGEMENT OF LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

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PHENOLOGY, NATURAL ENEMIES, AND MANAGEMENT OF LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

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VITA

Elly Marie Maxwell was born June 23, 1981 in Midland, Michigan to Casey and Roxane Maxwell. She graduated from Freeland Jr./Sr. High School in 1999 and enrolled in Michigan State University (MSU) that fall. Part of her undergraduate experience involved a study abroad program in Costa Rica where she studied environmental science at multiple biological stations. After working three years in the small fruit entomology lab with Dr. Rufus Isaacs, she graduated from MSU in 2003 with a B.S. in Entomology. She then worked an internship with Rose Exterminator in Saginaw, Michigan. Elly was accepted into the Entomology and Plant Pathology Department as a graduate student in August 2003.

THESIS ABSTRACT

PHENOLOGY, NATURAL ENEMIES, AND MANAGEMENT OF LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

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This thesis first looks at the seasonal abundance of the three key pests of cole crops, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), diamondback moth; *Trichoplusia ni* (Hübner) (Lepidoptera: Plutellidae), cabbage looper; and *Pieris rapae* (L.), imported cabbageworm in central Alabama on cabbage and collards. It also does a survey of the parasitoid complex of these pests. The only parasitoid recovered from *P. xylostella* was an ichneumonid wasp, *Diadegma insular* (Cresson) reaching parasitism rates of 57%. Very sporadic populations of *T. ni* resulted in very few parasitoids being recovered. Lastly, the dominant parasitoid of *P. rapae* was the pteromalid wasp, *Pteromalus puparum* (L.) which reached parasitism rates of 42.5%.

The second objective of this thesis is to evaluate two species-specific pheromone-based experimental attracticide formulations against these pests: Last Call^{TM} DBM for *P. xylostella* and Last Call^{TM} CL for *T. ni*. Laboratory toxicity experiments were first used to confirm the effectiveness in killing conspecific males. In replicated small plots of cabbage and collards, an attracticide treatment receiving the two Last Call ^{TM} formulations, each applied multiple times at the rate of 1600 droplets per acre was compared against *Bacillus thuringiensis* subspecies *kursatki* (*Bt*) spray at action threshold and a negative untreated control. Efficacy was measured by comparing among the three treatments the following parameters: male capture in pheromone-baited traps, larval counts in plots, and crop damage rating at harvest. The results show that LastCall^{TM} provided acceptable pest control comparable to *Bt* in three of the four seasons. Efficacy of LastCall^{TM} was shown to be dependent upon lepidopteran population densities, which fluctuated from season to season. In general, LastCall^{TM} was effective at low to moderate population densities of the three species, such as typically occurs in the fall, but not in the spring when high *P. rapae* population pressure typically occurs in central Alabama. In no case did we record significant trap shutdown in LastCall^{TM} plots suggesting that elimination of males by the toxicant (permethrin), rather than interruption of sexual communication, was probably the main mechanism behind the success of the attracticide formulations.

The third objective was to evaluate several reduced-risk insecticides for the management of these pests. The following formulated sprays were evaluated: Dipel® (*Bacillus thuringiensis* subspecies *kursatki*), XenTari® (*B. thuringiensis* subspecies *aizawai*), Dipel+XenTari (a premixed test formulation consisting of both subspecies of *B.*

thuringiensis), Entrust® (a formulation of spinosad), and Novaluron (insect growth regulator). An action threshold of 0.5 cabbage looper equivalents (CLE) per plant was used to determine the need for insecticide applications. Insecticide efficacy was determined by comparing densities of larvae and immatures (larvae + pupae) of each pest species, crop damage ratings, densities of key non-target arthropods, and number of insecticide applications in plots treated with each material versus untreated control plots. All five reduced-risk insecticide formulations were effective in reducing infestations of the three pests and in providing marketable cabbage and collards in Alabama. No significant effects of insecticide treatments were recorded in the numbers of spiders and lady beetles found per plant. The results also suggest that the 0.5 CLE action threshold can be used to produce marketable cabbage and collards with only minimal applications of reduced-risk insecticides.

The main goal of this project was to evaluate some componanents of an integrated pest management program to control these pests. Hopefully this information can be used to help growers in Alabama build a successful integrated management program for future use.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Henry Fadamiro for accepting me as his graduate student and for his support and patience throughout my years at Auburn. I want to extend another thanks to my committee members: Dr. Micky Eubanks, Dr. Joe Kloepper, and Dr. Wheeler Foshee. I would like to acknowledge Dr. William Moar, Dr. Wayne Clark, and Dr. Henry Fadamiro for the teaching experience I gained from working with them. Finally, a special thanks to my parents, Casey and Roxane for their support and encouragement throughtout my life.

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INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Cole Crop Production

Cole crops, *Brassica oleracea* (L.), including cabbage, collards, broccoli, kale, brussels sprouts, and cauliflower, are an important component of diets in many parts of the world, including the United States. Nationally, cabbage is the most common cole crop: 80,290 acres of cabbage were planted and 76,850 were harvested in 2003 translating to a yield of 302 cwt per acre (NASS 2004). In the year 2002, national cabbage production was valued at $301,482,000 (AASS 2003).

Cabbage and collards are the key cole crops grown in Alabama. Growers in the state utilize both spring and fall plantings for both crops, and often grow them in rotation with other vegetables (Kemble 1999). Approximately 1500 acres of cabbage and collards are grown in the state of Alabama (Kemble personal communication). Despite the relatively low in the state of Alabama, the high value of the crop (~9,000 US$/ha) and the low tolerance of damage accepted for fresh market demands for efficient pest management programs to be developed and implemented.

Cabbage and collards are the cole crops grown most commonly in the state of Alabama. Cabbage and collards are grown primarily in the northeastern corner of the state in Jackson and DeKalb counties. The remaining acreage is in Baldwin County and the Wiregrass area including Coffee, Dale, Henry, Geneva, and Houston Counties. Cole crops are also very common in home gardens, and even on a small scale, the associated pests of these crops can be devastating.

1.2 Non-Lepidopteran Pests

There is a highly diverse insect pest complex associated with *Brassica* crops and it is represented by several insect orders (Lamb 1989). Several species of aphids attack cole crops in Alabama (Kemble 1999). Aphid alates (winged) move onto the plants early in the season. Populations gradually increase through the season peaking prior to harvest. Cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae*) and turnip aphid (*Lipaphis erysimi*) are all fairly common on cabbage in Alabama. The majority of aphids stay on the wrapper leaves and cause minimal damage to the plants. turnip mosaic virus is transmitted to the plants by turnip aphid. Perhaps the most destructive problem associated with aphids is contamination. They can be present in large number and are hard to remove before market, and of course, aphid-covered cabbage is not favored for fresh market sale (Kemble 1999).

Cabbage maggot, *Delia radicum,* is a minor problem in Alabama. The adults look like small black houseflies. The maggot larvae are small, legless, and are yellow-white in color. Cabbage maggots chew into root hairs creating slimy tunnels on cabbage root systems (Bennett et al. 2000).

Several species of flea beetles, *Phyllotrela spp.* are known to feed on cabbage. Most commonly are the striped flea beetles, the western black flea beetle, and the crucifer flea beetle (Hines and Hutchison 2001b). Adult flea beetles cause the most plant injury. The adults overwinter and emerge in mid-spring. They oviposit on the soil and the larvae emerge to feed on the foliage and roots of the plants. They drop from the plant to pupate in the soil (Sorenson 1983). The beetles are a greater problem for growers in the spring months (personal observation) in central Alabama. The adult beetles chew many small irregular shaped holes in the leaves.

The Harlequin bug, *Murgantia histrionica* (Hahn), is another pest frequently found in cole crops in Alabama. It is a brightly colored red and black stinkbug with piercing-sucking mouthparts. It feeds mostly on the veins of leaves causing wilting. The eggs are barrel shaped, black and white, and are laid in clusters (Kemble 1999). Harlequin bugs typically reach high populations late in the season in the spring and throughout the fall.

1.3 Lepidopteran Pests

The three key lepidopteran pests of cole crops are: diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae); imported cabbageworm, *Pieris rapae* L. (Lepidoptera: Pieridae); and cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). These pests are often responsible for direct damage to the marketable part of the plant and associated frass (Harcourt et al. 1955, Shelton et al.1982, Talekar and Shelton 1993, Tabashnik 1994). The three caterpillars are not equal in the amount of damage inflicted on fresh market cabbage (Shelton et al. 1982). They are however similar in their feeding behavior and biology, and commonly are treated as a single caterpillar complex (Mahr et al. 1993).

Their life cycles are similar in that they all oviposit on the foliage of cole crops. The eggs hatch into larvae which begin feeding immediately on the surface of the leaves. All the larval instars are completed on the plants, and pupation occurs on the underside and in the crevices on the leaves. The adults also occur in close proximity to the plants, flying around to mate and lay more eggs again on the plants. *P. xylostella* has the capacity reaching up to 16 generations per year (Sarnthony et al. 1989). *Trichoplusia ni* and *P. rapae* also have multiple generations per year in most parts of the world, although not nearly as many as *P. xylostella*.

The relative abundance of these three caterpillar populations varies depending on location and season. For instance, in the northwestern United States, abundance of *P. rapae* was greatest followed by *P. xylostella*, and *T. ni* was least abundant, and frequently not observed (Biever et al. 1991). In the north central part of the country, *T. ni* is reported to be the most damaging to fresh market cabbage (Hines and Hutchinson 2001a). In the south central United States, *P. xylostella* was noted to be the most abundant lepidopteran pest (Legaspi et al. 2000). This shows a varying abundance of these pests in different regions of the United States, and underscores the importance of studying their relative occurrence in different regions. Additional minor lepidopteran pests in North America include: beet arymworm, *Spodoptera exigua*; cabbage webworm, *Hellula rogatalis,* cross striped cabbageworm, *Evergestis rimosalis;* and cutworms, *Agrotis spp.*

Plutella xylostella

Plutella xylostella is the most destructive pest of cruciferous plants throughout the world and the annual cost for managing *P. xylostella* is estimated to be $1 billion US dollars (Talekar and Shelton 1993). Although the native land of *P. xylostella* is debated by scientists, it is now accepted as cosmopolitan, damaging crucifers all over the world (Wagener 2004). The fact that it goes through generations quickly and that it has been the target of many pesticide sprays has contributed to the development of resistance by the pest complex to most conventional insecticides worldwide (Zhao and You 2001). In addition, *P. xylostella* has been reported to rapidly evolve resistance to *Bacillus thuringiensis* subspecies *kurstaki* (Bt) in the field (Ferre and van Rie 2002) as well as to the spinosyn class (Sayyed 2004). Resistance to *Bt* has also been reported in field populations of *P. xylostella* in Hawaii, Malaysia, the Philippines, Japan, Central America, Florida, and Thailand (Tabashnik 1994, Telekar and Shelton 1993).

The sex pheromone of *P. xylostella* is well documented. It consists of three components: (Z)-11-hexadecanal, (Z)-11-hexadecen-1-o1 acetate, and (Z)-11-hexadecanol. (Tamaki et al. 1977).

Trichoplusia ni

In addition to *P. xylostella* having resistance issues, *T. ni* has also been the target of a myriad of pesticide sprays. Therefore, the potential of *T. ni* to evolve resistance to *Bt* is feared, and was demonstrated among laboratory colonies (Estada and Ferre 1994). In addition, *T. ni*, has also been reported to evolve resistance to *Bacillus thuringiensis kurstaki* in greenhouse situations in Bristish Columbia (Janmaat and Myers 2003). .

Phermonal communication of *T. ni* is also well understood. It was first isolated by Berger (1965). (Z)-7-dodecen-1-o1 acetate and (Z)-7-dodecen-1-o1 are the components of the sex pheromone (Bjostad et al. 1984).

Pieris rapae

Compared to the other pests, *P. rapae* has shown the least amount of resistance development. It is not usually the target of insecticide sprays, but rather controlled by the same pesticides that are sprayed to target *T. ni* and *P. xylostella.* Pheromonal communication of *P. rapae* is not understood, and it is unclear whether they use sex pheromones to seek mates.

1.4 Management of Lepidopteran Pests

1.4.1 Monitoring and Thresholds

The most commonly used sampling method for cabbage and its three lepidopterous pests is the cabbage looper equivalent (CLE) method (Shelton et al. 1982, 1983). The CLE method was proven to be the most accurate and effective method for estimating pest populations, although it does take more time than other methods (Shelton et al. 1983, Hines and Hutchinson 2001a). It is used as a basis to determine the threshold for insecticide application. The CLE method accounts for the varying levels of feeding damage caused by the three species. It assigns a relative feeding ratio to each of the three pests to standardize the damage potential each individual caterpillar can inflict. In this method, 1 CLE = 20 *P. xylostella* larvae = 1.5 *P. rapae* larva = 1 *T. ni* larva (Shelton et al. 1982, 1983). For instance, the standard for spraying broccoli is 1.0 CLE (Maltais et al. 1994) while 0.5 CLE is a commonly used threshold in cabbage and collards (Shelton et al., 1982; 1983).

Plutella xylostella and *T. ni* synthetic sex pheromone is successful for monitoring populations of adult moths in the field (Baker et al. 1982, Shirai and Nakamura 1995, Ronald 1997, Reddy and Guerroer 2001, Wang et al. 2004). Pheromone lures placed in wing traps is a successful way to capture adult male moths and therefore monitor populations, and will be used in this project.

At harvest time the method of Greene et al. (1969) was used to evaluate damage rating. In this method cabbage plants were rated based on insect feeding damage on a scale of 1 to 6 as follows: 1 = no apparent insect damage on head or inner wrapper leaves; 2 = no head damage, but minor feeding on wrapper leaves with 0-1% leaf area consumed; 3 = no damage on head, but moderate feeding damage on wrapper leaves with 2-5% leaf area consumed; 4 = minor feeding on head (but no feeding through outer head leaves), but moderate feeding on wrapper or outer leaves with 6-10% leaf area consumed; 5 = moderate to heavy feeding damage on wrapper and head leaves and a moderate number of feeding scars on head with 11-30% leaf area consumed; and 6 = severe feeding damage to head and wrapper leaves with heads having numerous feeding scars with ≥ 30% leaf area consumed (Greene et al. 1969). A similar method was used to assess marketability of collards in fall 2004 and spring 2005 with damage rating based solely on the percent of leaf area consumed (since collards is not a head-producing plant). A damage rating of ≤ 3 is considerable marketable under normal conditions, whereas a damage rating of ≤ 4 is marketable under exceptional market conditions (Leibee et al., 1995).

1.4.2 Chemical Control

Traditionally, more attention has been paid to conventional programs than biological based control programs for management of the caterpillar complex (Taleker and Shelton 1993, Biever et al. 1994, Xu et al. 2004). Vegetable growers in North America have managed the cabbage caterpillar complex in their fields using calendar-based applications of broad-spectrum insecticides including carbamates (e.g., Sevin and

Lannate), organophosphates (e.g., Thiodan), and pyrethroids (e.g., Danitol) (Hines and Hutchison 2001a, Liu et al. 2002). However, many of these insecticides have now been lost due to governmental regulation (Food Quality Protection Act, 1996) or the development of pest resistance.

Because of insecticide resistance concerns with *P. xylostella* especially, the demand for alternatives to conventional pesticide and even *Bt* is very important. An integrated approach to pest management was introduced nearly half a century ago (Stern et al. 1959). There is a growing understanding that the best way to slow resistance development to any one tactic is to combine multiple control methods in this system. To be able to continually depend on the use of *Bt* sprays depends on the prevention of resistance in target pest populations (Ferre and van Rie 2002).

1.4.3 Biocontrol

The most widely used biologically-based control strategy in cole crops is formulated sprays of *Bacillus thuringiensis* (*Bt*). *Bacillus thuringiensis* is now the standard insecticide sprayed against the cabbage caterpillar complex in Alabama and several other parts of the United States. As mentioned before, resistance among *P. xylostella* populations to *Bt* is common, and therefore other tactics to rotate or combine with these sprays are necessary.

The natural enemies of the caterpillar complex have been well studied worldwide. There is a fairly strong understanding of these beneficial insects, especially the parasitoids of the *P. xylostella*. There are dozens of different wasp parasitoids of *P.*

xylostella from four hymenopteran families (Ichneumonidae, Braconidae, Pteromalidae, and Eulopidae), with 7 braconid wasps in the genus *Diadegma* alone (Wagner et al., 2004). Although worldwide *Diadegma semiclausum* Helen is the most abundant parasitoid, (Talekar and Shelton 1993, Shi et al. 2004, Wagener 2004), *Diadegma insulare* (Cresson) is the most important and abundant parasitoid of *P. xylostella* in the United States (Lasota and Kok 1986, Biever et al. 1991, Mitchell et al. 1997, Godin and Boivin 1998, Legaspi et al. 2000, Xu et al 2004, Shi et al. 2004). *Diadegma insulare* accounted for 98% of the parasitoids reared from field collected *P. xylostella* larvae in the state of Texas, although alone it did not provide sufficient control of the caterpillar pest (Legaspi et al, 2000). It was the only parasitoid we recovered s from populations of *P. xylostella* in Alabama (Fadamiro and Maxwell, unpublished data). However, Harvey and Eubanks (2003) recovered *Cotesia plutellae* Kurdjumov (Hymenoptera: Brachonidae) from *P. xylostella* in nearby field sites. The success and importance of *Diadegma* spp. was illustrated when *D. semiclausum* out-competed *C. plutellae* when the two were compared, supporting the idea that it is a successful and steadfast control for *P. xylostella* (Shi et al. 2004). Therefore, the demand to conserve these parasitoids is very important in a successful integrated program.

Unfortunately, most of the synthetic insecticides commonly used in *Brassica* fields are detrimental to *D. insulare* (Xu et al. 2004) and other parasitoids. Therefore, the incorporation of compatible chemical control methods is crucial. It is imperative that research in the area of reduced risk insecticides (including bacteria-derived insecticides and insect growth regulators) continue; both for the conservation of the natural enemy guild and for protection of the environment and human health.

Parasitic wasps have been extensively studied for their contribution to control of the caterpillar complex; however, little understanding of the role of predators such as carabid beetles and spiders is known (Talekar and Shelton 1993). In Australia, carabids, lady beetles, rove beetles, green lacewings, and spiders, are known to play a role in predation of the lepidopteran pests of cole crops (Furlong et al. 2004). Carabids do contribute to larval mortality of both *P. xylostella* and *P. rapae* (Schellhorn and Sork 1997, Suenaga and Hamamura 2001). Field boundaries are important in that they serve as reservoirs of beneficial carabid beetles (Wratten et al. 1998). Certain species of carabid beetle larvae have been shown to climb plants to forage and also consume the larvae of *P. xylostella* in Japan (Suenaga and Hamamura 1998). Predator activity is known to increase as shelter areas (i.e. weed growth, ground cover, etc.) in and around cole crop fields are available (Cranshaw 1984), implying that it may be beneficial to leave refuge areas to maximize natural enemy control. However, there is no evidence that interplanting necteriferous plants among cabbage increases parasitoid abundance in cabbage fields (Al-Doghairi and Cranshaw 2004).

Population dynamics of the cruciferous caterpillar complex and associated natural enemies have been studied in various regions of North America (Pimental, 1961; Harcourt et al. 1963; Oatman and Platner 1969, Elsey and Rabb 1970, Biever 1972, Harding 1976, Ru and Workman 1979, Andalaro et al. 1982, Latheef and Irwin 1983, Chamberlin and Kok 1986, Kok and McAvoy 1989, Biever et al. 1991). However, information on abundance and occurrence of these three pests and their associated parasitoids in the state of Alabama is limited.

1.4.4 Semiochemical-Based Control

Pheromones are utilized in pest control in multiple ways. Mass trapping and mating disruption are the most common applications for pheromone-based control. Mating disruption is accomplished by releasing large amount of pheromone from many point sources (Gaston et al. 1967, Farka et al., 1974).

Attracticide technology which incorporates an attractant (sex pheromone, floral, food) with a toxicant has been evaluated again multiple pests. Attracticide tactics were shown by Losel et al. (2000) and Charmillot et al. (1997, 2000) to be successful in controlling codling moth, *Cydia pomonella* in European apple orchards. It also was shown to successfully control the pink bollworm, *Pectinophora gossypiella* (Hofer and Angst 1995) and the light brown apple moth (Suckling and Brockerhoff 1999).

The LastCall^{TM} formulations used in the laboratory and field experiments were formulated and supplied by IPM Tech Inc., Portland, OR (now known as IPM Development Company, Marylhurst) and consisted (in addition to pheromone and toxicant) of a clear viscous paste (gel) with a base proprietary product plus other inert ingredients. Two species-specific experimental formulations were evaluated: i) Last Call ^{TM} DBM for *P. xylostella* consisting of 0.16% pheromone and 6% permethrin by weight), and ii) Last Call^{TM} CL for *T. ni* consisting of 1.6% pheromone and 6% permethrin by weight. The sex pheromone of *P. xylostella* consists of three components: (*Z)*-11-hexadecanal, (*Z)*-11-hexadecen-1-ol acetate, and (*Z)*-11-hexadecanol (Tamaki et al. 1977), while (*Z*)-7-dodecen-1-ol acetate and (*Z*)-7-dodecen-1-ol are the components of *T.*

ni sex pheromone (Berger 1966, Bjostad et al. 1984). The sex pheromone of each species was purchased from Bedoukian Research Inc. (Danbury, CT) and used as attractant in the proprietary formulations (IPM Tech Inc., Portland, OR). The Last Call^{TM} formulations were dispensed from an applicator tube with a calibrated pump that deposits metered droplets. Each 50 µl droplet of the gel formulation weighed ~ 50 mg and consisted of pheromone and permethrin as active ingredients.

