PREDICTING BOLL WEEVIL ERADICATION INDUCED PEST OUTBREAKS IN TEXAS COTTON

A Thesis

by

JAMES JOSEPH BUTLER

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

MASTER OF SCIENCE

August 2004

Major Subject: Entomology

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Approved as to style and content by:

Allen Knutson (Co-Chair of Committee)	Roel Lopez (Member)
Julio Bernal (Co-Chair of Committee)	Kevin Heinz (Head of Department)

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ABSTRACT

Predicting Boll Weevil Eradication Induced Pest Outbreaks in Texas Cotton.

(August 2004)

James Joseph Butler, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Allen Knutson
Dr. Julio Bernal

The boll weevil (*Anthonomus grandis grandis* Boheman) is currently under eradication in the U.S. The eradication program is implemented by means of area-wide applications of malathion ULV. Frequent applications of this insecticide result in high mortality of many beneficial insects, and a greater risk of secondary pest outbreaks. Notable among the latter are the outbreaks of beet armyworm (*Spodoptera exigua* Hübner) and cotton aphid (*Aphis gossypii* Glover) in the Lower Rio Grande Valley in 1995.

The present study (i) compared densities of beneficial and pest insect and spider populations between cotton fields in eradication and non-eradication areas; (ii) evaluated the use of beneficial cotton arthropod population densities as indicators of pest damage risks from cotton aphid, beet armyworm, bollworm (*Helicoverpa zea* Boddie), and other worms (*Estigmene acrea* Drury, *Pseudoplusia includens* Walker, and *Trichoplusia ni* Hübner); and (iii) evaluated the effects of malathion ULV on the red imported fire ant (*Solenopsis invicta* Buren) a key arthropod predator in cotton agroecosystems. Studies were conducted in central Texas, in the vicinities of College Station and Dallas, during 2002 and 2003.

Results showed that a majority of cotton predators were negatively impacted by malathion ULV applications. However, convergent lady beetle (*Hippodamia convergens* Guérin-Méneville) densities were greater in active eradication fields than inactive fields. Stepwise regression analyses identified densities of lacewing (*Chrysoperla carnea* Stephens) larvae and lady beetle larvae (*H. convergens*, *Coleomegilla maculata* De Geer, *Harmonia axyridis* Pallas, and *Coccinella septempunctata* L.) as predictors of cotton aphid density, and density of total spiders as predictors of bollworm density. Predictors of beet armyworm or other worm densities could not be determined. This study demonstrated malathion ULV was highly toxic to fire ants, and could repel ants from treated surfaces. Malathion ULV reduced the number of foraging fire ants in the cotton canopy for three weeks and reduced fire ant predation of beet armyworm eggs.

Predictors of secondary pest densities have been suggested which, if utilized, may help to prevent the occurrence of secondary pest outbreaks under boll weevil eradication. Validation of these predictors should be preformed before implementing them into an eradication program.

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CHAPTER I

INTRODUCTION

Background

The boll weevil (*Anthonomus grandis grandis* Boheman) has been a major pest of cotton in the U.S. since it was first reported near Brownsville, Texas, in 1892 (Haney 2001). Once established, the boll weevil rapidly spread across the southern U.S. By 1922 it had spread to the Atlantic coast, and by 1981 it reached California (TBWEF 2004). To date, the boll weevil has caused an estimated $22 billion in economic losses (Kaplan 2003). Consequently, much effort has been expended on boll weevil control including chemical control. Extensive use of insecticides such as calcium arsenate and pyrethroids led to problems such as increased cost of pest control and secondary pest outbreaks (Brazzel et al. 2001). The term secondary pest outbreak is used in this thesis in a narrow sense to include all pests except boll weevil, which is the target of eradication efforts based on ultra-low volume aerial malathion applications. Cotton fields treated with calcium arsenate early in the season had higher numbers of bollworms *Helicoverpa zea* (Boddie) late in the season than untreated cotton fields (Sherman 1930, Ewing and Ivy 1943). The high cost of boll weevil damage and the difficulty in controlling this pest led to a 1958 resolution at the National Cotton Council annual meeting that called for the development of technology "to eliminate the boll weevil as a pest of U.S. cotton at the earliest possible date." (Brazzel et al. 2001). The resolution led to the establishment of boll weevil eradication programs throughout the southern United States.

This thesis follows the style and format of the Journal of Economic Entomology.

Boll Weevil Eradication in Texas. The Texas Boll Weevil Eradication Foundation (hereafter referred to as TBWEF) was established in 1993 by the Texas Legislature and was charged with organizing and conducting boll weevil eradication in Texas (TBWEF 2004). TBWEF designated fourteen eradication zones within Texas. Eradication efforts in a zone are initiated after growers vote to participate. A total of thirteen zones have been involved in the eradication program since its inception. Twelve zones were active in the 2003 growing season (TBWEF 2004). The eradication program consists of three stages: diapause, full season, and maintenance (TBWEF 2004). All cotton fields are mapped and assigned a unique identification number that is used for the duration of the program. Diapause stage begins at the end of a growing season and involves repeated applications of malathion to all fields within a zone to reduce the number of boll weevils that enter diapause and overwinter. The Texas boll weevil eradication program utilizes ultra low volume applications of Fyfanon (Cheminova Inc., Wayne, NJ), an oil-based formulation containing 96.5% malathion (hereafter referred to as malathion ULV), to eradicate the boll weevil. The full season stage begins the following growing season after the diapause stage and involves season-long malathion ULV applications beginning in the spring to kill adult boll weevils emerging from over wintering habitats and continuing through the growing season to kill adults before they reproduce. Pheromone traps are placed around each field and checked once per week, and the number of captured boll weevils is recorded. Applications are made when an action threshold of two weevils per 16.2 ha (40 acres) is reached. Fields with boll weevil populations exceeding the threshold are aerially sprayed with malathion ULV at a rate of 876.9 ml/ha (12 oz/ac). In situations where aerial applications are not feasible, ground applications of 1169.2 ml/ha (16 oz/ac) are made using a mist blower mounted on a vehicle. Early in the full season stage many fields receive weekly applications, but after two or more years, boll weevil numbers typically decrease significantly, and consequently, so does the number of malathion ULV applications. The maintance stage begins when field sampling indicates no boll weevil reproduction is detected within a zone, at which time the zone is declared "functionally eradicated." Trapping continues during the maintenance stage to detect any resurgence or re-introduction of boll weevils in the eradicated zone (TBWEF 2004).

The Northern Blacklands zone in northeastern Texas has yet to implement the eradication program, while the eradication program in the adjacent Southern Blacklands zone began in 2001 with diapause spraying. While the program seeks to provide long term economic and environmental benefits (Brazzel et al. 2001), the risk of secondary pest outbreaks is of great concern. This is because area-wide repeated applications of malathion ULV directed against boll weevil are thought to reduce populations of beneficial insects that suppress other pests of cotton. The presence of an active zone adjacent to an inactive zone presents a unique opportunity to evaluate the effects of malathion ULV on cotton insect communities. This opportunity was exploited during the course of this research

Known Effects of Malathion on Selected Beneficial Insects. The Southern Blacklands zone consists of approximately 37,000 ha of cotton in a 65 county area. In 2003, fields within this eradication zone were treated with ULV malathion an average of 12.5 times during the season (TBWEF 2004). Frequent and repeated applications of malathion ULV over such large areas increase the risk of non-target effects on natural enemies. Adverse effects on predators and parasitoids can lead to outbreaks of secondary pests such as beet armyworms (*Spodoptera exigua* Hübner) and cotton aphids (*Aphis gossypii* Glover) (Doutt and Smith 1971, DeBach and Rosen 1991, Trichilo and Wilson 1993). A better understanding of the impact of ULV malathion on natural enemies is needed to anticipate pest outbreaks due to the disruption of biological control.

Toxicity of malathion to beneficial insects has been extensively studied in the laboratory. Predatory insects such as *Hippodamia convergens* (Guérin-Méneville) (Bartlett 1963, 1964, England 1997, Elzen et al. 1998), *Chrysoperla carnea* (Stephens) (Bartlett 1963, 1964, Elzen et al. 1998), and *Geocoris punctipes* (Say) (Elzen et al. 1998, Tillman and Mulrooney 2001) have all been found to be highly susceptible to malathion. Malathion was also found to be highly toxic to the parasitoids *Cotesia marginiventris* (Cresson) (England 1997, Tillman and Mulrooney 2001), *Bracon mellitor* (Say) (Tillman and Mulrooney 2001), and *Cardiochiles nigriceps* (Viereck) (Tillman and Williams 1997, Tillman and Mulrooney 2001). However, all of these studies were conducted under laboratory conditions. Area-wide field studies of the impact of malathion ULV on natural enemy communities have not been conducted and published in the scientific literature.

A wide range of predators have been found in Texas cotton including fire ants (Whitcomb and Bell 1964, Sterling et al. 1979). Predators and parasitoids play an important role in suppressing pest densities in cotton agroecosystems (Whitcomb 1980, Sterling et al. 1989, Kidd and Rummel 1997). Moreover, a complex of generalist predators including the red imported fire ants, lady beetles, and spiders are the major component of biological control of cotton pests (Sterling et al. 1989).

Fire Ants as Beneficial insects in Cotton. The red imported fire ant, *Solenopsis invicta* Buren (hereafter referred to as fire ant), has diverse roles in cotton fields, including predation of other insects (Fillman and Sterling 1983, Kaplan and Eubanks 2002, Diaz et al. 2004) and “tending” of cotton aphids by protecting them from other predators (Sterling et al. 1979, Reilly and Sterling 1983, Kaplan and Eubanks 2002, Eubanks et al. 2002). Fire ants prey upon lepidopteran eggs and caterpillars, including bollworm and beat armyworm (McDaniel and Sterling 1979, Lofgren 1986, Diaz et al. 2004). Fire ant densities in cotton canopies were lower in 2002, the first year of the eradication program, relative to the previous year’s density (Rodrigo Diaz, Texas A&M University, unpublished data). This suggested that malathion ULV may kill or repel fire ants, so that fewer ants foraged in the cotton canopy. However, no study on the impact of malathion ULV on fire ants in cotton canopies has been reported.

Effects of Malathion ULV Applications on Insect Pest Populations. Applications of insecticides for boll weevil eradication may have negative consequences on secondary pests. Two common secondary pests known to reach outbreak densities during boll weevil eradication include beet armyworm and cotton aphid (Stewart et al. 1996, Ruberson et al. 1994, Layton and Long 2001). A highly destructive beet armyworm outbreak occurred in 1995 during the first full season of boll weevil eradication in the Lower Rio Grande Valley of Texas. Summy et al. (1996) surveyed the region after the outbreak and found high densities of beet armyworm and other lepidopteran and homopteran pests, and lower densities of green lacewings in cotton fields in the eradication zone relative to cotton fields in the adjacent Lower Rio Grande Valley in Tamaulipas, Mexico, where early season spraying against cotton aphids and malathion ULV sprays against boll weevil did not occur. Ruberson et al. (1994) examined the numbers of applications to control beet armyworm in Georgia cotton fields from 1980 to 1992. They found that during the years of boll weevil eradication (1987-1990), there was a substantial increase in the number of insecticide applications against beet armyworm relative to other years. Layton and Long (2001) found that cotton aphid populations were considerably higher in areas entering the second year of boll weevil eradication compared to non-eradication areas in Mississippi. These observations indicate the need to identify conditions that may favor secondary outbreaks in order to develop damage prevention strategies.

Texas boll weevil eradication personnel are well aware of the negative impact of malathion ULV on beneficial insects. Their procedures attempt to reduce the risk of beet armyworms outbreaks by monitoring adult densities using pheromone traps and modifying the action threshold to reduce the number of fields requiring malathion treatment. Information on beet armyworm densities is obtained from trap captures and reports from growers, consultants and Cooperative Extension agents. Although this practice may help reduce the frequency of outbreaks by this pest, by the time action thresholds are modified and implemented, economic damage may have already occurred, and pest densities may have exceed levels at which biological control is effective. The potential for secondary pest outbreaks needs to be detected in advance so that malathion ULV spraying can be modified before pest densities cause damage. Monitoring beneficial arthropod populations in addition to pest populations, may allow malathion ULV treatments to be adjusted to enhance the contribution of beneficial insects in reducing the risk of secondary pest outbreaks.

Research Objectives. The overall objective of this research was to contribute to identifying arthropodan predators or insect parasitoids whose densities may indicate the likelihood of outbreaks of beet armyworm, cotton aphid, and bollworm; as well as assess the impact malathion ULV on foraging of fire ants in cotton canopies. The specific objectives were to: (1) identify common predator or parasitoid species that can be used as indicators of potential secondary pest outbreaks under boll weevil eradication programs, and; (2) quantify the effects of malathion ULV applications on foraging activity of the red imported fire ant in the cotton canopy.

CHAPTER II

PREDICTING BOLL WEEVIL ERADICATION INDUCED PEST OUTBREAKS IN TEXAS COTTON

Introduction

The boll weevil (*Anthonomus grandis grandis* Boheman) has been a major pest of U.S. cotton since it was first reported near Brownsville, Texas, in 1892 (Haney 2001). Once established, its spread across the southern U.S. was rapid and devastating. By 1922 it had spread to the Atlantic Coast, and by 1981 its western range included California (TBWEF 2004). Since invading the U.S., the boll weevil has caused an estimated $22 billion in economic losses (Kaplan 2003). Currently the boll weevil is under eradication throughout the U.S. cotton belt.

The Texas Boll Weevil Eradication Foundation (hereafter referred to as TBWEF), established in 1993, uses Fyfanon ® (Cheminova Inc. Wayne, NJ), an oil-based formulation containing 96.5% malathion (hereafter referred to as malathion ULV), to eradicate the boll weevil. Fyfanon is applied as an ultra low volume (ULV) formulation at 876.9 ml/ha (12 oz/ac) aerially. The eradication program generally uses a threshold of two weevils in pheromone traps per 16.2 ha (40 ac) to trigger an application of malathion ULV. Early in a program many fields within an active eradication zone are treated weekly with malathion ULV throughout the season (TBWEF 2004). Multiple insecticide applications over a large area, as practiced in boll weevil eradication, can negatively impact beneficial cotton insect populations, and increase the risks of secondary pest outbreaks of lepidopteran and aphid pests (Sherman 1930, Ewing and Ivy 1943, Doutt and Smith 1971, DeBach and Rosen 1991, Trichilo and Wilson 1993).

Laboratory studies have found many beneficial cotton insects to be highly susceptible to malathion. These predators include *Hippodamia convergens* (Guérin-Méneville) (Bartlett 1963, 1964, England 1997, Elzen et al. 1998), *Chrysoperla carnea* (Stephens) (Bartlett 1963, 1964, Elzen et al. 1998), and *Geocoris punctipes* (Say) (Elzen et al. 1998, Tillman and Mulrooney 2001), and the parasitoids *Cotesia marginiventris* (Cresson) (England 1997, Tillman and Mulrooney 2001), *Bracon mellitor* (Say) (Tillman and Mulrooney 2001), and *Cardiochiles nigriceps* (Viereck) (Tillman and Williams 1997, Tillman and Mulrooney 2001). One recent field study found that a single application of malathion ULV substantially reduced the density of lady beetle larvae (Primarily *Hippodamia* spp.), adult *Scymnus* lady beetles, and spiders, although these populations recovered after a week, probably because of immigration from surrounding untreated areas (Sparks and Norman 2001).

Two of the most common secondary pests in Texas cotton are the cotton aphid (*Aphis gossypii* Glover) and the beet armyworm (*Spodoptera exigua* Hübner). Several studies have been conducted on secondary pest population dynamics under boll weevil eradication. Layton and Long (2001) found that cotton aphid populations were considerably higher in active eradication versus inactive eradication fields in Mississippi. Ruberson et al. (1994) reviewed the number of insecticide applications against beet armyworm in Georgia cotton fields from 1980 to 1992. They found that during the years of boll weevil eradication (1987-1990) the number of applications against beet armyworm were greater than in the years prior to (1980-1986) and after (1991-1992) eradication. The most notable example of a secondary pest outbreak associated with boll weevil eradication was a beet armyworm outbreak that occurred in 1995 during the first full season of boll weevil eradication in the Lower Rio Grande Valley of Texas. Summy et al. (1996) surveyed the region after the outbreak and found higher densities of beet armyworm and lower densities of green lacewing (*C. carnea*) in cotton fields relative to fields in the adjacent Lower Rio Grande Valley of Tamaulipas, Mexico, where early season applications against aphids, and malathion ULV applications against boll weevil eradication did not take place.

Texas Boll Weevil Eradication Foundation personnel are aware of the negative impacts of malathion ULV on beneficial insects and attempt to reduce the risks of secondary pest outbreaks by monitoring pest densities and raising boll weevil thresholds when necessary, to reduce the frequency of malathion ULV applications. However, since this response is triggered by pests already in a field, crop loss may have already occurred. The potential for secondary pest outbreaks must be recognized in advance so that malathion ULV application frequencies can be reduced before losses occur. A more effective method for preventing secondary pest outbreaks may be to detect negative impacts on densities of natural enemies of the secondary pests of interest. Estimates of the densities of natural enemies can be used to predict the densities of pests at a later date (Driesche and Bellows 1996). For example, ratios of predator and prey mites (Nyrop 1988), sticky trap catches of leafminers and their parasitoids (Robin and Mitchell

1987), and ratios of parasitized and non-parasitized eggs (Hoffman et al. 1991) have been employed to predict future pest densities.

In cotton systems, the beat bucket is an effective and rapid technique for sampling a wide range of foliage-active arthropods (Knutson and Wilson 1999). This study sampled cotton arthropods using a beat bucket to evaluate natural enemy densities as predictors of subsequent pest densities. The primary objective of this study was to determine the risk of secondary pest outbreaks in Texas cotton fields under boll weevil eradication by identifying potential indicators of subsequent secondary pest population densities. Specifically, the main objectives of this study were to (1) evaluate the effects of malathion ULV on natural enemy populations, and (2) identify natural enemy species, or groups of species, that can best indicate the potential for secondary pest outbreaks under boll weevil eradication.

Methods and Materials

Study Sites. Studies were conducted in central Texas in commercial cotton fields within the Northern Blacklands, an inactive eradication zone, and in the Southern Blacklands, an active eradication zone during the 2002 and 2003 cotton growing seasons (TBWEF 2004). Fields in the Northern Zone were 75-100 miles from sample fields in the Southern Zone. The zones are adjacent and have similar cropping patterns, in which cotton, corn, sorghum, and wheat are the predominant crops. In 2002, twenty-four fields were sampled weekly for insects and spiders from mid-May to mid-August. Eight of these fields were located in the Northern Blacklands and 16 fields in the Southern Blacklands. Four of the inactive fields were located in Hill County, near Malone, and four in Navarro County, near Frost. Eight of the active fields were located in Williamson County, near Taylor, and eight in the Brazos Valley (Brazos, Robertson, and Burleson counties). In 2003, the number of sampled fields was increased to 32 to better represent the range of environmental conditions in each of the zones. The eight inactive fields in the Northern Blacklands were located near Malone and Frost, TX. In the Southern Blacklands, ten of the active fields were located near Taylor, and fourteen in the Brazos Valley. Sampled fields were located at least one mile apart.