The advantage to baiting the droplets with a sex pheromone makes the formulations species specific. An additional advantage that might favor the attractiveness of attracticide-baited traps might be the fact that droplets emit pheromones before the females are present or begin calling (Krupke et al., 2002). Also, with males contacting droplets, Haynes et at. (1986) showed that even sub-lethal insecticide doses to the pink bollworm, *Pectinophora gossypiella* impaired the ability of male moths to locate sources of pheromone.

1.5 Research Objectives

a. Determine seasonal abundance and phenology of cabbage caterpillar pest complex and associated natural enemies in central Alabama.
b. Evaluate the efficacy of attracticide for suppression of *Plutella xylostella* and *Trichoplusia ni* in cole crops using pheromone-based attracticide formulations in comparision to a grower standard (Bt spray) and a negative control.
c. Field evaluation of reduced risk insecticides against cabbage-caterpillar complex in Alabama.

CHAPTER II :

SEASONAL OCCURRENCE OF THE LEPIDOPTERAN PESTS OF COLE CROPS AND ASSOCIATED PARASITOIDS IN ALABAMA

2.1 Introduction

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), imported cabbageworm *Pieris rapae* (L.) (Lepidoptera: Pieridae) and cabbage looper, *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) are the three key lepidopterous pests of cole crops worldwide. They have similar feeding habits and host ranges do similar damage to the foliage of cole crops, and are therefore treated as a single caterpillar complex (Mahr et al. 1993). They are responsible for damage directly to the marketable part of the plant (Harcourt et al. 1955; Shelton et al. 1982 Talekar and Shelton 1993; Tabashnik 1994).

Despite the relatively few numbers of acres of cabbage and collards grown in the state of Alabama, the high value of the crop (~9,000 US$/ha) demands for efficient pest management programs to be developed and implemented. Broad-spectrum insecticides, such as organophosphates, pyrethroids, and carbamates have been used traditionally for control of these pests (Hines and Hutchison 2001, Liu et al. 2002). Unfortunately, resistant populations, non-target effects, and government regulations (Food Quality Protection Act, 1996) have directed most growers in the state of Alabama to rely on *Bacillus thuringiensis* sprays as a standard for control of these pests (Kemble personal communication). *Bacillus thuringiensis* is a good method for control because it is specific to lepidopteran insects, targeting only insects in that order and thus preserving the natural enemy complex. The pests and their natural enemies have been studied throughout the U.S. In the northwestern United States, abundance of *P. rapae* was greatest followed by *P. xylostealla*, and *T. ni* was least abundant, and frequently not even observed (Biever et al., 1991). In the midwest part of the country (Minnesota) *T. ni* is reported to be the most damaging to market cabbage (Hines and Hutchinson 2001). In the south central United States, *P. xylostella* was noted to be the most abundant lepidopteran pest (Legaspi et al., 2000). This shows a varying abundance of the pests having regional difference throughout the United States, and indicated the importance of studying the relative occurrence in different areas independently.

The natural enemies of the caterpillar complex have been well studied worldwide. There is a fairly strong understanding of these beneficial insects, especially the parasitoids of *P. xylostella*. There are dozens of different wasp parasitoids of *P. xylostella* from four hymenopteran families (Ichneumonidae, Braconidae, Pteromalidae, and Eulopidae), with 7 braconid wasps in the genus *Diadegma* alone (Wagner et al. 2004). Although worldwide *Diadegma semiclausum* Hellen is the most abundant parasitoid, (Talekar and Shelton 1993, Shi et al. 2004, Wagener, 2004), *Diadegma insulare* (Cresson) is the most important and abundant parasitoid of *P. xylostella* in the United States (Mahr et al. 1993, Lasota and Kok 1986, Biever et al. 1991, Mitchell et al. 1997, Godin and Boivin 1998, Legaspi et al. 2000, Xu et al 2004 Shi et al. 2004). *D. insulare* accounted for 98% of the parasitoids reared from field collected *P. xylostella* larvae in the state of Texas, although alone it did not provide sufficient control of the caterpillar pest (Legaspi et al, 2000).

Population dynamics of these three caterpillar pest species have been studied in various regions of North America (Pimental 1961, Harcourt 1963, Oatman and Platner 1969, Elsey and Rabb 1970, Biever 1972, Harding 1976, Ru and Workman 1979, Andalaro et al. 1982, Latheef and Irwin 1983, Chamberlin and Kok 1986, Kok and McAvoy 1989, Biever et al. 1991). However, information on abundance and occurrence of these three pests and their associated parasitoids in the state of Alabama is limited.

In this experiment, we intend to determine the seasonal abundance of the pest complex and its associated parasitoid guild. With the knowledge of pest composition and the natural enemy complex, growers in the state will be better able to utilize the biocontrol agents as part of an integrated pest management system. This study will be the first to describe seasonal parasitism of Lepidoptera in cabbage grown under commercial conditions in this production area.

2.2 Materials and Methods

2.2.1 General Methods

All data was collected at the E.V. Smith Agricultural Research Station in central Alabama. Eggs, larvae, and pupae of the three species were transported to the lab in a Styrofoam container via an air conditioned car and reared individually on freshly picked and washed collard leaves in Petri dishes in laboratory conditions. Emergence of the adults as well as parasitoids was recorded in the laboratory.

2.2.2 Preliminary Season

The year 2003 was used as a preliminary season where the objectives were to evaluate the type, activity, and abundance of parasitoids of the cabbage caterpillar complex. Eggs, larvae and pupae of the three pests were randomly collected and reared in laboratory conditions in individual Petri dishes on cabbage leaves. ‘Vates’ collards and ‘Bravo’ cabbage were grown.

2.2.3 Pest Phenology Experiment

The three lepidopteran pests were monitored at the E.V. Smith Agricultural Research Station. Populations of adult males of *P. xylostella* and *T. ni* were monitored weekly by placing in the center of each plot two wing traps baited with the commercial pheromone lures one for each species (IPM Tech Inc., Portland, OR). The two traps were spaced apart by ~ 10 m. Since no pheromone-baited traps are currently available for monitoring *P. rapae*, adult population of this species was monitored weekly using a visual method. This was done weekly by an observer (E. Maxwell) standing in the center of each plot and counting the number of adult *P. rapae* seen in the plot during a 5-minute observation period.

In spring 2004, ‘Vates’ collards were grown. In fall 2005, ‘Rio Verde’ cabbage and ‘Top bunch’ collards were grown. In spring 2005 ‘Bravo’ cabbage and “Vates” collards were grown.

Each season, 50 plants were sampled randomly weekly in each plot. Eggs, larvae, and pupae of the three species were transported to the laboratory in a Styrofoam container via an air conditioned car and reared individually on freshly picked and washed collard leaves in Petri dishes in laboratory conditions.

Emergence of the adults as well as parasitoids was recorded in the laboratory. In the case of excessive *P. rapae* egg densities, only one was removed from each plant.

2.3 **Results and Discussion**

Populations of all three species varied by season. In general, adult, larvae and pupal populations of all species were greater in the spring seasons than in the fall. *Plutella xylostella* showed to be the most consistent and abundant pest (Fig. 1) across all seasons. *Pieris rapae* was present in all growing seasons, but was very abundant in spring 2004 (Fig. 1, Fig. 2). Populations of *T. ni* were sporadic across the three seasons, and were the highest in the spring 2005 (Fig 1, Fig 4). *Trichoplusia ni* populations were not present in spring 2004, and were only found in the adult stage in Spring Fall 2004 at small numbers (Fig. 1).

Pieris rapae had the most diverse parasitoid complex. Three species of parasitoids were recovered including *Pteromalus puparum, Phyrxe vulgaris* (Fallen), and *Brachymeria ovata.* The most common was *Pteromalus puparum*, which in the preliminary season late in the summer was collected at rates as high as 86%. In fall of 2004 parasitism rates were also high, at 41.0% overall and reaching a maximum of 57% the week of December 2 . Rates of parasitism of *P. rapae* increased as the season progressed (Fig. 3), and were far more abundant in the fall seasons than the spring seasons.

The only parasitoid recovered from *P. xylostella* was *Diadegma insulare,* but it was recovered in very high percentages, especially later in the season. It was common in both the fall and the spring seasons reaching up to 77% parasitism midseason spring 2005.

Throughout much of our study, *T. ni* was not very abundant. With the exception of fall 2003 and spring 2005, significant levels at the station were not recorded. In spring 2005 the populations of this pest were high, but still parasitism rates were very low suggesting perhaps the inexistence of the pest in previous seasons reduced the parasitoid populations. The only parasitoids recovered from larvae and pupae of *T. ni* was *Voria ruralis* and *Litomastix truncatellum*, both at very low rates. A single *Trichogramma* spp wasp was recovered in the prelimilary season. a very high number of individuals in each parasitized pupa.

In many cases, the level of parasitoid activity we observed would not provide sufficient protection of cruciferous crops from the pest complex. To increase the effectiveness of the parasitoids it is very important that a grower continue to use reduced-risk insecticides that do not have nontarget effects. Judicious use of *Bacillus thuringiensis* or other bacterial derived insecticides have no effect on these parasitoids. Additionally, seasonal inoculations of some of these species might increase populations that are currently very small. The data generated in this study serves as a baseline study, the first data of its sort collected in Alabama, to assist in the development of a biological control management strategy that takes advantage of the parsitoid complex for control of the lepidopterous pests of cole crops.

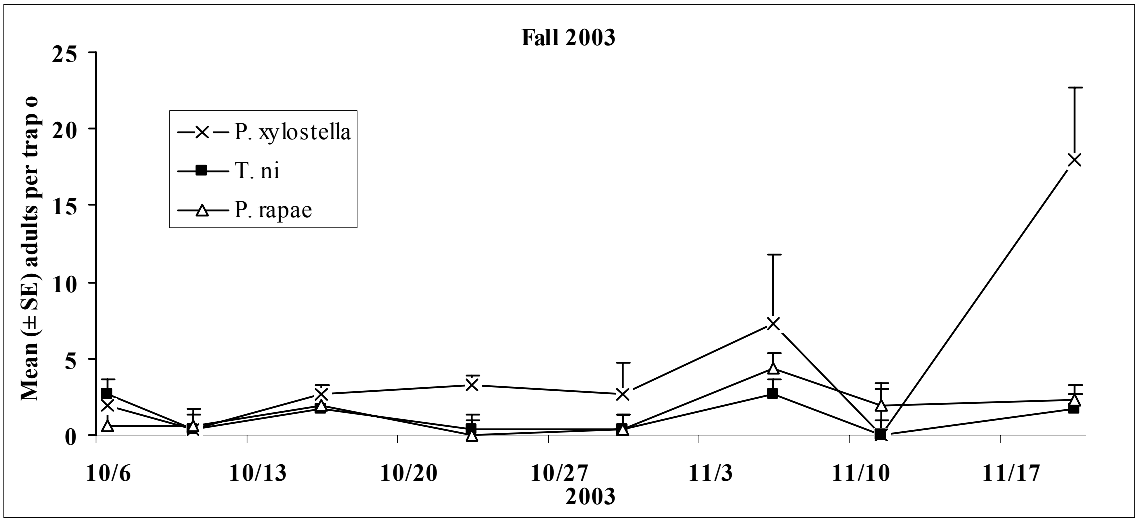

Figure 1a. Seasonal mean (± SE) number adults of lepidopteran species sampled. *Plutella xylopsella* and *Trichoplusia ni* were sampled using pheromone traps checked weekly, whereas *Pieris rapae* was monitored with 5 minute visual counts week during fall 2003 (A).

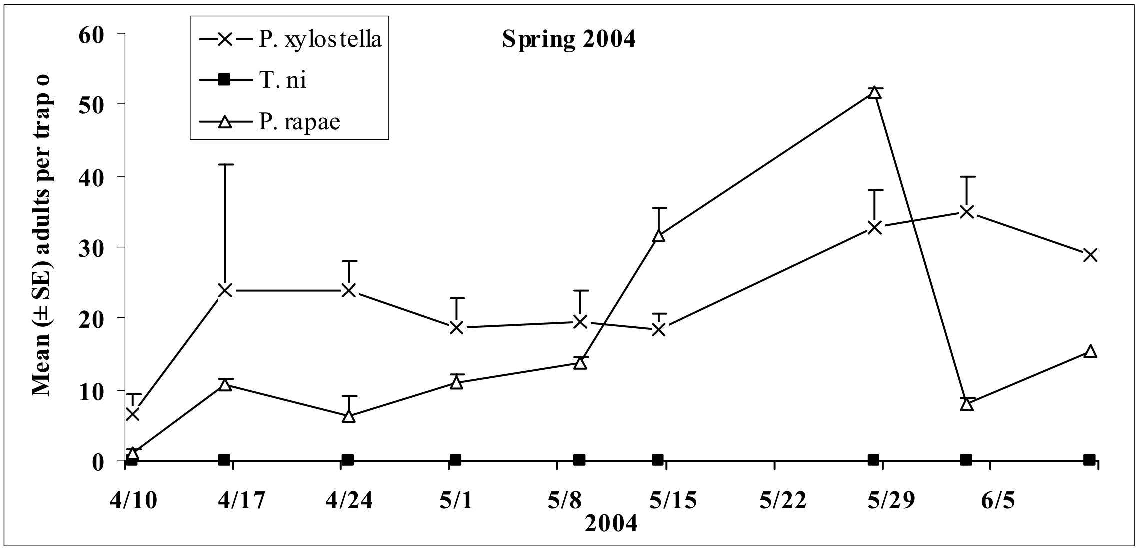

Figure 1b. Seasonal mean (± SE) number adults of lepidopteran species sampled. *Plutella xylopsella* and *Trichoplusia ni* were sampled using pheromone traps checked weekly, whereas *Pieris rapae* was monitored with 5 minute visual counts week during spring 2004.

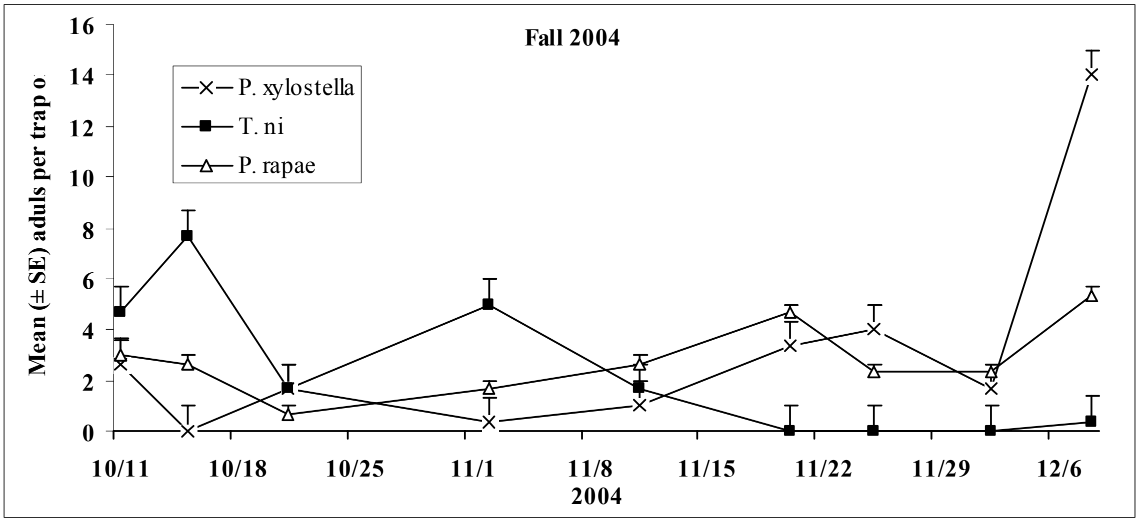

Figure 1c. Seasonal mean (± SE) number adults of lepidopteran species sampled. *Plutella xylopsella* and *Trichoplusia ni* were sampled using pheromone traps checked weekly, whereas *Pieris rapae* was monitored with 5 minute visual counts week during fall 2004.

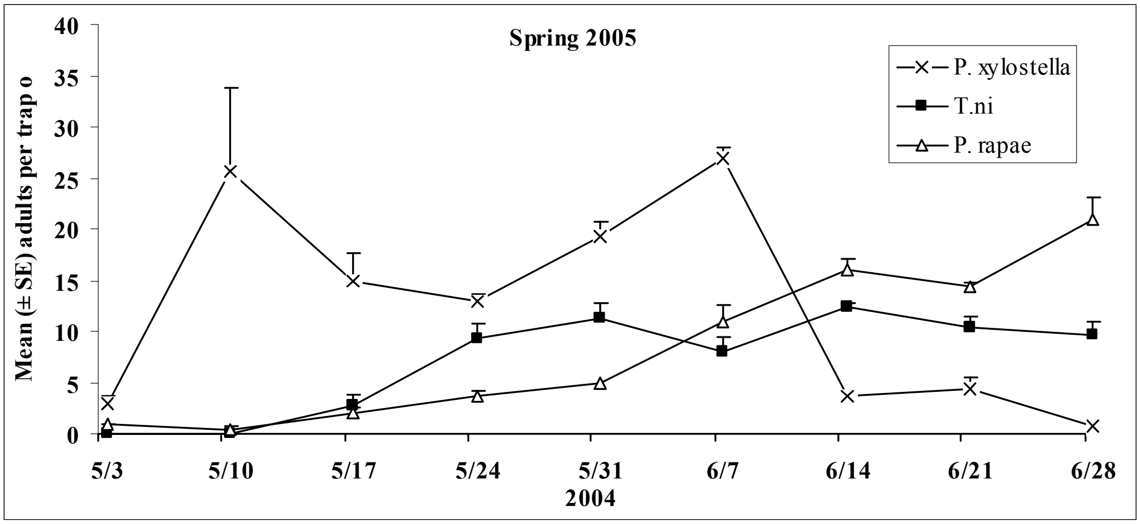

Figure 1.d. Seasonal mean (± SE) number adults of lepidopteran species sampled. *Plutella xylopsella* and *Trichoplusia ni* were sampled using pheromone traps checked weekly, whereas *Pieris rapae* was monitored with 5 minute visual counts week during spring 2005.

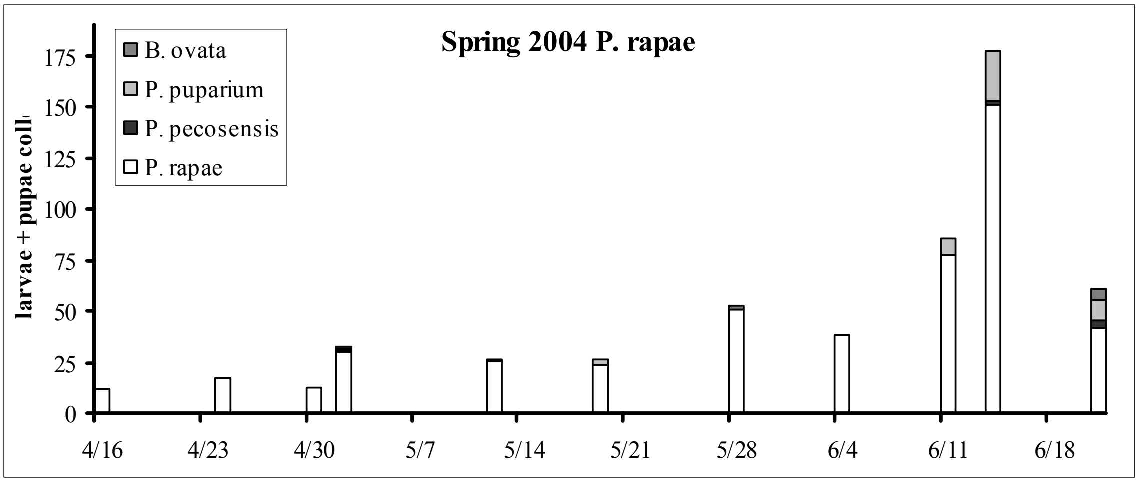

Figure 2.a. Total count of larvae and pupae collected per week of *P. rapae* and associated parasitoids during spring 2004.

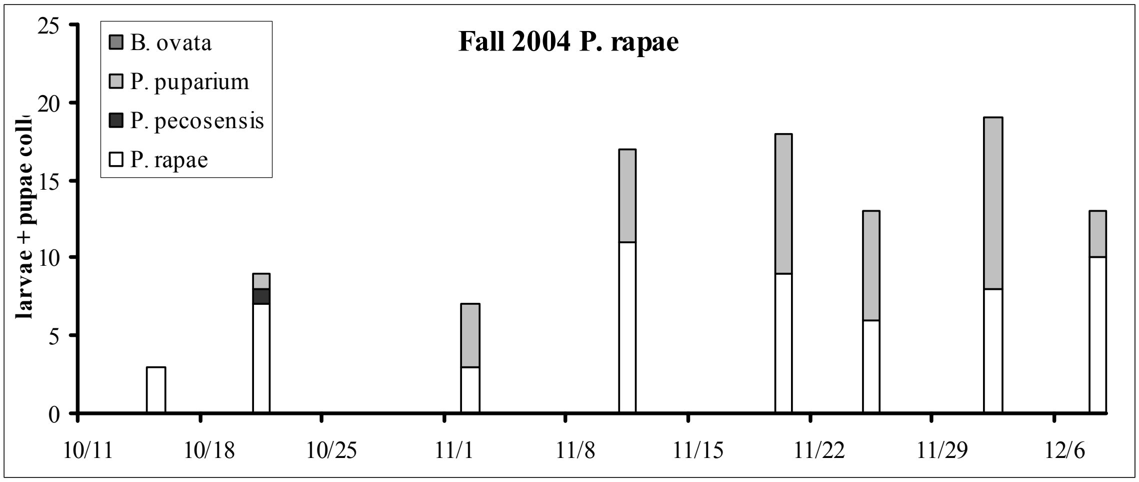

Figure 2.b. Total count of larvae and pupae collected per week of *P. rapae* and associated parasitoids during fall 2004.

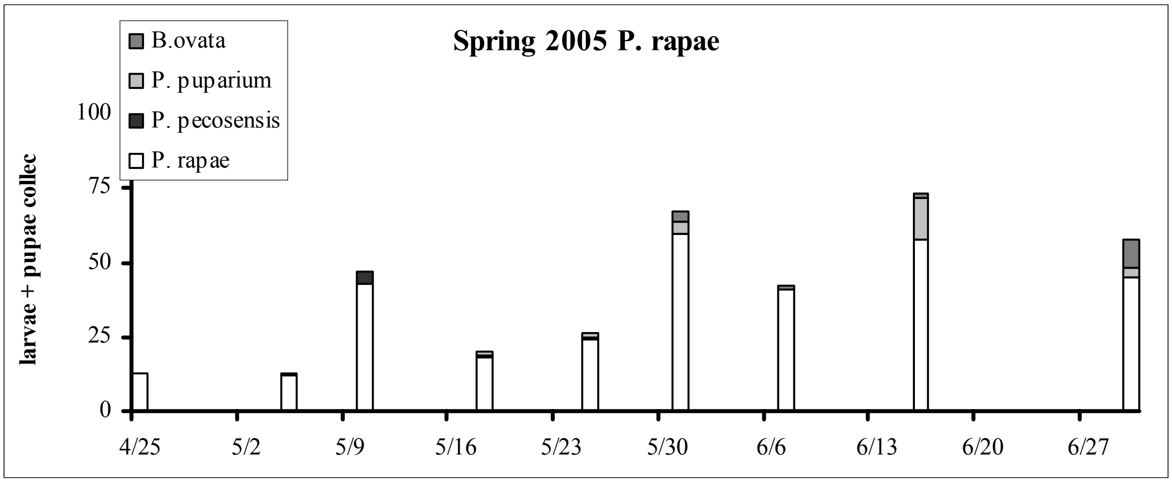

Figure 2.c. Total count of larvae and pupae collected per week of *P. rapae* and associated parasitoids during spring 2005.

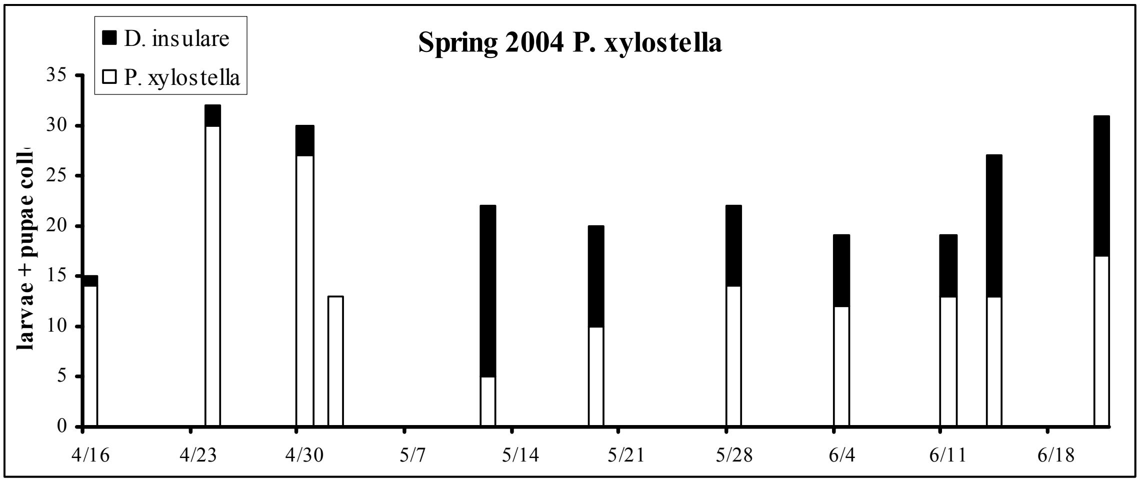

Figure 3.a. Total count of larvae and pupae collected per week of *P. xylostella* and associated parasitoids during spring 2004.

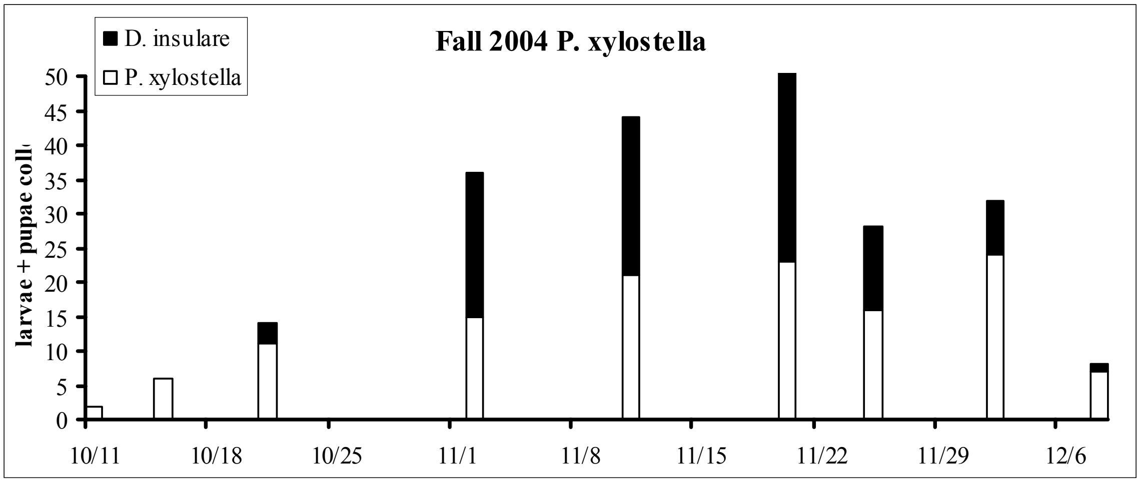

Figure 3.b. Total count of larvae and pupae collected per week of *P. xylostella* and associated parasitoids during fall 2004.

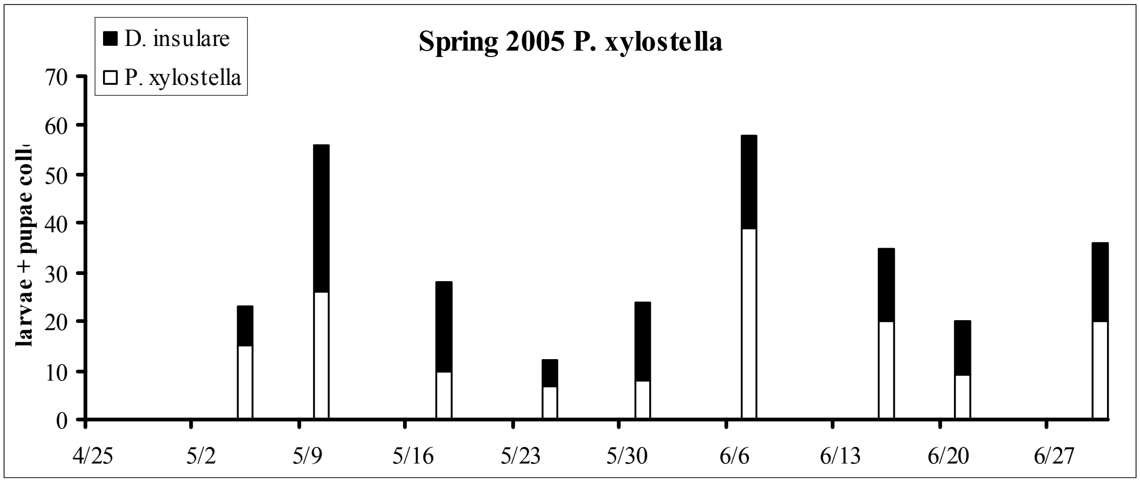

Figure 3.c. Total count of larvae and pupae collected per week of *P. xylostella* and associated parasitoids during spring 2005.

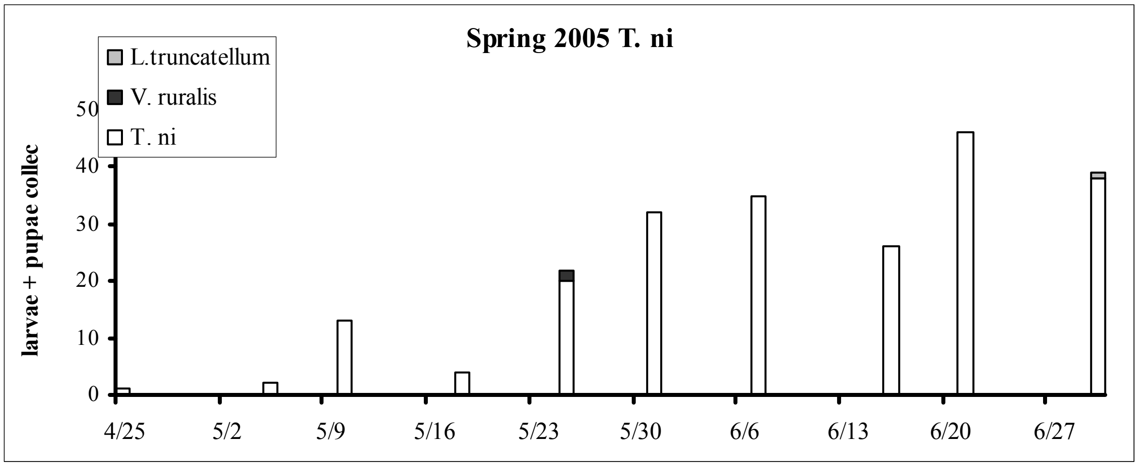

Figure 4. Total count of larvae and pupae collected per week of *T. ni* and associated parasitoids during spring 2005.

CHAPTER III :

SUPPRESSION OF *PLUTELLA XYLOSTELLA* AND *TRICHOPLUSIA NI* IN COLE CROPS BY USING PHEROMONE-BASED ATTRACTICIDE FORMULATIONS

3.1 Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), cabbage looper (CL), *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and imported cabbageworm (ICW), *Pieris rapae* L. (Lepidoptera: Pieridae) are the key pests of cruciferous plants (*Brassica spp.)* in North America. Lepidopteran larvae feed on the foliage and cause direct damage to the marketable part of cole crops (Harcourt et al. 1955, Shelton et al. 1982, Talekar and Shelton 1993, Tabashnik 1994). The three species are usually managed together as a single caterpillar complex, commonly referred to as the cabbage caterpillar complex (Shelton et al. 1982, Mahr et al. 1993, Hines and Hutchison 2001).

Traditionally, vegetable growers in North America have managed the cabbage caterpillar complex in their fields using calendar-based applications of broad-spectrum insecticides including carbamates (e.g., Sevin and Lannate), organophosphates (e.g., Thiodan), and pyrethroids (e.g., Danitol) (Hines and Hutchison 2001, Liu et al. 2002). However, many of these insecticides have now been lost due to governmental regulation (Food Quality Protection Act, 1996) or the development of pest resistance. The most widely used biologically-based control strategy in cole crops is formulated sprays of *Bacillus thuringiensis* (*Bt*). *Bacillus thuringiensis* is now the standard insecticide sprayed against the cabbage caterpillar complex in Alabama and several other parts of the United States. However, *Bt*-resistant field populations of *P. xylostella* have been reported in various locations world-wide (Mahr et al. 1993, Rueda and Shelton 1995, Tabashnik et al. 1997). Furthermore, *Bt* sprays are directed against pest larvae and thus allow some level of feeding damage to take place before mortality occurs. These concerns have prompted renewed interests in the development of alternative pest management tactics against the cabbage caterpillar complex. Integrating *Bt* sprays with another biologically based tactic (such as the use of semiochemicals) may have the potential of reducing the risk of resistance to *Bt*, and increase its efficacy.

Semiochemical-based strategies, including mating disruption and attracticides (attract-and-kill or lure and kill) can potentially be used to manage two of the three key lepidopteran pests of cole crops: *P. xylostella* and *T. ni*. The sex pheromone of both species have been characterized (Berger 1996, Tamaki et al. 1977, Bjostad et al. 1984), and studies have demonstrated the efficacy of mating disruption of *P. xylostella* and *T. ni* (Gaston et al. 1967, Farka et al. 1974, Mitchell et al. 1997, Mitchell 2002). Recently, there has been some focused interest in the potential use of semiochemicals as attracticides for pest management. The attracticide technology incorporates an attractant (e.g., synthetic pheromones) with an insecticide (e.g., permethrin), and its utility is based on the attraction of individuals of the target species to a hydrophobic matrix containing an insecticide where they are killed without insecticide run-off or drift, thereby limiting contamination of the crop and the ecosystem. The tactic is considered useful and promising because, unlike mass trapping, traps do not have to be deployed and serviced, and unlike mating disruption, males are actually killed, rather than confused. This technology has shown significant promise for the control of several key lepidopteran pest species including *Pectinophora gossypiella* (Saunders) (Haynes et al. 1986, Hofer and Angst 1995), *Epiphyas postvittana* (Walker) (Brockerhoff and Suckling 1999), *Cydia pomonella* (L.) (Charmillot and Hover 1997, Charmillot et al. 2000, Losel et al. 2000, Krupke et al. 2002, Evenden and McLaughlin 2005), and *Grapholita molesta* (Busck) (Evenden and McLaughlin 2004a,b, 2005). Mitchell (2002) reported the initial development of attracticide formulations against *P. xylostella* and *T. ni*, while Mullan (2003) evaluated some experimental attracticide formulations against *T. ni* in commercial vegetable greenhouses. The results of both studies demonstrated the potential of the attracticide technology for managing both pests in cole crops (Mitchell 2002, Mullan 2003).

In this paper, we report on laboratory and field experiments conducted to further evaluate the potential of attracticide formulations for managing *P. xylostella* and *T. ni* on cole crops. We tested two species-specific pheromone-based commercial attracticide formulations (one for *P. xylostella* and one for *T. ni*) supplied by IPM Tech Inc., Portland, OR (now known as IPM Development Company, Marylhurst, OR), which holds the global license rights to an attracticide matrix gel (LastCall^{TM}). Laboratory experiments were conducted to determine the toxicity of LastCall^{TM} formulations to laboratory-reared *P. xylostella* and *T. ni* males. We then evaluated over multiple field seasons the efficacy of LastCall^{TM} formulations for suppression of lepidopteran pest infestation and damage in crucifer plots, in comparison to *Bt* and a negative untreated control. Ultimately, we were interested in determining whether or not the attracticide technology could provide acceptable control of the cabbage caterpillar complex and yield marketable crops comparable to *Bt* despite the fact that LastCall^{TM} formulations are not currently available against *P. rapae*, a key member of the cabbage caterpillar complex in Alabama.

3.2 Materials and Methods

3.2.1 *Attracticide formulations*

The LastCall^{TM} formulations used in the laboratory and field experiments were formulated and supplied by IPM Tech Inc., Portland, OR (now known as IPM Development Company, Marylhurst) and consisted (in addition to pheromone and toxicant) of a clear viscous paste (gel) with a base proprietary product plus other inert ingredients. Two species-specific experimental formulations were evaluated: i) Last Call ^{TM} DBM for *P. xylostella* consisting of 0.16% pheromone and 6% permethrin by weight), and ii) Last Call ^{TM} CL for *T. ni* consisting of 1.6% pheromone and 6% permethrin by weight. The sex pheromone of *P. xylostella* consists of three components: (*Z)*-11-hexadecanal, (*Z)*-11-hexadecen-1-ol acetate, and (*Z)*-11-hexadecanol (Tamaki et al. 1977), while (*Z*)-7-dodecen-1-ol acetate and (*Z*)-7-dodecen-1-ol are the components of *T. ni* sex pheromone (Berger 1966, Bjostad et al. 1984). The sex pheromone of each species was purchased from Bedoukian Research Inc. (Danbury, CT) and used as attractant in the proprietary formulations (IPM Tech Inc., Portland, OR). The Last Call ^{TM} formulations were dispensed from an applicator tube with a calibrated pump that deposits metered droplets. Each 50 µl droplet of the gel formulation weighed ~ 50 mg and consisted of pheromone and permethrin as active ingredients. Formulations were kept in a -20º C freezer until use.

3.2.2 Laboratory experiments

We conducted simple "touch" toxicity tests to determine the toxicity of both experimental attracticide formulations by comparing exposure of adult *P. xylostella* and *T. ni* to conspecific Last Call™ formulations versus a double blank formulation (with no insecticide or pheromone). Both lepidopteran species were reared in the laboratory on modified pinto bean diet using standard rearing protocols for each species (McEwen and Hervey 1960, Guy et al. 1985, Shelton and Collins 2002). Pupae of each species were harvested from diet, sexed, and held individually in 1 oz plastic cups until adult emergence. Prior to the tests, moths were chilled briefly in the refrigerator (at 5 º C for ~15 min) to reduce activity. For each species, 24 newly-emerged (1 day old) males were treated with their conspecific Last Call™ formulation by using a toothpick to apply (touch) 1 drop of the gel to the back of the thorax. Treated moths were placed individually in 1 oz plastic cups (with lid) and provided with a 25% sugar solution (using cotton dental wicks). The cups were arranged in a random order on a tray and placed in a fume cupboard maintained at ~ 25º C and a photoperiod of 14:10 (L:D). The control treatment for each species consisted of another set of males exposed to a double blank formulation (no permethrin, no pheromone). Effect of contact with Last Call™ was determined by checking for male mortality at 2 and 24 h after exposure, scoring individual male as alive (apparently fully functional) or dead. Significant differences in the toxicity of Last Call™ and double blank formulations to males of each species were established using a χ^2 2 × 2 test of independence with Yates' correction for continuity (Parker 1979).

3.2.3 Field evaluation of attracticide formulations

We conducted field experiments in replicated small plots of cabbage and collards to evaluate the efficacy of LastCall^{TM} formulations in controlling *P. xylostella* and *T. ni* infestations. This study was conducted over four growing seasons (fall 2003, spring 2004, fall 2004, and spring 2005) at the E.V. Smith Agricultural Research Station in Shorter, central Alabama. Plots were 27.4 m by 18.2 m with plants spaced 45 cm apart within a row and 90 cm between rows for a total of 600 plants per plot. Plots were separated by 33.5 m and each plot was then subdivided into two equal subplots consisting of 300 cabbage plants and 300 collard plants. Plots were initially bare ground and established by transplanting cabbage (*Brassica oleraceae* L. var. *capitata* L.) and collard (*Brassica oleraceae* L. var. *acephala* L.) seedlings obtained from a nursery in western Georgia (Lewis Taylor Farms, Ty Ty, Georgia) following a preseason fire ant (*Solenopis invicta* Buren) treatment with Amdro® (active ingredient = hydramethylnon, BASF Corporation, Research Triangle Park, NC). In fall of 2003 'Bravo' cabbage and 'Vates' collards were mechanically transplanted on 24 September 2003. In spring of 2004 'Bravo' cabbage and 'Vates' collards were mechanically transplanted on 2 April 2004. In fall 2004, 'Rio Verde' cabbage and 'Top bunch' collards were mechanically transplanted on 3 October 2004. In spring 2005, 'Bravo' cabbage and 'Vates' collards were mechanically transplanted on 22 April 2005. Standard field preparation and crop production practices (i.e., irrigation, herbicide, fertilizer) were used to establish and maintain cabbage and collard plants in all four growing seasons. In each season, three treatments were evaluated: i) Attracticide treatment involving applications of *P. xylostella* and *T. ni* LastCall^{TM} formulations; ii) *Bt* spray at action threshold, and iii) Untreated control. Treatments were arranged in a randomized complete block design with three replicates (blocks) in each season (the experiment was replicated in three blocks with each block containing three plots, one for each treatment). Blocks were separated by ~ 60 m. In each season, each attracticide plot received multiple applications of the two formulations: LastCall^{TM} DBM and LastCall^{TM} CL. One droplet (50 mg) of each LastCall^{TM} formulation was applied to an unmarketable outer leaf of 100 plants per plot (50 plants per subplot), translating to a low rate of about 1600 droplets per acre. This application rate translated to ~ 0.14 g and 1.4 g of pheromone/acre for *P. xylostella* and *T. ni*, respectively, and was shown to be effective in preliminary experiments (Mitchell 2002, J. McLaughlin, unpublished data). The treated plants were evenly distributed in the plot. LastCall^{TM} applications were made at 2 week intervals during the first month of each growing season to accommodate early season leaf drop, and at 3-4 week intervals thereafter, for a total of 3-4 applications per season.