Estimating Predator and Lepidopteran Pest Densities. Populations of predatory insects, spiders, and large larvae of lepidopteran pests were sampled using the beat bucket method (Knutson and Wilson 1999). In 2002, each field was divided into four equal plots and samples were taken separately from each plot. In 2003, a sampling area of approximately ca. 0.4 ha was marked within each field, and subsequently divided into quadrants. Fields varied in size from 4.5 hectares to 21 hectares. Within each quadrant, sets of three plants (2002) or four plants (2003) separated by 1 meter were randomly selected along a diagonal transect and sampled by placing an individual plant inside the beat bucket and shaken it vigorously for 3 seconds to dislodge all arthropods. The plant was then removed from the beat bucket and all insects and spiders shaken from the plant were funneled into a collection cup at the bottom of the beat bucket. In 2002, a total of 240 plants per field were sampled weekly, whereas, 160 plants per field per week were sampled in 2003. During 2002, sampling sets were spaced to sample the entire field, whereas, during 2003, sample locations were separated by five meters within a plot. Arthropods collected in beat bucket samples were sorted in the laboratory according to the categories listed in Table 1. Insect categories were created based on beneficial and pest arthropods commonly collected using beat bucket sampling techniques (Knutson and Wilson 1999)

Estimating Cotton Aphid Densities. Cotton aphid densities were estimated by visual counts during both years. Counts included all life stages and were made on one terminal leaf per plant in each of 20 plants per plot. Plants were selected randomly along the diagonal transect (see above).

Estimating Parasitoid Densities. Parasitoids densities were estimated from captures on yellow sticky traps (Trece Pherocon AM, Adair, OK) in 2003. One trap was placed in each of the four corners of each plot. Traps were mounted on bamboo stakes and positioned immediately above the cotton plant canopy. Traps were collected every two weeks, wrapped in clear plastic for later examination, and replaced with new traps. Parasitoid populations were monitored from 2 July 2003 to 4 August 2003. Parasitoids captured on the sticky traps were identified in the laboratory as Tachinidae, Ichneumonidae, *Cotesia* spp., or non-*Cotesia* Braconidae.

Agronomic and Environmental Variables. Since agronomic and climatic variables influence pest and natural enemy populations (Prasifka et al. in press), weekly maximum and minimum temperature, and precipitation (National Climatic Data Center 2004), cumulative number of malathion ULV treatments, cumulative number of other insecticide treatments, planting date, and field perimeter-area ratio of surrounding vegetation types (2003 only) were also measured (Table. 1).

Statistical Analyses. The densities of all arthropods listed in Table 1 were subjected to repeated measures analysis (Zar 1999) to determine if differences between active and inactive eradication zones were significant. Sample sizes were unequal between eradication zones; therefore data were weighted by using the number of fields sampled in each zone as a covariate in the analyses. Because precipitation within fields reduced the efficiency of beat bucket sampling, any observations that were made under heavy precipitation were removed from the data set prior to analyses.

To assess the relationships of predator, agronomic, and environmental variables with pest densities, data were divided into two sets, cumulative densities throughout the season and mid-season predator/late season pest densities (see below), and subjected to correlation and stepwise regression analyses using methods similar to those of Prasifka et al. (2004). Cumulative densities of both pest and predator species for each field quadrant were calculated using sampling data. Cumulative pest densities have been used in many sequential sampling plans (Hoffmann et al. 1991, Meikle et al. 2000, Elliott et al. 2003). However, this analysis predicts densities of pests by using densities of natural enemies for the same time period. The objective of this study was to predict densities in advance, therefore, a subsequent analysis (hereafter "mid-late") was conducted using total sum numbers for mid-season (1st bloom – 1st cracked boll) predators to predict total sum numbers for late season (1st cracked boll – harvest) pests.

Many variables had a non-normal distribution, and therefore, the nonparametric Spearman's rank correlation was utilized to test for significant correlations between pest densities and predator, agronomic, and environmental variables for three time periods

Table 1. List of variables included in correlation and regression analyses. Variable name is followed by description of variable. * indicates arthropods categories sampled using a beat bucket.

Variable Name	Variable Description
Date	Calendar date sample was taken
Week	Calendar week sample was taken
Zone	Eradication zone, either active or inactive
Field	Field sampled
Quad	Quadrant sampled
Date Planted	Planting date of cotton
Cotton	Ratio of perimeter of surrounding cotton to total field area
Corn	Ratio of perimeter of surrounding corn to total field area
Sorghum	Ratio of perimeter of surrounding sorghum to total field area
Other Vegetation	Ratio of perimeter of surrounding non-crop to total field area
Malathion	Cumulative number of malathion ULV applications
Other Insecticides	Cumulative number of insecticide applications other than malathion ULV
Precipitation	Weekly precipitation, as recorded by nearest weather station
Maximum Temperature	Weekly maximum temperature, as recorded by nearest weather station
Minimum Temperature	Weekly minimum temperature, as recorded by nearest weather station
Aphids	Cotton aphid, *Aphis gossypii* Glover
Tachinidae	Parasitoids of the family Tachinidae
Ichneumonidae	Parasitoids of the family Ichneumonidae
Braconidae	Parasitoids of the family Braconidae, excluding the genus *Cotesia*
Cotesia	Parasitoids of the genus *Cotesia*
Total Predators*	Sum of all predators sampled (see below)
Total Insect Predators*	Sum of all insect predators sampled(see below)
Total Spiders*	Sum of all spiders sampled (see below)
Orius Nymphs*	Immatures of the genus *Orius*
Orius Adults*	Adults of the genus *Orius*
Fleahoppers*	*Pseudatomoscelis seriatus* (Reuter)
Crab Spiders*	Spiders of the family Thomisidae
Jumping Spiders*	Spiders of the family Salticidae
Lynx Spiders*	Spiders of the family Oxyopidae
Other Spiders*	Spiders other than Thomisidae, Salticidae, or Oxyopidae
Fire Ants*	Red imported fire ants - *Solenopsis invicta* (Buren)
Big-Eyed Bug Nymphs*	Nymphs of *Geocoris punctipes* (Say)
Big-Eyed Bug Adults*	Adults of *Geocoris punctipes* (Say)
Lacewing Larvae*	Larvae of *Chrysoperla carnea* (Stephens)
Lacewing Adults*	Adults of *Chrysoperla carnea* (Stephens)
Damsel Bugs*	Adults of the genus *Nabis*
Scymnus Larvae*	Larvae of the genus *Scymnus*
Scymnus Adults*	Adults of the genus *Scymnus*
Sevenspotted Lady Beetles*	Adults of *Coccinella septempunctata* (L.)
Convergent Lady Beetles*	Adults of *Hippodamia convergens* (Guérin-Méneville)
Pink Lady Beetles*	Adults of *Coleomegilla maculata* (De Geer)
Asian Lady Beetles*	Adults of *Harmonia axyridis* (Pallas)
Lady Beetle Larvae*	Larvae of *H. convergens* ,*H. axyridis, C. maculata*, and *C. septempunctata*
Syrphid Larvae*	Larvae of the family Syrphidae
Syrphid Adults*	Adults of the family Syrphidae
Beat Army Worms*	Larvae of *Spodoptera exigua* (Hübner)
Bollworms*	Larvae of *Helicoverpa zea* (Boddie)
Other Worms*	Larvae lepidopterans other than *Spodoptera exigua* and *Helicoverpa zea* [Mostly *Estigmene acrea* (Drury), *Pseudoplusia includens* (Walker), *Trichoplusia ni* (Hübner)]

corresponding to early season (fruit bud initiation to 1st bloom), middle season (1st bloom to 1st cracked boll), and late season (1st cracked boll to harvest). Only variables that were significantly correlated to the densities of the pest of interest were included as potential predictors of pest outbreaks in subsequent stepwise regression analyses. Secondary pests used as dependent variables were cotton aphid, beet armyworm, bollworm, and the sum of other lepidopteran pests which included loopers (*Trichoplusia ni* Hübner and *Pseudoplusia includens* Walker) and saltmash caterpillars (*Estigmene acrea* Drury). These pest species or groups were chosen because of their outbreak potential during boll weevil eradication (Ruberson et al. 1994, Summy et al. 1996) and because they were commonly found in field samples.

Stepwise regression analyses were conducted with each pest species as the dependent variable to determine which independent variables were the strongest predictors of pest densities. Data were standardized to mean number per plant, except for cotton aphids, which were mean number per leaf. Data from predator densities, agronomic, and environmental conditions were used as independent variables. For each season, separate regressions were run on each pest for four time periods, early, middle, and late season and for mid-season predators to late-season pests, i.e. mid-late regressions.

Results

The mean number of malathion ULV applications per field was 10.5 ± 0.8 in 2002 and 4.3 ± 0.5 in 2003 in the active eradication zone. No malathion ULV applications were made in the inactive eradication zone.(Northern Blackland Zone). The mean number of applications of other insecticides was 2.7 ± 0.4 per field in the active eradication zone and 2.9 ± 0.2 per field in the inactive zone for 2002. For 2003, the mean number of other insecticide applications was 1.4 ± 0.2 per field in the active eradication zone and 2.6 ± 0.2 per fields in the inactive eradication zone.

Pest and Natural Enemy Population Differences between Zones. Mean densities of pest and natural enemies were calculated for both the active and inactive eradication zones in 2002 and 2003 (Figs. 1-12) Cotton aphid densities were significantly greater in active eradication fields than in inactive fields during both years ($P \leq 0.015$) (Fig. 1). Beet armyworm densities were significantly greater in the eradication zone late in the 2002 season ($P = 0.0009$), but not in 2003 ($P = 0.621$). Densities of bollworms, other worms and total worms were significantly greater in active eradication fields than in inactive fields in 2002 ($P \leq 0.001$), but not in 2003 ($P = 0.664$, $P = 0.131$, $P = 0.083$ respectively) (Figs. 1, 2).

Densities of total predators, total insect predators, and total spiders were significantly greater in inactive eradication versus active eradication fields for both years ($P \leq 0.002$) (Fig. 3). Densities of Tachinidae, Braconidae, and specifically, *Cotesia* parasitoids were significantly greater in inactive eradication fields compared to active fields in 2003 ($P \leq 0.012$), while densities of Ichneumonidae were not significantly different between eradication zones ($P = 0.805$) (Fig. 4). Densities of 16 of the insect and spider species/groups were significantly lower in active vs. inactive eradication fields (Figs. 4-12) for at least one year, whereas, densities of lacewing adults, damsel bugs, sevenspotted lady beetles, pink lady beetles, Syrphidae larvae, and Syrphidae adults were not significantly different in either year (Figs. 6-11). Densities of convergent lady beetle adults were significantly greater in active eradication fields in both years ($P \leq 0.003$) (Fig. 10). Lady beetle larvae densities were significantly greater in active eradication fields in 2002 ($P = 0.004$), but not in 2003 ($P = 0.547$) (Fig. 10).

Pest -Natural Enemy Population Dynamics. Cumulative population densities of cotton aphids were positively correlated with the densities of many beneficial arthropods in early and middle season for both years (Table 2). However, cotton aphid densities in late season were negatively correlated to densities of these beneficial arthropods. Densities of lacewing larvae, convergent lady beetle adults, and lady beetle larvae, key predators of cotton aphids, were positively correlated with that of cotton aphids during early and middle season for both years. Middle season densities of lacewing larvae were negatively correlated with late season densities of cotton aphids for both years. Middle season convergent lady beetle densities were positively correlated with late season cotton aphid densities for both years. Middle season densities of lady beetle larvae were negatively correlated in 2002 and positively correlated in 2003 with late season cotton aphid densities (Table 2).

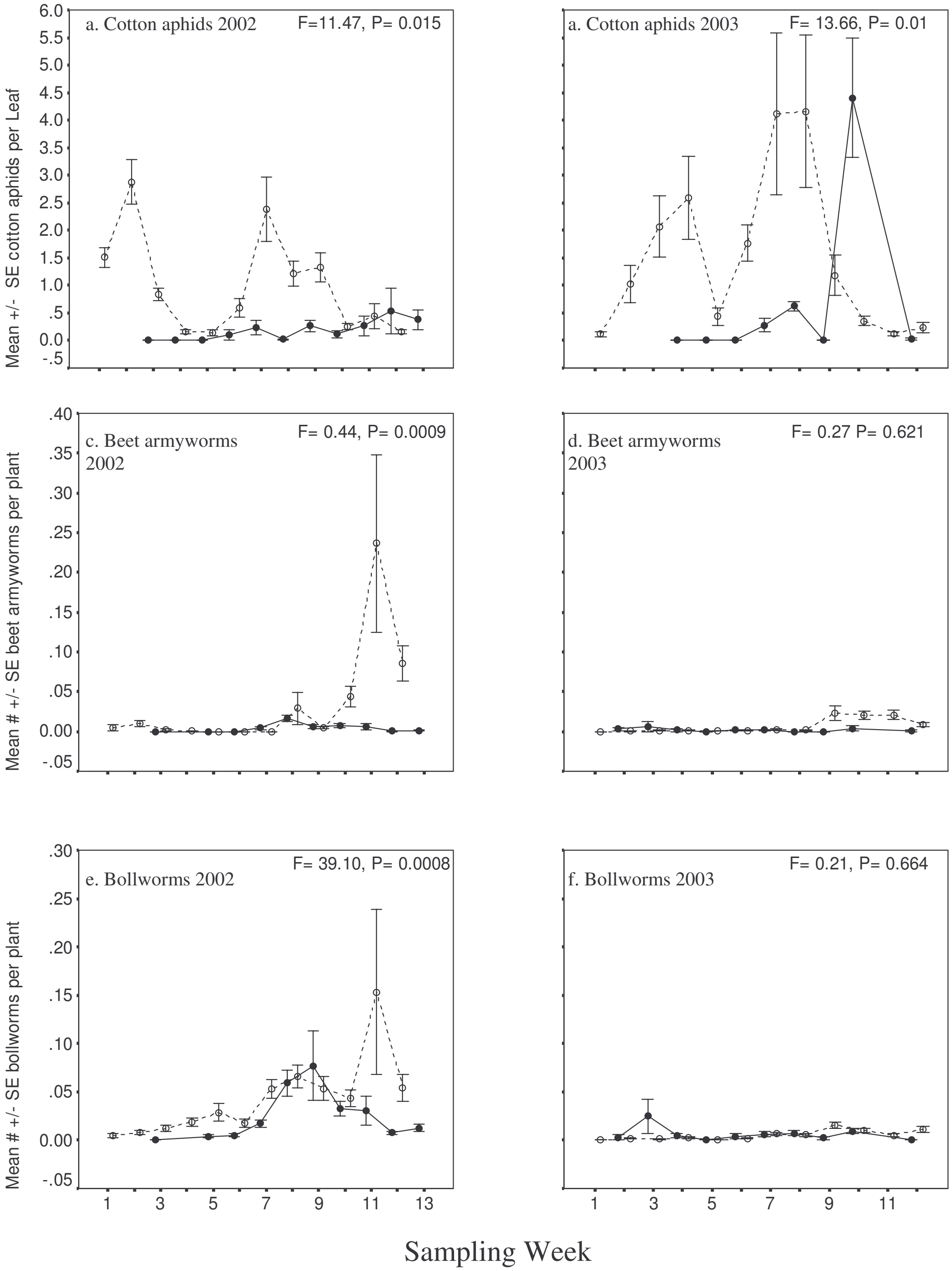

Fig. 1. Mean density of cotton aphids, *Aphis gossypii* Glover (a, b), beet armyworms, *Spodoptera exigua* Hübner (c, d), and bollworms, *Helicoverpa zea* Boddie (e, f) collected during 2002 and 2003. Solid black lines represent data from fields outside an active boll weevil eradication zone; dashed lines represent fields inside an active zone.

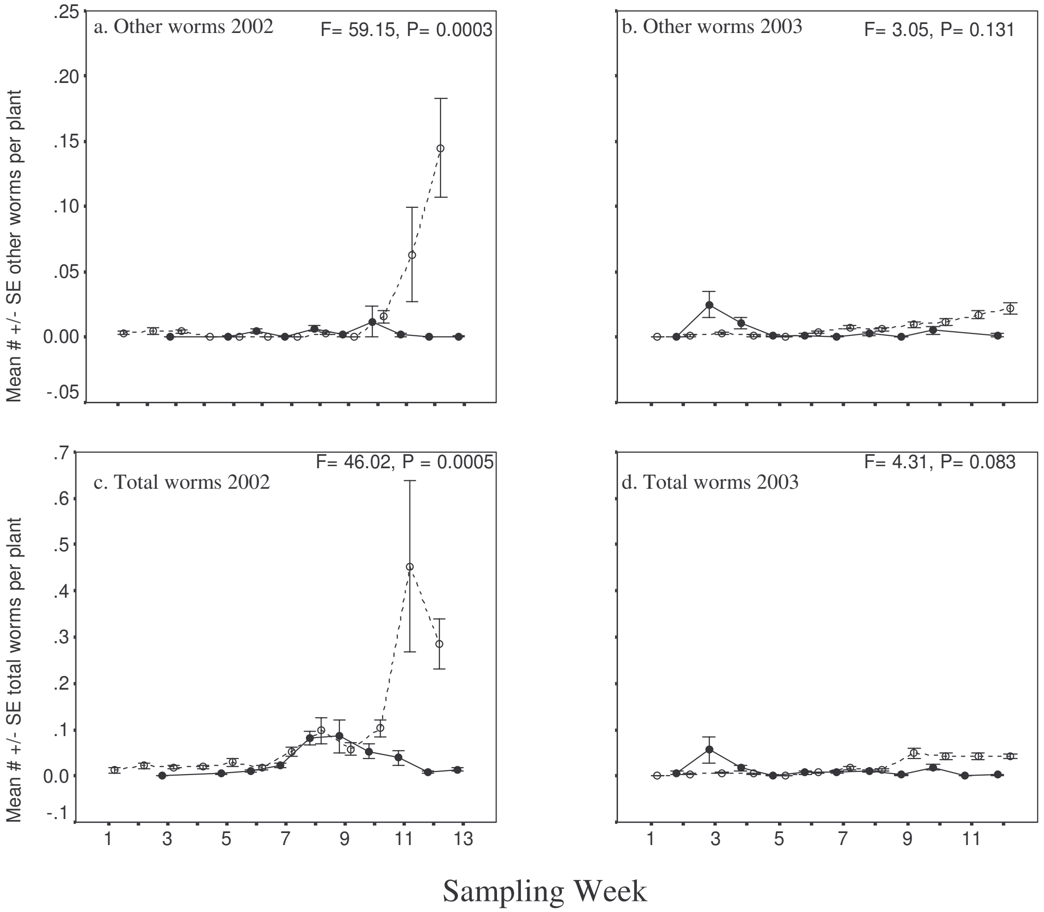

Fig. 2. Mean densities of "other worms" (a, b), and "total worms" (c, d) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to "other worms" and "total worms".