Plots were evaluated weekly for pest infestation by sampling ten randomly selected plants per plot, 5 from each subplot, for larvae of *P. xylostella, T. ni,* and *P. rapae.* The species, numbers, and size (instar) of caterpillars per plant were counted and recorded. Eggs and pupae of the three species were also sampled, but only larval data are presented in this paper. For the *Bt* treatment, Dipel® (a formulation of *B. thuringiensis* subspecies *kurstaki)* was applied, since it is currently the most commonly used microbial insecticide on Alabama vegetable crops (Kemble, personal comm.)

Applications of Dipel® (Valent Biosciences Libertyville, IL) were made only when larval counts exceeded a threshold of 0.5 cabbage looper equivalents (CLE) per plant (Shelton et al. 1982, 1983). The CLE method accounts for the varying levels of feeding damage caused by the three species. In this method, 1 CLE = 20 *P. xylostella* larvae = 1.5 *P. rapae* larva = 1 *T. ni* larva (Shelton et al. 1982 1983). Dipel® applications were made at the recommended rate of 1 pound per acre with a CO_2 pressurized backpack sprayer using a 3-ft boom with 3 nozzles calibrated to deliver about 25 gpa at 40 psi. On average, each *Bt* plot received 2-3 applications of Dipel® per season. Populations of adult males of *P. xylostella* and *T. ni* were monitored weekly by placing in the center of each plot two wing traps baited with the commercial pheromone lures one for each species (IPM Tech Inc., Portland, OR). The two traps were spaced apart by ~ 10 m. Since no pheromone-baited traps are currently available for monitoring *P. rapae*, adult population of this species was monitored weekly using a visual scheme. This was done weekly by an observer (E. Maxwell) standing in the center of each plot and counting the number of adult *P. rapae* seen in the plot during a 5-minute observation period.

At harvest, ten plants were randomly selected from each subplot and rated for caterpillar feeding damage and marketability using the method of Greene et al. (1969). In this method cabbage plants were rated based on insect feeding damage on a scale of 1 to 6 as follows: 1 = no apparent insect damage on head or inner wrapper leaves; 2 = no head damage, but minor feeding on wrapper leaves with 0-1% leaf area consumed; 3 = no damage on head, but moderate feeding damage on wrapper leaves with 2-5% leaf area consumed; 4 = minor feeding on head (but no feeding through outer head leaves), but moderate feeding on wrapper or outer leaves with 6-10% leaf area consumed; 5 =

moderate to heavy feeding damage on wrapper and head leaves and a moderate number of feeding scars on head with 11-30% leaf area consumed; and 6 = severe feeding damage to head and wrapper leaves with heads having numerous feeding scars with $\geq$ 30% leaf area consumed (Greene et al. 1969). A similar method was used to assess marketability of collards with damage rating based solely on the percent of leaf area consumed (since collard is not a head-producing plant). A damage rating of ≤ 3 is considered marketable under normal conditions (Leibee et al. 1995).

For each field season, mean seasonal numbers of larvae and adults of each species and mean damage rating at harvest were calculated for each treatment. Data were transformed by using the square-root method $\sqrt{(x + 0.5)}$ and analyzed for significant treatment effects by using analysis of variance (ANOVA) with the replicate plots (or subplots) considered as blocks. Means were compared using the Tukey-Kramer HSD comparison for all pairs (JMPIN Version 4.0.2, SAS Institute Inc., 1988). Significant differences were established at the 95% confidence level ($P < 0.05$).

3.3 Results

3.3.1 Laboratory experiments.

LastCall^{TM} formulations were significantly more toxic to adult *P. xylostella* and *T. ni* than the double blank formulation both at 2 h and 24 h exposure periods (Table 3.1). Approximately 71% of *P. xylostella* males and 63% of *T. ni* males were killed within 2 h of exposure to conspecific LastCall^{TM} formulations, whereas none of the males exposed to the double blank control died within this period. At the end of a 24 h exposure period to the formulations, 96% of *P. xylostella* males and 100% of *T. ni* males were killed, compared to the significantly lower 8% mortality recorded for males of both species exposed to the double blank control.

3.3.2 Field evaluation of attracticide formulations

In general, attracticide formulations provided significant suppression of *P. xylostella* and *T. ni* populations and crop damage. However, the efficacy of LastCall^{TM} varied from season to season and was shown to be highly dependent upon the population densities of the three lepidopteran species, which also fluctuated from season to season. Generally, no significant block effects were detected on any of the key parameters (variables), suggesting that the blocks (replicate plots) were similar in pest abundance and treatment efficacy.

In fall 2003, moderate larval infestations (≤ 0.5 larva per plant per week) of *P. xylostella* and *P. rapae* were recorded in the cabbage and collard subplots, while *T. ni* larval infestation was very low averaging less than 0.1 larva per plant per week (Figs. 5A & 6A). Male *P. xylostella* capture was relatively low throughout the fall increasing near the end of the season (Fig. 7A). Also, low numbers of *T. ni* were trapped in untreated control plots (Fig. 8A), while moderate numbers of adult *P. rapae* were recorded in visual counts (Fig. 9A). Fewer *P. xylostella* males were captured in pheromone traps located in LastCall^{TM} plots (seasonal mean ± SE = 3.4 ± 0.9) than in *Bt* (seasonal mean ± SE = 5.4 ± 0.9) or untreated (seasonal mean ± SE = 6.6 ± 1.4) plots. However, this ~ 49% reduction of trap capture in LastCall^{TM} plots relative to untreated plots was not significant ($F = 2.6$, df = 2, $P = 0.08$). Similarly, trap captures of *T. ni* in LastCall^{TM} plots were not significantly different than captures in the other two treatments ($F = 0.1$, df = 2, $P = 0.91$). Nonetheless, *P. xylostella* larval counts were significantly lower in cabbage subplots treated with LastCall^{TM} or *Bt* compared to untreated subplots ($F = 8.3$, df = 2, $P = 0.0003$), whereas no significant differences were recorded among the treatments in collard subplots ($F = 1.8$, df = 2, $P = 0.17$). Larval counts of *T. ni* in cabbage and collard subplots were too low to detect any significant differences among treatments (cabbage: $F = 1.0$, df = 2, $P = 0.37$; collard: $F = 0.49$, df = 2, $P = 0.61$). As expected, significantly lower numbers of *P. rapae* larvae were found in cabbage and collard subplots treated with *Bt* compared to untreated subplots (cabbage: $F = 5.5$, df = 2, $P = 0.004$; collard: $F = 2.9$, df = 2, $P = 0.05$), whereas larval counts were not significantly different between LastCall^{TM} subplots and untreated or *Bt* subplots. Significantly higher damage ratings were recorded in untreated subplots than in LastCall^{TM} or *Bt* subplots (cabbage: $F = 14.9$, df = 2, $P < 0.0001$; collard: $F = 14.5$, df = 2, $P < 0.0001$); marketable cabbage and collards were produced in both LastCall^{TM} and *Bt* subplots, but not in untreated subplots (Fig. 10A).

In spring 2004, *P. rapae* infestation was very high both in terms of larval pressure (Figs. 5B & 6B) and adult visual counts (Fig. 9B). Similarly, high numbers of male *P. xylostella* were captured in pheromone traps (Fig. 7B), while larval pressure was moderate (Figs. 5B & 6B). In contrast, *T. ni* larval pressure was extremely low in the subplots (Figs. 5B & 6B), while adult trap catch was virtually zero. Traps captures of male *P. xylostella* in pheromone traps were not significantly different among the three treatments ($F = 1.0$, df = 2, $P = 0.36$), although slighty lower in LastCall^{TM} plots. Significantly higher numbers of *P. xylostella* larvae were found in untreated cabbage subplots than in LastCall^{TM} or *Bt* subplots (*F* = 5.6, df = 2, *P* = 0.0004), whereas significant differences were not recorded among the treatments in collard subplots (*F* = 2.8, df = 2, *P* = 0.06). As observed in fall 2003, *T. ni* infestation was too low to detect any significant difference between treatments (cabbage: *F* = 1.0, df = 2, *P* = 0.37; collard: *F* = 1.0, df = 2, *P* = 0.37). Expectedly, *P. rapae* larval counts were significantly lower in cabbage and collard subplots treated with *Bt* compared to untreated or LastCall^{TM} subplots (cabbage: *F* = 26.8, df = 2, *P* < 0.0001; collard: *F* = 21.7, df = 2, *P* < 0.0001). Damage ratings were significantly higher in untreated and LastCall^{TM} subplots than in *Bt* subplots (cabbage: *F* = 190, df = 2, *P* < 0.0001; collard: *F* = 273, df = 2, *P* < 0.0001), and marketable cabbage and collards were produced only in the *Bt* subplots (Fig. 10B).

The results obtained in fall 2004 were generally similar to those of fall 2003 in terms of pest pressure, species abundance, and treatment efficacy. In this season, *P. xylostella* and *P. rapae* pressure was moderate (Figs. 5C, 6C, 7C & 9C). *Trichopulsia ni* larval infestation was not detected in the subplots (Figs. 5C & 6C), although low numbers of males were captured in pheromone traps (Fig. 8B). A modest (~ 34%) reduction in trap capture was recorded in LastCall^{TM} plots in comparison with untreated control plots, but this was not significant (*F* = 0.15, df = 2, *P* = 0.87). Similarly, the ~ 43.5% reduction in trap capture of *T. ni* males in LastCall^{TM} plots was not significant (*F* = 2.3, df = 2, *P* = 0.11). Larval counts of *P. xylostella* were significantly lower in subplots treated with *Bt*, compared to LastCall^{TM} or untreated subplots (cabbage: *F* = 6.3, df = 2, *P* = 0.002; collard: *F* = 6.3, df = 2, *P* = 0.002). Similarly, numbers of *P. rapae* larvae found in subplots treated with *Bt* were significantly lower than in LastCall^{TM} or untreated subplots (cabbage: *F* = 3.4, df = 2, *P* = 0.04; collard: *F* = 5.3, df = 2, *P* = 0.005). Nevertheless, damage ratings were significantly lower in LastCall^{TM} and *Bt* subplots than in untreated subplots (cabbage: $F = 3.2$, df = 2, $P = 0.05$; collard: $F = 210$, df = 2, $P < 0.0001$); marketable crops were produced both in LastCall^{TM} and *Bt* subplots but not in untreated subplots (Fig. 10C).

In spring 2005, moderate to high infestations of all three species were recorded in the subplots both in terms of larval pressure (Figs. 5D & 6D) and adult counts (Figs. 7D, 4D & 5D), resulting in a relatively higher total pest pressure than in the previous seasons. A modest (but not significant) reduction (~ 26%) was recorded in trap captures of male *P. xylostella* was recorded in LastCall^{TM} plots, compared to untreated plots ($F = 0.72$, df = 2, $P = 0.49$). Also, no significant differences in *T. ni* male trap capture were recorded among the treatments ($F = 0.02$, df = 2, $P = 0.98$). Seasonal mean numbers of *P. xylostella* larvae were significantly lower in LastCall^{TM} and *Bt* subplots than in untreated subplots (cabbage: $F = 8.8$, df = 2, $P = 0.0002$; collard: $F = 6.4$, df = 2, $P = 0.0002$). Similarly, significantly lower numbers of *T. ni* larvae were found in cabbage subplots treated with LastCall^{TM} or *Bt* than in untreated subplots ($F = 5.8$, df = 2, $P = 0.003$), whereas no significant differences were recorded among treatments in collard subplots ($F = 1.0$, df = 2, $P = 0.37$). For *P. rapae*, larval counts were significantly lower in subplots treated with *Bt* than in untreated or LastCall^{TM}-treated subplots (cabbage: $F = 4.6$, df = 2, $P = 0.01$; collard: $F = 3.2$, df = 2, $P = 0.04$). Significantly lower damage ratings were recorded in LastCall^{TM} and *Bt* subplots than in untreated subplots (cabbage: $F = 14.9$, df = 2, $P < 0.0001$; collard: $F = 14.5$, df = 2, $P < 0.0001$). Marketable cabbage and collards were produced in the LastCall^{TM} and *Bt* subplots, but *Bt* treatment resulted in a slightly better cabbage marketability rating than LastCall^{TM} (Fig. 10D). In general, similar results were obtained for cabbage and collard subplots.

3.4 Discussion

The goal of this study was to evaluate the efficacy of attracticide technology as a pest management tactic against lepidopteran pests of cole crops. First, we demonstrated in laboratory toxicity experiments the effectiveness of both commercial attracticide (LastCall^{TM}) formulations in killing *P. xylostella* and *T. ni* males. The results of our field experiments further confirmed the potential utility of both LastCall^{TM} formulations in suppression of infestations of *P. xylostella* and *T. ni* on cabbage and collards. In the current study, LastCall^{TM} provided acceptable control of *P. xylostella* and *T. ni* and yielded marketable crops similar to *Bt* in all but one of the four seasons (spring 2004). The results show that the efficacy of LastCall^{TM} was dependent upon the population densities of the three lepidopteran species, which fluctuated from season to season. In our study plots in central Alabama, *P. xylostella* was observed in moderate numbers in all four seasons, while population densities of *P. rapae* and *T. ni* were greater in the spring than in the fall.

The failure of LastCall^{TM} in yielding marketable produce in spring 2004 could be attributed to several reasons. First, *P. xylostella* infestation as indicated by adult trap captures was 2- to 3-fold greater in spring 2004 than in any of the other seasons (~ 24 males per trap per week recorded in spring 2004). In addition, LastCall^{TM} failure may be due to the relatively high *P. rapae* infestation recorded in spring 2004 coupled with the fact that this species was not the target of the LastCall^{TM} treatments. Several authors have also attributed attracticide failure to very high initial pest populations (Charmillot et al.

2000, Krukpe et al. 2002, Evenden and McLaughlin 2004b). Krukpe et al. (2002) postulated that the effectiveness of attracticide formulations is likely to decrease as the population density of female increases, since more females will increase the competition provided by natural sources of pheromones. Disruption of sexual communication (measured by trap shutdown) has been proposed as a key operative mechanism of the attracticide tactic (Mitchell 2002, Evenden and McLaughlin 2004a, b, Evenden et al. 2005). However, we did not record significant trap shutdown in LastCall^{TM} plots, yet LastCall^{TM} was effective in producing marketable cabbage and collards. This suggests that elimination of males by the toxicant (permethrin), rather than male confusion or interruption of sexual communication due to multiple pheromone sources, was probably the main mechanism behind the success of the attracticide tactic in this study (Brockerhoff and Suckling 1999, Charmillot et al. 2000).

Our field study covering four growing seasons each with varying pest pressure has yielded some insights on the potential efficacy of the attracticide tactic against lepidopteran pests of cole crops and allowed us to make some predictions regarding some of the factors that may influence the effectiveness of this relatively novel technology. The results of our study suggest that LastCall^{TM}, at the recommended application rate of 1600 droplets per formulation per acre, is effective at low to moderate population densities of *P. xylostella* and *T. ni* typically observed in the fall in central Alabama. The relatively greater spring population densities of *P. xylostella* and *T. ni* coupled with extremely high *P. rapae* pressure commonly observed in the spring in central Alabama may likely result in the failure of the present pheromone-based attracticide system against lepidopteran crucifer pests, which does not target *P. rapae*. It is possible that the use of a higher application rate (e.g., 3000 droplets per formulation per acre) or frequency may likely enhance the efficacy of LastCall^{TM} even at high pest population densities; however this possibility needs to be investigated. Attracticides utilizing floral attractants instead of pheromone are currently under development (IPM Development Company, Marylhurst, OR), and may hold promise for utilization against *P. rapae*. Floral attractants often attract both sexes and therefore have the potential to increase the utility of attracticide tactic against other species. Furthermore, the development of attracticide formulations that use botanical insecticides (e.g., pyrethrum) instead of synthetic insecticides will likely make the tactic acceptable to organic farmers, or those interested in sustainable crop production.

In summary, the attracticide technology is potentially effective against lepidopteran pests of cole crops and can be of use in an integrated pest management program against the cabbage caterpillar complex, either as a stand alone tactic utilizing multiple applications within a season or in rotation with *Bt* or other tactics. The advantages of this tactic include species specificity, little or no impact on non-target beneficial arthropods, and requirement of less pheromone per acre than mating disruption (Charmillot et al. 2000, Mitchell 2002, Evenden and McLaughlin 2004a, b). Additionally, because the attracticide technology targets the adult males of these insect populations, it is compatible and complementary with tactics aimed at eggs or larvae of *P. xylostella* and *T. ni.* However, it remains to be seen whether or not this tactic will provide a cost-effective alternative to *Bt* or conventional insecticides.

Table 3.1. Percent mortality of male *Plutella xylostella* and *Trichopulsia ni* after exposure to attracticide (Last Call ^{TM}) or double blank formulations. Twenty four males were exposed to each formulation. Percent within the same insect column having no letters in common are significant at *P* < 0.05.

Formulation treatment	% *P. xyllostella* males dead		% *T. ni* males dead	
	2 h exposure	24 h exposure	2 h exposure	24 h exposure
Attracticide (Last Call ^{TM})	70.8 a	95.8 a	62.5 a	100 a
Double blank control	0 b	8.3 b	0 b	8.3 b

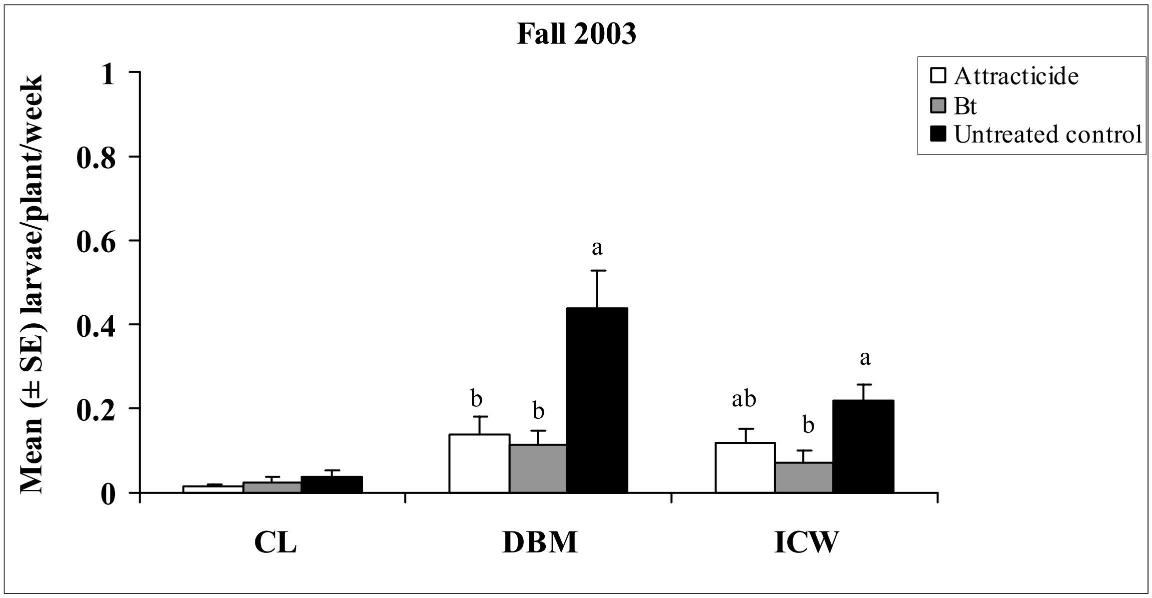

Figure 5.a. Seasonal mean (± SE) number of larvae of the three lepidopteran pest species sampled per plant per week in cabbage subplots treated with different treatments during fall 2003. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

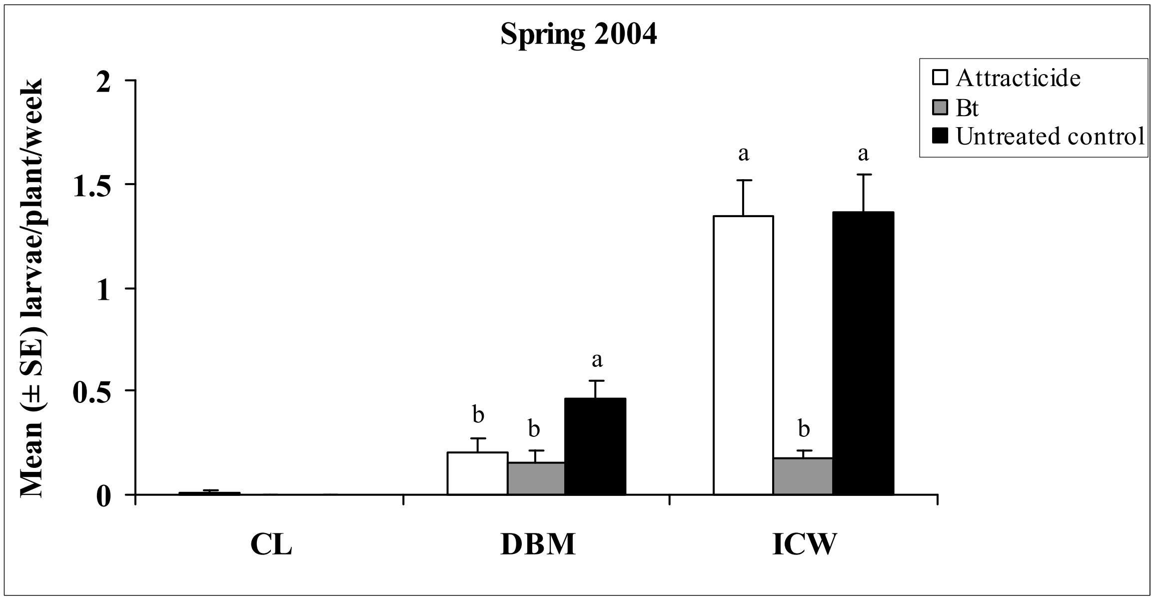

Figure 5.b. Seasonal mean (± SE) number of larvae of the three lepidopteran pest species sampled per plant per week in cabbage subplots treated with different treatments during spring 2004. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

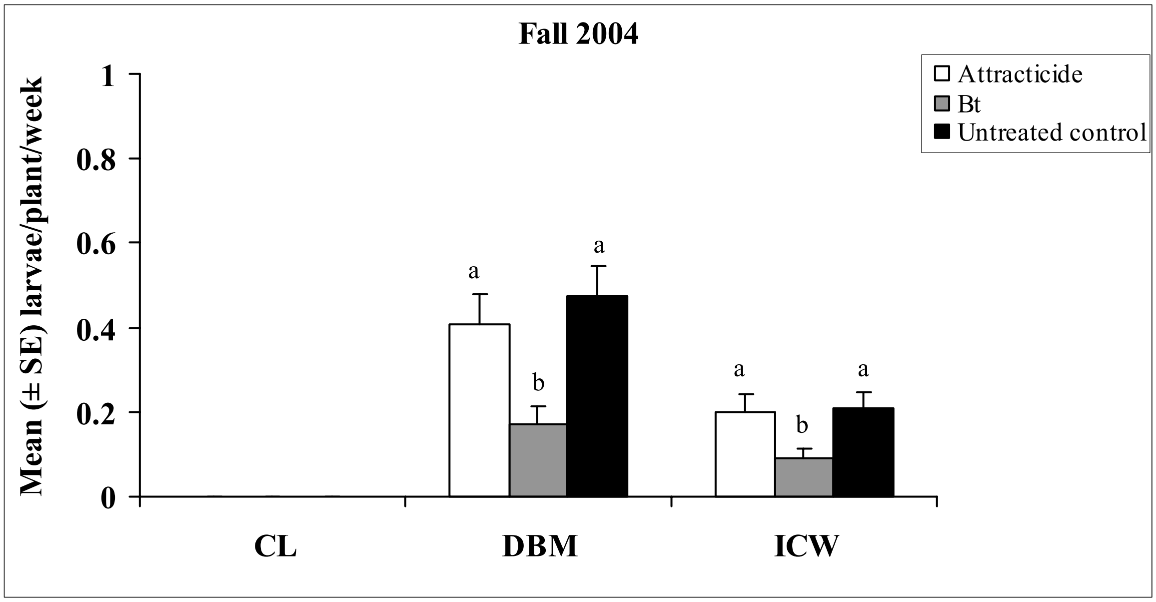

Figure 5.c. Seasonal mean (± SE) number of larvae of the three lepidopteran pest species sampled per plant per week in cabbage subplots treated with different treatments during fall 2004. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

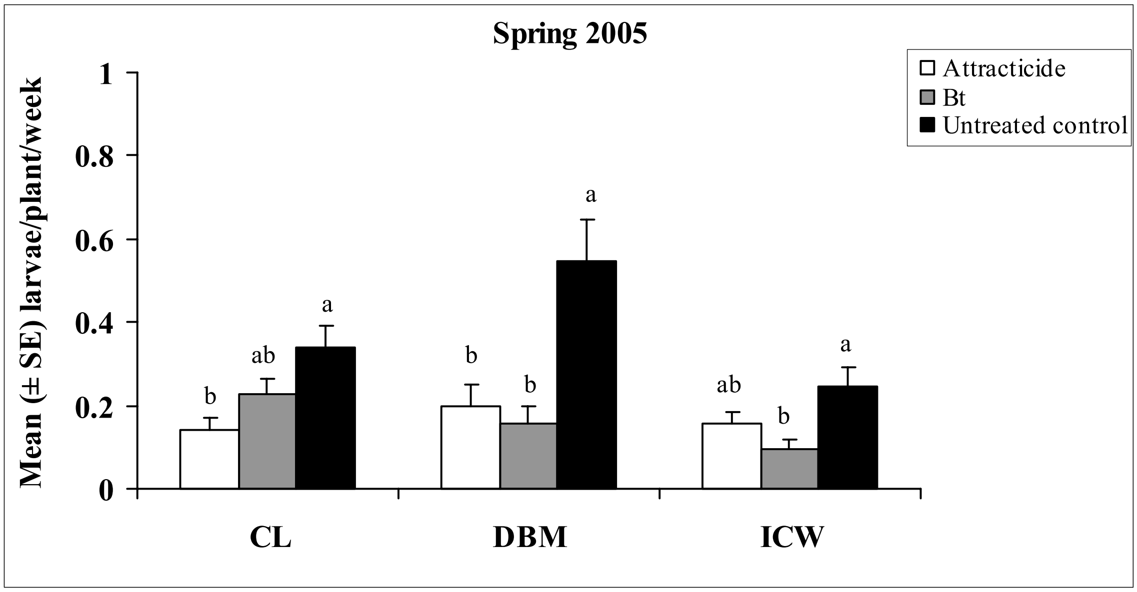

Figure 5.d. Seasonal mean (± SE) number of larvae of the three lepidopteran pest species sampled per plant per week in cabbage subplots treated with different treatments during fall 2004. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

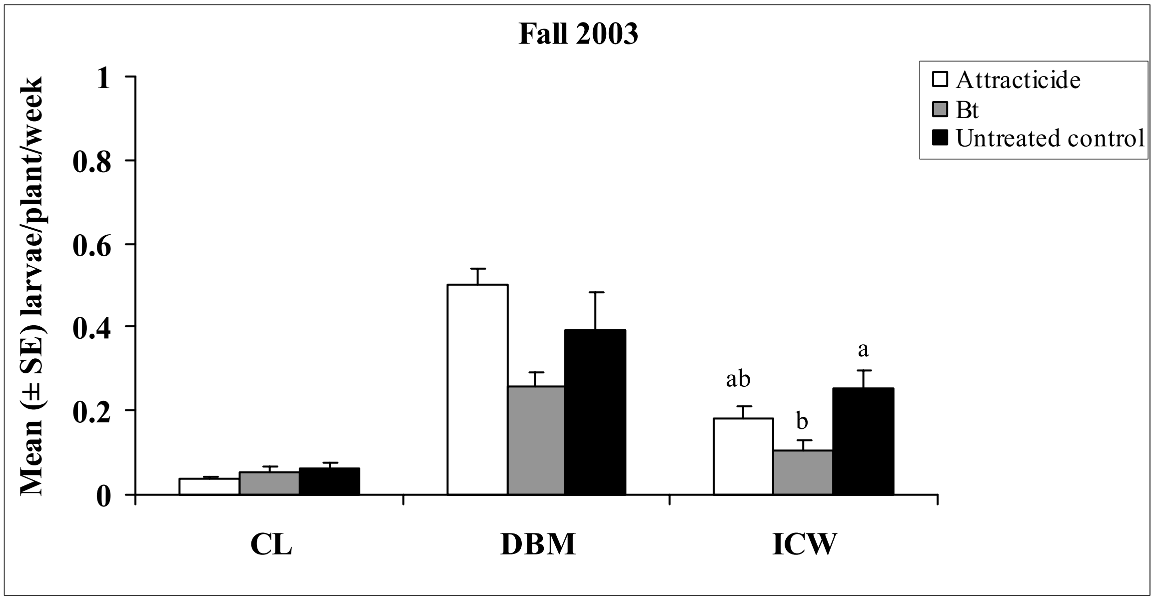

Figure 6.a. Seasonal mean (± SE) number of larvae of the three lepidopteran species sampled per plant per week in collard subplots treated with different treatments during fall 2003. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

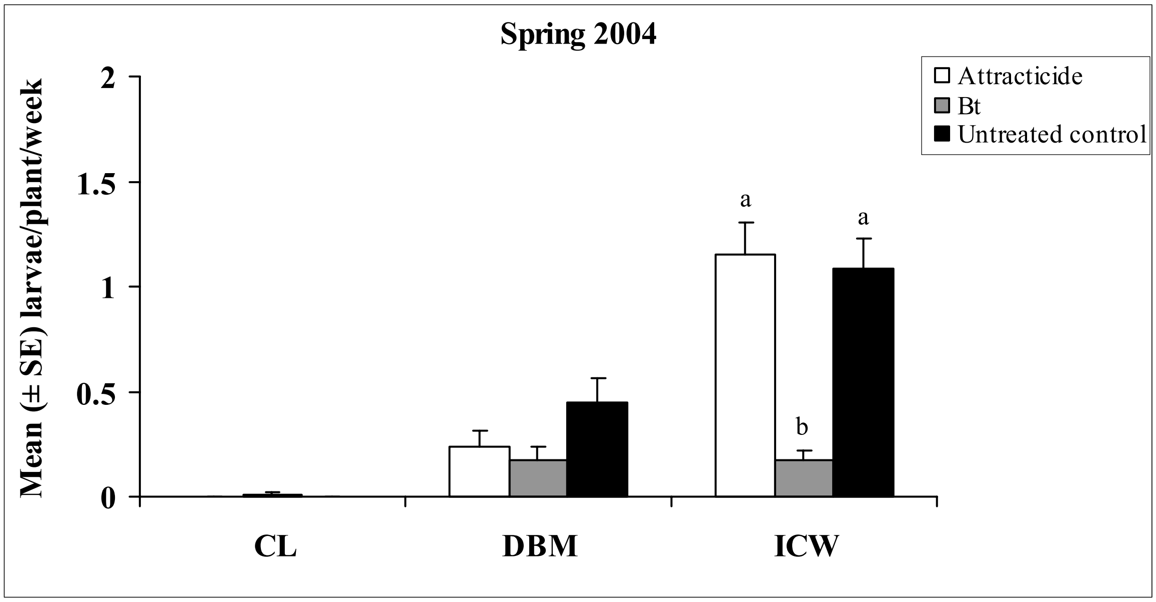

Figure 6.b. Seasonal mean (± SE) number of larvae of the three lepidopteran species sampled per plant per week in collard subplots treated with different treatments during spring 2004. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

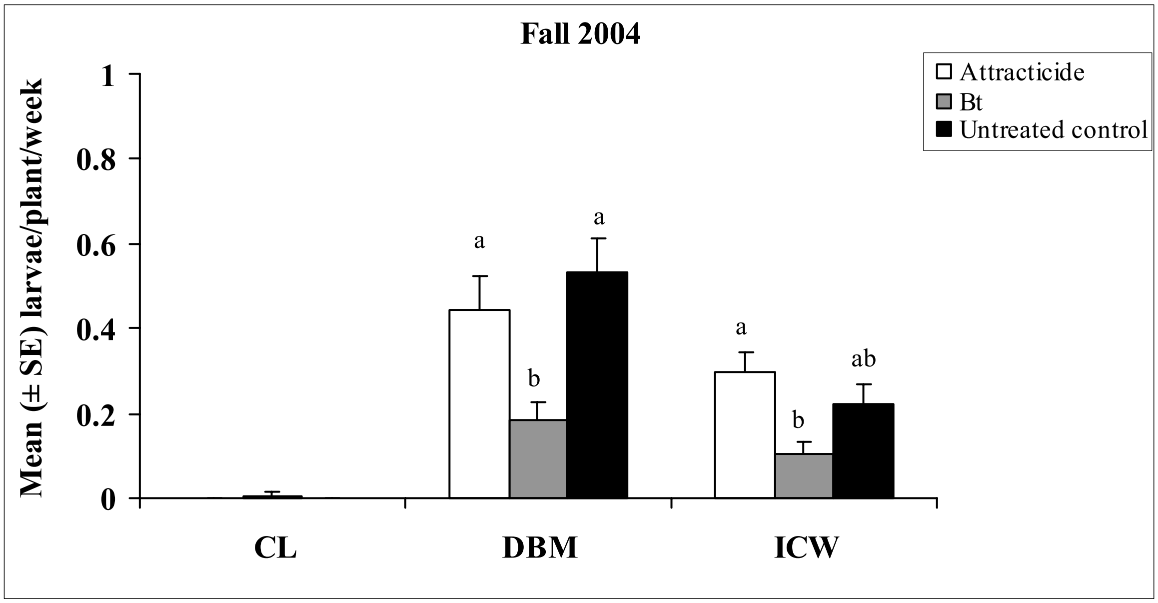

Figure 6.c. Seasonal mean (± SE) number of larvae of the three lepidopteran species sampled per plant per week in collard subplots treated with different treatments during fall 2004. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

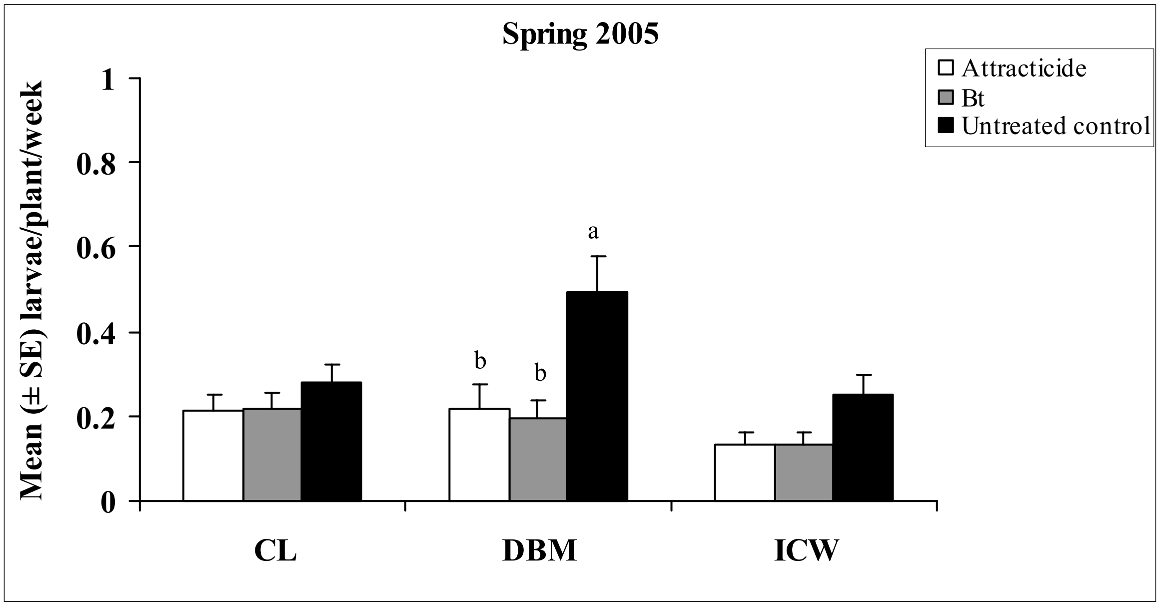

Figure 6.d. Seasonal mean (± SE) number of larvae of the three lepidopteran species sampled per plant per week in collard subplots treated with different treatments during spring 2005. DBM = diamondback moth, *Plutella xylostella*; CL = cabbage looper *Trichopulsia ni*; ICW = imported cabbageworm, *Pieris rapae.* Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

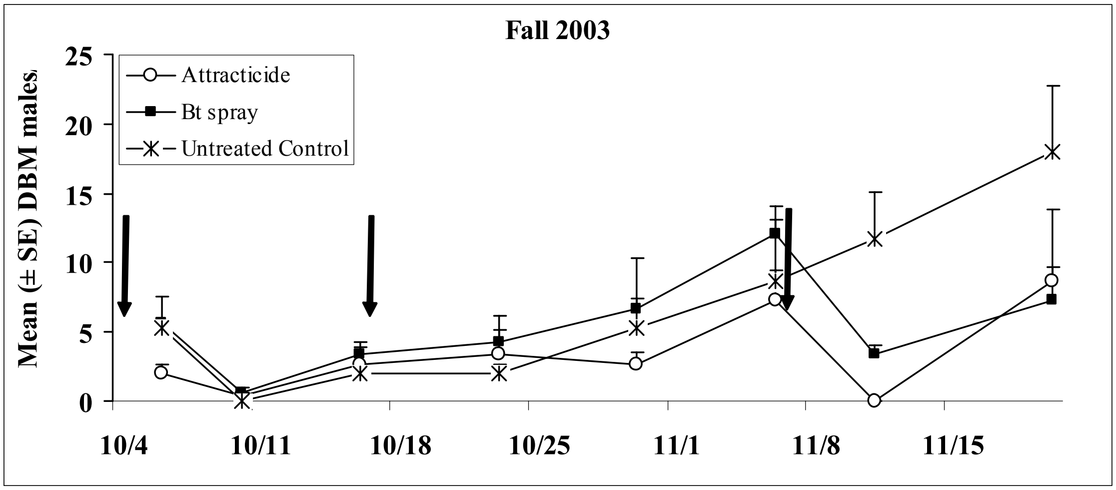

Figure 7.a. Mean (± SE) trap capture of diamondback moth (DBM), *Plutella xylostella* in pheromone baited wing traps for each of three treatments fall 2003.

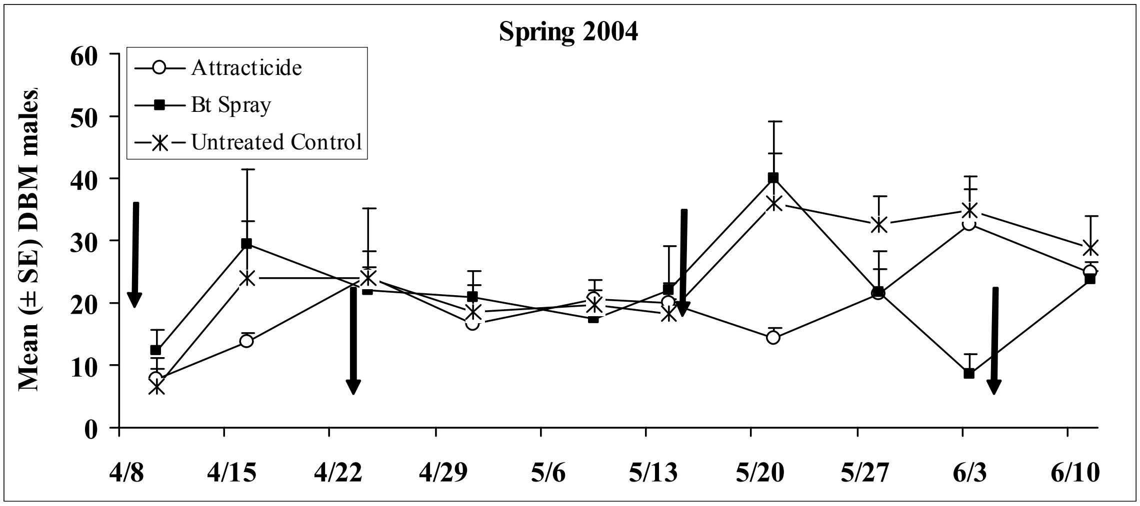

Figure 7.b. Mean (± SE) trap capture of diamondback moth (DBM), *Plutella xylostella* in pheromone baited wing traps for each of three treatments spring 2004.

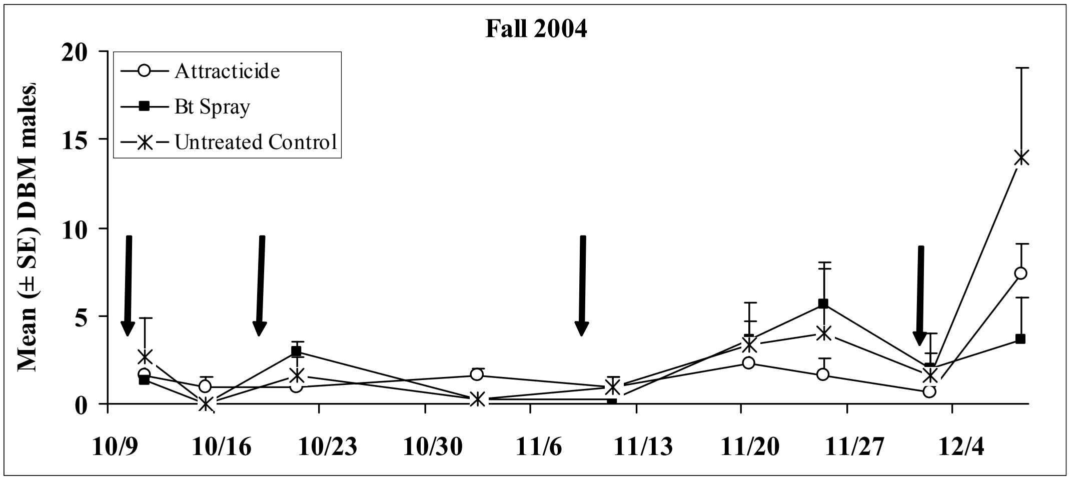

Figure 7.c. Mean (± SE) trap capture of diamondback moth (DBM), *Plutella xylostella* in pheromone baited wing traps for each of three treatments fall 2004.