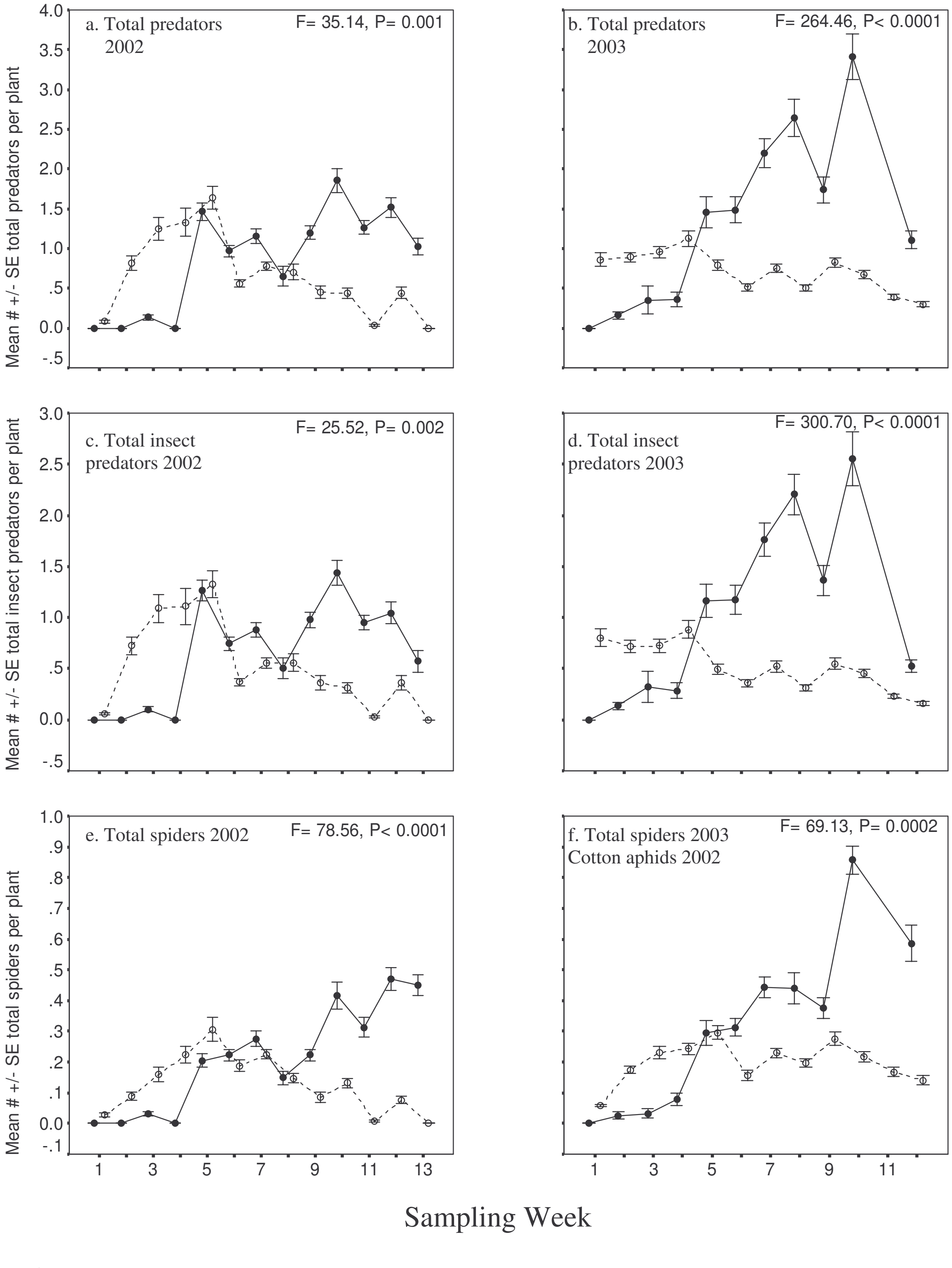

Fig. 3. Mean densities of "total predators" (a, b), "total insect predators" (c, d), and "total spiders" (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to "total predators", "total insect predators", and "total spiders".

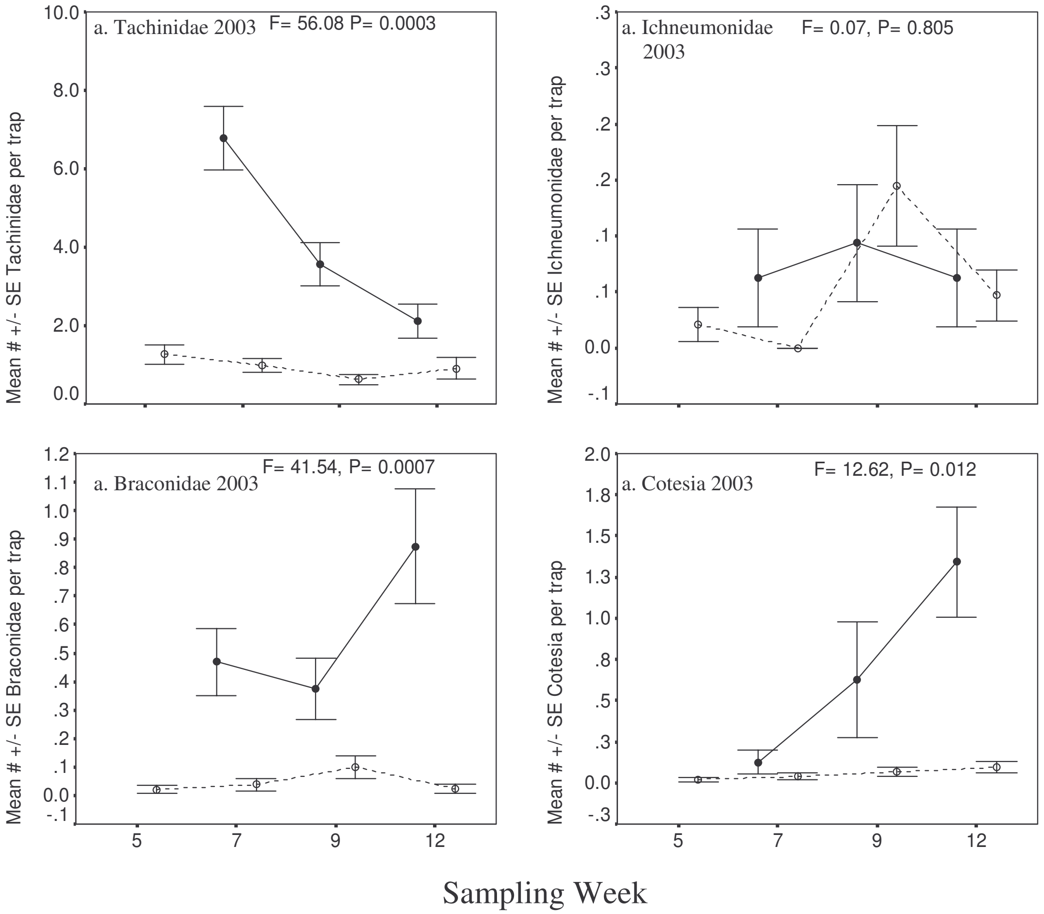

Fig. 4. Mean densities of Tachinidae (a), Ichneumonidae (b), Braconidae (c), and *Cotesia* (d) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to Tachinidae, Ichneumonidae, Braconidae, and *Cotesia*.

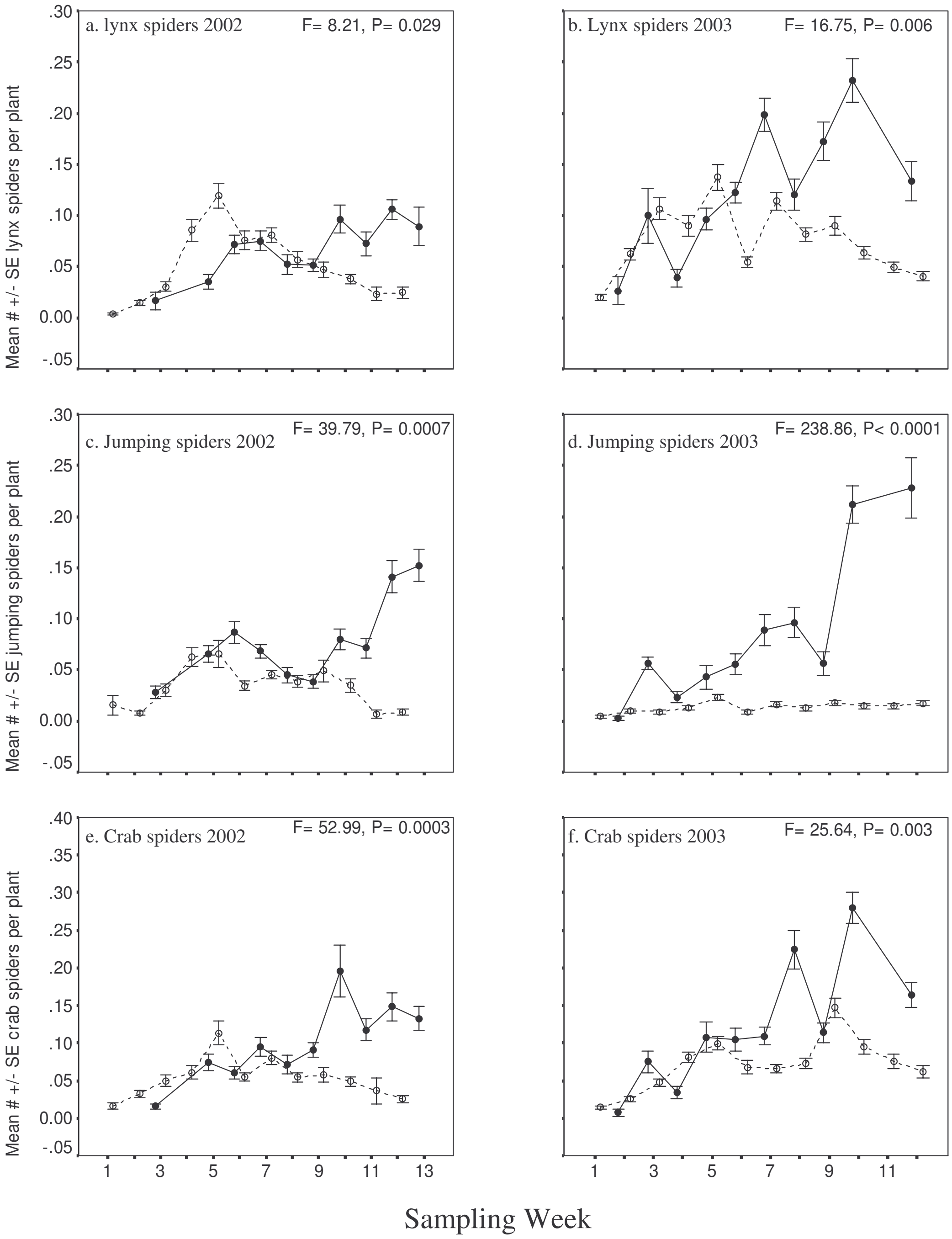

Fig. 5. Mean densities of lynx (a, b), jumping (c, d), and crab (e, f) spiders collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to lynx, jumping, and crab spiders.

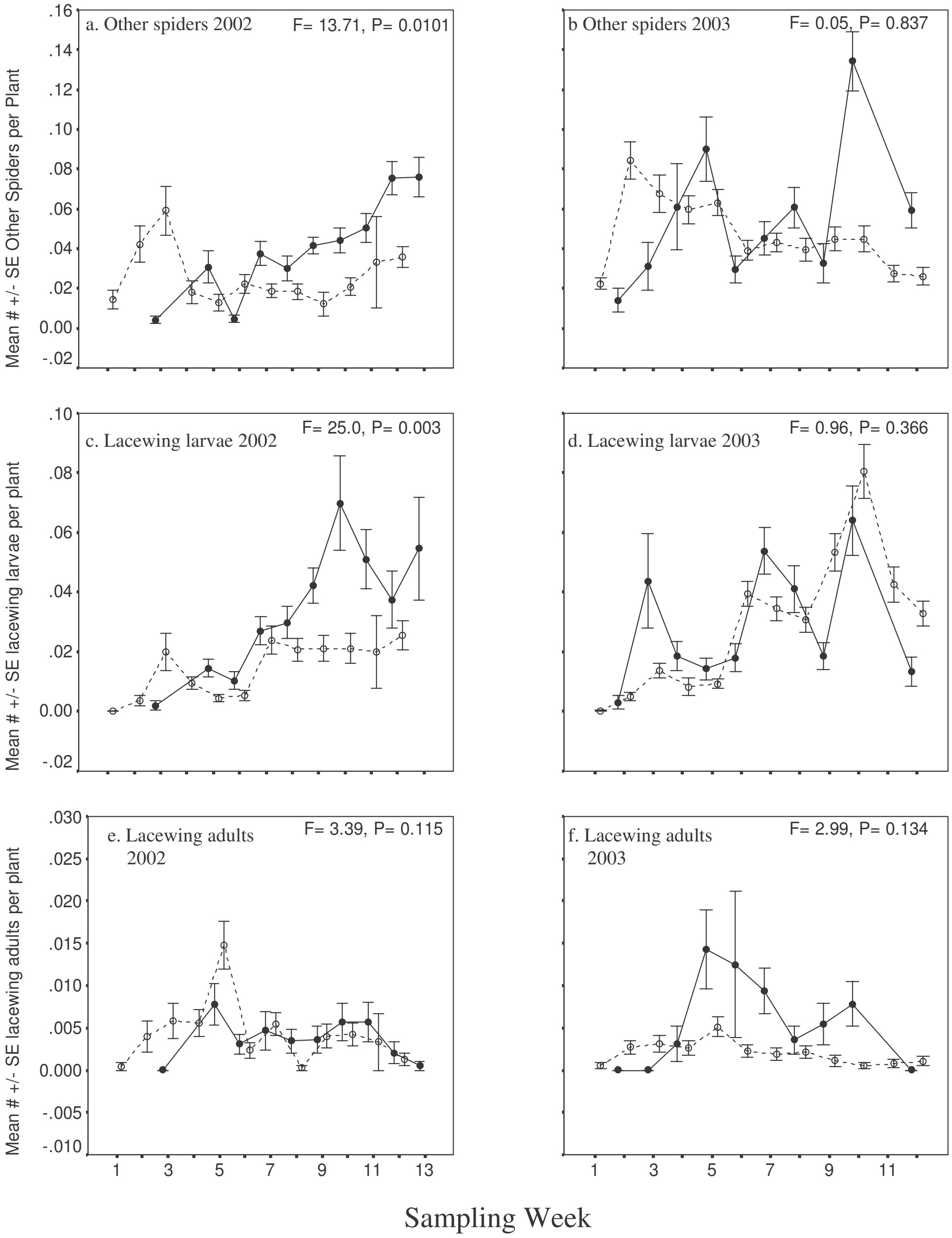

Fig. 6. Mean densities of "other spiders" (a, b), lacewing larvae, *Chrysoperla carnea* Stephens (c, d), and lacewing adults, *C. carnea* (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to "other spiders".

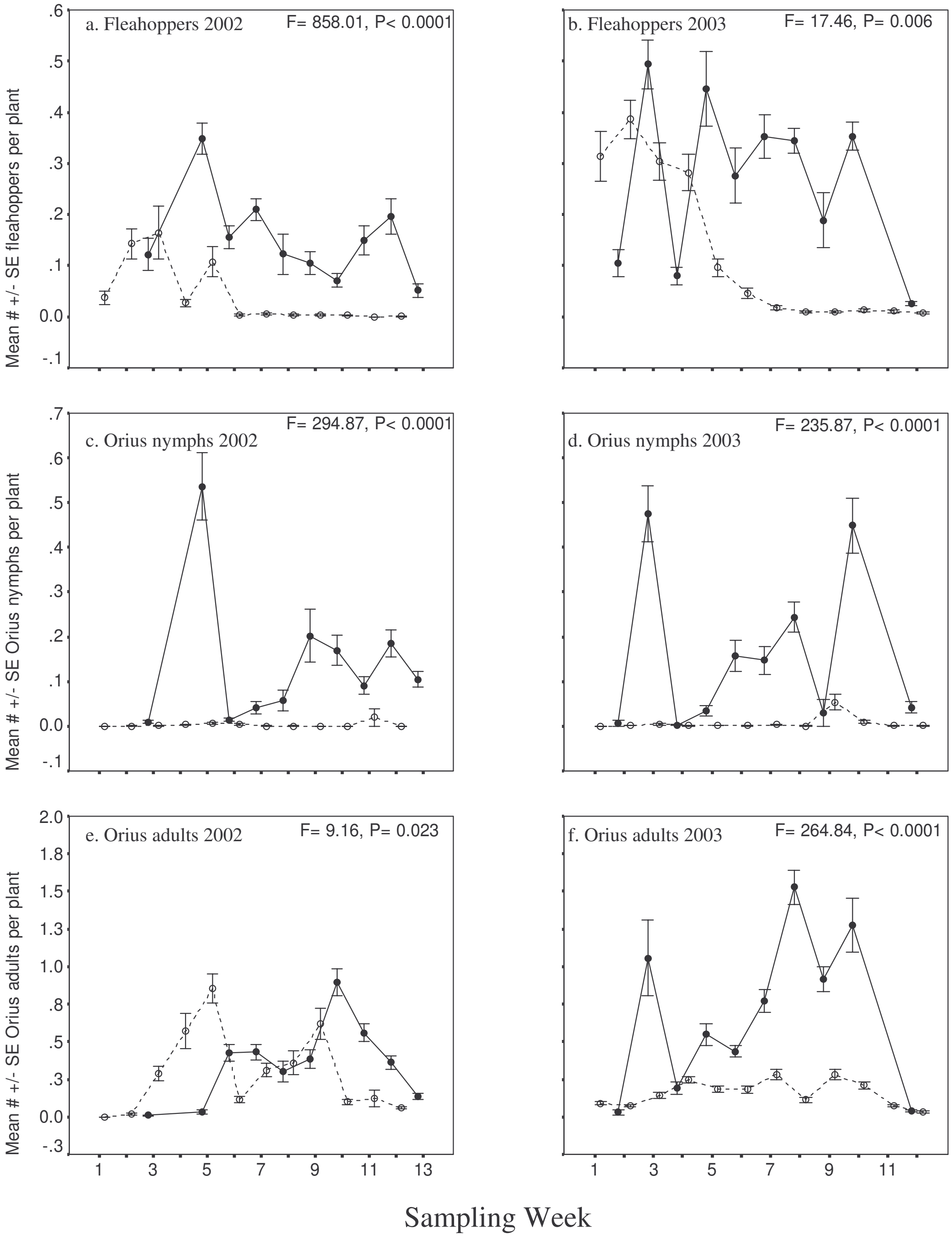

Fig. 7. Mean densities of fleahoppers, *Pseudatomoscelis seriatus* Reuter (a, b), *Orius* nymphs (c, d), and *Orius* adults (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to *Orius* nymphs, and *Orius* adults.