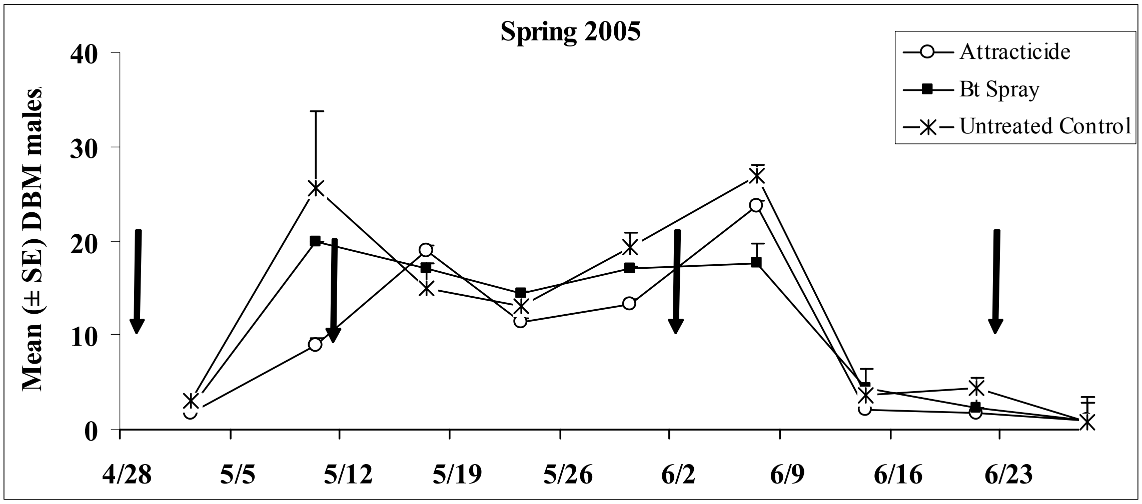

Figure 7.d. Mean (± SE) trap capture of diamondback moth (DBM), *Plutella xylostella* in pheromone baited wing traps for each of three treatments spring 2005.

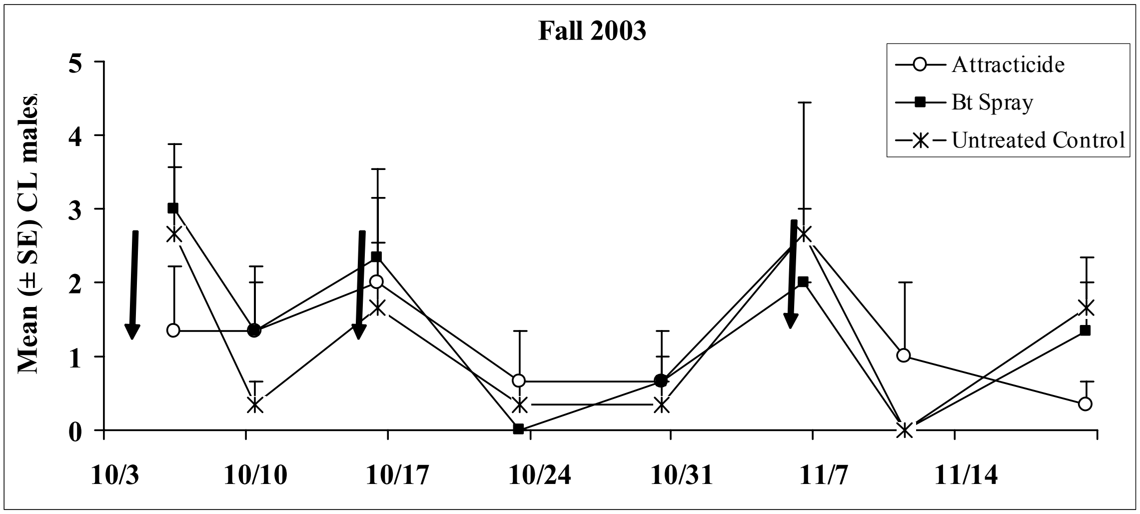

Figure 8.a. Mean (± SE) trap capture of cabbage looper (CL), *Trichopulsia ni* in pheromone baited wing traps for each of three treatments during fall 2003.

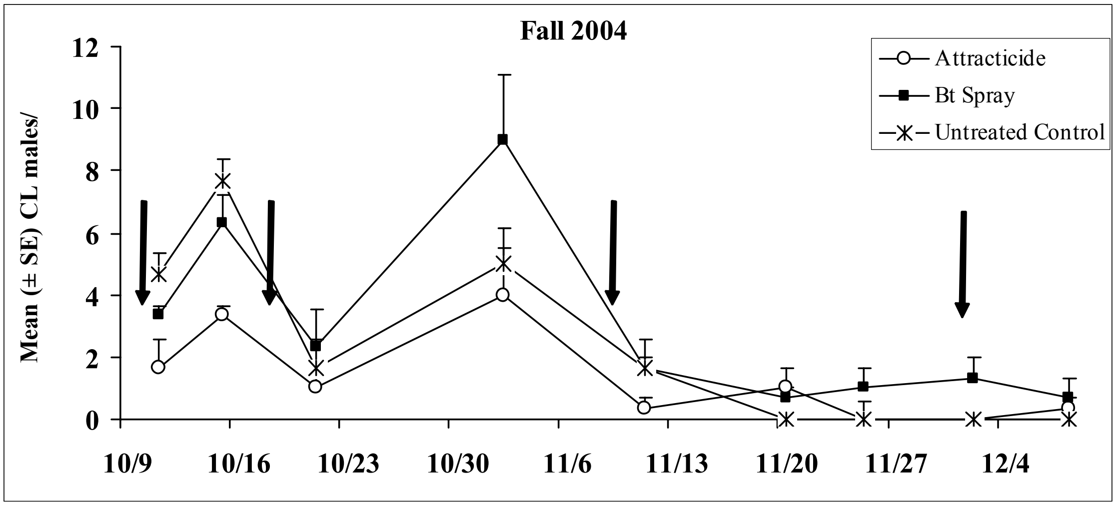

Figure 8.b. Mean (± SE) trap capture of cabbage looper (CL), *Trichopulsia ni* in pheromone baited wing traps for each of three treatments during spring 2004.

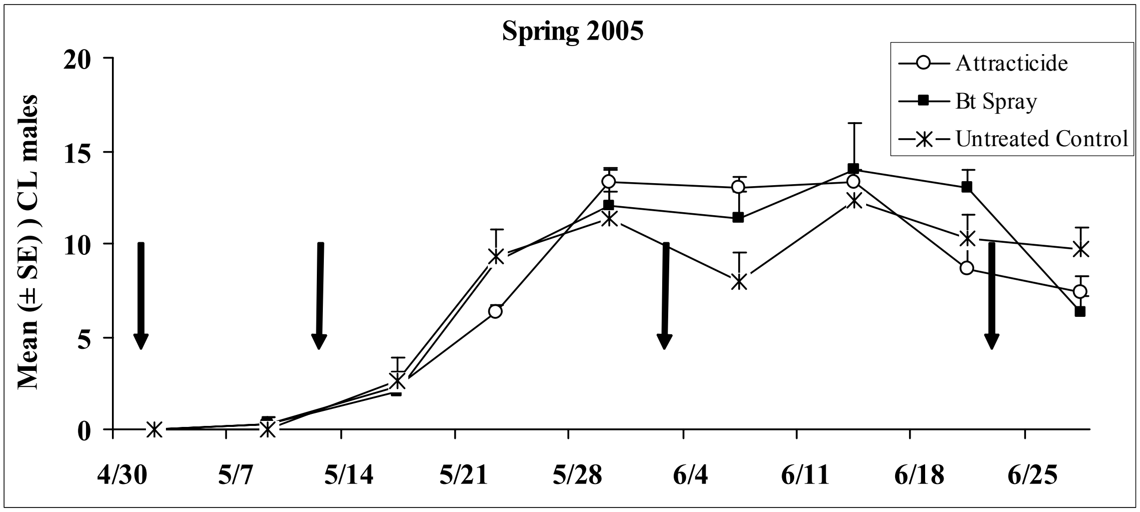

Figure 8.c. Mean (± SE) trap capture of cabbage looper (CL), *Trichopulsia ni* in pheromone baited wing traps for each of three treatments during spring 2005.

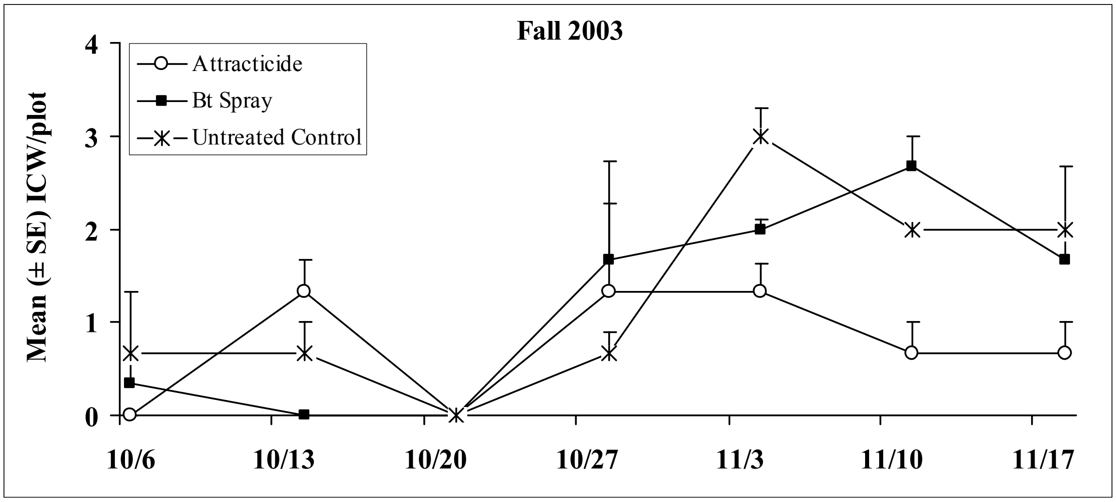

Figure 9.a. Mean (± SE) number of adult imported cabbageworm (ICW), *Pieris rapae* counted during 5-min observation period in untreated and treated plots during fall 2003.

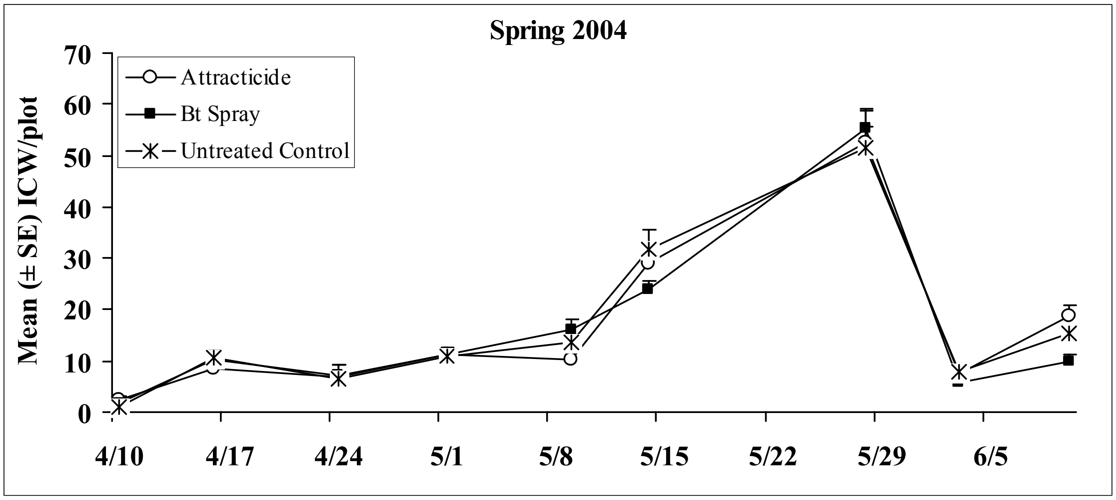

Figure 9.b. Mean (± SE) number of adult imported cabbageworm (ICW), *Pieris rapae* counted during 5-min observation period in untreated and treated plots during spring 2004.

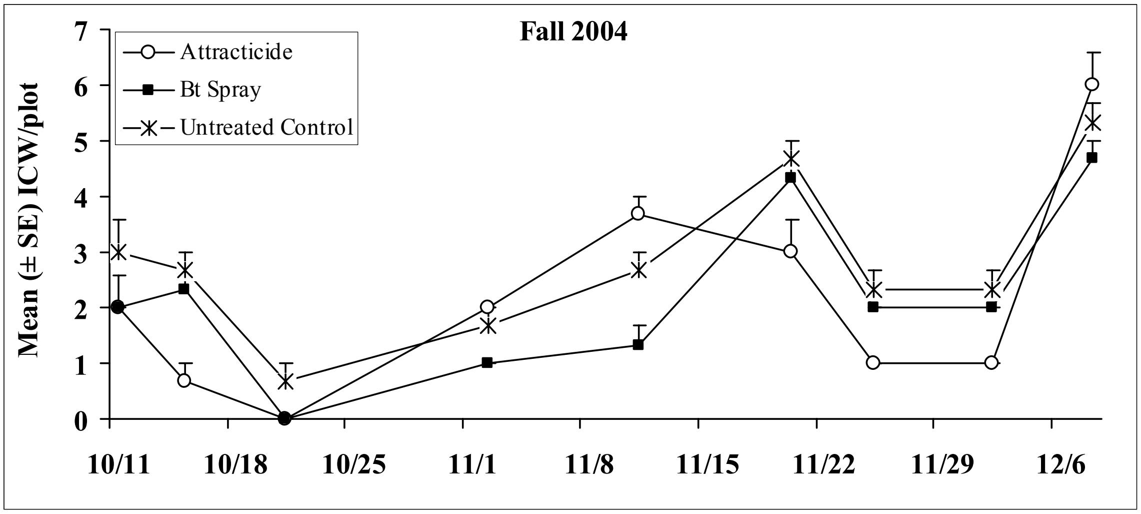

Figure 9.c. Mean (± SE) number of adult imported cabbageworm (ICW), *Pieris rapae* counted during 5-min observation period in untreated and treated plots during fall 2004.

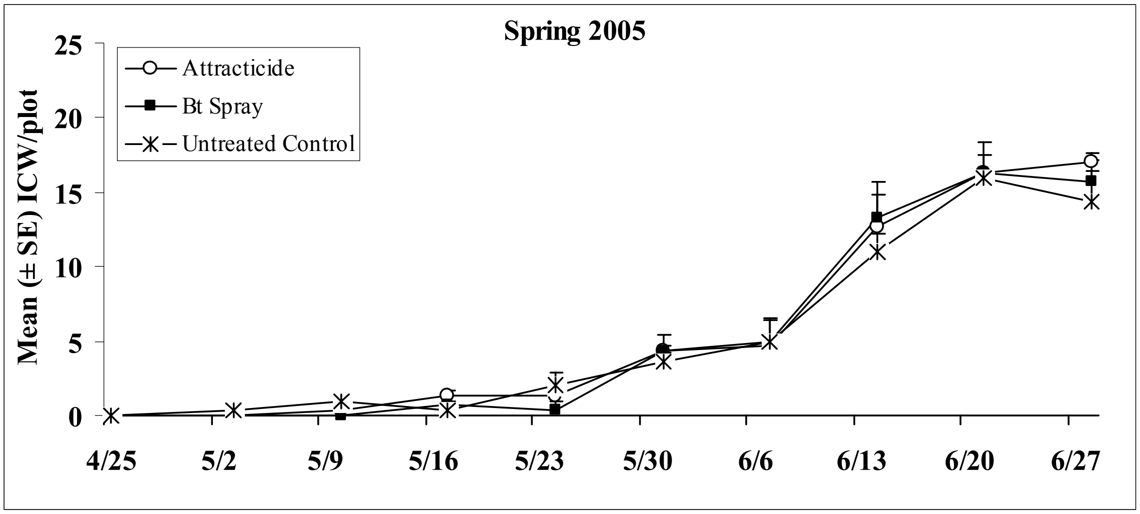

Figure 9.d. Mean (± SE) number of adult imported cabbageworm (ICW), *Pieris rapae* counted during 5-min observation period in untreated and treated plots during spring 2005.

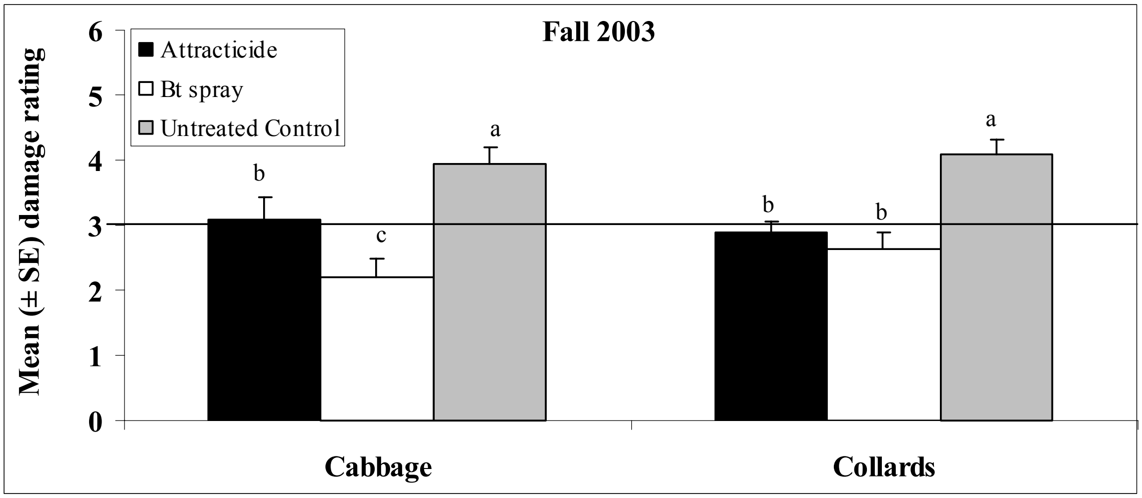

Figure 10.a. Mean (± SE) damage ratings of plants harvested from plots of each of three treatments in fall 2003. Line indicates marketability threshold of 3 above which produce is considered unmarketable (Leibee 1995). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

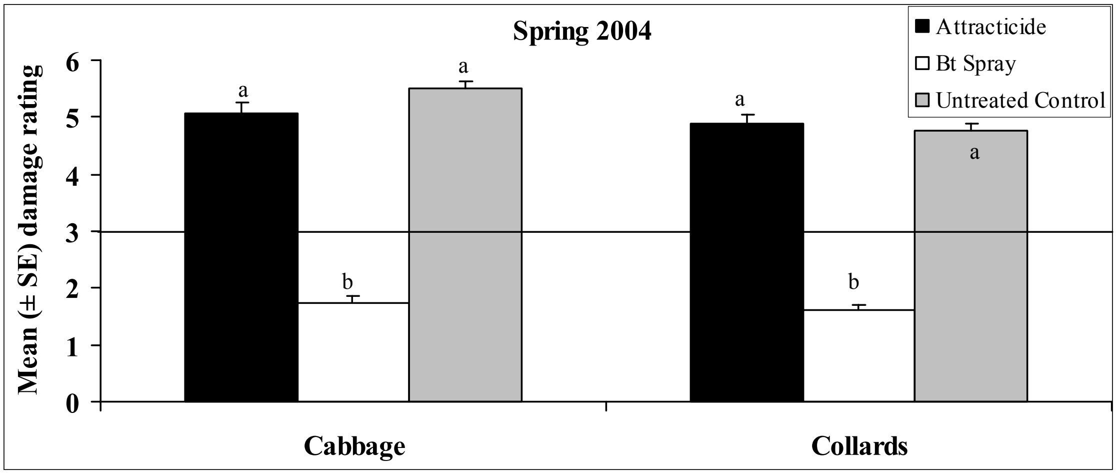

Figure 10.b. Mean (± SE) damage ratings of plants harvested from plots of each of three treatments in spring 2004. Line indicates marketability threshold of 3 above which produce is considered unmarketable (Leibee 1995). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

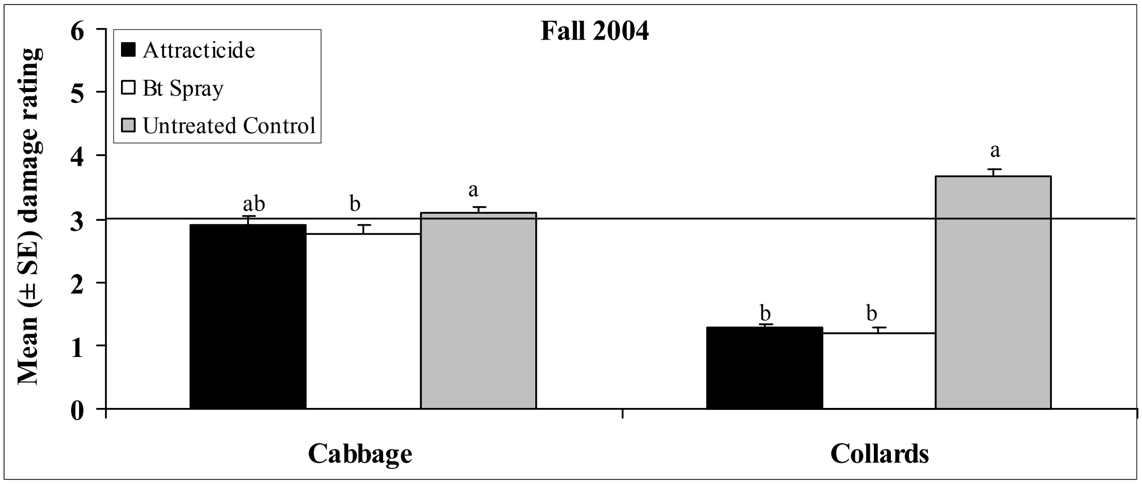

Figure 10.c. Mean (± SE) damage ratings of plants harvested from plots of each of three treatments in fall 2004. Line indicates marketability threshold of 3 above which produce is considered unmarketable (Leibee 1995). Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

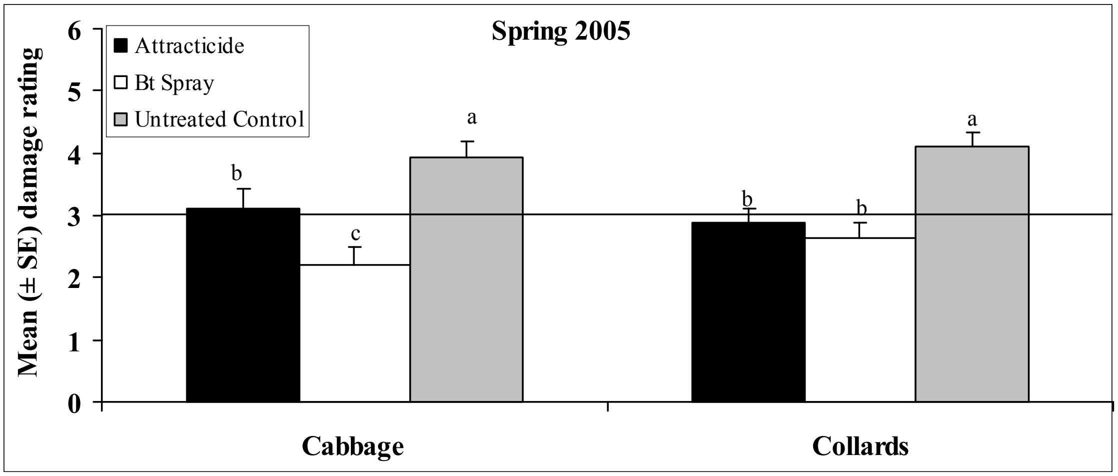

Figure 10.d. Mean (± SE) damage ratings of plants harvested from plots of each of three treatments in spring 2005. Line indicates marketability threshold of 3 above which produce is considered unmarketable (Leibee 1995). Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

CHAPTER IV :

EVALUATION OF SEVERAL REDUCED-RISK INSECTICIDES IN COMBINATION WITH AN ACTION THRESHOLD FOR MANAGING LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

4.1 Introduction

Cole crops, *Brassica oleracea* (L.), including cabbage, collards, broccoli, kale, brussels sprouts, and cauliflower, are an important component of diets in many parts of the world.