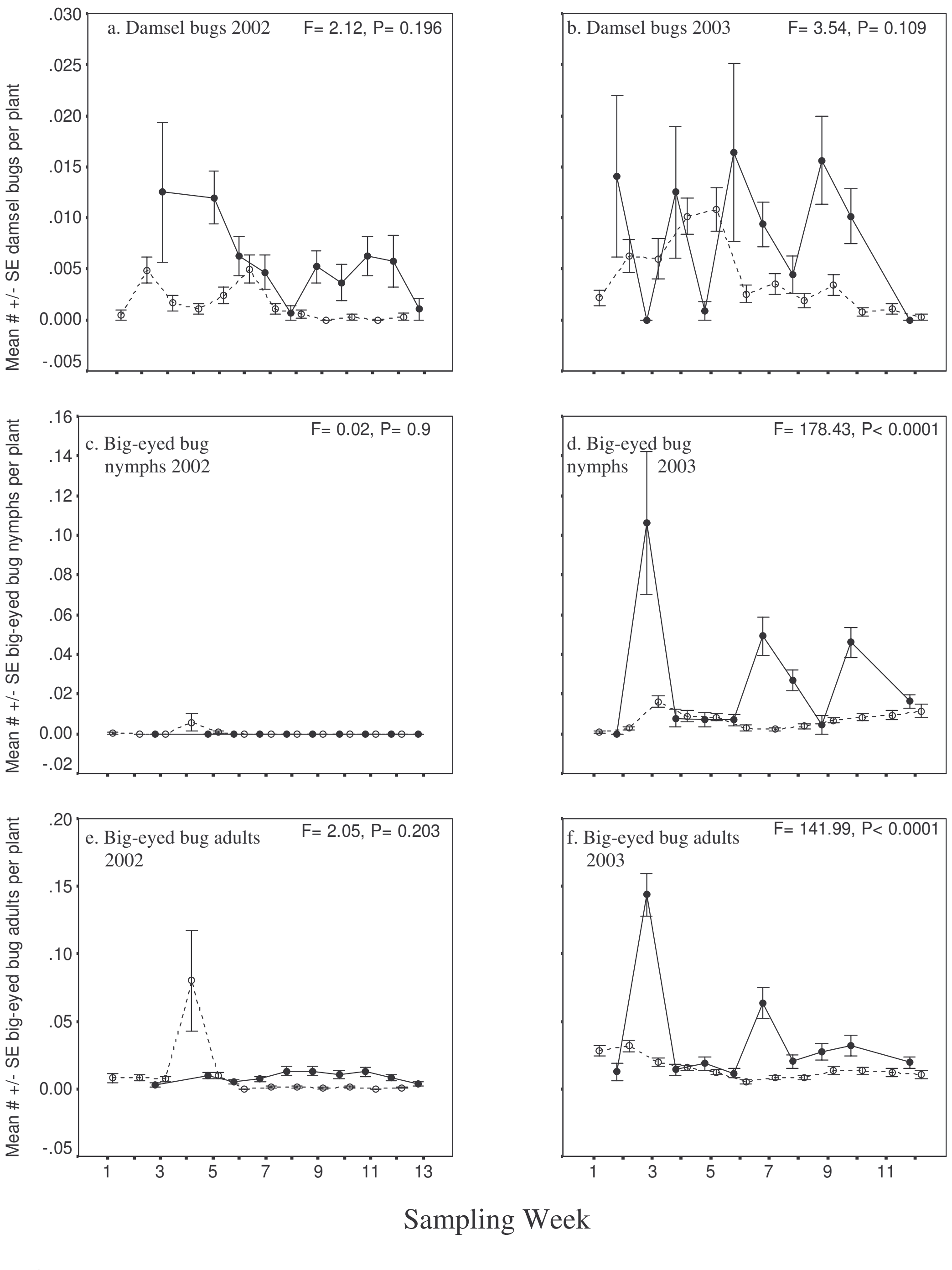

Fig. 8. Mean densities of damsel bugs, (a, b), big-eyed bug nymphs, *Geocoris punctipes* Say (c, d), and big-eyed bug adults, *G. punctipes* (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to damsel bugs.

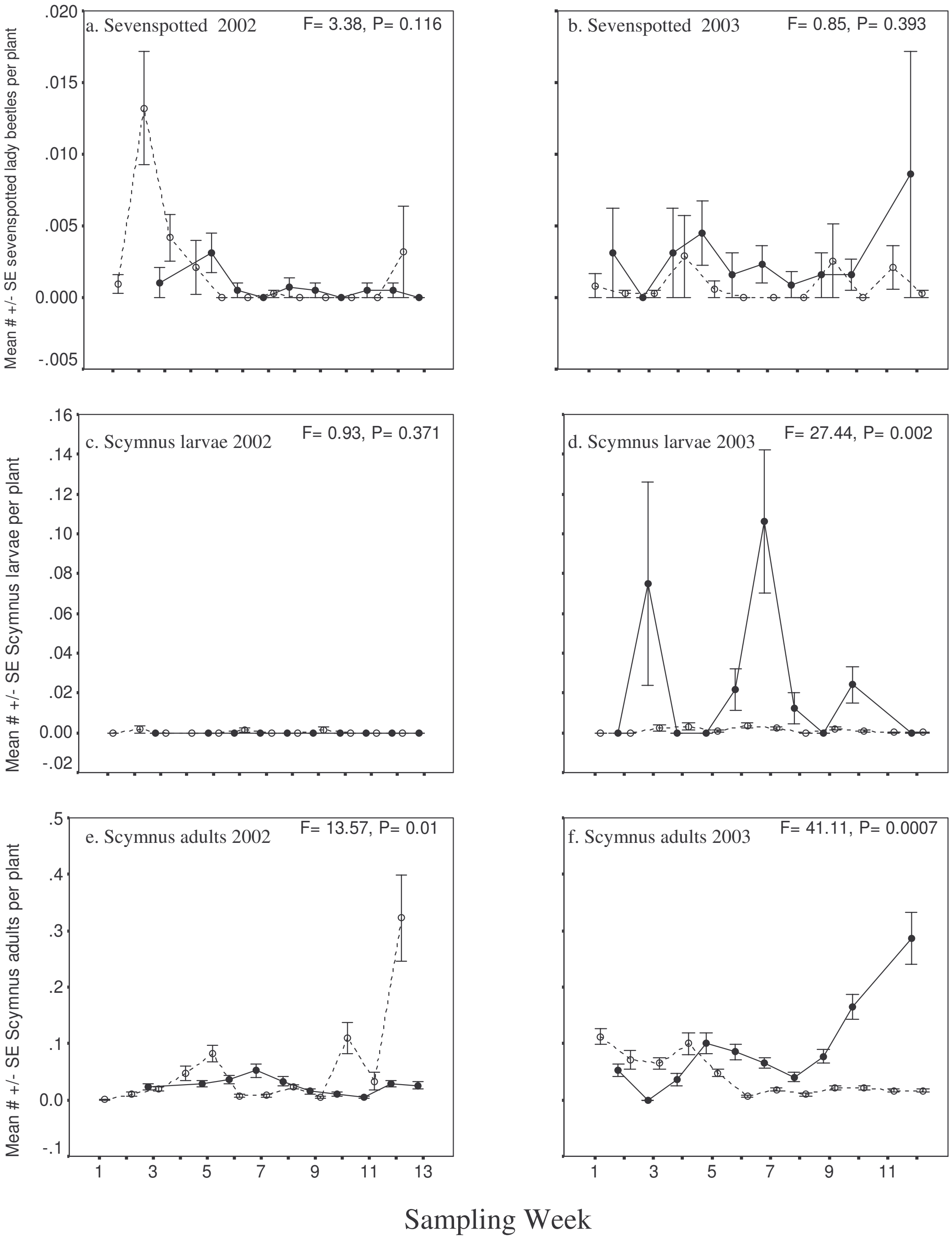

Fig. 9. Mean densities of sevenspotted lady beetles, *Coccinella septempunctata* L. (a, b), *Scymnus* larvae (c, d), and *Scymnus* adults (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to *Scymnus* larvae, and *Scymnus* adults.

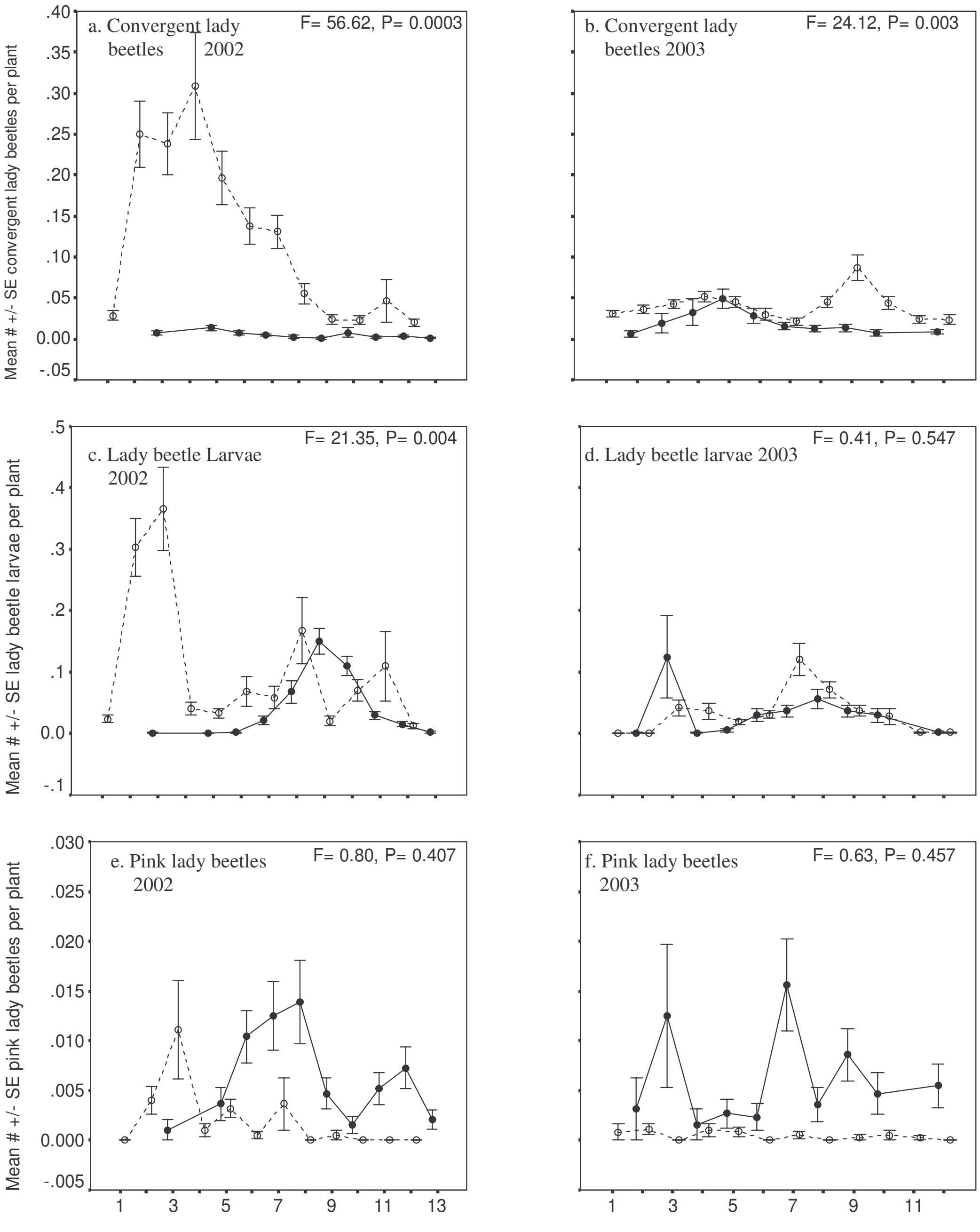

Fig. 10. Mean densities of convergent lady beetles, *Hippodamia convergens* Guérin-Méneville (a, b), lady beetle larvae (c, d), and pink lady beetles, *Coleomegilla maculata* De Geer (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to lady beetle larvae.

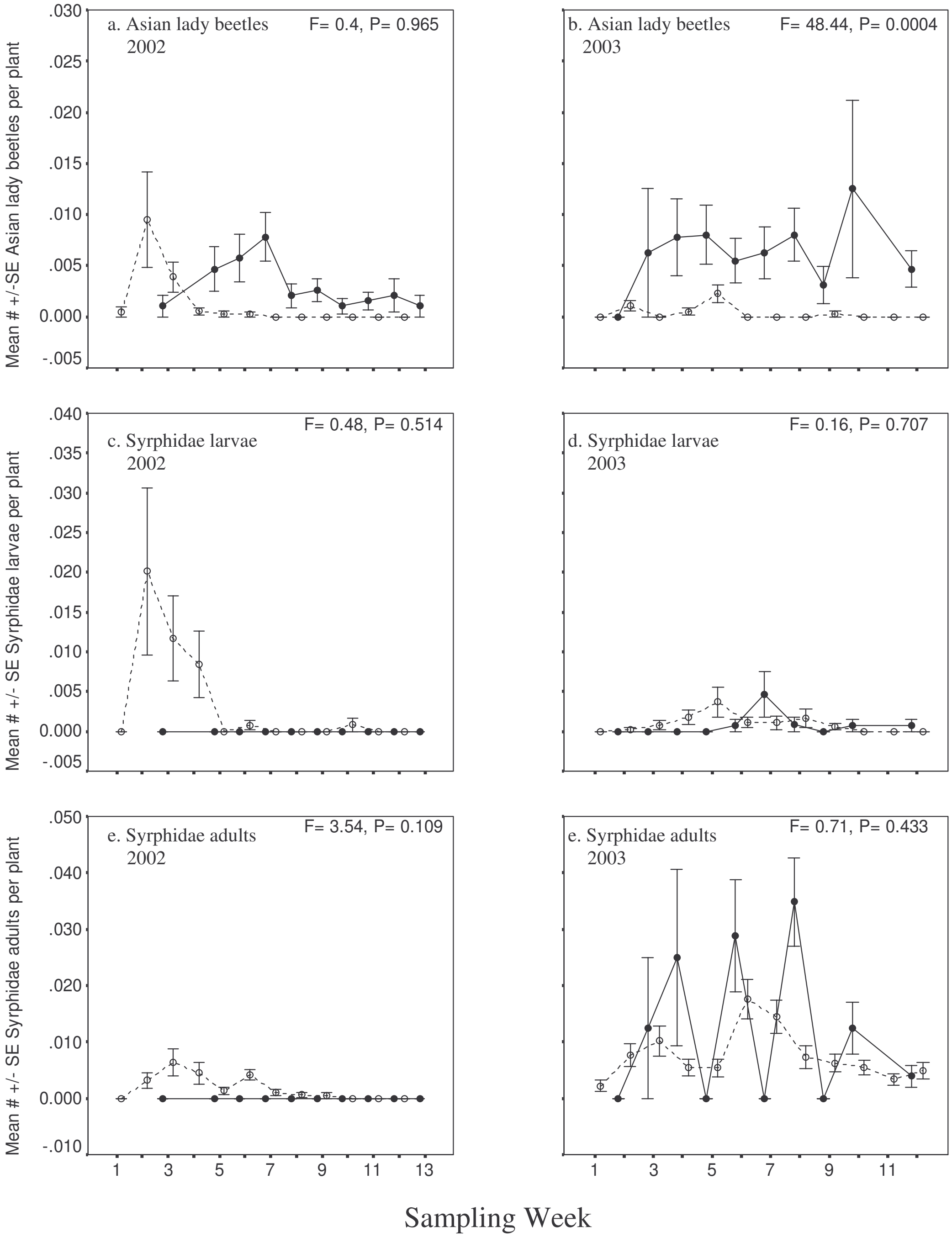

Fig. 11. Mean densities of Asian lady beetles, *Harmonia axyridis* Pallas (a, b), Syrphidae larvae (c, d), and Syrphidae adults (e, f) collected during 2002 and 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone. Refer to Table 1 for descriptions and scientific names corresponding to Syrphidae larvae and Syrphidae adults.

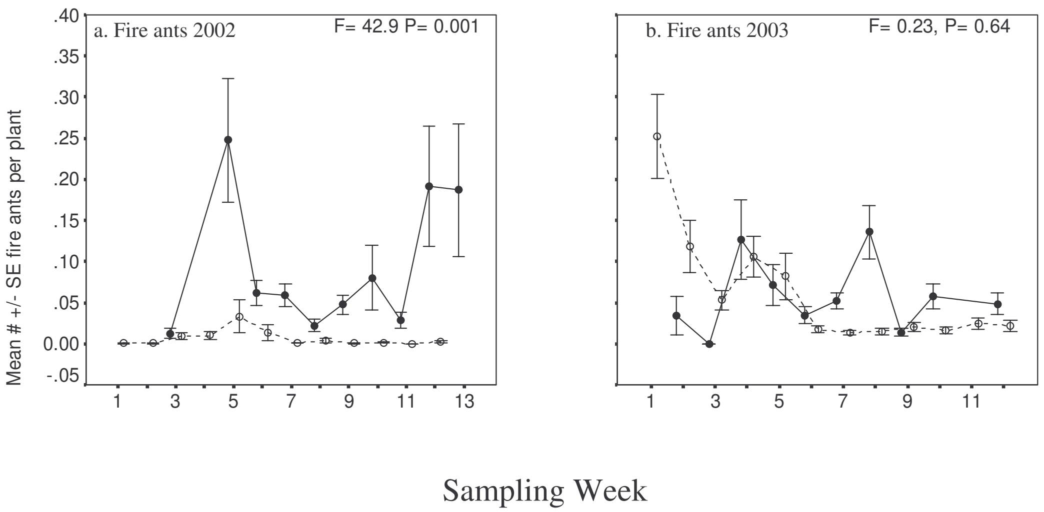

Fig. 12. Mean densities of fire ants, *Solenopsis invicta* Buren, collected during (a) 2002 and (b) 2003. Solid black lines correspond to densities outside of an active boll weevil eradication zone and dashed lines correspond to densities inside an active boll weevil eradication zone.

Table 2. Spearman's rank correlation coefficients between cotton aphid, *Aphis gossypii* Glover, and predator species during the early, middle, and late seasons of cotton development; and between mid season predators and late season pests. Refer to Table 1 for descriptions of variables and scientific names.