Cabbage and collards are the key cole crops grown in Alabama. Growers in the state utilize both spring and fall plantings for both crops, and often grow them in rotation with other vegetables (Kemble 1999). The key lepidopteran pests of cole crops in Alabama include the diamondback moth, *Plutella xylostella* (L.), imported cabbageworm, *Pieris rapae* (L.), and cabbage looper, *Trichoplusia ni* (Hübner) (Kemble 1999). *Plutella xylostella* and *P. rapae* are often the most abundant pests in many parts of Alabama, while infestations of *T. ni* are sporadic in nature (personal observation).

Caterpillars of the three lepidopteran species do direct damage to the marketable part of the plant by chewing holes in the foliage and producing frass (Harcourt et al. 1955; Shelton et al. 1982; Talekar & Shelton 1993; Tabashnik 1994), and are usually managed as a single caterpillar complex (Mahr et al. 1993). Tolerance of damage from these caterpillars is extremely low, basically zero to trace amounts of insect damage or frass in the final product (Morisak et al. 1984). In order to avoid significant economic loss, vegetable producers have typically managed these pests using an expensive therapeutic approach involving calendar-based applications of conventional insecticides, including various organophosphate, carbamate, and pyrethroid formulations. For instance, approximately 30,000 pounds of insecticide active ingredient are utilized annually for collard production in Alabama (Williams & Dangler 1992). Excessive and indiscriminate use of conventional insecticides has resulted in the development of pest resistance to insecticides (Hines & Hutchison 2001; Liu et al. 2002).

Globally, formulated sprays of microbial insecticides such as *Bacillus thuringiensis* and spinosad have been widely used as an alternative to chemical insecticides. However, development of pest resistance to microbial insecticides has been reported in several locations. For instance, resistance to *Bacillus thuringiensis* subspecies *kursatki* have been detected in field populations of *P. xylostella* in various locations in the mainland U.S. (Mahr et al. 1993; Shelton et al. 1993; Tang et al. 1997), and in several other locations throughout the world including Hawaii, Malaysia, the Philippines, Japan, Central America, and Thailand (Talekar & Shelton 1993; Rueda & Shelton, 1995; Tabashnik 1997). Similarly, field populations of *P. xylostella* collected in Malaysia have been reported to show resistance to spinosad (Sayyed 2004). The problem of insecticide resistance is not limited to *P. xylostella*. Resistance to *B. thuringiensis* has been demonstrated in laboratory populations of *T. ni* (Estada & Ferre 1994) and in greenhouse populations in British Columbia (Janmaat & Myers 2003).

Traditionally, more attention has been paid to insecticide-based control programs than biological control for management of lepidopteran pests of cole crops (Taleker & Shelton 1993, Biever et al. 1994, Xu et al. 2004). Although successful integrated pest management (IPM) programs have been developed and implemented in many parts of the world (Biever et al. 1994), it appears that insecticide-based control will remain the major tactic for managing caterpillar pests of cole crops for the foreseeable future (Xu et al. 2004).

Over the past several years, numerous biologically-based insecticides with novel modes of action have been developed and shown to have a high level of efficacy on lepidopteran pests of cole crops (Eger & Lindenbery 1998; Liu and Sparks 1999, Hill & Foster 2000; Hines & Hutchison, 2001). These include microbial insecticides (e.g., several formulations of spinosad and *B. thuringiensis*) and insect growth regulators. These new materials are termed “reduced-risk insecticides” because of their narrow spectrum of activity and low toxicity to humans and non-target organisms, and are considered IPM-compatible. Although reduced-risk insecticides are increasingly being used by vegetable growers worldwide, little has been done to evaluate these materials in Alabama. The objective of this study was to evaluate the efficacy of several reduced-risk insecticides against lepidopteran pests of cole crops in Alabama. The materials evaluated included three formulations of *B. thuringiensis* (Dipel®, XenTari®, and Dipel+XenTari mixture) (Valent Biosciences Libertyville, IL), Entrust® (Dow AgroSciences, Indianapolis, IN), and Novaluron (Crompton (now Chemtura), Middlebury, CT). Dipel® is a formulation of *B. thuringiensis* subspecies *kursatki* and is the most commonly used microbial insecticide on Alabama vegetable crops (Joseph Kemble, personal communication). XenTari® is a formulated spray of *B. thuringiensis* subspecies *aizawai*, while Dipel+XenTari is a premixed test formulation consisting of both subspecies of *B. thuringiensis*. Entrust® is a naturalyte insect control product formulated for the organic grower. The active ingredient, spinosad, is developed from a fermentation by-product of the soil-borne actinomycete bacterium, *Saccharopolyspora spinosa* (Liu et al. 1999). Novaluron is an insect growth regulator (IGR) that works by inhibiting chitin synthesis. It is currently labeled in the U.S. as Diamond® for use on cotton and Rimon® for use on apples, potatoes and sweet potato, and the registrant plans to label Novaluron for use on cole crops in the near future (K. Griffith, personal communication). These materials were evaluated over multiple field seasons (2004-2005) in central Alabama.

4.2 Materials and Methods

4.2.1 *General Methodology*

This research was conducted over three growing seasons; spring 2004, fall 2004, and spring 2005 at the E.V. Smith Agricultural Research Station in Shorter, AL. Treatments were arranged in a randomized complete block design with three replicates in each spring season and four in the fall 2004 season. All seedlings were obtained from a nursery in western Georgia (Lewis Taylor Farms; Ty Ty, Georgia) and were planted bareground following a preseason fire ant (*Solenopis invicta*) treatment with Amdro® (active ingredient = hydramethylnon, BASF Corporation, Research Triangle Park, NC). Standard field preparation and crop production practices (i.e., irrigation) were used to establish cabbage or collard plants in all three field seasons.

In spring of 2004 ‘Bravo’ cabbage was mechanically transplanted on 30 March 2004. Plots were 13.7 m by 9.1 m with plants spaced 45 cm apart within a row and 90 cm between rows for a total of 300 plants per plot. Plots were separated by a 15.2 m alley. The following four reduced-risk insecticides were compared: Dipel®, Xentari®, Dipel+Xentari combination, and Entrust®. In fall 2004, ‘Top bunch’ collards were mechanically transplanted 2 October 2004. Plots consisted of two 10–m rows, 100 cm apart with plants spaced 45 cm apart within a row and 90 cm between rows for a total of 40 plants per plot. Five reduced-risk insecticides were compared: Dipel®, Xentari®, Dipel+Xentari combination, Entrust®, and Novaluron. In spring 2005, ‘Vates’ collards were mechanically transplanted at the E.V. Smith Agricultural Research Station on 22 April 2005. The plot dimensions and treatments evaluated were as described for fall 2004.

Plots were evaluated weekly for pest infestation by sampling ten randomly selected plants per plot for larvae of *P. xylostella, P. rapae, and T. ni.* Eggs and pupae of the three species were also sampled. The number of immatures of each species was calculated by summing the number of larvae and pupae. Treatment applications were made only when larval counts exceeded a threshold of 0.5 cabbage looper equivalents (CLE) per plant (Shelton et al., 1982; 1983). The CLE method accounts for the varying levels of feeding damage caused by the three species. In this method, 1 CLE = 20 *P. xylostella* larvae = 1.5 *P. rapae* larva = 1 *T. ni* larva (Shelton et al., 1982; 1983). In addition, plants were also sampled for aphids (number of plants with aphid infestation) and key non-target predatory insects in our fields, mainly spiders and lady beetle adults (Coccinelidae). Treatment applications were made with a CO_2 pressurized backpack sprayer using a 3-ft boom with 3 nozzles calibrated to deliver about 25 gpa at 40 psi. Insecticides were applied at the recommended rates. Dipel®, Xentari®, and Dipel+Xentari were applied at the rate of 1 pound per acre, Entrust® at 2 oz per acre, and Novaluron applied at the rate of 12 fluid ounces per acre. Using the action threshold of 0.5 CLE, the average number of insecticide applications varied by treatment and season and ranged from 1.3 to 5 applications per season (Table 4.1).

At harvest, ten plants were randomly selected from each plot and rated for caterpillar feeding damage and marketability was quantified using the method of Greene et al. (1969). In this method cabbage plants grown in spring 2004 were rated based on insect feeding damage on a scale of 1 to 6 as follows: 1 = no apparent insect damage on head or inner wrapper leaves; 2 = no head damage, but minor feeding on wrapper leaves with 0-1% leaf area consumed; 3 = no damage on head, but moderate feeding damage on wrapper leaves with 2-5% leaf area consumed; 4 = minor feeding on head (but no feeding through outer head leaves), but moderate feeding on wrapper or outer leaves with 6-10% leaf area consumed; 5 = moderate to heavy feeding damage on wrapper and head leaves and a moderate number of feeding scars on head with 11-30% leaf area consumed; and 6 = severe feeding damage to head and wrapper leaves with heads having numerous feeding scars with ≥ 30% leaf area consumed (Greene et al. 1969). A similar method was used to assess marketability of collards in fall 2004 and spring 2005 with damage rating based solely on the percent of leaf area consumed (since collards is not a head-producing plant). A damage rating of ≤ 3 is considerable marketable under normal conditions, whereas a damage rating of ≤ 4 is marketable under exceptional market conditions (Leibee et al., 1995).

4.2.2 *Statistical Analysis*

For each season, mean seasonal larval and immature counts of each lepidopteran species, number of plants with aphid infestation, numbers of key non-target beneficial arthropods (i.e., spiders and lady beetle adults), and mean damage rating at harvest were calculated for each treatment. Data were transformed using the square-root method $\sqrt{(x + 0.5)}$ and analyzed for significant treatment effects by using analysis of variance (ANOVA) with the plots considered as blocks. Means were compared using the Tukey-Kramer HSD comparison for all pairs (JMPIN Version 4.0.2, SAS Institute Inc., 1988). Significant differences were established at the 95% confidence level ($P < 0.05$).

4.2 Results

Infestation levels of the three lepidopteran pests varied with growing season. Moderate to high populations of *P. xylostella* and *P. rapae* were recorded during all three field seasons, while *T. ni* population was recorded only in spring 2005. In general, relatively higher populations of the lepidopteran pests were recorded in both spring seasons compared with the fall season. This was also reflected in the number of applications per insecticide treatment made during each season which averaged 3.2, 1.3, and 4.3 for spring 2004, fall 2004, and spring 2005, respectively (Table 4.1). In both spring seasons, caterpillar pest pressure as measured by CLE per plant per week in untreated control plots began two weeks after planting and moderate caterpillar pressure was observed through harvest in spring 2004 (Fig. 11). Extremely high caterpillar pressure was recorded late in spring 2005 with CLEs greater than 3.5 per plant per week recorded in the last two weeks of the season (Fig. 11). In the lone fall season (fall 2004), however, caterpillar pest infestation did not commence until six weeks after planting averaging less than 0.5 CLE per plant per week for the remainder of the season (Fig. 11). In general, no significant block (plot) effects were detected ($P > 0.005$) for any of the dependent variables in any of the seasons, suggesting that the plots were similar in pest abundance and treatment efficacy.

In spring 2004, all four reduced-risk insecticides resulted in significant reductions in the number of *P. xylostella* larvae ($F = 9.5$, df = 4, $P = < 0.0001$) and immatures ($F = 8.9$, df = 4, $P = < 0.0001$), and *P. rapae* larvae ($F = 3.3$, df = 4, $P = < 0.0001$) and immatures ($F = 20.3$, df = 4, $P = < 0.0001$) compared with the untreated control (Fig. 12A). However, significantly higher numbers of *P. rapae* immatures were recorded for Dipel® compared with the other insecticide treatments. Significantly higher damage ratings were recorded in untreated control plots than in any of the treatments ($F = 65.3$, df = 4, $P = < 0.0001$; Fig. 13A). Comparing the treatments, mean damage ratings were significantly lower in Entrust® than in Dipel+Xentari combination. No significant effects of insecticide treatments were recorded in the number of plants with aphids ($F = 0.3$, df = 4, $P = 0.89$), and in the numbers of spiders ($F = 0.7$, df = 4, $P = 0.62$) or lady beetles ($F = 1.2$, df = 4, $P = 0.30$) found per plant (Fig. 14A).

In fall 2004, a significant treatment effect was recorded for *P. xylostella* larvae ($F = 2.3$, df = 5, $P = 0.04$). However, only Entrust® resulted in significant reduction in *P. xylostella* larvae compared with the untreated control; no significant differences were recorded for the other treatments (Fig. 12B). With the exception of Dipel®, all treatments significantly reduced *P. xylostella* immatures ($F = 4.4$, df = 5, $P = 0.0006$) and *P. rapae* larvae ($F = 5.3$, df = 5, $P < 0.0001$). Nonetheless, a significantly higher density of *P.*

rapae immatures was recorded in the untreated control than in any of the treatments (F = 11.3, df = 5, $P < 0.0001$). All five treatments had significantly lower mean damage ratings in comparison with the untreated control (F = 38.7, df = 5, $P < 0.0001$; Fig. 13B). No significant effects of treatments were recorded in the number of plants with aphids (F = 1.8, df = 4, P = 0.10), and in the numbers of spiders (F = 1.5, df = 4, P = 0.20) or lady beetles (F = 0.7, df = 4, P = 0.62) found per plant (Fig. 14B).

In spring 2005, *T. ni* was collected in the field, whereas it was not present during the previous two seasons (Fig. 12C). In general, all treatments resulted in significant reductions in pest populations (Fig. 12C). All treatments except Dipel® significantly reduced densities of *T. ni* larvae (F = 3.3, df = 5, P = 0.006) and immatures (F = 3.7, df = 5, P = 0.003) compared with the untreated control (Fig. 12C). For *P. xylostella,* significantly lower numbers of larvae (F = 8.1, df = 5, $P < 0.0001$) and immatures (F = 9.7, df = 5, $P < 0.0001$) were recorded for all treatments compared with the untreated control. Similar significant treatment effects were recorded for *P. rapae* larvae (F = 3.9, df = 5, P = 0.002) and immatures (F = 4.1, df = 5, P = 0.001); however *P. rapae* larval counts in plots treated with the Dipel+Xentari formulation were not significantly lower than larval counts in untreated control plots (Fig. 12C). A mean damage rating of 5.4 was recorded in the untreated control which was significantly higher (F = 101.4, df = 5, $P <$ 0.0001) than damage ratings in any of the five treatments (Fig. 13C). In all three seasons, mean damage ratings recorded in the treated plots were never above the marketability threshold of 3 (Green et al. 1969). No significant differences were recorded among the treatments in the number of plants with aphids (F = 0.26, df = 4, P = 0.93), numbers of spiders per plant (F = 1.2, df = 4, P = 0.30), and numbers of lady beetles per plant (F =

0.8, df = 4, *P* = 0.55) (Fig. 14C), suggesting little or no effects of insecticide treatments on the key non-target predators in our plots.

4.3 Discussion

The goal of this study was to evaluate the efficacy of various reduced-risk insecticides in providing acceptable control of lepidopteran pests of cole crops in Alabama. In all three seasons, all materials tested resulted in the production of marketable produce with considerably lower pest pressure and crop damage ratings compared with untreated control plots which never yielded marketable produce. These results indicate that all five reduced-risk insecticides were effective in controlling lepidopteran pests of cole crops in Alabama. The results also suggest that the 0.5 CLE action threshold recommended by Shelton et al. (1982, 1983) can be used to produce marketable cabbage and collards in Alabama with only minimal applications of reduced-risk insecticides, particularly in locations with minor or no endemic populations of *T. ni*. Although resistance evaluation was not the primary goal of this study, our results confirming the high efficacy of the various microbial insecticides tested in this study may suggest that *P. xylostella* resistance to *B. thuringiensis* is currently not a major problem in central Alabama, considering that vegetable growers in this region have been applying Dipel® in their fields for years.

Although we did not always find significant differences among the reduced-risk insecticides tested in this study, Entrust® consistently produced the lowest mean damage ratings (although not always significant) with the least mean number of applications per season (Table 4.1). The relatively higher efficacy of Entrust® recorded in this study may be due to its broad spectrum of activity and multiple mode of entry. Entrust® differs from the other materials evaluated in this study in that it successfully kills insects from several orders, whereas the other treatments are selective to lepidopterans only (Cisneros 2002). In addition, spinosad, the active ingredient in Entrust® has both contact and ingestion activity (Eger & Lindenber 1998; Liu et al. 1999), whereas the other reduced-risk insecticides must be eaten by the insects in order to be effective. It is thought that the broad spectrum activity of Entrust® will probably ensure some control of non-lepidopteran pests such as cruciferous flea beetles, harlequin bugs, aphids, and other minor pests that the other chemicals were not effective against. However, we did not observe in the current study a significant reduction in aphid-infested plants in Entrust®-treated plots compared to the other treatments or control. On the other hand, spinosad has also been reported as toxic to beneficial insects such as *Diadegma insulare* (Cressons) (Hymenoptera: Ichneumonidae) (Xu et al. 2004), a very common and effective parasitoid of *P. xylostella* in North America (Mahr et al. 1993). Hill & Foster (2000) showed a 100% *D. insulare* mortality rate after 8 hours of exposure to spinosad-treated brassica leaves, while Cisneros et al. (2002) recorded up to 98% mortality of predators exposed to high concentrations of this microbial insecticide. However, we did not record any significant effect of Entrust® or any of the other treatments on numbers of spiders and lady beetles, the two most important predators in our fields. Entrust® thus appears to be a promising tool for use in cole crop pest management and insecticide resistance management programs, considering that the active ingredient, spinosad has not been reported to share cross-resistance mechanisms with any other group of insecticides (Liu & Yue 2000, Wei et al. 2001). In general, Xentari® was second to Entrust® in producing acceptable damage ratings. However, the fact that this material had the highest average number of applications per season suggests that it may not provide economically acceptable control compared to the other treatments.

Significant variations in the populations of the three lepidopteran pests were recorded from season to season. In general, lepidopteran pest pressure was higher in both spring seasons than in the fall. Significant *P. xylostella* pressure was recorded in both spring seasons and in the fall, whereas *P. rapae* pressure was highest in spring 2004 followed by spring 2005. Furthermore, we recorded during spring 2004 ~ 60 flying *P. rapae* adults per plot in 5-min visual observations compared to ~ 8 flying adults in fall 2004, suggesting that this pest may be more severe in the spring than in the fall. The detection of *T. ni* in spring 2005 may have exacerbated total pest pressure during this season resulting in above threshold CLEs and the need to apply insecticides at a much higher frequency than in the first two seasons. This is especially likely since *T. ni* is the most voracious and damaging of the three pests (Shelton et al. 1982; Hines & Hutchison 2003). The reason for the detection of *T. ni* only in spring 2005 may be due to later planting date for this season. In summary, our results confirmed the efficacy of the tested reduced-risk insecticides in managing direct pests of cole crops in Alabama in a threshold-based IPM program. These reduced-risk insecticides offer a wide range of pest management options available to vegetable growers and should be used wisely or in rotation with one another to minimize selection for resistance to any one given material. Obviously, the longevity of these new insecticides as effective IPM tools will depend on their judicious use, compatibility with natural enemies, and cost effectiveness, among other factors.