	2002				2003			
	Cumulative				Cumulative			
Variable	Early	Middle	Late	Mid-late	Early	Middle	Late	Mid-late
Total Predators	0.637**	0.496**	-0.014	-0.269**	0.513**	0.407**	0.157**	0.360**
Total Insect Predators	0.590**	0.475**	0.046	-0.297**	0.491**	0.402**	0.194**	0.298**
Total Spiders	0.306**	.0363**	-0.153**	-0.057	0.382**	0.172**	-0.093**	0.293**
Orius Nymphs	-0.188**	-0.604**	-0.739**	-0.502**	0.073	-0.286**	-0.112*	0.540**
Orius Adults	0.494**	0.475**	-0.056	-0.148	0.185**	0.082	-0.048	0.274**
Fleahoppers	0.200**	-0.283**	-0.454**	-0.326**	0.373**	0.269**	0.074	0.348**
Crab Spiders	0'.360**	0.348**	-0.163**	-0.068	0.410**	0.233**	-0.003	0.292**
Jumping Spiders	0.170*	0.121*	-0.279**	-0.165	0.215**	-0.126**	-0.186**	0.361**
Lynx Spiders	0.392**	0.473**	0.078**	0.109	0.317**	0.133**	-0.102*	0.278**
Other Spiders	0.076	0.128*	-0.178**	-0.114	0.264**	0.190**	0.042	0.021
Fire Ants	0.106	-0.498**	-0.461**	-0.341**	0.406**	0.448**	0.393**	0.152
Big-Eyed Bug Nymphs	0.104	0.168**	0.190**	0.087	0.139**	-0.081	-0.247**	0.355**
Big-Eyed Bug Adults	0.017	0.162**	-0.209**	-0.316**	0.05	0.041	-0.226**	0.355**
Lacewing Larvae	0.273**	0.218**	-0.140*	-0.439**	0.190**	0.261**	0.223**	-0.212*
Lacewing Adults	0.287**	0.343**	0.150**	-0.067	0.126*	-0.04	-0.053	0.187*
Damsel Bugs	0.061	-0.118*	-0.386	-0.249*	0.115*	0.058	-0.093	0.244**
Scymnus Larvae	0.076	0.137**	0.180**	0.199	0.075	0.027	0.005	0.461**
Scymnus Adults	0.204**	0.116*	0.209**	-0.158	0.270**	0.304**	0.212**	0.284**
Sevenspotted Lady Beetles	0.275**	0.181**	0.179**	-0.022	0.02	-0.093*	0.039	0.208*
Convergent Lady Beetles	0.547**	0.717**	0.711**	0.393**	0.266**	0.499**	0.507**	0.099
Pink Lady beetles	-0.037	-0.102	-0.475**	-0.368**	0.088	-0.067	-0.053	0.329**
Asian Lady Beetles	0.177**	-0.037	-0.247**	-0.324**	0.009	-0.174**	-0.102*	0.365**
Lady Beetle Larvae	0.635**	0.725**	0.465**	-0.255*	0.340**	0.483**	0.552**	0.05
Syrphid Larvae	0.144*	0.327**	0.386**	0.027	0.256**	0.296**	0.343**	0.162
Syrphid Adults	0.109	0.464**	0.485**	0.16	0.138**	0.153**	-0.001	0.336**

.* indicates significance at 0.05, ** indicate significance at 0.01.

Cumulative population densities of beet armyworms in early and middle season were correlated with the densities of a few beneficial arthropods for both years (Table 3). However, cumulative densities of beet armyworms in late season were negatively correlated with the densities of many beneficial arthropods. Cumulative densities during late season of *Orius* nymphs, fleahoppers, and jumping spiders were negatively correlated with beet armyworm densities in late season for both years, whereas, convergent lady beetles were positively correlated. Middle season densities of total predators, total insect predators, *Orius* nymphs, fleahoppers, jumping spiders, fire ants, and *Scymnus* adults were all negatively correlated with late season densities of beet armyworms for both years (Table 3).

Cumulative densities of bollworms were positively correlated with many beneficial arthropods in early and middle season for both years (Table 4). Cumulative densities of bollworms in late season were negatively correlated with densities of total spiders, *Orius* nymphs, lynx spiders, big-eyed bug nymphs and adults, and *Scymnus* adults during 2002. However, densities of these same groups were positively correlated with cumulative densities of bollworms during late season 2003. Middle season densities of total spiders were the only group correlated (negatively) with late season densities of bollworms during 2002, yet they were not correlated during 2003. Middle season densities of total predators, total insect predators, *Orius* adults, fleahoppers, *Scymnus* adults, and convergent lady beetles were negatively correlated with late season densities of bollworms during 2003 (Table 4).

Table 3. Spearman's rank correlation coefficients between beet armyworm, *Spodoptera exigua* Hübner, and predator species during the early, middle, and late seasons of cotton development; and between mid season predators and late season pests. Refer to Table 1 for descriptions of variables and scientific names.

	2002				2003			
	Cumulative				Cumulative			
Variable	Early	Middle	Late	Mid-late	Early	Middle	Late	Mid-late
Total Predators	0.637**	0.177**	0.007	-0.276**	0.095	0.023	-0.242**	-0.417**
Total Insect Predators	0.590**	0.190**	0.052	-0.220*	0.096	0.032	-0.302**	-0.429**
Total Spiders	0.306**	0.013	-0.107	-0.346**	0.057	-0.056	0.06	-0.087
Orius Nymphs	-0.037	-0.089	-0.161**	-0.390**	0.285**	0.01	-0.291**	-0.309**
Orius Adults	0.122	0.181**	0.042	-0.075	0.169**	0.067	-0.368**	-0.414**
Fleahoppers	0.182**	0.024	-0.191**	-0.267**	0.012	-0.083	-0.234**	-0.324**
Crab Spiders	0.038	0.045	-0.128*	-0.328**	0.094	-0.036	0.009	-0.133
Jumping Spiders	0.130*	0.003	-0.146*	-0.391**	0.109*	0.007	-0.149**	-0.180*
Lynx Spiders	0.180**	0.028	-0.077	-0.096	0.074	-0.05	0.027	-0.026
Other Spiders	0.068	0.083	0.069	-0.183	-0.001	-0.073	0.232**	0.134
Fire Ants	0.145*	-0.024	-0.059	-0.237*	0.067	0.027	-0.186**	-0.217*
Big-Eyed Bug Nymphs	-0.028	-0.012	-0.124*	0.019	0.082	-0.065	-0.085	-0.131
Big-Eyed Bug Adults	-0.015	-0.106*	-0.113*	-0.166	0.01	-0.067	0.038	-0.157
Lacewing Larvae	0.150*	0.032	0.064	-0.159	0.133**	-0.037	0.239**	-0.001
Lacewing Adults	-0.071	0.011	-0.008	-0.112	-0.042	0.048	-0.095*	-0.14
Damsel Bugs	0.155*	-0.008	0.01	-0.03	0.073	-0.012	-0.191**	-0.216*
Scymnus Larvae	0.021	0.208**	-0.003	0.105	0.264**	0.125**	-0.241**	-0.280**
Scymnus Adults	0.074	0.012	0.072	-0.221*	-0.03	0.041	-0.175**	-0.269**
Sevenspotted Lady Beetles	0.200**	0.125*	-0.075	-0.134	0.077	0.08	-0.140**	-0.146
Convergent Lady Beetles	0.300**	0.201**	0.148**	0.322**	0.028	0.08	0.149**	-0.143
Pink Lady beetles	-0.088	0.018	0.04	-0.14	0.126*	0.039	-0.095*	-0.275**
Asian Lady Beetles	0.097	0.006	-0.09	-0.14	0.106*	-0.01	-0.205**	-0.331**
Lady Beetle Larvae	0.310**	0.238**	0.108	-0.155	0.145**	0.114*	0.219**	0.038
Syrphid Larvae	-0.036	-0.083	-0.018	-0.007	0.096	0.089	0.013	-0.01
Syrphid Adults	-0.011	-0.047	-0.012	0.126	0.028	0.106*	-0.230**	-0.235**

.* indicates significance at 0.05, ** indicate significance at 0.01.

Table 4. Spearman's rank correlation coefficients between bollworm, *Helicoverpa zea* Boddie, and predator species during the early, middle, and late seasons of cotton development; and between mid season predators and late season pests. Refer to Table 1 for descriptions of variables and scientific names.

	2002				2003			
	Cumulative				Cumulative			
Variable	Early	Middle	Late	Mid-late	Early	Middle	Late	Mid-late
Total Predators	0.487**	0.361**	-0.109	-0.054	0.103*	0.175**	0.033	-0.192*
Total Insect Predators	0.502**	.0387**	0.014	-0.001	0.069	0.130**	-0.015	-0.255**
Total Spiders	0.193**	0.159**	-0.387**	-0.218*	0.225**	0.234**	0.126**	0.114
Orius Nymphs	-0.066	-0.266**	-0.166**	0.01	0.152**	0.23**	0.302**	-0.094
Orius Adults	0.371**	0.401**	0.112*	0.04	0.144**	0.119**	-0.104*	-0.304**
Fleahoppers	0.209**	-0.111*	-0.323**	-0.002	0.08	0.131**	0.071	-0.175*
Crab Spiders	0.246**	0.222**	-0.359**	-0.104	0.140**	0.166**	0.05	0.067
Jumping Spiders	0.075	-0.06	-0.423**	-0.138	0.148**	0.198**	0.059	-0.024
Lynx Spiders	0.318**	0.221**	-0.182**	-0.176	0.181**	0.193**	0.177**	0.155
Other Spiders	-0.049	0.56	-0.242**	-0.093	0.202**	0.203**	0.090*	0.079
Fire Ants	0.08	-0.201**	-0.028	0.105	-0.127*	-0.083	-0.118**	0.003
Big-Eyed Bug Nymphs	0.097	-0.033	-0.189*	-0.187	0.215**	0.268**	0.242**	-0.004
Big-Eyed Bug Adults	0.185**	0.009	-0.112*	0.01	0.202**	0.188**	0.156**	0.161
Lacewing Larvae	0.337**	0.197**	0.061	0.056	0.164**	0.079	0.001	-0.049
Lacewing Adults	0.248**	0.143**	0.095	0.184	0.002	0.065	0.064	-0.023
Damsel Bugs	0.227**	0.092	-0.011	0.009	0.048	0.234**	0.197**	-0.034
Scymnus Larvae	0.148*	0.129*	0.06	0.163	0.121*	0.05	0.148**	-0.01
Scymnus Adults	0.186**	0.003	-0.194**	-0.131	-0.025	0.172**	0.124**	-0.185*
Sevenspotted Lady Beetles	0.099	0.005	0.021	0.001	0.034	0.034	-0.026	-0.086
Convergent Lady Beetles	0.461**	0.473**	0.181**	0.078	0.056	0.053	-0.03	-0.179*
Pink Lady beetles	-0.089	-0.118*	-0.182**	-0.061	0.155**	-0.004	-0.057	-0.032
Asian Lady Beetles	0.399**	0.155**	0.018	-0.049	0.043	0.074	0.062	-0.091
Lady Beetle Larvae	0.501**	0.482**	0.049	-0.036	0.111*	0.086	0.007	-0.123
Syrphid Larvae	0.165*	0.015	-0.105	0.199	-0.061	-0.116*	-0.212**	-0.086
Syrphid Adults	0.286**	0.172**	-0.067	-0.089	0.119*	0.298**	0.514**	0.217*

.* indicates significance at 0.05, ** indicate significance at 0.01

Cumulative densities of other worms were positively correlated with densities of many beneficial arthropods during early and middle season for both years (Table 5). Cumulative densities of other worms in late season were negatively correlated with densities of *Orius* nymphs, jumping spiders, and pink lady beetles and positively correlated to convergent lady beetles for both years. Cumulative densities in late season for *Scymnus* larvae and adults and sevenspotted lady beetles were positively correlated in 2002, and negatively correlated in 2003 with densities of other worms. Late season densities of other worms were negatively correlated with middle season densities of total predators, total insect predators, *Orius* nymphs, fleahoppers, *Scymnus* adults, pink lady beetles, and Asian lady beetles for both years. Middle season densities of convergent lady beetles were positively correlated in 2002 and negatively correlated in 2003 with densities in late season of other worms (Table 5).

Analysis of Potential Indicators of Pest Densities. Significant variables in early, middle, and late season 2002 cumulative data regressions explained 36 to 55% of the variation in cotton aphid densities (Table 6). Lacewing larvae and other insecticide applications were the only variables common between these time periods (Table 6). Significant variables in cumulative data regressions for early middle, and late season 2003 explained 66 to 74% of the variation in cotton aphid densities, and lady beetle larvae was the only predictor common between these time periods. Total predators was a predictor for middle season aphid densities in 2002, and total insect predators was a significant predictor for middle and late season aphid densities in 2003. Middle season densities of fire ants, convergent lady beetles, and big-eyed-bug adults explained 46% of

Table 5. Spearman's rank correlation coefficients between worms other than beet armyworm and bollworm, and predator species during the early, middle, and late seasons of cotton development; and between mid season predators and late season pests. Refer to Table 1 for descriptions of variables and scientific names.

		2002				2003		
		Cumulative				Cumulative		
Variable	Early	Middle	Late	Mid-late	Early	Middle	Late	Mid-late
Total Predators	0.276**	0.278**	0.055	-0.446**	0.184**	0.135**	-0.138**	-0.381**
Total Insect Predators	0.295**	0.303**	0.108	-0.369**	0.167**	0.145**	-0.192**	-0.491**
Total Spiders	0.122	0.081	-0.1	-0.495**	0.157**	0.054	0.109*	0.161
Orius Nymphs	-0.075	0.036	-0.212**	-0.474**	0.124*	-0.003	-0.252**	-0.407**
Orius Adults	0.151*	0.255**	0.058	-0.191	0.153**	0.090*	-0.276**	-0.532**
Fleahoppers	0.158*	0.162**	-0.160**	-0.501**	0.097*	0.092*	-0.008	-0.220*
Crab Spiders	0.162*	0.133*	-0.131*	-0.552**	0.110*	0.097*	0.044	-0.026
Jumping Spiders	0.131*	0.113*	-0.150**	-0.463**	0.054	-0.076	-0.119**	0.036
Lynx Spiders	0.172*	0.102	-0.015	-0.125	0.112*	0.055	0.155**	0.269**
Other Spiders	-0.001	0.003	0.031	-0.261	0.138**	0.066	0.195**	0.201*
Fire Ants	0.127	0.022	-0.147**	-0.386**	0.035	0.004	0.042	0.084
Big-Eyed Bug Nymphs	0.063	-0.015	0.036	0.230*	0.134**	0.032	0.086	-0.038
Big-Eyed Bug Adults	0.145*	0.159**	0.02	-0.261*	0.110*	0.107*	0.062	-0.104
Lacewing Larvae	0.201**	0.179**	0.074	-0.159	0.273**	0.262**	0.246**	-0.012
Lacewing Adults	0.211**	0.234**	0.008	-0.244*	0.055	0.001	-0.09	-0.1
Damsel Bugs	0.057	0.001	-0.038	-0.053	0.143**	0.118**	-0.04	-0.049
Scymnus Larvae	0.147*	0.311**	0.219**	0.043	0.149**	0.063	-0.236**	-0.356**
Scymnus Adults	0.180**	0.102	0.125*	-0.385**	-0.099*	-0.039	-0.216**	-0.204*
Sevenspotted Lady Beetles	-0.002	-0.036	0.129*	-0.035	0.123*	0.25	-0.125**	-0.128
Convergent Lady Beetles	0.185**	0.190**	0.313**	0.346**	0.123*	0.276**	0.158**	-0.346**
Pink Lady beetles	-0.055	-0.009	-0.181**	-0.347**	0.152**	0.031	-0.202**	-0.281**
Asian Lady Beetles	0.177**	0.124*	0.064	-0.271**	0.122*	-0.024	-0.129**	-0.174*
Lady Beetle Larvae	0.339**	0.188**	0.203**	0.056	0.279**	0.292**	0.07	-0.366**
Syrphid Larvae	-0.032	-0.052	0.03	0.03	0.08	0.052	0.041	-0.144
Syrphid Adults	-0.001	0.001	0.071	0.225*	0.158**	0.227**	-0.079	-0.123

.* indicates significance at 0.05, ** indicate significance at 0.01.

Table 6. Best fit stepwise regression models for cotton aphid, *Aphis gossypii* Glover, densities at early, middle, and late season. Variables included in best fit stepwise regression models are listed in order of strength. Refer to Table 1 for descriptions of variables and scientific names.

Year	Phenological Stage of Cotton	Variables in Best Fit Regression Model	R^2	SE	P<
2002	Early	other insecticides, field, lacewing larvae, area, max temp., zone	0.453	2.625	0.001
	Middle	field, lacewing larvae, week, other insecticides, total predators, fire ants, crab spiders, jumping spiders, other spiders, quadrant, big-eyed bug adults	0.548	4.436	0.001
	Late	damsel bugs, other insecticides, lacewing larvae	0.365	3.345	0.001
	Mid-late	fire ants, convergent lady beetles, big-eyed bug adults	0.461	2.132	0.001
2003	Early	lady beetle larvae, *Scymnus* adults, sorghum, convergent lady beetles, fire ants, damsel bugs, syrphid larvae, plant stage, planting date	0.74	4.697	0.001
	Middle	total insect predators, fleahoppers, *Orius* adults, lady beetle larvae, crab spiders, precipitation, other insecticides, fields, syrphid larvae, big-eyed bug adults	0.658	14.569	0.001
	Late	total insect predators, crab spiders, *Orius* adults, lady beetle larvae, *Orius* nymphs, syrphid adults, *Scymnus* larvae	0.733	18.308	0.001
	Mid-late	jumping spiders, *Scymnus* larvae, total spiders, malathion, Braconidae	0.674	2.164	0.001

the late season variation in cotton aphid densities in 2002. In 2003, malathion applications and middle season densities of jumping spiders, *Scymnus* larvae, total spiders, and Braconidae explained 67% of the aphid density variation in late season.

Densities of damsel bugs, convergent lady beetles, fleahoppers, and fire ants explained 43% of the variation in beet armyworm densities during the early season in 2002. Other insecticides, minimum temperature, and field explained up to 11% of the variation in beet armyworm densities during middle season 2002. Corn was the only predictor of beet armyworm densities during middle season 2003. Malathion applications and densities of jumping spiders explained 17% of the variation in beet armyworm densities for late season 2002. Lacewing larvae densities, malathion applications and cotton explained 11% of the variation in beet armyworm densities for late season 2003 (Table 7). Middle season applications of malathion explained 11% of the variation for beet armyworms in 2002. Malathion applications, precipitation, maximum temperature, and densities of syrphid larvae middle season explained 32% of the beet armyworm variation in late season 2003 (Table 7).

Other insecticides and densities of lady beetle larvae, *Scymnus* adults, and Asian lady beetles explained 40% of the variation in bollworm densities during early season 2002. In 2003, Corn, malathion, and densities of other spiders, big-eyed bugs, and *Orius* nymphs explained 14% of the variation in bollworm densities during early season. Other insecticides, malathion applications, and densities of crab spiders, *Scymnus* larvae, *Orius* nymphs, and lady beetle larvae explained 53% of the variation in bollworm densities

Table 7. Best fit stepwise regression models for beet armyworms, *Spodoptera exigua* Hübner, densities at early, middle, and late season. Variables included in best fit stepwise regression models are listed in order of strength. Refer to Table 1 for descriptions of variables and scientific names.

Year	Phenological Stage of Cotton	Variables in best fitting Step Wise Regression Model	R^2	SE	P
2002	Early	damsel bugs, other insecticides, area, convergent lady beetles, fleahoppers, fire ants	0.43	0.033	0.001
	Middle	other insecticides, date, min. temp., field	0.11	0.082	0.001
	Late	malathion, date, jumping spiders	0.17	0.153	0.001
	Mid-late	malathion	0.117	0.151	0.001
2003	Early	*Scymnus* larvae, precipitation, pink lady beetles	0.19	0.006	0.001
	Middle	corn	0.05	0.013	0.001
	Late	lacewing larvae, malathion, cotton	0.11	0.101	0.001
	Mid-late	malathion, precipitation, max. temp. syrphid adults	0.322	0.075	0.001

during middle season 2002. However, corn, minimum temperature, and densities of other spiders, syrphid adults, and fire ants explained 35% of the variation in bollworm densities during middle season 2003. During late season 2002, malathion applications, and densities of total spiders, fleahoppers, jumping spiders, and pink lady beetles explained 51% of the variation in bollworm densities. In late season 2003, corn and densities of syrphid adults, lynx spiders, *Orius* adults, *Scymnus* adults, big-eyed bug nymphs, and total insect predators explained 51% of the variation in bollworm densities. Middle season densities of total spiders explained 5% of the variation in late season bollworm densities during 2002. However, precipitation and densities of syrphid adults explained 22% of the variation in late season bollworm densities during 2003 (Table 8).

Significant variables in early and middle season cumulative data regressions for other worms explained up to 38% of the variation in other worm densities, with lady beetle larvae, as the only predictor common between time periods (Table 9). Pink lady beetle and seven spotted lady beetle densities explained 23% of the variation in other worm densities during late season 2002. Whereas, corn, and densities of lacewing larvae, *Scymnus* adults, *Orius* adults and total predators explained 25% of the variation in other worm densities during late season 2003 (Table 9). Middle season densities of total spiders and minimum temperature explained 23% of the variation in late season other worm densities in 2002, whereas, precipitation and lynx spider densities explained 31% of the variation in late season other worm densities in 2003 (Table 9).

Table 8. Best fit stepwise regression models for bollworms, *Helicoverpa zea* Boddie, densities at early, middle, and late season. Variables included in best fit stepwise regression models are listed in order of strength. Refer to Table 1 for descriptions of variables and scientific names.

Year	Phenological Stage of Cotton	Variables in best fitting Step Wise Regression Model	R^2	SE	P
2002	Early	lady beetle larvae, other insecticides, area, date, *Scymnus* Adults, Asian lady beetles	0.4	0.031	0.001
	Middle	area, other insecticides, field, week, crab spiders, malathion, *Scymnus* larvae, *Orius* nymphs, lady Beetle larvae	0.531	0.137	0.001
	Late	total spiders, field, date, malathion, fleahoppers, jumping spiders, pink lady beetles	0.511	0.209	0.001
	Mid-late	total spiders	0.049	0.122	0.001
2003	Early	corn, other spiders, malathion, big-eyed bugs, *Orius* nymphs	0.141	0.009	0.001
	Middle	corn, field, other spiders, planting date, syrphid adults, fire ants, min. temp.	0.347	0.334	0.001
	Late	syrphid adults, lynx spiders, corn, *Orius* adults, date, week, *Scymnus* adults, big-eyed bug nymphs, total insect predators	0.511	0.042	0.001
	Mid-late	precipitation, syrphid adults	0.22	0.031	0.001

Table 9. Best fit stepwise regression models for densities of caterpillars other than beet armyworm and bollworms at early, middle, and late season. Variables included in best fit stepwise regression models are listed in order of strength. Refer to Table 1 for descriptions of variables and scientific names.

Year	Phenological Stage of Cotton	Variables in best fitting Step Wise Regression Model	R^2	SE	P
2002	Early	lady beetle larvae, convergent lady beetles, field, area, zone, date, Asian lady beetles	0.383	0.016	0.001
	Middle	*Scymnus* larvae, lady beetle larvae, fleahoppers, jumping spiders, field, date, week, total predators	0.358	0.016	0.001
	Late	quadrant, pink lady beetles, sevenspotted lady beetle, week	0.119	0.059	0.001
	Mid-late	min. Temp., total spiders	0.229	0.192	0.001
2003	Early	crab spiders, syrphid adults, lady beetle larvae, big-eyed bug adults, fleahoppers, other spiders, plant stage, lacewing larvae, *Orius* nymphs, damsel bugs	0.208	0.009	0.001
	Middle	lacewing larvae, lady beetle larvae, syrphid adults, field, week	0.73	0.019	0.001
	Late	lacewing larvae, corn, *Scymnus* adults, week, *Orius* adults, total predators	0.249	0.039	0.001
	Mid-late	precipitation, lynx spiders	0.308	0.053	0.001

Discussion

The results of this study demonstrated that repeated applications of malathion ULV, as used in the Texas boll weevil eradication program, had significant impacts on the densities of most cotton arthropods observed in this study. In active eradication fields, densities of cotton aphids, beet armyworms, bollworms, and other worms were significantly higher than in fields within the inactive eradication zone. Densities of most natural enemies were significantly lower in active fields versus inactive eradication fields. However, densities of convergent lady beetle adults and lady beetle larvae were higher in active fields, probably in response to the increase in cotton aphid densities. Predator and pest densities were variably correlated, and in the majority of cases, predators were significantly correlated to densities of at least one pest species, with some positive and some negative correlations.

Cumulative and mid-late regression analyses suggested that densities of lacewing larvae, lady beetle larvae, fire ants, and big-eyed bugs have potential to be used as indicators of cotton aphid outbreaks. The number of malathion ULV applications and jumping spider's density were indicators of beet armyworm densities in 2002. Regression analyses suggested that the number of malathion ULV applications applied by mid-season was an indicator for beet armyworms late season. Cumulative regression analyses suggested that total spiders, fleahoppers, jumping spiders, and pink lady beetles were l indicators for densities of bollworm. Also, the densities of total spiders and syrphid adults in mid-season were indicators of bollworm densities in late-season. Cumulative regression analysis indicated that densities of pink lady beetle and sevenspotted lady beetle were indicators of densities of other worms. Mid-late regression for other worms suggested that mid-season densities of total spiders and lynx spiders were indicators of densities of other worms during the late season. Densities of beet armyworm, bollworm, and other worms were low in 2003 and consequently few variables were significant.

Malathion and natural enemies. Previous laboratory studies showed that many important predators in cotton are highly susceptible to malathion including *H. convergens*, *G. punctipes*, *C. carnea,* and *O. insidiosus* (Bartlett 1963, 1964; England 1997; Elzen et al. 1998, Tillman and Mulrooney 2001). In this study, *G. punctipes*, *C. carnea,* and *O. insidiosus* densities were significantly lower in active versus inactive fields. In contrast, *H. convergens* and total lady beetle larvae were significantly greater in active eradication fields. Higher densities of cotton aphids, an important food source for lady beetles, were significantly higher in active eradication fields, which may partially explain the higher densities of *H. convergens* despite malathion ULV applications. Moreover, lady beetles are strong fliers and found in diverse habitats including wheat and sorghum; they may have migrated from surrounding habitats where applications of malathion ULV were not made and quickly re-colonized treated fields (Gordon 1985, Knutson et al. 1993, Norman et al. 2000). However, the survival of large numbers of convergent lady larvae in malathion treated fields suggests the larvae are more tolerant to malathion than adults or that this species has developed resistance to malathion that has not been detected in laboratory studies of malathion toxicity (Elzen et al. 1998).

Previous laboratory studies on various cotton insect parasitoids showed that *C. marginiventris, B. mellitor,* and *C. nigriceps* are highly susceptible to malathion (England 1997, Tillman and Williams 1997, Tillman and Mulrooney 2001). In this study, densities of Braconidae, and specifically *Cotesia* sp., were significantly lower in active versus inactive eradication fields. Cotesia spp. are important parasitoids of beet armyworm in cotton (Ruberson et al. 1994). Densities of Tachinidae flies, parasitoids of beet armyworms and bollworms (Arnaud 1978, Ruberson et al. 1994, Stapel et al. 1997), were also found to be significantly lower in active eradication fields. Ichneumonidae densities, in contrast, were not significantly different between active and inactive eradication fields.

Spiders are important components of the beneficial cotton arthropod community (Fuchs and Harding 1976, Lopez et al. 1996) where they are predators of beet armyworm (Ruberson et al. 1994), bollworm (McDaniel and Sterling 1979, 1981), fleahoppers (Breene et al. 1988, 1989), and other beneficial insects (Whitcomb and Bell 1964). In this study, spider densities were similar in early season between boll weevil eradication zones, though densities declined after mid-season in active eradication fields, while densities continued to increase in inactive fields. Sparks and Norman (2001) found that a single application of malathion ULV significantly reduced spider densities relative to pre-application densities, and densities returned to pre-application levels one week later. However, they applied malathion ULV to a single field, whereas, boll weevil eradication uses area-wide applications of malathion ULV. Mulrooney et al. (2003) found that residues of malathion ULV on leaf surface remained active against boll weevil for 4 d, and the duration was longer with multiple applications. Leggett (1992) found that spider densities decreased for two weeks following an application of malathion compared to untreated fields, after which, densities increased above levels in untreated fields. The present study showed a steady decline in spider densities with repeated applications of malathion ULV.

The red imported fire ant, *Solenopsis invicta* Buren, is ubiquitous in the southeastern and central cotton growing region of the U.S. Fire ants suppress predation of cotton aphids by lady beetles and lacewing larvae (Kaplan and Eubanks 2002), but are also important predators of bollworms and beet armyworms (McDaniel and Sterling 1982, Fillman and Sterling 1983, Diaz et al. 2004), boll weevil (Fillman and Sterling 1983), and fleahoppers (Breene et al. 1990, Nyffeler et al. 1992). Diaz et al. (2004) found that fire ants were the most common predator of beet armyworm and bollworm eggs in cotton in central Texas. Other studies have also found that fire ants are important predators of bollworm eggs (McDaniel and Sterling 1979, 1982) and larvae in cotton (McDaniel et al. 1981). In this study, densities of fire ants in the cotton canopy were significantly lower in active versus inactive eradication fields. This suggests that malathion ULV has a negative impact on fire ant foraging activity in cotton canopies. Chapter III of this thesis summarizes results from tests evaluating the effects of malathion ULV on fire ant survival, abundance, and foraging in cotton canopies, and shows that malathion ULV has a significantly negative impact on fire ant activity in cotton canopies. Malathion ULV applications may suppress foraging of fire ants in the cotton canopy and reduce predation of fire ants on beet armyworm.

Indicators of Pest Outbreaks. Secondary pest outbreaks due to insecticide use in cotton have been well documented (Luck et al. 1977). Fields treated with calcium arsenate early in the season had higher densities of bollworms late in the season than untreated fields (Sherman 1930). Evleens et al. 1973 experimentally generated an outbreak of *S. exigua* using applications of dimethoate for *Lygus hesperus* Knight control. The present study focused on boll weevil eradication, which involves frequent applications of malathion ULV applied to many cotton fields over tens of thousands of hectares. Some risks associated with boll weevil eradication have been documented. Layton and Long (2001) found higher numbers of cotton aphids in active eradication fields, a finding consistent with ours. The present study found significantly higher densities of cotton aphids in early and mid-season in active versus inactive eradication fields. Ruberson et al. (1994) reviewed the number of applications to control beet armyworms in Georgia cotton from 1980 to 1992 and found that during the years of boll weevil eradication (1987-1990) a substantial increase in the number of applications occurred relative to years when eradication was not active. In this study, significantly higher densities of beet armyworm were found later in the season in fields under eradication in 2002, though no field in this study was treated for beet armyworms because densities did not reach economic thresholds. These findings coincide with those of previous studies showing that applications of malathion ULV negatively affect beneficial cotton insect populations.

The results of this study suggest that densities of lacewing larvae and lady beetle larvae are potential indicators of cotton aphid densities. Both of these predators feed upon cotton aphids (Ridgway and Kinzer 1974, Sterling et al. 1989, Lopez et al. 1996). While these were both predictors within early and mid season regression models, it is important to note that models for 2002 pointed to lacewing larvae, and models for 2003 to lady beetle larvae. Further work needs to be done to validate these indicator species as predictors of cotton aphid densities. Differences in environmental factors, arthropod communities, and applications of malathion ULV should be evaluated to further understand why lacewing larvae were a strong predictor in 2002, and lady beetle larvae were a strong predictor in 2003.

The cumulative number of applications of malathion ULV was found to be indicators of beet armyworm densities in late season for both years. Regression analyses for bollworm densities did not indicate any single predictor common between growing seasons, although both models predicted greater than 50% of the variation in bollworm densities. However, total spiders and jumping spiders were predictors of bollworm densities in 2002 and lynx spiders in 2003; therefore, spiders warrant further examination as predictors of bollworm densities. In other studies, spiders were identified as important predators of bollworm larvae (McDaniel and Sterling 1979, 1981), and this study found a strong decline in spider densities after mid-season in fields under eradication. Concurrently lynx spiders and total spiders were significant variables in models for mid to late season densities of other worms. This indicates that spiders are important predictors of subsequent worm densities, regardless of species, and therefore warrant further examination as indicators of lepidopteran worm densities.

Conclusion. Multiple applications of malathion ULV for boll weevil eradication significantly impacted arthropod communities in cotton fields. Densities of most arthropod declined while densities of convergent lady beetle, cotton aphid, and lepidopteran caterpillars increased in fields treated repeatedly with malathion ULV. Densities of pests such as cotton aphid, beet armyworm, and bollworm were all higher in active eradication fields, and it appears that biological control of these pests is negatively impacted by malathion ULV. This study determined that lacewing larvae and lady beetle larvae may be potential indicators of cotton aphid outbreaks. Non-malathion insecticide applications were significantly correlated to cotton aphid densities, probably as a result of being applied specifically for cotton aphid control. This study failed to find reliable candidates for indicators of outbreaks of beet armyworm and other worms. Total spiders may be a good candidate for predicting bollworm outbreaks, although no single group of spiders was determined to be a good indicator.

Risks of secondary pest outbreaks increase under frequent area-wide applications of malathion ULV for boll weevil eradication. Malathion has a negative impact on the community of predatory arthropods in cotton, which increases the potential for secondary pest outbreaks. Monitoring natural enemy populations may alert pest managers to the potential for secondary pest outbreaks, thus preventing outbreaks from occurring. While it is not practical to sample all predatory arthropods in cotton, this study identified some potential candidates, and future research should focus on examining these candidates as potential indicators of secondary pest outbreaks.

CHAPTER III

EFFECT OF MALATHION ULV ON SURVIVAL AND FORAGING ACTIVITY OF THE RED IMPORTED FIRE ANT, *Solenopsis invicta* Buren, (HYMENOPTERA: FORMICIDAE), UNDER BOLL WEEVIL ERADICATION

Introduction

In contrast with traditionally negative portrayals of red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (hereafter fire ant), recent research increasingly demonstrates the beneficial roles played by fire ants in cotton fields. Upon arrival in Alabama between 1933 and 1945 (Callcott and Collins 1996), fire ants soon spread across the southern United States, and recent estimates place its distribution at approximately 114 million ha in eleven states (Callcott and Collins 1996). With their venomous sting, high densities, aggressive behavior, and their preference for human-inhabited environments, fire ants have become major pests in urban and rural areas (Porter and Savignano 1990). Fire ants damage plants (Taber 2000), and prey upon pests (Vinson 1997, McDaniel and Sterling 1982, Fillman and Sterling 1983) and their natural enemies (Risch and Carroll 1986, Lofgren 1986, Vinson 1994, Eubanks et al. 2002). In cotton fields, in particular, fire ant play dual roles. Early in the growing season fire ants promote cotton aphid, *Aphis gossypii* (Glover), population growth by “tending” aphid colonies, protecting them from natural enemies (Sterling et al. 1979, Reilly and Sterling 1983, Kaplan and Eubanks 2002, Eubanks et al. 2002). During mid- and late season they feed upon such pests as tobacco budworm *Heliothis virescens* (F.)

(McDaniel and Sterling 1979, 1982; Agnew and Sterling 1982), bollworm *Helicoverpa zea* (Boddie) (Nuessly and Sterling 1994), and beet armyworm *Spodoptera exigua* (Hübner) (Lofgren 1986, Diaz et al. 2004). Chapter II of this study showed that densities of fire ants were significantly lower in fields under boll weevil eradication, raising the question of whether predation of pests by fire ants was negatively impacted by applications of malathion ULV as made under boll weevil eradication.

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) was the most economically damaging pest in the United States before being eradicated from much of the US. Since its detection in the U.S., the boll weevil has caused an estimated $22 billion in economic losses (Kaplan 2003) and consequently, boll weevil eradication programs were implemented throughout the southern U.S. (National Cotton Council 2004). This program relies upon aerial applications of ultra low volume (ULV) malathion (Fyfanon ®, 96% AI) (Cheminova Inc., Wayne, New Jersey) applied at a rate of 876.9 ml/ha (12 oz/ac) (hereafter malathion ULV) (TBWEF 2004). Generally, in the first full season of eradication, each field within an eradication zone is sprayed on average once per week, followed by a substantial decline in number of sprays over the next 3-4 seasons. Such applications pose a problem because area-wide applications of malathion ULV for boll weevil eradication have been associated with an increase in populations of secondary pests (Summy et al. 1996, Layton and Long 2001).

Laboratory bioassays have shown malathion is highly toxic to many beneficial insects common in cotton, including *Orius insidiosus* (Say), *Geocoris punctipes* (Say), *Hippodamia convergens* (Guérin-Méneville), and *Chrysoperla carnea* (Stephens) (Bartlett 1963, 1964; England 1997; Elzen et al. 1998; Tillman and Mulrooney 2001). However, no studies have been undertaken to measure the toxicity of malathion ULV to fire ants. The potential impact of malathion ULV on fire ants came to light in recent studies. Substantial decreases in fire ant densities were found during the first full season of boll weevil eradication in one area compared to the previous season's densities (Rodrigo Diaz, unpublished data). In addition, field studies reported in Chapter II of this thesis demonstrated a significant decrease in fire ant densities in cotton plant canopies within a boll weevil eradication zone, compared to canopies from fields outside the eradication zone.