Table 4.1. Mean number of applications of each reduced-risk insecticide treatment per plot during each season. Treatment applications were made only when weekly larval counts exceeded a threshold of 0.5 cabbage looper equivalents (CLE) per plant.

Treatment/ Formulation	Spring 2004	Fall 2004	Spring 2005
Dipel DF	2.67 ± 0.19	1.50 ± 0.14	4.33 ± 0.19
Xentari DF	2.33 ± 0.19	1.25 ± 0.13	5.00 ± 0.00
Dipel+Xentari DF	2.67 ± 0.19	1.25 ± 0.13	4.67 ± 0.19
Entrust 80WP	2.33 ± 0.19	1.25 ± 0.13	3.67 ± 0.29
Novaluron	---	1.25 ± 0.13	4.00 ± 0.00

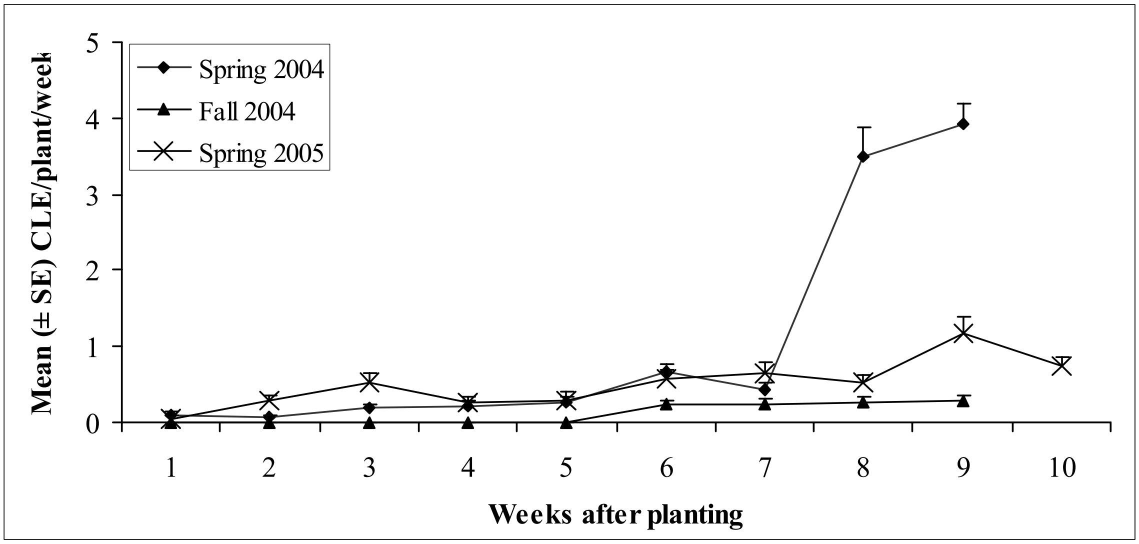

Figure 11. Caterpillar pressure expressed as mean (± SE) number of cabbage looper equivalents (CLE) per plant recorded weekly after planting in untreated control plots during spring 2004, fall 2004, and spring 2005. Planting dates for spring 2004, fall 2004, and spring 2005 were March 30 2004, October 2 2004, and April 22 2005, respectively.

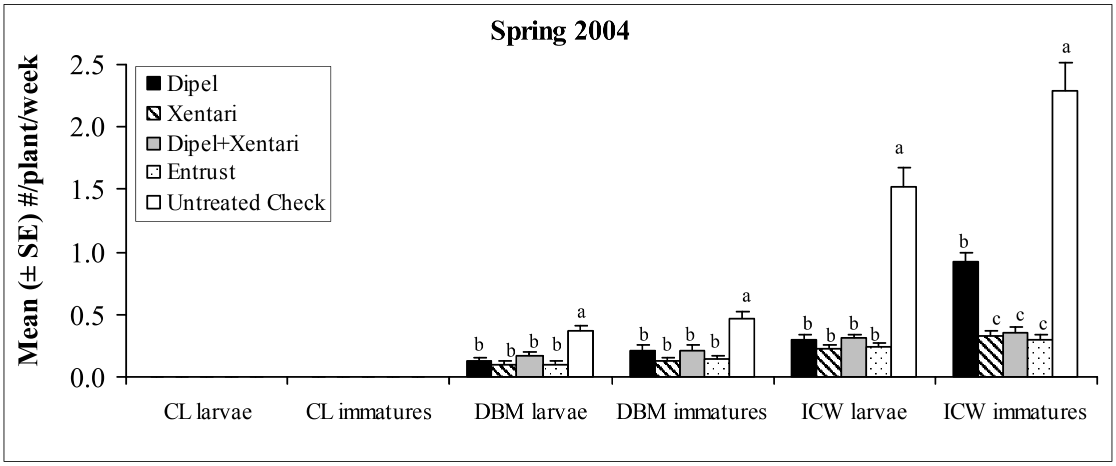

Figure 12.a. Seasonal mean (± SE) number of larvae and immatures of lepidopteran species sampled per plant per week in plots treated with different reduced-risk insecticides during spring 2004. Key: CL = cabbage looper (*Trichopulsia ni*); DBM = diamondback moth (*Plutella xylostella*); ICW = imported cabbageworm (*Pieris rapae*). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

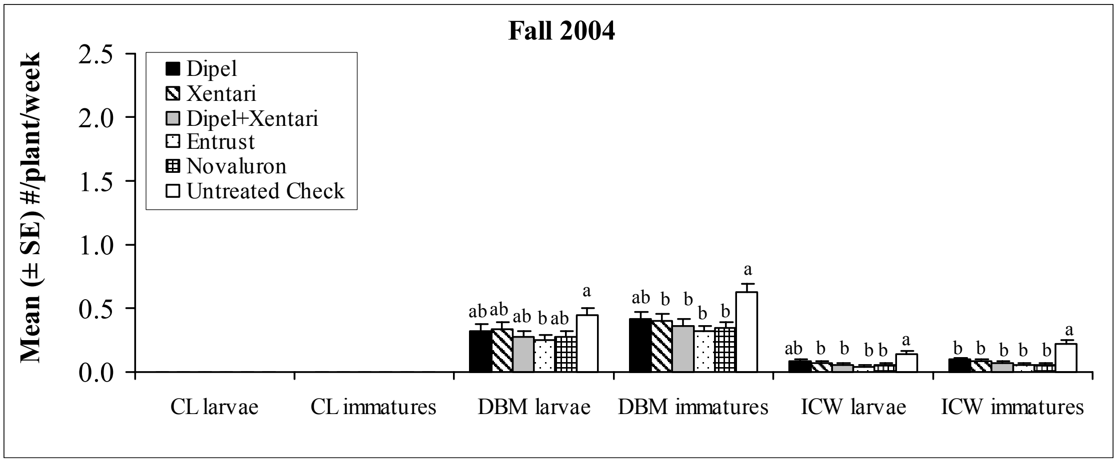

Figure 12.b. Seasonal mean (± SE) number of larvae and immatures of lepidopteran species sampled per plant per week in plots treated with different reduced-risk insecticides during fall 2004. Key: CL = cabbage looper (*Trichopulsia ni*); DBM = diamondback moth (*Plutella xylostella*); ICW = imported cabbageworm (*Pieris rapae*). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

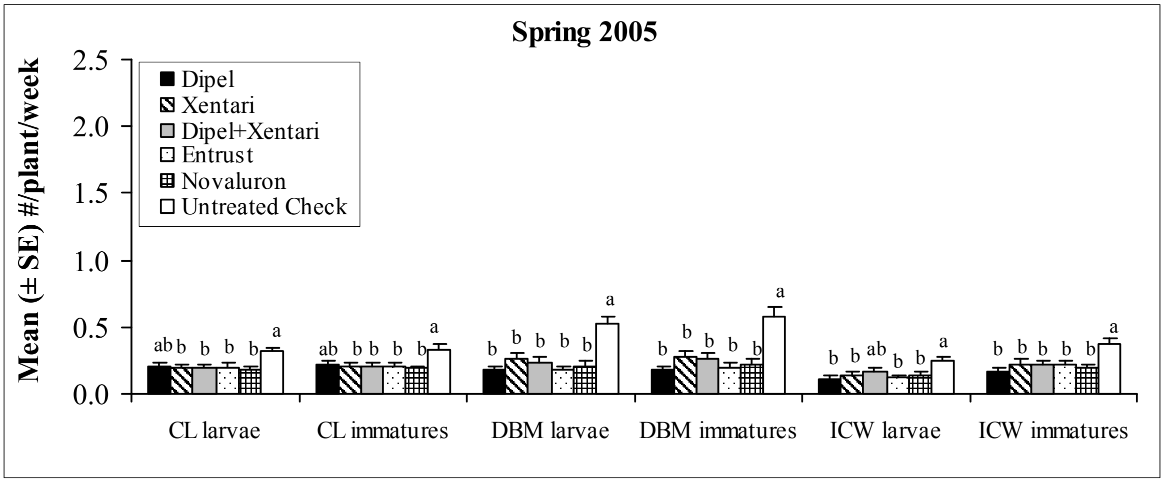

Figure 12.c. Seasonal mean (± SE) number of larvae and immatures of lepidopteran species sampled per plant per week in plots treated with different reduced-risk insecticides during spring 2005. Key: CL = cabbage looper (*Trichopulsia ni*); DBM = diamondback moth (*Plutella xylostella*); ICW = imported cabbageworm (*Pieris rapae*). Means followed by the same letter are not significantly different ($P < 0.05$, Tukey-Kramer HSD).

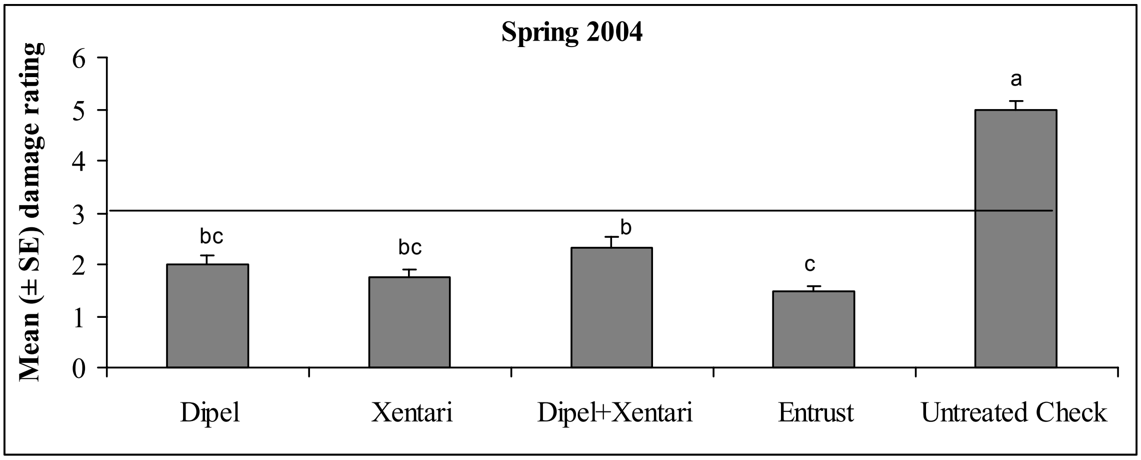

Figure 13.a. Mean (± SE) damage ratings of plants harvested from plots treated with different reduced-risk insecticides during spring 2004. Line indicates marketability threshold of 3 above which produce is considered unmarketable. Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

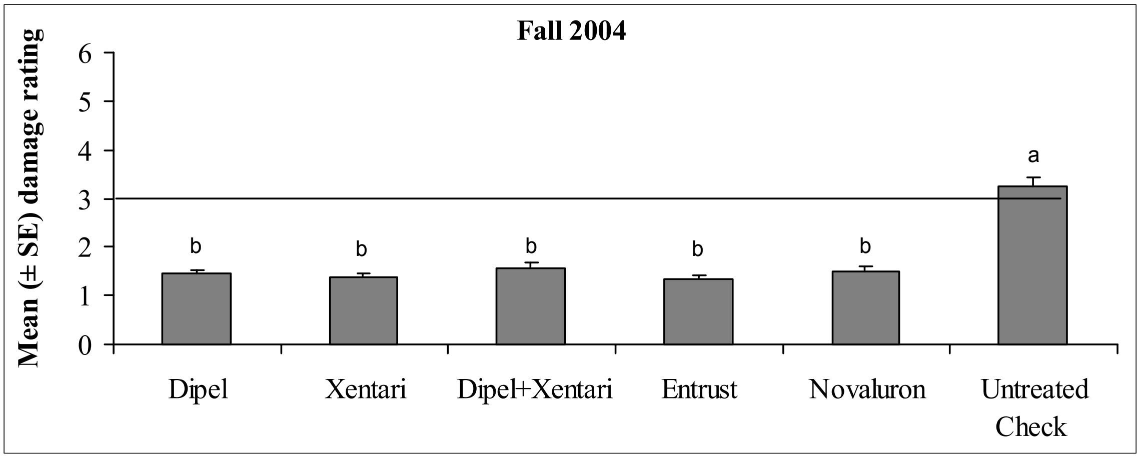

Figure 13.b. Mean (± SE) damage ratings of plants harvested from plots treated with different reduced-risk insecticides during fall 2004. Line indicates marketability threshold of 3 above which produce is considered unmarketable. Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

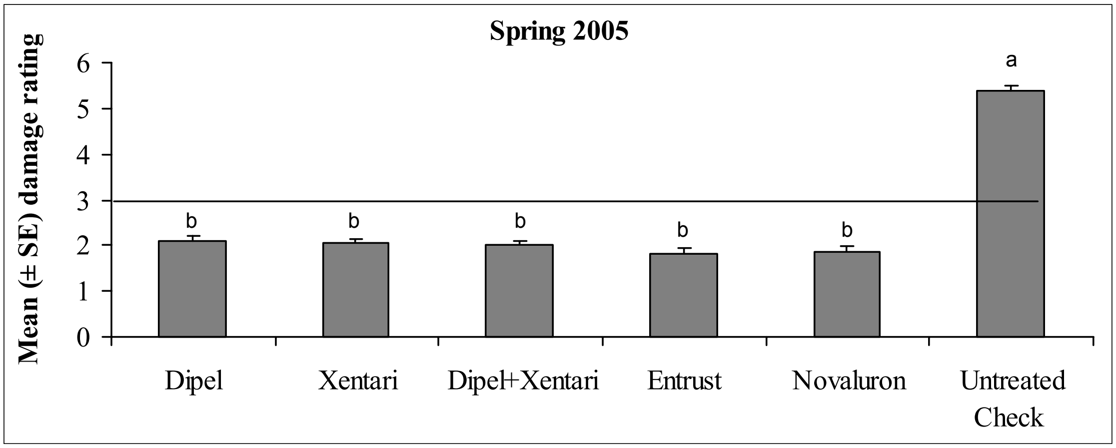

Figure 13.c. Mean (± SE) damage ratings of plants harvested from plots treated with different reduced-risk insecticides during spring 2005. Line indicates marketability threshold of 3 above which produce is considered unmarketable. Means followed by the same letter are not significantly different (*P* < 0.05, Tukey-Kramer HSD).

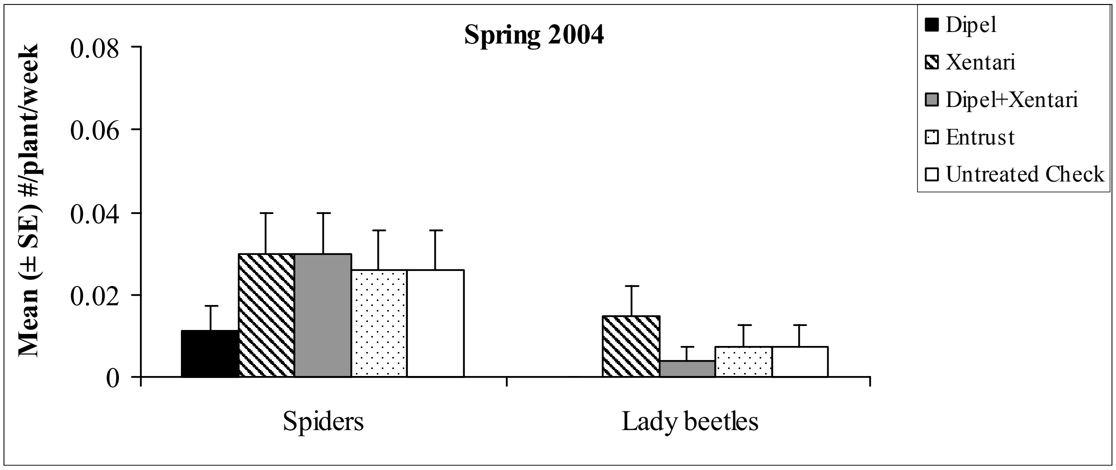

Figure 14.a. Seasonal mean (± SE) number of non-target spiders and lady beetle adults found per plant per week in plots treated with different reduced-risk insecticides during spring 2004. Means are not significantly different (*P* > 0.05, Tukey-Kramer HSD).

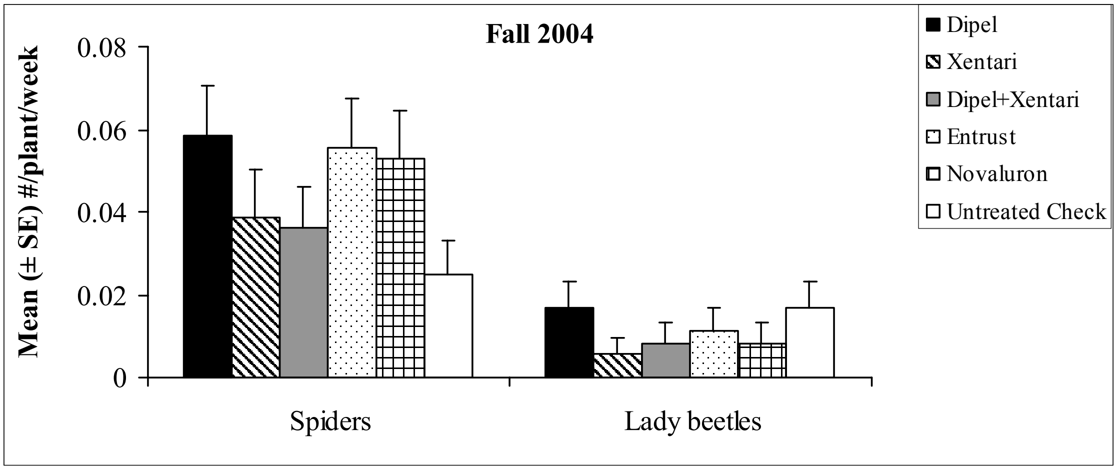

Figure 14.b. Seasonal mean (± SE) number of non-target spiders and lady beetle adults found per plant per week in plots treated with different reduced-risk insecticides during fall 2004. Means are not significantly different ($P > 0.05$, Tukey-Kramer HSD).

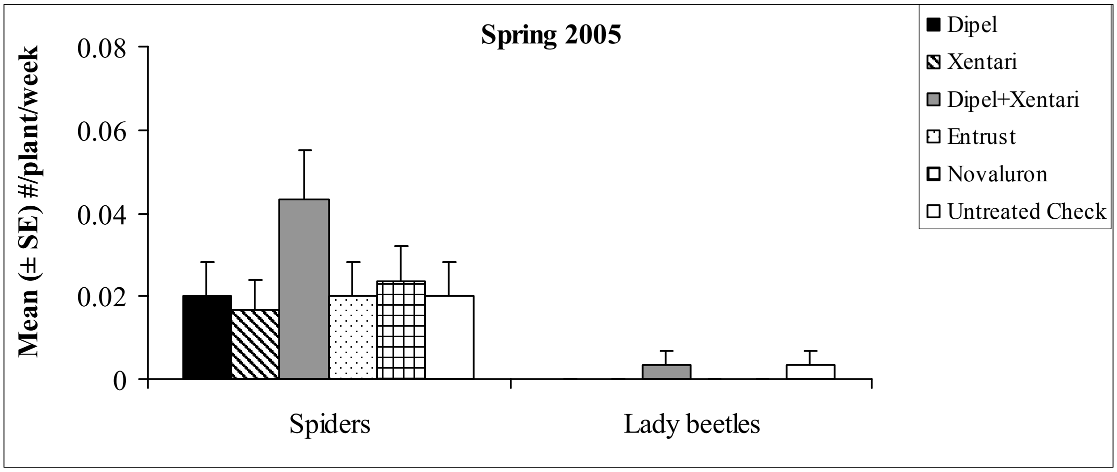

Figure 14.c. Seasonal mean (± SE) number of non-target spiders and lady beetle adults found per plant per week in plots treated with different reduced-risk insecticides during spring 2005. Means are not significantly different (*P* > 0.05, Tukey-Kramer HSD).

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