Malathion ULV may impact fire ant activity in cotton in at least two ways. First, it may be toxic to fire ants, killing foraging ants within treated cotton canopies, thus reducing fire ant densities through increased mortality. Second, it may repel fire ants, therefore reducing fire ant abundance, foraging and predation activity in malathion ULV treated cotton canopies. The objective of this study was to evaluate the impacts of malathion ULV applications on red imported fire ant activity and predation of beet armyworm eggs in cotton agroecosystems. To address these objectives, this chapter describes a series of laboratory, greenhouse, and field experiments.

Materials and Methods

Insects. Four polygyne fire ant colonies of similar size (ca. 15,000 workers) were collected from an open field ca. 8 km east of Caldwell, TX. Colonies were transferred into individual containers and maintained under greenhouse conditions and provided 5 ml honey + water (75% + 25%) and five yellow mealworms (*Tenebrio molito*r L.) per day (purchased from Fluker Farms, Port Allen, LA).

Each colony was maintained in a 28.3 liter plastic box (Rubbermaid, Wooster, OH) of dimensions (17.8 x 37.2 x 58.0 cm). Boxes were filled 5 cm deep with blasting sand #5 (Texblast, Eagle Lake, TX) for a substrate. The inner surface of the boxes was covered with fluon (Fluoropolymers USA Inc., Chadds Ford, PA) to prevent fire ants from escaping. These fire ant colonies were used first in the experiment assessing fire ant mortality and subsequently in the experiment assessing fire ant repellency.

Fire Ant Mortality Following Exposure to Malathion Treated Leaves. The purpose of this experiment was to assess mortality of fire ants exposed to malathion treated cotton leaves. Cotton leaves were collected from commercial cotton fields near Mumford, TX, that had been aerially treated with malathion ULV at a rate of 876.9 ml/ha (12 oz/ac) by the Texas Boll Weevil Eradication Program. Within 3 h after treatment, six fully expanded terminal leaves from four fields (n = 24), were collected and transported to the laboratory. Cautionary measures were utilized when collecting and handling malathion treated leaves. Twenty-four fully expanded terminal leaves collected from greenhouse-grown cotton plants served as control. A 10 cm × 10 cm square was cut from each leaf and used to line the interior of 50 ml polypropylene conical vials (Falcon BD Biosciences, San Jose, CA). Twenty worker ants from each fire ant colony described above, were placed in each of, six vials with malathion ULV treated leaves and six with control leaves. The vials were sealed with a plastic screw-top lid. Ants were exposed in these vials for one hour and then transferred to clean vials for the rest of the observation period. The numbers of dead ants were counted at this time (1 h) and 11 hours later (12 h). Two identical trials were conducted in this manner. A third trial was conducted in a similar manner, with the exception that the 50 ml vials were replaced by 50 mm Petri dishes (Sigma-Aldrich Co, St. Louis, MO) with a 40 mm diameter mesh window in the lid to allow any insecticide fumes to escape.

Mixed model analysis of variance (ANOVA) on arcsine-square root transformed proportions of dead fire ant workers was used to test for significant differences in mortality between treatments. Treatment was designated as fixed factor, while colony was designated as a random factor. Separate analyses were run for each trial due to differences between dates and methods.

Fire Ant Repellency. The purpose of this experiment was to determine if fire ant foraging decreased on wood barrier sections treated with malathion ULV compared to untreated sections. Repellency tests were conducted in the 28.3 l plastic boxes containing a fire ant colony described above. Each box was fitted with two threaded metal rods (1/4" x 26 cm long) with wing nuts and lock washers to provide tension at the center of the box. These rods held a birchwood barrier (33.0 cm x 6.4 cm) in place, which divided the container lengthwise into two equal sides. Each fire ant colony was located in one side of the box; and food and water were provided in the opposite side. This arrangement forced foraging fire ants to climb over the birchwood barrier to reach food and water.

Malathion ULV was applied to each of the wooden barriers using a Micron ULVA+ sprayer (Micron Sprayers Ltd., Herefordshire, UK) to simulate field applications. This sprayer is a hand-held spinning disc sprayer capable of depositing 50-100 μm droplets which is within the size range of the droplets applied by the Boll Weevil Eradication Program (Wolfenbarger 2001). The sprayer was calibrated to deposit 876.9 ml/ha (12 oz/ac) of malathion ULV the rate used by the boll weevil eradication program (Wolfenbarger 2001). Two hours before the experiment (1000 h), eight sections (5 cm × 5 cm) were delineated on one side of each wood barrier. Four sections were treated with malathion and four sections were left untreated to serve as controls. To selectively treat the four malathion ULV sections, all eight sections were first covered with packaging tape and the tape was subsequently removed from areas to be treated with malathion ULV. Malathion ULV was applied to the barriers using the ULVA+ ® applicator described above. Fifteen min after the application of malathion ULV, the packaging tape covering the untreated (control) sections was removed. Barriers were then placed back within each plastic box arena for the repellency tests.

Repellency was assessed by counting the number of fire ants present in each section at the time of observation. Observations were taken every 15 min for 4 h (16 observations) after placement of the barrier and food provision. Digital photos were taken to facilitate counting fire ants walking on barriers at each observation. The experiment was repeated on eight dates (one day apart) on the same colony, for a total of 64 trials.

The cumulative number of fire ants (i.e. over 4 h) within each section (malathion ULV treated, or untreated) was calculated for each date, and repeated measures ANOVA (Zar 1999) was used to test for differences between treatments. Treatment was designated as a fixed factor, and date, section (independent of treatment), and colony were designated as random factors. Student's *t*-tests were used to test for significant differences between numbers of foraging fire ants walking on malathion ULV treated sections and control sections on individual dates.

Fire Ant Abundance and Foraging Activity in the Field. The purpose of this study was to determine malathion ULV effects on fire ant abundance and foraging activity in cotton canopies. Experiments were conducted at the Texas &M Agricultural Research and Extension Center, Dallas, TX, which is within the inactive Northern Blacklands boll weevil eradication zone, and thus not subject to malathion ULV applications. Two tests were conducted, one with two applications of malathion ULV (Trial 1), and the other with a single application of malathion ULV (Trial 2), to determine if multiple applications had affected fire ant abundance and foraging differently than a single application. The cotton field was not irrigated, and the plants were in the pre-bloom stage (corresponding to early-season in Chapter II) when the experiments were initiated. The southern side of the field was used for Trial 1 and the northern side of the field for Trial 2, with ~50 m separating the trials

Eight plots measuring 12 m × 9 rows of cotton were marked for each trial. Plots were separated from each other by 12 m. Four plots received malathion ULV treatment and four plots were left untreated as controls. Eight plants in each plot, four from the third row, and four from the fifth row, were selected to monitor fire ant activity. These plants within each row were separated by 3 m. A 50 ml plastic vial (Falcon conical tubes, BD Biosciences, San Jose, CA) was attached open side up to the main terminal of each plant using gardening wire. On the first day of each trial a piece (ca. 5 cm) of processed beef frank and one jelly bean candy were placed inside each vial beginning On the first day of each trial, a piece (ca. 5 cm) of processed beef frank and one jelly bean candy were placed inside each vial beginning at 1200 h. Malathion ULV was applied to treatment plots using the micron ULVA+ ® sprayer described above; control plots were left untreated. Observations on foraging activity were made on a 4 cm area just below the bottom of the vial on the main stem of the plant. To prevent contamination with malathion ULV, all vials were sealed, and all baited plants within control plots were covered with plastic bags (33 gallon trash bags, Glad Inc., USA) before malathion ULV application.

Malathion ULV was first applied to Trial 1 on 03 July 2003, and fire ant activity was measured at -4, 4, 12, 24, 36, and 48 h after treatment. Malathion ULV was applied again to Trial 1 on 10 July 2002, and for the first and only time to Trial 2. Observations of fire ant abundance and activity (see below) were then made at -9, -6, -3, 3, 6, 9, 24, 36, and 48 h after treatment for both trials. Data were collected weekly beginning 48 h after the last application of malathion ULV in both trials and consisted of one morning (0800 h) and one evening (1900 h) observation of the same day every 7 days; two observations were made daily because fire ants are most active in the cotton canopy at these times. Fire ant abundance was measured by counting the number of fire ants in the food-baited vials fastened to the plant terminals. At the same time, fire ant foraging activity was recorded as the number of fire ants observed walking in the 4 cm marked stem area, beneath the food-baited vials, for 30 s.

Repeated measures ANOVA (Zar 1999) on log (x+1) transformed data were used to test for differences in fire ant abundance and activity between malathion ULV treated and control plots across sampling dates. ANOVA on log (x+1) transformed data was used to test for significant differences within individual sampling dates. Treatment effects were examined with Fisher's Least Significant Difference means separation test (LSD) and an α of 0.05.

Fire Ant Predation of Beet Armyworm Eggs. Predation of beet armyworm eggs was assessed in the field plots described in the previous section. Diaz et al. (2004) determined that while many insects, spiders, and mites predate *in situ* on beet armyworm eggs on cotton plants, only fire ants physically remove eggs from plants Therefore, this study used the number of eggs removed by fire ants from individual beet armyworm egg masses to assess predation of beet armyworm eggs by fire ants. Twenty-eight d after a application of malathion ULV, beet armyworm egg masses were placed on cotton plants to test for differences in predation by fire ants between plots treated with malathion ULV or left untreated. Plots employed in Trial 1 (above) were used in this study. Four beet armyworm egg masses were placed on the terminal of four plants in each plot at 1900 h. Plants were selected from 1 m to the left of plants used in Trial 1, on the fifth row. The number of eggs per egg mass was adjusted to 20-25 eggs. Egg masses were glued to a terminal leaf using gum Arabic adhesive (Diaz et al. 2004). All egg masses were collected 12 h after placing them on cotton plants, and the number of missing eggs was recorded for each mass. The percentage of beet armyworm eggs missing per egg mass were arcsine square-root transformed for analysis. Comparisons were made between malathion ULV treated and control plots using ANOVA methods (Zar 1999). Treatment effects were examined with Fisher's Least Significant Difference means separation test (LSD) and an α of 0.05.

Results

Fire Ant Mortality. Fire ant worker mortality was significantly greater in vials containing malathion ULV treated leaves than in vials with control leaves in all three trials (Fig. 13). Mean ant mortality was 98.5 % (95% CI = 97.5% to 99.5%) after 1 hr exposure to malathion ULV treated leaves and 4.0% (95% CI = 3.0% to 5.0%) on control leaves and differences were significant ($P< 0.001$) (Fig. 13a). Mean mortality 12 h after exposure was significantly greater ($P< 0.001$) on malathion ULV treated leaves, with 100% mortality on treated leaves and 11.3% mortality (95% CI = 9.02% to 13.5%) on control leaves (Fig. 13b).

Fire Ant Repellency. Overall, fire ant activity on the birchwood barrier was significantly lower on surfaces treated with malathion ULV compared to surfaces left untreated ($P = 0.01$) (Fig. 14), though differences were not significant on some

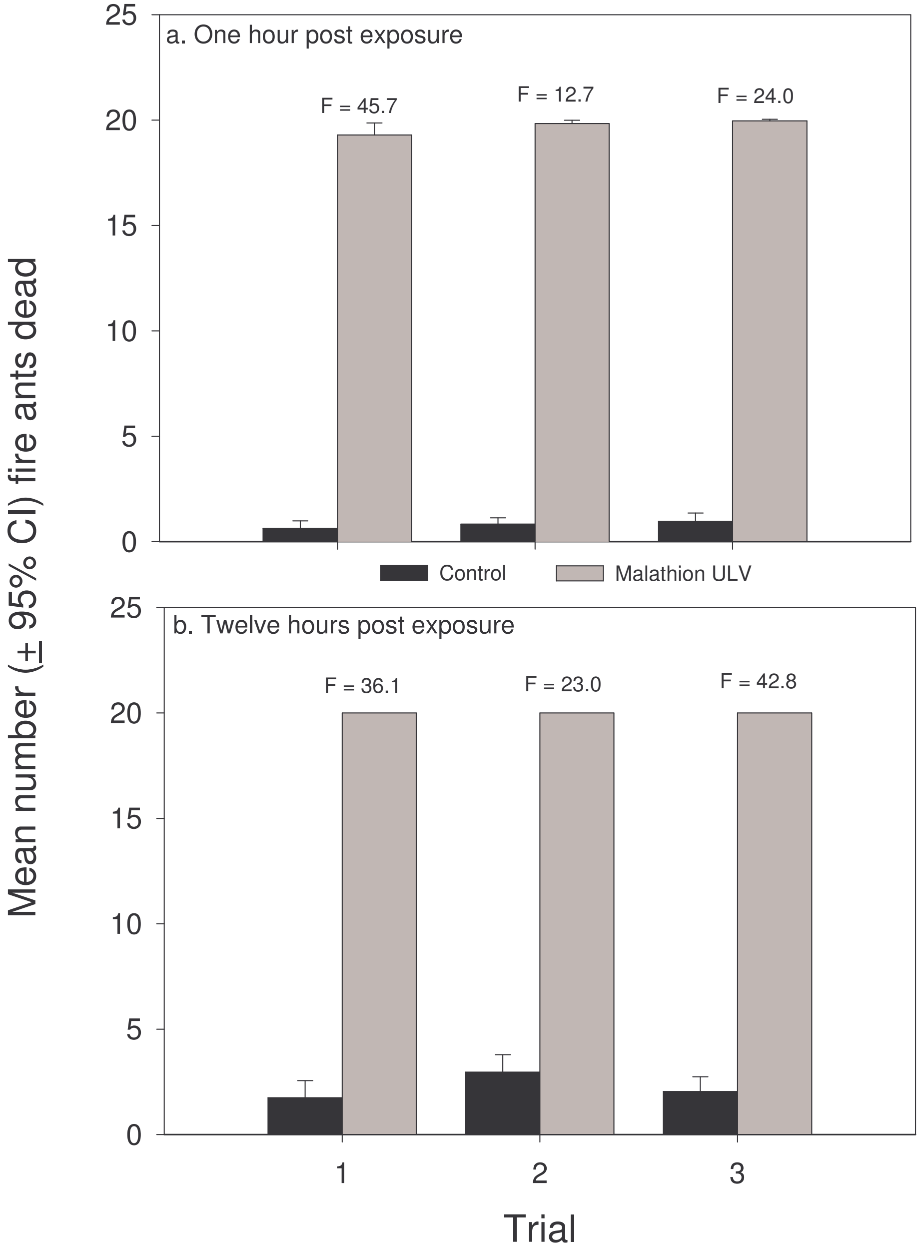

Fig. 13. Number of *Solenopsis invicta* dying after exposure for (a) 1 h and (b) 12 h to leaves either treated with malathion ULV in the field 3 h earlier or left untreated in three independent trials. Trials 1 and 2 involved sealed plastic vials, while Trial 3 involved Petri dishes with mesh screening. For each trial, differences were significant in both time periods using mixed model ANOVA (F -values inset) ($P < 0.001$, df = 1, 46) for each trial.

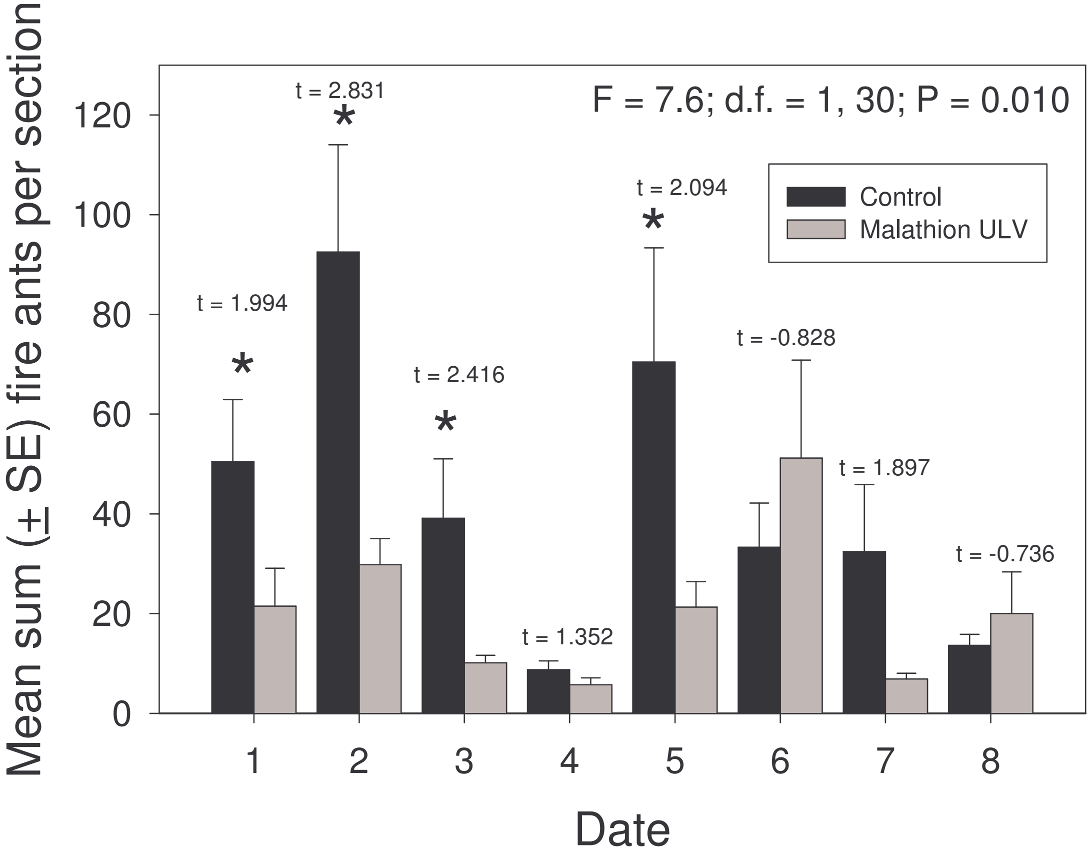

Fig. 14. Mean cumulative number (16 observations over 4 h) of *Solenopsis invicta* observed on birchwood barrier surfaces treated with malathion ULV or left untreated. Differences were significant across all dates combined (*F* statistics inset). Asterisks indicate significant differences according to *t*-tests on individual dates (P < 0.001, df = 1, 30 for each date)); differences are not significant on dates lacking asterisk (*t*-values inset, P ≥ 0.067, df = 1, 30 for each date).

individual dates. Data from dates 1, 2, 3, and 5 showed significant differences ($P \leq 0.045$) (control mean = 63.14 ± 9.11 ants; malathion ULV treated mean = 20.1 ± 2.7 ants); while data from dates 4, 6, 7, and 8 did not show significant differences ($P \geq 0.067$) (control mean = 22.0 ± 4.2 ants; malathion ULV treated mean = 21.0 ± 5.7 ants) (Fig. 14).

Fire Ant Abundance and Foraging Activity in the Field. In both trials, fire ant abundance, measured as mean number of fire ants captured in baited vials, was significantly greater in control plots compared to malathion ULV treated plots across all dates ($P < 0.001$ for both trials) (Fig. 15 a, b). In Trial 1, the mean number of ants was 6.9 ± 1.0 per baited vial in malathion ULV treated plots compared to 22.1 ± 1.2 per vial in control plots across all dates (Fig. 15a). In Trial 2, the mean number of ants was 2.5 ± 0.5 per vial in malathion ULV treated plots compared to 10.8 ± 0.7 per vial in control plots (Fig. 15b).

In Trial 1, the mean numbers of fire ants per vial were not significantly different between treatments 4 h prior to application of malathion ULV, but were significantly less in the malathion ULV treated plots from 0 h to 550 h ($P < 0.0001$) (Fig. 15a). Fire ant abundance in Trial 2 followed a similar pattern as ant density per vial was not significantly different between treatments 9 h prior to application of malathion ULV, but were significantly less in the malathion ULV treatment 0 h to 500 h after application ($P < 0.0001$) (Fig. 15b).

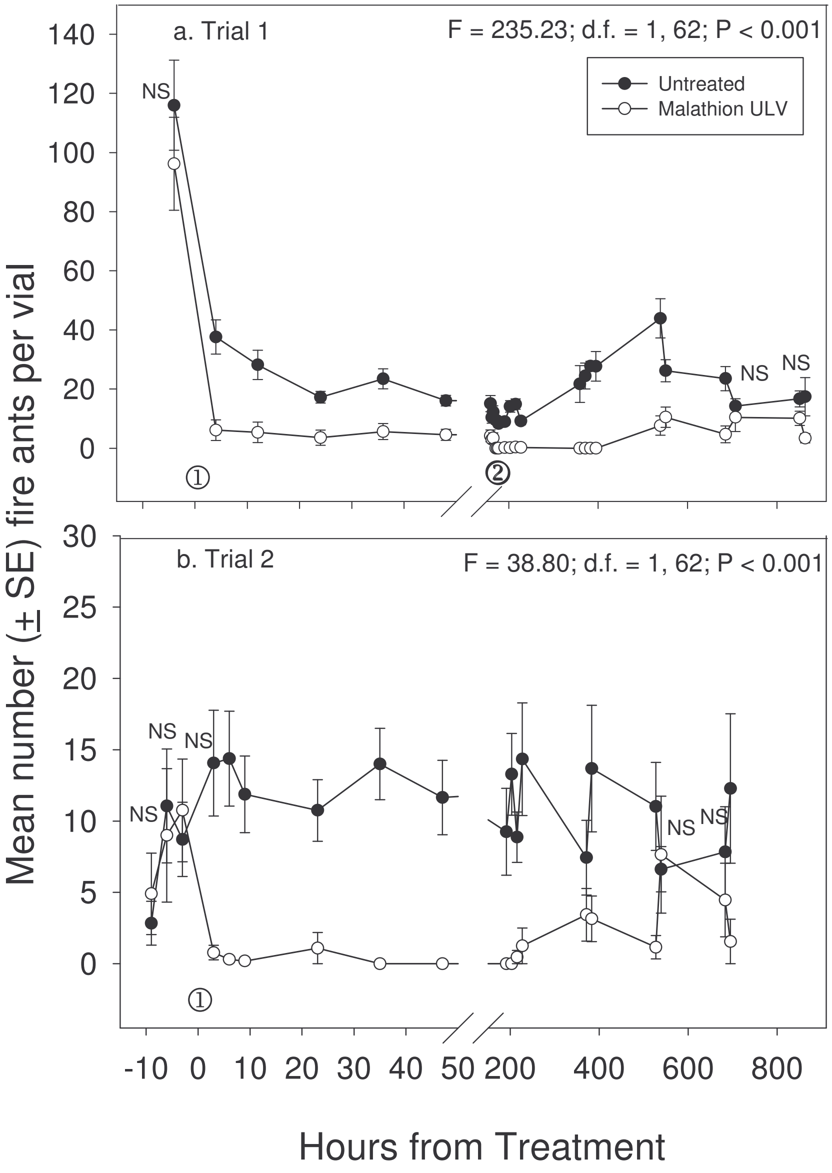

Fig 15. Mean number of fire ants within baited vials in cotton treated with malathion ULV or left untreated, in two independent trials, (a) Trial 1, and (b) Trial 2. In both trials, mean number of fire ants were not significantly different between treatments before application of malathion ULV (①). After application, fire ant numbers in malathion treated plots were significantly less ($P < 0.01$) than numbers in untreated plots through ca. 700 hrs in Trial 1 and 500 hrs in Trial 2. Applications of malathion ULV are indicated with ① and ② respectively. NS indicates a lack of significant difference in mean numbers of fire ants at α 0.05.

Fire ant activity, measured as the number of ants on a 4 cm segment of a main cotton stem, was significantly less in the malathion ULV treated plots across all dates in both trials ($P < 0.0001$) (Fig. 16a, b).

In Trial 1, the mean number of ants walking on the stem was 1.5 ± 0.2 in the malathion ULV treated treatment compared to 5.8 ± 0.3 in control plots across all dates (Fig. 16a). In Trial 2, the mean number of ants walking on the stem was 0.7 ± 1.1 in malathion ULV treated plots compared to 3.5 ± 0.2 in control plots across all dates (Fig. 16b). In Trial 1, ant activity was similar between treatments 4 hours prior to application, but was significantly less in the malathion ULV treatment 4 h to 700 h (29 d) ($P < 0.0001$) (Fig. 16a). In Trial 2, fire ant activity was similar between plots 9 hours prior to application, and significantly less in malathion ULV treated plots from 3h to 520h (21 d) ($P < 0.0001$) post application (Fig 16b)

Fire Ant Predation of Beet Armyworm Eggs. A significantly greater percentage of beet armyworm eggs was removed from egg masses in control plots (90.6% ± 2.9) relative to egg masses in malathion ULV treated plots (36.4% ± 3.3) ($F = 38.56$; $df = 1, 94$; $P < 0.0001$).

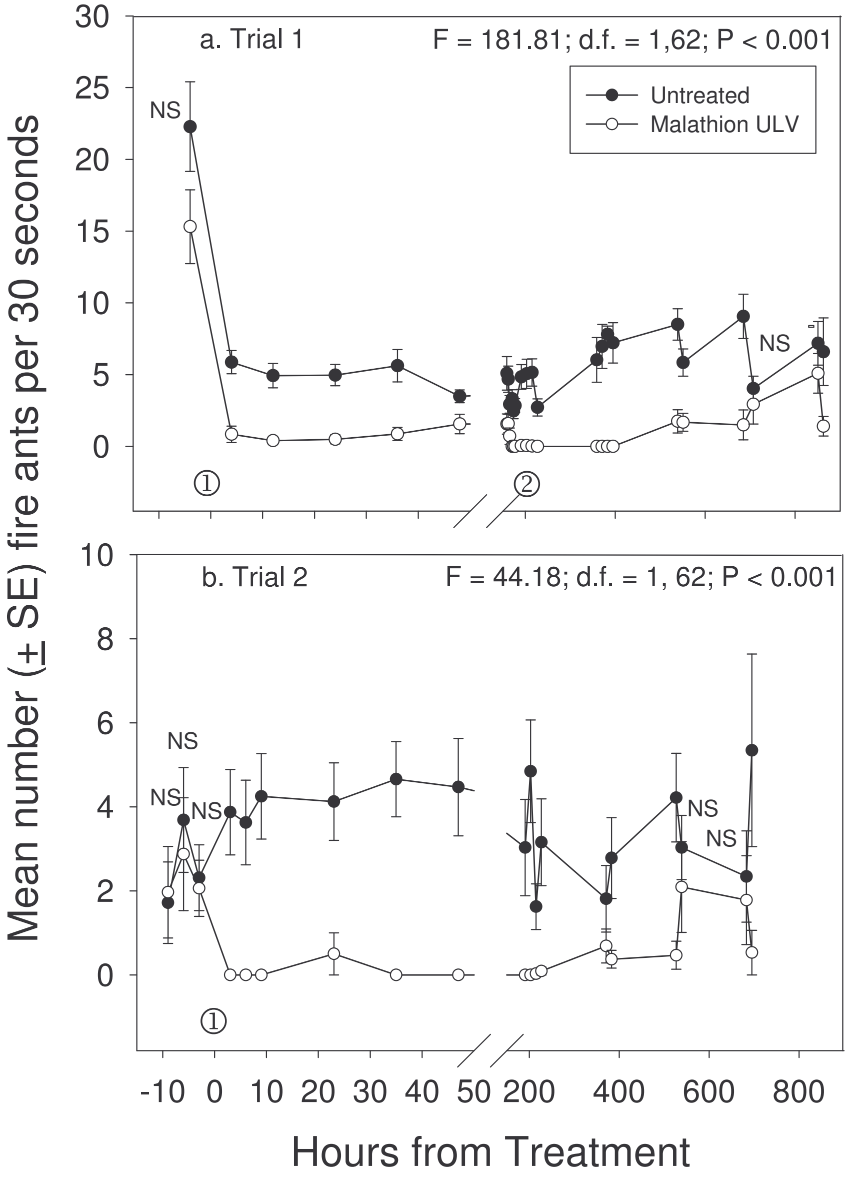

Fig 16. Mean number of fire ants walking on a 4 cm stem segment within 30 s in plots treated with malathion ULV or left untreated, in two independent trials, (a) Trial 1, and (b) Trial 2. In both trials, mean number of fire ants were not significantly different between treatments before application of malathion ULV (①). After application of malathion ULV, fire ant numbers in malathion treated plots were significantly less ($P < 0.01$) than numbers in untreated plots through ca. 700 hrs in Trial 1 and 500 hrs in Trial 2. Applications of malathion ULV are indicated with ① and ② respectively. NS indicates a lack of significant difference in mean numbers of fire ants at α 0.05.

Discussion

Malathion ULV, as applied by the Texas Boll Weevil Eradication Program, significantly increased fire ant mortality under laboratory conditions, had a repellent effect on fire ants under greenhouse conditions, and decreased fire ant abundance, foraging, and predatory activity under field conditions. In the laboratory, fire ant mortality was 24-fold and 10-fold greater 1 h and 12 h, respectively, after exposure to malathion ULV treated leaves compared to control leaves. In the greenhouse, significantly more fire ants foraging for food were observed on untreated surfaces relative to malathion ULV treated surfaces. In the field, one or two applications of malathion ULV reduced the abundance of fire ants foraging in the cotton canopy for 21-29 days after application. Predation of beet armyworm eggs was 2.5-fold greater in untreated cotton relative to cotton treated with malathion ULV.

In this study, residues of malathion ULV on cotton leaves following aerial applications were found to be highly toxic to fire ants and resulted in 98.5% mortality of ants after one h exposure. Acephate and chlorpyrifos are highly toxic to fire ants and are organophosphate insecticides as is malathion (Seagraves and McPherson 2003). Laboratory studies have shown that malathion is highly toxic to beneficial insects common in cotton agroecosystems such as *H. convergens, C. carnea, O. insidiosus,* and *G. punctipes* (Bartlett 1963, 1964, England et al. 1997, Elzen 1998). However, none of these studies evaluated the toxicity of malathion to fire ants. The demonstrated susceptibility of fire ants and of other generalist predators in cotton to malathion will likely influence the dynamics of pest populations through reduced levels of predation.

Due to the evident high toxicity of malathion ULV to fire ants, which are important predators in cotton fields (McDaniel and Sterling 1979, Agnew and Sterling 1982, Fillman and Sterling 1983, Lofgren 1986, Nuessly and Sterling 1994, Kaplan and Eubanks 2002), it will be important to monitor fields under boll weevil eradication for reductions in fire ant densities, and possible impacts on pest predation levels.

Malathion ULV significantly decreased the numbers of fire ants crossing a treated surface while foraging for food. In contrast, Pranschke et al. (2003) found that two formulations of bifenthrin had no repellency effect on fire ants. However, Pranschke et al. defined repellency as an absence of ants on treated surfaces, whereas the present study quantified numbers of fire ants on surfaces over time. While the present study found a significant reduction in activity on malathion ULV treated surfaces, had we used repellency criteria similar to Pranschke et al. (2003), we likely would have failed to confirm that malathion was repellent to fire ants. The results of this study indicate that malathion ULV has a repellent effect on foraging fire ants, though the effect is not strong enough to completely exclude fire ants from foraging across treated surfaces. In fields treated with malathion ULV, it is likely that fire ants will continue foraging, though at levels lower than in untreated fields.

Treatment with malathion ULV significantly decreased fire ant abundance, foraging, and predatory activity in cotton canopies up to 3 wk after an application. Mulrooney et al. (2003) found that malathion ULV residues accumulated with each application. Moreover, a single application of malathion ULV was found to reduce densities of lady beetle larvae, adult *Scymnus* lady beetles, and spiders, relative to pre-treatment densities (Sparks and Norman 2001), though these returned to pre-application densities within one week. However, fire ants were not monitored in that study. Results of this study showed that there is no difference in the duration of the effects on fire ant activity between one and two treatments of malathion ULV. In both cases, fire ant abundance and foraging activity in the cotton canopy were significantly reduced for 21-29 days following the final application. In the first full season of boll weevil eradication, fields are typically treated with malathion ULV ca. once per week, with a reduction in frequency over the subsequent 4-5 yr. With weekly applications, it is likely that suppression of fire ant densities would continue throughout the season, an effect likely recorded in Chapter II (Fig 6). Suppression of fire ants in this manner may lead to increased densities of pests typically preyed upon by fire ants. While a suppression of fire ants may allow an increase in natural enemies due to lessened intraguild predation and prey competition (Lofgren 1986, Porter 1991, Vinson 1994, Cook 2003, Diaz et al. 2004), many of the insects this would benefit are also susceptible to malathion ULV. Therefore, use of malathion ULV for boll weevil eradication poses the risk of increased pest densities due to reductions in the densities of various natural enemies, including fire ants, spiders, and lacewings.

Conclusion. Although fire ant abundance and foraging in the field are reduced following applications of malathion ULV, it is unclear whether this is due to mortality, repellency, or both. In any case, application of malathion for boll weevil eradication decreases fire ant activity in the cotton canopy, thus impacting the dual role of fire ants in cotton fields. Middle to late season applications of malathion ULV may hinder the beneficial role fire ants have in controlling cotton pests such as bollworm and beet armyworms (McDaniel and Sterling 1979, Lofgen 1986, Diaz 2004). Suppression of fire ant foraging activity, and subsequent reduction in predation of beet armyworm eggs due to malathion ULV may partially explain the higher number of insecticide applications for beet armyworm control in Georgia during years of boll weevil eradication (Ruberson et al. 1994). However, this is not the only factor associated with beet armyworm outbreaks as they have occurred in cotton in California and west Texas, two areas not subject to boll weevil eradication efforts based on malathion ULV applications (Stewart et al. 1996). The repeated applications of malathion used in boll weevil eradication have the potential to disrupt biological control of aphid and lepidopteran pests in cotton. Due to the increase risk of outbreaks of these secondary pests, it is imperative that fields under boll weevil eradication be monitored closely for the possibility of increased pest densities.

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

Cotton production, worth ca. $906 million, is an important component of the Texas economy (National Cotton Council 2004). Yield loss due to pest damage, worth an estimated $53 million in bale loss during 2002, is a major problem in Texas (Williams 2004). The boll weevil has been the leading cotton pest, causing an estimated $22 billion in accrued economic losses since it first entered the U.S. (Kaplan 2003). Currently, the boll weevil is the focus of eradication efforts in several U.S. states, and has been eradicated from the western U.S. and a number of southeastern states (National Cotton Council 2004). However, frequent, area-wide applications of malathion ULV for boll weevil eradication pose some risks, including an increased potential for secondary pest outbreaks. Currently, secondary pests are monitored throughout the season, though economic loss is possible before an outbreak occurs.

Secondary pest outbreaks due to insecticide use are a well known phenomenon, and in cotton are believed to result from disruption of biological control following decimation of populations of natural enemies. The risk of secondary pest outbreaks is especially significant when malathion ULV is applied frequently and over extensive areas for boll weevil eradication. Malathion ULV is known to be highly toxic to a number of beneficial cotton insects, and the negative impact boll weevil eradication has on them likely reduces their effectiveness as biological control agents of secondary pests.

Results presented in Chapter II showed an increase in densities of cotton aphid, beet armyworm, bollworm, and other worms (primarily loopers and saltmarsh caterpillars) in fields under boll weevil eradication, compared to non-eradication fields. Concurrently, population densities of many predatory insects and spiders sampled were significantly lower in active boll weevil eradication fields relative to inactive fields. However, *H. convergens* adults, and the complex of *H. convergens, C. septempunctata, C. maculata,* and *H. axyridis* larvae, were significantly greater in active eradication fields, compared to inactive fields. This was mostly like due to increased densities of cotton aphid which is a common prey of lady beetles. Spider densities were similar between active boll weevil eradication fields and inactive fields in early season. however, during middle and late season, densities in eradication fields decreased, whereas spider densities in non-eradication fields increased. This suggested that as beneficial insect and spider densities decreased, so did suppression levels of pests such as beet armyworm and bollworm. If the relationships between beneficial insect and spider populations and pests are not monitored, then the potential for serious pest outbreaks likely increases.

Correlation and regression analyses pointed to many significant relationships between predator and pest densities. Regression analyses suggested that densities of lacewing larvae and lady beetle larvae may be good indicators of cotton aphid densities throughout the season. Densities of beet armyworm and other worms were low in both seasons of study, making it difficult to identify suitable candidates for indicators of beet armyworm or other worm densities. However, regression analyses suggested that the number of malathion ULV treatments may be a predictor of beet armyworm densities and total spiders is an important predictor of other worm densities. Regression analyses for bollworm suggested that total spider density may be a potential indicator of bollworm density.

Fire ants are important in the cotton community where they perform dual roles. Fire ants “tend” cotton aphid, but they also prey upon pests, such as beet armyworm and bollworm, as well as other beneficial insects through intraguild predation. This study found that malathion ULV applications negatively impacted fire ant foraging and predation in the cotton canopy. Malathion ULV was found to be acutely toxic to fire ant under laboratory conditions and reduced the number of foraging fire ants on treated surfaces, while a repellent effect also reduced the number of foraging fire ants on treated surfaces. In the field, this study showed that cotton plants treated with malathion ULV had significantly fewer fire ants in the cotton canopy for up to three weeks after treatment compared to untreated cotton plants. Also, predation of beet armyworm eggs was significantly reduced in malathion ULV treated plots relative to untreated plots. This suggested that boll weevil eradication negatively impacted fire ant abundance and foraging activity in the cotton canopy.

The Texas Boll Weevil Eradication Foundation frequently and intensively applies malathion ULV for boll weevil eradication. Therefore, it is important to closely monitor fields under eradication for outbreaks of pests which are biologically controlled by generalist predators. Results from this study suggest several predator groups which may be useful indicators that biological control is being disrupted by malathion treatments and that the risk for secondary pest outbreaks is increasing. Further research is needed to validate these groups as potential indicators of pest outbreaks under boll weevil eradication.

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